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SPECTROPHOTOMETRIC DETERMINATION OF FAMOTIDINE USING P-CHLORANILIC ACID

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تم استخدام تفاعل الفاموتيدين مع حمض الكلورانيليك فى الاسيتونيتريل للتحليل الكمى للفاموتيدين وذلك بطريقة بسيطة ودقيقة بقياس شدة امتصاص اللول الأحمر الناتج من التفاعل عند طول موجه ٥٢١ نانوميتر.

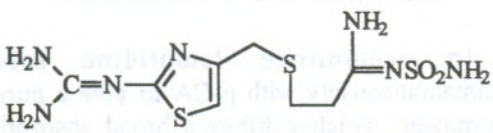
ولقد تم أيضا قياس قيمة المشتق التفاضلى الأول لناتج التفاعل مع حمض الكلورانيليك عند طول موجة ٣٢٣ نانوميتر. لقد حددت العوامل التجريبية الملائمة للتفاعل. وقد تبين أن قانون بيير متبع فى مدى تركيز يتراوح بين ٢٥-٢٤٠ ميكرجرام لكل ملياليتر وبين ٢-٣٠ ميكروجرام لكل ملياليتر للطريقة اللونية وطريقةِ المشتق التفاضلى الأول على التتابع.

أستخدمت هذه الطريقة فى تحليل الفاموتيدين فى الأقراص وتم تطبيق طريقة المشتق التفاضلى لتقدير الفاموتيدين فى البول المطعم , ولقد كلن الأسترجاع كميا ولم يزد الانحراف القياسى النسبة عن ٢٪.

Simple and accurate spectrophotometric method for the analysis of famotidine have been presented. The method was based on the interaction of famotidine and p-chloranilic acid (p-CA) in acetonitrile to give a stable and purple colored product measured at 521 nm. The first derivative value of the interaction product with p-CA was also measured at 323 nm. The optimum experimental parameters have been worked out. Beer's law was obeyed from 25 to 240 μg/ml and from 2 to 30 μg/ml for the direct absorbance and first derivative measurements respectively. The method enabled the assay of famotidine in its tablets. The derivative method was applied for the determination of famotidine in tablets and spiked urine. Recovery was quantitative and relative standard deviation did not exceed 2 %.

INTRODUCTION

Famotidine is a potent inhibitor of histamine H_2 receptors, it supresses both the acid concentration and volume of gastric secretion. It is used in conditions where inhibition of gastric acid secretion may be beneficial such as gastric and duodenal ulcers.[1] It has little, if any, anti- and rogenic effect and the potential for drug interactions is much less than with cemetiditie, otherwise its advese effects are similar.[2,3]



Famotidine

Famotidine in pharmaceutical formulations and in biological fluids was determined by several analytical techniques including spectrophotometry,[4-9] flow-injection analysis,[10] liquid chromatography,[11-17] capillary electrophoresis,[18] polarography,[19] and potentiometry.[2]

p-Chloranilic (p-CA) was used for the spectrophotometric determination ot basic nitrogenous compounds such as alkaloids,[20] pentazocin,[21] phenothiazines,[22] some antihistamine drugs,[23] triamterene,[24] and meclobemide.[25]

The present work describes the use of p-CA for rapid determination of famotidine by two methods, direct spectrophotometric method which measures the intensity of the purple

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colour at 521 nm, and derivative method based on recording the first derivative spectrum of the reaction product at 300-350 nm and measuring the first derivative value at 323 nm.

EXPERIMENTAL

Apparatus

A Jasco (Tokyo, Japan) Uvidec Model 320 spectrophotometer with 1 cm quartz cell was used for measuring the absorbance. Perkin-Elmer (USA) Model Lambda 3B UV-VIS spectrophotometer with 1 cm quartz cell was used for recording the spectra and measuring the first derivative values. Scan speed 120 nm/min, and ordinate maximum and minimum $\pm$ 1.

Chemicals and reagents

P-Chloranilic acid (p-CA) (Sigma Chemical Co., USA) solution, 3 mg/ml, in acetonitrile. Famotidine was purchased from Sigma Chemical Co., St Louis, MO, USA. Antodine tablet (contain 20 mg famotidine) was purchased from local market. All solvents were of analytical grade.

Preparation of standard solution

About 75 mg, accurately weighed of famotidine was transferred into 25-ml volumetric flask, dissolved in methanol to provide standard of concentration 3 mg/ml. From this solution a series of dilutions were prepared to obtain a range of 0.2-2.5 mg/ml solutions.

Preparation of sample solutions

Tablets

Twenty tablets were weighed, finely powdered and mixed thoroughly. An accurately weighed quantity of the powder, equivalent to about 50 mg of famotidine was transferred into 50-ml volumetric flask, shaked with methanol for about 2 min completed to the mark with the same solvent and filtered.

