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SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF METOCLOPRAMIDE IN PHARMACEUTICAL FORMS

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A UV-Vis spectrophotometry method was developed for the determination of metoclopramide hydrochloride in pure and several pharmaceutical preparations, such as Permosan tablets, Meclodin syrups, and Plasil ampoules. The method is based on the diazotization reaction of metoclopramide hydrochloride with sodium nitrate and hydrochloric acid to yield the diazonium salt, which is then reacted with 3,5-dimethyl phenol in the presence of sodium hydroxide to form a yellow azo dye. Calibration curves were linear in the range from 0.3 to 6.5 μg/mL, with a correlation coefficient of 0.9993. The limits of detection and quantification were determined and found to be 0.18 and 0.61 μg/mL, respectively. Accuracy and precision were also determined by calculating the relative error, relative standard deviation, and recoveries. No interference was found from additive substances in pharmaceutical preparations. The proposed method has been successfully applied to determine metoclopramide hydrochloride concentrations in different pharmaceutical formulation samples.

Keywords: *metoclopramide hydrochloride, diazotization, pharmaceutical preparations, spectrophotometry.*

Introduction. Antibiotics play a vital role in the treatment of many infectious diseases that can be a major cause of human death, such as tuberculosis, gonorrhea, and urinary tract infection [1]. They are a potent drugs class and have been extensively used for killing or reducing the growth of bacteria [2–4]. However, antibiotics are a growing public health concern worldwide due to their side effects and indiscriminate prescription [5]. Antibiotics have several different modes of action, including inhibition of nucleic acid and peptidoglycan synthesis, which has a negative impact on cell replication and ultimately leads to cell death. There is a growing awareness of drug-resistant microbes, and together with the lack of development of new antibiotics the future health implications may present difficulties [6]. 4-Amino-5-chloro-N-[2-(diethylamino)ethyl]-2-methoxybenzamide (metoclopramide) is mostly used to treat a wide range of functional gastrointestinal disorders [7]. It can also be used for the treatment of nausea and vomiting, and the prevention of cancer chemotherapy-induced emesis [8].

A wide range of analytical methods have been used for the determination of metoclopramide hydrochloride (MCP) in biological and pharmaceutical forms, such as high-performance liquid chromatography (HPLC) [9, 10], spectrophotometric [8, 11–16], and spectrofluorimetric [17]. In the study by Kahali et al., RP-HPLC has been used to determine MCP after extraction from the solid dispersion [18]. Determination of MCP in different pharmaceutical preparation was successfully carried out by Al-Shirifi et al. using UV-Vis [19]. Several chromatographic methods have been combined with mass spectrometry for quantitative analysis of MCP in plasma, such as liquid chromatography–mass spectrometry (LC–MS) [20] and hydrophilic interaction chromatography–tandem mass spectrometry (HILIC–MS/MS) [21]. Al-Haideri et al. have prepared selective membrane electrodes to determine MCP in pure forms and pharmaceutical preparations using the direct and standard addition methods [22].

The yields in this study were higher compared to those of other studies. Most studies have only focused on the sensitivity of the proposed method and used one sample type (tablets) without further investigating or optimizing

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the methodologies, such as the effect of the order of addition of reactants and the stability of the colored complex. The effect of contaminant interference on the absorbance of the complex formation has also not been investigated previous [12, 13, 15].

The specific aim of this study was to demonstrate a simple, fast, and cost-effective analytical method that can be used for the determination of metoclopramide hydrochloride in different pharmaceutical forms. The UV-Vis method presented is versatile, stable, and highly sensitive in comparison with those obtained by other few studies [12, 15, 16].

