

SPECTROPHOTOMETRIC DETERMINATION OF METOCLOPRAMIDE HYDROCHLORIDE IN BULK AND PHARMACEUTICAL PREPARATIONS BY DIAZOTIZATION-COUPPLING REACTION

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ABSTRACT

Objective: Develop a simple, rapid, sensitive, selective, and accurate method for the spectrophotometric determination of metoclopramide hydrochloride (MCP-HCl) in bulk and dosage forms.

Methods: The method is based on diazotization of primary amine group of (MCP-HCl) with sodium nitrite and hydrochloric acid followed by coupling with 2,5-dimethoxyaniline (DMA) in aqueous mildly acidic medium to form a stable orange azo dye, showed a maximum absorption at 486 nm.

Results: Beer's law was obeyed over the concentration range of 0.1-12 ppm with a molar absorptivity $4.55 \times 10^4 \text{ L.mol}^{-1}.\text{cm}^{-1}$. Sandell's sensitivity, limit of detection (LOD), and limit of quantification (LOQ) are $0.008 \mu\text{g.cm}^{-2}$, 0.016 ppm, and 0.054 ppm respectively, the recoveries range 99.15-100.80%.

Conclusions: The method has been successfully applied to the determination of (MCP-HCl) in its pharmaceutical preparations tablet, syrup, injection and drop with very good recoveries.

Keywords: Spectrophotometric; Metoclopramide hydrochloride; Diazotization-coupling; 2,5-dimethoxyaniline; Pharmaceutical preparations

INTRODUCTION

Metoclopramide Hydrochloride MCP-HCl is a white or almost white, crystalline powder which is very soluble in water. Its chemical name 4-amino-5-chloro-N-[2-(diethylamino)ethyl]-2-methoxybenzamide hydrochloride. Its action and use is dopamine receptor antagonist and antiemetic [1]. It is also used in the prevention of cancer chemotherapy-induced emesis [2]. There are many pharmaceutical preparations for MCP-HCl from different companies due to its wide applications in both clinical and experimental medicine [3]. So that needed an accurate and simple methods for its quantitative determination. The British Pharmacopoeia BP [1] recommend a non aqueous acid-base titration with potentiometric detection of the end-point for the evaluation of the raw material. For dosage forms: tablet and injection recommend a chromatographic method while for oral solution recommend spectrophotometric method based on azo-coupling reaction with N-(1-naphthyl)ethylenediamine dihydrochloride. Various analytical methods have been developed for the determination of this drug. These methods include chromatographic [4,7], electrochemical [8,11], spectrophotometric [12,16], spectrofluorimetric [17,18], and flow injection analysis [19,22]. The aim of present work was to develop simple, sensitive, and selective spectrophotometric method based on diazotization-coupling reaction with the new reagent 2,5-dimethoxyaniline (DMA) for the determination of MCP-HCl in bulk as well as pharmaceutical formulations.

MATERIALS AND METHODS

Instruments

All spectral and absorbance measurements were carried out on a double-beam UV-Visible spectrophotometer (Shimadzu, Japan) model UV-1650 PC with quartz cell of 1 cm path length, which connected to computer have the software UV-Prob version 2.21.

Reagents

All chemicals used were of analytical reagent grade purity. Standard reference metoclopramide hydrochloride was obtained from (State Company for Drug Industries and Medical Appliance, SDI, Samara, Iraq). Pharmaceutical preparations containing MCP-HCl obtained from the commercial market.

Solutions

All aqueous solution were prepared using deionized water. MCP-HCl standard aqueous solution 250 ppm. Sodium nitrite (BDH)

aqueous solution 0.01M. Hydrochloric acid (BDH) aqueous solution 1M. Sulfamic acid (BDH) aqueous solution 0.2 M. 2,5-dimethoxyaniline (Sigma-Aldrich) 0.01 M prepared using absolute ethanol (GCC). Dosage form aqueous solutions 100 ppm.

