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Charge Transfer Spectrophotometric Determination of Metronidazole in Pharmaceutical Formulations by Normal and Reverse Flow Injection Analysis Coupled with Solid-Phase Reactor Containing Immobilized $FePO_4$

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Abstract

Two rapid, simpleand sensitive flow injection methods were developed for the estimation of metronidazole (MRZ) in pharmaceutical formulations. The proposed methods were based on charge transfer reaction between metol (N-methyl-p-aminophenol sulfate) as a π-acceptorand reduced MRZ as an n-donor to produce a blue colored chargetransfer complex. Method A depends on the reaction of reduced MRZ with metol (MT) in the presence of $NaIO_4$ using two lines manifold to form blue colored product exhibiting absorption maxima at 700 nm.While method B depends on charge transfer reaction of reduced MRZ with MT in presence of a solid phase reactorcontainingfixed$FePO_4$ on cellulose acetateusing reverse flow injection manifold to form a blue colored productwhich was measured spectrophotometrically at690 nm.Various experimental parameters for both methods were studied. Beer's law was obeyed in the ranges of2.5-200 and 2.5-150 µg mL^{-1},with r^2 of 0.9995 and 0.9972;whilethe detection limit values were2.53 and 2.12µg mL^{-1} for methods A and B, respectively. Both of the suggested methods were successfully applied for the estimation of MRZ in commercial formulations. The results of the developed methods were compared with those obtained by the British pharmacopeia method, showinghigh accuracy and precision.

Keywords: Flow injection, Metronidazole and $FePO_4$ solid phase reactor.

التقدير الطيفي لانتقال الشحنة للميترونيدازول في المركبات الصيدلانية بواسطة التحليل بالحقن الجرياني العادي والعكسي مقترناً مع مفاعل طور الصلب يحتوي على فوسفات الحديديك مثبتة

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الخلاصة

تم تطوير طريقتين بسيطه وسريعة وحساسة بالحقن الجرياني الطيفي لتقدير الميترونيدازول (MRZ) في المستحضرات الصيدلانية. وكانت الطرق المقترحة تعتمد علي تفاعل انتقال الشحنة بين الميتول (N-methyl-p-aminophenol sulfate) ككاشف لوني (المستقبل π -)وMRZالمختزل (n - المانح)لاعطاءمعقد انتقاالشحنةذو لون ازرق. وتعتمدالطريقةA علىتفاعل MRZ مع metol (MT)بوجود

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$NaIO_4$ باستخدام منظومة من خطينلتكويناتجازرق اللوناظهر الحد الأقصى للامتصاص عند 700 nm. بينماتعتمد الطريقةB علىتفاعل انتقال الشحنة بينMRZ مع (MT) بوجود مفاعل الطور الصلبالذييحتوي على$FePO_4$مثبت على السيللوز اسيتيت باستخدام منظومة الحقن الجرياني العكسي لتكويناتج ازرق اللونالذي قدر طيفياًعند 690 nm. تم دراسة الظروف التجريبية المختلفة لكلا الطريقتين. وقد اعطى قانون بيرمدى 2.5−200 و 2.5−150 ميكروغرام مل$^{-1}$ ، معr^2 0.9991 و 0.9946 وكان حد الكشف 2.53 و 2.12 ميكروغرام مل$^{-1}$ للطريقتينA و B على التوالي. تم تطبيق الطريقتين المقترحة بنجاح لتقديرMRZ في المستحضراتالتجاريه. تمت مقارنه نتائج الطرق المطورة مع تلك التي تم الحصول عليها بواسطة طريقه Britishpharmacopeiaبضبط و بدقه عالية.

Introduction

Metronidazole (MRZ)is chemically known as 2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethanol and has a molecular formula of $C_6H_9N_3O_3$(Figure-1) [1]. MRZ that belongs to5-Nitroimidazolesfamily can be used in antibacterial treatment [2]. The discovery of the antitrichomonal properties of the antibiotic azomycin led to the investigation of nitroimidazoles as antiparasitic agents [3].

MRZ is a nitro imidazole antiprotozoal and antibacterial drug used againstanaerobic organismsand amoebozoa infections [4, 5]. The clinical studies of MRZ showed that it was activefortreatment of amoebic liver abscess, amoebic invasive dysentery,and colon, small intestine, and vaginal infections, as well asthe treatment of *Helicobacter pylori* (peptic ulcer diseases) [6]. Officially recommended procedures for the estimation of MRZ include high-performance liquid chromatography (HPLC) [7-11],spectrophotometry\[12-16], flow injection analysis [17, 18] and polarographic analysis [19].

The present work describes a sensitive and simple two flow injection spectrophotometric methods for the determination of MRZ in its pure form along withpharmaceuticals formulations. Method Adepends on the chargetransfer complexation of MRZ molecule with MTto form the colored product in the presence of $NaIO_4$using two lines flow injection manifold,whilethe resulting colored was measured at 700 nm.Method B employs one line reverse flow injection manifold coupled with solid phase reactor containing immobilized $FePO_4$ and the response was measured at 690 nm.

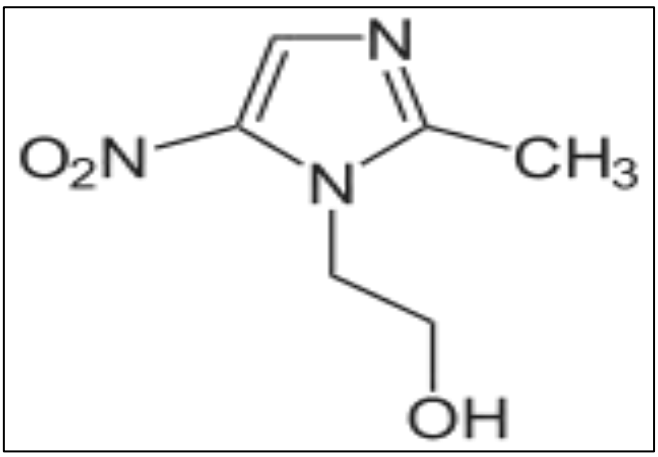


Figure 1-structure of MRZ.

