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Spectrophotometric Determination of Metochlopramide in itsPharmaceutical Preparations using Diazotization Coupling Reaction

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ABSTRACT: Simple and direct spectrophotometric method for quantitative estimation of metoclopramide hydrochloride (MTP) in pure and pharmaceutical dosage forms was adopted. The established method is based on a diazotization coupling reaction between diazotized MTP with new coupling reagent, 7-iodo-8-hydroxyquinoline5-sulphonic acid in alkaline medium. An intense red, water-soluble dye that is very stable and has a maximum absorption at 510 nm had been obtained. Regression analysis of Beer's law plot showed good correlation in the concentration range 0.4-12µgml^{-1} with a molar absorbtivity of 1.91×10^{5} L mol^{-1}cm^{-1} and Sandell's sensitivity of 0.0018µg.cm^{-2}. The proposed method was successfully applied to the determination of MTP in its pharmaceutical formulations.

1. INTRODUCTION

Metoclopramide hydrochloride(Figure1), which is chemically named as 4-amino-5-chloro-2-methoxy-N-(2-diethylamino-ethyl) hydrochloride, is a dopamine-receptor antagonist. It isa prokinetic and antiemetic drug used in disorders of decreased gastrointestinal motility as wellas in gastro esophageal reflux disease, dyspepsia, and for the prevention of cancer chemotherapy-induced vomiting[1].Metoclopramide is also beneficial for diabetic patients who have poor emptying of their stomachs. Treating gastro paresis can decrease symptoms of nausea, vomiting, and stomach/abdominal fullness[2].

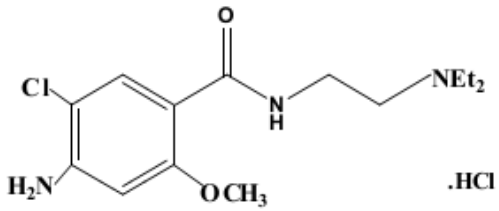


Fig.1: Metoclopramide hydrochloride

In literature survey, several methods for the analysis and determination of MTP in different samples were reported, include reversed phase ultra performance liquid chromatography (RP-UPLC)[3],flow injection based on $KMnO_4$-HCHO chemiluminescence in a micellar medium[4],Liquid chromatography-Mass spectrometry(LC-MS)[5],spectrofluorimetric using Eu^{3+} ion doped in sol–gel matrix[6],high-performance liquid chromatography[7],Stripping voltammetry[8,9],and spectrometry[10-16].However the direct spectrophotometric methods which reported for the analysis of MTP are still few. This research describes new spectrophotometric method for determination of MTP by the diazotization-coupling reaction with new reagent(7-iodo-8-hydroxyquinoline5-sulphonic acid)in alkaline medium. The method was found to be useful for assay our target drug, because it produced stable coupling organic product rapidly, with high sensitivity. In addition this

method gave good recoveries when applied for the determination of MTP hydrochloride in pure and pharmaceutical preparations.

2.EXPERIMENTAL

2.1. Apparatus

All spectral and absorbance measurementswere performed on an Optima SpectrophotometerUV-VIS (Japan) double-beam and using 1 cmquartz cells.

2.2. Preparation of solutions

- Metoclopramide hydrochloridestock standardsolution (1000 μg mL^{-1}= 2.8×10^{-3} M) wasprepared by dissolving 0.1000 g of pure MTP(SDI) in distilled water and made up to 100 mLvolumetric flask with the same solvent. Workingstandard solutions were prepared by suitabledilution of the stock standard solution withdistilled water.
- Sodium nitrite solution (2.82×10^{-3} M) wasprepared by dissolving 0.0486 g of sodiumnitrite (Merck) in distilled water and diluting tothe mark in 250 mL volumetric flask.
- Hydrochloric acid solution (1M) was prepared by diluting 43mL of 11.64 M of concentrated hydrochloric acid (BDH) with distilled water in500 mL volumetric flask.
- 7-Iodo-8-hydroxyquinoline 5-sulphonic acid solution (2.848×10^{-4}M)was prepared by dissolving 0.1000 g of reagent(BDH) in distilled water and diluting to the mark in 100 mL volumetric flask with the same solvent.
- Ammonium hydroxide solution (1M) was prepared by diluting 8.5mL of 11.77Mof concentrated ammonium hydroxide (Fluka) with distilled water in 100 mL volumetric flask and working solutions were prepared by appropriate dilution of the stock solution.
- Samples of metoclopramide hydrochloride solution obtained from commercial sources:

Tablets samples (MTP): An accurately weighed amount (20 powdered tablets of MTP/Actavis, UK-10mg and/or Meclodine/SDI,Iraq-5mg) equivalent to 50 mg of the pure drug was dissolved into a 100 mL of distilled water and completed to the mark with the same solvent to obtain 500 μg $mL^{-1}$of MTP. The flask with its contents was shacked well and filtered. A sample of 200 μg of MTP in a final volume of 25 mL was taken and the measurements were carried out as described under general procedure.

2.3. General procedure

An increasing amount of a standard solution (1000 μg mL^{-1}=2.82×10^{-3} M) containing 10-300μg of MTP was transferred into series of 25 mL volumetric flasks. In order to diazotize MTP, equimolar of sodium nitrite solution (2.82×10^{-3} M) was addedwith2mL of 0.1 M hydrochloric acid solution. The solution was shaken carefully then, 2mL of 2.848×10^{-4} M of 7-iodo-8-hydroxyquinoline 5-sulphonic acid was transferred and 5mL of 0.1M ammonium hydroxide solution was added and the contents were diluted to the mark with distilled water.After 5min, the absorbance of the red colored azo dye was measured at 510nm against theanalogous reagent blank.

2.4. Results and discussion

2.4.1. Absorption spectra

When a very diluted aqueous solution of diazotized MTP was mixed with 7-iodo-8-hydroxyquinoline 5-sulphonic acid in alkaline medium, an intense red dye produced directly, which became stable after 5min and remain stable for 2 hour at least and have a maximum absorption at 510nm . Figure2 shows the spectra of the product formed .

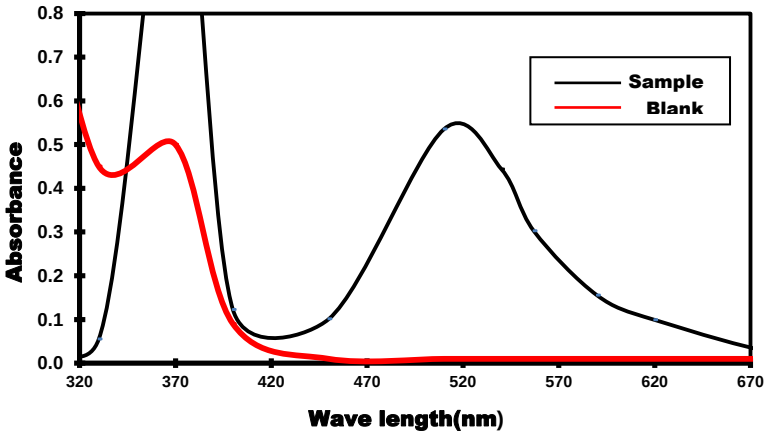


Fig.2: Absorbance spectra of MTP (8 µg mL^{-1}) measured against blank and the reagent blank measured against distilled water

2.4.2.Study of the experimental conditions

The effects of various parameters on formation of the product and the absorption intensity were studied and optimized . A 8µg mL^{-1} of MTP was used in all optimization experiments.

- **Effect of acid used in diazotization process**

Acidic medium is very essential for accomplishment the diazotization reaction. For that reason the effects of different prepared acids solutions (0.1M) were examined such as hydrochloric acid, nitric acid, sulfuric acid and acetic acid. The results showed that nitric acid gave more sensitivity but less stable response than HCl, therefore hydrochloric acid was the most suitable acidic medium and was used in all subsequent experiments (Figure3).