Recommended Procedure and Calibration Graph

Transfer increasing volumes of working MCP-HCl solution, covering the range 2.5-300 μg (0.1-12 ppm), into a series of 25-mL volumetric flasks. Add 0.1 mL of 1M HCl and the mixtures are shaken. Then 1 mL of 0.01 M sodium nitrite solution is added and the mixtures are allowed to stand for 2 minutes. Then 2 mL of 0.2 M sulphamic acid solution is added and the mixtures allowed to stand for 2 minutes. After that 3 mL of 0.01 M (DMA) was added and the volumes completed to the mark with deionized water. After 10 minutes measure the absorbance against a reagent blank, prepared in the same manner but containing no MCP-HCl at 486 nm using 1-cm cells.

Procedure for dosage forms

Tablet: Five tablets (5mg and 10 mg MCP-HCl/tablet) were weighed and finely powdered. A portion of the powder equivalent to 10mg of the drug was weighed and dissolved in deionized water then transferred into 100 mL volumetric flask and completed to the mark with the same solvent. The solution shaken well, filtered and an aliquot of the filtered drug solution was then treated as done in a recommended procedure.

Syrups: The content of two containers of MCP-HCl syrup (5mg MCP-HCl/5mL) was mixed well and 10 mL of the syrup mixture was quantitatively transferred into 100 mL volumetric flask and completed to the mark with deionized water. An aliquot of this solution was then treated as done in recommended procedure.

Oral drop: The content of three containers of MCP-HCl oral drops (4 mg MCP-HCl/mL) was mixed well. An accurate volume 2.5 mL of MCP drops (4mg MCP-HCl/mL) was transferred into a 100 mL volumetric flask and completed to the mark with deionized water, then it was proceeded as described under recommended procedure.

Injection: The contents of ten injection tubes (2mL/10mg) mixed well. An aliquot containing 2mL was measured accurately and transferred to a 100 mL volumetric flask and completed to the mark with deionized water. An aliquot of this solution was then treated as done in recommended procedure.

RESULTS AND DISCUSSION

Chemistry

Azo dye derivatization are the most widely applied reaction for the chemical derivatization of drugs. In this method, the drug contain a free primary amino group will diazotated and coupled with a coupling component contain a powerful electron-releasing group, generally -OH, -NR₂, or -NH₂. Typically, coupling with phenols is carried out in mildly alkaline solution, and with amines in mildly acidic solution [23,24]. These group not affected on the rate of reaction only, but act as an auxochrome which enhance the spectral properties. Among the auxochrome groups, its found that amino group and its derivatives exert a grater effect than hydroxyl group [25].

Choice of coupling reagent

A critical study of several coupling reagents, most of which have not previously been used, was made. The reagents tested are: m-hydroxy benzoic acid, 2,4-dimethyl phenol, m-amino phenol, 2,5-dimethoxyaniline, 1,2-phenylenediamine, 1,3-phenylenediamine, and o-Tolidine. Useful analytical results obtained by 2,5-dimethoxyaniline. This reagent gave a stable water-soluble azo dye with MCP-HCl. High intensity of the azo dye formed with a good color contrast and relatively rapid coupling rates. Therefore, this reagent was selected and the optimum conditions of its reaction with MCP-HCl was further studied.

Spectral characteristics

Absorption spectrum of the orange azo product with maximum absorption at 486 nm is shown in Fig.1. The reagent blank has practically negligible absorption at this wavelength. Hence, all measurements were made at this wavelength against reagent blank.

