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RESEARCH ARTICLE

Visible Spectrophotometric Determination of Metochlopramide Hydrochloride at Trace Levels in Various Samples after Cloud Point Extraction Using Derivatization Reaction

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Abstract: Two new methods have been established based on extraction and determination of drug metochlopramide hydrochloride (MCP.HCl) in different samples, by using surfactant as extracting medium and visible spectrophotometer as a detection system. The methods are depended on the reaction of diazonium MCP.HCl with (A) 8-hydroxyquinoline (8-HQ) and (B) phenylephrine hydrochloride (PHE) in alkaline media to form azo dyes products which can be extracted into micelles of triton x-114 and drug MCP.HCl determined in the visible region at λ_{max} of 530 and 490 nm respectively. All parameters that influence on diazotization coupling reaction between the drug and reagents as well as for extraction efficiency of the colored products are examined by using the conventional optimization. The results have shown that the enrichment factors of 217.7 and 26.98 fold, achieving the detection limit of 0.019 and 0.24 μgmL^{-1}, with linear range of 0.1-0.7 μgmL^{-1} (r = 0.9996) and 0.5-11.0 μgmL^{-1} (r=0.9998) for the drug MCP.HCl in method A and B respectively. The mean percent recovery was 98.20±2.71% in drug vial sample and 99.37±2.41 % in tap water and the precision (RSD%) ranged between 3.53-1.21% and 0.97-0.11% for MCP.HCl in method A and B, respectively. The developed methods were compared with reported methods published in chemical literature and used for the determination of MCP.HCl in different samples.

Keywords: Metochlopramide hydrochloride, 8-Hydroxyquinoline, Phenylephrine hydrochloride, Diazotization-coupling reaction, Cloud point extraction, Visible- spectrophotometry

Introduction

The determination of medicaments in the biological and environmental samples, not only in drug formulations, has become of urgent issues for the time being. On the contrary of the rational use of the drug, the misuse of medicaments is becoming a familiar with peoples especially for those countries that do not have strict and restrictive legislation in dealing with drugs which lead to the risk of dire consequences for the people. Thus cases of abuse of drugs when submit to the laboratories cause a problem to the analysts in obtaining rapid and

reliable results[1]. Consequently, standard and selective analytical protocol is a must, for the quantification of drugs in the biological samples for the purposes of quality control and more significantly for the clinical diagnosis[2]. On the other hand, there are numerous human and veterinary medicaments are released to the environment by various routes which ultimately enter surface waters and this fact was confirmed by many studies[3,4]. Thus this problem undoubtedly poses environmental risks in which humans, animals and microorganisms are affected. Since these contaminants occur at low concentration levels, so they require a separation from their matrices and determination by using sophisticated instrumentation characterized with high selectivity and reliability.

Several analytical techniques have been used for the determination of the drugs in the above matrices including RP-HPLC[5,6], SPE-HPLC[7] HPLC/MS[8,9], HPLC/MS/MS[10,11] UPLC-MS/MS[12], GC/MS[13], voltammetric and polarographic methods[14-17] and FIA-CL[18,19]. Although these techniques are sophisticated and have a high detection power but they are relatively expensive and not always available in all laboratories. Recently, the combination of UV-Vis spectrophotometry with cloud point extraction (CPE) has proved to offer a promising alternative method of improving conventional analytical procedure toward easy, simple, eco-friendly and inexpensive routine analyses of the pharmaceuticals in different matrices instead of using expensive instrumentations[20-23].

The efforts in this trend are continuing to design new CPE procedures according to the successful attempts that are made for the first time in our research groups in exploiting the cloud point extraction (CPE) and the adoption of derivatization reactions in converting organic compounds (drugs and pesticides) to the colored derivatives that capable to be extracted by using surfactant as an extracting medium[24-27]. In the present work, drug metoclopramide hydrochloride (Figure 1) is selected as a model for designing new CPE procedures after using two coupling agents for its extraction and determination by visible spectrophotometry. The drug is chemically IUPAC named as 4-amino-5-chloro-*N*-(2-(diethylamino)ethyl)-2-methoxybenzamide and widely used in prevention and relief of nausea and vomiting as well as in combination with chemotherapy, where drugs such as cisplatin and other cytotoxic agents, are highly emetic[28,29].

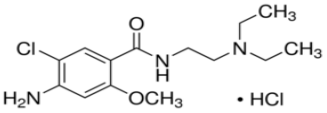


Figure 1. Chemical structure of metoclopramide hydrochloride ($C_{14}H_{23}Cl_2N_3O_2$.HCl; M.wt.354.3 g mol^{-1})

Experimental

The main instrument employed in this work was a Shimadzu double-beam UV-Vis spectrophotometer model UV-1800 (Kyoto, Japan) equipped with 5 mm optical path cell for scanning the absorption spectrum of the resulting colored products beside the absorbance measurements of the target drug under study. Thermostatic water bath model WNB7-45 experts (England) was used throughout the CPE experiments. For solution pH measurements, a portable pH/mV/C meter HI 83141 (HANNA, Romania) was used.

Reagents and materials

The chemicals used in this work were of high purity and used as received. Doubly distilled water was used for the preparation of all solutions and for the final rinsing of glassware. A pure grade (95.5%) of metoclopramide hydrochloride (MCP) was obtained from Sigma Aldrich (USA).

The diazonium salt of MCP stock solution (1000 μgmL^{-1}) was daily prepared by dissolving 0.10 g in minimum amount of water and then 1.5 mL of HCl (1.0 M) added with stirring at 5 °C in ice bath. After 5 min, a 0.0240 g of sodium nitrite (Sigma-Aldrich, USA) was added to the mixture while keeping in ice bath and shaking well for 5 min, then the solution made up to the mark in a 100 mL volumetric flask with water. The working solutions were daily prepared by appropriate dilutions in water. 8-hydroxyquinoline (8-HQ,) at concentration of 1.0 mM was prepared by dissolving 0.0145 g in a little amount of ethanol and diluted to 100 mL with same solvent in 100 mL volumetric flask. An amount of 2.4 mM phenylephrine hydrochloride (PHE, 95.5%, Sigma-Aldrich, USA) was prepared by dissolving 0.05 g in a minimum amount of water diluted to 100 mL with water in 100 mL volumetric flask. Triton x-114 (purity >99.9%), was purchased from AMRESCO LLC (Solon, USA). A 10% (v/v) of triton x-114 is prepared by diluting 10 mL with water in a 100 mL volumetric flask. An amount of 0.5 M of sodium hydroxide (BDH), sodium carbonate (BDH) and potassium hydroxide (Riedel De-Haenag, Germany) were prepared by dissolving an appropriate amount of base in water. An amount of 0.5 M ammonia (BDH, England) solution was prepared from concentrated solution (13.4 M) by transferring 3.73 mL into a 100 mL volumetric flask and diluted to mark with water. A 1.0 M of HCl (BDH, UK) is prepared from concentrated HCl solution (11.98 M) by transferring 8.35 mL into 100 mL volumetric flask and diluted to mark with water.