Experimental Work

Apparatus: Shimadzu spectrophotometer UV/VIS 260 digital double beam (Japan), with the use of 1 cm path length and 50 µL volumeflow cell. A peristaltic pump (Shennchen, Lab M1, China) andan injection valve (Knauer, Germany). Flexible vinyl and Teflontubing (0.5 mm i.d.) was used for manifold design.

Materials: All chemical reagents in the present study were supplied in a pure form. Pure MRZ (M.Wt. 171.16 g.mol^{-1}) was obtained from Iraq-Samara(SDI), while thebrand namestablets containingMRZwereobtained from local market sources.

Iron (III) phosphate $FePO_4$ (Merck, Chemicals Ltd., Germany).

Reduction of nitro group and preparation of the standard solution

Pure MRZ powder (100 mg)was dissolved in 20 mL of methanol. 10 mL of 5 N Hydrochloric acid (37 % w/w, BDH, England) was added to the methanolic solution of MRZ and 0.5 g of zinc powder (BDH, England) was addedat room temperature. The solution was filtered using a Whatman filter paper (No 41) after standing for 20 minutes to remove the insoluble matter,then the volume was made up to 100 mL with methanol.

Procedure of pharmaceutical forms (Tablets)

An equivalent to 100 mg of MRZ was weighed, powderedto ten tablets of MRZ,and then dissolved in 20 mL of methanol. The resulting filtrate was treated as described above for theestimation of MRZ.

Metol (MT) reagent solution (0.1)

Metol reagent solution was prepared by mixing0.861 g of MTreagent (MT, Merck, Chemicals Ltd., Germany, M.Wt.344.38 g mol^{-1}) in distilled water and the solution was made up to 50 ml and stored in a dark flask.

Sodium periodate solution (0.1 M)

2.1391 g of$NaIO_4$(BHD, England,M. wt. 213.91 g mol-1, purity99%) was dissolved in 5 mL distilled water and the volume was completed to 100 mL in a volumetric flask.

Preparation of solid -phase reactor containing immobilized $FePO_4$ (F-SPR)

In light of previous studies [20], a new method for the preparation of immobilized $FePO_4$ was successfully used to prepare a solid phase reactor which could be used in many oxidation methods, such as spectrophotometric flow injection analyses. The immobilizingsteps for the preparation of F-SPR were carried out by dissolving 0.5 g of cellulose acetate (CA) in 5 mL of acetone and 0.5 mL of dimethylformamide with continuous stirring.Then, 1.5 g of $FePO_4$ was added aftermanual homogenization by stirringuntil homogenous mixture viscosity was increased. A few minutes later,distilled waterwas usedfor washing and rigid oxidizing material was formed. Different sizes(0.15 – 1.18 μM) wereobtained by crushingof the driedfixed $FePO_4$into the desired particle size which was selected by sieving on sieves with known mesh sizes (Retsch GmbH & Co.KG, Germany). Finally, the $FePO_4$ particles werepackedinto different lengths of glass tubes (2 mm i.d.)for the preparation of F-SPR.To hold the packed particles in place, small sponge pieces were inserted at the ends of the tubes.

Flow injection procedure: In method, (A) 150 μL of reduced MRZ (100 μg mL^{-1}) was injected into the stream of 0.01 M of MTat a total flow rate of 3 mL min^{-1}, then merged at Y-link with second line of $NaIO_4$solution (0.03 M).Afterwards,the solution was passed through75 cm reaction coil length (Figure- 2-A). Whilein method B (rFIA), one channel manifold (Figure- 2-B) was used. Therefore, 150μL of the MT (0.015 M) was injected into the carrier stream of reduced MRZ (100 μg mL^{-1}) which was then oxidized through 10 cm F-SPR containing immobilized $FePO_4$ at a flow rate of 1.9 $mLmin^{-1}$. The colored product absorbance measurementwas carried outat 700 and 690 nm formethodsA and B, respectively.

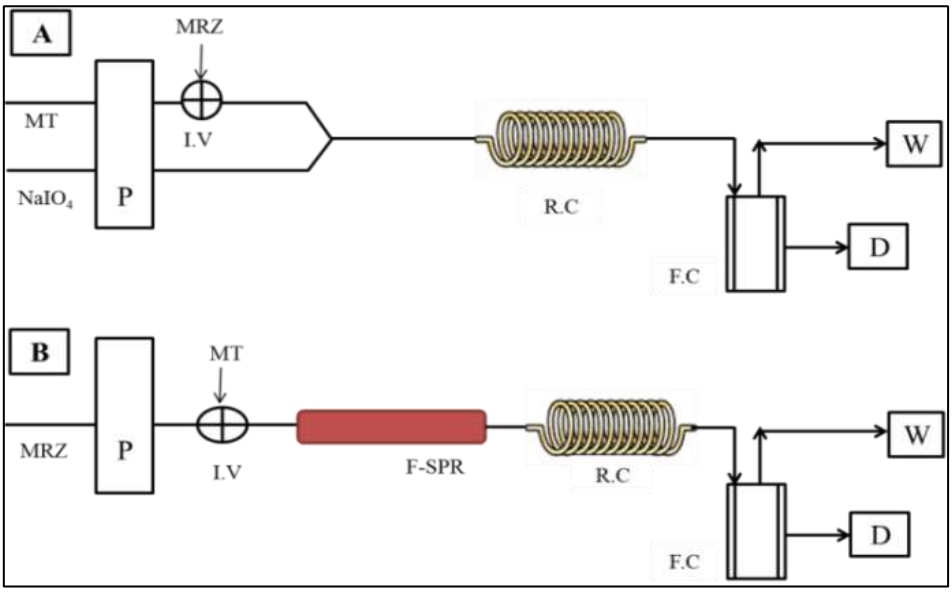


Figure 2-Schematic manifold diagram, A and B, for methods A and B respectively;I.V, Injection valve; RC, reaction coil; P, Peristaltic pump; D, Detector; F.C, Flow Cell; W, waste; MRZ, reduced metronidazole; MT, metol solution and F-SPR, fixed $FePO_4$ on CAsolid phase reactor.