Materials and Methods. *Chemicals and reagents.* All chemicals were used without any further purification, and all solutions were prepared by distilled water. Metoclopramide hydrochloride, magnesium stearate, glucose, lactose, sucrose, acatia, cellulose, and meclodin syrup were supplied by State Company for Drugs Industry and Medical Appliances (S.D.I.), Samarra, Iraq. Hydrochloric acid and sodium hydroxide were obtained from British Drug Houses (BDH). Sodium nitrate and 3,5-dimethylphenol were provided through Sigma Aldrich, USA. Permosan tablets and plasil ampoules were supplied by the United Arab Emirates (U.A.E.). Solutions of 1 M hydrochloric acid and sodium hydroxide were prepared. 3×10^{-4} and 1.88×10^{-3} M solutions of sodium nitrate and 3,5-dimethylphenol were prepared, respectively.

Preparation of standard solutions. A stock solution of MCP was prepared by dissolving 0.01 g in 100 mL of distilled water. The correlation between the absorbance and the concentrations of standard mixture solutions was determined for the concentration range from 0.3 to 6.5 μg/mL.

General procedure. A wide range of MCP concentrations was prepared from a stock solution and were used to prepare the controls and the calibration. All starting materials of the diazotization reaction were placed in the flasks in the following order: 3×10^{-4} M of sodium nitrate (0.6 mL) + 1 M of hydrochloric acid (0.5 mL) + 1.88×10^{-3} M of 3,5-dimethylphenol (1.2 mL) + 1 M of sodium hydroxide (0.5 mL). The absorbance of all solutions was measured at λ_{max} = 435 nm. The limit of detection (LOD) and limit of quantification (LOQ) were determined and calculated by considering the standard deviation to the slope:

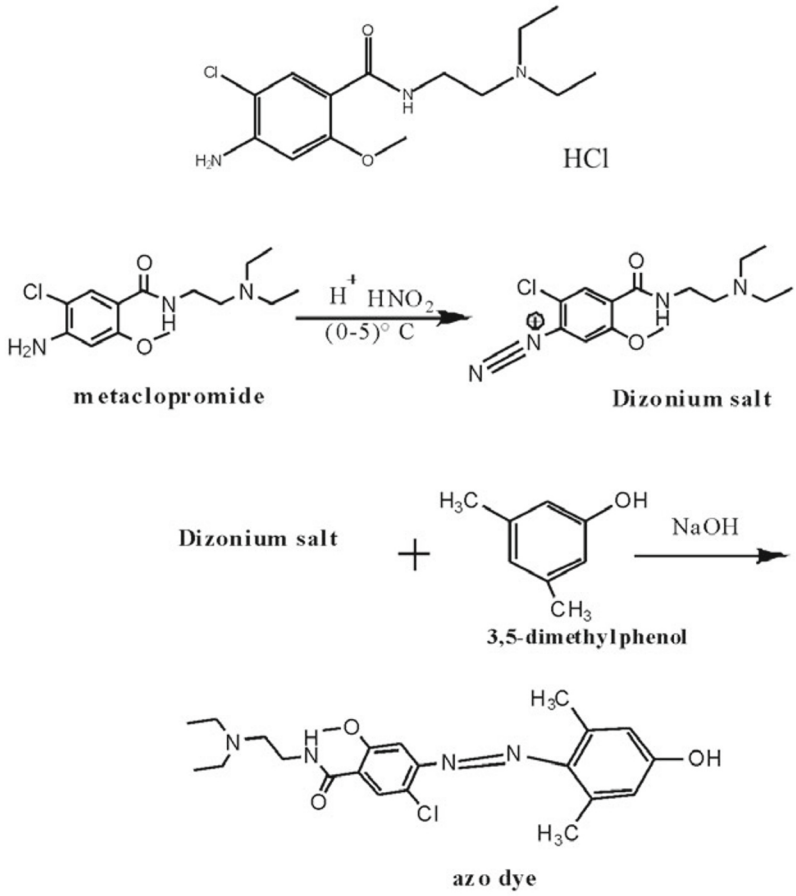
$$\text{LOD} = (\text{STEYX/Slope}) \times 3, \quad \text{LOQ} = (\text{STEYX/Slope}) \times 10 .$$

Apparatus. A double-beam spectrophotometer UV-Vis instrument was used for all measurements (Kyoto-Shimadzu Model 1800, Japan). The absorbance was recorded for all samples and blank backgrounds using a 1-mm quartz cell measuring 200–800 nm across.

