Spectrophotometric Assay of Metoclopramide Hydrochloride in Pharmaceutical Preparations via Oxidative Coupling Reaction with Promazine Hydrochloride and N-Bromosuccinimide

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الخلاصـــة

تم تطوير طريقة طيفية بسيطة وسريعة وحساسة لتقدير الميتوكلوبراميد هيدروكلوريد بهيئته النقية وفي مستحضراته الصيدلانية. اعتمدت الطريقة على تفاعل الاقتران التأكسدي العضوي للميتوكلوبراميد هيدروكلوريد مع الكاشف بروموازين هيدروكلوريد بوجود N-بروموسكسينميد في وسط حامضي لتكوين ناتج أزرق-مخضر يقاس امتصاصه عند 600 نانوميتر. وكانت حدود تطبيق قانون بير 0.4-28 مايكروغرام / مللتر بدقة وتوافق مرضيين، إذ بلغ معدل نسبة الاسترجاع 100.92% والانحراف القياسي النسبي اقل من 2.0%. بلغت الامتصاصية المولارية 14917 لتر. مول$^{-1}$. سم$^{-1}$ بحدود كشف 0.11 مايكروغرام/مللتر وتقدير كمي 0.37 مايكروغرام/مللتر. أظهرت النتائج عدم حدوث تداخل في الطريقة المطورة من قبل مواد السوّاغ بوصفها مضافات في المستحضرات الصيدلانية. طبقت الطريقة بنجاح في تقدير الميتوكلوبراميد هيدروكلوريد في المستحضرات الصيدلانية، إذ كانت النتائج متفقة مع طريقتي شركة ادوية سامراء (S.D.I.) ودستور الادوية البريطاني القياسيتين وكذلك مع المحتوى الاصيل للمستحضرات الصيدلانية.

ABSTRACT

Simple, rapid and sensitive spectrophotometric method for the determination of metoclopramide hydrochloride in a bulk sample and in dosage forms is described. The method depends on the oxidative coupling reaction of metoclopramide. HCl with promazine. HCl in the presence of N-bromosuccinimide to form a bluish-green product which absorbs at 600 nm. Beer's law is obeyed in the concentration range 0.4–28.0 μg/ml with good accuracy (average recovery) 100.92% and precision (RSD) is less than 2.0%. The molar absorptivity is 14917 $l.mol^{-1}.cm^{-1}$ with detection and quantification limits 0.11 μg/ml and 0.37 μg/ml respectively. Common excipients used as additives in pharmaceuticals do not interfere in the proposed method. The method is successfully employed for the determined of metoclopramide.HCl in pharmaceutical preparations and the results agree favourably with S.D.I. and British pharmacopoeia methods and also with certified values.

Introduction

Metoclopramide hydrochloride, [4-amino-5-chloro-N-(2-diethyl-aminoethyl)-2-methoxybenzamide hydrochloride] acts centrally by blocking dopamine D_2 receptors in the CTZ and peripherally by enhancing the action of acetylcholine at nuscarinic nerve endings in the gut. It increases gastric peristalsis while relaxing the pylorus and first part of the duodenum. Metoclopramide is effective and polar drug for many types of vomiting, postoperative, drug induced, disease associated especially migraine), radiation sickness, it is of little benefit in the treatment of motion sickness. It is also effective in vomiting induced by highly emetic anticancer drugs (1,2). Many analytical methods have been developed for the analysis of metoclopramide, based on spectrophotometric (2-5), fluorometric (6), flow-injection-chemiluminescent (7), 'H-NMR spectroscopic (8) or chromatographic techniques (9-11). The British pharmacopoeia 1998 reported a potentiometric method using 0.1M perchloric acid for the determination of metoclopramide HCl powder and a UV method for tablets and ampoules (12). The potentiometric method requires about 250mg of drug, whereas, the UV method is liable to interferences from tablet excipients, and requires pre-extraction with chloroform.

Phenothiazine and its derivatives have been successfully utilized as chromogenic agent (active coupling agents) for the spectrophotometric determination of many organic compounds and drugs including aniline and its substituents (12, 13), sulphonamide drugs (14, 15), folic acid (16), chloramphenicol (17), benzocaine (18).