Results and Discussion

Absorption spectra

A spectrophotometric method for the determination of MRZ has been reported [21] by charge transfer complex of MRZ with the MT to give a blue colored product in the presence of an oxidizing reagent. The reported reaction was the base for developing nFIA (method A) and rFIA (method B).

Before applying the proposed reaction by means of nFLA or rFIA,the blank and colored product absorption spectra were measuredmanually to obtain the bestparameters for the colored productformation. The test wascarried out in 10 mL flask containing 40 µg mL^{-1} of reduced MRZ, 0.5 mL of $NaIO_4$ (0.03M) and 0.5 mL (0.01 M) of MT for method A. While method B was based on the use of 30 µg mL^{-1} of reduced MRZ, 1 mL (0.01 M) of MT and 0.06 g of immobilized $FePO_4$ particles (1.18 mm). As soon as the solutions were mixed and swirled, the blue colored product was formed. The flasks were made up to the volume with distilled water and then filtered.

Depending on the optimum conditions, the absorbance spectrum of the colored product versus reagent blank was recorded between 250 and 1000 nm. The λ_{max}values werefound to be 700 and 690 nm) for methods A and B, respectively(Figure-3, which will be used in all subsequent experiments.

The proposed reaction was used for developing normal flow injection method (A) and a reverse flow injection method (B),coupled with one line packed F-SPR containingfixed $FePO_4$. The reaction mechanism may be suggested and established by depending on the previously reported mechanismwherethe nitro compound is first reduced to the corresponding amino derivative.The reaction mechanism is dependenton the reaction of MRZ amino group (n-donor) with the oxidized metol (π-acceptor) to form charge transfer complex, which subsequently forms a blue colored product that was measured at 700 and 690 nm for methods A and B, respectively. On the basis of the literature survey, tentative reaction mechanisms for MRZ and MT complexes in the presence of $NaIO_4$ or $FePO_4$ are proposed and given in schemes 1[21-23]. It can be seen that the charge transfer complex was formed in the ratio of 1:1 (Drug: Reagent).

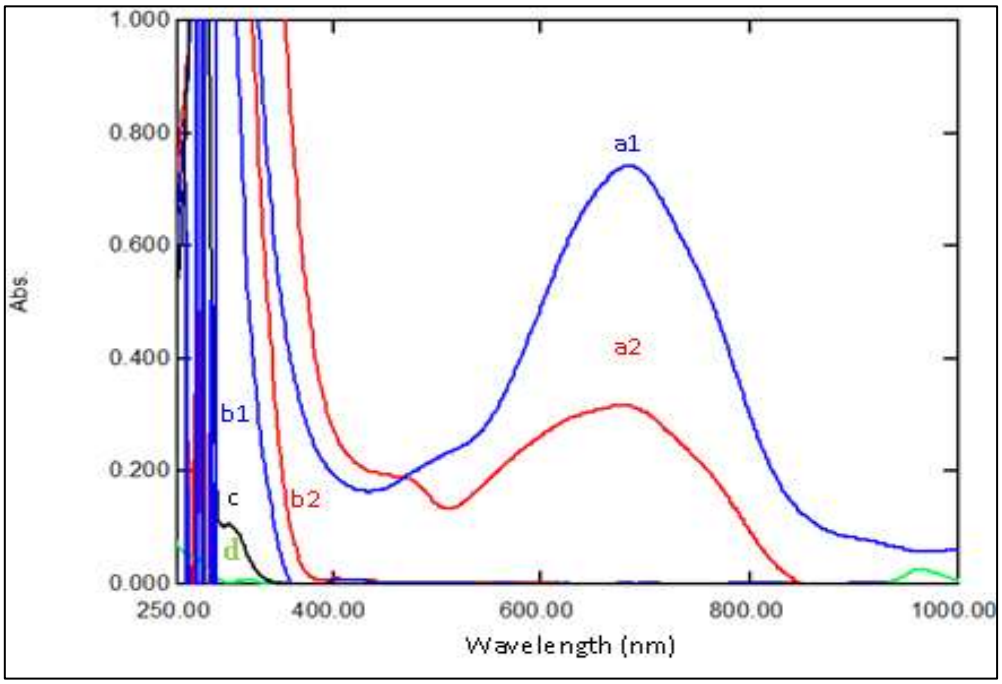
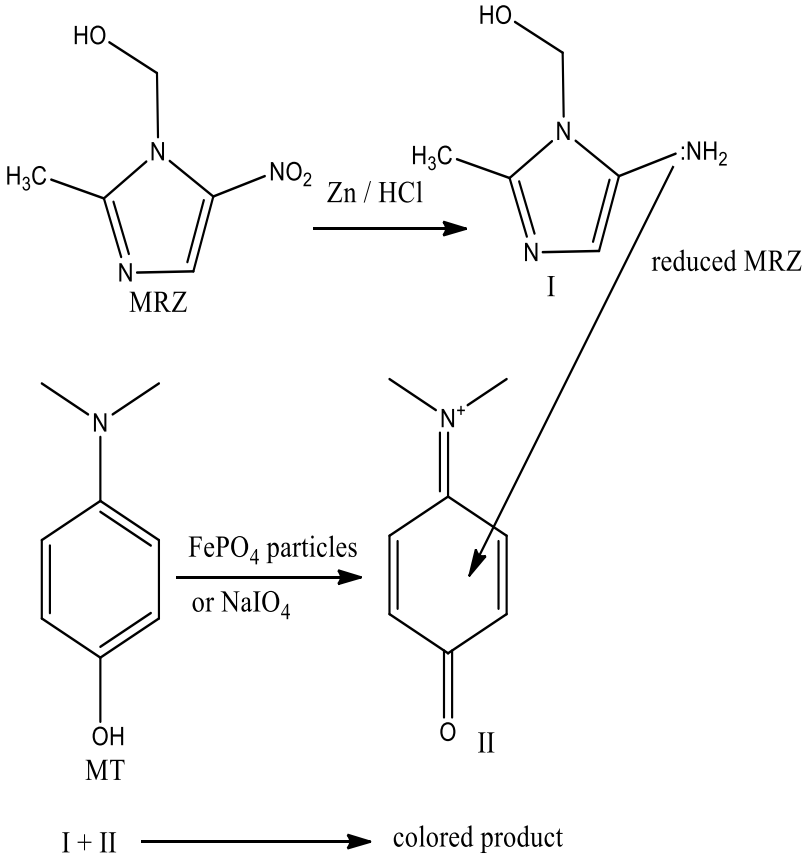


