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Determination of Famotidine in Pharmaceutical Preparations by Flow Injection Spectrophotometric Method and using organic reagent (Pyro catechol)

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ABSTRACT

Simple accurate and sensitive batch and flow injection spectrophotometric method was depicted for the determination of famotidine (FAM) in pharmaceutical formulation. The proposed method based on oxidative coupling reaction between famotidine and the organic reagent -pyro catechol- in the presence of ferric ammonium sulfate as oxidant agent to give the water soluble blue colored product with high absorptivity at a wavelength of 580nm.The molar ratio of FAM and pyro catechol was 1:1. The Optimum conditions and analytical parameters of batch reaction method were evaluated The linearity of spectrophotometric method was in the range of (15-450) μg.ml^{-1} with a correlation coefficient of 0.9979. The detection limit was 10.425 μg.ml^{-1}. The flow injection method the linear range (100- 1500) μg. mL^{-1}, and the detection limit 87.22μg .mL^{-1}. The proposed method was applied successfully to the assay of famotidine in pharmaceutical preparation.

Key words: Famotidine, Flow Injection, Spectrophotometric determination, famotidine,

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INTRODUCTION

Famotidine (FAM), 3-[[[2-[(aminoiminomethyl)amino]-4- thiazolyl] methyl]thio]-N-(aminosulfony [1] .

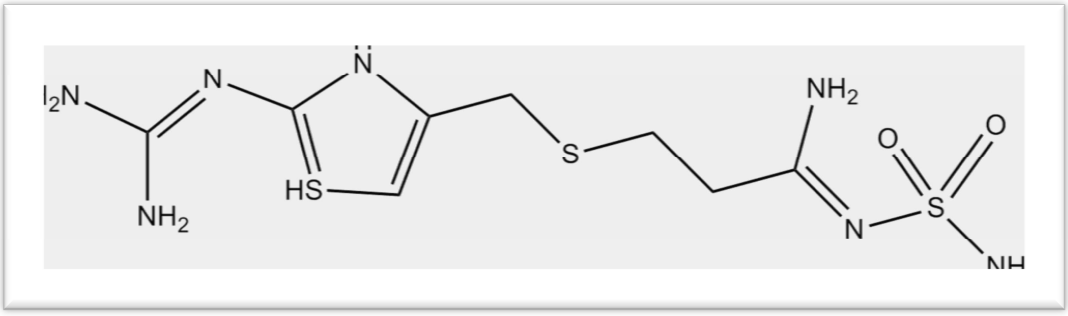


Fig (1): Structure of FAM

The chemical formula of C8H15N7O2S3 (molecular weight = 337.45 g / mol) , pKa value of 7.1 in water at 25°C and melting point of 161-163oC [2]. Famotidine is pale yellowish-white, crystalline powder figure [1]. It is sensitive to light, freely soluble in dimethylformamide and in glacial acetic, slightly soluble in methanol, very slightly soluble in water, [3]. Famotidine ligand is a highly effective long acting histameric H2-receptor antagonist drug widely used for the treatment of duodenal ulceration, benign gastric ulcer and hyper acid secreting conditions such as gastro-esophageal reflux, heart-burn, and Zollinger-Ellison syndrome [1-4]. Due to the presence of amino, amido and

thioether groups in its structure, this drug possesses chelating properties and may interactvery effectively with the essential metal ions present in blood plasma and different tissues [1]. Famotidine inhibits many of the isoenzymes of the hepatic CYP450 enzyme system. Other actions of famotidine include an increase in gastric bacterial flora such as nitrate-reducing organisms. Famotidine is given to surgery patients before operations to prevent postoperative nausea and to reduce the risk of aspiration pneumonitis. Famotidine is also given to some patients who take NSAIDs, to prevent peptic ulcers [3]. The drug is official in the USP which specifies non-aqueous titration for assay of raw material and HPLC method for tablet analysis [5,6].The reference official methods for assaying famotidine in its tablet formulation involve the use of HPLC techniques[7,8].Other procedures techniques has been studied in determining Famotidine in different biological and pharmaceutical preparations including differential pulse paleography [4] , spectrophotometric procedures [7-13], Spectrofluorimetry [14], calorimetry [15], potentiometry [16-17], chromatography [18-20]. Electrochemically, and other special procedures such as Square wave adsorptive stripping voltammetric [20-22], Square wave voltammetry [23], . The present study describes the development of method based on oxidative coupling reaction between famotidine and the organic reagent -pyro catechol- in the presence of ferric ammonium sulfate as oxidant agent to give the water soluble blue colored product with high absorptivity at a wavelength of 580nm.throgh the literature survey on the drug determination, some of them have used the colorimetric reagent in the determination procedure but no previous study have used the pyrochaticol as the organic reagent. The assay procedure is fast, accurate, simple and has been applied for the determination of famotidine in pure and pharmaceutical preparations.