Spiked urine

Spiked urine samples of three different concentrations of famotidine were prepared by mixing aliquots of standard famotidine solution in three separate 50 ml volumetric flasks with 10 ml urine. The spiked urine was mixed well with methanol and the solution was completed to the mark with methanol to obtain concentrations of 40, 200, 500 μg famotidine per milliliter. The solutions were filtered if necessary. A blank solution was prepared similarly to contain 10 ml urine without famotidine.

General procedure

a- Direct absorbance measurement

One milliliter, accurately measured, of standard or sample solution of famotidine was transferred into 10 ml volumetric flask. One milliliter of p-CA reagent solution was added. The solution was mixed and completed to the mark with acetonitrile. The absorption intensity was measured at 521 nm against a blank treated similarly.

b-Derivative spectrophotometric method

Half milliliter, accurately measured, of standard or sample solution of famotidine was transferred to 10 ml volumetric flask. Half milliliter of the p-CA reagent solution was added. The solution was mixed and completed to the mark with acetonitrile. The first derivative spectrum of the solution was recorded from 300-350 nm and the D^1 value was measured at 323 nm (Fig. 2).

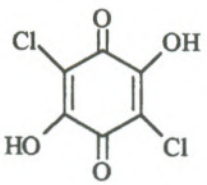
Stochiometric relationship

Job's method of continuous variation was employed. Equimolar solutions ($4x10^{-4}$ M) of p-CA and famotidine were prepared. Series of 10 ml portions of mixtures of the drug and p-CA were made up containing different proportions in 10 ml volumetric flask. The absorbances of these solutions were measured at 521 nm against a reagent blank treated similarly.

RESULTS AND DISCUSSION

In acetonitrile famotidine reacts instantantaneously with p-CA to give a purple chromogen, which exhibits a broad absorption maximum at 521 nm and a sharp peak at 317 nm while p—CA in acetonitrile shows a broad

maximum at about 455 nm (Fig. 1). Chloranilic acid exists in three ionic forms,[21] the neutral yellow orange H_2A at very low pH, the dark purple HA^- which is stable at pH 3 and the pale violet $A^=$ which is stable at high pH. The purple coloured reaction product obtained indicates that HA^- may be the form of p-CA involved in the reaction with famotidine. In this reaction, a proton transfer from p-CA to the basic center of famotidine may take place (Scheme 1). The obtained ion pair salt can be dissociated, in presence of solvent of high dielectric constant, To the purple anion HA^-. The first derivative spectrum of the reaction product was recorded from 300-350 (Fig. 2).



p-CA (H_2A)

$$H_2A = H^+ + HA^- \text{ (purple)}$$
$$B + H^+ = BH^+ \text{ (colorless)}$$

Scheme 1

Optimization of variables

A study was carried out to obtain the optimum concentration of p-CA required for the reaction. Different concentrations of p-CA were added to the same concentration of famotidine, the absorption intensity and D^1 values were followed. It was found that the best and constant readings were obtained when 1 ml of p-CA solution (3 mg/ml) in acetonitrile was utilized for measuring the absorbance at 521 nm (Fig. 3) and 0.5 ml of the same solution for measuring D^1 values.

In order to determine the most suitable solvent that would give the highest colour intensity and D^1 values, investigations were carried out. Different solvents were examined such as, ethanol, methanol, isopropanol, acetone, acetonitrile, dioxan, dimethyl-formamide, dimethylsulphoxide, dichloroethane, and chloroform. Among the solvents examined acetonitrile was the best solvent to be used in case of colour development and also in measuring the D^1 value.

The effect of reaction time on developing the colour of the reaction product was studied. The absorbance values were monitored at 5 minutes intervals for a period of two hours. It was found that the optimum reaction time was attained immediately and the coloured product remained stable for several hours. Fig. 4 shows that the developed colour remained stable for at least 2 hours.

Under the previously established conditions, concentration of famotidine was found to be proportional to absorbance and D^1 values for the reaction product of p-CA with famotidine. Beer's law was valid over the concentration range of 25-240 and 2-30 μg/ml famotidine for the direct absorbance and first derivative measurement methods respectively. Regression equations derived using the least square method are:

$$A_{521} = 0.0037 + 0.0039\ C \qquad r = 0.9989$$
$$D^1_{323} = -0.0010 + 0.0026\ C \qquad r = 0.9999$$

where C= Concentration in μg/ml

The low values of the intercepts indicate that the blank reading at 521 and 323 nm was low. The good correlation coefficients indicate that these two methods are suitable for quantitative analysis of famotidine. The limit of detection was calculated,[26] for the spectrophotometric and first derivative methods. The value of limit of detection (8.5, 0.008 μg/ml for direct spectrophotometric and first derivative methods respectively) revealed that the proposed method is sensitive.

