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Application of ninhydrin to spectrophotometric determination of famotidine in drug formulations

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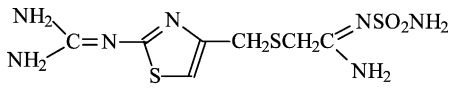
Abstract

A simple and fast spectrophotometric procedure has been developed for the determination of famotidine. The method is based on the interaction of ninhydrin with primary amines present in the famotidine. This reaction produces a blue coloured product which absorbed maximally at 590 nm. The effects of variables such as reagent concentration and reaction time were investigated to optimize the procedure. Beer's law was obeyed in the concentration range of 5–30 μg ml^{-1} with molar absorptivity of 6.99×10^3 l mol^{-1} cm^{-1}. The results were validated statistically. The proposed method has been applied to the determination of famotidine in tablets with satisfactory results.

Keywords: Famotidine; Ninhydrin; Pharmaceutical formulations; Spectrophotometry

1. Introduction

Famotidine (I) is relatively a new histamine H_2 receptor antagonist. It is widely used for the treatment of duodenal ulcers, benign gastric ulcer and hyper acid secretory conditions such as Zollinger–Ellison syndrome [1]. Therapeutic trials have shown that famotidine is twenty times more effective than cimetidine in inhibiting gastric acid secretion in humans [2]. The drug is official in European Pharmacopoeia [3] and US [4] which described the potentiometric titration and HPLC methods for assay of famotidine in pharmaceutical formulations. Thin layer chromatographic [3,4] and high performance thin layer chromatographic methods [5] have been reported for purity control of famotidine.



(I)

A review of literature revealed that several HPLC methods have been described for the determination of famotidine in biological fluids and pharmaceutical formulations [6–16]. These methods have enough sensitivity to determine lower concentration of the drug and hence, it is justified to use them for assay of such a lower concentration in biological fluids. However, it is always required to develop analytical methods using low cost techniques. UV–Vis spectrophotometry is still considered a convenient and economical technique for routine analysis of the drug in pharmaceutical formulations in third world countries.

The famotidine was determined spectrophotometrically [17] based on the reaction of drug with 1,4-benzoquinone at pH 5.2. Beer's law was obeyed in the concentration range of 40–160 μg ml^{-1} at 502 nm. Another spectrophotometric method [18] was described which involved the interaction of famotidine with *p*-chloranilic acid in acetonitrile and subsequent measurement at 521 nm. The first derivative value of interaction product with *p*-chloranilic acid was also measured at 323 nm. Several other spectrophotometric methods [19] have also been reported in the literature based on the charge transfer complexation with chloranil, 2,3-dichloro 5,6-dicyano 1,4-benzoquinone and dichloronitrophenol and absorbance measurements were made at 458, 460 and 425 nm, respectively. Extractive spectrophotometric methods [20,21] for its determination have

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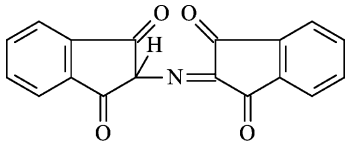
been discussed which described extractable 1:1 ion-pair complex between famotidine and each of bromocresol green and bromothymol blue. The detection limits were found to be 2.0 and 0.7 μg ml^{-1} for bromocresol green and bromothymol blue, respectively. A stability indicating method was developed based on measuring the peak height of the second derivative at 304 nm [22]. The drug content in pharmaceuticals was also estimated [23] by reaction with sodium nitroprusside in alkaline medium resulting in the formation of red coloured complex which absorbed maximally at 498 nm. Famotidine was determined in tablets by difference spectrophotometric and quadratic polynomial coefficient methods [24].

A differential pulse voltammetric method [26] was suggested based on its electrochemical oxidation at a glassy carbon or platinum electrode. It was also determined by potentiometric titration [27] with a solution of palladium (II) chloride at pH 3.6 using a silver electrode.



(II)

The present communication describes a spectrophotometric method for the determination of famotidine based on the reaction of amino group of the drug with ninhydrin in *N*,*N*′-dimethylformamide medium.

2. Experimental

2.1. Apparatus

All spectrophotometric measurements were carried out using a spectronic 20D^{+} spectrophotometer (Milton Roy, USA). A water bath shaker (NSW 133, India) was used to control the heating temperature.

2.2. Materials and reagents

All reagents used were of analytical reagent grade. A 0.1% famotidine (Wockhardt India Ltd) solution was prepared in *N*,*N*′-dimethylformamide (DMF) and further diluted according to the need with DMF.

A 0.06 M ninhydrin (E. Merck) solution was also prepared in DMF.

