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The Spectrophotometric Determination of Famotidine Drug via Coupling with Diazotized Metochlopramide Hydrochloride

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Abstract:

A new, simple and sensitive spectrophotometric method was described for the determination of famotidine (FAM) as a pure material and in pharmaceutical formulation. This method was based on diazotization and coupling reaction between famotidine and diazotized solution of metochlopramide hydrochloride (DMPH) in the presence of phosphate buffer solution to give a compound of azo dye having orange color soluble in water with high absorptivity at a wave length of 478 nm. The data shows that FAM and DMPH combine in the molar ratio of 1:1 at PH 7.0 .The method obeys Beer's law over concentration range of 1-40 $\mu g.ml^{-1}$ of famotidine with a correlation coefficient of 0.9955 and a detection limit of 0.10 $\mu g.ml^{-1}$. The apparent molar absorptivity referred to famotidine has been found to be 2.0×10^4 L. $mol^{-1}cm.^{-1}$. The proposed method was applied successfully to the assay of famotidine in pharmaceutical preparation.

Key words: Famotidine, Spectrophotometric determination, Metochlopramide, Diazotization and coupling.

Introduction:

Famotidine (FAM), 3-[[[2-[(Aminoiminomethyl) amino] – 4-thiazolyl] methyl] thio] -*N*-(aminosulfonyl) propanimidamide (Figure 1) (Molecular weight: 337.5 $g.mol^{-1}$) [1], is a histamine H_2-receptor antagonist (H_2-RA) which competitively inhibits the action of histamine on the H_2-receptors of parietal cells and thereby reduces the gastric acid secretion under daytime and nocturnal basal conditions. It is widely used in the management of gastrointestinal disorders, such as aspiration syndrome, dyspepsia, gastro- oesophageal reflux disease, peptic ulcer and Zollinger-Ellison syndrome. FMT is official in the British Pharmacopoeia [1] and in the United States Pharmacopoeia [2]. Various methods have been reported for the determination of famotidine as a pure drug and in pharmaceutical preparations. These methods include titrimetric [3], spectrophotometric, [4-17] and HPLC [18-25]. It is always required to develop a simple, fast, and inexpensive analytical method that can

be readily adopted for routine analysis at relatively low-cost to the different requirements of analytical problems. The present study describes the development of method based on diazotization and coupling reaction between diazotized metochlopramide hydrochloride (DMPH) reagent with famotidine in an alkaline medium. The orange product was spectrophotometrically measured at 478 nm. Thorough survey of literature on the famotidine revealed that quantification using diazotization reaction has not been reported yet. The analytical procedure is simple, fast, accurate, and has been applied for the determination of famotidine in pure and pharmaceutical preparations.

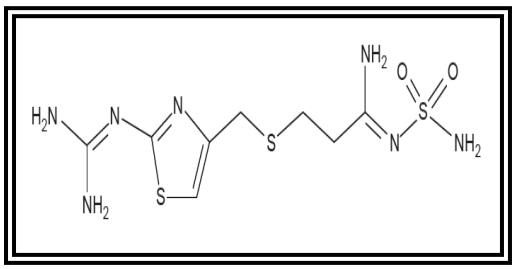


Fig (1): Structure of FAM

Materials and Methods:

Apparatus

A Shimadzu UV-VIS 260 digital double-beam recording spectrophotometer (Kyoto, Japan) was used for all spectral and absorbance measurements with a 1 cm matched quartz cuvettes.

Chemicals and reagents:

Chemicals and reagents of analytical grade used in this study. The standard material of FAM and excipients usually used in pharmaceutical tablets were provided from the State Company for Drug Industries and Medical Appliances (SDI), Samarra-Iraq.

Pharmaceutical tablets:

Pharmaceutical tablets were obtained from commercial sources.

Famodar Tablets: 20 mg famotidine for each tablet (Dar Al Adwa, Na'ur-Jordan).

Peptifam Tablets: 20 mg famotidine for each tablet (The United Pharmaceutical Manufacturing, Amman - Jordan).

Ulceran Tablets: 20 mg famotidine for each tablet (Medochemie, Limassol-Cyprus).

Solutions:

Famotidine stock solution (1000 µg. ml.$^{-1}$ = 2.96 × 10^{-3}M):

A 0.100 gm amount of pure famotidine (SDI) was dissolved in distilled water then completed to 100 ml in a volumetric flask with the same solvent. More dilute solutions were prepared by suitable dilution of the stock standard solution with distilled water.