Recommended CPE procedure

Method A

In 10 mL volumetric flasks, an amount of MCP standard or sample solutions to the range of 0.3-0.7 $\mu g\ mL^{-1}$, 0.02 mL of 1.0 mM 8-HQ and 0.01 mL of 0.5 M KOH were added. The content of each flask was kept aside for 5 min at room temperature to complete the reaction. Then, 0.4 mL of triton x-114 (10%) was added to each flask, mixed well and diluted to mark with water. The content of each flask was transferred into a 10 mL centrifuging tubes and kept in the thermostatic bath at 55 °C for 10 min. Separation of the phases was done by centrifugation at 3500 rpm for 20 min. The aqueous phase was easily removed by pipette. The surfactant-rich phase that contains the colored product was dissolved in 1.0 mL ethanol and the absorbance of the product measured at λ_{max} of 530 nm against a reagent blank prepared under similar conditions. The remaining MCP in aqueous solution was determined by traditional spectrophotometry at λ_{max} of 274 nm in order to determine the distribution ratio (D) and extraction efficiency (%E).

Method B

To a 10 mL volumetric flasks, 0.18 mL of 2.4 mM PHE was ipette to each flask followed an amount of diazotized MCP standard (0.5-11.0 μgmL^{-1}) or sample solutions in the range of a calibration curve, 0.01 mL of 0.5 M NaOH solution were added, mixed well and the solutions were kept aside for 15 min for complete reaction. After the formation of azo dye product, 1.4 mL of 10% TX=114 was added, mixed well and each flask diluted to mark with water. The content of each flask was transferred into a 10 mL centrifuging tubes and kept in the thermostatic bath at 55 °C for 10 min, After the separation of the two phases, each tube was put in a centrifuge at 3500 rpm for 20 min, then SRP of each tube was dissolved in 1.0 mL of ethanol and the azo dye was measured at wavelength maximum of 490 nm *vs.* blank prepared in a similar manner without MCP.HCl solution. The remaining MCP in aqueous solution was determined by traditional spectrophotometry at λ_{max} of 274 nm in order to determine the distribution ratio (D) and extraction efficiency (%E).

Preparation of samples

Pharmaceuticals

Three selected medicaments from different producers purchased from the drugstores in Baghdad/Iraq in the form of ampoules containing 10 mg per 2 mL of active MCP.HCl were analyzed via the direct dilution of the ampoules with water and subjected to recommended CPE procedure for MCP (method A and B) and the content of drug was measured spectrohotometrically at λ_{max} of 530 and 490 nm respectively for five repeated measurements.

Serum

Blood samples were randomly selected from five normal volunteers who were not taking any drug, via withdrawing 5 mL from the vein using a medical syringe. Each sample was transferred immediately into a sterilized plastic centrifugal tube, kept aside for 15 min and centrifuged at 6000 rpm for 10 min to separate the serum from the whole blood. To ensure a complete separation of blood matrix from each sample, 50 µg of trichloroacetic acid was added and the content re-centrifuged for 10 min and pure serum samples were kept in refrigerator until analyzed. All serum were thawed and spiked with 0.7 µg mL^{-1} diazotized MCP and subjected to the recommended CPE procedure (method A). The content of MCP drug in the colored product was determined spectrophotometrically at 530 nm.

Urine

Urine samples were randomly selected from five normal volunteers who were not taking any drug, via collecting 10 mL from each person. Each sample was transferred immediately into a glass centrifugal tube and centrifuged at 6000 rpm for 10, filtered and the filtrate of each sample was gently heated to remove all residual protein and salts. Each urine sample was spiked with 0.1, 0.3 and 0.7 µgmL^{-1} diazotized MCP and subjected to the recommended CPE procedure (method A). The content of MCP drug in the colored product was determined spectrophotometrically at 530 nm.

Water samples

About one liter of various water samples was randomly collected from different sources such as Tigris River in Baghdad, water well from local house and Baltic Sea which passes in Denmark. The samples were kept in refrigerator until analyzed. 5.0 mL of water was taken from each sample in a centrifugal tube, then centrifuged at 3500 rpm for 15 min, filtered and 1.0 mL of each filtrate was spiked with 1.0, 3.0 and 6.0 µgmL^{-1} of diazotized MCP.HCl followed the recommended CPE procedure for MCP.HCl drug (Method B). The content of MCP drug in the colored product was determined spectrophotometrically at 490 nm.

Statistical analysis

Minitab version 17 (Minitab Inc., State College, PA, USA)[30] and Excel 2010 (Microsoft Office®) were was to carry out all statistical calculations such as regression and correlation analysis, ANOVA and significance tests.

Results and Discussion

Preliminary studies

The first attempt of this study was focused on the possibility the reaction of diazotized MCP drug with 8-HQ or PHE reagents in basic medium by taking an aliquot of 10 mL containing a fixed concentrated of diazotized MCP (0.3 µgmL^{-1}), $1.0x10^{-5}$ M 8-HQ and $3.0x10^{-3}$ M KOH method (A) or (3.0 µgmL^{-1}) MCP, $4.5x10^{-5}$ M PHE and $3.0x10^{-3}$ M NaOH (Method B). Each mixture was left for 5 min for the complete reaction and then 1.0 mL of 10% triton x-114

was added. The solution was subjected to heat in a controlled-temperature water bath at a temperature of 60 ^{o}C for 15 min and the cloud point layer which contains a colored product was separated by centrifuge, dissolved in 1.0 mL ethanol then scanned spectrophotometrically from 300-1100 nm against the reagent blank. The results revealed that the two azo dye products gave maximum absorption signals at 530 and 490 nm for method A and B respectively as shown in Figures 2a and 3a. The absorption spectra of the two azo dye products formed in method A and B were also recorded against the corresponding reagent blank after obtaining optimum conditions according to the recommended CPE procedures (A and B). Figure 2b shows the absorption spectrum of the extracted MCP- 8HQ product against reagent blank (320 nm), exhibiting again λ_{max} of 530 nm and giving a molar absorptivity of $1.29x10^{6}$ L.mol^{-1} cm^{-1}. Figure 3b shows the absorption spectrum of the extracted MCP-PHE product against blank (340 nm), exhibiting λ_{max} of 490 nm and giving a molar absorptivity of $1.64x10^{5}$ L.mol^{-1} cm^{-1}. While a pure MCP solution displays absorption maximum at 274. Consequently, the wavelength maxima at 530 and 490 nm for the two colored-products were used throughout this study for micro amounts of MCP.HCl drug.

