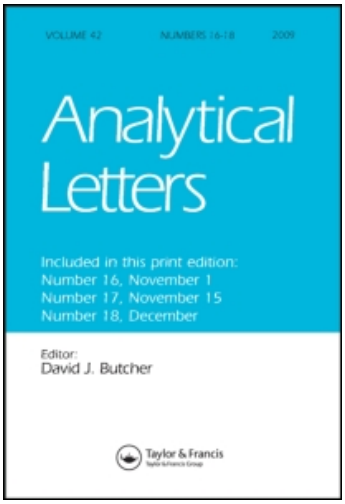

Analytical Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597227

SPECTROPHOTOMETRIC DETERMINATION OF FAMOTIDINE THROUGH OXIDATION WITH *N*-BROMOSUCCINIMIDE AND CERRIC SULPHATE

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Online publication date: 20 August 2002

To cite this Article Amin, A. S. , Shama, S. A. , Ahmed, I. S. and Gouda, E. A.(2002) 'SPECTROPHOTOMETRIC DETERMINATION OF FAMOTIDINE THROUGH OXIDATION WITH *N*-BROMOSUCCINIMIDE AND CERRIC SULPHATE', Analytical Letters, 35: 11, 1851 — 1862
To link to this Article: DOI: 10.1081/AL-120013588
URL: http://dx.doi.org/10.1081/AL-120013588

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ANALYTICAL LETTERS
Vol. 35, No. 11, pp. 1851–1862, 2002

MOLECULAR SPECTROMETRY

SPECTROPHOTOMETRIC DETERMINATION OF FAMOTIDINE THROUGH OXIDATION WITH *N*-BROMOSUCCINIMIDE AND CERRIC SULPHATE

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ABSTRACT

Three simple, accurate, sensitive and selective spectrophotometric methods (A, B and C) for the determination of famotidine (Fam) in bulk sample, in dosage forms and in the presence of its oxidative metabolites are described. The first method A is based on oxidation of the drug by *N*-bromosuccinimide (NBS) and determination of the unreacted NBS by measuring the decrease in absorbance of Amaranth dye (AM) at a suitable λ_{max} (521 nm). The methods B and C involve addition of excess cerric sulphate and determination of the unreacted Ce(IV) by decrease the red colour of chromotrope 2R (C2R) at λ_{max} 528 nm for method B or decrease the orange pink colour of rhodamine 6G (Rh6G) at λ_{max} 526 nm for method C. Regression analysis of

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DOI: 10.1081/AL-120013588
0003-2719 (Print); 1532-236X (Online)

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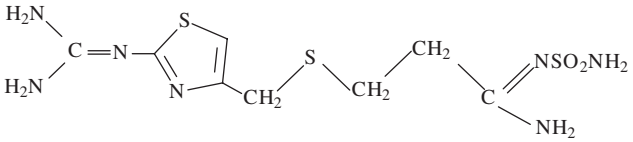
Beer-Lambert plots showed good correlation in the concentration ranges 0.1–2.4 μg mL^{-1} for method A and 0.1–2.2 μg mL^{-1} for methods B and C. The apparent molar absorptivity, Sandell sensitivity, detection and quantitation limits were calculated. For more accurate results, Ringbom optimum concentration ranges were 0.2–2.2 μg mL^{-1} for method A and 0.2–2.0 μg mL^{-1} for methods B and C. The stoichiometric ratio between the drug (Fam) and the oxidant (NBS or Ce^{4+}) was estimated. The validity of the proposed methods were tested by analysing pure and dosage forms containing famotidine and in the presence of its oxidative degradates. Statistical treatment of the results reflects that the proposed procedures are precise, accurate and easily applicable for the determination of famotidine in pure form, in pharmaceutical preparations and in the presence of its oxidative degradates.