MATERIALS AND METHODS

Apparatus

Digital double-beam recording spectrophotometer using 1 cm quartz cells. The FIA system comprised a peristaltic pump (Labor technic-Analytic, CH-8152, gltbrugg – zurich , All spectral and absorbance measurements were performed on a Shimadzu UV -VIS 260 (Tokyo ,Switzerland, six channels) with poly vinyl chloride flow tubes of 0.8 mm i.d., an injection valve (Rheodyne, Altex 210, Supelco-USA), a 50 μL flow cells and Shimadzu UV-VIS 260 spectrophotometer (Tokyo, Japan) as the detector. Flexible Teflon tubes of 0.5 mm. were used for reaction coils and to transport reagents solutions. T-link was also used to mix two streams of reagents measurements.

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 (l dw N u -Jordan).
*Ullcerin 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 g m amount of pure famotidine (SDI) was dissolved in hot 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.

Pyrocatechol (PC) is[C6H6O2, M. w t =110.11g.mol^{-1}] from (Sherman Chemicals Ltd. , Down ham Mills, Totten ham ,London).

The solutions of PC is 5×10^{-3}M for batch and FIA procedure respectively. These were freshly prepared by dissolving (0.055 g) of PC and diluting with distilled water in 100mL volumetric flask. Ferric ammonium sulfate[Fe(NH4)2(SO4)2.6H_2O] M. w t=392.16g.mol^{-1}] from (H& W) This solution as oxidizing agent was prepared by dissolving 0.0392 g of ferric ammonium sulfate and diluting to 100 mL with distilled water volumetric flask to obtain (1×10^{-3}M) [Fe(NH4)2(SO4)2).6H2O] for batch and FIA methods.

Samples Preparation:

Tablets were accurately weighted and finely powder equivalent to 0.1g of Drug was dissolved in 100mL of distilled water. The solution was filtered into 100 mL volumetric flask, the residue was washed and diluted to volume with as described above to obtain 500 μ g. m l^{-1} of. Further appropriate solutions of pharmaceutical preparations for batch and FI A procedures were made by using distilled water.

Procedure.

General batch procedure

working solution In a 10 ml calibrated flask, transfer increasing volumes of FAM (reduction solution (100 µg.ml$^{-1)}$ to cover the range of the calibration graph 15-450 µ g.ml^{-1} of FAM. Add 1 ml of 5×10^{-3}M PC and 1 ml of 1×10^{-3} M ferric ammonium sulfate solution and shake well. Dilute the solution to the mark with distilled water and allow the reaction mixture to stand for 10 min at room temperature. Measure the absorbance at 580 nm against a reagent blank prepared in the same way but no containing famotidine. The color of the formed product is stable for 80 min. For optimization of conditions and in all subsequent experiments, a solution of is a final volume of 10 ml (i.e.100µg) was used.

General FI A procedure

Different concentrations of reduced FAM (100-1500µ g. m L^{-1}) were injected into the carrier stream of solution (PC,5×10^{-3} M). which was mixed then with the oxidizing solution (1×10^{-3}M) $Fe(NH_4)_2(SO_4)_2.6H_2O$.

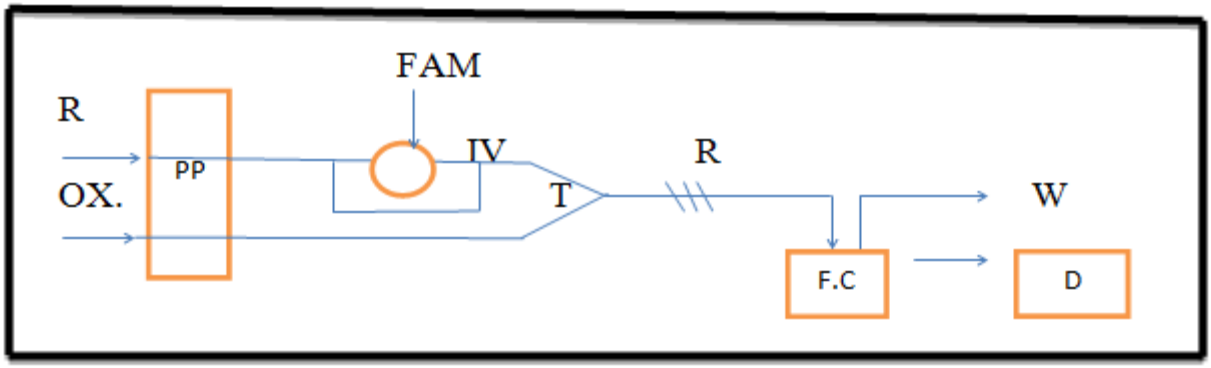


Figure 1 - FIA manifold for determination of Famotidine. Where: R =5×10^{-3} M pyro catechol, oxidant agent 1×10^{-3}M ferric ammonium sulfate