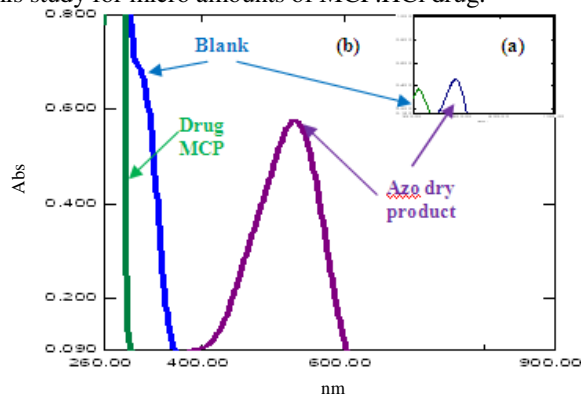


Figure 2(a). Absorption spectra of the azo dye product (A) against reagent blank before CPE and **(b)** The azo dye product (B) after CPE at optimum conditions: 0.3 µgmL^{-1} MCP.HCl, $2.0x10^{-6}$ M 8-HQ, 0.5 mM KOH, 0.4% TX-114

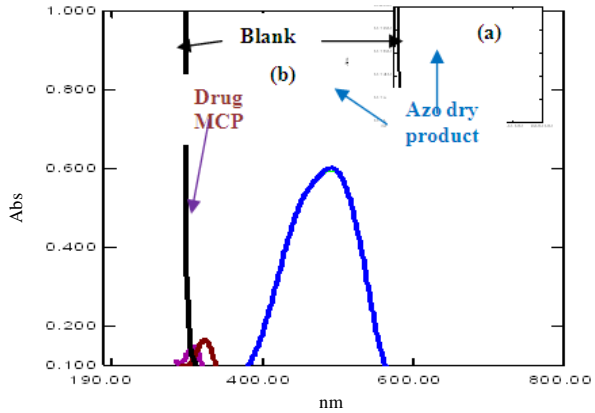


Figure 3(a). Absorption spectra of the azo dye product (B) against reagent blank before CPE and **(b)** The azo dye product (B) after CPE at optimum conditions: 3.0 µgmL^{-1} MCP.HCl; $4.32.0x10^{-5}$ PHE, 0.5 mM NaOH, 1.4% TX-114

The impact of different alkaline media such NaOH, KOH, NH_4OH and Na_2CO_3 at 3.0 mM concentration level was tested to select the best type of alkaline medium that gives the best absorption signal for the azo dye products by taking the same concentrations of the diazonium drug and reagents as mentioned above and the absorbance of the colored-product was measured at 530 and 490 nm respectively. It was shown that the best alkaline mediums were KOH and NaOH for method A and B respectively because they gave the best sensitivity of the azo dye products.

The influence of reaction time for the formation of the azo dye products was also examined between 0-30 min by using the same concentration of diazonium MCP and reagents without subjecting the reaction mixture to CPE. It was appeared that the best time giving a better absorption signal for the colored products was 5 and 15 min for method A and B respectively. All these findings were adopted in the optimization conditions of CPE for both reactions.

Optimization of CPE

Number of significant experimental factors that potentially affecting the extraction efficiency, enrichment factor and sensitivity of detection have been carried out for both derivatization reaction systems. These factor such as; the effect of reagents concentration, alkaline medium, amount of surfactant, equilibration temperature and incubation time have been systematically optimized in detail by using the univariant method in which one factor is altered while the others fixed. In the following experiments, a specified concentration of diazotized MCP.HCl standard solution was taken to 10 mL volumetric flasks into which the reagents added and diluted to mark with water followed CPE procedure. The absorbance of a series of solutions was measured at 530 and 490 nm versus reagent blank for method A and B respectively.

Effect of reagent concentration

The influence of concentration of 8-HQ and PHE was studied using different volumes of 1.0 mM and 2.4 mM of solutions respectively. Figure 4 shows that the optimum volume of 1.0 mM 8-HQ was of 0.02 mL ($2.0x10^{-3}$ mM in 10 mL final solution) and 0.8 mL of 2.4 mM PHE (0.0432 mM in 10 mL final solution) were enough to give maximum absorbance, high stability of the colored products and subsequently the best extraction efficiency for the determination of MCP drug in the two reaction systems. At lower or higher concentrations of each reagent than optimal, less intensely colored products were observed so any excessive amount of reagent was not needed. Therefore, 0.02 mL of 1.0 mM 8-HQ and 1.8 mL of 2.4 mM PHE in 10 mL solution were used in further experiments.

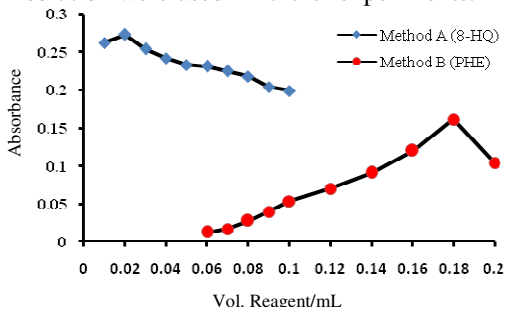


Figure 4. Effect of reagent concentration on the absorbance of the azo dye products [*Conditions: method A; MCP (0.3 μgmL^{-1}); 8-HQ (0.01-1.1.0 mL of 1 mM); KOH (3.0 mM) reaction time (5 min); TX-114 (1.0%); CP temperature (60 ^{o}C); incubation time (15 min). Method B: MCP (3.0 μgmL^{-1}); PHE (0.06-0.2 mL of 2.4 mM); NaOH (3.0 mM); reaction time (15 min); TX-114 (1.0%); CP temperature (60 ^{o}C); incubation time (20 min*)]

Effect of alkaline medium

In preliminary tests, it was shown that the coupling of the two reagents with diazonium salt of MCP drug occur in alkaline medium to form azo dyes and needs too strong basic medium to produce intense colored dye such NaOH and KOH. Therefore, the effect of different volumes of 0.5 M KOH and NaOH was investigated by varying the volume between (0.01-0.1 mL) keeping other variables fixed. The results are displayed in Figure 5. The data revealed that the optimum volume was 0.01 mL of 0.5 M KOH and NaOH (0.5 mM in 10 mL solution) gave a maximum absorption signal of the colored azo dyes for method A and B respectively, which is used in the next experiments.

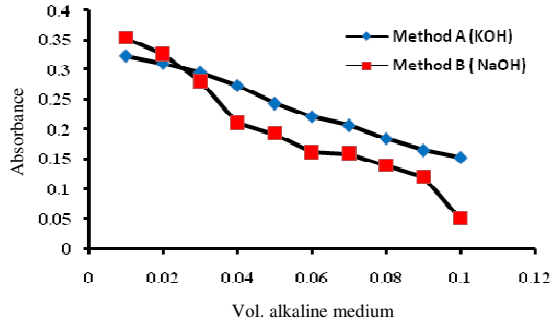


Figure 5. Effect of alkaline medium concentration on the absorbance of the azo dye products [*Conditions: method A; MCP (0.3 µgmL^{-1}); 8-HQ (2.0x10^{-3} mM); KOH(0.01-0.1 mL of 0.5 M) reaction time (5 min); TX-114 (1.0%); CP temperature (60 ^{o}C); incubation time (15 min). Method B: MCP (3.0 µg mL^{-1}); PHE (0.0432 mM); NaOH (0.01-1.0 mL of 0.5 M); reaction time (15 min); TX-114 (1.0%); CP temperature (60 ^{o}C); incubation time (20 min*)]

Effect of triton x-114 amount

Different volumes ranges of triton x-114 (10% v/v) were used in this study at previously optimum conditions as shown in Figure 6. It was shown that the absorbance for both azo dyes increases gradually with increasing the triton x-114 amount and reached maximum at 0.4 and 1.4 mL of 10% TX-114 for method A and B respectively. Thus, these values were selected as optimal volumes and used in the proposed methods (A and B) for the detection of MCP drug.