Figure 3-Absorption spectra of colored product; a1: with $NaIO_4$; a2: in presence of $FePO_4$ particles,measured against reagent blank b1 and b2 for method A and B, respectively, c: MRZ in methanol, d; reduced MRZ (5µg mL^{-1}).



Scheme 1- Suggestedreaction mechanism of the charge transfer complex between MT and reduced MRZ in presence of $FePO_4$ particles or $NaIO_4$.

Optimization of the experimental conditions

The effectof different parameters (physical and chemical)was studied for both methods (A and B). The optimizationconditions were carried out by changing one parameter and keeping all the others constant. Table-1summarizesthe preliminary conditions for both suggested methods.

Table 1-The experimental conditions for the proposed methods

Parameter	**Value**	
	Method A	**Method B**
MRZ concentration (μg mL^{-1})	100	100
MTconcentration (M)	0.015	0.01
Total flow rate ($mL.min^{-1}$)	3.4	1.6
Sample volume (μL)	100	100
$NaIO_4$concentration (M)	0.01	-------
Reaction coil (cm)	50	25
FeO_4 : CA (w:w, g) Ratio	----------	1 : 0.25
F-SPR length (cm)		8
Size of theparticles (mm)		1.18
Weightof the particles (g)		0.05
λ_{max} (nm)	700	690

Chemical optimization of method A

Various types of 0.01M oxidant ($K_3[Fe(CN)_6$, $NaOI_4$, Cr^{+6}, $K_2S_2O_8$ andKIO_3]) were optimizedin order to select the most appropriate oxidizing agent for method A. The maximum responsewas obtainedby the use of $NaOI_4$ (Figure-4-a).Therefore, itwill be used in next studies for method A.

The effect of various $NaOI_4$concentrations (0.001 to 0.07 M)was examined. It was found that the response was increased with increasing$NaOI_4$concentration up to 0.03M. However, any level beyond this concentration (0.03 M) led to the reductionof theresponse (Figure-4-b). Therefore, the $NaOI_4$(0.03M) was selected in the next studies for the estimation of MRZ.

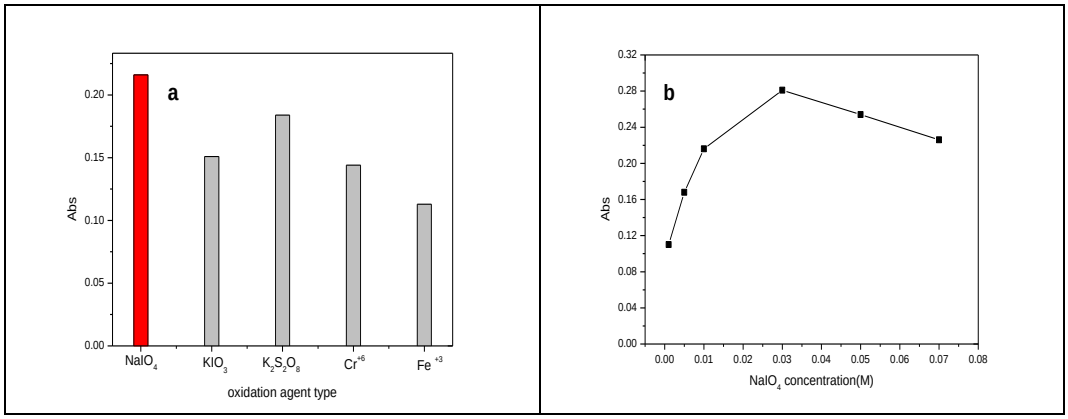


Figure 4- a, Type of oxidant agent and b, the effect of $NaIO_4$ concentration (M) for method A.

Optimization of solid-phase reactor (F-SPR) conditions for method B Effects of solid-phase reactor composition

The proportion of $FePO_4$fixedin CA has asignificant role in the activity of the F-SPR. Various weight ratios of fixedoxidizer in CA were used for the developing of the F-SPR materials; 0.25:1, 0.5:1, 0.5:1. 5, 0.5:2 and 1: 2 (CA: $FePO_4$, w: w, g). It was found that the ratio of 0.5:1.5 g provided the reproducibility and highest response for F-SPR (Figure-5). Thus,it will be used in next studies for method B.