Procedure for the determination of MCP in pharmaceutical preparations. Stock solutions of two MCP containing pharmaceuticals were prepared. Permosan tablets were powdered and dissolved in 100 mL of distilled water to prepare 100 μg/mL solutions. Each 0.12 g of tablet contained 10 mg of the active drug (MCP). Each 10 mL of the tablet slurry contained 10 mg of the active MCP; 100 μg/mL solutions of both Meclodin syrup and Plasil ampoule were prepared. Each 10 mL of syrup and 2 mL of ampoule contained 10 mg of active MCP. Three different concentrations (2, 3, and 4 μg/mL) of each was prepared from the stock solutions above and added to the optimal conditions of the parameters mentioned before (0.6 mL $NaNO_2$ + 0.5 mL HCl + 1.2 mL 3,5-dimethylphenol + 0.5 mL NaOH). Then each of these was measured three times.

Procedure for standard additions. Stock solutions (100 μg/mL) of each sample of the Permosan tablet, Meclodin syrup, and Plasil ampoule were prepared; 0.1 mL of each sample was added to seven 10-mL volumetric flasks. After that, different volumes of 0, 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3 mL were taken from 100 μg/mL of the standard solutions of pure MCP and added to each flask. Finally, the absorbance of all samples was measured at 435 nm to find the unknown concentration of MCP.

Results and Discussion. Various parameters were studied to optimize the method by maximizing the spectral absorbance for the azo MCP complex. The reaction conditions of interest were the effect of the sodium nitrate volume, sodium hydroxide volume, hydrochloric acid volume, 3,5-dimethylphenol volume, cooling time, and order of addition. Comparisons were also made to understand the effect of the type of acid and base on the lambda max of the complex formed. Further experiments were carried out to check the impact of the order of addition of reactants on the color development. The chemical structure of metoclopramide hydrochloride are follow and the schematic of the chemical reaction used for the formation of azo dye:



To identify the optimal acid to analyze the reaction product, the following acids were tested: HCl, H_2SO_4, CH_3COOH, and HNO_3. Similarly, the following bases were tested: KOH, NaOH, and NH_4OH. HCl and NaOH (both 1 M) were found to be the most suitable for this purpose (Fig. 1a,b).

To investigate the effect of acid and base volumes on the diazotization process, ranges of HCl volumes 0.1–2.0 mL and NaOH 0.2–2.0 mL were measured. The results showed that using 0.5 mL of both HCl and NaOH can give higher absorbance (Fig. 1c,d).

Different volumes (0.3–0.6 mL) of $NaNO_2$ solution were used in this study to increase the sensitivity. The volume of 0.6 mL was found to give the highest absorbance of sodium nitrate (Fig. 1e).

The effect of the 3,5-dimethylphenol volume on the absorbance was studied by using different volumes of 0.1–2.0 mL. There was a rise in the absorbance of 3,5-dimethylphenol linearly over the first 1 mL, and then it settled at a constant value up to 2 mL (Fig. 1f). Therefore, 1.2 mL was selected as the optimal volume for the high absorbance of the compound.

Changes in the absorbance of the reaction product with cooling time were also studied. There were significant differences observed between the absorbance of the three cooling times tested. They showed that 10 min was the optimal time for cooling the reaction, which must be stopped at that time to obtain the maximum absorbance value (Fig. 1g).

The stability of color complex was studied by measuring the absorbance of the azo product. As shown in Fig. 1h, the color product settles at a constant value for up to 2 h.

The order of addition of reactants was investigated to study the influence of the sequence of addition on the color development. The results suggest that the order metoclopramide + $NaNO_2$ + HCl + 3,5-dimethylphenol + NaOH is better than using other orders (Table 1).