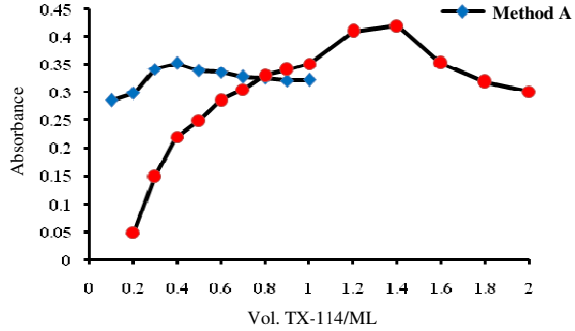


Figure 6. Effect of triton x-114 amount on the absorbance of the azo dye products [*Conditions: method A; MCP (0.3 µgmL^{-1}); 8-HQ (2.0x10^{-3} mM); KOH (0.5 mM) reaction time (5 min); TX-114 (0.1-1.0 of 10%); CP temperature (60 ^{o}C); incubation time (15 min). Method B: MCP (3.0 µg mL^{-1}); PHE (0.0432 mM); NaOH (0.5 mM); reaction time (15 min); TX-114 (0.2-2.0 of 10%); CP temperature (60 ^{o}C); incubation time (20 min*)]

Effect of equilibration temperature

Figure 7 shows the variation on the absorption signal via varying the temperature between 25 to 70 ^{o}C keeping other parameters fixed. It found that the maximum absorption signal of the target analyte (MCP) in both methods was achieved at 55 ^{o}C, due to a high number of micelles formed in cloud point layer leading the entire transfer of the azo dye products into a surfactant-rich phase which maximizes the sensitivity. A remarkable decrease of the absorbance response was observed thereafter, probably due to the instability or dissociation of the azo dye products at a higher temperature than optimal. Therefore, a temperature of 55 °C was selected and used as optimal in the general CPE procedures of both methods.

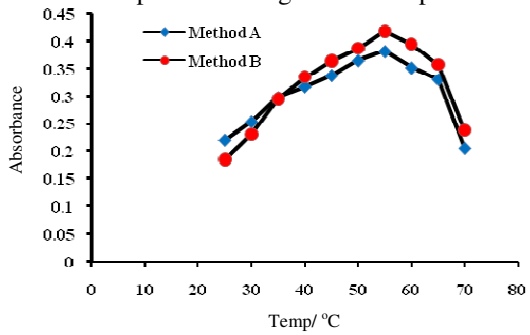


Figure 7. Effect of equilibration temperature on the absorbance of the azo dye products [*Conditions: method A; MCP (0.3 µgmL^{-1}); 8-HQ (2.0x10^{-3} mM); KOH (0.5 mM) reaction time (5 min); TX-114 (0.4%) CP temperature (25-70 ^{o}C); incubation time (15 min). Method B: MCP (3.0 µgmL^{-1}); PHE (0.0432 mM); NaOH (0.5 mM); reaction time (15 min); TX-114 (1.4%); CP temperature (25-70 ^{o}C); incubation time (20 min)]*

Effect of incubation time

The impact of incubation time on the absorption signals of the two azo dyes products was investigated using different heating times starting from 5 min until 50 min at cloud point temperature of 55 ^{o}C. The maximum absorption signal was achieved at 10 min for both MCP-8HQ and MCP-PHE products and gradually decreased thereafter as showed in Figure 8. It was also noted that the centrifugation speed and time of 20 min at 3500 rpm were suitable for complete the separation of the two phases.

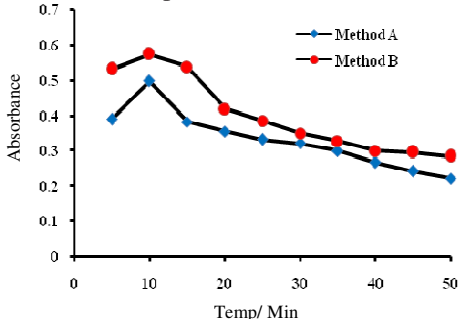


Figure 8. Effect of equilibration temperature on the absorbance of the azo dye products [*Conditions: method A; MCP (0.3 µgmL^{-1}); 8-HQ (2.0x10^{-3} mM); KOH (0.5 mM) reaction time (5 min); TX-114 (0.4%) CP temperature (55 ^{o}C); incubation time (5-50 min). Method B: MCP (3.0 µgmL^{-1}); PHE (0.0432 mM); NaOH (0.5 mM); reaction time (15 min); TX-114 (1.4%); CP temperature (55 ^{o}C); incubation time (5-50 min)]*

Table 1 shows a summary of the optimum values of the experimental variables for the determination of MCP.HCl drug spectrophotometrically at λ_{max} of 530 and 490 nm by the two proposed methods after CPE.

Table 1. The summary of optimum experimental conditions for the extraction of colored products by CPE for method A and B

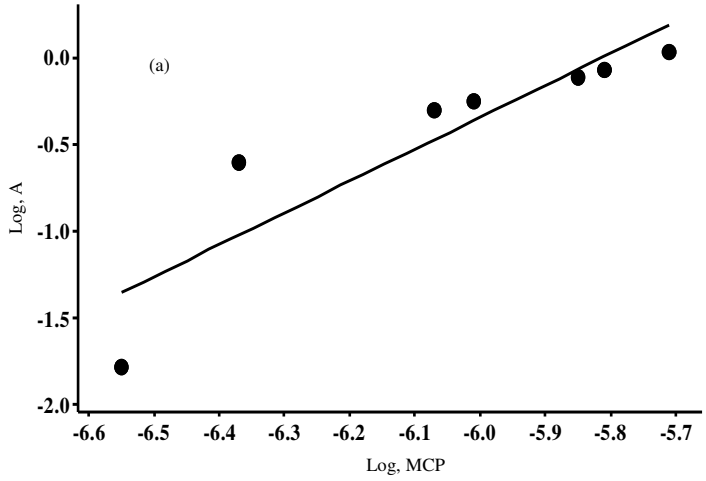
Variable	Method A(λ_{max} = 530)	Method B(λ_{max} = 490)
8-HQ	0.02 mL of 1.0 mM	-
PHE		0.18 mL of 2.4 mM
KOH	mL of 0.5 M	-
NaOH		0.01 mL of 0.5 M
Reaction time	5 min	15 min
TX-114	0.4 mL of 10% TX=114	1.4 mL of 10% TX=114
Temperature	55 ºC	55 ºC
Incubation time	10 min	10 min
λ_{max}	530	490

Stiochoimetry of the reaction

The molar ratio of the two azo dyes products was determined by using the limiting logarithmic method[31]. In this method, two sets of experiments were prepared followed the recommended CPE procedures of both methods. The first set was done by varying the concentration of the each reagent with fixed concentration of MCP, while the second set was conducted by varying the concentration of MCP with fixed concentration of each reagent. The logarithms of the obtained absorbance were plotted as a function of the logarithms of the concentrations of each reagent and MCP in the first and second sets of experiments, respectively.