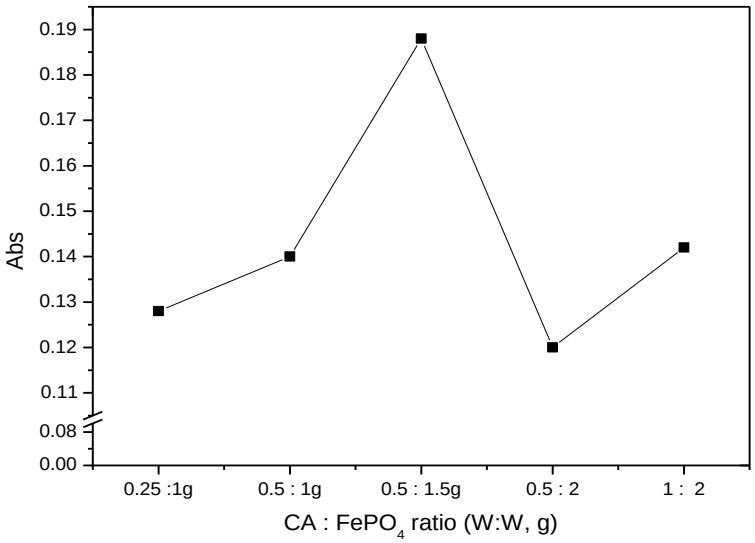


Figure 5-The composition ratio effect (CA: $FePO_4$) on the absorbance of colored product.

Effect of solid-phase particles size

Various particles sizesof fixed $FePO_4$wereinvestigated (0.15 -1.18 mm). It can be seen in Figure- 6 that the responseraiseswith increasing the particles size up to 1 mm; therefore,1 mm particle size was selected and used in the next studies for method B.

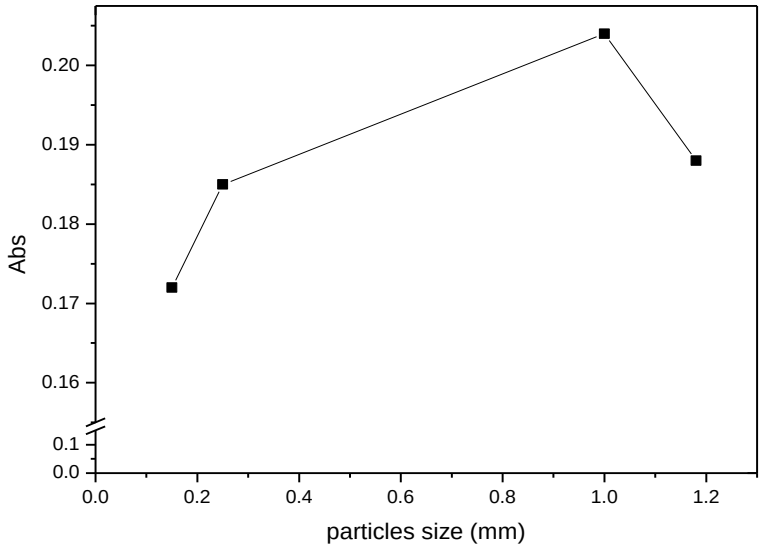


Figure 6-The particles size effect on the absorbance of colored product.

Effect of solid-phase reactor length

The effect of reactor length (F-SPR) on the response was optimized by changing the length of the reactor in the range of 4-12 cm. It was found that theemploymentof10 cm reactor length gave thehighest response, as presented in Figure-7. By comparing the stability of the response, the length of 10 cm was selected and used in the next studies for method B.

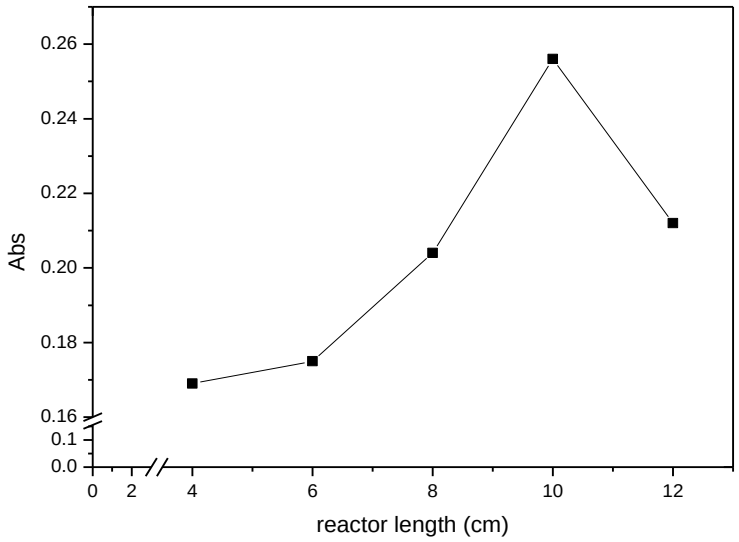


Figure 7-The reactor length effect on the absorbance of the colored product.

Effect of solid-phase particles weight (degree of packing)

The effect of particles weight of the F-SPR (0.04-0.1 g) was studied using variousweights of the fixed $FePO_4$ on CA. It can be seen (Figure-8) that the weight of 0.091 g gave the highest response.

Therefore, 0.091 g, as an optimum degree of packing (particle weight), was selected and used in the next studies for method B.