Application

The direct absorbance method was successufuly applied for the determination of famotidine in powder and tablets. When this method was applied to determination of famotidine in spiked urine, high recovery values were obtained with high standard deviations. So, the derivative spectrophotometric measurement was applied for the determination of famotidine in spiked urine. It may eliminate interferences in addition to being more sensitive. The results of analysis, shown in Table 1, are in good agreement with the reported method.[7] Student t-test and f-test showed that, they did not exceed

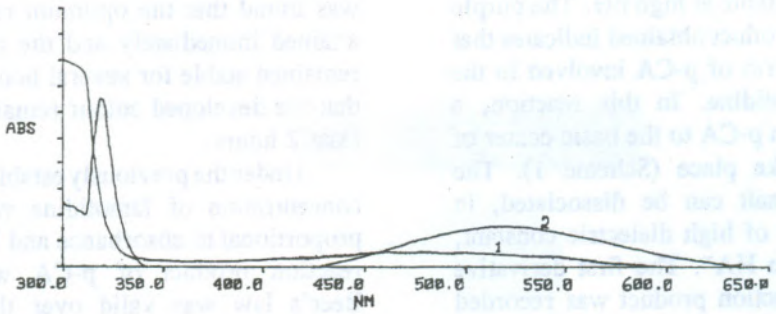


Fig. 1: Absorption spectra of p-chloranilic acid solution (1) and its reaction product with famotidine (2), 50 μg/ml

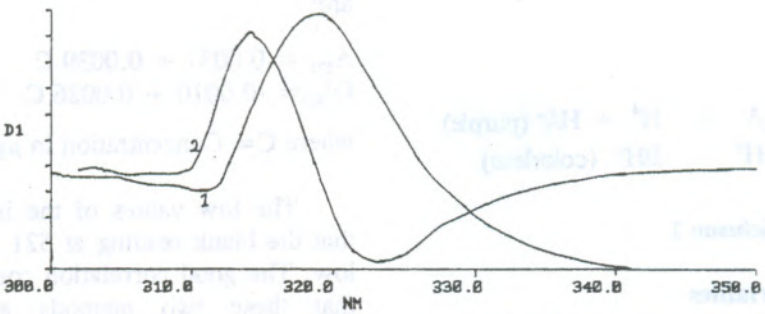


Fig. 2: Absorption spectrum (1) and first derivative spectrum (2) of reaction product of p-chloranilic acid with famotidine, 25 μg/ml.

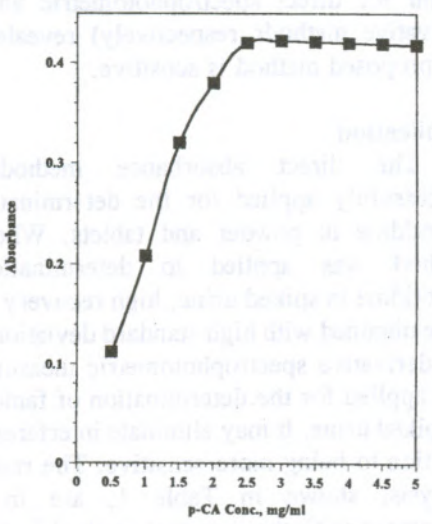


Fig. 3: Effect of p-CA concentration on its reaction product with famotidine, 100 μg/ml.

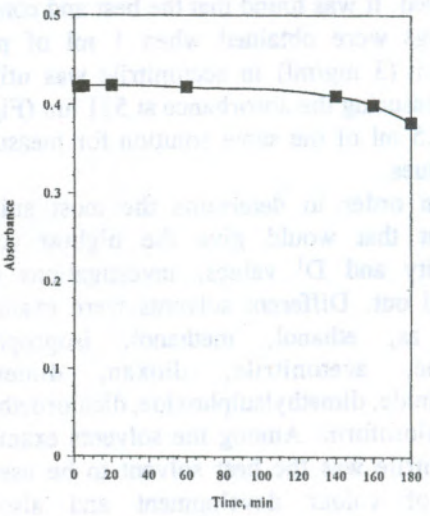


Fig. 4: Stability time of the reaction product of p-CA with famotidine, 100 μg/ml.

Table 1: Analysis of famotidine in its tablet and in spiked urine by colorimetric and first derivative spectrophotometric methods.

