Spectrophotometric determination of metoclopramide hydrochloride in bulk and in pharmaceutical preparations

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Abstract

A rapid, simple, reproducible and sensitive spectrophotometric method for assay of metoclopramide hydrochloride was investigated. The method is based on the interaction of metoclopramide hydrochloride with 2,4-dinitro-1-flourobenzene reagent to give a highly coloured species with maximum absorption at 315 nm in aqueous solution in the presence of sodium hydroxide. Beer's law was obeyed in the range of 1.0-28 μg/ml with a molar absorptivity of 8.007×10^3 $l.mol^{-1}.cm^{-1}$ and the limit of detection was 0.01858 μg/ml. The accuracy (average recovery %) was 101.45 % and the relative standard deviation (RSD) was better than 1.3%. Also, it was found that the product formed was in ratio of 1:1 species with stability constant of 2.975×10^6 L/mol. The method was applied successfully to the assay of metoclopramide hydrochloride in pharmaceutical formulations and was agreed well with its certified value and with those of standard addition and British pharmacopoeia methods.

الخلاصة

تم تطوير طريقة طيفية سهلة لتقدير كميات مايكروغرامية من عقار الميتوكلوبراميد هيدروكلوريـد. تعتمد الطريقة على التفاعل بين الميتوكلوبراميد هيدروكلوريد والكاشف 4,2-ثنائي نيتـرو-1- فلـوروبنزين بوجود هيدروكسيد الصوديوم في الوسط المائي. لقد وجد أن الناتج يمتلك طيفـا امتـصاصيا لـه أقـصى امتصاص عند 315 نانوميتر. أمكن تطبيق قانون بير ضمن مدى التراكيز 1-28 مايكروغرام/مللتر في حين كانت الامتصاصية المولارية 1.5623×10^4 لتر.مول$^{-1}$سم$^{-1}$ وحد الكشف 0.01858 مـايكروغرام/مللتـر ومعدل نسبة الاسترجاع 101.45% في حين كان ألانحراف القياسي النسبي أفضل من 1.3%. كذلك وجد أن ناتج يتكون بنسبة 1:1 وأن ثابت الاستقرار 2.975×10^6 لتر.مول$^{-1}$.

Keywords: Spectrophotometry; 2,4-dinitro-1-fluorobenzene; metoclopramide
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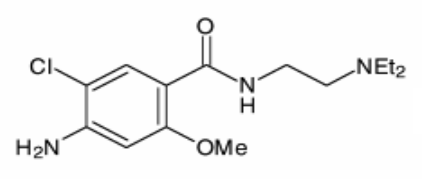
Introduction

Metoclopramide hydrochloride, [4-amino-5-chloro-N-(2-diehylaminoethyl)-2-methoxybenzamide hydrochloride] [I] 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[1], also it is used to relieve certain stomach and esophagus problems such as diabetic gastroparesis and gastroesophageal reflux disorder[2].



$HCl.H_2O$

[I] $C_{14}H_{22}ClN_3O_2.HCl.H_2O$ M.Wt. = 354.3

Many analytical techniques have been employed for the determination of metoclopramide hydrochloride. The generally used analytical techniques are fluorometric[3], ^{1}H-NMR spectroscopic[4], or chromatographic techniques[5-8]. Of course, the above mentioned techniques are sensitive but expensive. Spectrophotometry is the technique of choice even today due to its inherent simplicity.

In the literature, many spectrophotometric procedures have been applied for determination of metoclopramide hydrochloride using different reagents including phenothiazine as coupling reagent and ferric nitrate as oxidizing reagent [9], o-phenanthroline or bipyridyl in the presence of Fe III or Ce IV as oxidizing reagents [10], dibenzoyl methane [11], aniline as coupling reagent[12] and p-dimethylaminocinnamaldehyde[13] in addition to other spectrophotometric methods[14-18]. Some of these methods are time-consuming, extraction procedures or heating and require strictly controlled reaction conditions. Others are less sensitive. The British Pharmacopoeia 1998 reported a potentiometric method using perchloric acid for determination of metoclopramide hydrochloride powder and a spectrophotometric method for tablets and ampoules[19]. The potentiometric method requires about 250 mg of drug, whereas the spectrophotometric method is liable to interferences from tablet excipients, and requires pre-extraction with chloroform.[20] However; 2,4-dinitrobenzene-l-fluorobenzene (DNFB), the so called Sanger's reagent, has been used as a chromogenic reagent for the spectrophotometric determination of amino acids and primary and secondary amines [21-25], amino acid nitrogen in plasma and urine [26-27], isoniazid [28], various aminoglycoside antibiotics (gentamicin, tobramycin, amikacin) [29], phenols [30], and the enzyme amidase [31]. It has also been used in high-performance liquid chromatography for the determination of amines and aminoglycosides and in thin-layer chromatography [(32-34]. These methods are lack from selectivity. In this paper, the employment of DNFB as a