The slopes of the fitting lines in both sets of experiments were calculated. In each method, two straight lines were obtained. The values of the slopes of these lines were of 1.838 and 0.858 by method A (Figure 9), indicating the molar ratio of reaction with MCP/8-HQ was considered to be 2:1. Whilst in method B, the values of the slopes of these lines were 1.906 and 0.881 (Figure 10), indicating the molar ratio of reaction with MCP/PHE was also considered to be 2:1

log A = 10.68 + 1.838 log [MCP]



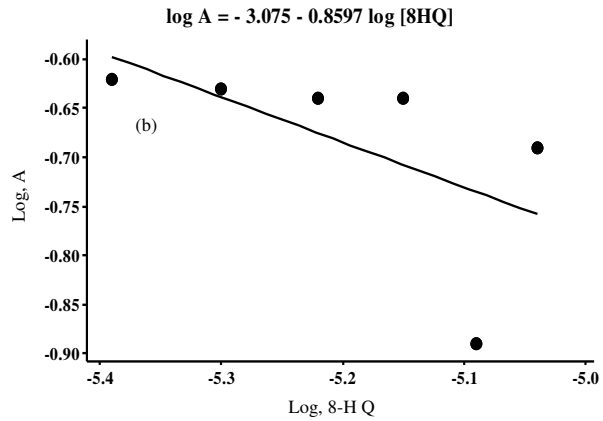


Figure 9(a). Limiting logarithmic plots for the molar reactivity of MCP with 8-HQ: logarithm of absorbance *vs*. log MCP] at which [8-HQ] is kept constant; **(b).** Logarithm absorbance *vs*. log [8-HQ] at which [MCP] is kept constant

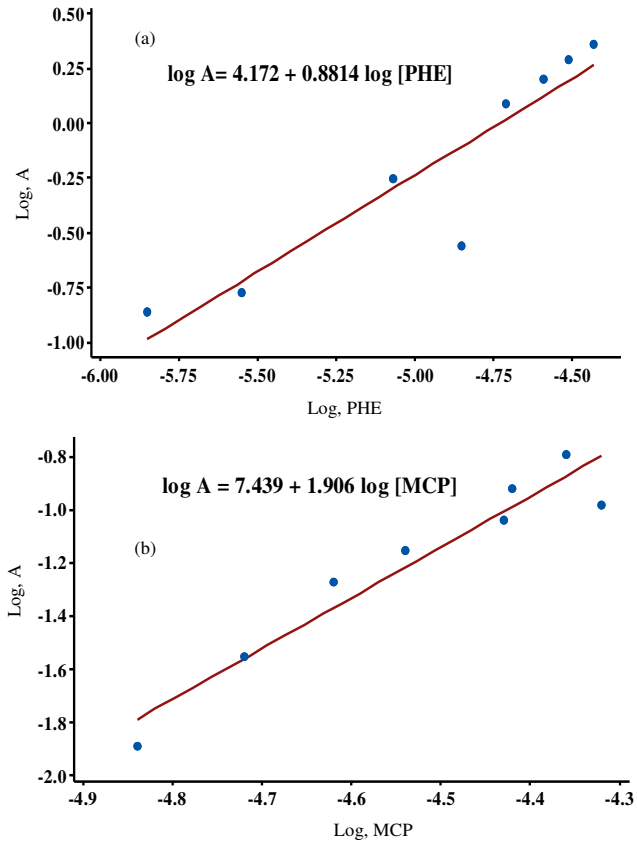
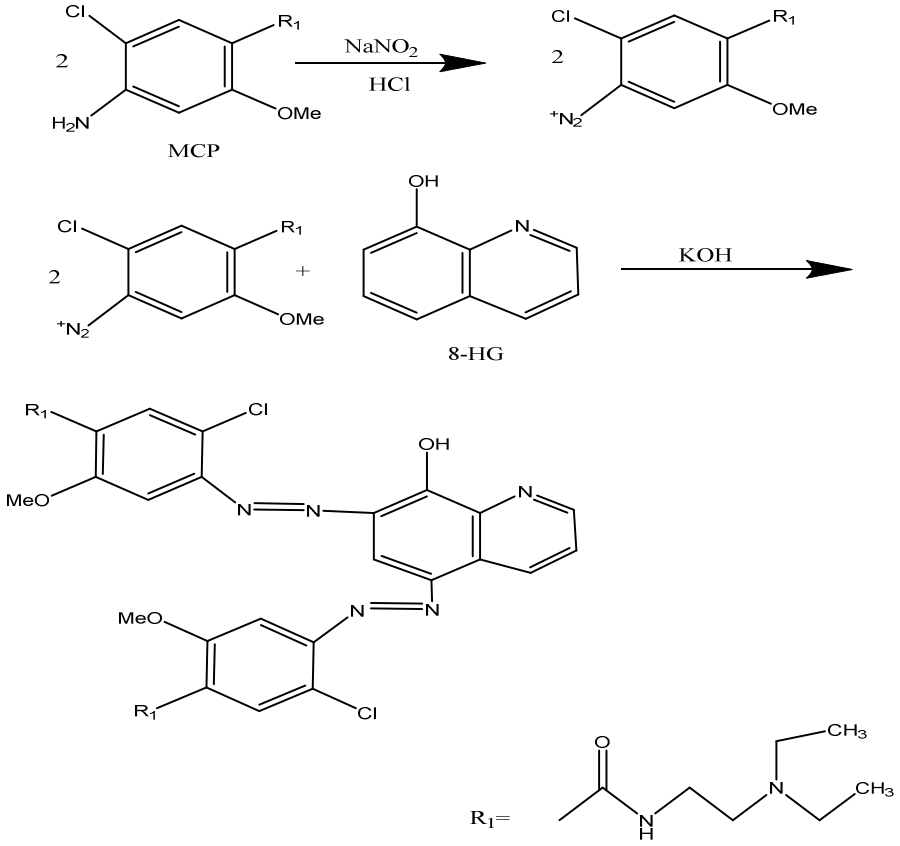


Figure 10(a). Limiting logarithmic plots for the molar reactivity of MCP with 8-HQ: logarithm of absorbance *vs*. log MCP] at which [PHE] is kept constant; **(b).** Logarithm absorbance *vs*. log [PHE] at which [MCP] is kept constant