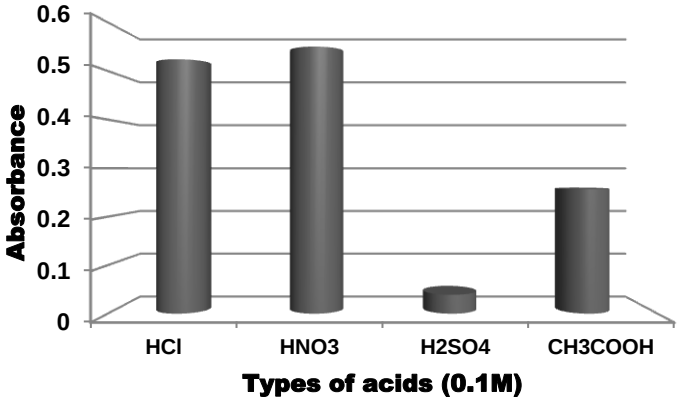


Fig.3: Effect of the type of acid

Consequently the effect of various volumes of hydrochloric acid (0.1M) were optimized on the maximum absorbance by changeable the volume of HCl and fixing the other parameters(MTP and $NaNO_2$ (2.28×10^{-3} M)) and highest absorbance was obtained with 2mL of acid and was chosen for further use (Figure 4).

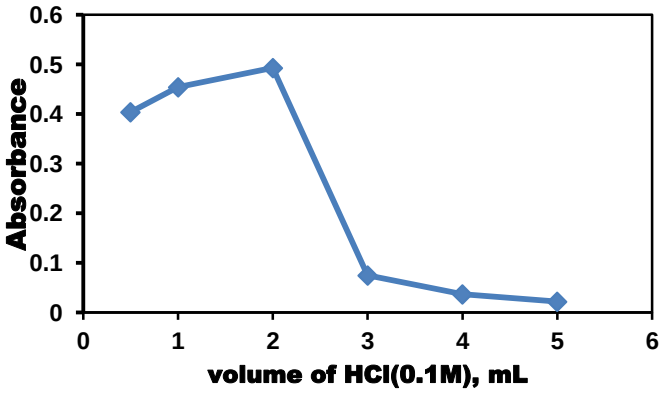


Fig.4: Effect of the volume of HCl (0.1M)

•Effect of the basic medium(type and volume)

After experimentation of a different medium in order to increase intensity of the reaction, the experiments indicated that the alkaline medium is necessary for developing a more intense color. Accordingly, a different

alkaline solutions(0.1M) of sodium hydroxide, potassium hydroxide, ammonium hydroxide and sodium carbonate were examined .With regard to Figure 5, it was clear that the ammonium hydroxide was the appropriate alkaline medium for a maximum absorbance and was used in all next experiments.

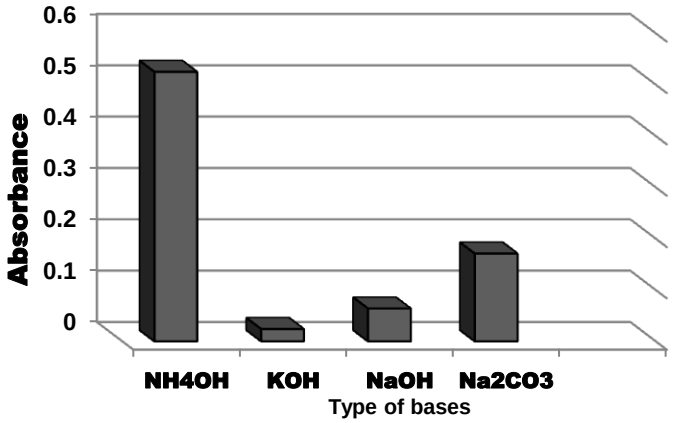


Fig. 5: Effect of bases

Effect of different volumes of ammonium hydroxide (0.1M) was studied on the intensity of colored product by varying the volume of the base solution between (0.3-7mL) with fixing the other parameters. A volume of 5mL of ammonium hydroxide (0.1M) was enough to obtain the maximum absorbance (Figure6).