chromogenic reagent for simple, sensitive, selective, rapid, accurate, precise and an inexpensive method for determination of metoclopramide hydrochloride in bulk and pharmaceutical preparations is described.

Materials and Methods

Apparatus and reagents

Shimadzu (UV-210) Double Beam Spectrophotometer with 1.0 cm silica cells was used to measure the absorbance and graduated pipettes were employed. Analytical grade chemicals and distilled water were used. Metoclopramide hydrochloride (State Company for Drug Industries and Medical Appliances, Sammara-Iraq) standard solution (100µg/ml) was prepared in distilled water to get a stock solution, which was diluted further as required, while 1×10^{-2}M of DNFB (Sigma Co.) and 1×10^{-3}M of sodium hydroxide and hydrochloric acid were prepared in distilled water.

General procedures

To an aliquot of standard solution containing 0.25 to 7.0 ml (100µg/ml) of metoclopramide hydrochloride was transferred quantitatively into a series of 25-ml standard flasks. To this solution 0.2 ml of 1×10^{-2}M DNFB and 1ml of 1×10^{-3} M sodium hydroxide were added and the solution was heated for 20 min in a water bath adjusted at 40°C, then cooled to room temperature and diluted to the mark with distilled water. The resulting absorbance of the yellow colour was measured at 315 nm against all reagents except metoclopramide hydrochloride as a blank. A calibration curve was prepared (Fig.1). The colour reaction obeys Beer's law from 1 to 28 µg/ml of metoclopramide hydrochloride.

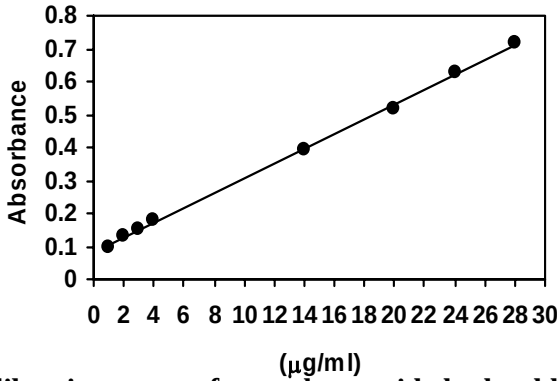


Figure 1. Calibration curve of metoclopramide hydrochloride with DNFB.

Procedure for the determination of metoclopramide hydrochloride in pharmaceutical preparations

Ten tablets (each tablet containing 10 mg metoclopramide hydrochloride) were accurately weighed and pulverized. A portion of the fine and homogenized powder equivalent to 10 mg was accurately weighed and dissolved in about 60 ml of distilled water. The solution was shaken thoroughly for about 10 min. and the residue was filtered through Whatmann no. 42 filter paper into 100 ml volumetric flask. The filtrate was diluted to the mark by repeated washing with distilled water. The filtrate was diluted to get a 100µg/ml solution of metoclopramide hydrochloride. An aliquot containing 2.5µg to 7µg/ml was taken and the procedure as described above was followed. The absorbance was measured at 315 nm. The quantity per tablet was calculated from the standard calibration curve.

For the analysis of ampoule, 2ml vial containing 10mg/2ml of metoclopramide hydrochloride was transferred into 100 ml volumetric flask and diluted up to the mark with distilled water. Working standard was prepared by suitable dilution and the general procedure was used for metoclopramide hydrochloride for its determination.

Results and Discussion

Absorption spectrum of the coloured complex

Metoclopramide hydrochloride reacts with DNFB and sodium hydroxide when heated for 20 min at 40°C to give a yellow colored product, the absorption spectrum of which under optimum condition shows a maximum at 315 nm, whereas the reagent blank gave maximum absorption at 250 nm (Fig. 2).