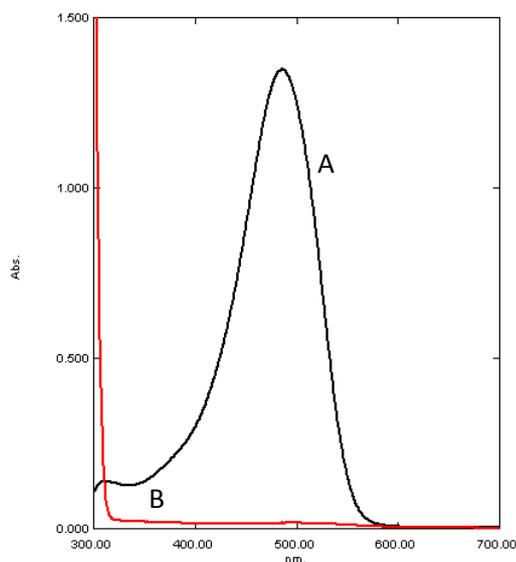


Fig. 1: Absorption spectra of A: (10 ppm) of MCP-HCl treated as described under procedure and measured against DMA reagent blank and B: the DMA reagent blank measured against distilled water.

Optimization of reaction conditions

The effects of the various parameters on the absorption intensity of the azo dye were studied and the reaction conditions are optimized.

Effect of acid: Different amounts (0.05-5 mL of 1M) of different acids (have been examined. A 0.1mL of 1M HCl give the best results.

Effect of Sodium Nitrite Concentration and Time: The effect of sodium nitrite concentration was tested by using different volumes (0.1-3 mL) of 0.01 M NaNO₂ solution. It was found that the addition of 1 mL of NaNO₂ solution was required with 2 minute reaction time to obtain a maximum absorbance.

Effect of Sulfamic acid Concentration and Time: The excess of nitrous acid is removed by the addition of sulfamic acid solution. The effect of its concentration was tested by using different volumes (0.1-4 mL) of 0.2 M sulfamic acid solution. It was found that the addition of 2 mL of sulfamic acid solution was required with 2 minute reaction time to obtain a maximum absorbance.

Effect of Reagent Concentration: The effect of reagent concentration was tested by using different volumes (1-5 mL) of 0.01 M 2,5-dimethoxyaniline solution. The results showed that 3 mL of reagent is sufficient for production of maximum and reproducible color intensity.

Effect of Time: The effect of time on the formation of the azo product was investigated by allowing the reaction to proceed for varying times. The results showed that the azo-dye reached maximum absorbance after 10 minutes and remains stable at least for 120 minutes.

Effect of Temperature: The effect of temperature on the absorption was investigated at different temperatures (1-80° C). The results revealed that the absorbance relatively stable in the temperature range (1-40° C). At higher temperatures, the absorbance value decreased, which was probably due to the dissociation of azo-dye.

Calibration Curve and Sensitivity

Under optimum conditions studied above, standard calibration curves for MCP-HCl were constructed Fig.2, and different parameters of the analytical performance of the proposed method are summarized in Table 1

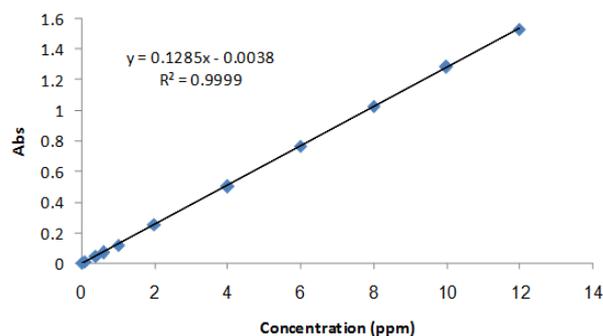


Fig. 2: Calibration graph for MCP-HCl determination using DMA as coupling reagent

Nature and stability constant of the dye product

The stoichiometric ratios were determined using Job's method of continuous variation [26]. The results obtained show a 1:1 drug to reagent product was formed Fig.3. The formation of dye may probably occur as given in scheme.1. The stability constants of the dye product are calculated using equation cited in [27]. The result show that the stability constant is $6.58 \times 10^9 \text{ M}^{-1}$, which indicate a stable dye products are formed through the reaction of MCP-HCl drug with DMA.