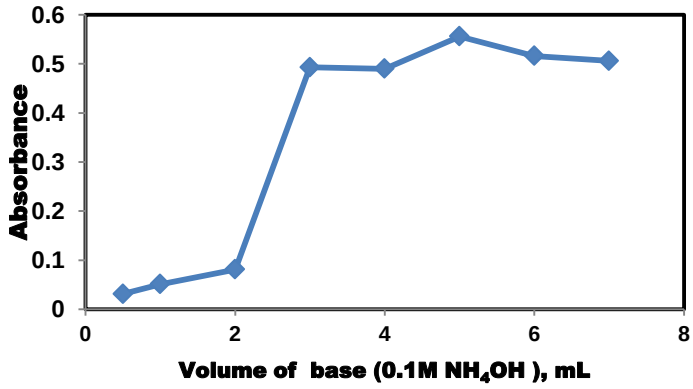


Fig.6:Effect the volume of base

- **Effect of coupling reagent concentration**

Until now the literature contains no method used 7-iodo-8-hydroxyquinoline 5-sulphonic acid as a chromogenic coupling agent for determination of drugs, therefore this reagent and in addition for its sensitivity was used for our adopted reaction. Different volumes of reagents (7-iodo-8-hydroxyquinoline 5-sulphonic acid (2.848×10^{-4}M) was studied in the range of (0.5-4mL) with fixing the volumes of HCl and NH_4OH. The greatest absorbance intensity was obtained with2mL of 7-iodo-8-hydroxyquinoline 5-sulphonic acid Figure 7).

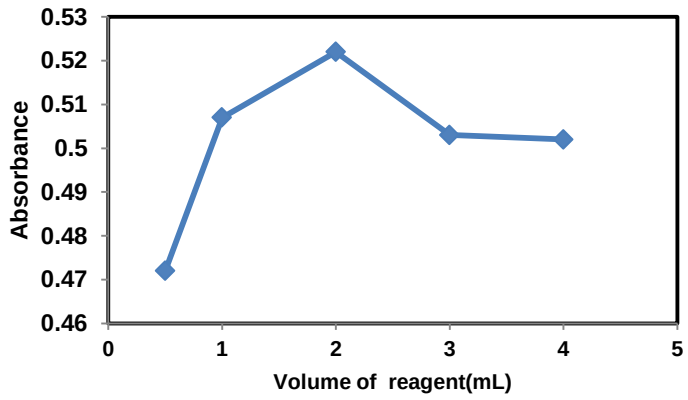


Fig.7:Effect of the volume of reagent (7-iodo-8-hydroxyquinoline 5-sulphonic acid(2.848×10^{-4}M)

- **Order of addition of reagents**

The order of reagents addition is very important, so different orders of addition of reagents were examined and it was found that the order of addition of reagents by mixing MTP with sodium nitrite then HCl, 7-iodo-8-hydroxyquinoline 5-sulphonic acid and ammonium hydroxide gave the highest absorbance and was used in all later experiments.

- **Effect of reaction time and color stability**

The resultant colored product of the proposed method was found to be formed rapidly and immediately, but the color intensity reached a maximum after 7-iodo-8-hydroxyquinoline 5-sulphonic acid solution has

been reacted with diazotized MTP ,in alkaline medium for 5min, therefore a 5 min development time was selected as optimum in the general procedure. The color obtained was stable for 2hr.

2.5. Composition of the product

Continuous variation and mole ratio methods [17], had been established under the recommended optimum conditions in order to study the composition of the formed complex between diazotized MTP and 7-iodo-8-hydroxyquinoline 5-sulphonic acid. The results obtained in (Figure 8) and (Figure 9) show that a 1:1 azo dye was formed between diazotized MTP and reagent at 510nm.