Sample of Famotidine	Drug content, mg	Recovery, % ± SD*		
		Direct method	D' method	reported method
Bulk drug	20	98.51±0.92 f= 2.61 t= 0.42	99.13±0.87 f= 1.60 t= 1.33	98.20±1.10[a]
Antodine tablet	20	97.37±0.83 f= 1.92 t= 0.39	98.5±0.82 f= 1.97 t= 0.82	97.92±1.15[a]
Spiked urine	2 10 25	**	1.2.10±1.52 101.80±1.13 100.60±0.99	

Theoretical values for t= 2.228 and f= 5.05 (at p=0.05).
* :Average of four determinations.
**: High recovery values.
[a] : Reference 7.

the theoretical values (at 95 % confidence level) and so, there is no significance difference between the proposed and reported methods. This indicates the accuracy and precision of the proposed method. The first derivative method is about ten times more sensitive than the direct spectrophotometric one. So, it was applied successfully for the determination of famotidine in tablets and spiked urine.

Mechanism of reaction

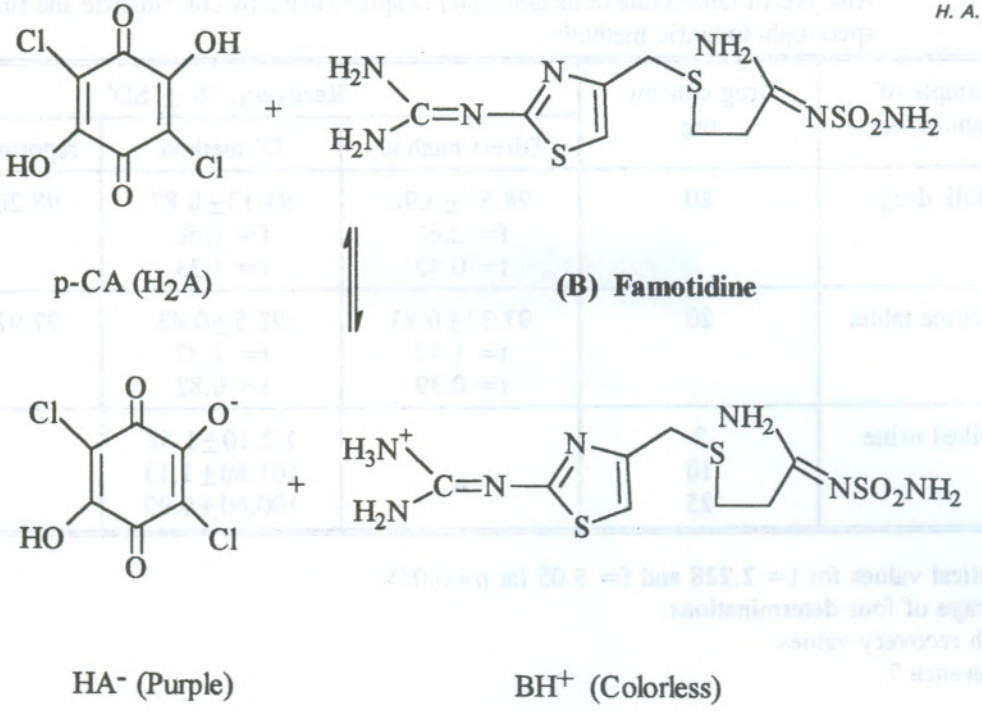
Some reports (23-25, 27) claim that the reaction of p-CA (H_2A) with certain basic compounds (**B**) is probably due to charge transfer complex formation between these bases as n-donor and p-CA as π-acceptor with subsequent formation of a coloured radical anions of p-CA as follows:

$$B \rightleftharpoons \underset{\text{Intermediate}}{[B \rightarrow H_2A]} \rightarrow \underset{\text{radical anion}}{B^+ + H_2A^-}$$

However, the reaction pathway may be explained as a proton transfer from p-CA to the basic center of the drug (Scheme 1) which is most probable. The obtained ion pair salt was dissociated in presence of acetonitrile to give the anion form of p-CA (HA^-) which is purple colored. This explanation was confirmed by IR, electron spin resonance, H^1-NMR as well as C^{13}-NMR.[28]

The reaction of famotidine with p-CA is obviously so fast, sensitive, and the purple chromogen is more stable when compared with charge transfer reaction of similar basic drugs with π-acceptors. Job's method of continuous variation was carried out to determine the ratio of famotidine to p-CA. It was found that the ratio is 1:1, indicating that, in famotidine, only one basic site participates in the reaction with p-CA. These findings suggest that the interaction of p-CA with famotidine could be an ion pair formation rather than a charge transfer complex interaction (Scheme 2).

In conclusion, the proposed method has the advantages of sensitivity simplicity, accuracy, precision and time saving. These advantages make it suitable for analysis of famotidine in dosage form and urine, and for application in quality control.



Scheme 2

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