Journal of Positive School Psychology http://journalppw.com
2022, Vol. 6, No. 9, 1812-1823

Spectrophotometric Determination Of Anti-Ulcer Drug (Famotidine) By Brady Reagent

Fatima Abdali Hassoni , Muthana Saleh Mashkour*

Department of Chemistry, Faculty of Science, Kufa University.

Abstract:

It was described how to determine famotidine utilizing a novel, simple, and sensitive spectrophotometric method, both as a pure chemical and in pharmaceutical formulation. To create a strongly colored chromogen with maximum absorbance (max) at 608 nm, 2,4-dinitrophenylhydrazine (2,4-DNPH) and famotidine are combined in an alkaline solution after being oxidized. The amount of Famotidine was then measured using spectrophotometry. Other analytical criteria, such as the ideal reaction circumstances, were evaluated as well. These variables include the amounts of the bases, the 2,4-DNPH and potassium iodate, the coupling reaction time, temperature, and the sequence in which the components of the finished product are added.Beer's law is followed at concentrations between 25-250 $\mu g.mL^{-1}$with The correlation coefficient(R^2)0.9986 , molar absorptivity 3337.808($L.mol^{-1}.cm^{-1}$) , Sandell's sensitivity 0.1010($\mu g.\ cm^{-2}$) limit of detection (LOD)(0.006 $\mu g.ml^{-1}$) and limit of quantification (LOQ)(0.018 $\mu g.ml^{-1}$) were calculated. he suggested method for locating famotidine in pharmaceutical formulations worked well.

Key Word: Famotidine, 2,4DNPH, azo coupling reaction, anti-ulcer drug

Introduction

Famotidine(FMT),3-[2-(diaminomethyleneamino)thiazol-4-ylmethylthio]-N sulfamoylpropionamidine [1] Famotidine is a recently developed histamine H2 receptor antagonist. It is widely utilized to treat hyperacid secretory conditions such Zollinger-Ellison syndrome, benign gastric ulcers, duodenal ulcers, [2]. Clinical studies have shown that famotidine is twenty times more effective than cimetidine at inhibiting stomach acid output in people.. [3]. The medicine has been approved by the European Pharmacopoeia. [4] and US [5] which covered the methods for famotidine assay in pharmaceutical formulations using HPLC and potentiometric titration. thin-layer chromatographic in nature [4،5] and high performance thin layer chromatographic methods [6] Several HPLC approaches have been published for the determination of famotidine in biological fluids and pharmaceutical formulations. According to a review of the literature, several HPLC procedures have been described for famotidine purity testing. [7-10] it is chemical structure, (Figure 1) .

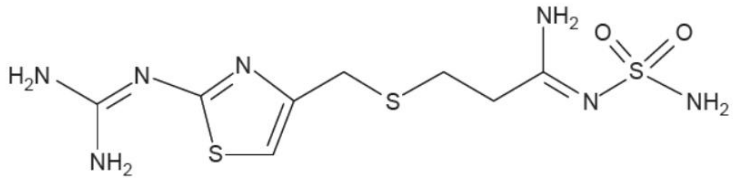


Figure (1): The chemical structure of Famotidine

Cimetidine, ranitidine, and famotidine are common histamine H2-receptor antagonists used for prevention of the aspiration of stomach content syndrome. [11, 12] These medicines are also given to individuals undergoing major surgery in order to prevent stress ulcers (such as cardiovascular surgery, neurosurgery, and organ transplantation). [13]. It is always preferable to choose and develop an easy-to-use, accurate, precise, and affordable method for the detection of pharmaceuticals in pharmaceutical dosage forms and pathological samples because assay method development is crucial for the pharmaceutical industries and pathological laboratories.[14]. In order to support formulation studies, develop drug syntheses, test finished goods prior to distribution, and keep track of the stability of bulk pharmaceuticals and formed products, analytical data can be used. [15] The investigation of the interaction between electromagnetic light and chemical molecules or atoms is known as absorption spectroscopy. [16] In pharmaceutical analysis, techniques like UV, visible, infrared, and atomic absorption are widely used.. [17]. and can be used Despite the fact that colorimetric techniques typically depend on the functional group (NH2, OH, or SH) in the drug molecule, they are sometimes used as stability indicators. [18] There are a number of colorimetric topics that require in-depth analysis. To begin with, the colorant ought to be selective. 2,4-Dinitrophenylhydrazine, one of the important organic compounds, has the chemical formula [C6H3(NO2)2NHNH2]. The solid molecule 2,4-Dnphh has an orange-red hue. Given that it has two nitro groups in both the orthogonal and paragonal locations, its chemical makeup (Figure 2) demonstrates that it is a substituted hydrazine. [19]