Key Words: Famotidine; Spectrophotometry; Oxidation reaction; *N*-Bromo-succinimide; Cerric sulphate; Pharmaceutical analysis

INTRODUCTION

Famotidine, 3-[(2-guanidinethiazole-4-yl)methylthio-]*N*-sulfamoyl propion-amidine, is a relatively new histamine H_2-receptor antagonist. It is now widely used for the treatment of duodenal ulcers, benign gastric ulcer, reflux oesophagitis and hyper-acid secretory conditions. Famotidine is rapidly but incompletely absorbed (40–45% bioavailability). As therapeutic doses of famotidine recommended in patients are low (40 mg daily), these doses produce very low therapeutic concentrations in plasma (78 ng mL^{-1}). A small proportion of famotidine is metabolised in the liver to famotidine *S*-oxide, but most is excreted unchanged in the urine. Famotidine sulfoxide is the only metabolite identified in humans. The drug is eliminated by renal (65–70%) and metabolic (30–35%) routes.[1]



Famotidine

Several methods have been reported for determination of famotidine including chromatography,[2,3] HPLC,[4–6] polarography,[7] voltammetry,[8] potentiometry,[9] spectrofluorimetry,[10] and spectrophotometry.[11–18] The spectrophotometric methods suffer from disadvantages such as lack selectivity and have low sensitivity, take long reaction time for colour development, and require prior extraction of the coloured product. This work describes three visible spectrophotometric methods, which are superior to the reported ones, for their selectivity and high sensitivity. Azo dye such as Amaranth and chromotrope 2R, and xanthene dye such as rhodamine 6G are well known for their high absorptivity and they will have been utilized for estimation of excess oxidant (*N*-bromosuccinimde and cerric sulphate). The present work aims to demonstrate a simple, rapid, accurate, sensitive and selective spectrophotometric methods suitable and convenient for the determination of famotidine in pure form, in dosage forms and in the presence of its oxidative degradates, where modern and expensive apparatus such as GLC, HPLC and HPTLC are not available.

EXPERIMENTAL

Apparatus

All the absorption spectral measurements were made using JASCO V-530 (UV–VIS) spectrophotometer (Japan), with scanning speed 400 nm/min and band width 2.0 nm, equipped with 10 mm matched quartz cells.

Materials and Reagents

All chemicals used were of analytical or pharmacopoeia grade purity, and water was doubly distilled. Pure famotidine was obtained from Amoun Pharmaceutical Company, Egypt. Stock famotidine solution (200 $\mu g\,mL^{-1}$) was prepared by dissolving 20 mg in water and adjusted to 100 mL with water. Working solutions of lower concentration were prepared by serial dilutions. Aqueous solutions of Amaranth (Merck, 2.0 mM), Chromotrope 2R (Aldrich, 5.0 mM) and Rhodamine 6G (BDH, 1.0 mM) were prepared by dissolving an appropriate weight in 100 mL water. A solution of cerium(IV) sulphate (May and Baker, 3.0 mM) was prepared by dissolving known weight of $Ce(SO_4)_2$ in least amount of warm 1.0 M H_2SO_4 in a 250 mL calibrated flask, then adjusted with the same acid to the volume. An aqueous solution of *N*-bromosuccinimide (Aldrich, 100 $\mu g\,mL^{-1}$) was freshly

prepared. A solution of 5.0 M HCl was prepared and standardized as recommended previously[19] prior to use.

Recommended Procedure

Method A

To each 10 mL volumetric flask containing 1.0–24 $\mu g\,mL^{-1}$ Fam solution, 2.0 mL of 100 $\mu g\,mL^{-1}$ NBS, 1.25 mL of 5.0 M HCl and 1.0 mL of 1.0% KBr were transferred and the solutions were diluted to 7.0 mL. After 5.0 min, 0.70 mL of 2.0×10^{-3} M Amaranth dye was added, mixed throughout and volume adjusted with water. The absorbance was measured at 521 nm against a blank solution prepared in the same manner without drug. Calibration graph was prepared by plotting absorbance of the dye against the drug concentration. The amount of drug in any sample was calculated from its calibration curve.