Fe(NH)2(SO4)2.6H2, PP = Peristaltic Pump, IV = Injection Valve, T =T-link, RC = Reaction Coil, FC = Flow Cell, D = Detector and W = West

Statistical analysis

Statistical analysis was conducted with Statistical Product Differences in application methods (Batch and FIA) of pharmaceutical formulations containing(FAM)was the proposed method is compared with standard method using independent sample T-test at a 95% confidence level .

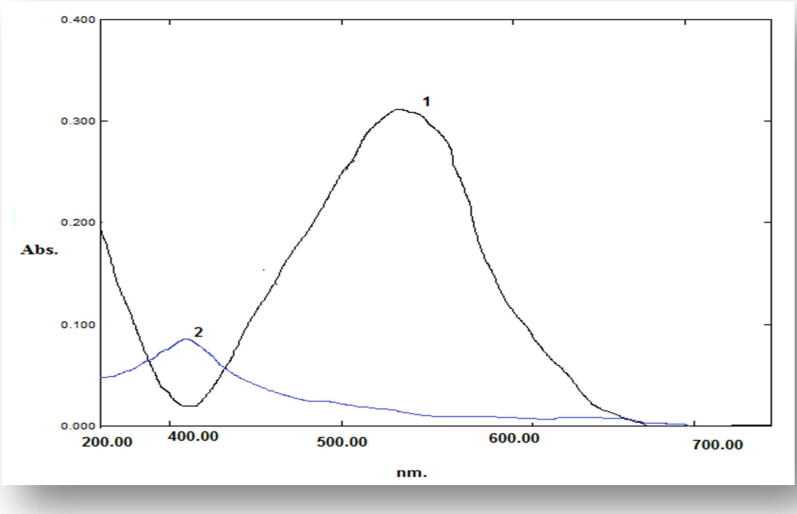


Figure 2- Absorption spectra of the(1) product obtained by the reaction of *PC* with *100*µg. mL^{-1} of *FAM* in the presence ferric ammonium sulfate, all versus reagent blank, versus distilled water

Discussion Results Preliminary Studies When a very dilute aqueous solution of (FAM) was mixed with PC reagent and ferric ammonium sulfate solutions, an intense blue colure product formed. This product has a maximum absorption Figure- 2], in contrast to the reagent blank which shows no absorption at the same

Optimization of the experimental conditions
The effects of various parameters on the absorption intensity of the formed product were optimized.

A- Batch method
In the subsequent experiments, 100 µg.ml^{-1} of famotidine was taken in 10 mL final volume (i.e. 16 µg. mL^{-1}) and the absorbance of a series of solutions were measured by varying one and fixing the other parameters at 580 nm versus reagents blanks.

The effects of different volumes of 5×10^{-3}M PC, (0.5-4mL) and 1×10^{-3}M Fe(NH4)2(SO4)2.6H2O (0.5-4mL), were examined on the maximum absorbance of the formed product.