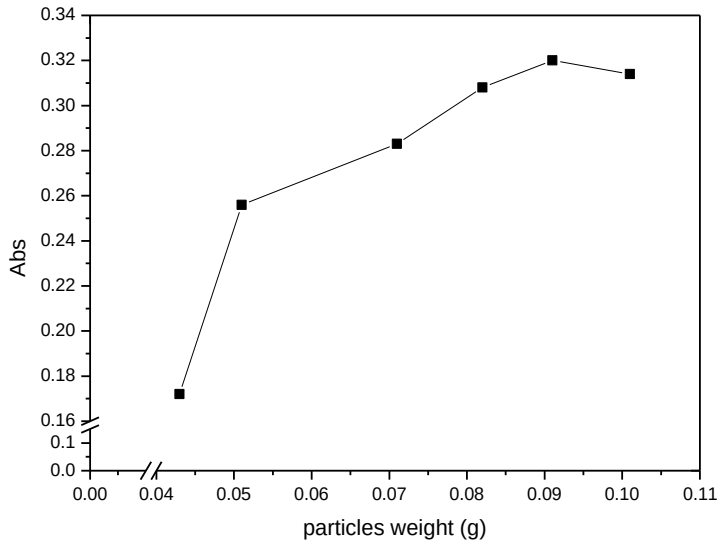


Figure 8-The effects of $FePO_4$particlesweight

The reagent concentration was varied in the range (0.001-0.005)% in order to maximize the peak height.

Optimization of chemical and physical conditions for both methods

The effect of MT concentration

In order to maximize the absorbance of charge transfer product, the reagent (MT) concentration was examined for both methods in a range of 0.005-0.025 M. It can be seen that the response washeightened as the MT concentration was increased up to 0.01 and 0.015 M for methods A and B, respectively (Figure-9). Therefore, 0.01 and 0.015 M were selected as the best concentrations for methods A and B, respectively.

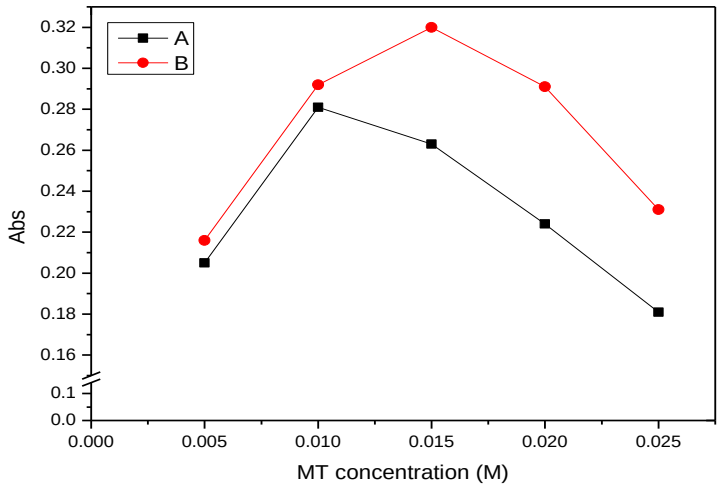


Figure 9-Metol (MT) concentration effect on the formation of charge transfer complex for methods A and B.

The effect of total flow rate

The effect of total flow rate on the response of the colored product was also examined for methods A and B in the range of 1.2 to 3.6 and 0.6 to 2 mL min^{-1}, respectively(Figure-10). When the flow rate

was increased, the signal was heightened up to 3 and 1.9 mL min^{-1} for methods A and B, respectively. Therefore, the flow rates of 3 and 1.9 mL min^{-1} were selected as optimum flow rates for methods A and B, respectively, which will be used in the next studies.

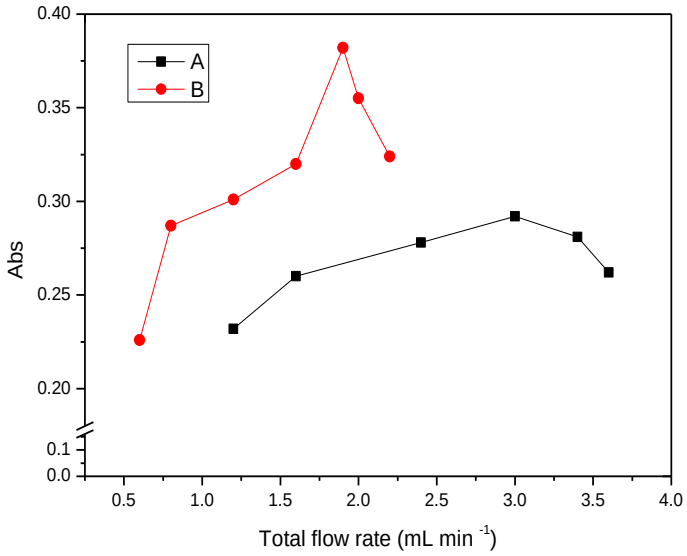


Figure 10-Total flow rate effect for A and B methods.

Effect of injection sample volume

The injected volume (75 to 200 µL) into the carrier stream was evaluated since it has an important rolein the response value. It can be seen that 150 µL as an injected volume gave the best response for both methods A and B (Figure-11). Therefore, were selected this volume for the next studies.

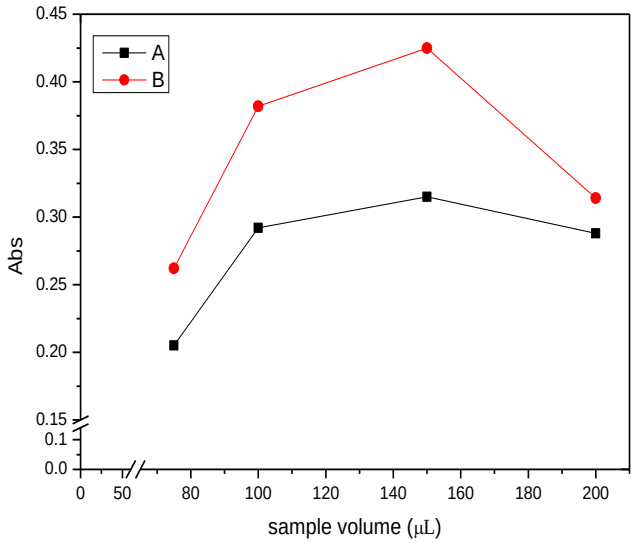


Figure 11-Sample volume effect on the formation of the colored product for A and B methods.