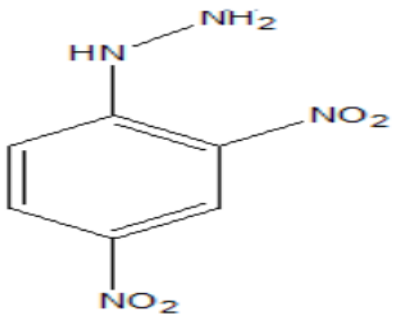


Figure (2): The chemical structure of 2,4-Dnphh

2,4-Dinitro phenyl hydrazine is dissolved in methanol with a little amount of strong sulfuric acid to create Brady's reagent, also referred to as Borche's reagent. [20]. 2,4-Dinitrophenylhydrazine is a crucial reagent that is used to generate the corresponding 2,4-Dinitrophenyl-hydrazone (addition-elimination) reactions for the quantitative spectrophotometric determination of various compounds via a variety of reactions, including oxidative coupling, diazotization, and coupling reaction. [21]. Pharmaceutical preparation analysis is crucial for the pharmaceutical industry since it defines the typical requirements of these preparations and evaluates how closely they adhere to the pharmacopoeia. Pharmaceutical analytical chemistry is a rapidly growing field. [22-23] Development of simple, rapid, and economical analytical techniques that may be promptly used for routine analysis at a relatively low cost to the diverse requirements of analytical problems is constantly important. [24,25].

Experimental

Instruments

UV-1650PC UV-Visible Spectrophotometer, SHIMADZU, Japan (Double beam(, 303 PD UV-Visible Spectrophotometer, Apel, Japan (Single beam) and pH meter, Spinbot, thephaw

Materials and Reagents

All of the chemicals and reagents employed were of analytical grade, and China was the source of the famotidine powder in its purest form.

Preparation of solutions:

1-Standard Famotidine solution(1000 μg.m^{-1}) :

By combining 0.1 grams of powdered medication with the required amount of deionized water, transferring the mixture to a volumetric flask measuring 100 ml, and topping off the volume to the mark with deionized water, you can create a solution of famotidine with a concentration of (1000 μg.m^{-1}).

2- 2,4-Dinitrophenylhydrazine reagent solution (2,4-DNPH) 4.5×10^{-3} M :

The detector solution (2,4-DNPH) is made with 0.009 g of 2,4-DNPH in 0.20 ml of concentrated sulphuric acid (H_2SO_4) at a concentration of (4.5×10^{-3} M), transferred to a 10 ml volume flask, filled to the mark with deionized water, kept out of the light, and used right immediately.

3-Potassium iodate oxidized solution (KIO_3) 0.01 M:

By dissolving 0.025g of KIO_3 in the necessary volume of deionized water, and then transferring the solution to a 10 ml volumetric flask and topping it off with deionized water, the oxidizing agent solution (KIO_3) is created at a concentration of (0.01M).

4- Sodium hydroxide solution (NaOH) 10 M :

By dissolving 4 g of sodium hydroxide in the necessary amount of deionized water, the basic solution sodium hydroxide (NaOH) is created with a concentration (10 M). and then poured into a 10 ml volumetric flask before adding deionized water to fill the flask to the mark.

5-Preparation of Solutions for the analysis of Famotidine in pharmaceutical preparations

i. In Tablet

One 20mg tablet's worth of material was precisely weighed, ground into a fine powder, dissolved in a quantity of deionized water, and agitated for 10 minutes to ensure that the medication was completely dissolved. The resulting mixture was then poured into a 20 mL volumetric flask and diluted with deionized water to the appropriate mark to obtain 1000 μg. mL^{-1}.. Using filter paper, the solutions were filtered. through the appropriate dilution and evaluated using the research procedure. Additionally, the same procedures are followed to dissolve the 40 mg pill in 40 ml.

Analytical procedure

After 10 minutes to stabilize the color, 0.50 ml of (10 M) sodium hydroxide was added to 1 ml of standard famotidine solution in the presence of 0.75 ml of potassium iodate solution in basic medium. The mixture was then diluted with deionized water in a 10 ml volumetric flask to produce a green color product. At 608 nm, absorbance was measured in comparison to a reagent blank that had undergone the identical preparation but was devoid of any medication.After being oxidized, 2,4-DNPH underwent an oxidative coupling procedure before being coupled with famotidine in an alkaline medium.The best conditions for determining the presence of Famotidine in pharmaceutical preparations were established by testing the impact of various variables on the color development.

RESULTS AND DISCUSSION

Spectrum of absorption and proposed reaction

In the primary test, the reaction of oxidized 2,4-DNPH with famotidine produced a colorful product with an absorption spectrum that had a

maximum absorption (max) at 608 nm. The hardly noticeable absorbance of the slightly yellowish blank solution at the maximum concentration at which the medication was tested. Figure (3) displays the maximum absorption of 2,4-DNPH (354 nm), FAM (265 nm), and the color product for the reaction of FAM and 2,4-DNPH.