Methods B and C

These methods depend on oxidation of Fam performed by adding 1.0–22 $\mu g\,mL^{-1}$ of Fam to an excess volume (1.0 mL) 3.0 mM $Ce(SO_4)_2$ containing 1.0 M H_2SO_4. The solution was boiled in a water bath for 5.0 min. The mixture was cooled and 0.35 mL of 5.0 mM C2R was used for method B, or 0.30 mL of 1.0 mM Rh6G was mixed to warm solution and then cooled for method C. The volume was completed to 10 mL with water. The decrease in colour intensities of C2R or Rh6G were measured spectrophotometrically at their corresponding maximum wavelengths 528 or 526 nm, respectively. The concentration range was determined in each case by plotting the concentration of Fam against absorbance at the corresponding λ_{max}.

Preparation of Degradation Products

A suitable amount (0.1 g) of Fam was dissolved in 10 mL 0.1 M HCl and then 1.0 mL of 12 % H_2O_2 was added. The solution was boiled in water bath for 45 min and then diluted in 100 mL volumetric flask to the mark with water. The stock solution was diluted quantitatively to obtain degraded sample of the required concentrations.

Procedure for Dosage Forms

At least 10 tablets of the drug were weighed into a small dish, powdered and mixed well. A portion equivalent to 20 mg was weighed and dissolved in 100 mL water, shaken well and filtered through a sintered glass crucible G_4. A 10 mL aliquot of the test solution (200 μg mL^{-1} of Fam) was diluted to 100 mL in volumetric flask. One millilitre of this solution was then treated as described above in procedure A, B and C.

RESULTS AND DISCUSSION

We have developed simple spectrophotometric methods for the determination of famotidine. The presence of the sulphur atom makes this compound liable to atmospheric oxidation forming *S*-oxide derivative. The structural activity relationship shows that, these oxidative degradates (*S*-oxide) are inactive as antipeptic ulcer. For this reason the establishment of methods that quantitatively determine the pure drug in presence of its degradates are of great pharmaceutical value.

Method A

This method involves two steps namely:

1. Reaction of the drug with an excess of oxidant (NBS) giving products involving oxidation.
2. Estimation of excess oxidant by measuring the decrease in red colour of Amaranth dye spectrophotometrically at λ_{max} 521 nm.

Several experiments were done to achieve the optimum parameters, i.e., effect of acid concentration, time, KBr concentration, sequence of additions, and effect of dye concentration. It was found that 1.25 mL of 5.0 M HCl, 1.0 mL of 1.0% KBr and 0.70 mL of 2.0 mM Amaranth dye are required for maximum colour development. The reaction takes place completely in presence of KBr after 5.0 min of mixing. Fam–NBS–HCl–KBr is the optimum sequence of addition. The effect of reaction time after the addition of dye indicated that shaking for 1 min is sufficient to give reliable results. NBS reacts with Fam with consumption of 10 moles of NBS per each mole of Fam giving a mixture of products. The remaining oxidant reduces the intensity of red colour of amaranth dye through disruption of the conjugation system in the dye. The remaining colour stays constant in absorbance for at least 48 h then slightly decrease afterwards.

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Methods B and C

These methods involve two steps:

1. Oxidation of Fam with excess $Ce(SO_4)_2$ in acid medium with heating.
2. Determination of the unreacted oxidant (Ce^{4+}) by measuring the decrease in absorbance of C2R or Rh6G at a suitable λ_{max} 528 or 526 nm for the two methods, respectively.

Investigations were carried out to achieve maximum colour development in the quantitative determination of Fam. The influence of each of the following variables on the reaction was tested. The most suitable acid to be used with $Ce(SO_4)_2$ was found to be sulphuric acid of 1.0 M concentration presented as 10% (v/v) of total volume in reaction mixture. The oxidation process of Fam with $Ce(SO_4)_2$ is catalyzed by heat and reach maximum at 100°C. The time required to complete the reaction is 5.0 min. After oxidation process, the solution must be cooled at least for 3.0 min before addition of C2R for method B. However for method C, the addition of Rh6G to the hot solution give maximum colour intensity. The optimum volume of dye used for the production of maximum and reproducible colour intensity is 0.35 mL of 5.0 mM of C2R for method B or 0.30 mL of 1.0 mM Rh6G for method C. The effect of time after the addition of dye indicated that shaking for 1 min is sufficient to give reliable results in case of using C2R, whereas in case of using Rh6G the solution must be shaken for 3 min to give reliable results. $Ce(SO_4)_2$ reacts with Fam with consumption of 34 moles of $Ce(SO_4)_2$ per each mole of Fam, giving a mixture of products. The remaining Ce^{4+} reduces the colour intensity of C2R or Rh6G through disruption of the conjugation system in the dye. The remaining colour stays constant in absorbance for at least 48 h then slightly decrease afterwards.