The λ_{max} of metoclopramide hydrochloride solutions (2, 4, and 5.5 µg/mL) after diazotization and coupling with dimethylphenol was determined using UV-Vis spectrophotometry (Fig. 2).

A positive linear correlation was found between the absorbance and the concentration of metoclopramide hydrochloride in the range from 0.3 to 6.5 µg/mL using the optimal reaction conditions of materials (Fig. 3). The correlation

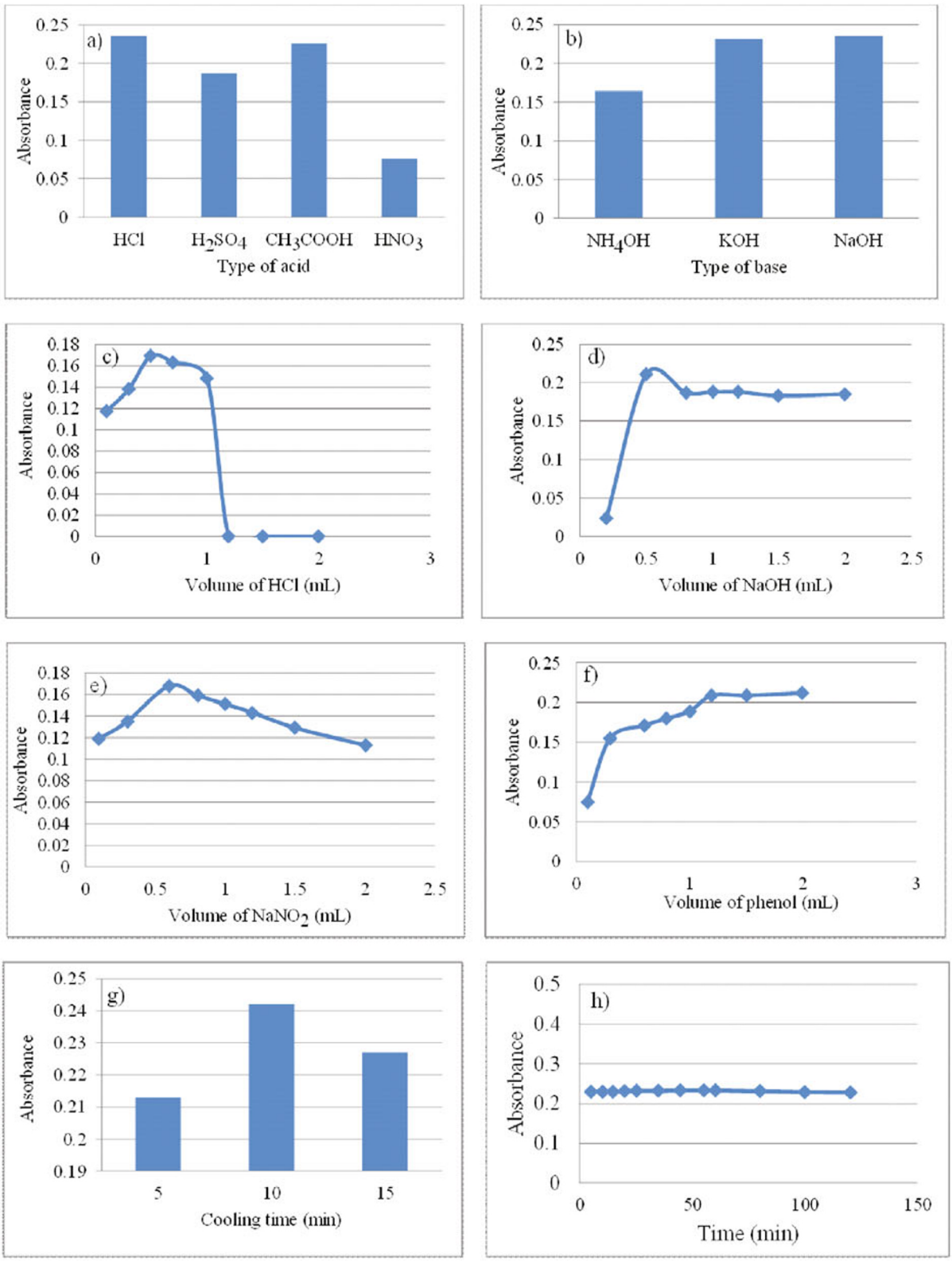


Fig. 1. Optimization of the reaction conditions: a) type of acid, b) type of base, c) V of HCl, d) V of NaOH, e) V of $NaNO_2$, f) V of phenol, g) cooling time, and h) stability.

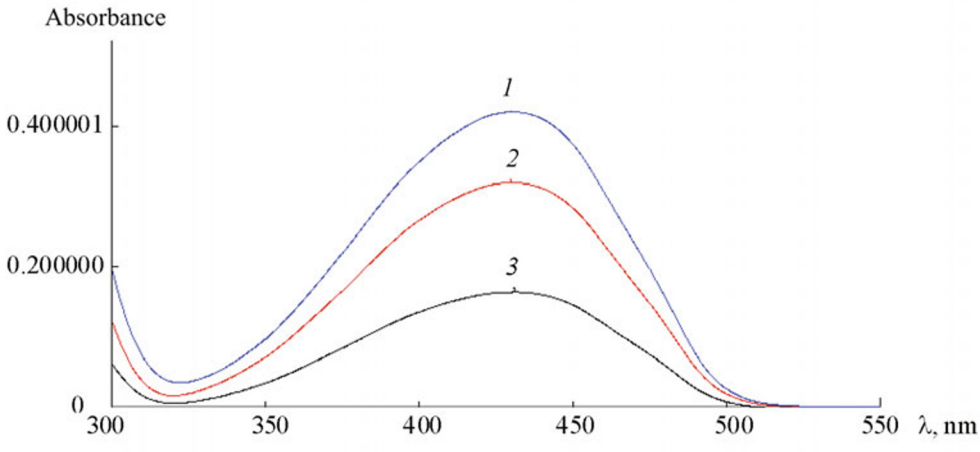