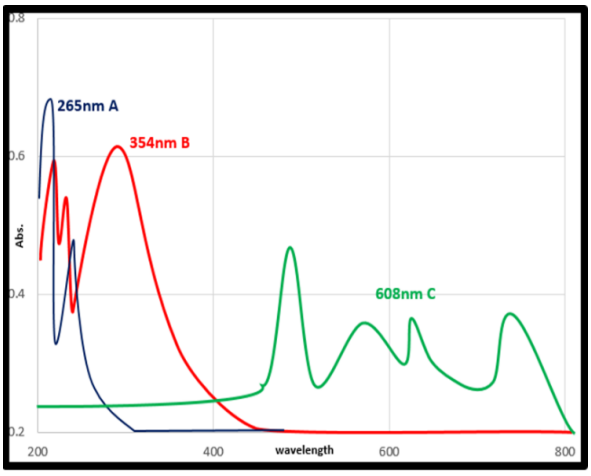


Figure (3): Absorption spectra A:(λmax) of 2,4-DNPH (354nm) ,B: (λmax) of FAM (265nm) and C: (λmax) of Color product for reaction FAM and 2,4-DNPH.

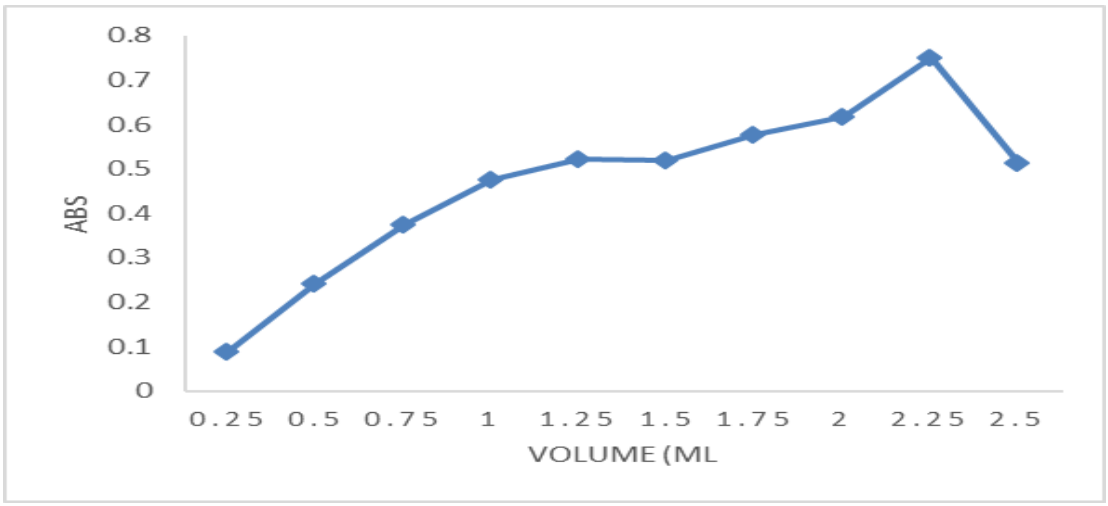
Optimization of The Experimental Conditions

Univariate method

The impact of various parameters on the production of color products were methodically examined by altering each parameter separately while keeping the others constant. These variables include the volumes of 2,4-DNPH and potassium iodate, the quantity of base, the coupling reaction time, temperature, and the order in which the constituents of the final product are introduced.

1.Effect Volume Reagent of the 2,4-DNPH on the Reaction of FAM drug:

Different volumes of the reagent solution ranged between (0.25 - 2.50 ml) to the volumetric flasks with installations of the other components as a drug, oxidizing agent KIO_3, and sodium hydroxide the volume is completed to 10 ml with deionized water, and the effect amount of 2,4-DNPH (4.5×10-3 M) on the measured absorbance of the formed colored product was carried out. The color intensity of the reaction product between the reactants is directly (4).



Figure(4): Effect volume reagent of (2,4-DNPH) on the reaction with FAM drug

2.Effect Volume of the Potassium Iodate on the Reaction of FAM drug:

In Figure (5), the measured absorbance values are shown against various oxidant volumes (0.25 - 2.50 ml), with 0.75 mL of KIO_3 solution

producing the highest absorption. Therefore, 0.75 mL of solution was sufficient for the work that followed.