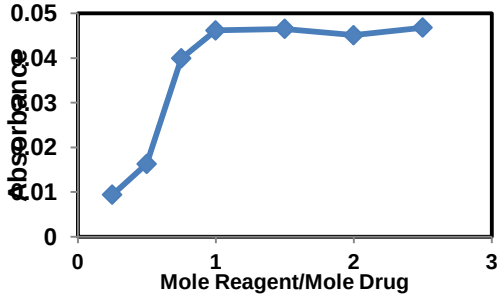


Fig.8: Mole ratio plot

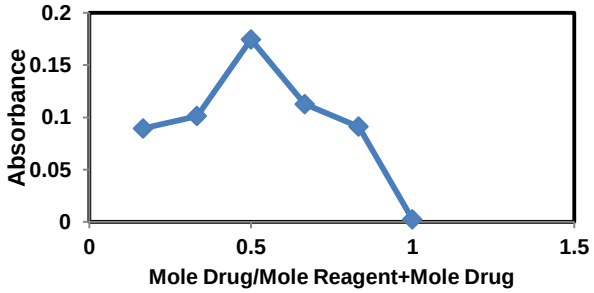
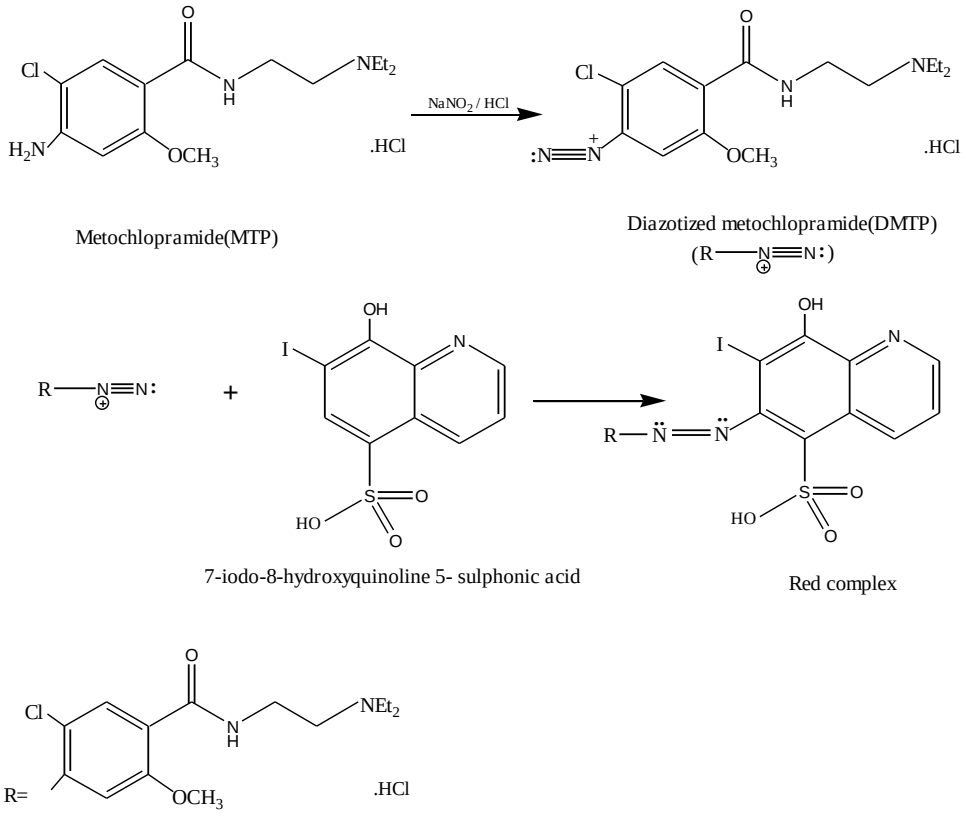


Fig.9: Continuous variation plot

2.6. Mechanism of reaction

Usually two steps are required to accomplish the diazotization coupling reaction. The first step is conversion the amino compound (MTP) to diazo compound by reaction with nitrous acid ($NaNO_2$/HCl), while the second step involves a coupling between diazotized drug and the coupling reagents[18]. The red dye product was only formed in alkaline medium (ammonium hydroxide). According to mentioned mole ratio and continuous variation results, and the obtained ratios, the reactions pathway were postulated to proceed as shown in scheme1[19,20].



Scheme 1: proposed mechanism of the proposed reaction

2.7. Determination of stability constant of complex

The apparent stability[21] constant of the method was calculated by comparing the absorbance of a solution containing stoichiometric amount (1.128×10^{-3}M) of diazotized MTP and reagent (7-iodo-8-hydroxyquinoline5-sulphonic acid)(A_S) with that of a solution containing a five- fold excess of reagents(A_m) and according to analytical procedure. The average stability constants were (K) = 2.716×10^4 L mol^{-1}, where [$K = (1-\alpha) / \alpha^2 C$; $\alpha = (A_m - A_s) / A_m$].

2.8. Analytical characteristics and validation of method

Calibration graph (Figure10) for the proposed method, were obtained by the procedure described before in which a sequence of standard solutions were analyzed in triplicates to test the linearity. All the analytical characteristics, like the slope (a), the intercept (b), and the correlation coefficient(r) were validated by a least-squares regression analysis and are incorporated in Table (1). The linearity for method was also obtained and the value of molar absorbtivity insures that the method was sensitive for determination of MTP.

