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A New Visible Spectrophotometric Approach for Mutual Determination of Amoxicillin and Metoclopramide Hydrochloride in Pharmaceuticals After Cloud Point Extraction

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Abstract: A new approach has been developed and validated for the mutual determination of the drugs of amoxicillin (AMX) and Metoclopramide hydrochloride (MCP. HCl) in pharmaceuticals. The method is based on the reaction of diazotized Metoclopramide with amoxicillin in an alkaline medium to form an intense orange water-soluble product which can be easily extracted from micelles of a non-ionic surfactant (Triton X-114) and both drugs measured sequentially at the same absorption maximum of 479 nm. The optimization of all experimental variables was individually performed to obtain high extraction efficiency for both target medicaments. Under the optimized conditions, Beer's law was obeyed in the concentration range of 0.3-3.0 µg mL^{-1} (r=0.9995) for both AMX and MCP. The enrichment factors were found to 214 and 90.85 fold for AMX and MCP, led to obtaining the detection limits of 0.083 and 0.098 µg mL^{-1}, and a superb sensitivity in terms of the molar absorptivity of $2.35x10^5$ and $2.25x10^5$ $L.mol^{-1}.cm^{-1}$, respectively. The mean recovery percentage of 97.77±1.72% (in AMX capsule) and 98.20±1.95 (in MCP ampoule); the precision (RSD %) ranged between 2.35-10.8% and 0.20-3.43% were obtained for AMX and MCP respectively. The proposed method was validated and applied for determination of AMX and MCP in various samples of the pharmaceutical preparations.

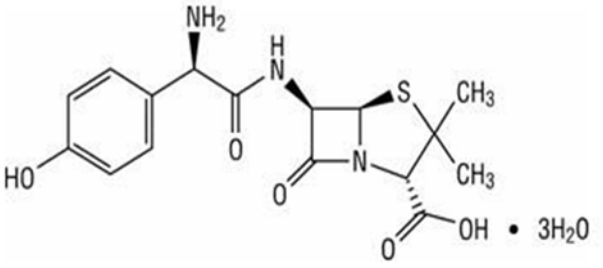
Keywords: Amoxicillin, Metoclopramide Hydrochloride, Diazotization Coupling Reaction, Cloud Poin Extraction, Visible Spectrophotometry

1. Introduction

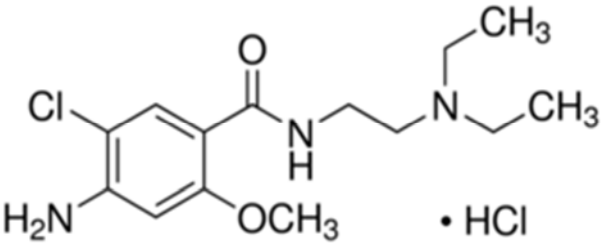
The chemical analysis of two analytes mutually in one reaction system with the same extraction and detection method has become a unique and attractive theme in contemporary analytical chemistry. This type of chemical analysis is in itself economic in terms of reducing analyst effort, time and simplifies the analytical procedures as well as reduces the costs involved in the use of chemicals in the analytical methods when determining two or more of the target analytes. These features are embodied by our recent published papers [1-3] which encouraged us and other researchers in our universities to be engaged in the development of new analytical methods based on other reactions systems that make us believe that this work will open new horizons in analytical chemistry. We have adopted in our preceding works, complex formation reactions in which the ligand and metal ions have been estimated in the same reaction system. We relied in this work on the diazotization –coupling reactions between two drugs to form a chromogenic product, namely amoxicillin (AMX) and metoclopramide hydrochloride (MCP.HCl), with a view to extract them via the cloud point extraction (CPE) and their mutual determination spectrophotometrically. The main reason for selecting the diazotization-coupling reaction system in this research work goes back to the increasing of its popularity in the estimation

of the drugs spectrophotometrically.

Amoxicillin (Figure 1a) chemically known as 6-(p-hydroxy-alpha-aminophenyl acetamido) penicillanic acid is commonly used as an antibacterial drug in the treatment of infections caused by gram-positive gram-negative bacteria [4]. whilst the metoclopramide hydrochloride (Figure 1b),4-amino-5-chloro- [2- (diethylamino)ethyl]-2 methoxybenzamide HCl, is 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 [5-6]. Human pharmacokinetic interactions of these drugs have not yet documented, but the clinicians advise patients not to use these drugs together because this reduces the action of each drug as well as AMX may induce diarrhoea in some patients [7]. Due to their wide use for the medical and clinical treatments, it is found that there are enormous research publications been dedicated to estimating these drugs in the pharmaceuticals and to a



(a)



(b)

Figure 1. *Chemical structure of (a) Amoxicillin ($C_{16}H_{25}N_3O_8S$; M.wt. 419.45 g mol^{-1}) and (b) Metoclopramide hydrochloride ($C_{14}H_{23}Cl_2N_3O_2$.HCl; M.wt.354.3 g mol^{-1}).*

little in bio-samples using different instrumental techniques. Spectrophotometric assay of AMX and MCP.HCl individually in pure or dosage forms were occupied the wide attentions among the reported methods. Most of these methods were adopted for the determination of these drugs using the azo-coupling reaction with various chemical reagents such as benzocaine [8], o-nitrophenol [9], p-nitrophenol [10], o-nitroaniline [11], p-amino benzoic acid and procain [12], sulphanilic acid [13] for AMX drug, and dibenzoyl methane [14] aniline [15], benzoylacetone [16], imipramine hydrochloride [17], p-dimethylaminocinnamaldehyde [18] phenol [19],8-hydroxyquinoline [20],diphenylamine [21], 2, 5 dimethoxyaniline [22] doxycycline hyclate [23], phenoxide [24] for MCP drug. Although these methods have adequate sensitivity, but they are not devoid of matrix interferences, which may be caused by certain medications additives present in pharmaceuticals for perhaps through their involvement in the diazotization coupling reaction. Therefore, the elimination of the interfering compounds from drug solution before its measurement is a must. Recently, cloud point extraction-spectrophotometric method has received a remarkable attention in quantifying many of the organic compounds of medicinal significance to resolve this dilemma [25-28]. These methods mostly depend on converting the drug compound into chelate or highly colored derivative compounds that can be extracted by using cloud point extraction, and then measured colorimetrically.