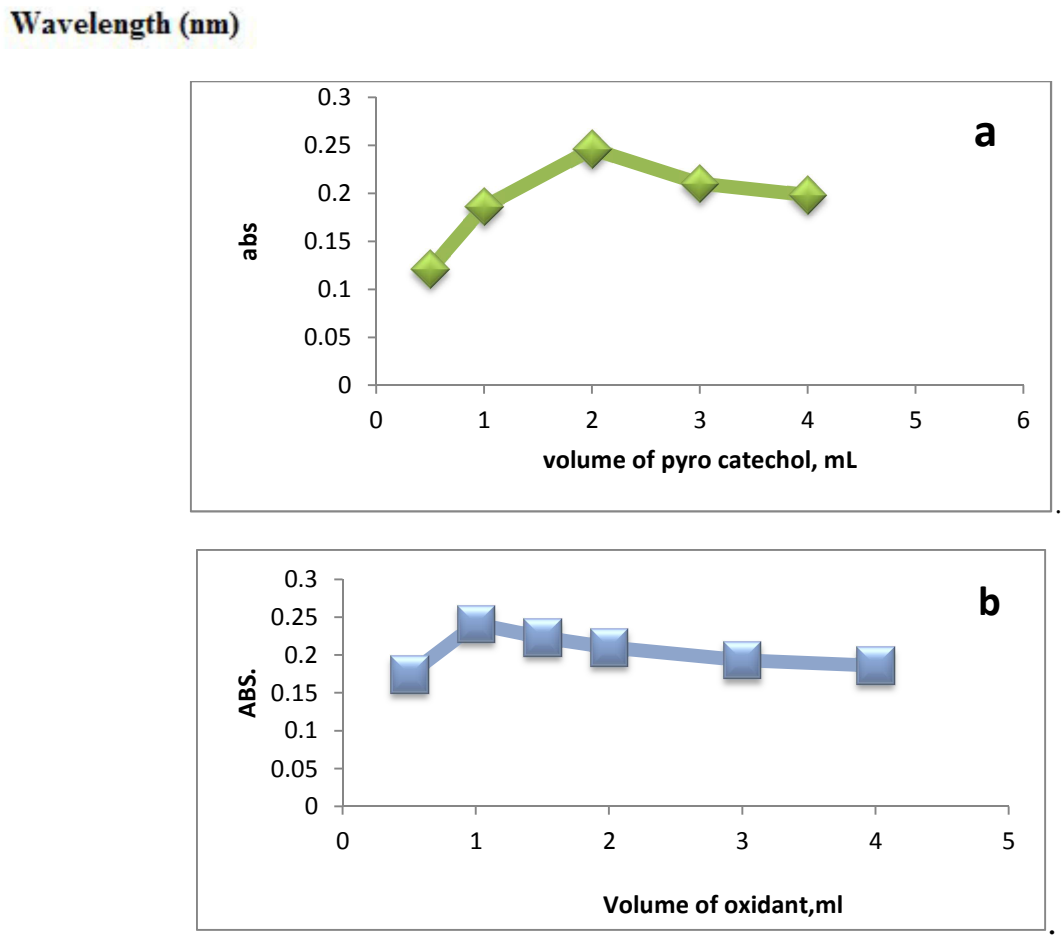
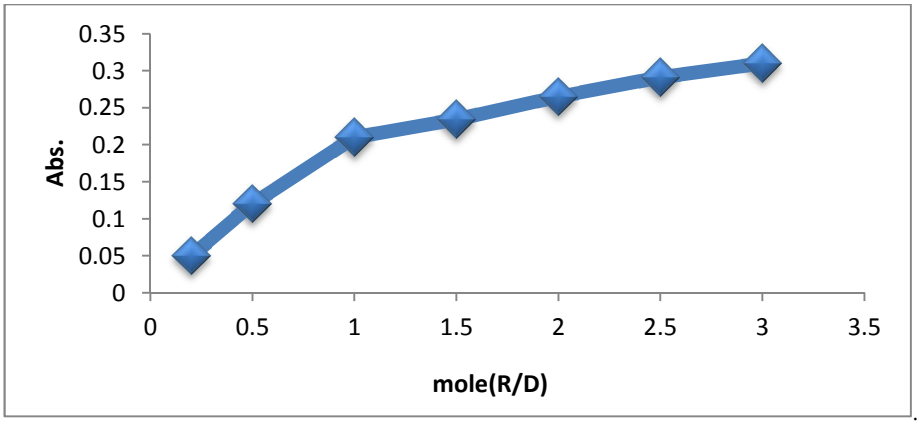


Figure-3 shows : a= that 2 mL of 5×10^{-3}M PC, and :b= 1ml of 1 ×10^{-3}M Fe$(NH_4)_2(SO_4)_2.6H_2O$ were enough to obtain the maximum absorbance

Order of addition.
The order of addition of reagents should be followed as given under the procedure, otherwise a less color intensity and stability was observed. The effect of temperature on the color intensity of the product was studied.