Effect of reaction coil length

The effect of mixing coil length on the response was optimized in range of 0 (without reaction coil) to 100 cm. According to the results (Figure-12), lengths of 75 and 50 cm were chosen as optimum lengths that gave the maximum absorbance for the colored product for methods A and B, respectively,andwill be used in next studies.

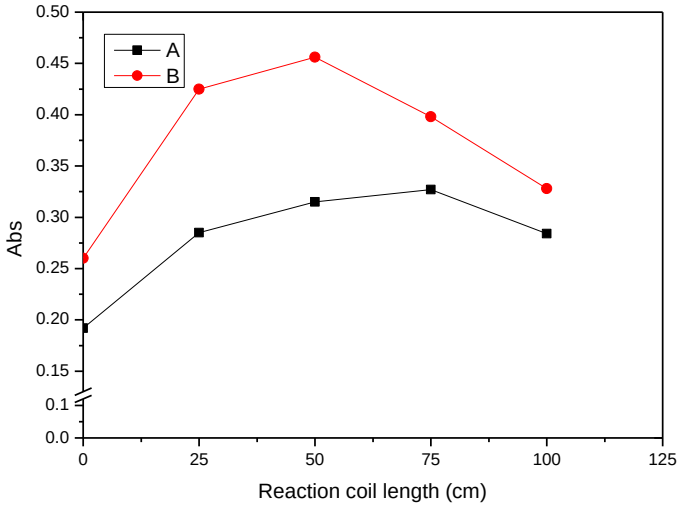


Figure 12-Reaction coil length effect on absorbance of charge transfer complex for A and B methods.

Selected Optimum Conditions

The optimum values of all investigated parameters are summarized in Table-2 for using the proposed methods (A and B) for the determination of MRZ.

Table 2-The optimum conditions for the estimation of MRZ for both methods A and B.

Parameter	Studied range	Method A	Method B
		Optimum value	
Type of oxidant	Different type	$NaIO_4$	-------
$NaIO_4$ concentration (M)	0.001-0.07	0.03	-------
MTconcentration (M)	0.005-0.025	0.01	0.015
Total flow rate ($mL.min^{-1}$)	0.6-3.6	3	1.9
Sample volume (µL)	75-200	150	150
Reaction coil (cm)	0-100	75	50
$FePO_4$: CA (w:w, g)ratio	Different ratios	----------	1.5:0.5
F-SPR length (cm)	4-12		10
Size of theparticles (mm)	0.15-1.18		1
Weight of theparticles (g)	0.04-0.1		0.09
λ_{max} (nm)	250 - 1100	700	690

Sampling frequency for both methods and F-SPR life-time

Depending on the optimum parameters, the sampling frequency was evaluated by recording the time from the sample injection to the maximum absorbance (27 and 37 seconds for A and B, respectively). 110 and 74samples hr^{-1}were achieved as practical sampling frequency for methods A and B, respectively. To examine the efficiency of the F-SPR (method B) containing immobilized $FePO_4$ on the CA,the experiment was performed with injection of MT (150 µL) into the MRZ stream at a flow-rate of 1.9 $mL.min^{-1}$ and then thepassagethrough F-SPR. The results indicated that 34 injections with RSD% of 4.37 could be achieved with good reproducibility (RSD ≤ 5) [24] as well as life time for F-SPR.

Calibration graph

The response of the colored product was recorded and plotted against the concentration of MRZ (Figure-13), depending on the selected parameters mentioned in Table-2. Two series of MRZ solutions

were prepared in the range of 2.5-200 and 2.5- 150 µg mL^{-1}. The detection limit was 2.53 and 2.12 µg mL^{-1} for methods A and B, respectively.Table-3 summarizesthe other analytical values of statistical treatments for the calibration graph[25].

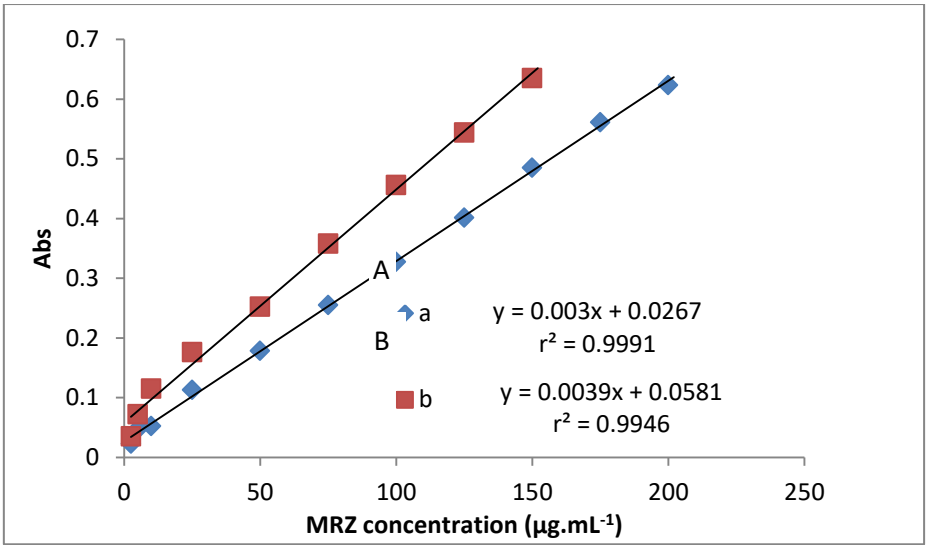


Figure 13-Calibration curve for estimation of MRZ for A and B methods**.**

Table 3-Analytical values of statistical treatments for the calibration graph

Parameter	**Method A**	**Method B**
	Value	
Leaner equation	0.003x + 0.0267	0.0039x + 0.0581
Coefficient correlation, r	0.9995	0.9972
Linearity percentage, r^2 %	99.91	99.46
Linearity (µg.mL^{-1})	2.5 - 200	2.5 - 150
Slope, b (mL.µg-1)	0.003	0.0039
Intercept, a	0.0267	0.0581
Sd of the residuals, $S_{y/x}$	0.007	0.017
Sd of the slope, S_b	$3.1x10^{-5}$	$1x10^{-4}$
Sd of the intercept, S_a	0.0033	0.008
LOD (µg.mL^{-1})	2.530	2.120
LOQ (µg.mL^{-1})	7.670	6.410
Sampling rate (per hour)	110	74