The objective of this study was to develop a simple, precise and accurate spectrophotometric method for the determination of metoclopramide. HCl, either in their pure form or in tablet, injection and syrup preparations. This analytical procedure was based on the oxidative coupling of metoclopramide. HCl with promazine. HCl in the presence of N-bromosuccinimide and in acidic medium to form an intense bluish-green coloured product.

Experimental

Apparatus

All spectral and absorbance measurements were carried out on a Shimaduz UV-visible 210 digital double beam spectrophotometer with 1-cm matched quartz cells.

Reagents

All of the chemicals used were of analytical grade and all of the solutions were prepared in distilled water. Metoclopramide.HCl and promazine.HCl pure materials were provided from S.D.I. Iraq, N-bromosuccinimide and hydrochloric acid (36%) were obtained from Fluka.

Solutions

A stock standard solution of 1000 μg/ml of metoclopramide.HCl was prepared by dissolving 0.1000g of pure material in 20ml of water and completed to 100ml with distilled water in a 100ml volumetric flask. Working solutions were obtained by further dilution of the stock solution with water. A 0.5% promazine.HCl solution, 0.001M N-bromosuccinimde solution and 0.1M hydrochloric acid solution were prepared in distilled water.

Recommended procedure

Appropriate aliquots of standard metocloproamide.HCl solution containing 10-700 μg were transferred into 25ml volumetric flasks, to which 1 ml of 0.5% promazine.HCl, 1ml of 0.1M hydrochloric acid and 5ml of 0.001M N-bromosuccinimde were added and the contents were diluted to the mark with distilled water and mixed well. The absorbances were measured after 5min at 600nm against a reagent blank treated similarly. A calibration graph was constructed by plotting the measured absorbance versus drug concentration (Fig. 1). The concentration of the unknown was computed from the regression equation.

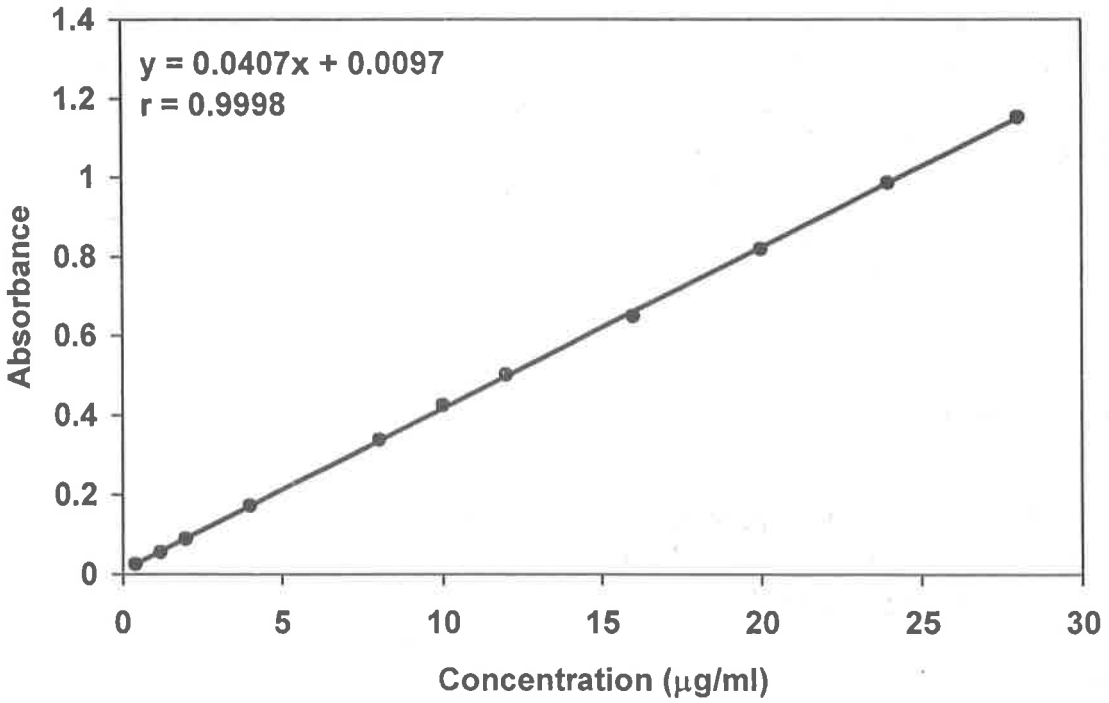


Fig. (1) Calibration graph for metoclopramide.

