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SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF FAMOTIDINE IN DRUG FORMULATIONS

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Abstract

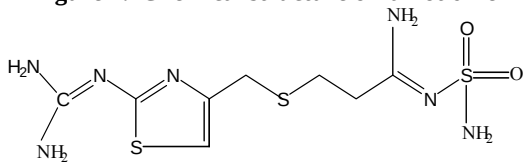
This paper describes two sensitive and simple spectrophotometric methods for determination of famotidine in pharmaceutical preparations. The first method is based on the hydrolisis of famotidine with sodium hydroxide assisted with microwave radiation, whereby the sulfide ion is produced. The sulfide was allowed to react with N, N – diethyl – p – phenylendiamine oxalate and Fe (III), and the blue color produced was measured at 671 nm. Beer's law was fulfilled in the concentration range 0.6 – 52.8 µg/ml, with a detection limit of 0.2 µg/ml. The second method is based upon the formation of a ternary complex between lead (II), eosin and famotidine in the presence of methyl cellulose as a surfactant. The ternary complex showed an absorption maximum at 541 nm. The method obeys Beer's law over concentration range of 7.4-100.0 µg/ml. The methods are simple, sensitive and accurate. The proposed method was applied on the famotidine determination in tablets and intravenous solution, shows recoveries in the range of 97.9-104.2 %. The results obtained by proposed methods were statistically validated.

***Keywords*:** Famotidine, spectrophotometry, pharmaceutical formulations, hydrolysis, ternary complex.

1. Introduction

Famotidine, 3-[[[2-[(aminoiminomethyl)amino]-4-thiazolyl]methyl]thiol]-N-aminosulfonyl) (Fig. 1), is a histamine H2-receptor antagonist that is a high potent inhibitor of gastric and acid secretion in humans and animals. Famotidine is useful in any situation where stomach irritation is an issue and ulceration is a concern. It is often used in the treatment of Helicobacter infection, inflammatory bowel disease, canine parvovirus, ingestion of a toxin that could be ulcerating, any disease involving protracted vomiting, or chronically in combination with medications which may have stomach irritating properties[1].

Figure 1. Chemical structure of famotidine



Various analytical methods such as high performance liquid chromatography[2-4], fluorimetry[5], capillary electrophoresis[6], polarography[7], voltammetry[8], and potentiometry[9] have been reported for the determination of famotidine. However, these methods are expensive and not available at most quality control laboratories. Visible spectrophotometry is still considered a convenient and economical technique for routine analysis of the drugs in pharmaceutical formulations. Famotidine has been determined spectrophotometrically based on reactions with ninhydrin[10], 1,4-benzoquinone[11], and other reagents[12-13].

Due to the presence of amino, amido and thioether groups in its structure, this drug possesses chelating properties and may interact with metal ions[14-15]. It also has sulfur atoms in its structure that allow the hydrolysis of the molecule with formation of sulfide ions. On the basis of these characteristics, in this paper we propose two new methods of spectrophotometric analysis. The first method is based on the formation of a complex between famotidine, eosin and Pb(II). The second method is based on the formation of ethylene blue by reaction of sulfide ion derived from famotidine, N,N-diethyl-p-phenylenediamine oxalate (N,N-DPPD oxalate) and Fe(III).

2. Material and methods

2.1. Apparatus: A Agilent 8453 UV-Vis spectrophotometer (Nortwalk, CT, USA) with glass cells (l = 1 cm) was used for all spectrophotometric measurements. A household microwave oven (Daewoo) with a nominal maximum power of 1200 W as marked was used.

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2.2. *Reagents and materials:* All reagents were of analytical reagent grade unless stated otherwise. Water was purified with a Nanopure system (Barnstead, USA).

Standard solution of famotidine: Famotidine pure sample was kindly provided by Elmor Laboratory Co., Valencia, Venezuela. Stock solution containing 1000 µg/ml of famotidine was prepared by exact weighing of the drug and dissolution in water. The solution was stable for at least 4 weeks if stored in the dark at 4 ºC. Working solutions were prepared daily by appropriate dilutions.

Eosin solution: Eosin solution (Fluka, Germany) was prepared as 0.1% w/v, aqueous solution.

Lead (II) solution: Lead solution (2000 µg/ml) was prepared dissolving 0,31969 g of lead (II) nitrate (Fluka, Germany) in deionized water and diluting to 1 l with deionized water.