Quantification

Beer–Lambert law limits, molar absorptivities, Sandell sensitivities, regression equations and correlation coefficients were calculated and shown in Table 1. The limits of detection ($K = 3$) and quantitation ($K = 10$) were established according to IUPAC definitions.[20] In order to determine the accuracy and precision of the methods, solutions containing three different concentrations of Fam were prepared and analysed in 6 replicates. The analytical results obtained from this investigation were summarized in Table 2.

Table 1. Optical and Regression Characteristics

Parameters	Methods		
	A	B	C
Beer's law limits ($\mu g\,mL^{-1}$)	0.1–2.4	0.1–2.2	0.1–2.2
Ringbom limits ($\mu g\,mL^{-1}$)	0.2–2.2	0.2–2.0	0.2–2.0
Molar absorptivity ($L\,mol^{-1}\,cm^{-1}$)	1.78×10^5	1.95×10^5	1.82×10^5
Sandell sensitivity ($ng\,cm^{-2}$)	1.88	1.73	1.85
Detection limits ($ng\,mL^{-1}$)	48	46	53
Quantitation limits ($ng\,mL^{-1}$)	88	70	98
Regression equation*:			
Slope (b)	0.529	0.577	0.538
Standard deviation of slope (s_b)	5.72×10^{-4}	6.28×10^{-4}	4.92×10^{-4}
Intercept (a)	1.66×10^{-3}	4.95×10^{-3}	2.78×10^{-3}
Standard deviation of intercept (s_a)	2.11×10^{-4}	7.35×10^{-4}	3.11×10^{-4}
Correlation coefficient (r)	0.9996	0.9995	0.9992

*With respect to $A = a + bC$ where C is concentration ($\mu g\,mL^{-1}$) and A is absorbance unit.

Table 2. Evaluation of the Accuracy and Precision of the Proposed Procedures

Method	Taken ($\mu g\ mL^{-1}$)	Recovery (%)	RSD[a] (%)	RE (%)	Confidence Limits[b]
A	0.5	101.2	0.80	0.84	0.506 ± 0.0042
	1.0	99.7	0.60	0.63	0.997 ± 0.0063
	2.0	99.6	0.35	0.37	1.993 ± 0.0073
B	0.5	101.4	1.00	1.05	0.507 ± 0.0052
	1.0	100.3	0.60	0.63	1.003 ± 0.0063
	2.0	99.4	0.40	0.42	1.988 ± 0.0084
C	0.5	101.6	1.20	1.26	0.508 ± 0.0063
	1.0	100.2	0.70	0.73	1.002 ± 0.0073
	2.0	99.5	0.45	0.47	1.990 ± 0.0094

[a]Relative standard deviation for six determinations.
[b]95% confidence limits and 5 degrees of freedom.

Table 3. Analysis of Fam in the Presence of Its Degradation Products

	Method A		Method B		Method C	
Exp. No.	Conc. of Degradation Product Added[a] ($\mu g\,mL^{-1}$)	Recovery[b] (%)	Conc. of Degradation Product Added[a] ($\mu g\,mL^{-1}$)	Recovery[b] (%)	Conc. of Degradation Product Added[a] ($\mu g\,mL^{-1}$)	Recovery[b] (%)
1	1.0	100.2	1.0	100.6	1.0	100.8
2	2.0	101.6	2.0	101.6	2.0	101.8
3	4.0	101.8	4.0	103.6	4.0	103.2
4	10	103.4	10	104.2	10	104.2

[a]Each mixture contains 2.0 $\mu g\,mL^{-1}$ of Fam.
[b]Each result is the average of three experiments.