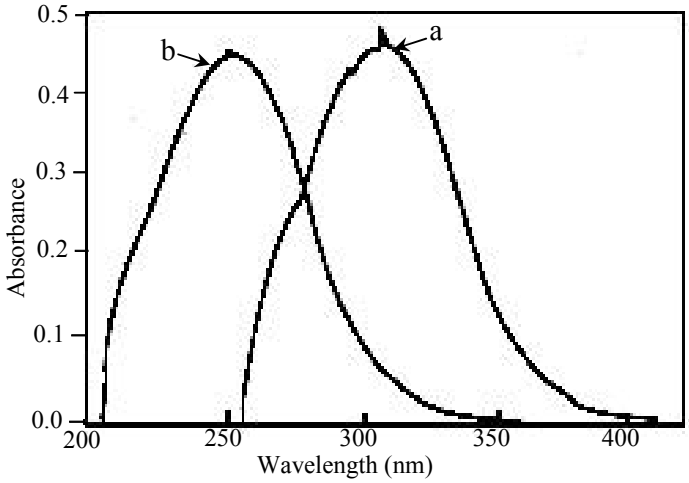


Figure 2. Absorption of spectrum of (a) metoclopramide hydrochloride (20µg/ml) with DNFB against reagent blank , and (b) reagent blank versus distilled water.

Optimization of variables

Effect of pH and buffers

The effect of pH ranged from 4 to 12 on the absorption of the product was studied using different concentrations of hydrochloric acid and sodium hydroxide. It was found that the product formed at pH of 6.9 using 1ml of 1×10^{-3}M NaOH. Therefore different buffers of pH 6.9 were prepared such as citrate, borate and phthalate to examine the sensitivity. It was found that these buffers decrease the absorbance, therefore the effect of different bases, such as sodium hydroxide, potassium hydroxide, sodium carbonate, sodium bicarbonate, sodium acetate and ammonium hydroxide with 1×10^{-3}M concentration, on the intensity of the coloured product were studied. However; it was found that 1ml of 1×10^{-3}sodium hydroxide was suitable to gave full colour development and it was used in all subsequent experiments, (Table 1).

Table 1: Effect of bases on the absorbance of metoclopramide hydrochloride-DNFB product

Type of base	Without	NaOH	KOH	Na_2CO_3	$NaHCO_3$	NH_4OH	CH_3CO_2Na
Absorbance	0.171	0.199	0.146	0.179	0.186	0.192	0.187

Effect of temperature and reaction time

Full coloured product was developed rapidly after the sequence addition of the reagents and the maximum absorbance was attained after 20 min at 40°C. The colour was stable for a period of more than 30 min after which it begun to fade.

Effect of the amount of DNFB reagent

The influence of DNFB concentration on the colour intensity was studied by measuring the absorbance at the specified wavelength in the standard procedure for solutions containing the same drug amount and sodium hydroxide concentration but varying amount of DNFB. A volume of 0.2 ml of 1×10^{-2} M in a total volume of 25 ml was found to be sufficient for full color development.

Effect of order of addition

To obtain optimum results the order of addition of reagents should be followed as given under the general procedure, otherwise a loss in colour intensity was observed.

Precision and accuracy

The accuracy and precision of the proposed method was established by measuring the content of metoclopramide hydrochloride in pure form at three different concentration levels (low, medium and high) for six replicates at 4, 14 and 24µg/ml, (Table 2). The values of relative standard deviation and mean percent recovery obtained by the proposed method can be considered to be very satisfactory.

Table 2 : Precision and accuracy of the proposed method

Amount added µg/ml	Recovery* (%)	Average recovery (%)	RSD*
4	100.0	101.45	0.824
14	102.7		1.276
24	101.66		0.741

* Average of six determinations.

Quantification and Analytical Data

The absorbances of the formed product conform with Beer's law in the concentration range 1-28 µg/ml. The molar absorptivity is 8.007×10^3 $l.mol^{-1}cm^{-1}$. The linearity was represented by the regression equation and the corresponding correlation coefficient for metoclopramide hydrochloride determined by the proposed method were shown in Table 3.

