SPECTROPHOTOMETRIC DETERMINATION OF METOCLOPRAMIDE HYDROCHLORIDE IN TABLETS BY DIAZOTIZATION AND COUPLING REACTION WITH PHENOL

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Abstract

A rapid and sensitive spectrophotometric method is proposed for the determination of metoclopramide hydrochloride. This method is based on the diazotization reaction of metoclopramide hydrochloride with sodium nitrite in hydrochloric acid medium; the formed diazonium salt is then coupled with phenol in sodium hydroxide medium, to form a yellow water-soluble mono azo dye. Beer's law is obeyed in the concentration range of 1 – 20 μg mL^{-1} at 463 nm with detection limit 0.406 μg mL^{-1}. The molar absorptivity and Sandell's sensitivity were found to be 2.42×10^4 L mol^{-1} cm^{-1} and 0.0015 μg cm^{-2}, respectively. Common excipients used as additives in tablets do not interfere in the proposed method. The method is successfully employed for the determination of metoclopramide hydrochloride in tablets and the results agree favorably with the official British Pharmacopoeia method.

التقدير الطيفي لهيدروكلوريد الميتوكلوبراميد في الأقراص بوساطة تفاعل الأزوتة والازدواج مع الفينول

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الخلاصة

اقترحت طريقة سريعة و حساسة لتقدير هيدروكلوريد الميتوكلوبراميد. تعتمد الطريقة على تفاعل الازوتة لهيدروكلوريد الميتوكلوبراميد مع نتريت الصوديوم في وسط حامض الهيدروكلوريك، ثم ازدواج ملح الديازونيوم الناتج مع الفينول في وسط هيدروكسيد الصوديوم لتكوين صبغة صفراء ذائبة في الماء. إذ أطاع قانون بير عند مدى التركيز 1 – 20 مايكروغرام $مل^{-1}$ هيدروكلوريد الميتوكلوبراميد عند 463 نانومتر مع حد كشف 0.406 مايكروغرام $مل^{-1}$. و قيمة الامتصاصية المولارية و حساسية ساندل مساوية إلى 2.42×10^4 لتر $مول^{-1}$ $سم^{-1}$ و 0.0015 مايكروغرام $سم^{-2}$ على التوالي. لم تتداخل المواد المعروفة التي تستعمل كمضافات للأقراص مع الطريقة المقترحة. طبقت الطريقة بنجاح في تقدير هيدروكلوريد الميتوكلوبراميد في الأقراص و كانت نتائج الطريقة متوافقة معنوياً مع الطريقة القياسية المعتمدة في دستور الأدوية البريطاني.

Introduction

Metoclopramide hydrochloride (MPH), 4-amino-5-chloro-N-[2-(diethylamino)ethyl]-2-methoxybenzamide hydrochloride, is a white crystalline powder[1]. It is used as an anti-emetic in the treatment of some forms of nausea and vomiting and to increase gastrointestinal motility. It is of little benefit in the prevention or treatment of motion sickness or in the treatment of nausea and vertigo due to Meniere disease or other labyrinth disturbance[2]. In this perspective, the wide applications of MPH in both clinical and experimental medicine have prompted extensive interest in its determination.

Current analytical methods employed for the determination of MPH can involve fluorimetry[3], spectrophotometry [4–7] , chroma-tography [8–11] , capillary electrophoresis [12], differential scanning calorimetry (DSC) and X-ray diffraction[13], gas chromatography–mass spectrometry (GC–MS)[14], potentiometry [15] , voltammetry [16], fast stripping continuous cyclic voltammetry[17] and square wave anodic stripping voltammetry[18].

The British Pharmacopoeia (BP) reported a potentiometric method for the determination of MPH powder and UV method for tablets[19]. The potentiometric method requires about 250 mg MPH. The UV method is liable to interferences from tablet excipients and requires pre-extraction of MPH with chloroform.