2.3. Procedure for calibration curve

Aliquots of 0.10–0.60 ml famotidine standard solution (0.25 mg ml^{-1}) were pipetted into a series of boiling test tubes. To each test tube 0.7 ml of ninhydrin solution was added, mixed well and heated on a water bath at 100 ± 1 °C for 5 min. After heating the solution, tubes were cooled to room temperature. The content of the tube was transferred to a 5-ml volumetric flask and diluting to volume with DMF. The absorbance was measured at 590 nm against reagent blank treated similarly. The concentration of famotidine was calculated either from calibration graph or regression equation.

2.4. Procedure for the assay of famotidine in pharmaceutical preparations

Ten tablets were accurately weighed and powdered. A portion equivalent to 50 mg famotidine was stirred with 20 ml DMF and let stand for 10 min. The residue was filtered on Whatman No. 42 filter paper and washed with DMF. The filtrate and washings were diluted to volume in a 50-ml volumetric flask. A suitable volume of this solution was further diluted to give a final concentration of 0.25 mg ml^{-1}. An aliquot of this solution was analysed for famotidine following the procedure described for the calibration curve.

3. Results and discussion

The possible use of ninhydrin for the detection and quantitative estimation of amino acids and imino acids depends on the formation of Ruhemann purple [28]. It was reported that in alkaline medium ninhydrin is converted to *o*-carboxyphenylglyoxal which would reduce ninhydrin to 2-hydroxyindan-1,3-dione. The primary amino group of famotidine reacted with 2-hydroxyindan-1,3-dione in DMF to form the amino compound which condensed with ninhydrin to give diketohydrindylidene-diketohydrindamine (II).

This compound (II) further interacts with amino group of the drug resulting in the formation of a blue coloured product which absorbed maximally at 590 nm.

The different experimental parameters influencing the intensity of the developed colour were extensively investigated to determine the optimal conditions for the assay procedure. All conditions studied were optimized in DMF medium. The optimum reaction time was determined by heating the reaction mixture on water bath at 100 ± 1 °C. It is apparent from Fig. 1 that complete colour development was attained after 4 min of heating and remained constant up to 10 min. Therefore, the optimum heating time was fixed at 5 min throughout the experiment. The effect of ninhydrin concentration on the colour development was investigated. For this purpose, different volumes of 0.06 M ninhydrin (0.1–1.0 ml) were added to a fixed amount of famotidine (25 μg ml^{-1}). The results are presented in Fig. 2 which showed that the highest and most stable absorbance was obtained with 0.6 ml, beyond which the

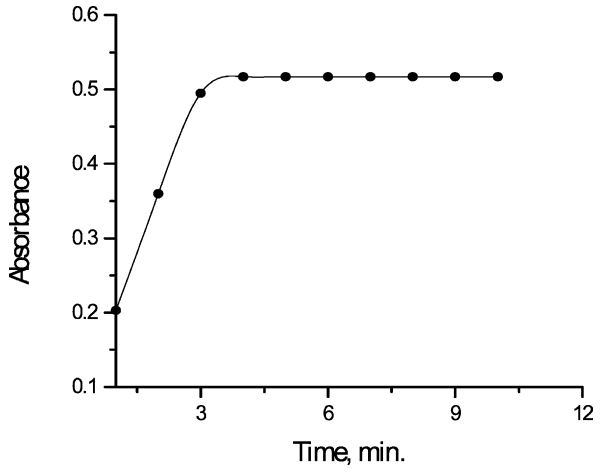


Fig. 1. Effect of heating time on the formation of coloured product (drug = 25 μg ml^{-1}).

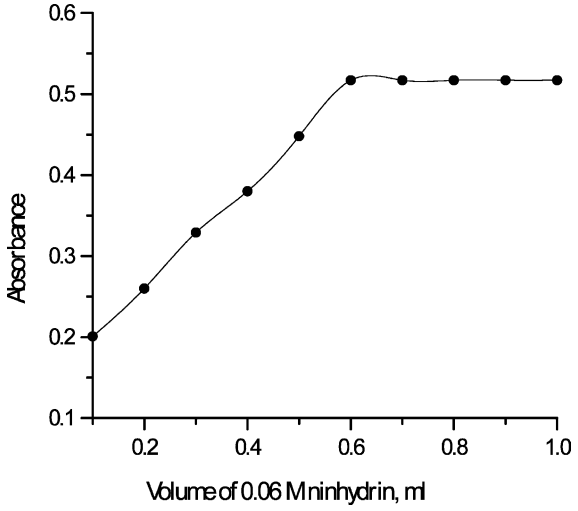


Fig. 2. Effect of ninhydrin concentration on the absorbance of the coloured product (drug = 25 μg ml^{-1}).