Fig. 2. UV-Vis spectra of the metoclopramide hydrochloride solution 5.5 (1), 4.0 (2), and 2.0 μg/mL (3) after diazotization and coupling with dimethylphenol.

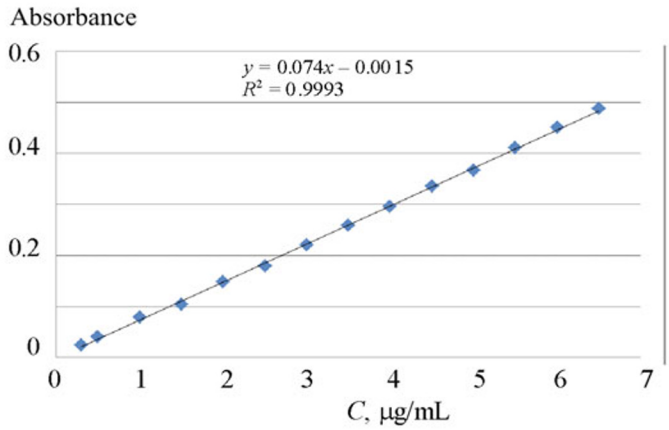


Fig. 3. Calibration curve of metoclopramide hydrochloride using 0.5 mL HCl, 0.6 mL $NaNO_2$, 1.2 mL 3,5-dimethylphenol, and 0.5 mL NaOH.

TABLE 1. Order Effect of Reactants on the Absorbance of the Azo-Dye Product

Order of addition					Absorbance, a.u.
MCP	HCl	$NaNO_2$	Phenol	NaOH	0.231
MCP	$NaNO_2$	HCl	Phenol	NaOH	0.237
$NaNO_2$	HCl	MCP	Phenol	NaOH	0.233

coefficient (R^2) value of the drug was found to be 0.9993. The LOD and LOQ were determined and found to be 0.18 and 0.61 μg/mL, respectively.