Table 4. Determination of Famotidine in Pharmaceutical Formulations Using the Proposed and Official Methods

Preparation	Supplier	Nominal Value	Recovery ± S.D.%[a]			Official Method
			A	B	C	
Antodine (tablets) B.N. 2377	Amoun[1]	20 (mg/tablet)	99.9 ± 0.8 $F^b = 3.06$ $t^b = 0.15$	99.6 ± 0.9 $F^b = 2.42$ $t^b = 0.29$	100.2 ± 1.0 $F^b = 1.96$ $t^b = 0.57$	99.8 ± 1.4
Famotin (tablets) B.N. 300941	Memphis[2]	20 (mg/tablet)	99.5 ± 0.7 $F^b = 3.45$ $t^b = 0.16$	99.9 ± 0.8 $F^b = 2.64$ $t^b = 0.48$	100.4 ± 0.9 $F^b = 2.09$ $t^b = 1.24$	99.6 ± 1.3
Peptec (tablets) B.N. 029	Julphar[3]	20 (mg/tablet)	100 .3 ± 0.8 $F^b = 1.89$ $t^b = 0.18$	100.2 ± 0.9 $F^b = 1.49$ $t^b = 0.34$	100.8 ± 0.7 $F^b = 2.47$ $t^b = 0.75$	100.4 ± 1.1

[a]The average of six determinations ± standard deviation; [b] the *t*- and *F*-values refer to comparison of the proposed method with the official method. Tabulated *t*-value for $P = 0.05$ and ten degrees of freedom is 2.23. Tabulalted *F*-value for $P = 0.05$ and $f_1 = f_2 = 5$ is 5.05.

[1]Amoun Pharmaceutical Company S.A.E. El-Obour City, Cairo, Egypt.

[2]Memphis Company for Pharmaceutical & Chemical Industries, Cairo, Egypt.

[3]Gulf Pharmaceutical Industries, Ras Al-Khaimah, U.A.E.

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Interferences

A systematic quantitative study was performed by measuring the absorbance of solutions containing $2.0\,\mu g\,mL^{-1}$ of Fam with varying excess of oxidative degradation products (1.0, 2.0, 4.0 and $10\,\mu g\,mL^{-1}$) using the recommended methods A, B and C. No significant interference was observed from common degradation products results from oxidation of famotidine which are likely to occur at normal storage condition, as shown in Table 3. Also, there was no interference from the additives and excipients commonly used such as glucose, lactose, fructose, calcium hydrogen phosphate, magnesium stearate and starch for the examined methods A, B and C.

Analytical Applications

The proposed methods were successfully applied to determine Fam in its dosage forms. The results obtained were compared statistically by Student's *t*-test (for accuracy), and variance ratio *F*-test (for precision) with the official method[21] [based on non-aqueous titration of the sample solution using 0.1 M $HClO_4$ as titrant and the end point is detected potentiometrically] at 95% confidence level as recorded in Table 4. The results showed that the *t*- and *F*- values were smaller than the critical values indicating that there was no significant difference between the proposed and official methods. The proposed methods were more accurate with high recoveries than the official method, so the proposed methods can be recommended for routine analysis of Fam in pure form and in dosage forms in the majority of drug quality control laboratories.

CONCLUSION

The proposed methods were advantageous over other reported visible spectrophotometric and colorimetric methods with respect to their high selectivity and sensitivity which allowed the determination of up to $0.1\,\mu g\,mL^{-1}$, simplicity, rapidity, reproducibility, precision and stability of coloured species for more than 48 h. Furthermore, the methods depend on simple reagents that are available, besides being less time consuming. The proposed methods may be applied for routine analysis and in quality control laboratories for the quantitative determination of the studied drug in raw materials, in pharmaceutical formulations and in the presence of its oxidative degradates.

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Received April 8, 2002
Accepted April 21, 2002