Table 1
Optical characteristics and statistical data for the regression equation

λ_{max} (nm)	590
Beer's law limit (μg ml^{-1})	5–30
Molar absorptivity (l mol^{-1} cm^{-1})	6.99×10^3
Sandell's sensitivity (μg ml^{-1} per 0.001 A)	4.83×10^{-2}
Regression equation [a]	$A = 0.006 + 0.0205$ C
Intercept (a)	6.00×10^{-2}
tS_a [b]	3.11×10^{-3}
Slope (b)	2.05×10^{-2}
tS_b [c]	1.49×10^{-4}
Correlation coefficient (γ)	0.9999
Variance (S_o^2)	2.69×10^{-6}
Linearity	0.3415
Detection limit ((μg ml^{-1})	0.16

[a] $A = a + bc$ where c is the concentration in μg ml^{-1}.
[b] Confidence interval of the intercept at 95% confidence level.
[c] Confidence interval of the slope at 95% confidence level.

absorbance became constant. A 0.7 ml of the reagent, therefore, was used as an optimum value for colour development.

3.1. Analytical data

In the above established working conditions, the main figures of merit of the proposed procedure for determination of famotidine have been established. The calibration graph obtained by plotting absorbance at 590 nm against famotidine concentration was found to be linear over Beer's law range given in the Table 1. The molar absorptivity, detection limit [29], variance, slope, intercept, correlation coefficient and linearity obtained by least squares treatment of the results are also given in Table 1. High value of correlation coefficient confirms the excellent linearity of the calibration curve. The value of detection limit and variance speak of good sensitivity of the proposed method and negligible scatter of the

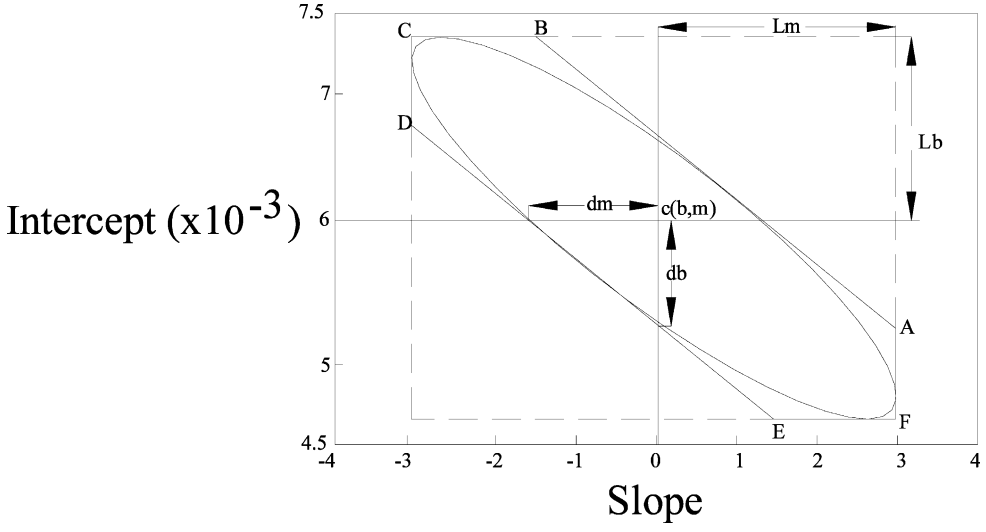


Fig. 3. Joint confidence region for the slope and intercept of line of regression.

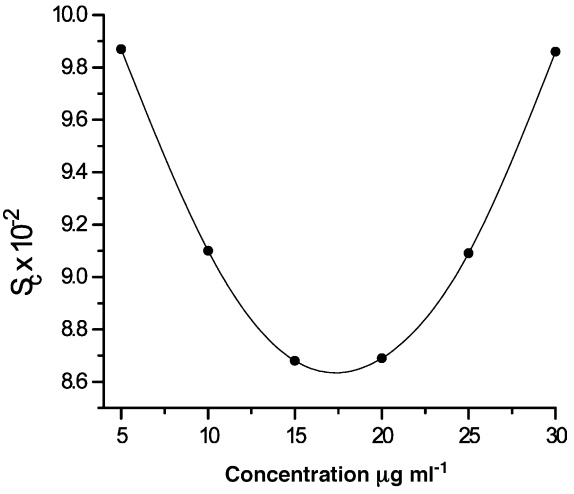


Fig. 4. Errors (S_c) in the determination of the concentration of famotidine.

calibration data around the line of regression. The value of intercept is 6.00×10^{-2} and hence it is required to judge if the intercept is significantly different from zero. This has been demonstrated by drawing a joint confidence region for slope and intercept following the method of Mandel and Linnig [30,31] which is an ellipse having the point of best fit as its centre. It is evident from Fig. 3 that points for which intercept is zero fell well within the ellipse. Therefore, it is concluded that the intercept is not significantly different from zero and the proposed method is free from constant error independent of the famotidine concentration.

The absolute error, Sc, in the determination of a given concentration of famotidine was calculated by statistical analysis of regression equation [30] Fig. 4 shows a graph of Sc vs. concentration of famotidine. It is apparent from the figure that the error is minimum for a concentration of about 17 μg ml^{-1} of famotidine.