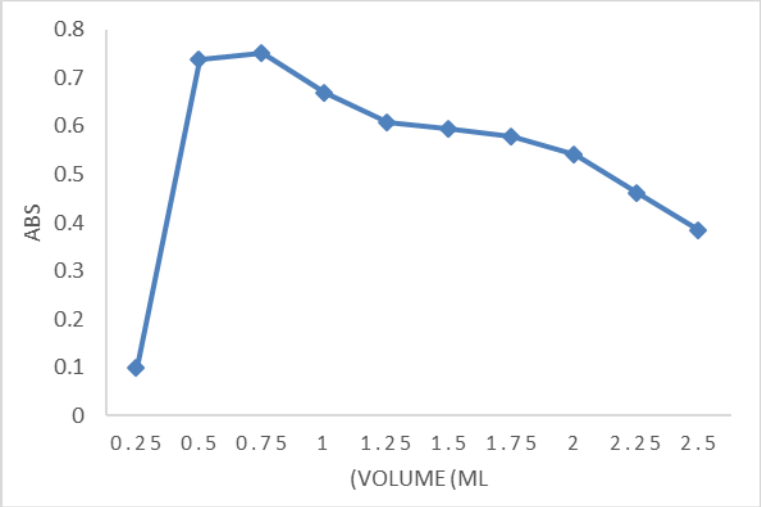


Figure (5): Effect volume of KIO_3 on the reaction (2,4-DNPH) with FAM drug

3.Effect Volume of Sodium Hydroxide on the Reaction of FAM drug:

The addition of 0.50 mL of sodium hydroxide (10 M) resulted in the maximum absorbance, according to the data shown in Figure 6. Various amounts of sodium hydroxide (10 M) were studied ranging from (0.25-2.50) mL. Therefore, 0.50 mL of NaOH(10M) was utilized in each experiment that followed.

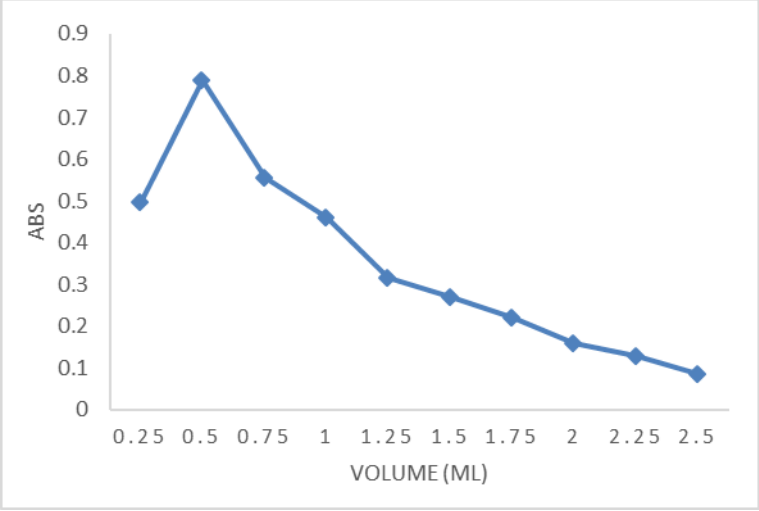


Figure (6): Effect volume of base (NaOH) on the reaction (2,4-DNPH) with FAM drug

4.Effect temperature on the Reaction of FAM drug:

It was investigated how temperature affected the intensity of the colors. As shown on Figure 7, the color formed at 30°C, which is the ideal temperature, and a maximum absorption was attained in practice. Therefore, it is applied in later experiments.

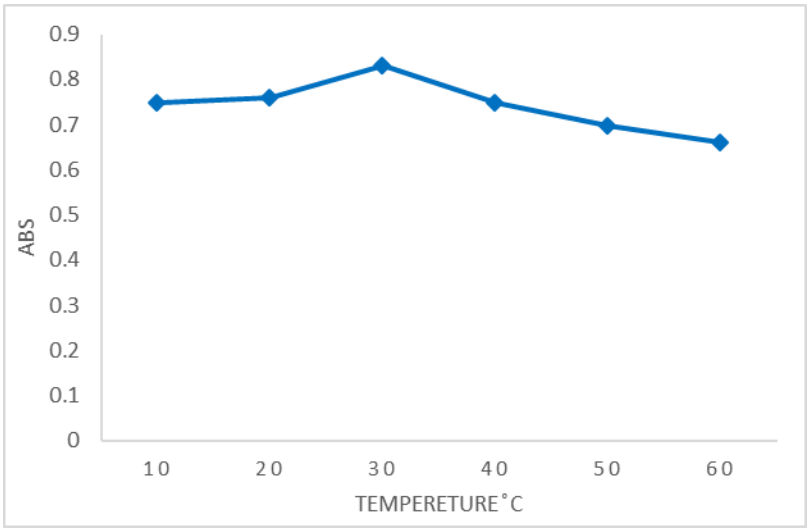


Figure (7): Effect temperature on the reaction (2,4-DNPH) with FAM drug

5.Effect Time on Coupling Reaction of FAM drug:

Absorbance measurements were taken at various intervals, from right away to after 60 minutes of waiting. According to Figure, the oxidative coupling reaction was finished in 15 minutes (8).