Table 1: Analytical features of the procedure developed for the determination of MCP-HCl

Parameter	Proposed Method
Regression equation	Y= 0.1285 X - 0.0038
Slope	0.1285
Correlation coefficient	0.9999
Linear Range (ppm)	0.1 - 12
Molar absorptivity (L.mol ⁻¹ .cm ⁻¹)	4.55×10^4
Limit of detection (LOD) (ppm)	0.016
Limit of quantification (LOQ) (ppm)	0.054
Sandell's sensitivity, S (μg cm ⁻²)	0.008
Reproducibility (%)	0.49
Recovery (%)	99.82

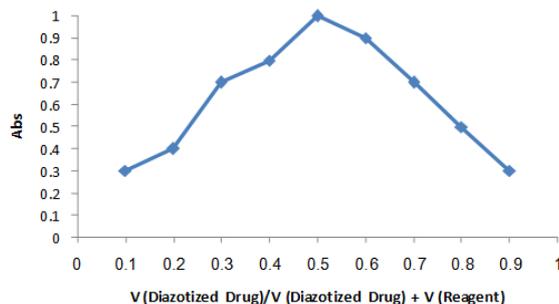
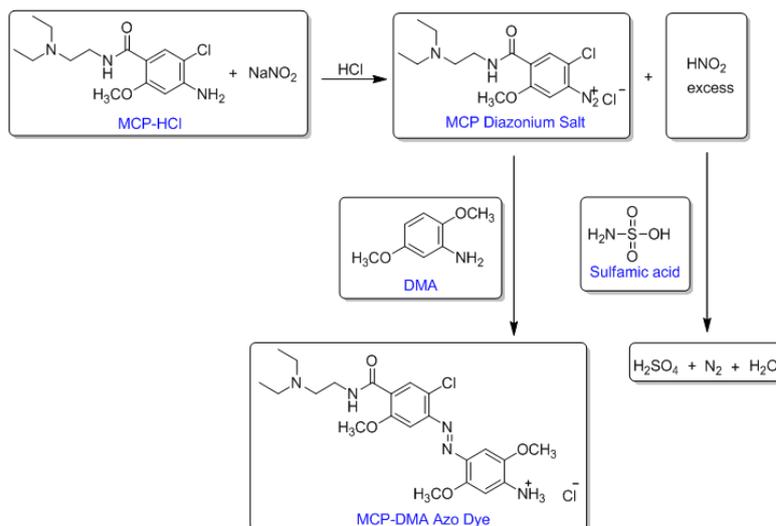


Fig. 3: Study of the mole ratio of the reaction between MCP-HCl and DMA.



Scheme 1: Proposed mechanism of the reaction between MCP-HCl and DMA.

Interference study

The extent of interference by common excipients frequently found with MCP-HCl drug in pharmaceutical formulations were determined. These excipients include Tween 80, PVP, acacia, merritol, lactose, sodium chloride, sucrose, benzoic acid, aspartate, microcrystalline cellulose, talc, starch, and magnesium stearate. The study done by measuring the absorption of a synthetic sample solutions containing 8 ppm of MCP-HCl and excess amounts (10-fold excess) of each excipient solution. An error of 5.0% in the absorbance readings was considered tolerable; none of these substances interfered seriously.

Pharmaceutical applications

The proposed method was applied to analysis four different dosage forms containing MCP-HCl in order to evaluate the analytical usefulness of the spectrophotometric method. The proposed methods were applied successfully to the analysis. Good results with good recoveries and reproducibility's were obtained based on three determinations for three different concentrations of each pharmaceutical preparation Table.2. Finally, statistical analysis [28], F- and t-test, show that there is no significant difference in accuracy between the proposed method and the official BP [29] method Table.3.

Table 2: Application of the proposed method to the determination of MCP-HCl in some dosage forms.