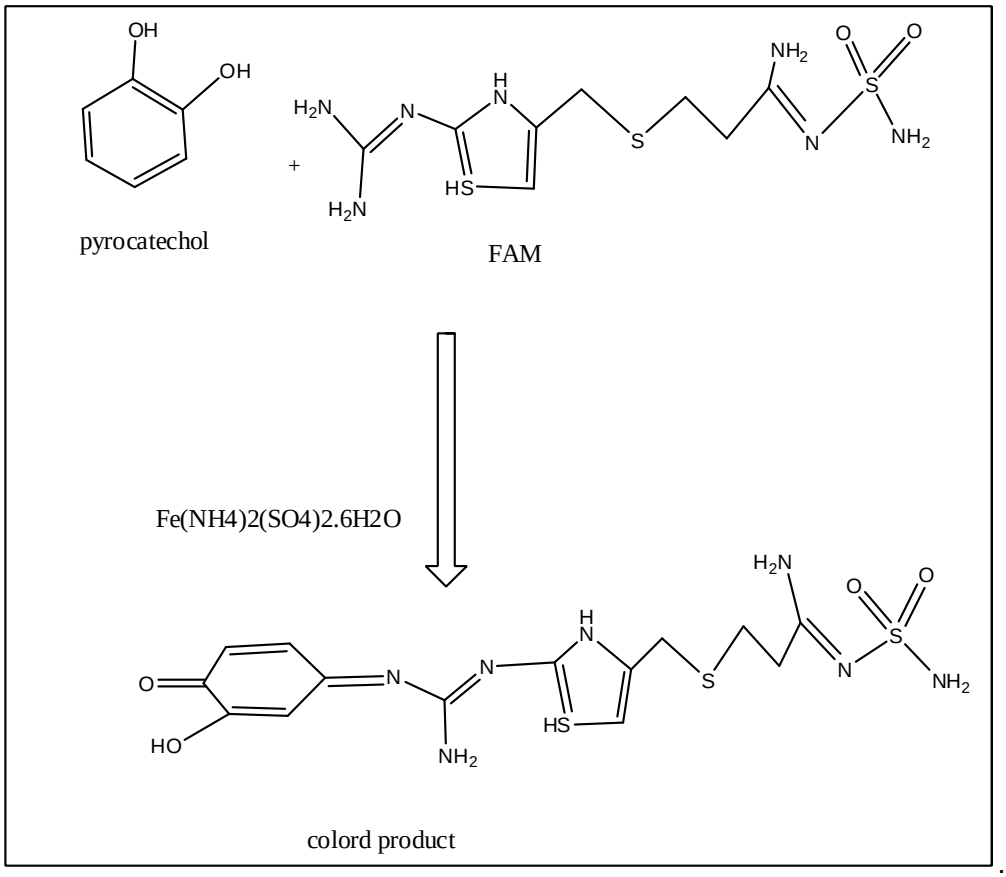
In practice, high absorbance was obtained when the color was developed at room temperature (25^0 C) that when the calibrated flasks were placed in ice bath (5 ^{0}C) or in water bath (45 ^{0}C). The stoichiometry of the product was investigated using the mole ratio method. The results obtained Figure-4 show that a 1:1 complex was formed between FAM (D) and PC (R).

Therefore, the formation of the product probably occurs according to the following equation (scheme-1).



(Figure 4)- Mole ratio plot using 1.4 ×10^{-3} M for both D, R and 1mLof 1× 10^{-3}M Fe(NH4)2(SO4)2.6H$_2$O

FAM, by virtue of the strong electron donating ability [24],react with PC(oxidized to o-benzoquinone by ferric ammonium sulfate), leading to the formation of oxidative coupling product [25], as shown in scheme (2).



Scheme 2: suggested reaction path

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 (20µg.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 1).

Table 1- Determination of 20µ g mL⁻ˡ of FAM in the presence of excipients

Excipient	Conc. Famotidine (µg mL⁻ˡ)		
200µ g m L⁻ˡ)	20µgmL⁻ˡ	E%	Rec%
Starch	20.27	1.35	101.35
Mg-stearate	20.244	1.22	101.22
Lactose	19.69	-1.83566	98.18
Pvp	20.27	-1.5	98.5

B- FIA method

The manifold used for the determination of famotidine is shown in Figure-1.

A two-channel FIA system was applied, in which the sample was injected into the PC stream, which was then mixed with a stream of oxidizing solution. The reagent and the oxidizing solution streams were pumped at the same flow rate to achieve effective mixing of the sample and reagents solutions.

The chemical and physical parameters were optimized by the un vitiated method with the purpose of maximizing the analytical frequency and reproducibility. According to the results of preliminary spectrophotometric studies for the FIA method.

Chemical variable

The effects of various concentrations (1×10^{-1}) - (1×10^{-2})M of ferric ammonium sulfate were studied ,and it was found that (1×10^{-3})M gave the best results and different concentrations of PC (1×10^{-3}) – (1×10^{-2})M were also investigated and (5×10^{-3})M was optimum(Figure5a,5b).