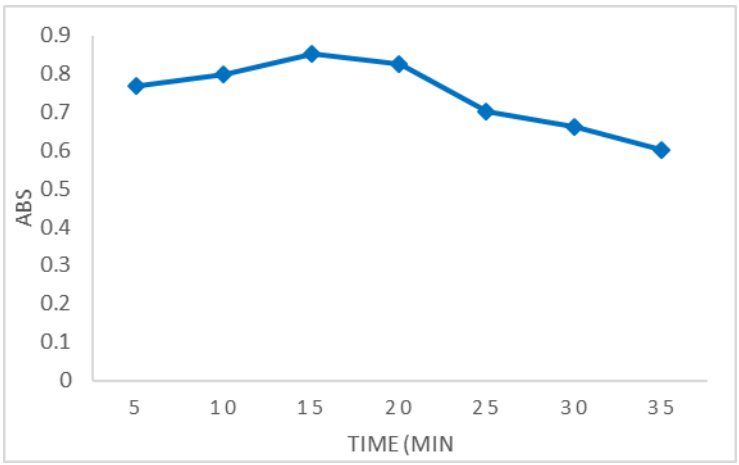


Figure (8): Effect time on the reaction (2,4-DNPH) with FAM drug

6.Order of Additions for Reaction 2,4-DNPH with FAM drug:

We looked at four processes with various component adding orders. The sequences (1) and (2) produced absorbance values that were higher, but the sequences (3) and (4) produced absorbance values that were lower since the blank value was more than the absorbance value of the solution. the movements

The hydrazine group of 2,4-DNPH is oxidized by KIO_3 to diazonium cation, which interacts with the drug in an alkaline media to produce a stable derivative of 2,4-DNPH (see Table 1). So, it was decided to use sequence 1 in the procedure under study.

Table (1) : Order of Additions for reaction 2,4-DNPH with FAM drug.

NO.	Addition	Absorption
1	2,4-DNPH + KIO_3+ Drug + NaOH	0.750
2	Drug + 2,4-DNPH + KIO_3 + NaOH	0.504
3	2,4-DNPH + Drug + KIO_3 + NaOH	0.333
4	Drug + NaOH + 2,4-DNPH + KIO_3	0.051

Calibration Curve and Analytical Data

In a series of volumetric flasks (10ml), (0.25-2.50 ml) of (1000 μg.ml^{-1}) of Famotidine were transferred, 0.75 ml of KIO_3 0.01 M and 2.25 ml of 2,4-DNPH reagent (4.5×10^{-3} M), 0.50 ml of sodium hydroxide solution equal to approximately (pH 13)were added at 30°C. After that the solutions were left for 10 min to complete the reaction, then the volumes were completed to the mark with deionized water. The absorbance was measured at 608 nm against the blank reagent. linear relationship was observed between the absorbance and concentration of Famotidine ranged from (25-250 μg.mL^{-1}) as shown in Figure (9). The correlation coefficient(R^2)0.9986 , molar absorptivity 3337.808(L.mol^{-1}.cm^{-1}) , Sandell's sensitivity 0.1010(μg. cm^{-2}) limit of detection (LOD) (0.006 μg.ml^{-1}) and limit of quantification (LOQ) (0.018 μg.ml^{-1}) were calculated.

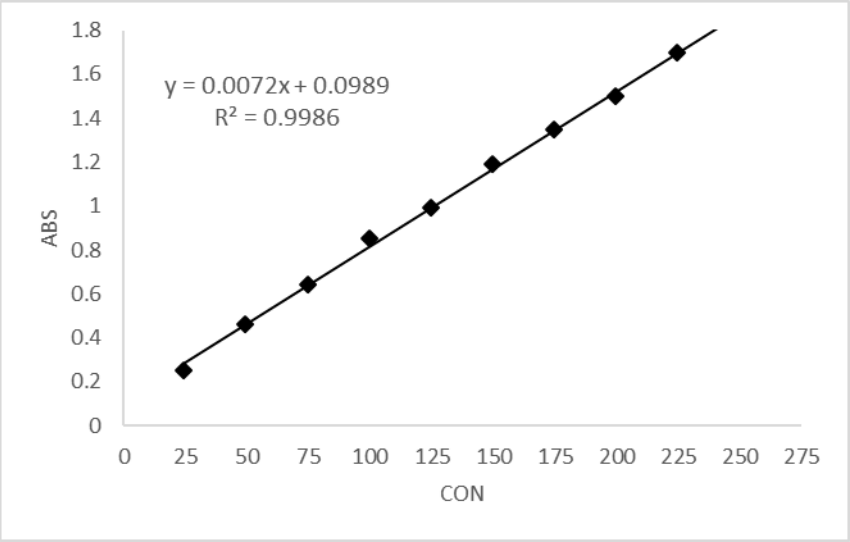


Figure (9): The Calibration Curve of (FAM) drug with (2,4-DNPH)

Accuracy and precision

to confirm the accuracy and precision of the procedure under study. First, we determined precision by performing five times of each experiment at the concentration of each (100 μg.ml^{-1}). The R.S.D. percent computation and figures for this medicine, which were equivalent to (1.853%), show the method's high level of precision. The results are shown in Table (2).