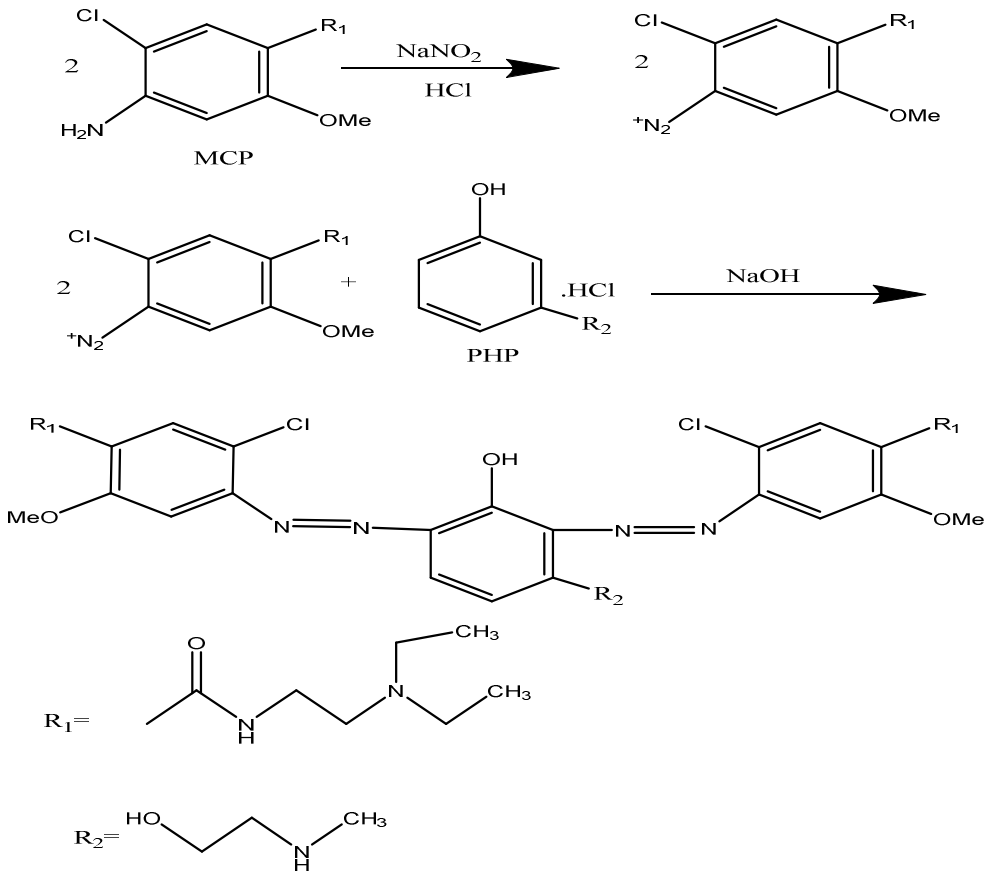
Based on the above result (Figures 9 and 10), the two reagents 8-HQ and PHE are coupled with the formed diazonim MCP in alkaline medium to give a deep red color azo dye which exhibited absorption maximum at 530 nm against reagent blank solution in the method A and a yellow azo dye with absorption maximum at 490 nm against reagent blank solution in the method B. Thus the most probable pathway for formation of the extracted azo dyes products are preceded by two steps as shown in the Scheme 1 and 2.

Analytical figures of merit

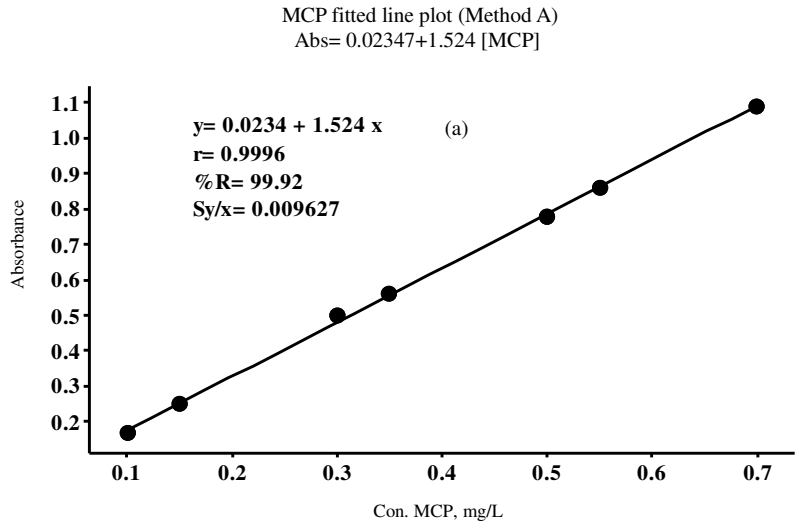
Under the optimized established conditions (Table 1), seven standard solutions of the drug MCP. HCl were individually prepared in order to obtain a concentration range from 0.1-0.7 μgmL^{-1} (method A) and 0.5-11.0 μgmL^{-1} (method B) followed the recommended CPE procedures (A and B). The fitted calibration curves resulting from plotting the absorbance *versus* MCP.HCl concentration are illusterated in the Figure 11. The statistical evaluation for the two calibration curves reveals that the linear regression equations for both analytes were statistically valid. This because of the ratios (MS_{reg}/MS_{error}) for 1 and 6 dof, larger than critical value ($F^{1}_{,7}$= 5.99 at α=0.05), indicating that the predication based on the regression line is satisfactory as listed in Table 2. These regression lines were used to estimate the drug MCP.HCl concentration in the selected samples which appears justified on statistical basis.



Scheme 1. The probable reaction mechanism of coupling 8-HQ with diazotized MCP in alkaline medium



Scheme 2. The probable reaction mechanism of coupling PHE with diazotized MCP in alkaline medium



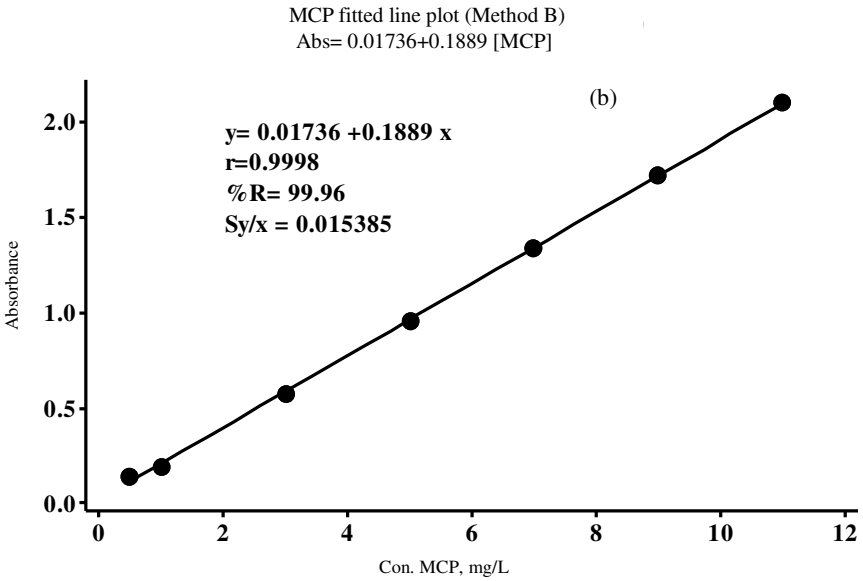


Figure 11. Calibration curves for MCP.HCl by the proposed methods

Table 2. Analysis of variance of regression line for MCP of method A and B

Method	Source	dof	SS	MS	F –value	Significance *p*-value
A	Regression	1	0.660314	0.660314	7125.47	0.000
	Residual (Error)	5	0.000463	0.000093		
	Total	6	0.660778			
B	Regression	1	3.42167	3.42167	14455.28	0.000
	Residual (Error)	5	0.00118	0.00024		
	Total	6	3.42285			