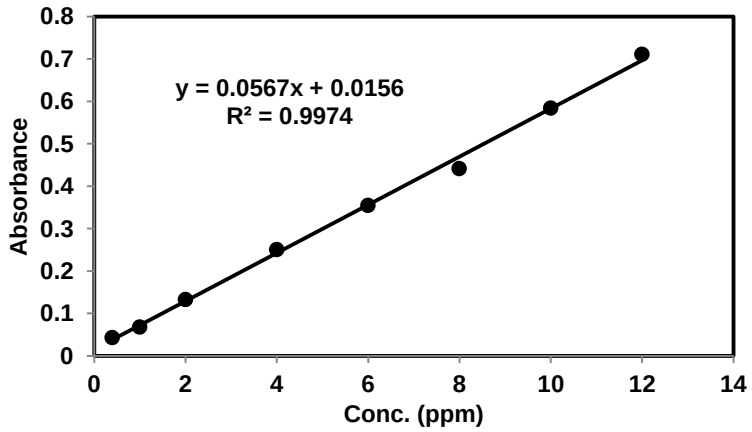


Fig.10: Calibration graphs of MTP

Table 1: Analytical parameters of spectrophotometric method

Parameters	Value
λ_{max}(nm)	510
Linearity range, µg mL^{-1}	0.4-12
Molar absorbtivity,ε (L mol^{-1} cm^{-1})	1.91×10^{5}
Sandell's sensitivity(µg mL^{-1})	0.0018
Regression equation	y = 0.056x + 0.015
Correlation coefficient, r	0.9987
Linearity percentage, r^{2}%	99.74
Slope, b	0.056
Intercept, a	0.015

Relative standard deviation (RSD%)	**<1.11**
Average of recovery%	**100.5**
Stability (hr)	**2**
Molar ratio (D:R)	**1:1**
Limit of detection(LOD) , µg mL^{-1}	**0.036**

2.9. Accuracy and precision of the proposed method

Using the optimum conditions obtained from previous experiments, the accuracy and precision of the proposed method was studied using two concentrations of MTP. Table2 shows the results of Error%, Rec.%, and RSD% of four replicates of each concentration.

Table 2: Accuracy and precision of the proposed methods

Conc. of MTP, µg mL^{-1}		E%*	Rec.%*	RSD%*
Present	**Found**			
4.00	**4.08**	**2.00**	**102.00**	**0.96**
10.00	**9.88**	**-1.20**	**98.80**	**1.11**

***Average of four determinations.**

2.10. Estimation of MTP in tablets

The proposed method was applied for the estimation of MTP in tablets by the analysis of one concentration for each sample using the recommended procedure, and the quantity per tablet was calculated from the standard calibration curve(direct method) and standard addition method (SAM).Solutions of tablets were prepared as given below the section 2.2, and the results obtained are summarized in Table3.

Table 3: Application of the proposed method for determination of MTP in pharmaceutical preparations

Drug form	Direct method					SAM method
	Conc. of MTP, µg mL^{-1}		E%*	RSD%*	Rec.%*	Rec.%
	Present	Found				
Meclodine/SDI,Iraq-tab.5mg	**6.00**	**5.93**	**-1.17**	**1.29**	**98.83**	**98.75**
Actavis,UK-tab.10mg	**4.00**	**3.77**	**-5.75**	**2.87**	**94.25**	**96.25**

***Average of four determinations**

The applicability of the proposed method for the estimation of MTP in tablets and using both direct and standard addition methods were statistically compared with those obtained by the British Pharmacopoeia(BP)procedure (HPLC method=official method)[22] using two tests the Student t-test and variance ratio F-test (confidence level at 95%)[23].The obtained results are summarized in Table4. In all cases, the calculated F and t values did not exceed the theoretical values, indicating that there is no considerable difference between the performance of the proposed method with official method as regard accuracy(t-test) and precision (F-test).