The aim at the current work is directed towards designing a new approach to the analyzing the two drugs (AMX and MCP) mutually in the same reaction system by adopting the diazotization coupling reaction, in the first attempt to determine both drugs together by the same analytical procedure in the pharmaceutical dosage forms. Since there are no reports on extracting these drugs using the CPE methodology, so the current method is founded on the diazotized MCP via the aromatic amino group that exists on MCP with nitrous acid ($NaNO_2$ / HCl) and diazonium salt thus formed and coupled with AMX drug to form an azo dye product which can easily be extracted into micelles of non-ionic surfactant which subsequently, the AMX drug first determined, and then MCP was back-determined colorimetrically at the same absorption maximum but with slight differences in the optimum experimental conditions for both drugs.

2. Materials and Methods

2.1. Apparatus

The main instrument employed in this work is 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 product beside the absorbance measurements of the two target drugs under study. Thermostatic water bath model WNB7-45 Experts (England) is used throughout the CPE experiments. For solution pH measurements, a portable pH/mV/C meter HI 83141 (HANNA, Romania) is used.

2.2. Reagents and Materials

The chemicals used for this work are of high purity and used as received. Doubly distilled water was used in the preparation of all solutions and for final rinsing of glass wares. A pure grade (95.5%) of amoxicillin trihydrate (AMX) was obtained from Sigma Aldrich (USA). A stock solution of 1000 µg mL^{-1} (or 0.0020 M) for the drug AMX was prepared by dissolving 0.1000 g in minimum amount of water and diluted to mark with water in a 100 mL volumetric flask. This solution was transferred to brown bottle and stored in the refrigerator. The working solutions were daily prepared by appropriate

dilutions in water. A 1.0 M of HCl (BDH, UK) was prepared from concentrated solution (12.1 M) by transferring 20.66 mL into 250 mL volumetric flask and diluted to mark with water. An amount of 5 mM diazotized metoclopramide hydrochloride (95.5%, Sigma, USA) solution was daily prepared by dissolving 0.1772 g of MCP in a minimum volume of distilled water, and then an amount of 3 mL of HCl (1.0 M) was added with stirring at 5°C in ice bath. After 5 min, a 0.0345 g of sodium nitrite (Sigma-Aldrich, USA) was added to the mixture while keeping in ice bath and shaking well for 5 min and the solution was made up to the mark in a 100 mL volumetric flask with water. An amount of 0.5 M of sodium hydroxide (BDH), sodium carbonate (BDH) and potassium hydroxide (Riedel De-Haenag,Germany) was prepared by dissolving an appropriate amount in water. An amount of 0.5 M ammonia (BDH, England) solution was prepared from from concentrated solution (13.4 M) by transferring 3.73 mL into 100 mL volumetric flask and diluted to mark with water. Triton X-114 (purity >99.9%), was purchased from AMRESCO LLC (Solon, USA). A 10% (v/v) of Triton X-114 was prepared by diluting 10 mL with water in a 100 mL volumetric flask.

2.3. Recommended CPE Procedure for AMX Drug

In 10 mL volumetric flasks, an amount of AMX standard or sample solutions to the range of 0.3-3.0 µg mL^{-1}, 0.18 mL of 0.5 mM of diazotized MCP and 0.06 mL of 0.5 M Na_2CO_3 were added. After the formation of an orange azo dye product, 1.0 mL of 10% Triton X-114 was added, 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 60°C for 25 min. Separation of the phases was conducted 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 479 nm against a reagent blank prepared under similar conditions. The remaining AMX 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).

2.4. Recommended CPE Procedure for MCP Drug

In 10 mL volumetric flasks, an amount of diazotized MCP standard or sample solutions to the range of 0.3-3.0 µg mL^{-1}, 0.08 mL of 1.0 mM of AMX and 0.02 mL of 0.5 M Na_2CO_3 were added. After the formation of an orange azo dye product, 0.6 mL of 10% Triton X-114 was added, 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 50°C for 20 min, and then the same steps were followed as in recommended CPE procedure for AMX. The remaining MCP in aqueous solution was determined by traditional spectrophotometry at λ_{max} of 272 nm in order to determine the distribution ratio (D) and extraction efficiency (%E).

2.5. Preparation of Pharmaceutical Samples

Two types of pharmaceuticals for amoxicillin namely capsules and vials were obtained from the drugstores in Baghdad as described in Table 9. The powder of ten capsules or vials were mixed, homogenized, and the content of one capsule (0.6077 g) which equivalent to 500 mg of active drug was dissolved in sufficient amount of water with continuous shaking and filtered. The filtrate solution was transferred into a 100 mL volumetric flask and diluted to mark with water. This solution contains 5000 µg mL^{-1} of AMX from which 100 µg mL^{-1} was prepared by dilution. 10 mL containing different concentrations of the prepared sample solution were transferred to centrifugal tubes and each solution followed the recommended CPE procedure for AMX and the content of drug was measured spectrophotometrically at λ_{max} of479 nm for five repeated measurements. Three selected medicaments from different producers in the form of ampoules containing 10 mg per 2 mL of active MCP.HCl were analysized via the direct dilution of the ampoules with water and subjected to recommended CPE procedure for MCP and the content of drug was measured spectrohotometrically at λ_{max} of 479 nm for five repeated measurements.