Methylcellulose solution: Methylcellulose solution (Riedel-de Haen) was prepared as 0,5% w/v in water with the aid of heat.

N,N-DPPD oxalate solution: Prepared by dissolving 0.5000 g of N,N-DPPD oxalate (Fluka, Germany) and diluting to 50 ml with 1:1 sulfuric acid.

Ammomium iron (III) sulfate solution 0.21 M: Prepared by dissolving 5.0000 g of $NH_4Fe(SO_4)_2.12H_2O$ and diluting to 50 ml with 1.0 M sulfuric acid.

2.3. Procedure

2.3.1. Method 1: Spectrophotometric method based on ternary complex formation

Appropriate volumes of the standard solutions were placed in a series of 10 ml volumetric flasks. A 2.0 ml of the buffer solution pH 3.0, 1.5 ml of methyl cellulose, 1.0 ml of eosin solution and 1.0 ml of lead (II) solution were added to the flasks, in this order. The mixture was diluted to volume with water and homogenized by shaking. Each solution was allowed to stand for 8 min at room temperature (25 ± 3 ºC). The absorbance was measured at 500 nm against a reagent blank treated similarly

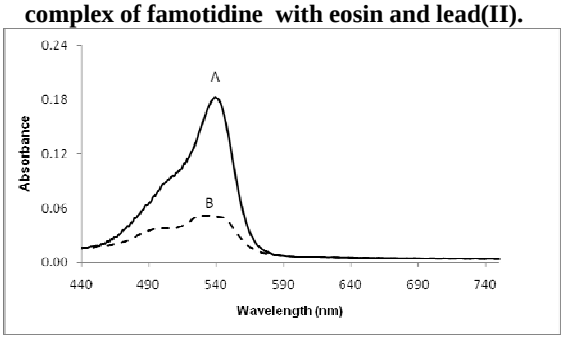
2.3.2. Method 2: Spectrophotometric method based on ethylene blue formation

To a PTFE reactor, a 1.0 ml volume of the sample solution, 1.0 ml of water and 6.0 g of NaOH were added. The reactor was placed inside the domestic microwave oven irradiated at 1100 W power for 60 s. The content of the reactor were transferred to a 100 ml volumetric flask and was diluted to volume with deionized water. 2.0 ml of the diluted solution was placed in a 50 ml volumetric flask, to which 5.0 ml of 0.05 M H_2SO_4, 0.5 ml of N,N-DPPD oxalate solution and 0.5 ml of $NH_4Fe(SO_4)_2$ was sequentially added. The mixture was diluted to a final 50 ml volume with deionized water, shaken and allowed to stand for 10 min. The absorbance was measured at 670 nm against a reagent blank.

3. Results and discussion

3.1 Method 1: The reactions of formation of ternary complex in which a central metal ion is coordinated to two ligands have been widely used in spectrophotometric analysis. These reactions have found extensive application in the determination of different drugs[16]. The particularity of the ternary complex in this study is that the first ligand is famotidine, the second ligand is eosin, and the ion metal is lead (II). The ternary complex has higher value of molar absortivity than blank solution formed by eosin and lead (II) producing a red color with maximum absorbance value at 541 nm (Fig. 2). Different metals were also studied to choose the most suitable one for formation of ternary complex, e.g. Fe(III), Cu(II), Ni(II), Pd(II) and Pb(II). The latter was found to have the highest absorbance value.

Figure 2. Absorption spectrum of the ternary complex of famotidine with eosin and lead(II).



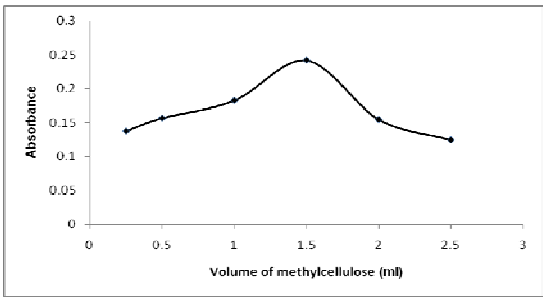
Preliminary experiments were carried out to optimize the main parameters affecting the development and stability of the colored complex investigated.