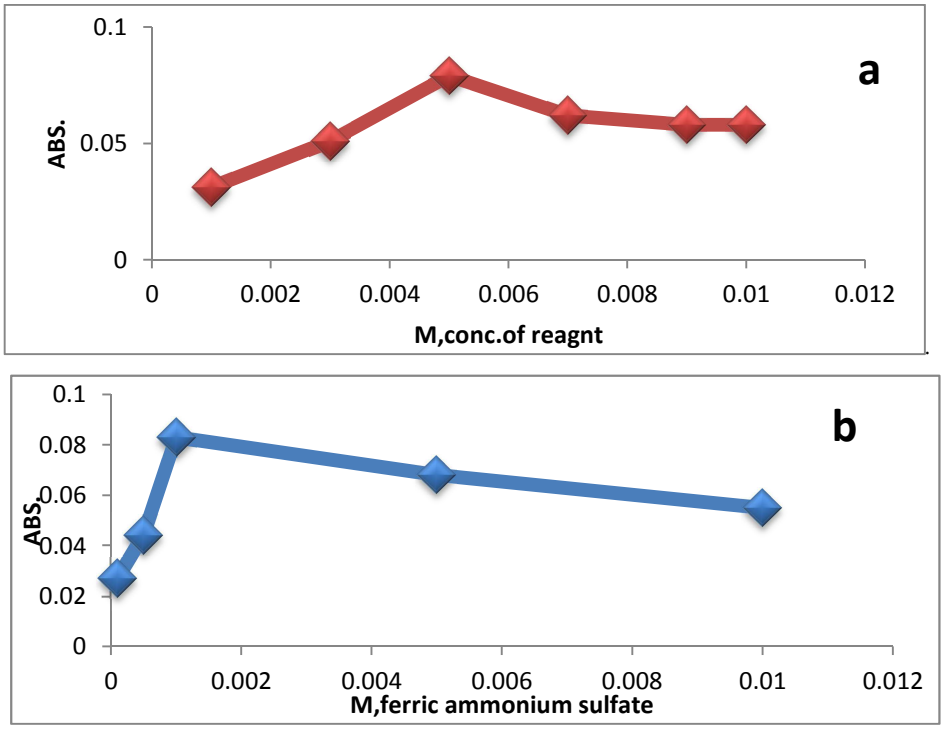


Figure 5- (a ,b) Chemical conditions of FIA procedure for the determination of FAM

The effects of flow rate in the analytical response was studied over the range mL min^{-1}.

Figure-6 shows that the absorbance increased up to 1.5mL min^{-1} and then decreased, therefore, this flow rate was selected.

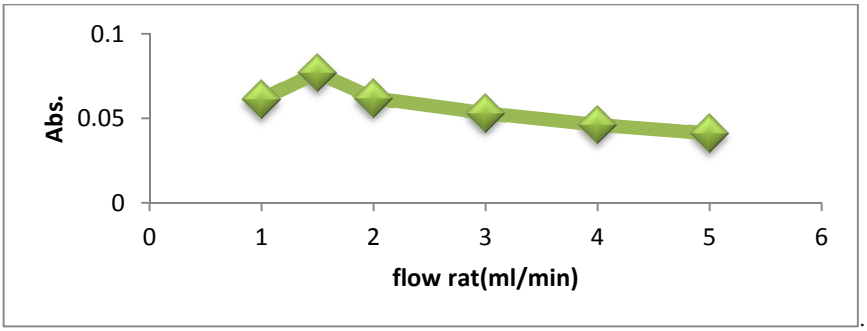


Figure 6- The effects of flow rate in the analytical

The reaction coil length is very essential parameter that effected on the sensitivity of the colored reaction product, therefore, it was investigated in the range (25-250)cm. The results obtained showed that a coil length of 80 cm gave the highest absorbance as shown in Figure- 8 and was used in all subsequent experiments. The injected sample volume in the range (100-250) µ L was evaluated by changing the length of sample loop in the injection valve, while other variable remained fixed. The absorbance increased with increasing the volume of sample injected up to 200 µ L (Figure- 7), which was chosen. The flow system selected provided a sampling rate of 30 samples h^{-1}.