2.6. Statistical Analysis

Minitab version 17 (Minitab Inc., State College, PA, USA) (29) and Excel 2010 (Microsoft Office®) were employed to carry out all statistical calculations such as regression and correlation analysis, ANOVA and significance tests.

3. Results and Discussion

3.1. Absorption Spectra

In an attempt to ascertain the occurrence of reaction between two drugs in the reaction system, certain amounts of standard solution of AMX and diazotized MCP were mixed in the presence of an alkali medium; an intense orange product was immediately formed showing an absorption maximum at 479 nm (Figure 2A) which was adopted in the optimization conditions of CPE for the two drugs. The absorption spectrum of the azo dye product formed was also recorded against the corresponding reagent blank between 270 to 1100 nm after obtaining optimum conditions according to the recommended CPE procedure using a Shimadzu model UV-1800 equipped with 1.0- cm quartz cell. Figure 2B shows the absorption spectra of azo dye product and the individual pure drugs solutions. It was observed that the absorption maximum of the colored product solution containing $2.24x10^{-5}$ M of AMX and $4.50x10^{-5}$ M of diazotized MCP in 1.0 mL of 10% TX-114 occurred again at 479 nm, giving the molar absorptivities of $2.35x10^5$ and $2.25x10^5$ $L.mol^{-1}.cm^{-1}$ for AMX and MCP drugs respectively. Whilst the individual pure AMX and MCP solutions display absorption maximum at 274 and 272 for AMX and MCP drugs respectively. Thus, the wavelength maximum at 479 nm for the AMX-MCP azo dye product was used throughout this study for micro amounts

determination of both drugs.

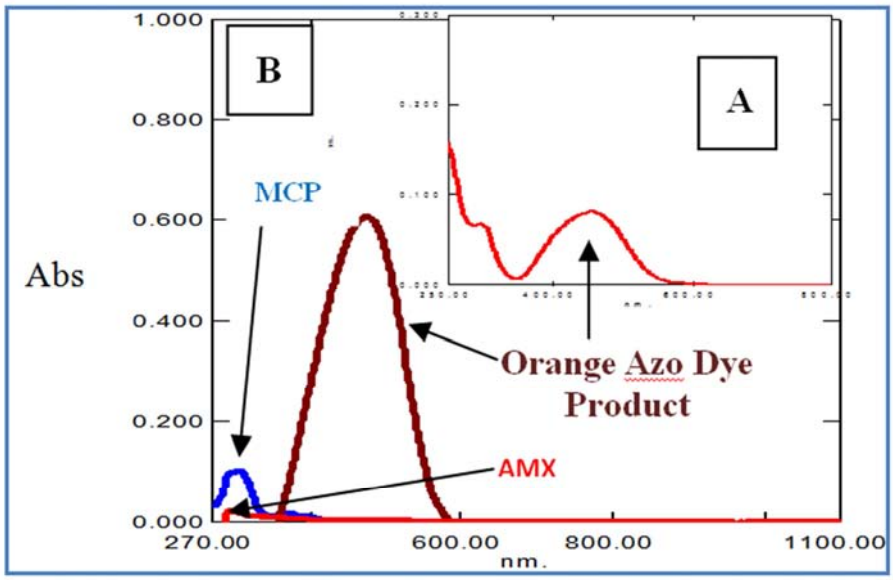


Figure 2. *Absorption spectra of azo dye product (A) before CPE and (B) 2.24x10^{-5} M (approx.1.0 µg mL^{-1}) of AMX or 2.5x10^{-5} M (approx 0.9 µg mL^{-1}) of MCP treated according to recommended CPE.*

3.2. Optimization of CPE Methodology

A group of experiments has been conducted to study the effect of several variables that affect the extraction efficiency of the CPE and maximize the sensitivity of the detection system for both drugs under study using a classical optimization. The variables such as the concentration of alkaline medium, concentration of each reagent, Triton X-114 amount, equilibration temperature and incubation time. It was previously reported that HCl was more satisfactory acid compared with other acids such HNO_3, H_2SO_4, H_3PO_4, and CH_3COOH for diazotization reaction of MCP [30]and 3.0 mL of 1.0 M HCl was found necessary for complete diazotization of this drug [31-32].

3.2.1. The Effect of Alkaline Medium

It was found that the coupling reaction between the drug AMX and diazotized MCP formed in alkaline medium [31]. Consequently, few of the alkaline solutions were tested such as NaOH, KOH, NH_4OH and Na_2CO_3 in two series of the separated experiments by taking 10 mL solution containing 1.0 µg mL^{-1} of AMX and 1x0^{-4} M of diazotized MCP, or 0.88 µg mL^{-1} diazotized MCP and 7.15x10^{-6} M AMX, then 3.0x10^{-3}M of alkaline solution and 0.5% TX-114 were added to each solution. The cloud point extraction was conducted at 65 ºC at 20 min. The results summarized in Table 1 revealed that sodium carbonate was the best alkaline medium for azo coupling reaction between the two drugs used in all subsequent experiments. The effect of different volumes of 0.5 M Na_2CO_3 was investigated by varying its volume between (0.01-0.1 mL) keeping other parameters constant. The results are depicted in Figure 3. It found that the best extraction efficiency for the azo dye product was obtained with 0.06 and 0.02 mL of 0.5 M Na_2CO_3 which equivalent to 3.0x10^{-3}M and 1.0x10^{-3}M $Na_2CO_3$for the determination of AMX and MCP respectively, from which they were chosen as optimal in the next experiments.