Diazotized metochlopramide hydrochloride (DMPH) (5 × 10^{-3}M):

Prepared by dissolving 0.1772 g of MPH (SDI) in a minimum volume of distilled water, 3 ml of 1M hydrochloric acid was added in a 100 ml volumetric flask .The mixture was cooled to 0-5 °C for 5 min using an ice–bath. A weight of 0.0345 g amount of sodium nitrite was added and the mixture was stirred. After 5 min the volume was made up to the mark with distilled water [26,27]. More dilute solutions were prepared by suitable dilution with distilled water.

Hydrochloric acid (BDH) (1M):

Prepared by diluting 43 ml of 11.64 M of concentrated hydrochloric acid with distilled water in 500 ml volumetric flask.

Acetic acid (Fluka) (0.1 M):

Prepared by diluting 2.8 ml of 17.41 M of concentrated acetic acid with distilled water in 500 ml volumetric flask.

Sodium hydroxide solution (BDH) (0.1 M):

1.0 gm amount of NaOH was dissolved in a 250 ml volumetric flask with distilled water.

Ammonium acetate (BDH) (0.1M):

7.709 gm amount of CH_3COONH_4 was dissolved in a 1000 ml volumetric flask with distilled water.

Disodium hydrogen phosphate (BDH) (0.1M):

14.1960 gm amount of Na_2HPO_4 was dissolved in a 1000 ml volumetric flask with distilled water.

Acetate buffer solutions (BDH) (PH 5 and 6):

Acetate buffer solutions were prepared by mixing a Suitable amount of 0.1M CH_3COONH_4 with 0.1M CH_3COOH. The PH of buffer solutions then adjusted with PH-Meter.

Phosphate buffer solutions(PH7-11):

Phosphate buffer solutions were prepared by mixing a Suitable amount of 0.1M Na_2HPO_4 either with 0.1M HCl (to prepare buffer solutions have a PH value equal to 7, 8, and 9) ,or with 0.1M NaOH (to prepare buffer solutions have a PH value equal to 10 and 11). The PH of buffer solutions then adjusted with PH-Meter.

Recommended procedure and calibration graph:

Into a series of 25 ml calibrated flasks, add 1.5ml of 5×10^{-3} M DMPH solution, followed by 0.75ml of buffer solution. Then transfer increasing volume of famotidine drug solutions (500 $\mu g.ml^{-1}$).Dilute the solution to the mark with distilled water and allow the reaction mixture to stand for 30 min in ice bath. Measure the absorbance at λmax 478 against a blank reagent prepared in the same way but containing no FAM drug (For high concentrations of absorbance of higher than 1 absorbance unit, a dilution of solutions were performed and the absorbances were multiplied by a factor). The colour of the resulting dye is stable for about 30 min. The calibration graph as shown in figure (2) was linear over the concentration range of 1-40 $\mu g.ml^{-1}$.