Dizotization and coupling reactions were used for determination of MPH by diazotization reaction of MPH and coupling with different coupling agents such as N-(1-naphthyle)ethylenediamine dihydrochloride[20], terbutaline sulfate, 1-naphthylamine[21], thymol [22] , resorcinol [23], dibenzolyl methane [24] and aniline [25]. These reactions required removing of excess of sodium nitrite by sulfamic acid or ammonium sulfamate, also required using methanolic coupling reagent, diluting the azo dye by methanol and high concentration of sodium hydroxide as alkaline medium for coupling reaction.

In this work, a rapid and sensitive method using spectrophotometric detection at 463 nm was proposed for determination of MPH in tablets. The method is based on the diazotization reaction of MPH with sodium nitrite in hydrochloric acid medium; the formed diazonium salt is then coupled with phenol in sodium hydroxide medium to form a yellow water-soluble mono azo dye. This method does not need to get rid of excess sodium nitrite (by addition sulfamic acid or ammonium sulfamate) because of the low concentration of sodium nitrite used in equimolar solution of MPH and sodium nitrite. The proposed method has been successfully applied to the determination of MPH in tablets. The method is safe, simple, sensitive, selective and accurate.

Experimental

Apparatus

A Shimadzu UV-VIS 260 (Tokyo, Japan) digital double-beam recording spectrophoto-meter was used for all spectral and absorbance measurements with matched 1-cm quartz cells.

Reagents

All chemicals were of analytical reagent grade.

1- MPH stock standard solution 1000 μg mL^{-1} was prepared by dissolving 0.1000 g of pure MPH (SDI) in distilled water and diluting to the marked in 100 mL volumetric flask. Working standard solution 100 μg mL^{-1} was prepared by diluting 10 mL of this stock standard solution with distilled water in 100 mL volumetric flask.

2- Sodium nitrite solution 5×10^{-3} M was prepared by dissolving 0.0690 g of sodium nitrite (Merck) in distilled water and diluting to the marked in 200 mL volumetric flask. Then, 2.82×10^{-4} M was prepared by diluting 14.1 mL of sodium nitrite solution (5×10^{-3} M) with distilled water in 250 mL volumetric flask.

3- Hydrochloric acid solution 1 M was prepared by diluting 43 mL of 11.64 M of concentrated hydrochloric acid (BHD) with distilled water in 500 mL volumetric flask.

4- Phenol solution 1% w/v was prepared by dissolving 1.0000 g of phenol (BHD) in distilled water and diluting to the marked in 100 mL volumetric flask.

5- Sodium hydroxide solution 1 M was prepared by dissolving 10.0000 g of sodium hydroxide (BHD) in distilled water and diluting to the marked in 250 mL volumetric flask.

Pharmaceutical preparations of metoclopramide hydrochloride

Pharmaceutical preparations were obtained from commercial sources.

1- Meclodin tablets (SDI, Iraq): 5 mg metoclopramide hydrochloride for each tablet.

2- Meclodin tablets (SDI, Iraq): 10 mg metoclopramide hydrochloride for each tablet.
3- Metoclopramide tablets (Ajanta Pharma Limited, India): 10 mg metoclopramide hydrochloride for each tablet.

Analytical procedure for calibration

An aliquot of a standard solution (100 µg mL^{-1} = 2.82 × 10^{-4} M) containing 0.25 – 5.00 mL of MPH was transferred into a series of 25 mL calibrated flasks. To this solution was added equimolar of sodium nitrate solution (2.82 × 10^{-4} M) and the acidity was adjusted with 1 mL of 1 M hydrochloric acid solution. The solution was shaken thoroughly. Then, 3 mL of 1% phenol and 3 mL of 1 M sodium hydroxide solutions were added and the contents were diluted to the mark with distilled water and mixed well. After 5 min, the absorbance of the colored azo dye was measured at 463 nm against the corresponding reagent blank.

Procedure for the assay of tablets solution (1000 µg mL^{-1})

The average tablet weight was calculated from the contents of 20 tablets that had been finely powdered and weighed. A portion of this powder, equivalent to 100 mg of MPH, was accurately weighed. The sample was dissolved in distilled water and filtered into a 100 mL volumetric flask, the residue was washed and diluted to the marked with distilled water.
Further appropriate solution (100 µg mL^{-1}) was made by using distilled water. Two different concentrations of this tablets solution were analyzed in five replicate by analytical spectrophotometric procedure.