Table 1. *Effect of type of alkaline media.*

Type of alkaline medium 0.6 mL of 0.5M in 10 mL solution	Measured at Wavelength (nm)	Absorbance
NaOH	471	0.135
KOH	484	0.108
NH_4OH	397	0.196
Na_2CO_3	479	0.204

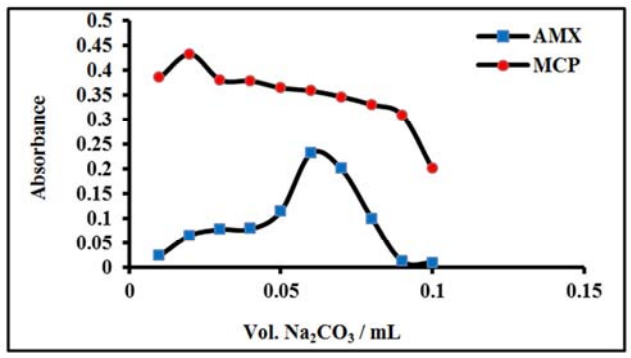


Figure 3. *Effect of volume of 0.5 M Na_2CO_3 on absorbance and extractability of azo dye product. [Conditions: For AMX: 1.0 µg mL^{-1}, diazotized MCP; 1.0x10^{-4}M, TX-114; 0.5%, CPT; 65ºC and 20 min incubation time. For MCP; 0.88 µg mL^{-1}, AMX; 7.15x10^{-6} M, 0.5% TX-114, CPT; 65ºC and 20 min incubation time].*

3.2.2. Effect of the Reagents Concentration

The effect of different concentrations of diazotized MCP on the formation the azo dye product and extraction efficiency of 1.0 µg mL^{-1} of AMX was conducted by varying the volume from 0.02 to 2.00 mL of 5 mM of diazotized MCP, while the concentration of AMX which affect the extraction of 0.88 µg

mL^{-1} MCP drug as the diazotized form was carried out by varying the volume from 0.01 to 0.1 mL of 1.1mM of AMX and keeping other conditions constant. Figure 4 displays that the optimum volume of 5 mM diazotized MCP) was of 0.18 mL (32 µg mL^{-1}) and 0.08 mL of $1.1x10^{-3}$ M AMX (4 µg mL^{-1}) were sufficient to give maximum absorbance, high stability the azo dyes and subsequently the best extraction efficiency for the determination of AMX and MCP drugs in the reaction system. At lower or higher concentrations of each reagent, less intensely colored product was observed so any excessive amount of reagent was not necessary. Therefore, 0.18mL of 5 mM diazotized MCP and 0.08 mL of $1.1x10^{-3}$M of AMX in 10 mL solution were used for further experiments.

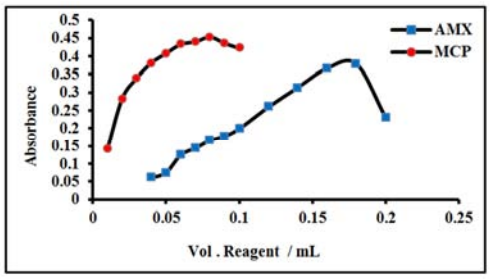


Figure 4. *Effect of the reagents concentration on absorbance and extractability of azo dye product [Conditions: For AMX: 1.0 µg mL^{-1}, Na_2CO_3; $3.0x10^{-3}$M, TX-114; 0.5% CPT; 65ºC and 20 min incubation time. For MCP; 0.88 µg mL^{-1}.Na_2CO_3; $1.0x10^{-3}$ M, TX-114; 0.5%, CPT; 65ºC and 20 min incubation time].*

3.2.3. Effect of Triton X-114 Amount

Most studies confirm that the amount of an nonionic surfactant-type TX-114 as an extracting medium plays an important role for maximizing the extraction efficiency by minimizing the phase volume ratio (Vs/Va) and therefore improving the pre-concentration factor of the CPE procedure [25, 33]. Therefore, the amount of TX-114 was investigated by varying the volume of 10% TX-114 between (0.2-2.0 mL) for AMX and (0.1-1.0 mL) for MCP. The results are presented in Figure 5. It was noticed that the absorbance values of AMX drug continued to increase dramatically and reached maximum at 1.0 mL of 10% TX-144 (i.e. 1.0% TX-114 in 10 mL solution), while there was a marginal increase in the absorbed values for MCP drug and reached maximum at 0.6 mL of 10% TX-114 (i.e. 0.6% TX-114 in 10 mL solution). These values were selected as optimal amount and used in the proposed methods for the detection of AMX and MCP.

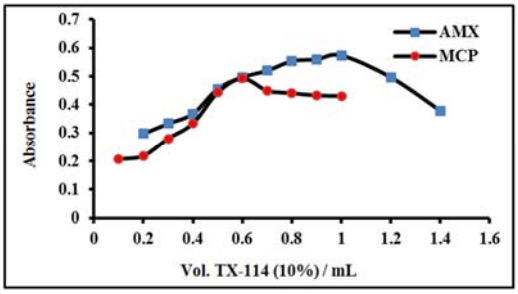


Figure 5. *Effect of the TX-114 amount on absorbance and extractability of azo dye product [Conditions: For AMX: 1.0 µg mL^{-1}, diazotized MCP ; $9x10^{-5}$ M, Na_2CO_3; $3.0x10^{-3}$M, CPT; 65ºC and 20 min incubation time. For MCP; 0.88 µg mL^{-1}, AMX; $9.53x10^{-6}$ M, Na_2CO_3; $1.0x10^{-3}$M,, CPT; 65ºC and 20 min incubation time].*