Table 3. Summary of optical characteristics and statistical data for the proposed method

Parameters	Values
λ_{max} (nm)	315
Beer's law (µg/ml)	1-28
Molar absorptivity ($l.mol^{-1}cm^{-1}$)	8.007×10^3
limit of detection(µg/ml)	0.01858
Regression equation (Y)*	
Slope (b)	0.0226
Intercept (a)	0.0816
Correlation coefficient (r)	0.9994
Relative standard Deviation (RSD%)**	1.276
Temperature(°C)	40
Development time(min.)	20
Stability period (min.)	30
Final pH	6.9

* Y=a+bC where C is the concentration of analyte (µg/ml) and Y is the absorbance unit.

** Calculated from six determinations.

Interference

The extent of interference by some excipients which often accompany pharmaceutical preparations were determined by measuring the absorbance of solutions containing 14µg/ml of metoclopramide hydrochloride and various amounts(in mg) of diverse species in final volume of 25 ml. It was found that the studied excipients do not interfere in the present method, even when present in large excess. An error of 5.0% in the absorbance readings was considered tolerable. Typical results are given in Table 4.

Table 4: Effect of excipients for assay of metoclopramide hydrochloride

excipients	Amount added (mg)	Relative error (E%)	Recovery (%)
Acacia	0.2	1.338	101.34
	2.5	-0.191	99.80
	4.0	-0.191	99.80
Glucose	0.2	2.865	102.86
	2.5	1.912	101.91
	4.0	5.736	105.7
Sodium chloride	0.2	-3.441	96.55
	2.5	2.485	102.48
	4.0	8.030	108.03
Starch	0.2	-2.672	97.32
	1.5	-2.485	97.51
	3.0	-6.500	93.50

Application

The proposed method was applied for the quality control of pure metoclopramide hydrochloride and in the pharmaceutical dosage form, as shown in Table 5. Statistical analyses

of the results using the t-test at 95% confidence level, showed that the calculated value (2.208) did not exceed the theoretical value (2.7). This demonstrated that there is no significant different between the official method[19] (analysis of tablet) and the proposed method, (Table 5).

The proposed procedure was applied for the determination of the drug in some pharmaceutical formulations by applying the standard addition technique (Fig.3). The results were compared with the official method[19], (Table 5).

Table5: Assay of metoclopramide hydrochloride drug in some pharmaceutical formulations by the proposed, standard addition and official methods

Procedure applied	Pharmaceutical preparation	Drug amount taken (µg/ml)	Recovery * (%)	Drug constant found (mg)	Average recovery (%)	Certified value (mg)
Proposed method	Tablet†	8	97.0	9.70	99.63	10
		14	98.6	9.86		
		24	103.3	10.33		
	Injection†	14	97.2	9.72	98.56	
		20	96.5	9.65		
		24	102.0	10.2		
Standard addition technique	Tablet	1	99.0	9.9	98.83	
		2	100.0	10		
		4	97.5	9.75		
	Injection	1	99.0	9.9	98.0	
		2	100.0	10		
		4	95.0	9.5		
Official method[19]	Tablet		102.0	10.02	-	

*Every reading is an average of six determinations.
†Meclodin, 10mg(provided from CID Co. Egypt).
†Plemazol, 10mg/2ml(provided from SDI Co. Iraq).

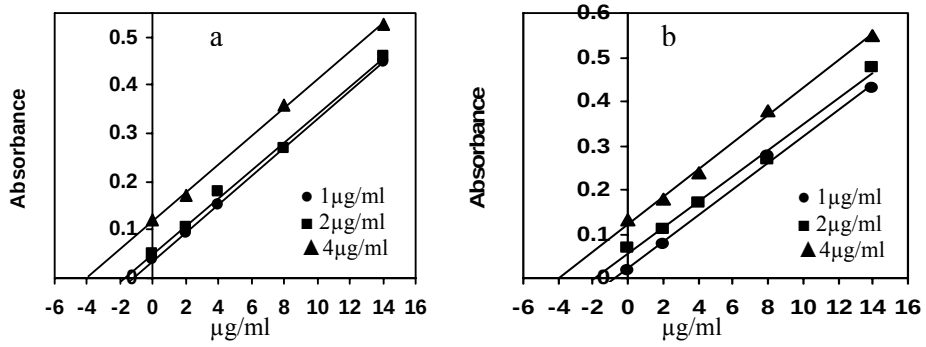


Figure 3: Standard addition technique for (a) tablet and (b) injection.