Sd; standard deviation

Accuracy and precision

The precision and accuracy forboth methods were evaluated by injections of pure drug solution at two various concentrations. Table- 4 indicates the statistical treatment of thestudy for both suggested methods.

Table 4-The Accuracy and precision of the proposed methods

MRZ concentration (μg mL^{-1})		Rec%	RE%	RSD% n=6
Present	Found *			
Method A				
50	50.43	100.86	0.86	0.96
125	124.77	99.816	-0.184	1.42
Method B				
50	49.72	99.44	-0.56	1.20
125	124.59	99.672	-0.328	1.89

Average of five determinations

Analysis of pharmaceutical formulations

Both suggested methods were applied for the estimation of MRZ using different pharmaceutical formulation tablets (*Negazole 500 mg Tablet Julphar, UAE and MEDAZOLE 500mg Tablet, S.D.I, IRAQ*).

Under the recommended procedure, the standard addition method (Table-5) was applied by preparing a series of solutions for each sample (50 and 100 μg mL^{-1}) via transferring the required volume (0.25 or 0,5 mL, of 1000 μg mL^{-1}) of commercial dosage to five volumetric flasks (10 mL), followed by the addition of various volumes (0 , 0.1, 0.2, 0.3 and 0.4 mL) of thereduced MRZ (1000 μg mL^{-1}). The results were mathematically treated for standard additions method and the results were summarized in Table-5.In order to examine the success and the efficiency of both methods (A and B) for the estimation of MRZ in pure and commercial tablets, the results were compared statistically with the standard method [26]. The suggested and standard methods obtained results whichwere compared statistically for theestimation of MRZ pharmaceutical formulations at 95% confidence level by means of the F-test and t-test. It was found (Table-6) that there is no significant difference between theproposed and standardmethods, while the F-test and t-test did not exceed the theoretical values.

Table 5-Application of the proposed methods (A and B) for the determination of MRZ in pharmaceutical preparations by applying standard addition method

Sample	MRZ concentration (μg mL^{-1})		Rec%	RSD%
	Present	Found		
	Method A			
*1	25	25.1	100.4	0.95
	50	48.83	97.66	1.12
	100	97.02	97.02	0.88
**2	25	24.96	99.84	1.34
	50	51.85	103.7	1.27
	100	102.97	102.97	1.62
1	Method B			
	25	25.02	100.08	1.25
	50	48.9	97.8	1.77
	100	101.2	101.2	1.54
2	25	24.87	99.48	0.86
	50	50.82	101.64	1.15
	100	99.05	99.05	1.72
1	Official method [26]			
	50	48.8	97.6	1.32
	100	99.8	99.8	1.08
	200	197	98.5	0.47
2	50	49.7	99.4	0.81
	100	102	102	0.66
	200	195.6	97.8	1.15

***Negazole 500 mg Tablet Julphar, UAE.**

**** MEDAZOLE 500mg Tablet, S.D.I IRAQ.**

Table 6-The comparison of the proposed methods with standard method using t- and F-statistical tests

Dosage form	Method A		Method B		Official method [26]	
	Rec %	$(Xi - \bar{X})_1^2$	Rec %	$(Xi - \bar{X})_1^2$	Rec %	$(Xi - \bar{X})_1^2$
Pure MRZ	100.34	0.002	99.56	0.04	99.87	0.21
***1**	98.36	3.72	99.65	0.01	98.633	0.61
****2**	102.17	3.53	100.10	0.11	99.733	0.10
Statistical values	$(\bar{X})$ = 100.29	$\Sigma(Xi - \bar{X})_1^2$ = 7.26	$(\bar{X})$ = 99.77	$\Sigma(Xi - \bar{X})_1^2$ = 0.17	$(\bar{X})$ = 99.41	$\Sigma(Xi - \bar{X})_1^2$ = 0.92
	S_1^2 = 3.63		S_1^2 = 0.08		S_2^2 = 0.46	
	*S p= 2.05		*S p = 0.27		**t_{tab} = 2.77 ***F_{tab} = 19.0	
t_{cal}	0.75		0.84			
F_{cal}	7.89		5.75			

***S p = pooled standard deviation**
Theoretical values at 95% confidence limit, n1=3, n2 = 2.
****t = 2.77, where t has degrees of freedom = (n1 + n2 – 2) = 3**
*****F = 19.0, where F has degrees of freedom = (n1 – 1) = 2, (n2 – 1) = 1**

Conclusions

The present study describes the successful evaluation of immobilized $FePO_4$ on cellulose acetate as the oxidizing agent and MT π acceptors as an analytical reagentfor the development of normal and reverse flow injection methods for the accurate estimation of MRZin pharmaceutical dosage forms. The rFIA (method B) coupled with anSPR containing immobilized $FePO_4$gives many advantages;it ishighlysensitive, simple, rapid, and does not need expensive sophisticated apparatus. The results obtained showed that the reproducibility of F-SPR (RSD % ≤ 5) as well as life timewere good, in addition to havingthe capacity for loading a desirable number of reagent injections (34injections). The proposed methods used inexpensive reagents with excellent shelf life and are available in any analytical laboratory.

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