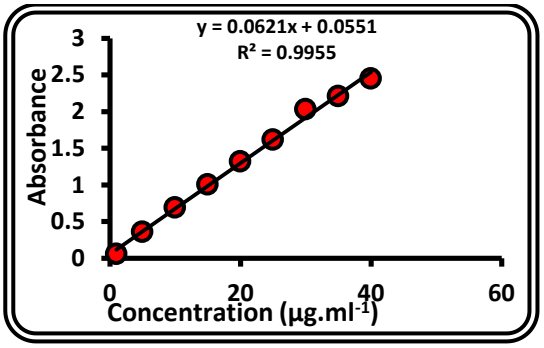


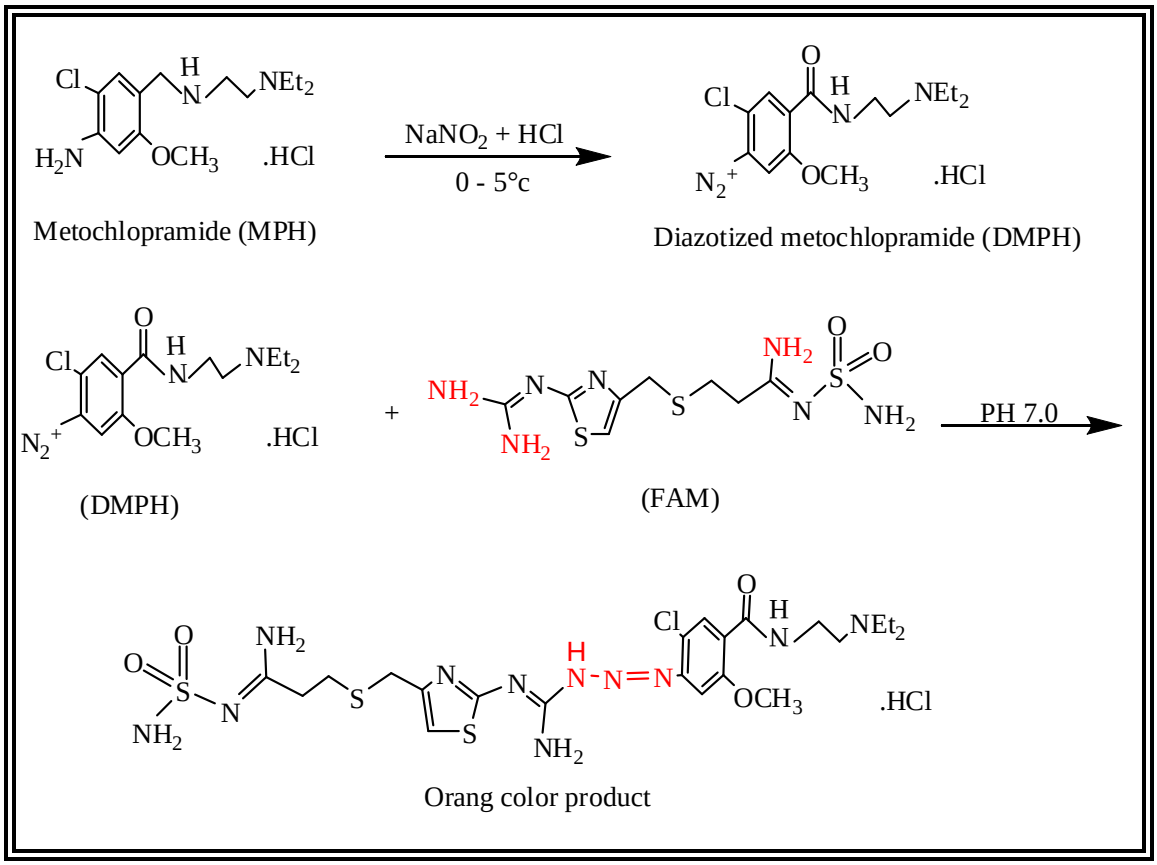
Fig (2): The calibration graph of FAM

Procedure for the assay of pharmaceutical preparations Tablets solution (250 $\mu g.ml^{-1}$):

Ten formulated tablets were accurately weighed and powdered. A quantity of powder equivalent to 20 mg of FAM was transferred to 100 ml volumetric flask and dissolved in distilled water and completed to the mark with the same solvent. The sample solution was then shaked well and filtered through Whatman filter paper No.41. More dilute solutions were prepared by suitable dilution with distilled water.

Results and Discussion:

The method involves the coupling reaction between famotidine with diazotized metochlopramide hydrochloride in phosphate buffer solution to give a deep orange coloured azo dye. The absorption spectrum of the colored dye is shown in figure (3).Two steps are involved in the reaction that produces the colored dye. The first step included the preparation of DMPH as mentioned before while the second step involved the coupling of the diazonium ion with famotidine in buffer solution (PH=7) to form the azo dye. The steps involved are shown in Scheme (1).



Scheme (1): Steps of the main reactions

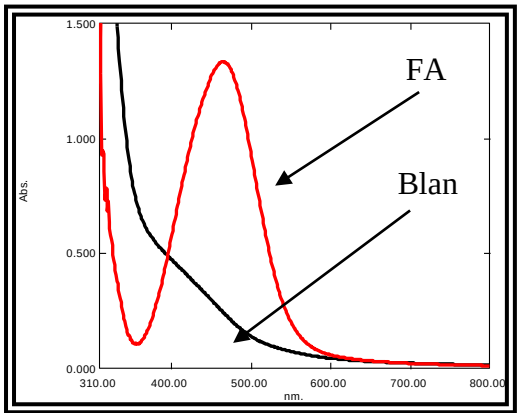


Fig (3): Absorption spectra of 20 µg.ml.$^{-1}$ of FAM treated as described under procedure and measured against blank and the reagent blank measured against distilled water.