The effect of pH was investigated by changing the pH in the range 3.0 – 6.0. Maximum absorbance value was achieved at pH 3.0, using 2.0 ml of 0.1 M acetate buffer. Other buffers having the same pH value such as phosphate and phthalate were tried and compared with acetate buffer. Among different buffers solution tested, the use of acetate buffer resulted in the highest absorbance value. Thus, 0.1 M acetate was chosen as buffer system in this investigation.

The selection of an appropriate surfactant is of major importance for the optimization of the spectrophotometric method. In this study three surfactant namely, triton X-100, methylcellulose

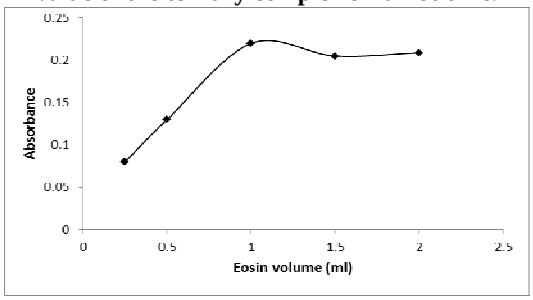
and sodium dodecylsulfate were tested. Among different surfactant studied, best results were obtained in the presence of methylcellulose. We investigated the effect of the 0.5% w/v methylcellulose volume on the absorbance of the complex. The response increased when the methylcellulose volume increased from 0.25 -1.5 ml while larger volumes produce decrease in the response (Fig. 3). The addition of 1.5 ml of 0.5% w/v methylcellulose ensured the maximum response for famotidine complex.

Figure 3. Effect of volume of 0.5% w/v methylcellulose on the absorbance value of the ternary complex of famotidine at pH 3.0.



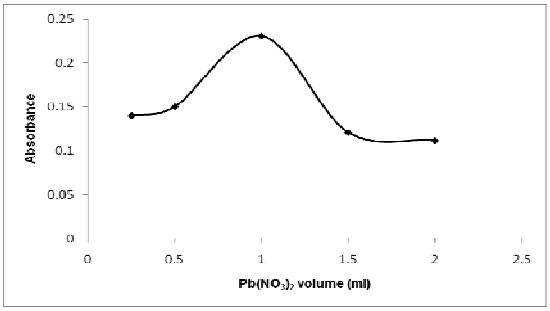
In order to set an optimal volume of the eosin reagent, a range of 0.25 - 2.0 ml of 0.1% w/v eosin volumes were tested. Increasing the volume of eosin in the range 0.25 – 1.0 ml improved the absorbance value of the ternary complex while the response remains constant at higher amounts of eosin (Fig. 4). For this reason 1.0 ml of 0.1% w/v eosin was used for the rest of the experiments.

Figure 4. **Effect of eosin volume on the absorbance value of the ternary complex of famotidine**.



The effect of volume of Pb(II) on the absorbance of the complex was also studied keeping the concentration of the famotidine and eosin constant. As shown in Fig. 5, it was observed that increasing the volume of 2000 µg/ml of Pb(II) result in a gradual increase in the absorbance up to 1.0 ml, after which the absorbance of complex began to decrease. Thus, 1.0 ml of 2000 µg/ml of Pb(II) which resulted in a final concentration of 200 µg/ml was used in this investigation.

Figure 5. Effect of volume of 2000 µg/ml lead(II) on the absorbance value of the ternary complex of famotidine at pH 3.0.



In order to examine the effect of temperature on the absorbance of the ternary complex, the experiment was carried out at temperature range 25 - 70 °C, using thermostatic water bath for 10 min. The solution was cooled to ambient temperature before measuring the absorbance. Maximum absorbance was obtained at ambient temperature (25 ± 2 °C). Higher temperatures decrease the absorbance sharply.

The sequence of addition of reactants can influence the formation of coloured product. Twelve procedures with different orders of component addition were examined. Thus, the sequence buffer-famotidine-methylcellulose-eosin-lead gave higher values of absorbance.

3.2 Method 2: The proposed method is based on the hydrolysis of famotidine with NaOH to give sulfide, which is then reacted with N,N-DPPD and Fe(III) to form ethylene blue. The reaction is an oxidative coupling of two aromatic rings[17]. The absorbance of the blue product at 671 nm is proportional to the concentration of famotidine.