Results and discussion

Preliminary studies

Throughout the preliminary study on the diazotization reaction of MPH, with sodium nitrite in hydrochloric acid medium; the formed diazonium salt is then coupled with phenol in sodium hydroxide medium, a yellow water-soluble azo dye was obtained with a maximum absorbance at 463 nm [Figure 1]. The absorbance of the azo dye solution measured versus reagent blank which has negligible absorbance at this wavelength.

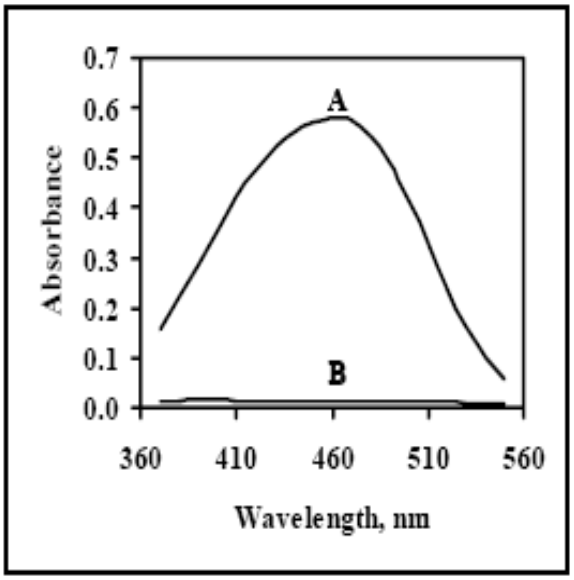


Figure 1: Absorption spectra of the azo dye against reagent blank (A) and reagent blank against distilled water (B)

Optimization of the experimental conditions

The effect of various variables on the color development was studied to establish the optimum conditions for the determination of MPH.
In the subsequent experiments, 2 mL of MPH solution (100 µg mL^{-1} = 2.82 × 10^{-4} M) with equimolar of sodium nitrite solution (2 mL of 2.82 × 10^{-4} M) was taken in 25 mL final volume and the absorbance of a series of solutions were measured by varying one and fixing the other parameters at 463 nm versus reagents blanks.
This method does not need to get rid of excess sodium nitrite (by addition of sulfamic acid or ammonium sulfamate) because of the low concentration of sodium nitrite in equimolar solution of MPH and sodium nitrite.
The effect of different volumes of 1 M hydrochloric acid solution (0.3 – 5.0 mL) (used in diazotization reaction of MPH), 1% phenol solution (0.5 – 6.0 mL) and 1 M sodium hydroxide solution (0.5 – 5.0 mL) were examined from the appearance the maximum absorbance of the azo dye. Figure (2) shows that 1 mL of hydrochloric acid solution (1 M), 3 mL of phenol solution (1% w/v) and 3 mL of sodium hydroxide solution (1 M) were enough to obtain the maximum absorbance.