Statistical analytical figures of merit of the proposed methods are summarized in Table 3. The enrichment factors were calculated as the ratio between the slope of a curve obtained using aqueous solutions submitted to the CPE procedure (y=1.5240x+0.0234x) or (y= 0.1889x+0.01736) and to that obtained without CPE (y=0.007x+0.005) and found to be of 217.70 and 26.98 fold for method A and B respectively. These enrichment factors allow to detect the drug in aqueous solutions spectrophotometrically at low detection limit of 0.019 (Method A) and 0.24 μgmL^{-1} (Method B) which based on the standard deviation of the response (residual standard deviation; $\sigma_{y/x}$) and the slope(s) of the calibration curve using the equation; LOD = $3\sigma_{y/x}/s$. The detection limits obtained by the proposed methods was in harmony with a few but much better than obtained with most reported methods in literature using different diazotization reactions with various chromogenic reagents as listed in Table 4. Moreover, the molar absorptivity (ε) of MCP.HCl was found to be of $1.29x10^6$ and $1.64x10^5$ $L.mol^{-1}cm^{-1}$ for method A and B respectively, indicating the excellent sensitivity of the proposed methods.

Table 3. Statistical data and analytical figures of merits for AMX by method A and B

Parameter	Method A	Method B
Product colour	Red	Orange
λ_{max}, nm	530	490
Regression equation (7 points)	y=1.5240x+0.0234	y= 0.1889x+0.01736
Standard deviation of regression line($S_{y/x}$)	0.009627	0.015385
Correlation coefficient (r)	0.9996	0.9998
Coefficient of determination (R^2)	99.92	99.96
C.L. for the slope (b± ts_b) at 95%	1.5524±0.12276	0.1889±.0.010663
C.L. for the intercept (a± ts_a) at 95%	0.0234±0.0526	0.01736±0.06817
Beer's law range, µg mL^{-1}	0.1-0.7	0.5-11.0
Limit of detection, µg mL^{-1}	0.019	0.24
Limit of quantitation, µg mL^{-1}	0.063	0.81
Sandell's sensitivity, µg cm^{-2} x10^{-3}	0.6587	5.531
Molar absorptivity, $L.mol^{-1}.cm^{-1}$	1.29x10^6	1.64x10^5
Composition of the colored product*	1:2	1:2
RSD% (n=5)	0.24 at 0.1 µgmL^{-1}	3.60 at 1.0 µgmL^{-1}
RSD% (n=5)	0.16 at 0.7 µgmL^{-1}	2.50 at 5.0 µgmL^{-1}
Preconcentration factor**	33.3	33.3
Enrichment factor	217.7	26.98
Recovery, %	97.23±2.04	98.00±3.95
Extraction efficiency, %E	99.66	98.40

**Limiting logarithmic method **Preconcentration factor was calculated the ratio of the original sample volume to that extracted volume of surfactant-rich phase*

Table 4. Reported methods for the determination of MCP.HCl by spectrophotometry after diazotization, oxidative coupling and charge transfer reactions[27]

Coupling reagent used/ Reaction type	λ_{max}, nm	Linearity µg mL^{-1}	LOD, µg mL^{-1}	Ref.
Dibenzoyl methane/ diazotization	440	-	-	32
Aniline/ diazotization	410	0.5-12.0	-	33
Benzoylacetone/ diazotization	437	0.8-13.2	0.033	34
Imipramine hydrochloride/diazotization	570	0.5-5.0	0.014	35
p-Dimethylaminocinnamaldehyde/ diazotization	553	4-24	1.120	36
Phenol/ diazotization	463	1-20	0.406	37
8-Hydroxyquinoline /diazotization	528	0.2-12	-	38
Diphenylamine / diazotization	530	0.3-7.5	0.220	39
2,5-Dimethoxyaniline (DMA) /diazotization	486	0.1-12	0.016	40
Doxycycline hyclate /diazotization	452	0.1-10	0.012	41
Phenoxide / diazotization	462	10-80	3.700	42
Malachite green in the presence of 0.01 M chloramine-T and 2 M H_2SO_4,	623	2-10	0.087	43
Folin–Ciocalteu/ complex formation	760	Up to 100	2.000	44
9-Chloroacridine / oxidative coupling	470	2-50	0.368	45
Pyrocatecolin presence of ammonium ceric sulphate /Oxidative coupling	500	5-35	-	46
8-HQ /Diazotization	530	0.1-0.7	0.019	This
PHE/ Diazotization	490	0.5-11.0	0.24	work

Accuracy and precision study

Since the certificate reference materials (CRM's) that define exactly the true value of MCP.HCl drug are not available, the validity of the proposed methods was evaluated via accuracy test in terms of recovery percentage. The experiments were conducted by spiking different concentrations of MCP.HCl standard solutions to the drug sample prepared from vial produced from GLAND PHRMA LIMITED, (India) and water sample for the method A and B respectively, keeping the concentration of the drug within the calibration ranges as showed in Table 5. Each spiked sample solution is subjected to the recommended CPE procedures (A and B) with repeated five time measurements. The results in Table 5 revealed that the accuracies of the proposed methods were within the acceptable confidence limits at α=0.05, indicative the suggested methods are unbiased and confirmed that they are relatively interferences-free, from drug excipients that might be added during pharmaceutical formulation and water matrices. Hence, the study of interferences from the drug or water matrices is almost an undue. Meanwhile, each spiked sample was repeated five times for precision test in terms of %RSD and found in the range of 1.21-0.3.53% and 0.11-0.97% for method A and B respectively, indicative a good precision.