The effect of various variables on the color development of the azo dye formed from the reaction of FAM (20 µg.ml^{-1}) with DMPH was investigated and the optimum conditions have been selected. All following experiments were achieved in an ice-bath to increase the stability of the azo dye.

The effect of hydrochloric acid (1M):

The diazotization coupling reaction occurred in an acidic medium and a hydrochloric acid of concentration 1M was selected, the effect of different volumes (1 − 5 ml) of 1 M of HCl were studied and 3 ml volume seems to be optimum for an intense azo dye color as shown in table (1).

Table (1): The effect of (1M) HCl

Vol. of HCl (1M)	1	2	3	4	5
Abs.	0.372	0.588	0.812	0.101	0.010

The effect of the coupling reagent (DMPH):

The effects of the different volumes (0.5 – 2.0 mL) of 5 mM DMPH solution were examined on the maximum formation of the colored product. Table (2) shows that 1.5 ml of the solution was optimum and was used in the subsequent experiments.

Table (2): The effect of the coupling reagent

Vol. of DMPH (5 mM), mL	**0.5**	**0.75**	**1**	**1.5**	**1.75**	**2**
Abs.	0.815	0.873	0.886	1.002	0.603	0.094

The effect of PH:

The effect of different PH (5-11) of buffer solutions was studied. Table (3) shows that PH =7 of the Na_2HPO_4 buffer solution was optimum and was used in the subsequent experiments.

Table (3): The effect of PH of buffer solution

PH	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Abs.	0.300	0.659	1.010	0.906	0.413	0.376	0.224

The effect of volume of buffer solution:

According to the optimum pH found in table (3), different volumes (0.5 to 2 ml) of buffers of pH 7 have been tested. The results shown in table (4) indicated that 0.75 ml of buffer solution was the optimum and recommended in the subsequent experiments.

Table (4): The effect of volume of buffer solution

Vol. of buffer solution (PH8.0),mL	**0.5**	**0.75**	**1**	**1.5**	**1.75**	**2**
Abs.	0.952	1.212	1.012	0.900	0.872	0.813

The effect of addition order:

Three orders of addition were examined .Table (5) shows that order No.2 was the optimum and recommended in the subsequent experiments.

Table (5): The effect of addition order

NO.	***Addition order***	***Abs.***
1	D + B + R	1.210
2	R + B + D	1.310
3	D + R + B	1.282

D = Drug, R = Reagent, B = Buffer

The effect of temperature:

The effect of temperature on the colour intensity of the product was studied. In practice the same absorbance was obtained when the colour was developed in an ice-bath at 5°C but when the calibrated flask was placed in a water-bath at 45°C or at room temperature (25°C) a loss in colour intensity and stability were observed, it is therefore recommended that the colour reaction should be carried out in an ice-bath at (5°C) as shown in the table (6).

Table (6): The effect of temperatures

Temperature, °C	**5**	**25**	**45**
Abs.	1.310	0.964	0.844

Effect of reaction time:

The color intensity reached a maximum after drug solution had been reacted immediately with DMPH in neutral medium and became stable after 30 min and remained stable for another 30 min (Table 7). Therefore, 30 min development time was selected as optimum in the analytical procedure.

Table (7): The effect of reaction time

Time (min.)	**5**	**10**	**15**	**20**	**25**	**30**	**35**
Abs.	**1.043**	**1.125**	**1.257**	**1.274**	**1.290**	**1.319**	**1.316**
Time (min.)	**40**	**45**	**50**	**60**	**70**	**90**	**120**
Abs.	**1.319**	**1.318**	**1.319**	**1.314**	**1.243**	**1.025**	**0.736**

Nature of the dye product:

The stoichiometry of the reaction between FAM and DMPH was investigated using the molar ratio and continuous variation methods with concentration of 7.4×10^{-4} M and 2.9×10^{-4} respectively; it was found that FAM reacted with DMPH in a ratio of 1:1 as shown in figure (4) and figure (5) respectively.