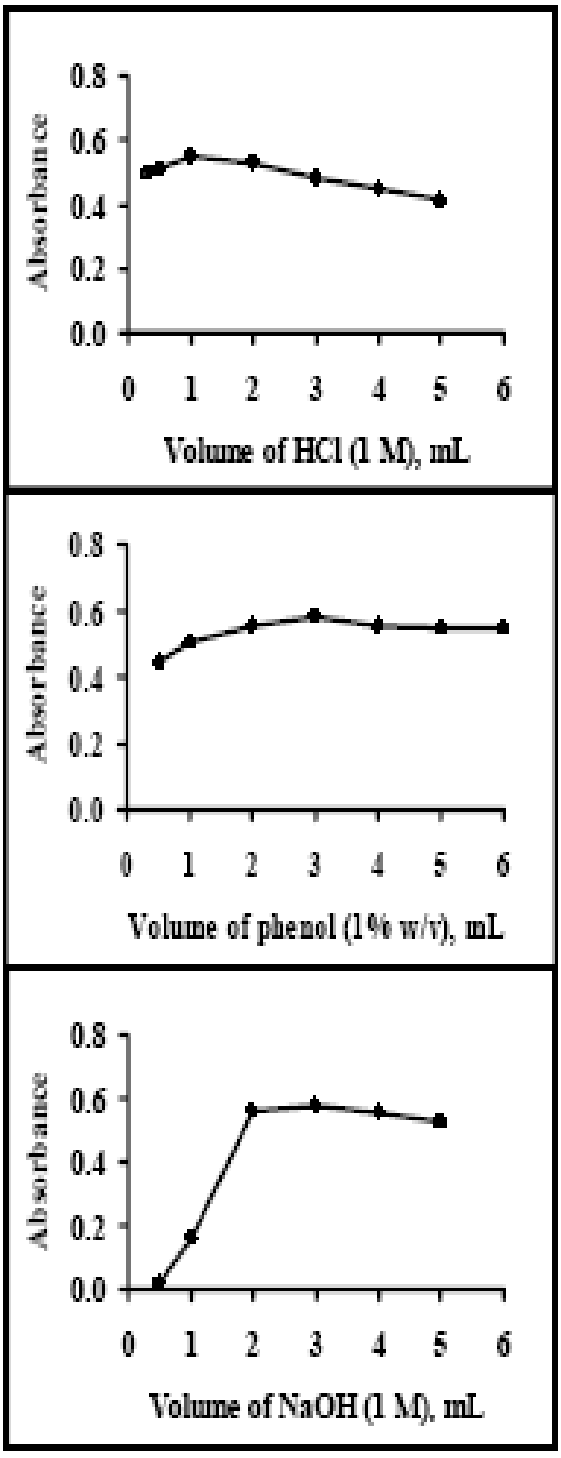


Figure 2: Optimum conditions for determination of MPH

The yellow azo dye was only formed in alkaline medium. Therefore, the effects of different alkaline solutions were studied such as sodium carbonate, potassium hydroxide, sodium hydroxide and ammonium hydroxide. It was found that sodium hydroxide is the most suitable alkaline medium to produce a maximum absorbance and was used in all subsequent experiments.

To obtain optimum results, the order of addition of reagents should be followed as given under the analytical procedure, otherwise a loss in color intensity and stability were observed.

The stability of the dye was studied for 2 h following the mixing of the reagents. The absorbance of the dye was sharply increased 2 min after mixing and remained constant for at least 2 h.

The effect of temperature on the diazotization and coupling reaction show that the absorbance of the azo dye remains constant in the range 0 – 30ºC and decrease at higher than 30 ºC. Therefore, it has been recommended to carry out reaction at room temperature (25ºC) and cooling to 0 – 5ºC was not necessary.

The stoichiometry of the product was studied applying the continuous variation method. Volumes of 1 – 6 mL of 2.82×10^{-4} M portions of MPH (V_D) were diazotized and coupled according to analytical procedure with the corresponding complementary volume of 2.82×10^{-4} M phenol solution (V_R) to give a total volume of 6 mL for $V_D + V_R$. The results obtained in Figure (3) shows that a 1 : 1 azo dye was formed between diazotized MPH and phenol.