Table (2) : (R.S.D%) for the studied method to determine drug (FAM)

X	$X - X^{-}$	$(X - X^{-})^2$
0.850	0.01	1×10^{-4}
0.853	0.013	1.69×10^{-4}
0.851	0.011	1.21×10^{-4}
0.822	0.018	3.24×10^{-4}
0.824	0.016	2.56×10^{-4}
$\sum_X = 4.2$		$\sum_{(X-X^{-})^2} = 9.7 \times 10^{-4}$

$$X^{-} = \frac{\sum X}{n} = \frac{4.2}{5} = 0.84$$

$$S.D = \sqrt{\frac{\sum(X-X^{-})^2}{n-1}} = \sqrt{\frac{9.7\times10^{-4}}{5-1}} = 0.015$$

$$R.S.D\% = \frac{S.D}{X^{-}} \times 100 = \frac{0.015}{0.84} \times 100 = 1.853\%$$

Recovery % = 101%

The Stoichiometry of the formed product

Both the Job's approach and the molar ratio method were used to determine the stoichiometry of the medication to reagent that resulted in the green color product. In both techniques (the concentration of each of the standard Famotidine solution and 2,4-DNPH reagent solution was equel). In Job's approach, various volumes of the drug solution, ranging from 0.1 to 0.9 ml, and various volumes of the reagent solution, ranging from 0.9 to 0.1 ml, were mixed in a series of volumetric flasks (10 ml), A 0.75 ml of potassium Iodate (0.01M) and 0.50 ml of sodium hydroxide solution were added and volumes were completed to the mark with deionized water. The absorbance was measured at 608 nm against the blank reagent. The results as it Figure (10) show that the ratio is 1:1.

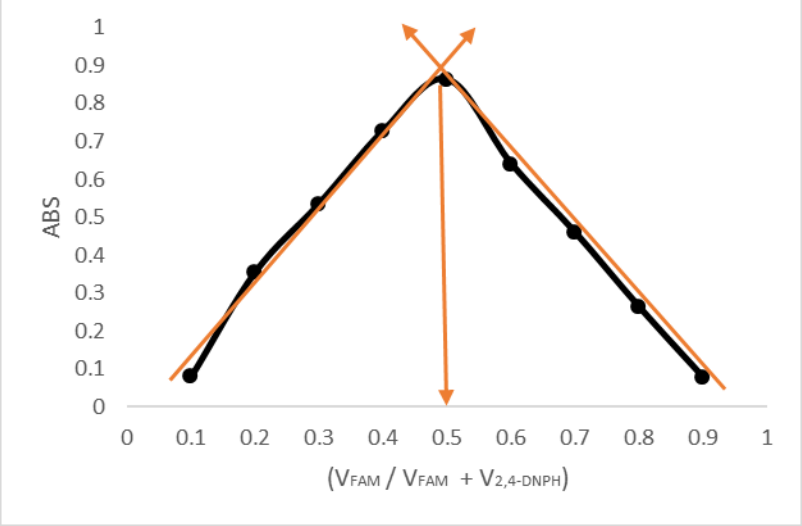


Figure (10): the continuous variation (Job's method) FAM drug with (2,4-DNPH)

In the molar ratio approach, 0.25-2.50 ml of the 2,4-DNPH reagent solution in various quantities were added to 1 ml of the reference drug solution in a series of volumetric flasks (10 ml)., 0.75 ml of potassium Iodate (0.01 M) and 0.50 ml of sodium hydroxide solution were added. Deionized water was used to fill the volumes to the mark, and the absorbance was measured at 608 nm in comparison to the blank reagent.. Molar ratio was found to be 1:1. Figure (11)

displays the results, which are consistent with those obtained using the Job's approach. The reaction is depicted in Scheme (1) below. The stability constant for the FAM medication was equal to (6.370 106) L, and the degree of disintegration was equal to (0.047). mol^{-1}