Procedure for dosage forms

For tablets. Twenty tablets were weighed and finely powdered. A portion equivalent to 100mg was weighed and dissolved in 100ml of water, shaken well and filtered through a sintered glass crucible (G_4). A 1.0 ml aliquot of the test solution was diluted to 100ml in a calibration flask and was treated as described above in the recommended procedure.

For injections. The contents of five ampoules were quantitatively transferred into 500 ml volumetric flask and completed to the mark with distilled water. The above-stated procedure was applied to determine the examined drug.

For Syrup. An accurately measured volume (25ml) of meclodin syrup equivalent to 25mg of drug was quantitatively transferred into 250ml volumetric flask and completed to the mark with distilled water. A suitable liquid was treated as described under the preparation of calibration graph.

Results and Discussion

Spectral characteristics

A bluish green product is formed when metoclopramide.HCl was allowed to react with promazine.HCl in the presence of N-bromosuccinimide in hydrochloric acid medium with maximum absorption of 600 nm as shown in Fig. 2.

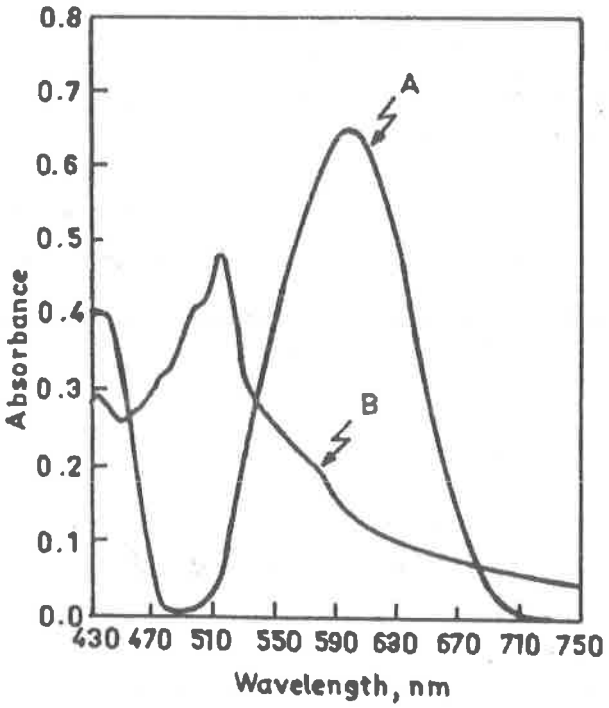


Fig. (2) Absorption spectra (A) of 16 μg/ml of metoclopramide treated as described under procedure and measuring against reagent blank and (B) the reagent blank measured against distilled water.

Optimum conditions for product formation

In order to establish the optimum conditions necessary for a rapid and quantitative formation of the coloured product with maximum stability and sensitivity, the investigators measured the absorbance of a series of solutions by varying one and fixing the other parameters at 600 nm.

Effect of pH

The effect of pH on the sensitivity of the colured reaction product was investigated in the range of 1-6 using different buffer solutions. Results showed that 0.1M hydrochloric acid (pH<1) gives clear bluish green colour with maximum intensity and stability and it was found that the optimum amount of hydrochloric acid was 1ml.

Effect of reagent and oxidant concentrations

The effect of promazine.HCl reagent was studied in the range of 0.1-2ml. A range of 0.7-1.5ml of 0.5% promazine.HCl was necessary to achieve the maximum colour intensity. Hence 1ml of the reagent was selected for further studies, as it gave excellent results. The maximum intensity of the bluish-green colour was achieved in the range of 4-6 ml of 0.001M N-bromosuceinimide , therefore, 5ml of the oxidant reagent was used.