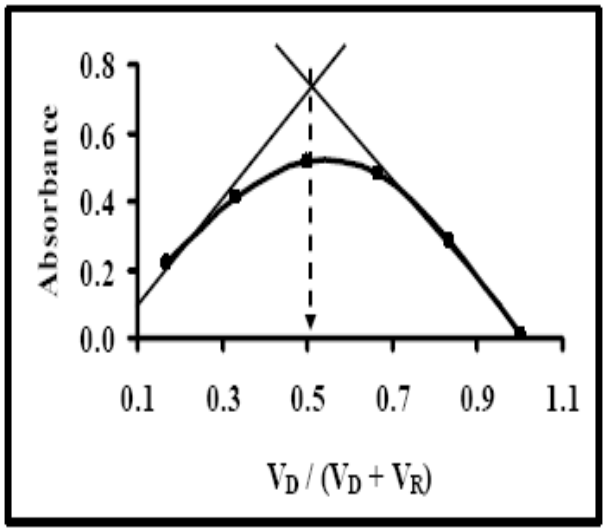
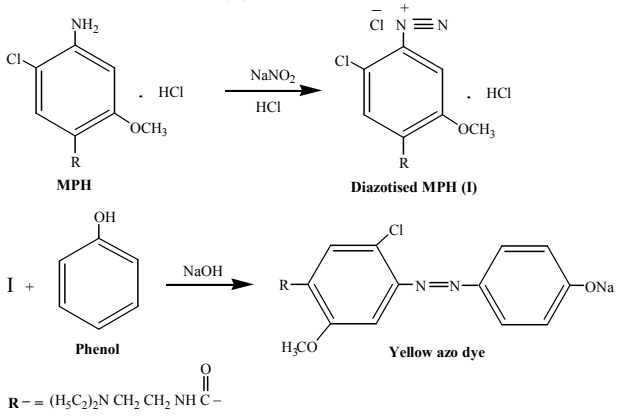


Figure 3: Continuous variation plot

For the diazotization process; it would be expected that NH_2 group in MPH would be readily diazotized in a hydrochloric acid medium. The diazonium group would then react with a molecule of phenol by electrophilic substitution at the 4-position of the coupling agent to produce an intense yellow azo dye in sodium hydroxide medium. An investigation of the continuous molar variation of diazotized

MPH and phenol showed that diazotized MPH interacts with phenol in the ratio of 1 : 1. A reaction sequence based on the above results is shown in Scheme (1).



Scheme 1: Reaction sequence

The formation constant of the reaction product (K_f) was calculated adopting the following formula[26]:

$$K_f = (A / A_m) / ([(1 - A) / A_m)]^{n+1} C^n n^n)$$

Where A is maximum absorbance, A_m is the absorbance corresponding to intersection of the two tangents of the curve in Figure 3, C is the concentration corresponding to maximum absorbance, n is the amount of the MPH in reaction product. Using this equation, K_f was found to be equal to 4.529×10^4 L mol^{-1}.

The Gibbs free energy of the reaction (ΔG) was also calculated adopting the following equation[26]:

$$\Delta G = -2.303\ R\ T \log K_f$$

Where R is the universal gas constant and T is the absolute temperature.

The value of ΔG was found to be –26.535 KJ mol^{-1}. The negative sign of ΔG points out to the spontaneous nature of the reaction.

In order to assess the possible analytical applications of the proposed method. The effect of some common excipients frequently found with MPH in pharmaceutical preparations such as lactose, starch, talc, magnesium stearate and polyvinylpirrolidone (PVP) was studied by analyzing synthetic sample solutions containing 8 µg m^{-1} of MPH and excess amounts (10-fold excess) of each excipient, none of these substances interfered seriously [Table (1)].

Table 1: Determination of 8 µg mL^{-1} of MPH in the presence of excipients

Excipient, 80 µg mL^{-1}	Concn. of MPH, µg mL^{-1} Found*	Erel.[a], %	Rec.[b], %	RSD[c], %
Lactose	7.960	– 0.500	99.500	0.291
Starch	8.161	+ 2.013	102.013	0.566
Talc	7.930	– 0.875	99.125	0.636
Mg stearate	8.131	+ 1.638	101.638	0.784
PVP	8.051	+ 0.638	100.638	0.701

*** Average of five determinations.**

[a] Erel. is relative error, [b] Rec. is recovery, [c] RSD is relative standard deviation

Analytical characteristics of the proposed method

The calibration graph was obtained by the analytical procedure described previous and a series of standard solutions were analyzed in triplicates to test the linearity. The molar absorptivity (ε), the Sandell sensitivity (S), the intercept (a), the slope (b), the correlation coefficient (r), the correlation of determination (r^2), were evaluated by a least-squares regression analysis and are included in Table (2). Beer's law plot (n = 11) was linear with very small intercept and good correlation coefficient in the concentration range in Table (2).

Statistical evaluation[27] of the regression line gave the values of standard deviations for residuals ($S_{y/x}$), intercept (S_a) and slope (S_b) at 95% confidence are shown in Table (2). These small figures point out to the high precision of the proposed method.