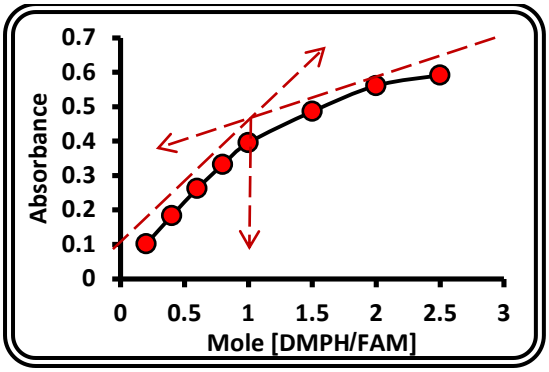


Fig (4): Mole ratio plot

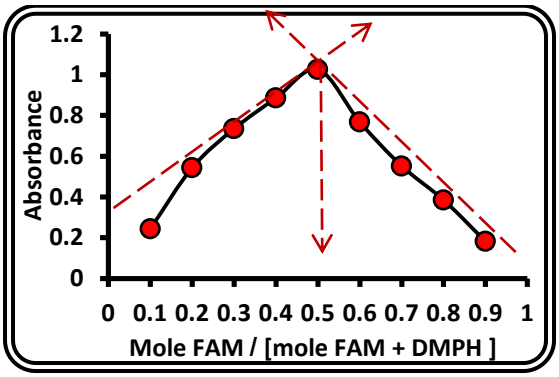


Fig (5): Continuous variation plot

Analytical data:

Analytical values of statistical treatments for the calibration graph are summarized in table (8).

Table (8): Analytical values of statistical treatments for the calibration graph

Parameter	*Value*
λ_{max} (nm)	478
Regression equation	Y = 0.0621x + 0.0551
Correlation coefficient(r)	0.9977
Correlation coefficient, r^2	0.9955
Linearity percentage	99.55
Dynamic range (µg.ml^{-1})	1-40
Molar absorptivity, ε (L.mol^{-1}.cm^{-1})	2.0958×10^4
Sandell's sensitivity, S (µg.cm^{-2})	0.0161
Limit of Detection (µg.mL^{-1})	0.106
Limit of Quantitation (µg.mL^{-1})	0.353

Accuracy and Precision:

The accuracy and precision of the determination of FAM were studied depending upon the value percentage of the relative error (E %), recovery (Rec. %), and relative standard deviation (RSD %) respectively. For five replicates of each concentration of FAM containing 10, 15, 20 and 35 µg.ml.$^{-1}$. The results in table (9) show a good accuracy and precision.

Table (9): Accuracy and precision of the proposed method

Conc., µg.mL $^{-1}$		*E%** $E\% = \frac{X - X^0}{X^0} \times 100$	*Rec.%** $\text{Rec.}\% = 100 + E\%$	*RSD%** $RSD\% = \frac{S}{X} \times 100$
Present	*Found*			
10	10.28	2.83	102.83	1.15
15	15.49	3.26	103.26	0.97
20	20.37	1.87	101.87	0.17
35	34.34	-1.90	98.10	0.16

***Average of five determinations, x = measured value, x° = true value**

The effect of interferences:

To evaluate the selectivity of the proposed method for the analysis of pharmaceutical preparations containing FAM, the interfering effect of excipients were examined by determining FAM in the presence of the interference and applying the analytical procedure. The excipients studied were: lactose, talc, starch, magnesium stearate, and polyvinylpirrolidone (PVP). For this study, a solution containing FAM (20 µg.ml^{-1}) and each one of the excipients was taken separately in concentrations ten-times greater than that of FAM was analyzed. Under the reaction conditions used all of the excipients do not interfere as the results shown in table (10).

Table (10): The effect of excipients on the recovery of FAM

Excipient (200 µg .ml^{-1})	*Conc. of FAM, µg. ml^{-1}*		*E%**	*Rec.%**
	Present	*Found*		
Lactose	20	19.85	-0.74	99.26
Mg stearate	20	19.97	-0.15	99.85
Starch	20	20.12	0.61	100.61
Talc	20	19.91	-0.47	99.53
PVP	20	20.20	1.02	101.02

***Average of five determinations**

Pharmaceutical applications:

The proposed method was applied for the determination of FAM in tablets by the analysis of three different concentrations of sample using the analytical procedure. The results obtained are summarized in table (11).