3.2.4. Effect of Equilibration Temperature and Incubation Time

The influence of these two parameters is considered of the most crucial steps in CPE, in order to ensure the efficient phase separation, which reflects certainly the magnitude of extraction efficiency of each target analyte. Figure 6 shows the variation on the absorption signal via varying the temperature between 25 to 70ºC at 20 min incubation time for both drugs

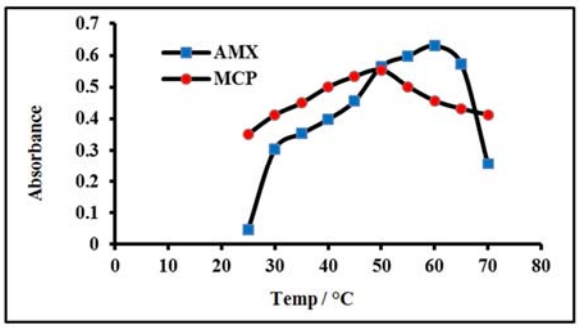


Figure 6. *Effect of equilibration temperature on the absorbance and extractability of azo dye product [Conditions: For AMX: 1.0 µg mL^{-1}, diazotized MCP ; $9x10^{-5}$ M, Na_2CO_3; $3.0x10^{-3}$M, TX-114; 1.0%, 20 min incubation time. For MCP; 0.88 µg mL^{-1}, AMX; $9.53x10^{-6}$M, Na_2CO_3; $1.0x10^{-3}$M, TX-114; 0.6%, 20 min incubation time].*

which proved that the maximum absorption signal of both target analysts was achieved at 60 and 50 ºC for AMX and MCP respectively because of high number of micelles formed in cloud point layer leading the entire transfer of the azo dye product into surfactant-rich phase that maximize the sensitivity. A significant decrease of the absorbance response was observed thereafter, probably due to the instability or dissociation of the azo dye product at higher temperature than optimal. 60 and 50°C were selected and used as optimal in the general CPE procedures of both analytes. Figure 7 illustrates the study of varying of incubation time from 5 to 40 min at optimum temperatures of both analytes. It was found that the incubation times of 25 and 20 min were quite enough for the maximum absorption signal of AMX and MCP in the azo dye product extraction respectively.

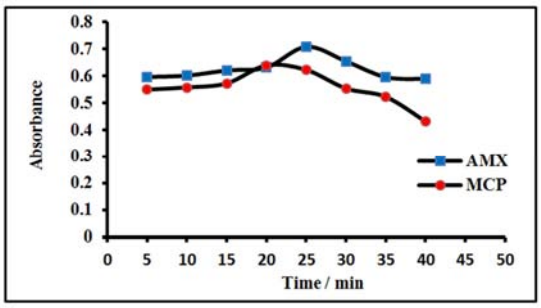


Figure 7. *Effect of incubation time on absorbance and extractability of azo dye product [Conditions: For AMX: 1.0 µg mL^{-1}, diazotized MCP ; $9x10^{-5}$ M, Na_2CO_3; $3.0x10^{-3}$M, TX-114; 1.0%,, CPT; 60ºC,. For MCP; 0.88 µg mL^{-1}, AMX; $9.53x10^{-6}$M, Na_2CO_3; $1.0x10^{-3}$M, TX-114;0.6 % CPT; 50 ºC].*

Table 2 shows a summary for the best values of the study of the experimental variables for the direct determination of AMX drug and back- determination of MCP drug spectrophotometrically through the azo dye formation after CPE method.

Table 2. *The summary of optimum experimental conditions for the extraction of azo dye product by CPE for both drug.*

item	Variable	AMX	MCP
1	Na_2CO_3*	$3.0x10^{-3}$M	$1.0x10^{-3}$M
2	diazotized MCP drug*	$9.0x10^{-5}$ M	-
3	AMX drug *	-	$9.53x10^{-6}$M
4	TX-114*	1.0%	0.6%
5	Temperature	60 ºC	50 ºC
6	Incubation time	25 min	20 min
7	λ_{max}	479	479

*Final concentrations in 10 mL solution that carried out by CPE

3.2.5. Order of Additions

The effect of order for additions of the reagents on the absorbance of each analyte by the general CPE was tested. Table 3 shows that the best order of addition is the number 1 for both target analytes due to giving a highest absorption signal among the others.

Table 3*. Effect of order of additions.*

No.	Addition	Absorbance at λ_{max}=479
1	Analyte + reagent+ Na_2CO_3+ TX-114	0.260
2	Analyte + Na_2CO_3+ reagent+ TX-114	0.037
3	Reagent++ Na_2CO_3+ Analyte+ TX-114	0.044
4	Reagent+Analyte+ Na_2CO_3+ TX-114	0.171

3.3. Structure of Azo Dye Product

It was reported that the analysis of the dependence log D = f (log $C_{REAGENT}$) allows calculating the composition of the azo dyes product by using the slope analysis method [34]. The results depicted in Figure 8 show that the slope for log D as a function of log [Diazotized MCP] is equal to 1.846, indicating that the azo coupling reaction system between AMX and diazotized MCP in alkaline medium with ratio of 1:2 (AMX: Diazotized MCP) extracted into the surfactant-rich phase. Thus the most probable pathway for formation of the extracted azo dye product is preceded by two steps as shown in the Figure 9.