Stoichiometric Relationship

The molar ratio of the product formed between the studied drug and the reagent used was investigated applying the molar ratio[34] and continuous variation (Job's) methods[35] using equimolar solutions of the drug and reagent. The results indicated that the product was formed in the ratio of 1:1 (Fig. 4). This finding supports that the interaction of the studied drug and the reagent used takes place at only one site which was the more sterically free terminal basic aliphatic amino group.

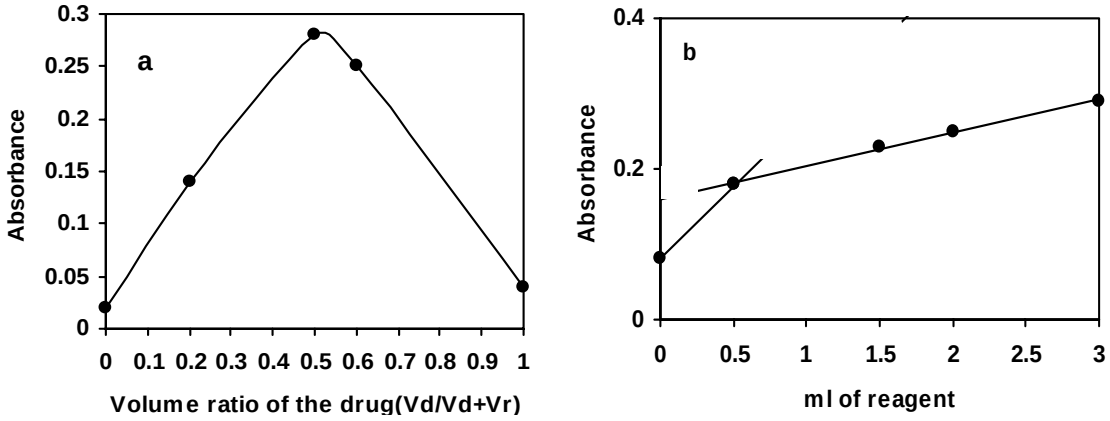
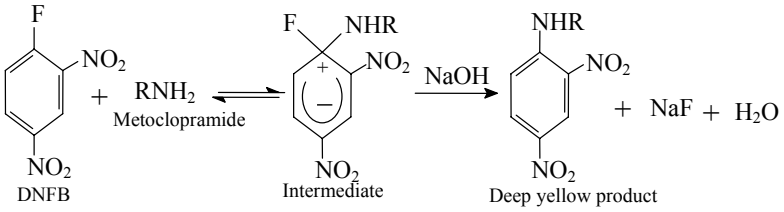
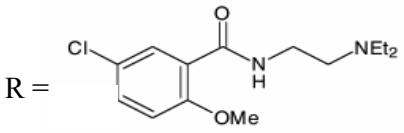


Figure 4: Continuous variation (a) and mole ratio(b) plots for the product of metoclopramide hydrochloride(0.4×10^{-3}M) with DNFBunder the optimum conditions .

Reaction mechanism:

The reaction of DNFB with drugs that own a free primary amine group results in the formation of coloured products[36,37]. This reaction was first introduced by Sanger[38] as means for determination of the DNA sequence. Based on the Job's method of continuous variation and mole ratio, it was found that metoclopramide interacted with the DNFB in ratio of 1:1. This result indicates that the reaction between the drug and the reagent used takes place at only one site which was the more sterically free terminal amino group. The reaction is typical nucleophilic substitution and proceeds through an intermediate product as follows:





Conclusion

An spectrophotometric method for the determination of metoclopramide hydrochloride was developed. The method is simple, reliable, sensitive and less time consuming. The statistical analysis is in good agreement with those of the official British Pharmacopoeia 1992. The colour reaction is selective for metoclopramide hydrochloride. The method can be successfully applied for the micro determination of

metoclopramide hydrochloride either in pure or in pharmaceutical preparations. The advantage of the present procedure is that it does not require many solvents. A significant advantage of a spectrophotometric determination is its application for the determination of individual compounds. This aspect of spectrophotometric analysis is of major interest in analytical pharmacy, since it offers a distinct possibility of quality control in the assay of pharmaceutical dosage formulations.

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