Table (11): The application of proposed method for determination of FAM in pharmaceutical preparation

Pharmaceutical Preparation	*Conc. of FAM, µg. mL^{-1}*		*E%**	*Rec.%**	*RSD%**
	Present	*Found*			
Peptifam (Tablet 20 mg)	5	4.92	-1.59	98.41	0.81
	10	9.90	-1.04	98.96	0.75
	15	14.93	-0.49	99.51	0.75
Ulceran (Tablet 20 mg)	5	4.95	-0.99	99.00	0.73
	10	10.03	0.26	100.26	0.32
	15	14.90	-0.65	99.35	0.09
Famodar (Tablet 20 mg)	5	4.95	-0.90	99.10	1.54
	10	10.04	0.36	100.36	1.26
	15	14.99	-0.03	99.96	1.24

***Average of five determinations.**

The evaluation of the proposed method:

The results obtained by the proposed method were compared with British Pharmacopoeia (BP) method (HPLC) [1,2] by applying the F-test and the t-test at 95% confidence level as shown in Table (12). The calculated value for F and t for famotidine did not exceed the critical value of F and t. These confirming that there are no significant differences between the proposed method with BP method with respect to precision and accuracy in the determination of famotidine in pharmaceutical preparations.

Table (12): The comparison of the proposed method with standard methods using t- and F-statistical tests

Drug form	*Proposed method*		*Standard method*		*Statistical values*
	Rec.% $(X_i)_1$	$(X_i-\bar{X})_1^2$	Rec.% $(X_i)_2$	$(X_i-\bar{X})_2^2$	
FAM pure	101.87	0.614	101.111	0.309	S_1^2 = 0.854
Peptifam (Tablet 20 mg)	98.408	0.303	100.833	0.420	S_2^2 = 1.483
Ulceran (Tablet 20 mg)	99.006	0.283	98.333	0.308	S = 1.081
Famodar (Tablet 20 mg)	99.096	0.506	101.666	1.929	t*= 0.026
	$(\bar{X}_i)_1$ = 100.239	$\sum(X_i-\bar{X})_1^2$ = 1.708	$(\bar{X}_i)_2$ = 100.216	$\sum(X_i-\bar{X})_2^2$ = 2.966	F*= 1.736

***Theoretical values at 95% confidence limit, $n_1 = n_2 = 3$, t = 2.776, where t has degrees of freedom = $n_1+n_2-2=4$ F = 19.000, where F has degrees of freedom = $n_1-1= n_2-1=2$**

Conclusion:

The proposed study describes method for estimation of FAM in pharmaceutical formulation. The method was validated and found to be simple, sensitive, and accurate. The method was successfully used for determination of FAM in pharmaceutical formulation.

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التقدير الطيفي لدواء الفاموتيدين عن طريق الازدواج مع الميتوكلوبرامايد هيدروكلورايد المؤزوت

مؤيد قاسم العبايچي* ***سعاد سلمان محمد**** ***أنفال جلال هادي****

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** قسم الكيمياء، كلية العلوم للبنات، جامعة بغداد، بغداد، العراق

الخلاصة:
تم وصف طريقة طيفية جديدة، بسيطة وحساسة لتقدير الفاموتيدين (FAM) كمادة نقية وفي المستحضرات الصيدلانية. تعتمد الطريقة على تفاعل الازوتة والازدواج بين الفاموتيدين و المحلول المؤزوت للميتوكلوبرامايد هيدروكلورايد (DMPH) بوجود محلول الفوسفات المنظم ليعطي مركب لصبغة ازو ذو لون برتقالي ذائب بالماء مع اعلى امتصاصية عند طول موجي 478 نانومتر. أظهرت النتائج بأن FAM و DMPH يتحدان بنسبة جزئية قدرها 1:1 عند PH 7.0. تخضع الطريقة لقانون بير عند تركيز يتراوح بين 1-40 مايكروغرام.مل $^{-1}$ من الفاموتيدين مع معامل ارتباط 0.9955 وحد كشف 0.10 مايكروغرام.مل$^{-1}$. وجد أن الامتصاصية المولية العائدة للفاموتيدين هي 2.0×10^4 لتر$^{-1}$.مول$^{-1}$.سم$^{-1}$. تم تطبيق الطريقة المقترحة بنجاح لتعين الفاموتيدين في المستحضرات الصيدلانية.

الكلمات المفتاحية: الفاموتيدين، النقي الطيفي، الميتوكلوبرامايد، الازوتة، الازدواج.