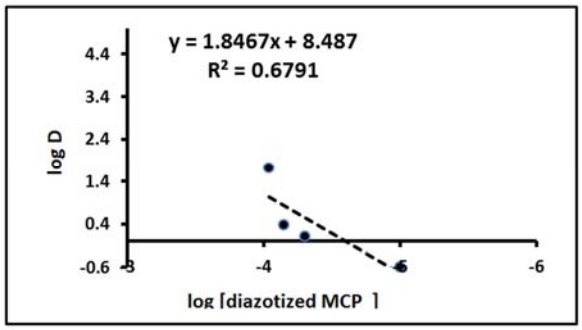


Figure 8. *Slope analysis graph for the determination the composition of azo coupling product in the diazotization reaction AMX with diazotized MCP.*

3.4. Validation of the Analytical Method

The validation of the proposed method for the direct detection of AMX and back-determination of MCP in pharmaceutical samples was conducted via testing the linearity, sensitivity, limits of detection (LOD) quantitation (LOQ), accuracy and precision and other important parameters to achieve the acceptance criteria that applicable for the analysis of both drugs in the samples under study.

3.4.1. Linearity

Nine standard solutions of the drugs AMX and MCP were individually prepared in order to obtain a concentration range from 0.3-3.0 µg mL^{-1} and then subjected to the recommended CPE procedures under the optimized established conditions (Table 2). The graphical presentations of the absorbance plot obtained for AMX and MCP against the concentration of each analyte solution are given in the Figure 10 (a) and (b).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 (MSreg/MSerror) for 1 and 7 dof, larger than critical value (F1, 7= 5.59 at α=0.05), indicating that the predication based on the regression line is satisfactory as listed in Table 4.

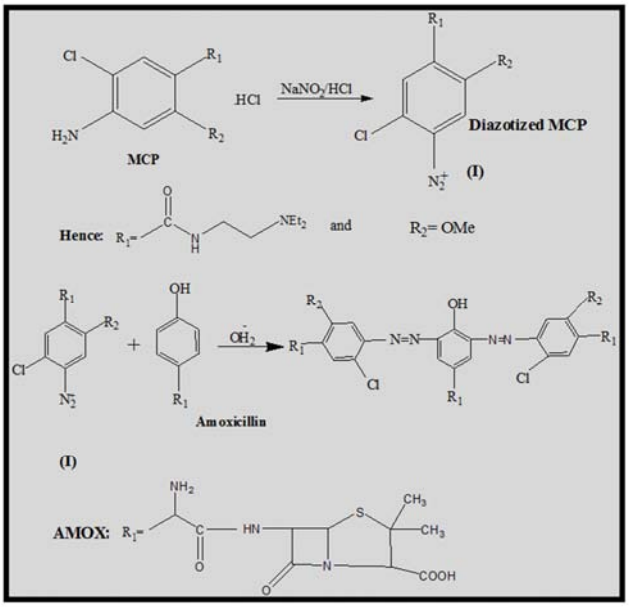
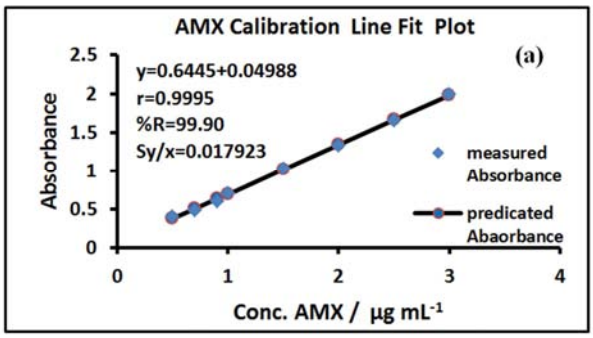


Figure 9. *The probable reaction mechanism between AMX and diazotized MCP in alkaline medium.*



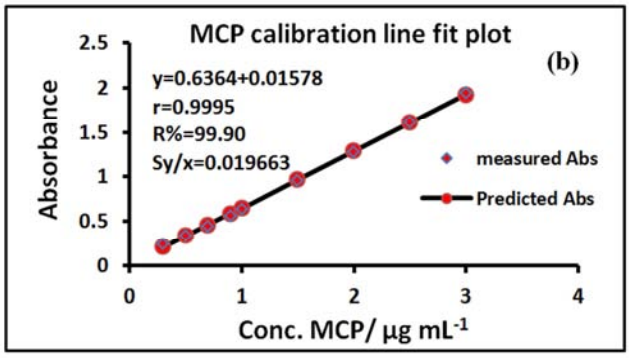


Figure 10. *Calibration curves for (a) AMX and (b) MCP by the proposed method.*

Table 4. *Analysis of Variance of regression line for AMX and MCP.*

Analyte	Source F (p-value)	dof	SS	MS	F	significance
AMX	Regression	1	2.93096	2.93096	9124.79	0.000
	Residual Error	7	0.00225	0.00032		
	Total	8	2.93321			
MCP	Regression	1	2.85722	2.85722	7390.24	0.000
	Residual Error	7	0.00271	0.00039		
	Total	8	2.85993			

Table 5. *Statistical data and analytical figures of merits for AMX and MCP by. CPE- Spectrophotometry.*

Parameter	AMX	MCP
Dye colour	Orange	Orange
λ_{max} (nm)	479	479
Regression equation (9 points)	y= 0.6445x+0.0498	y= 0.6364x+0.0158
Standard deviation of regession line ($S_{y/x}$)	0.0179223	0.019663
Correlation coefficient (r)	0.9995	0.9995
Coefficient of determination (R^2)	99.90	99.90
C.L. for the slope (b± ts_b) at 95%	0.6459±0.05509	0.6364±0.05252
C.L. for the intercept (a± ts_a) at 95%	0.0468±0.09551	0.0158±0.02020
Beer's law range (µg mL^{-1})	0.3-3.0	0.3-3.0
Limit of Detection (µg mL^{-1})	0.083	0.098
Limit of Quantitation (µg mL^{-1})	0.29	0.31
Sandell's sensitivity (µg cm^{-2}) $x10^{-3}$	0.00155	0.00157
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	$2.35x10^5$	$2.25x10^5$
Compsition of the colored product*	1:2	1:2
RSD% (n=5)	3.15 at 0.7 µg mL^{-1}	0.132 at 0.88 µg mL^{-1}
RSD% (n=5)	2.30 at 1.0 µg mL^{-1}	0.029 at 1.50 µg mL^{-1}
Preconcentration factor**	33.3	50.0
Enrichment factor***	214	90.85
Recovery (%)****	98.67±2.31	100.33±5.19
Extraction efficiency (%E)	98.17	98.40
Distribution ratio (D)	53.70	61.50