Table 5. Accuracy and precision test for MCP.HCl in pharmaceutical (Method A) and water samples (Method B)

Method	Sample	MCP Taken, µg mL^{-1}	MCP Found, µg mL^{-1}	Rec., %	Mean Rec%±C.L. at α=0.05	Accuracy, $\%E_{rel}$	Precision (%RSD) (n=5)
A	MCP	0.1	0.098	98.0		-2.0	3.53
	(Indian	0.3	0.287	95.6	98.20± 3.71	-4.4	1.74
	vial)	0.6	0.606	101.0		-1.0	1.21
	Spiking	1.0	1.03	103.0		3.0	0.97
B	water	3.0	2.87	95.6	99.37±2.41	-4.4	0.34
	sample	6.0	5.97	99.5		-0.5	0.11

Table 6. Determination of MCP.HCl drug in pharmaceuticals by the proposed method (A) and statistical comparison with quoted values

Commercial name and content	Practical content (mg/ 2 mL) (proposed method)	$t= (\overline{x}-\mu)\sqrt{n}/s$ Proposed method *Vs.* claimed value at 95% C.I.	$\%E_{rel}$	%RSD (n=3)
Metoclopramide – METAMID injection ((IBN HAYYAN PHARM Syrian), 10 mg/ 2 mL	9.80 9.54 10.2 9.85±0.332	t_{cal}=0.80 0.80< 4.303	-0.15	3.37
Metoclopramide, injection (GLAND PHRMA LIMITED, India), 10 mg /2 mL	9.80 9.56 10.1 9.82±0.271	t_{cal}=1.15 1.15<4.303	-0.18	2.76
Metoclopramide – injection (hamelnpharmaceuticals gmbh Langes Feld 13 Germany),10 mg/2 mL	9.70 9.70 10.4 9.93±0.404	t_{cal}=0.29 0.29<4.303	-0.07	4.06

Table 7. Determination of MCP.HCl drug in pharmaceutical by the proposed method (B) and statistical comparison with quoted values

Commercial name and content	Practical content, mg/2 mL (proposed method)	$t= (\bar{x}-\mu)\sqrt{n}/s$ Proposed method Vs. claimed value at 95% C.I.	$\%E_{rel}$	%RSD (n=3)
Metoclopramide - METAMID injection ((IBN HAYYAN PHARM Syrian), 10 mg/ 2 mL	9.80 9.96 9.97 9.91±0.10	t_{cal}=1.63 1.63< 4.303 p=0.224	-0.09	1.01
Metoclopramide, injection (GLAND PHRMA LIMITED, India), 10 mg /2 mL	9.90 9.96 9.96 9.94±0.034	t_{cal}=3.000 3.000<4.303 p=0.095	-0.06	0.34
Metoclopramide - injection (hamelnpharmaceuticals gmbh Langes Feld 13 Germany),10 mg/2 mL	9.90 9.77 9.98 9.95±0.0436	t_{cal}=1.99 1.99<4.303 p=0.185	-0.05	0.44

Determination of MCP.HCl drug in pharmaceuticals

Two proposed methods were applied to assay metochlopramide hydrochloride drug in the pharmaceutical vials produced in different countries and containing 10 mg/2 mL MCP.HCl as an active ingredient. These samples were prepared as described in the experimental part, from which each sample was submitted to the recommended CPE and MCP.HCl drug estimated spectrophotometrically at λ_{max} of 530 and 490 nm**.** The results presented in Table 6 and 7 revealed that the calculated *t*-values for MCP.HCl determination in different pharmaceuticals using 8-HQ and PHE as coupling reagents are less than *t*-tabulated (4.303) at 95% confidence interval and (n-1) degrees of freedom, so the null hypothesis Ho is maintained, concluding there is no evidence for systematic and random errors at the 95% confidence level and accordingly manufacturer's claims can be accepted.

Applications

According to analytical features provided by the proposed methods in aqueous solutions, the application of these methods was performed to test their truthfulness on the real samples.

Determination of MCP.HCl drug in biological samples

Since this medication like the other medicines is absorbed via the blood and excreted at a certain amount in the urine after its oral administration, it becomes necessary to estimate this drug in these vital samples. Therefore, the application of the method (A) was directed to the detection the drug MCP.HCl in the spiked human serum and urine taken from five normal volunteers as described in the experimental section. All serum samples were spiked with 0.7 μgmL^{-1} standard MCP.HCl solutions, while the urine samples were spiked with 0.1, 0.3 and 0.7 $\mu g\ mL^{-1}$, then followed the recommended CPE procedures. The results are presented in Table 8 and 9. The results revealed that good recoveries of the drug were obtained in the presence of serum or urine matrix, suggesting the established method (A) is unbiased, confirming it is relatively free from matrix interferences and with a good precision (RSD=1.04%) therefore it can be adopted in routine analysis of MCP.HCl in the clinical quality control laboratories.

Table 8. Determination of MCP.HCl in human serum by proposed method (A)

Sample	Mean MCP.HCl, µg mL^{-1}	Recovery, %	Mean Recovery±Sd, %	E_{rel}, %	Mean RSD, % (n=3)
1	0.720	102.8	100.92±1.05	2.8	1.04
2	0.703	100.4		0.4	
3	0.703	100.4		0.4	
4	0.704	100.5		0.5	
5	0.704	100.5		0.5	

Table 9. Determination of MCP.HCl in human urine by proposed method (A)

Sample	MCP.HCl added, µg mL^{-1}	MCP.HCl found, µg mL^{-1}	Recovery %	Mean Recovery±Sd, %	Mean RSD, % (n=3)
1	0.1	0.097	97.0	98.63±1.42	1.44
	0.3	0.298	99.3		
	0.7	0.697	99.6		
2	0.1	0.097	97.7	99.13±1.16	1.34
	0.3	0.298	99.3		
	0.7	0.702	100.2		
3	0.1	0.095	95.0	97.40±2.62	2.96
	0.3	0.291	97.0		
	0.7	0.703	100.4		
4	0.1	0.095	95.0	98.03±2.76	2.82
	0.3	0.296	98.7		
	0.7	0.702	100.2		
5	0.1	0.097	97.7	99.10±1.35	1.83
	0.3	0.297	99.2		
	0.7	0.703	100.4		

Determination of MCP.HCl drug in water

Method B was applied to the determination of MCP.HCl in various water samples as described in the experimental section to ensure its applicability and validity in these matrices. The results summarized in Table 10 indicated that acceptable recoveries were obtained in water matrix and therefore this method can used in the routine determination of the drug in the pollutant water.

Table 10. Determination of MCP.HCl in water samples by proposed method (B)

Sample	MCP.HCl added, µg mL^{-1}	MCP.HCl found, µg mL^{-1}	Recovery, %	Mean Recovery±Sd, %	E_{rel}, %	Mean RSD, % (n=3)
Tigris river	1.0	0.97	97.0	97.27±2.11	-3.0	2.17
	3.0	2.86	95.3		-4.7	
	6.0	5.97	99.5		-0.5	
Baltic sea	1.0	1.03	103	99.37±3.70	3.0	3.73
	3.0	2.87	95.6		-4.4	
	6.0	5.97	99.5		-0.5	
Well	1.0	1.03	103.0	99.33±3.75	3.0	3.78
	3.0	2.86	95.3		-4.7	
	6.0	5.97	99.5		-0.5	

Conclusion

Two methods have been developed for the determination of drug MCP.HCl in different matrices using CPE combined with visible spectrophotometry. The methods are straight forward, fast, accurate, precise, sensitive and eco-friendly methods compared with other reported methods (*c.f.* Table 4). They can be easily applied to the dosage form, biological and environmental samples, particularly for waste water flowing from the medicaments industries.

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