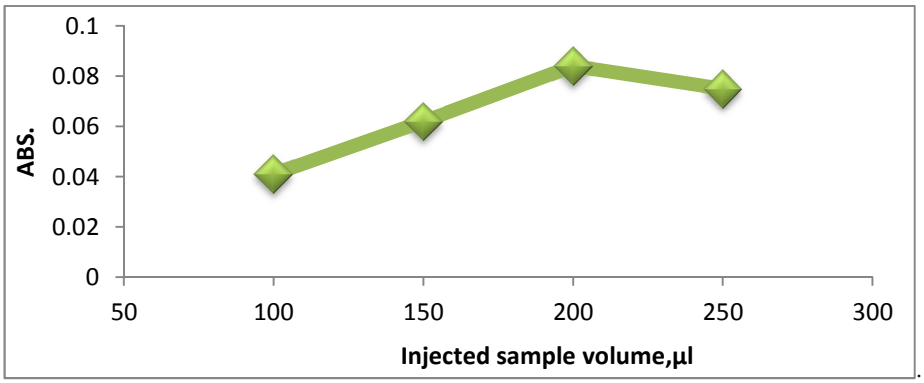


Figure 7- Physical conditions of FIA procedure for determination of famotidine
B. physical effect of FIA; injected sample volume of famotidine

Accuracy and precision

Accuracy and precision of the batch and FI spectrophotometric methods.

The accuracy and precision of the two methods were tested by analysis four replicate samples of FAM by batch and FIA spectrophotometric methods. The low values of percentage errors (E%) and standard deviation (RSD%) summarized in Table 3 indicated the high accuracy and precision of the two methods. different pharmaceutical formulations containing FAM and the results are summarized in Table 4. For all formulations examined, the assay results Pharmaceutical applications

Table 3- Accuracy and precision of the proposed methods

(DRUG)FAM	Conc., µgmL-1		E%*	REC.% *	RSD%
	present	Found			
Batch	30	30.4	1.52	101.5	0.28
	50	49.83	-0.33	99.66	0.57
	100	100.25	0.25	100.25	0.49
	150	149.41	-0.38	99.61	0.82
FIA	300	302.75	0.9	100.9	4.40
	400	401.5	0.37	100.3	1.64
	500	499	-0.2	99.8	1.04
	800	789	-0.01	99.9	1.18

**Average of four determinations*

The two proposed methods were applied successfully for the analysis of both methods were in good agreement with the declared content. The results obtained by two proposed methods were compared with BP method [26].

Table 4- Pharmaceutical applications for FAM using Tables (5) showed the statistical analysis according to t-test and F-value was for the proposed methods t*=0.361039, f *=4.292335 respectively at 95% confidence level. was

statistically compared, using the student t-test and variance ratio F test. The batch and FI methods were not significant not differ to standard method.

Table 4- Pharmaceutical applications for famotidine using the proposed methods

Method	Pharmaceutical Preparation	Conc. Of famotidin (μg mL-1) preparation Presence	Found	*E%	*Recovery%	*RSD%
Batch	Alcerane	15	14.9	-0.422	99.57	2.64
		40	41.08	2.72	102.72	1.51
		50	51.19	2.39	102.39	1.83
		100	102.44	2.44	102.44	0.59
	Faomdar	15	15.15	1.12635	101.05	1.68
		40	39.45	-1.1627	98.63	1.17
		50	49.32	-1.2088	98.79	1.09
		100	102.80	2.74	102.80	2.75
FIA	allcerane	300	301.20	0.40	100.40	1.85
		500	497.49	-0.50	99.49	3.05
		800	793.62	-0.84	99.15	1.93
		1000	1001.18	0.11	101.11	1.02
	Famodar	300	296.77	-1.67	98.92	-1.07
		500	507.71	1.54	101.54	-1.54
		800	802.27	0.28	100.28	0.28
		1000	1022.83	2.28	102.28	2.28

** Average of four determinations.*

Table (5) : The comparison of the proposed method (batch) with standard methods using t- and F-statistical tests

Drug form	Proposed method		Standard method		Statistical values
	Rec.% $(x_i)_1$	$(x-\bar{x}_i)_1^2$	$(X_I)_2$	$(x-\bar{x})2_2$	
FAM pure	100.255	0.748	101.111	0.549	
Ulceran (Tablet 20 mg)	101.78	0.436	98.333	4.1493	S_1^2 =0.7445 S_2^2 =3.1964 S=1.4044 t*=0.361039 f *=4.292335
Famodar (Tablet 20mg)	101.31	0.0388	101.666	1.6796	
	$(\overline{xi})_1$= 101.12	$\Sigma(xi-\bar{x})_{1=}^1$ 1.2228	$(\bar{x}_i)_2$= 100.37	$\Sigma(xi-\bar{x})_2^2$= 6.3779	

Table (6): The comparison of the proposed method (FIA) with standard methods using t − and F − statistical tests