The limit of detection (LOD) and limit of quantitation (LOQ) were determined using the formula: LOD or LOQ = k S_a / b, where k = 3 for LOD and 10 for LOQ. The LOD and LOQ values are shown in Table (2).

Table 2: Data for the calibration graph for MPH using the proposed method

Parameter	Value
Linearity range, μg mL^{-1}	1 – 20
r	0.9994
r^2	0.9988
ε, L mol^{-1} cm^{-1}	2.4199×10^4
S, μg cm^{-2}	0.0146
a	0.0020
b, mL μg^{-1}	0.0683
$S_{y/x}$	1.6029×10^{-2}
S_a	9.2469×10^{-3}
S_b	7.8126×10^{-4}
LOD, μg mL^{-1}	0.4062
LOQ, μg mL^{-1}	1.3539
Erel.%*	– 0.3017**
RSD%*	0.3610**

*** For 10 μg mL^{-1} of MPH.**
**** Average of five determinations.**

Accuracy and precision of the proposed method

The accuracy and precision of the proposed method were tested by analyzing five replicate samples of MPH by analytical procedure. The low value of the percentage error (Erel.%) are summarized in Table (2). The percentage relative standard deviation (RSD%) was found to be low. These values indicate the high accuracy and precision of the proposed method.

Pharmaceutical applications

In order of demonstrate the applicability of the proposed method to the determination of MPH, the method was applied to the analysis of MPH in various samples of tablets.

The proposed method were successfully applied to the analysis of different tablets containing MPH and the results are summarized in Table (3). When different tablets of MPH were analyzed by the proposed method, interference from the sample matrix posed no problem. For all the tablets examined, the assay results of proposed method were in good agreement with the declared content.

Table 3: Pharmaceutical applications for MPH using the proposed method

Pharma-ceutical prepara-tion	Concn. of MPH, μg mL^{-1}		Erel., %	Rec.%	RSD,%
	Present	Found*			
Meclodin Tablets- 5	4.000	3.870	– 3.250	96.750	0.513
	12.000	12.150	+ 1.250	101.250	0.636
Meclodin Tablets-10	4.000	4.110	+ 2.750	102.750	0.871
	12.000	11.880	– 1.000	99.000	1.123
Metoclo-pramide Tablets	4.000	3.910	– 2.250	97.750	1.564
	12.000	11.950	– 0.417	99.583	1.989

***Average of five determinations.**

The results obtained by the proposed method were compared with BP method[19] [Table (4)] by applying the F-test and the t-test at 95% confidence level. The calculated values for F-test (4.435) and t-test (0.342), did not exceed the critical values of $F_{3,3} = 9.277$ and $t = 2.447$ ($n_1 + n_2 - 2 = 6$). These confirming that there are no significant differences between the proposed method and BP method with respect to precision and accuracy in the determination of MPH in tablets.

Table 4: Comparison of the proposed method with BP method for determination of pharmaceutical tablets

Pharmaceutical tablets	Rec.*,%	
	Proposed method	BP method
Pure MPH	100.000	100.000
Meclodin-5 Tablets	99.000	98.000
Meclodin-10 Tablets	100.875	99.400
Metoclopramide Tablets	98.666	99.520

***Average of five determinations.**

Conclusions

The azo dye studied in this work is stable in sodium hydroxide medium and has spectrophotometric characteristics suitable for application to spectrophotometric determination of the drug (MPH).

It can be concluded that, the present method has the advantages of high sensitivity over the official method, since the minimum quantifiable limit was taken as 1 μg ml^{-1} for MPH by the proposed method. Concerning the published UV methods, necessitate pre-treatment procedures involving extraction of the active ingredient to avoid interference from tablet excipients. However, the present method is simple as there is no need for solvent extraction or separation steps before the analysis, since no interferences were observed from tablet excipients.

In addition to these advantages, the proposed method is accurate and precise as indicated by the good recoveries of MPH and low RSD values. There is no significant difference between the proposed method and BP method with respect to precision and accuracy.

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