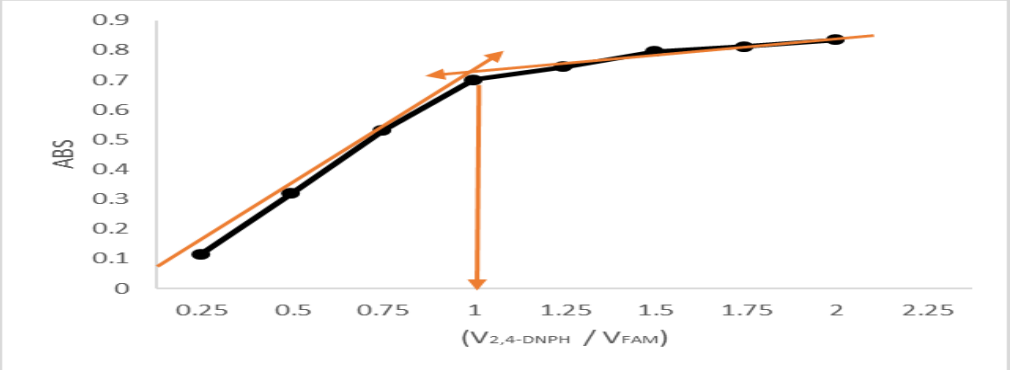
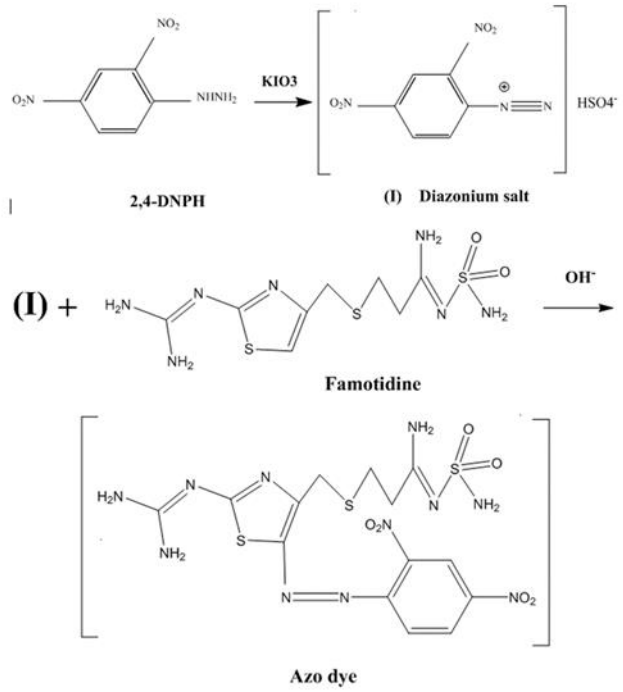


Figure (11): mole ratio of FAM drug with (2,4-DNPH)

Suggest steps for Reactions 2,4-DNPH with FAM drug in presence of oxidizing agent and alkaline medium:

The steps for reaction of 2,4 DNPH reagent with oxidizing agent KIO_3 to form azo coupling to react with FAM drug in alkaline medium to form color azo compound as shown in scheme 1..



Scheme (1): Steps for the Reactions 2,4-DNPH with FAM drug.

Effect of interference

By performing the measurement of Famotidine, the effects of certain common excipients, Hydroxy propyl cellulose, and Iron oxides were studied in order to gauge the analytical potential of the suggested approach. Excipients did not interfere with the experimental procedure,

according to experimental data, which are shown in Table (3).

Table (3): Effect of interferences on the reaction (2,4-DNPH) with FAM drug

Interference	Absorbance
Hydroxy propyl celluse	0
Iron oxides	0
Micro crystalline cellulose	0
Corn Starch	0
Magnesium sterate	0
Hypromellose	0
TiO_2	0
Talc	0

Application in Pharmaceutical Preparation of FAM drug:

The results of the application of the researched approach, which are shown in Table (4), were good. Real samples with known contents of the drug famotidine (20 mg/tablet) and another tablet (40 mg) were used to test the effectiveness of the method.

Table (4): analytical applications pharmaceutical of FAM drug

Sample	Concentration($\mu g.mL^{-1}$)		Error (%)	Recovery (%)	RSD (%)
	Taken	Found			
20 mg/tablet (Iraq)	60	59.91	-0.15	99.85	1.09
	80	79.88	-0.15	99.85	1.13
40 mg/tablet (Iraq)	40	40.09	0.22	100.22	0.64
	120	120.35	0.29	100.29	0.38

Conclusion

For the quantitative determination of (FAM) in pure form and pharmaceutical preparations, it was discovered that the oxidative coupling reaction between 2,4-DNPH after oxidation was followed by coupling with FAM in alkaline medium was a straightforward, sensitive, accurate, and affordable spectrophotometric method. The investigated approach has good linearity and accuracy.

Reference

1. Waller, D. G., Sampson, A., & Hitchings, A. Medical pharmacology and therapeutics E-Book. Elsevier Health Sciences. (2021).

2. Kanakapura, B. and Okram, Z. D. Application of Oxidizing Properties of Permanganate to the Determination of Famotidine in Pharmaceutical Formulations, J. Mex. Chem. Soc. 54(4): (2010) .182-191.