Table 4: The comparison of the proposed method with standard method using t- and F-statistical tests

Drug form	Proposed method		Official method(BP)
	Rec.% $(Xi)_1$ Direct method	Rec.% $(Xi)_1$ SAM	Rec. $(Xi)_2$
Pure MTP	100.49	100.00	100.500
Meclodine	98.83	98.75	102.500
Actavis	94.25	96.25	98.500
S**	2.782	1.955	(S_2^2=4.00)
t (2.776)*	0.992	1.357	$(n_1 + n_2 - 2) = 4$
F (19.000)*	2.869	1.097	$(n_1 - 1) = 2, (n_2 - 1) = 2$

*Theoretical value.

**s = pooled standard deviation $= \sqrt{\frac{(n_1-1)S_1^2+(n_2-1)S_2^2}{n_1+n_2-2}}$,t $= \frac{|\bar{X}_1-\bar{X}_2|}{S\sqrt{\left(\frac{1}{n_1}+\frac{1}{n_2}\right)}}$, S_1^2 = variation = $\frac{\sum(Xi-\bar{X})_1^2}{n_1-1}$ and $S_2^2 = \frac{\sum(Xi-\bar{X})_2^2}{n_2-1}$

In addition a paired t-test [23] was conducted between the samples from three sources determined by either method of analysis (using direct and standard addition methods in applications)with standard method(BP) as shown in Table 6. The t-value(t_{tab}) for n-1 degree of freedom = 4.303. Calculated t-value=2.307 (using standard addition method for analysis MTP tablets) for n-1 at α 0.05 (95%),two tailed indicate that since 2.307 <<4.303 therefore; it can be regarded that there is no difference in using the two methods. The recovery results of direct method were examined and the results were summarized in Table 5.

Table 5: Paired t-test for proposed methods with classical method

Sample	Recovery found		Xd	$\bar{X}d$	σ_{n-1}	$t_{cal} = \bar{X}\sqrt{n}\,/\sigma_{n-1}$ at 95%	t_{tab} at 95%
	SAM	Classical method					
Pure MTP	100.00	100.500	0.500	2.167	1.627	2.307(1.866)*	<<4.303
Meclodine	98.75	102.500	3.750				
Actavis	96.25	98.500	2.250				

Xd: difference between two methods, $\bar{X}d$:Differencemean, σ_{n-1}:Difference SD,$t_{critical} = t_{tab} = t_{\frac{\alpha}{2},n-1} = 4.303$,n=no. of sample

***For direct method**

Conclusions

This research offers new spectrophotmetric method using new reagent (7-iodo-8-hydroxyquinoline 5-sulphonic acid) as chromogenic agent for determination of MTP drug. The developed method is very effortless and effective for the determination of MTP in its dosage forms at a microgram concentration level and without requiring any complicated steps. In addition the present method, as compared with other expensive techniques such as HPLC-MS, GC, fluorimetry, electro sensors and capillary electrophoresis, are economical and cheap and have an excellent accuracy and precision.

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التقـدير الطيفـي للميتوكلوبرمايـد فـي المستحضـرات الصـيدلانية بوسـاطة تفاعـل الازوتة و الازدواج

أستاذ مساعد وسن عبد الامير الازري ، مدرس د.هند هادي عبدالله،مدرس غادة فاضل*

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الخلاصة

تم اعتماد طريقة تحليلية للتقدير الطيفي المباشر والبسيط لهيدروكلوريد الميتوكلوبرمايد بصورته النقية وفي المستحضرات الصيدلانية. اعتمدت الطريقة على تفاعل الازوتة والازدواج بين الميتوكلوبرمايد المؤزوت مع كااشف ازدواج جديدة :7– ايودو، 8–هيدروكسي كوينولين ،5–حامض السلفونيك في الوسط القاعدي لتكوين صبغة حمراء مستقرة وذائبة في الماء اعطت أقصى امتصاص عند طول موجي 510 نانومتر . يشير الرسم البياني للامتصاص مقابل التركيز بان قانون بير ينطبق ضمن المدى 0.4– 12 مكغم مل$^{-1}$ وكانت الممتصية المولارية 1.91×10^{5}لتر مول$^{-1}$سم$^{-1}$وحساسية ساندل 0.0018مكغم سم$^{-2}$. تم تطبيق الطريقة بنجاح في تقدير الميتوكلوبرمايد في المستحضرات الصيدلانية.