Time, Temperature and colour stability

The reaction between metoclopramide and promazine. HCl in the presence of N-bromosuccinimide was found to be instaneous. However, the reaction is complete within 5 min at room temperature, but 10 min was sufficient to get maximum intensity and stable colour for at least 2h. The effect of temperature on the colour intensity of the product was studied. In practice, the absorbance of the sample was low at 0 °C, whereas at 45 °C a high value for the blank was obtained. Therefore, it is recommended that the colour reaction be carried out at room temperature.

Order of addition of reagents

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

Optical characteristics

The Beer's law limits, molar absorptivity, Sandell sensitivity, regression equation and correlation coefficient obtained by linear square treatment of the results are given in Table 1. The detection and quantification limits were calculated from the standard deviation of the absorbance measurements obtained from a series of 10 blank solutions (19). The limits of detection and of quantification were well below the lower limit of Beer's law range. Beer's plot at 600 nm revealed very small intercept and a perfect linearity between the absorbance and the drug concentrations over the range 0.4-28 μg/ml and negative deviation from Beer's law was observed at higher concentration of metoclopramide. The values of molar absorptivity and Sandell sensitivity indicated that the proposed method is sensitive.

Accuracy and precision

The accuracy and precision of the method were established by performing six replicate analyses on solutions containing three different amounts (with in the Beer's law limits) of the drug. The mean recovery (100.92 %) and the relative standard deviation (<2.0) can be considered to be satisfactory (Table 1).

Table 1. Optical characteristics, precision and accuracy data.

Parameter	Value
Beer's law limits, μg ml^{-1}	0.4-28
Limit of detection, μg ml^{-1}	0.11
Limit of quantification, μg ml^{-1}	0.37
Molar absorptivity, l mol^{-1} cm^{-1}	14917
Sandel sensitivity, μg cm^{-2}	0.024
Regression equation*	
Slope (a)	0.0407
Intercept (b)	0.0097
Correlation coefficient, r	0.9998
Recovery, %**	100.92
Relative standard deviation, %**	<2.0

* y = ax + b where y is the absorbance for concentration, x in μg ml^{-1}

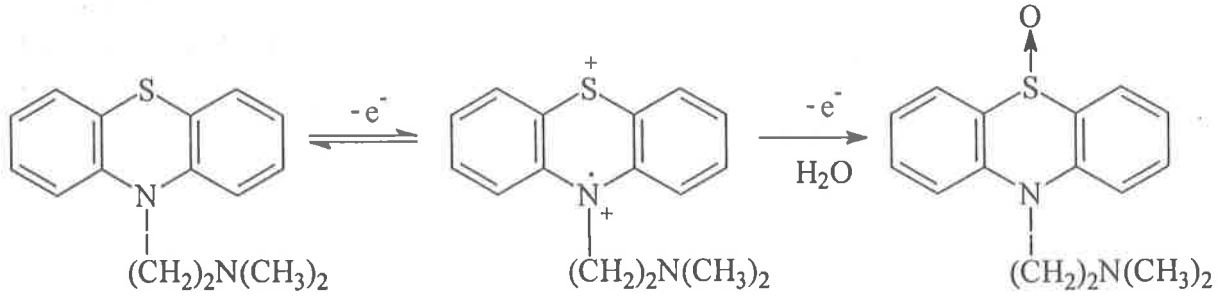
** For six replicate analyses within Beer's law limits.

