Spectrophotometric Determination of Mexiletine Hydrochloride In Pharmaceutical Preparations, Urine and Serum Using Complexing Reagents.

Fadhil M. Najib*, Ahmmad M. Abdullah and Dler M. Salh
Chemistry Department, College of Science, Sulaimani University
Kurdistan Region, Iraq
*E-Mail: fadhilmnster@gmail.com

(NJC)

Abstract

In this work mexiletine hydrochloride (MH) [1-(2,6-(dimethylphenoxy)-2-aminopropane hyrochloride] has been determined spectrophotometrically, using methyl orange (MO) and xylenol orange (XO). The method involved the addition of 1.5ml 0.1% (MO) or 1.2ml 0.05% (XO) reagents to a certain amount of MH, standard or samples, containing between (5-20 μgml^{-1}) MH. The mixture is shaken for (30 sec.) and diluted to about 23ml in case of MO and to 8ml in case of XO in volumetric flasks using distilled water. The pH was adjusted by adding 1ml phthalate buffer pH 2.8 to the MO mixture and finally completed to 25ml, or with NaOH and HCl to pH 5.5 in case of XO and completed to 10ml. The colored ion-pair formed between MH and the reagents were transferred into separating funnels and extracted using 5.5ml CH_2Cl_2 and were shaken for 30 – 60s. After separation, the organic or aqueous layers were used for constructing calibration curves for spectrophotometric measurements of MH at 429nm and 438nm in cases of MO and XO respectively. The blanks were carried out in exactly the same way throughout the whole procedure. Molar absorptivity(ε L.mol^{-1}.cm^{-1}), detection limit, limit of linearity(μg.ml^{-1}) and r^2 were: 4.2×10^3, 0.32, 4 and 0.9961 for (MH-MO) and 2.3×10^3, 1.35, 5 and 0.9961 for (MH-XO) respectively. The method was used with reasonable accuracy and precision of(1.6-3.6 E%) and ($\pm$1-3.6 S.D%) respectively, for the determination of (MH) in synthetic samples of real blood, urine and capsules.

**Keywords:** spectrophotometric determination of mexiletine hydrochloride, methyl orange, xylenol orange.

(MH   ) (Mexiletine Hydrochloride)

)   [1-methyl-2-(2,6-xylyloxy) ethylamine hydrochloride]

  0.1% MO   1.5ml   .((XO)   (MO)

  (5-20 µg.ml^{-1})   MH   0.05% XO   1.2ml

XO   8ml MO   23ml   .(30 s)

MO   1ml Phthalate Buffer   2.8   (pH)   .

.XO   10ml   pH= 5.5   NaOH   HCl   25ml

(MH)   (Ion-Pair)

(30 -60 s) CH_2Cl_2 5.5ml

(MO)   (438 nm)   (429 nm)   MH

. MH   (XO)

4.2*		(r^2)	($\mu g.ml^{-1}$)		(ε $L.mol^{-1}.cm^{-1}$)
0.9961	5	1.35	$2.3*10^4$	(MH-MO)	0.9961 4 0.35 10^3
(%S.D=3-4)	(%E=1.6-3)				. MH-XO

.   MH

Introduction

Mexiletine hydrochloride (MH) [1-(2,6-(dimethylphenoxy)-2-aminopropane hyrochloride] or (Mexitile) [1-9], have the following chemical structure (I).
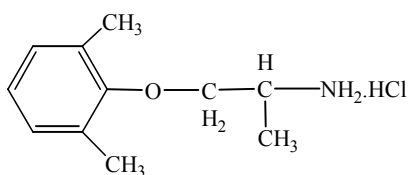
(I)

Mexiletine is one of the lidocaine derivatives that produces cardiac effects similar to lidocaine and are used for outpatient ventricular arrhythmias [1-4]. It has also shown significant efficacy in relieving chronic pain, especially pain due to diabetic neuropathy and nerve injury [5].

Gas-chromatography was the oldest method used for the determination of MH in biological fluids[10]. The method included at least four extraction and re-extraction steps. Later, many GC and HPLC methods for the determinations of this drug and its metabolites have been reported [11-21] attempting different modifications in the method to increase sensitivity, reducing steps of analysis, or other improvements.