To investigate the accuracy and precision of the method, different concentrations of the pure drug were taken using the optimal conditions as presented in Table 2. The data indicate that the proposed method is suitable for the determination of MCP in pharmaceutical sample forms.

The continuous variation test was used to analyze the stoichiometry of the product. Volumes ranging from 0.1 to 0.9 mL of MCP (V_D) were mixed within the range 0.9–0.1 mL of 3,5-dimethylphenol (V_R) (both 3.3×10^{-4} M). It is apparent from the data presented in Figure 4 that the ratio between the volume of MCP and the volume of 3,5-dimethylphenol is 1:1.

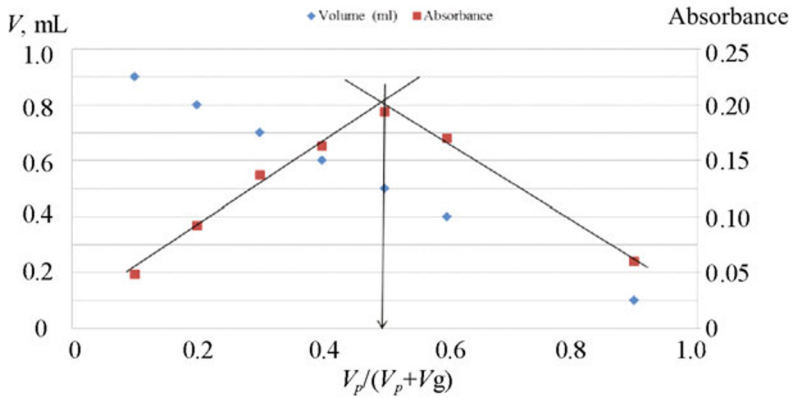


Fig. 4. The continuous variation plot of the complex.

TABLE 2. Statistical Analysis for the Determination of MCP (R.E. is the relative error and R.S.D. is the relative standard deviation)

MCP taken, μg/mL	MCP measured, μg/mL	% R.E.	% Recovery	% R.S.D. $n = 5$
2	1.899	–5.00	94.90	0.98
3	2.974	–0.87	99.13	0.90
4	3.96	–0.95	99.00	0.89

TABLE 3. Effect of Interferences on the Determination of 100 and 300 μg/mL of MCP

Interferences	Measured concentration in 100 μg/mL	% Recovery	Measured concentration in 300 μg/mL	% Recovery
Magnesium strearate	2.977	99.23	2.884	96.13
Glucose	2.924	97.46	2.844	94.80
Lactose	3.003	100.10	2.924	97.46
Sucrose	2.964	98.80	3.003	100.10
Acatia	2.924	97.46	2.924	97.46
Cellulose	2.924	97.46	2.870	95.66

Effect of interferences. The effect of interferences on the absorbance value of the reaction product was studied. This is achieved by adding some of the substances such as magnesium, glucose, lactose, sucrose, acatia, and cellulose to the sample solution containing 3 μg/mL of the drug. Different concentrations of interference solutions (100 and 300 μg/mL) were used to determine 3 μg/mL of MCP drug in the prepared solution. The results did not show any significant interference from these substances on the proposed method (see Table 3). However, the results showed some changes that happened in the absorbance of interferences and concentration of the MCP drug. It can be seen from Fig. 5 that the absorbance of interferences, concentration, and recovery of the MCP drug increased when a solution of 100 μg/mL was used compared to the 300 μg/mL one.