Table 2
Determination of famotidine in pharmaceutical preparations by standard addition method

Preparation [a]	Amount taken (μg ml^{-1})	Amount added (μg ml^{-1})	Total amount found (μg ml^{-1})	Recovery (%)	SAE [b]	RSD(%)
Topcid 40	10	10	19.66	98.33	0.0659	0.82
	10	15	25.08	100.32	0.0319	0.32
	20	10	29.94	99.81	0.0444	0.36
Famocid 40	10	10	19.68	98.41	0.0393	0.49
	10	15	25.008	100.03	0.0498	0.48
	20	10	29.96	99.89	0.0498	0.40
Famotin 40	10	10	19.85	99.26	0.0630	0.77
	10	15	24.98	99.93	0.0526	0.51
	20	10	29.86	99.53	0.0426	0.34
Famonite 40	10	10	19.92	99.63	0.0535	0.67
	10	15	24.97	99.90	0.0356	0.34
	20	10	29.91	99.70	0.0609	0.49
Facid 40	10	10	19.91	99.59	0.0709	0.87
	10	15	24.89	99.57	0.0430	0.42
	20	10	29.78	99.29	0.0674	0.55

[a] Six independent analyses.
[b] Standard analytical error.

Table 3
Determination of famotidine in commercial tablets by the proposed and reference method

Formulation	Proposed method		Reference method		t_{cal} [b]	F_{cal} [c]
	Recovery [a] (%)	RSD (%)	Recovery [a] (%)	RSD (%)		
Topcid 40	99.70	0.52	99.73	0.30	0.166	2.973
Famocid 40	100.14	0.65	100.04	0.38	0.430	2.937
Famotin 40	100.09	0.68	99.86	0.46	0.875	2.193
Famonite 40	100.14	0.66	100.04	0.38	0.411	3.021
Facid 40	100.00	0.71	99.91	0.49	0.668	2.126

[a] Average of 10 independent analyses.
[b] t-Value at 95% confidence level is 1.734 [32].
[c] F-value at 95% confidence level is 3.18 [32].

Table 4
Comparison of the proposed method with other existing spectrophotometric methods

Reagent	λ_{max} (nm)	Beer's law limit (μg ml^{-1})	Molar absorptivity (l mol^{-1} cm^{-1})	RSD (%)	References
Sodium nitroprusside	498	5–50	5.90×10^2	0.15	[23]
Chloranil	458	50–500	0.67×10^3		[19]
p-Chloranilic acid	521	25–240		< 2.0	[18]
1,4-Benzoquinone	502	40–160			[17]
DDQ [a]	460	40–450	0.82×10^3		[19]
DCNP [b]	425	10–100	3.70×10^3		[19]
BCG [c]	420	2–23.6	5.00×10^3	1.6–3.4	[20]
BTB [d]	420	0.7–8.1	1.20×10^4	1.8–3.1	[20]
Methanol	288	5–15			[25]
Ninhydrin	590	5–30	6.99×10^3	0.60–0.94	This work

[a] DDQ = 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone.
[b] DCNP = 2,4-Dichloro-6-nitrophenol.
[c] BCG = Bromocresol green.
[d] BTB = Bromothymol blue.

Reproducibility was measured for a series of ten independent determinations at two concentration levels. The percent relative standard deviation and standard analytical error (SAE) were found to be 0.94%, 0.059 and 0.60%, 0.057 for the determination of 20 and 30 μg ml^{-1} of famotidine, respectively.

The validity of the present method was tested by standard addition method. For this purpose, solutions containing three different concentrations of famotidine were prepared by adding a known amount of pure drug to the preanalyzed commercial dosage forms and determined in six replicates. The results are summarized in Table 2 which can be considered to be very satisfactory. The common excipients present in tablet formulations did not interfere in the determination of famotidine.

The commercial tablets were successfully analyzed for famotidine content by the proposed and reference methods [23]. The results (Table 3) obtained by both the methods were compared statistically. The calculated Student's *t*-and *F*-values did not exceed the theoretical ones at 95% confidence level which confirms the absence of any difference between the methods compared.

The proposed method is favourably compared with other existing spectrophotometric methods in terms of molar absorptivity and Beer's law limit (Table 4). It is evident from Table 4 that present method is more sensitive ($\varepsilon = 6.99 \times 10^3$ l mol^{-1} cm^{-1}) as compared to other reported methods except the method using BTB as ion pair formation reagent. However, the Beer's law limit of the proposed method is quite reasonable for the assay of famotidine in pharmaceutical dosage forms with low RSD (0.60–0.94%) in comparison to the methods involving extractable ion pair complexes [20]. Moreover, the present method is fast and directly applicable to the drug sample without prior separation or treatment.

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