*Slope analysis method ** Preconcentration factor was calculated the ratio of the original sample volume to that of extracted volume (of surfactant-rich phase) *** Enrichment factor was calculated experimentally by dividing the slop of calibration of the target analyte with CPE to the slope calibration of the target analyte without CPE.****in aqueous solution

3.4.2. Detection, Quantitation Limit and Sensitivity

The evaluation of these parameters was based on the regression line. Thus the limit of detection (LOD) and limit of quantitation (LOQ) are based on the standard deviation of the response (residual standard deviation; $S_{y/x}$)and the slope of the calibration curve using the following equations; LOD = $3\sigma_B/s$; LOQ = 10 σ_B/s, where (σ_B) is the standard deviation from the regression line and (s) its slope. Based on the results of the calibration curve for AMX and MCP drugs, detection limits of 0.083 and 0.098 µg mL^{-1} were calculated, while LOQ's were found to be of 0.29 and 0.31 µg mL^{-1} (Table 5). These low detection limits might be due to obtaining high enrichment factors (214 and 90.85) of both drugs by the proposed method compared to traditional UV-Vis spectrophotometry. Further,

good sensitivities in terms of molar absorptivity (ε) for each target analyte were nearly equal and found to be of $2.35x10^5$ and $2.25x10^5$ $L.mol^{-1}.cm^{-1}$ for AMX and MCP respectively. Concerning the detection limit of AMX drug, the detection limit obtained in the proposed method was better than the reported methods using diazotization reaction (Table 6). Whilst for MCP drug, our findings turned out to be better than that obtained by some authors. But, it is not better than the remainder reports using different coupling reagents as shown in Table 7. In addition, the determination of two drugs sequentially by exploiting the same procedure itself is unique to the chemical analysis method compared with the earlier reported work published in the chemical literature, which focused on estimating on target analyte (Tables 6 and 7)

3.4.3. Accuracy and Precision Study

An approach according to three-point calibration with 1.0, 1.5 and 2.0 µg mL^{-1} for AMX and/or 0.7, 1.0 and 1.5 µg mL^{-1} of MCP standard solutions were chosen in this validation study. All these standard solutions were spiked with accurate amounts of drug matrix solution containing 1.0 µg mL^{-1} for AMX (PAN capsule, France) and MCP (MCP – Hameln injection, Germany) followed the recommended CPE procedure and each solution was measured five times. The results summarized in Tables 8 and 9 have revealed that a satisfactory accuracy in terms of percent recoveries obtained were within average of 97.77± 1.72% for AMX and 98.20± 1.95% for MCP with the 95% confidence interval ranges from 96.05 % to 100.15 %, concluding the theoretical value of 100% is included. It confirmed that the systematic errors were relatively absent, concluding the presence of matrix constituents of these samples had no appreciable effect on the determination of these analytes as the requirement for mean recovery of this validation study is met. Thus the study of interferences from the drugs matrices is unnecessary. Meanwhile, each spiked sample was repeated five times for precision testing in terms of %RSD and found in the range of 2.35-4.35% for AMX and 0.20-3.43% for MCP, indicative a good precision of the method.

Table 6. *Reported methods for the determination of AMX by spectrophotometry after diazotization,oxidative coupling and charge transfer reactions.*

Coupling Reagent Used/ Reaction Type	λ_{max} (nm)	Linearity (µg m^{l-1})	LOD (µg m^{l-1})	Ref.
Benzocain /diazotization	455	2-16	0.240	8
o-nitroaniline/ diazotization	435	1-5	0.125	9
p-nitroaniline/ diazotization	478	0.5-100	0.104	10
o-nitroaniline /diazotization	435	25-400	5.100	11
p-amino benzoic acid procain / diazotization	435 450	0.4-10 0.4-14	0.187 0.192	12
Sulphanilic acid/ diazotization	455	0.3-30	0.150	13
2,4- dinitrophenylhydrazine (DNPH)	515	1-40	0.230	35
4-Aminoantipyrine /Oxidative coupling	510	1-60	0.173	36
N-bromosuccinamid (NBS)+ methylene blue/ oxidation	663	5-50	-	37
2, 4- dinitrophenylhydrazine/ Oxidative Coupling	520	4-33	0.090	38
N,N-dimethyl-p-phenylenediamine and potassium hexacyanoferrate (III)/ Oxidative coupling	600	2-40 10-70	0.637 4.900	39
Metol / Charge transfer	620	5-60	1.494	40
Metoclopramide hydrochloride/ diazotization	479	0.3-3.0	0.083	This work

Table 7. *Reported methods for the determination of of MCP.HCl by spectrophotometry after diazotization, oxidative coupling and charge transfer reactions.*