Applications of the proposed method in some pharmaceutical preparations. A spectrophotometric method for analysis of pharmaceutical preparations using UV-Vis has been developed in this study. This method determined the

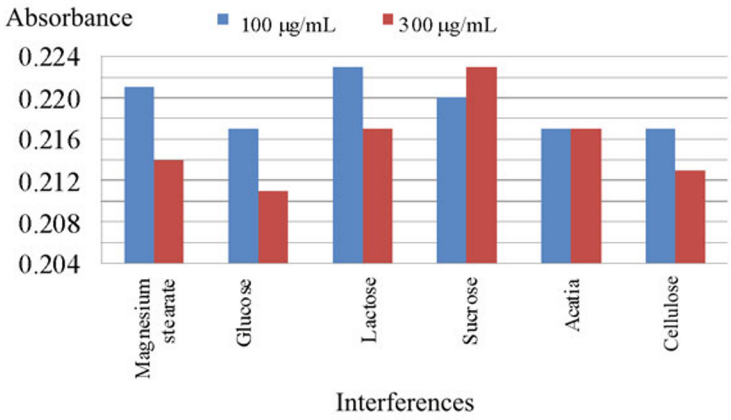


Fig. 5. Effect of the concentration of the interference solution on the absorbance value.

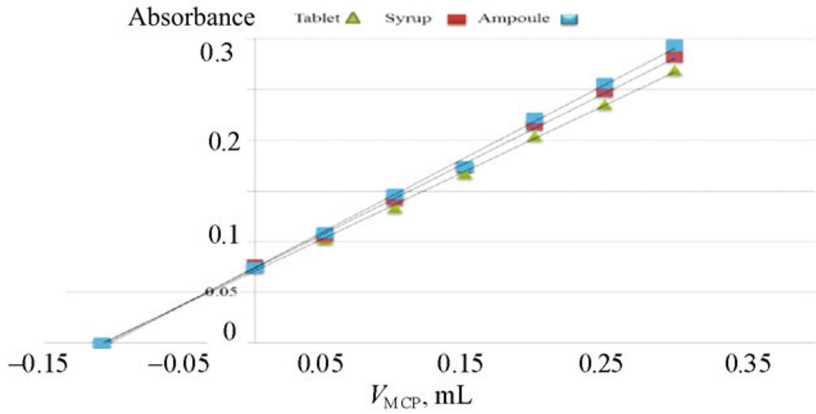


Fig. 6. Effect of standard additions of pure MCP on the absorbance of 1 µg/mL of Permosan tablet, Meclodin syrup, and Plasil ampoule samples.

TABLE 4. UV-Vis Spectrophotometric Determination of the MCP Drug in Pharmaceutical Samples

Sample	Taken, µg/mL	Measured, µg/mL	Recovery % ± S.D. Proposed method	% Recovery Standard method
Permosan (tablets)	2	1.904	95.20 ± 0.007	100.75
	3	2.897	96.56 ± 0.013	
	4	3.807	95.17 ± 0.033	
Meclodin (syrups)	2	1.933	96.65 ± 0.010	100.25
	3	2.870	96.66 ± 0.029	
	4	3.820	95.50 ± 0.031	
Plasil (ampoules)	2	1.942	97.10 ± 0.067	102.35
	3	2.928	97.60 ± 0.021	
	4	3.883	97.07 ± 0.036	

TABLE 5. Determination of MCP in Pharmaceutical Samples after Standard Additions of Pure MCP

Pharmaceutical samples containing MCP	Regression equation	Correlation coefficient	Found concentration of MCP after addition (μg/mL)	Recovery %
Permosan (tablets)	$y = 0.6542x + 0.0713$	0.9991	1.09	109.0
Meclodin (syrups)	$y = 0.6912x + 0.0739$	0.9992	1.04	104.0
Plasil (ampoules)	$y = 0.7208x + 0.0747$	0.9983	0.98	98.00

concentration of the MCP drug in different pharmaceutical formulations, such as tablet, syrup, and ampoules. A Premosan tablet, Meclodin syrup, and Plasil ampoule were chosen and analyzed in this study as pharmaceutically relevant samples. The concentrations of both the taken and measured samples were compared to indicate whether a matrix effect occurs. The results proved the efficiency of the proposed method in analyzing pharmaceutical preparations containing MCP (Table 4). As shown in this table, there are no significant differences in recoveries between the proposed method and the standard method.