Drug form	Proposed method		Standard method		Statistical Values
	Rec.% $(x_i)_1$	$(xi-\bar{x})_1^2$	Rec.% $(xi)_2$	$(X-\overline{X})_2^2$	
FAM pure	99.23	0.5184	101.111	0.549	
Ulceran (Tablet20mg)	100.66	0.5041	98.333	4.15	s_1^2=0.51575 s_2^2=3.1889 S=1.361S t*=0.378 f*=6.183
Famodar (Tablet 20 mg)	99.98	0.009	101.666	1.68	
	$(\bar{x}i)_1$ =99.95	$\Sigma(xi-\bar{x})_1^2$ =1.0315	$(\overline{xi})$= =100.37	$\Sigma(xi-\bar{x})_2^2$ =6.3779	

Theoretical values at 95% confidence limit,n1=n2=2,
t test has value=2.776 when t have degrees freedom n1+n2-2=4
F test has value=19.000 when F have degrees freedom=(n_1-1)=(n_2-1)=2

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REFERENCES

[1] Maples. H. D. , James. L. P. , Stowe .C. D. , *J. Clini. Pharma.*, (**2003**) .Vol. 43, no. 1, 7–14.
[2] Gebremariam ,T., Taddesse,A. *Inter. J. Chem.Natu Sci*; (**2014**) Vol. 2, No. 5 . 125-131
[3] A., Kumar, Yogesh Pandey. R. Garg, *Inter.J. Pharma Chem. Sci*. (**2015**)Vol. 4 (2) .
[4] Walash ,M., Khaled, M., El-Sayed Metwally, M., Reda Shabana Gebremariam, M, J. *Chin. Chem. Society*, (**2005**), *52*, 927-935
[5] FormulationsAruna K. vanka ; *International Journal of Pharmacy and Pharmaceutical Sciences*., **2013**; 5(1).
[6] Najma Sulthana*Medicinal chemistry*., **2013**; 3(1): 183-187.
[7] Helali, N, Monser, L. *J. Sep. Sci.* **2008**; 31: 276-282.

[8] R. Vijaya Lakshmi. Application of RP-HPLC Method for simultaneous determination of diclofenac sodium & famotidine in bulk & tables. Vol. 3 issue **2013** issue no: 2231-6876 no: 6366
[9] M. Ayad, A. Shalaby, H. Abdellatef, H. Elsaid, *J. Pharm. Biomed. Anal*. (**2002**), 29, 247 .
[10] J. Petkovic, D. Minic, Z. Koricanac, J. Jovanovic, *Pharmazie*; (**1998**), 58, 163 .
[11] S. Skrzypek, W. Ciesielski, A. Sokolowski, S. Yilmaz, D. Kazmierczak, Talanta (**2005**) ,66, 1146.
[12] Abu Zuhri, R. Shubietah, G. Badah, *J. Pharm. Biomed. Anal*. (**1999**) , 21, 459 .
[13] K. Kelani, A. Azziz, M. Hegazy, L. Abdel Fattah, *Spectros. Lett*. (**2002**), 35, 543 .
[14] Amin, S. Shama, I. Ahmed, E. Gouda, *Anal. Lett*. (**2002**), 35, 1851 .
[15] S. Nassr, M. Brunet, P. Lavoie, J. Brazier, *J. Liq. Chromatogr*. (**2002**), 24, 265 .
[16] N. Rahman, M. Kasihif, *Il Farmaco* (**2003**),58, 1045.
[17] N. Rahman, M. Kashif, *Anal. Sciences*, (**2003**),19, 907.
[18] M. Walash, M. Sharaf-El-Din, M. Metwally, M. Shabana, *J. Chinese Chem. Soc*. (**2005**) , 52, 71 .
[19] M. Baranska, K. Czarniecki, L. Proniewicz, *J. Mol. Struct*. (**2001**) ,27, 347 .
[20] T. Perez-Ruiz, C. Martinez-Lorenzo, V. Tomas, E. Bravo, R. Galera, *J. Pharm. Biomed. Anal*. (**2002**), 30,1055.
[21] B. Kamath , K. Shivram and A. Shah; *J. Pharm. Biom. Anal*. (**1994**) , 12, 343.
[22] B. Kamath, K. Shivram and S. Vangani, *Anal. Letters*; (**1993**) 26, 2183 .
[23] Najma, S.; Safila, N.; and Saeed, M.. *Med chem*. **2013** 3(1): 1
[24] Krishnavenl, G. and Sathyannarayana, P. *Int J Pharm Bio Sci*. **2013**, 4(3):655–662.
[25] Najma, S.; Mahwish, A.; Sana, S.; and Somia, G.. Simultaneous Determination of Moxifloxacin and H2Receptor Antagonist in Pharmaceutical Dosage Formulations by RP-HPLC: Application to in Vitro Drug Interactions, Quim. Nova. **2011**, 34(4):