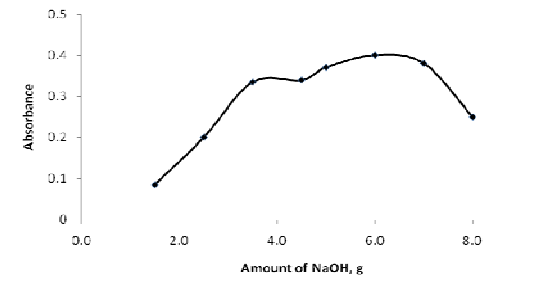
The effects of experimental parameters on the hydrolysis of famotidine, such as NaOH amount, the radiation power supplied, reaction hydrolysis time and total volume of reagents were studied using PTFE reactors.

To investigate the effect of microwave irradiation on the hydrolysis of famotidine, irradiation time of 15 s was used for microwave-assisted hydrolysis at 800 W irradiation powers. For the purpose of comparison, hydrolysis was also performed using a water bath with a reaction time of 30 min at 80 °C. The results show that although conventional heating is an appropriate way to make alkaline hydrolysis of famotidine, a relatively long hydrolysis time of about 30 min was still required for the completion of the reaction. Moreover, the absorbance obtained with the hydrolysis assisted by microwave radiation was higher than that obtained using the water bath. The experimental results obtained demonstrated that the speed of

alkaline hydrolysis can be significantly increased by using microwave heating.

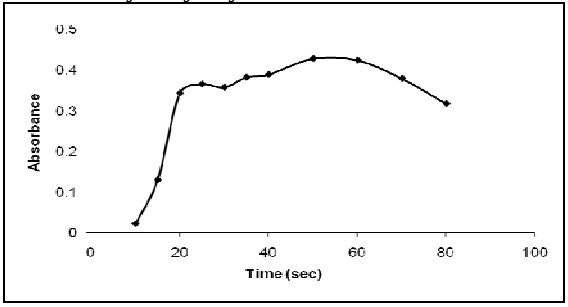
To study the effect of NaOH amount on the hydrolysis yield of famotidine, experiments were carried out at a power level of 800 W and using an irradiation time of 20 s. As shown in Fig. 6, an increase in NaOH provides an increase in the absorbance up to 6.0 g after which the absorbance of blue compound began to decrease. The addition of 6.0 g of NaOH ensured the maximum response for famotidine.

Figure 6. Effect of NaOH amount on the hydrolysis yield of famotidine.



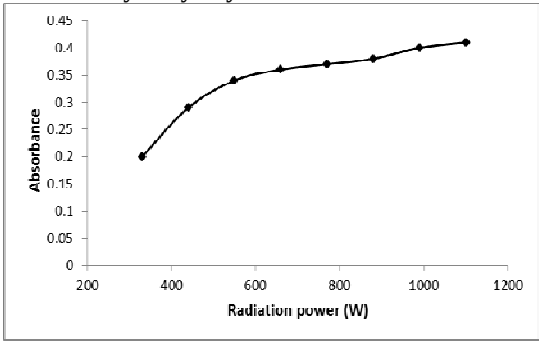
The effect of the irradiation time was studied among 10-90 s. The hydrolysis yield increased with time increased in the range 10-60 s. However, above 60 s, the hydrolysis yield decreases (Fig. 7). Thus, 60 s was chosen as irradiation time.

Figure 7. Effect of the irradiation time on the hydrolysis yield of famotidine.



The effect of radiation power on the hydrolysis of famotidine was studied in the range 330-1100 W, using an irradiation time of 60 s in the presence of 6 g of NaOH. Absorbance of ethylene blue increases with increased radiation power (Fig. 8). We chose a radiation power of 1100 W.

Figure 8. Effect of the radiation power on the hydrolysis yield of famotidine.



3.3 Application and validation of the proposed methods: Using the above mentioned spectrophotometric procedures, linear regression equations were obtained over the concentration ranges stated in Table 1. Two replicates were used for each of six prepared standards to obtain each calibration graph. The good linearity of the calibration graphs is clearly evident from the values of regression coefficients.

In order to evaluate the precision of the proposed methods, solutions containing three different concentrations of the stated drugs were prepared and analysed in five replicates. The analytical results obtained from this investigation are summarized in Table 1. The low values of the relative standard deviation (R.S.D.%) indicate the high precision of the proposed methods (Table 1).