Coupling Reagent Used/ Reaction Type	λ_{max} (nm)	Linearity (µg m^{l-1})	LOD (µg m^{l-1})	Ref.
dibenzoyl methane/ diazotization	440	-	-	14
Aniline/ diazotization	410	0.5–12.0	-	15
Benzoylacetone/ diazotization	437	0.8-13.2	0.033	16
Imipramine hydrochloride/diazotization	570	0.5-5.0	0.014	17
p-dimethylaminocinnamaldehyde/ diazotization	553	4-24	1.120	18
Phenol/ diazotization	463	1-20	0.406	19
8-hydroxyquinoline /diazotization	528	0.2-12	-	20
Diphenylamine / diazotization	530	0.3-7.5	0.220	21
2,5-dimethoxyaniline (DMA) /diazotization	486	0.1-12	0.016	22
doxycycline hyclate /diazotization	452	0.1-10	0.012	23
Phenoxide / diazotization	462	10-80	3.700	24
malachite green in the presence of 0.01M chloramine-T and 2M H_2SO_4,	623	2-10	0.087	41
Folin–Ciocalteu/ complex formation	760	Up to 100	2.000	42
9-chloroacridine / oxidative coupling	470	2-50	0.368	43
Pyrocatecolin presence of ammonium ceric sulphate /Oxidative coupling	500	5-35	-	44
Amoxicillin /diazotization	479	0.3-3.0	0.098	This work

Table 8. *Accuracy and precision test for AMX capsule by proposed method.*

AMX Taken (µg mL^{-1})	AMX Found (µg mL^{-1})	Recovery (%)	Mean Rec%±C.L at 95%	Er%	RSD% (n=5)
Sample	1.0	-			
1.0	1.94	97.00	97.77± 1.72	-3.00	4.35
1.5	2.45	98.00		-2.00	3.67
2.0	2.95	98.33		-1.67	2.35

Table 9. *Accuracy and precision test for MCP in German Ampoule.*

MCP Taken (µg mL^{-1})	MCP Found (µg mL^{-1})	Recovery (%)	Mean R%±C.L at 95%	Er%	RSD% (n=5)
sample	1.0	-			
0.7	1.693	99.0	98.20± 1.95	-1.0	3.43
1.0	1.945	95.5		-0.5	1.26
1.5	2.516	101.1		1.1	0.20

3.4.4. Applications

i. Determination of AMX drug in pharmaceuticals

The proposed method was applied to the AMX determination in five selected pharmaceutical drugs (one capsule and four vials) produced in different countries containing 500 mg amoxicillin as an active ingredient. The samples were prepared as stated in experimental section from which each sample was subjected to the recommended CPE and AMX drug detected spectrophotometrically at λ_{max} of 479 nm. The results are presented in Table 10. The results revealed that the calculated t-values for AMX determination using MCP drug as diazotization agent in different pharmaceuticals 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.

Table 10. *Determination of AMX drug in tablet and injection samples by the proposed method and statistical comparison with quoted values.*

Commercial name, and content	Practical Content (mg) (proposed method)	t= (x-µ)√n/s proposed method Vs. Claimed value at 95% C.I.	%E$_{rel}$	%RSD (n=3)
Amoxicillin, capsule (Iranian) farabi pharmaceutical,500mg	492 503 483 492.67±10.02	t_{cal}=**1.27** 1.27<4.303	-1.47	2.03
Amoxicillin - AMITRON (Barcelona)LDP Laboratorios TORLAN S.A (Spain), vial 500 mg	488.0 492.0 487.0 486.33±6.03	t_{cal}=**3.93** 3.93<4.303	-2.73	1.24
Amoxicillin (PANPHARMA S.A.,France), vial 500 mg	498.0 483.7 493.8 491.93±7.35	t_{cal}=**1.92** 1.92<4.303	-1.61	1.49
Amoxicillin- cox PHARMACEUTICAL LTD (England), vial 500 mg	491.5 485.0 478.0 484.83±6.75	t_{cal}=**3.89** 3.89<4.303	-3.03	1.39
Amoxicillin Pharma Roth (Germany), vial 500 mg	484.0 489.3 498.2 490.50±7.18	t_{cal}=**2.29** 2.29<4.303	-1.90	1.46

ii. Back-Determination of MCP drug in pharmaceuticals

Three selected pharmaceuticals from different producers in the form of ampoules containing 10 mg per 2 mL of active MCP medicament were examined to test the applicability of proposed method using AMX as mediating agent. Each sample was simply diluted with water to obtain an appropriate concentration from which it was subjected to the recommended CPE procedure and MCP drug determined spectrophotometrically at at λ_{max} of 479 nm. The statistical analysis results exhibited in Table 11 proved again that the calculated t-values for MCP determination in different pharmaceuticals 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.

4. Conclusions

This work presents a new mode of chemical analysis by the combined CPE-spectrophotometry compared with our previous published works, for the mutual determination of the two drugs that participate in chemical derivatization via using azo coupling reaction. The proposed method gave distinct features which appeared the acceptable analytical figures of merit and high reliability compared with other published methods (Tables 6 and 7). Furthermore, the prospect advantages of the established method are time-saving, reducing the amount of reagents used as well as minimizing analyst effort. However, the shortcoming of this method lies in difficulty of estimating these drugs in biological samples (blood and urine) because the presence of some of the

constituents in these matrices may be involved in the azo-coupling reaction which leads to the deterioration of the sensitivity and detection limit of the drugs under study. However, this method can be easily applied to environmental samples, particularly waste water flowing from the medicaments industries.

Table 11. *Determination of MCP drug in the injection samples by the proposed method 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	9.64 9.95 9.66 9.75±0.173	t_{cal}=2.50 2.50< 4.303	-2.5	1.77
Metoclopramide, injection (GLAND PHRMA LIMITED, Indian), 10 mg /2 mL	9.64 9.84 10.0 9.83±0.180	t_{cal}=1.66 1.66<4.303	-1.7	1.83
Metoclopramide - injection (hamelnpharmaceuticals gmbh Langes Feld 13 Germany),10 mg/2 mL	9.90 9.54 10.1 9.85±0.284	t_{cal}=0.94 0.94<4.303	-1.5	2.88

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