Proposed reaction sequence

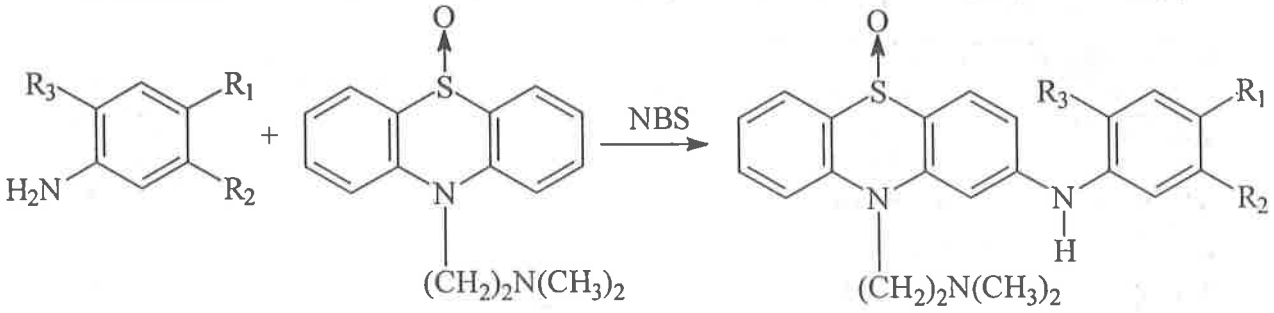
The stoichiometry of the reaction between metoclopramide and promazine.HCl was studied by Job's method of continuous variation (20). The results obtained showed the existence of a 1:1 metoclopramide: promazine.HCl reagent at 600nm. Therefore, the reaction sequence may be pestulated as follows:

Promazine hydrochloride easily undergoes oxidation, like other phenothiazines, in an acidic medium by oxidizing agents (21, 22). The oxidation reaction process is in two steps. The first, reversible, step produces a coloured cation-radical. In the second step (irreversible), the presence of excess oxidant leads to the generation of the colourless promazine sulfoxide (active coupling species), which couples with metoclopramide to give a bluish green coloured product (23) as shown in the following reaction Scheme.

Metoclopramide



Promazine



where $R_1 = -\overset{O}{\overset{\|}{C}}-NH-(CH_2)_2-N(C_2H_5)_2$, $R_2 = -OCH_3$, $R_3 = -Cl$

NBS = N-Bromosuccinimide

The average stability constant of the dye product was 7.30×10^4 $l.mol^{-1}$ which indicates a stable dye product is formed.

Study of interferences

To test the efficiency and selectivity of the proposed analytical method to pharmaceutical preparations, a systematic study under the optimum experimental conditions was made for the effect of additives and excipients (e.g. lactose, glucose, fructose, starch, magnesium stearate, talc and sodium chloride) that are usually present in dosage forms. The criterion of interference was an error of not more than ± 4.0% in the absorbance. In this study, a wide range of concentrations was used in which the determination of the 500 µg/25 ml of a drug was performed. Results showed that there was no interference from additives or excipients for the examined method up to 200-fold excess .

Analytical applications

The proposed method was successfully applied to determine metoclopramide in their pharmaceutical preparations. The obtained results were compared statistically by a Student's t-test for accuracy, and a variance ratio F-test for precision (24) with the official methods (25, 26) at the 95% confidence limits for six and three degree of freedom respectively, as recorded in Table 2. The results showed that the t- and F-values were less than the critical value, indicating that there was no significant difference between the proposed and official methods.

Table 2. Analysis of metoclopramide pharmaceuticals by the proposed and official methods

Pharmaceutical preparation	Supplier	Certified value (mg)	Proposed method		Official method
			Amount found[a] (mg)	Recovery[a] (%)	Recovery (%)
Meclodin tablets	S.D.I. Iraq	10 mg	10.32	103.19	99.80[c]
Meclopran tablets	Alexandria Co. for Pharmaceuticals Alexandria-Egypt	10 mg	10.19	101.86	100.57[c]
Meclopran injection	Alexandria Co. for Pharmaceuticals Alexandria-Egypt	10 mg/2ml	9.90	98.96	101.23[d]
Meclodin syrup	S.D.I. Iraq	100 mg	102.56	102.56	101.94[c]

[a] for three determinations of 4, 8, 16 μg/ml
[c] S.D.I. standard methods
[d] British pharmacopocia B.p.
[e] The calculated and tabulated values of t and F at the 95% confidence limit are 0.72, 4.18 and 2.45, 9.28.

Conclusion

The proposed method for the spectrophotometric determination of metoclopramide in pharmaceutical preparations is simple, rapid and sensitive. The oxidative coupled product formed is fairly soluble and quite stable. The method does not require heating or extraction and time interval for formation of coupled product and does not interfere with associated excipients and additives. Statistical analysis of the results indicates that the method has good precision and accuracy.

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