Preparation	Concentration taken (ppm)	Concentration found (ppm)	Recovery (%)	RSD ^a (%)
MECLODIN Tablets 5 mg	2	1.98	98.75	0.33
	6	6.02	100.36	0.15
	10	9.84	98.35	0.14
Metoclopramide Tablets 10mg	2	2.03	101.48	0.84
	6	5.91	98.55	0.12
	10	9.91	99.13	0.37
MECLODINE Oral Drops 4 mg/mL	2	1.99	99.92	0.47
	6	6.10	101.66	0.65
	10	9.90	98.74	1.33
METOCAL INJECTION 10mg/2mL	2	2.01	100.70	0.64
	6	6.10	101.01	0.21
	10	10.10	100.68	0.21
Primperan Injection 10mg/2mL	2	2.01	100.31	0.72
	6	5.99	99.84	0.39
	10	9.97	99.67	0.14
CLOPRAM Syrup 5mL/5mg	2	1.95	97.59	0.74
	6	5.88	98.03	0.47
	10	10.22	102.24	0.29

a. For three determinations.

Table 3: Application of the proposed and official methods to the determination of MCP-HCl in pure and some dosage forms.

Preparation	Proposed method		Official method
	Recovery ^a (%)	RSD ^a %	Recovery ^a (%)
Metoclopramide HCl Pure	99.71	0.7	99.90
MECLODIN Tablets 5 mg	99.15	0.2	100.75
Metoclopramide Tablets 10mg	99.72	0.44	100.26
MECLODINE Oral Drops 4 mg/mL	100.12	0.81	100.25
METOCAL INJECTION 0mg/2mL	100.80	0.35	100.50
Primperan Injection 10mg/2mL	99.94	0.41	100.75
CLOPRAM Syrup 5mL/5mg	99.30	0.5	99.89

Unpaired t test = (2.053) F test= (2.341)

The value of t (tabulated) at 95% confidence level and for 12 degrees of freedom, two tailed is 2.18.

The value of F (tabulated) at 95% confidence level and for 6, 6 degrees of freedom, two tailed is 5.82.

a For three determinations.

Comparison of Methods

Table 4 shows the characteristics of spectrophotometric methods based on diazotization-coupling reaction used for the determination of MCP-HCl. We found that reagents (coupler)

with amino group are more sensitive than hydroxyl group (comparison between phenol and aniline). Also, the presence of methoxy group enhance the sensitivity. This is very clear when we compare aniline with our proposed reagent 2,5-dimethoxyaniline.

Table 4: Characteristics of methods for the determination of MCP-HCl based on diazotization-coupling reaction

Reagent	λ_{max} /nm	$\epsilon \times 10^4$	Linear range/ppm	Remarks	Ref.
Aniline	410	3.53	0.5-12.0	B. M. heating	[30]
8-hydroxyquinoline	528	3.10	0.2-12	B. M.	[31]
2,4-dihydroxyacetophenone	450	2.48	0.4-12	B. M.	[32]
1-naphthol	550	3.49	0.4 - 18	B. M.	[33]
Phenol	463	2.42	1 - 20	B. M.	[34]
Phloroglucinol	424	4.30	0.2-16	B. M.	[35]
Benzoylacetone	411	2.97	-	B. M.	[36]
Imipramine hydrochloride	570	4.50	0.5-5	6 M HCl	[37]
2-naphthol	553	2.74	1-10	B. M.	[38]
Dibenzoyl methane	440	2.85	-	B. M.	[39]
Citrazinic acid	465	1.92	-	B. M.	[40]
2,5-dimethoxyaniline	486	4.55	0.1-12	Mildly acid medium	This work

ϵ = Molar absorptivity/L.mol⁻¹.cm⁻¹
B. M. = Basic Medium

CONCLUSION

The proposed method is found to be simple, rapid, selective and highly sensitive than most of the spectrophotometric methods available in literature. The statistical parameters and the recovery study data clearly indicate the reproducibility and accuracy of the method. Thus the method can be adopted as an excellent spectrophotometric method.

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