Table 1. Analytical parameters

	Method 1 (ternary complex formation)	Method 2 (Ethylene blue formation)
λ max (nm)	541	671
Linearity range (µg/ml)	7.4 – 100.0	0.6 – 52.8
Correlation coefficient	0.998	0.999
Intercept (a)	0.0280	0.0019
Slope (b)	0.0077	0.0133
Detection limit (µg/ml)	2.2	0.2
Quantification limit (µg/ml)	7.4	0.6
Repeatability (R.S.D.,%), n = 10		
	4.8 (8.0 µg/ml)	4.5 (6.6 µg/ml)
	2.4 (40.0 µg/ml)	2.6 (26.4 µg/ml)
	0.8 (100.0 µg/ml)	2.3 (52.8 µg/ml)

The influence of commonly used tablet and lyophilized powder excipients (lactose, starch, magnesium stearate, talc, mannitol, titanium dioxide and microcrystalline cellulose) was investigated before the determination of the drug in dosage forms using the standard addition methodology. Student's t-test shows the similarity of the slope of standard calibration and standard addition calibration and therefore no interference could be observed with the proposed methods.

Samples of commercial dosage forms were fortified with different levels of famotidine. Table 2 shows results of the study of recovery. Validation of the proposed method for these samples was tested by using a recovery test (Student *t*-test)[18]. As the *P*-values calculated in all cases are greater than 0.05, the null hypothesis appears to be valid, *i.e.*, recoveries are close to 100% (Table 2).

Table 2. Results of recovery assays to check the accuracy of the proposed method.

Method	Sample	Spiked (µg/ml)	Found [#] (µg/ml)	Recovery (%)	*t*	*P**
Method 1	Tablets-40 mg	12.0	12.06 ± 0.13	100.5	1.05	0.35
		18.0	17.87 ± 0.14	99.3	2.01	0.12
		24.0	23.87 ± 0.28	99.5	1.07	0.34
	Tablets-10 mg	10.0	10.09 ± 0.16	100.9	0.94	0.39
		15.0	15.04 ± 0.12	100.3	0.85	0.44
	Powder-20 mg	20.0	20.03 ± 0.17	100.2	1.04	0.35
		10.0	10.06 ± 0.13	100.6	1.05	0.35
	powder-20 mg	15.0	14.94 ± 0.14	99.6	1.72	0.15
		20.0	19.98 ± 0.45	99.9	0.21	0.84
Method 2	Tablets-40 mg	6.0	6.25 ± 0.25	104.2	2.24	0.09
		9.6	9.51 ± 0.55	99.1	0.34	0.75
		12.0	12.33 ± 0.31	102.7	2.43	0.07
	Tablets-10 mg	6.0	6.17 ± 0.14	102.8	2.70	0.06
		9.6	9.40 ± 0.42	97.9	1.08	0.34
	Powder-20 mg	12.0	12.43 ± 0.50	103.9	1.83	0.15
		8.0	8.12 ± 0.30	101.5	0.92	0.41
	powder-20 mg	12.8	12.85 ± 0.45	100.4	0.27	0.79
		16.0	16.21 ± 0.31	101.3	1.51	0.20

[#] Average value ± standard deviation of five determinations.
* P value of the one-sample comparison test.

The applicability of the proposed methods was tested by the determination of famotidine in tablets and lyophilized powder. The results obtained were presented in Table 3. The values reported by manufacturers are located within the confidence intervals at 95% confidence level, indicating that there is no significant difference between the values experimental and nominal. These results demonstrate the usefulness of the proposed methods for this application.

Table 3. Determination of famotidine in pharmaceutical formulations by the suggested spectrophotometric methods.

Preparations	Nominal composition	Found (mg)* Method 1	Found (mg)* Method 2
Tablets	40 mg	39.93 ± 0.30	41.25 ± 1.36
Tablets	10 mg	9.96 ± 0.57	9.93 ± 0.50
Lyophilized powder	20 mg	20.05 ± 0.63	19.88 ± 0.70

*Average value ± 95% confidence interval of five determinations.

4. Conclusion

Two new spectrophotometric methods for the determination of famotidine in dosage forms are presented. They are quite simple and do not require tedious extraction procedure. The methods have wider linear range with good accuracy and precision. The present methods are useful and convenient for quality control and routine determination of drugs in pharmaceutical dosage forms.

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