Standard additions. The matrix effect on the determination of MCP in pharmaceutical preparations was investigated. The measured concentrations of MCP in all mixtures were checked by comparing with those of the preparation samples before addition. The results in Table 5 show clear and high recoveries. The results also showed that the behavior of absorbance over the added volume for MCP in the tablet is quite similar to that found of syrup and ampoule (Fig. 6).

Conclusions. This study set out to determine the metoclopramide hydrochloride in pure and pharmaceutical preparations. The proposed method was developed by optimizing the conditions, including type, concentration, and volumes of acid, base, and other reagents. The findings of this investigation indicated that UV-Vis spectrophotometery can be used for the determination of metoclopramide hydrochloride in tablets, syrups, and ampoules. The results also showed that the method is simple, fast, and has good linearity.

REFERENCES

1. World Health Organization. *WHO Report on Surveillance of Antibiotic Consumption* (2018).
2. P. To, *Adherence to Medical Plans for Active and Healthy Ageing* (2016); doi: 10.13140/RG.2.1.5114.4804
3. B. Aswani et al., *World J. Pharm. Res.*, **5**, 983–999 (2013).
4. T. M. Bakheet and A. J. Doig, *BMC Bioinform*., **11** (2010).
5. A. M. Anderson et al., *Am. J. Health-System Pharm.*, **76**, 175–181 (2019).
6. S. Markland et al., *Cross Sec. Stud.*, **10**, 1–12 (2019).
7. M. Hassannezhad, M. Hosseini, M. R. Ganjali, and M. Arvand, *Chem. Select*, **4**, 7616–7626 (2019).
8. O. Z. Devi, K. Basavaiah, K. B. Vinay, and H. D. Revanasiddappa, *J. Food Drug Anal.*, **20**, 454–463 (2012).
9. M. J. Hussan, M. Q. Al-abachi, and M. A. Mustafa, *Nat. J. Chem.*, **17**, 19–39 (2005).
10. A. Khan et al., *Pak. J. Pharm. Sci.*, **25**, 135–140 (2012).
11. A. A. Jawad and K. H. Kadhim, *Int. J. Pharm. Pharm. Sci.*, **5**, 294–298 (2013).
12. U. A. Deokate and A. M. Gorde, *Int. J. Pharm. Pharm. Sci.*, **6**, 394–397 (2014).
13. N. P. Dudhane, S. S. Vidhate, B. H. Borkar, R. T. Lohiya, and M. J. Umekar, *J. Pharm. Sci. Res.*, **2**, 48–52 (2010).
14. J. Shah, M. R. Jan, M. A. Khan, and S. Amin, *J. Anal. Chem.*, **60**, 633–635 (2005).
15. R. Sinan and S. S. Abed, *Spectrophotom. Iraq. J. Sci.*, **50**, 136–143 (2009).
16. O. Zenita Devi, K. Basavaiah, K. B. Vinay, and H. D. Revanasiddappa, *Arab. J. Chem*., **9**, S64–S72 (2016).
17. M. S.Attia and M. M. Aboaly, *Talanta*, **82**, 78–84 (2010).
18. N. Kahali and J. Khanam, *J. Appl. Pharm. Sci.*, **8**, 018–026 (2018).
19. A. N. Alshirifi and M. H. Abbas, *Int. J. Chem. Sci.*, **13**, 1093–1108 (2015).
20. M. Yan, H. Li, B. De M.Chen, X. L. Liu, and Y. G. Zhu, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, **878**, 883–887 (2010).
21. H. W. Lee et al., *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, **877**, 1716–1720 (2009).
22. A.-M. A. Al-Haideri, N. I.Abdulla, and I. K. Malih, *Iraq. J. Pharm. Sci.*, **21**, 70–77 (2012).