3. Lilia, A.; Niebel, P.; Ricardo, M.; Jair, M. and Avismelsi, P. Spectrophotometric Method for The Determination of Famotidine in Drug Formulations, IJAPA. 2(1):(2012) . 24-29.

4. Monograph, T. European Pharmacopoeia. European Directorate for the Quality of Medicine & Health Care of the Council of Europe (EDQM), edn,9, (2017). 3104-3105.

5. Rahman, N., & Kashif, M.. Application of ninhydrin to spectrophotometric determination of famotidine in drug formulations. Il Farmaco, 58(10), (2003). 1045-1050.

6. Okram, Z.; Kanakapura, B.; Pavagada, J. R. and Kanakapura, B. V. Simple and Sensitive UV Spectrophotometric Methods for Determination of Famotidine in Tablet Formulations, Farmacia. 59(5): (2011). 647-658.

7. Najma, S.; Safila, N.; and Saeed, M. RP-HPLC Method for the Simultaneous Determination of Captopril and H2-Receptor Antagonist: Application to Interaction Studies, Med chem..3(1): (2013) .183-187.

8. Nita, Adriana, et al. "HPLC-UV Method for Determination of Famotidine from Pharmaceutical Products." Rev. Chim 69 (2018): 297-299.

9. Kontou, Marina, and Anastasia Zotou. "Use of a monolithic column for the development and validation of a HPLC method for the determination of famotidine, cimetidine and nizatidine in biological fluids." Journal of Applied Bioanalysis 3.4 (2017): 1856.

10. Alamgir, M. Y. K. M., et al. "HPLC determination of metformin, famotidine and ranitidine by derivatization with benzoin from drugs and biological samples." Pharm Anal Acta 8.546 (2017): 2.

11. Elbashir, Abdalla Ahmed, and Shahd Moutasim Merghani. "Spectrophotometric determination of ranitidine hydrochloride (RNH) in pharmaceutical formulation using 9-fluorenylmethyl chloroformate (FMOC-Cl)." Asian Journal of Pharmaceutical Research and Development 6.6 (2018): 7-14.

12. Mikawa, K., Akamatsu, H., Nishina, K., Shiga, M., Maekawa, N., Obara, H., & Niwa, Y. The effects of cimetidine, ranitidine, and famotidine on human neutrophil functions. Anesthesia & Analgesia, 89(1), (1999). 218-224.

13. Marino, P. L. Marino's the ICU Book. (2017).

14. J.B. Murimi – Worstell, J.M. Ballreich, M.J. Seamans and G. C. Alexander "US Pharmacopeia (USP) monograph standards, generic entry and prescription drug costs" Plos one 2019 Vol. 14 Issue 11 pages e0225109.

15. D. G. Watson " Pharmaceutical analysis E-book: a textbook for pharmacy student and pharmaceutical chemists" Publisher: Elsevier Health Sciences 2020.

16. Abelian, Anush, et al. 'Pharmaceutical chemistry.' Remington. Academic Press, 2021. 105-128..

17. M. L. C. Passos and M. L. M. F. S. Saraiva "Detection in UV-visible spectrophotometry" Measurement 2019 Vol. 135 Pages 896-904.

18. Fadhel S. R., Abdulla N. I. Sulaiman I. D. Spectrophotometric determination of carbamazepine via oxidative coupling reaction with 2,4-dinitrophenyl hydrazine. Ibn Al-Haitham Jour. for Pure & Appl. Sci.29(1). (2016). 226-238

19. S. A. Zakaria, Z. Talal and N. S.Othman "Using 2,4-dinitrophenylhydrazine in

spectrophotometric determination" Samarra Journal of pure and Applied Science 2022 Vol. 4 Issue 2 Pages 107-117.

20. Habeeb, E. D., & Sulaiman, I. D. Spectrophotometric determination of propranolol hydrochloride via oxidative coupling reaction with 2, 4-dinitrophenyl hydrazine. International Journal of Drug Delivery Technology,11(1), (2019). 29-35.

21. Mesquita, C. S., Oliveira, R., Bento, F., Geraldo, D., Rodrigues, J. V., & Marcos, J. C. Simplified 2, 4-dinitrophenylhydrazine spectrophotometric assay for quantification of carbonyls in oxidized proteins. Analytical biochemistry, 458, (2014). 69-71.

22. Kar, Ashutosh. Pharmaceutical drug analysis. New Age International, 2005..

23. Watson, David G. Pharmaceutical analysis E-book: a textbook for pharmacy students and pharmaceutical chemists. Elsevier Health Sciences, 2020.

24. Teleb, Said M., et al. "Chemical and biological studies on charge-transfer complexes of cimetidine with various electron acceptors." Journal of Molecular Structure 1202 (2020): 127256.

25. Magda, M.; Ayad, A. S. H. E.; Abdellatef, H. E. M.; J. Pharm. Biomed. Anal. 2002, 29, 247.