Beckett and Chidomere have attempted the improvement of MH analysis and its metabolic products in urine, by applying only one step extraction and obtaining linearity down to (6 or 40 ng ml^{-1}MH) [11]. Other workers [12-21] have applied between 2-4 steps pretreatments and lowered limit of linearity down to 4 ng.ml^{-1}. More recently, capillary zone electrophoresis was developed for separation of 14 antiepileptic drugs and MH was quantified under conditions of optimum separation[22].

Extensive search in the literature has shown only three spectrophotometric methods for the determination of MH [23-25] in which two of them[23,25] where in the UV region. In one assay, the first and second-order derivative measurements with the use of "peak-zero" and "peak-peak" techniques were applied [23].

Visible spectrophotometric technique, however, was developed for the determination of this drug in capsules using bromothymol blue [24], depending on the ion-pair formation. The review presented in this study showed that most of the methods applied were rather complex and expensive, such as GC and HPLC. Less importance was given to the easy

spectrophotometric method, which becomes the aim of the present study.

Preliminary practical tests on many reagents revealed that methyl orange and xylenol orange were two suitable reagents to form colored species with the drug MH and were exploited for its quantitative determination in capsules, ampoules, serum, and urine samples.

Experimental
Apparatus:

All spectral and absorbance measurements were taken with CECIL (3021) spectrophotometer, with 1cm quartz cells. Other equipments were: Hanna pH-meter with combined glass electrode (910600) Orion Comb pH, Hermle Z 200A-Centrifuge, Tafesa Water bath (Hannover-W.Germany), Water bath Thermostat Shaker (GFL 1083) and Micro pipettes (variable and fixed).

Chemicals, reagents, and drugs:

Both A.R. and general purpose reagents were used from [Fluka, Rohm and Haas, GCC (Gainland Chemical Company), and Merck] without further purification. Ordinary distilled water prepared in all glass still and stored in polyethylene container was used.

Mexiletine hydrochloride ampoule(250mg/10ml),[Boehringer Ingelheim], was taken as a stock solution, since it was in its pure form, and no pure powder of the drug could be obtained. other concentrations were prepared by usual dilution.

Methyl orange (MO) and Xylenol orange (XO): 0.05% , 0.1% and 1% were prepared by dissolving 0.05 or 0.1 or 1g sodium salts of the reagents in 100ml distilled water(D.W) in volumetric flasks.

Phthalate buffer (pH=2.8) was prepared by mixing 50ml of 0.1M (potassium hydrogen phthalate), with 28.9 ml of 0.1M HCl [26], and pH was adjusted with a pH meter.

Sodium acetate, acetic acid buffer (pH=5.6) was prepared by mixing 4.8ml of 0.2M acetic acid with 45.2ml 0.2M sodium acetate [26], diluted to 100ml in a volumetric flask by D.W, and pH was adjusted with a pH meter.

Citrate buffer (pH=5.49) was prepared by mixing 25ml of 2M NaOH with 10ml of 2M citric acid [26], and diluted to 100ml in a volumetric flask with D.W, and pH was adjusted with NaOH or citric acid. Phosphate buffer (pH=5.8) was prepared by mixing 4ml of 0.2M di-sodium hydrogen phosphate (Na_2HPO_4) with 46ml of 0.2M sodium di-hydrogen phosphate[26], diluted to 100ml in a volumetric flask with D.W, followed by pH adjustment.

Different solutions of other compounds were prepared for interference studies by dissolving the appropriate weights of the corresponding salts in D.W and completing to 250 ml in a volumetric flask with D.W. in the usual way.

Deproteinization and sample treatment:
Five mls of venous blood or Urine samples were drawn and the blood samples were allowed to stand for 15 minutes at room temperature until it had clotted. The serum was separated by centrifugation at 3000 rpm for 10 minutes. Three mls of 0.15M $Ba(OH)_2$ were added to 1ml of the serum or urine in a test tube followed by 3ml of 2.5% $ZnSO_4.7H_2O$. The solution was well mixed, closed and centrifuged. The clear supernatant liquid was used for the determination of MH [27].

The recommended procedures:

A volume of [1.5ml 0.1% (MO) reagent] or [1.2ml 0.05% (XO) reagent] were added to a certain amount of MH standard or samples containing between (4-20 μgml^{-1}) or (5-20 μgml^{-1}) MH in cases of MO and XO, respectively. The mixture was shaken for 30 sec. and diluted to about 23ml in case of MO and to 8ml in case

of XO in volumetric flasks using D.W. The pH was adjusted by adding 1ml phthalate buffer (pH 2.8) to the MO mixture and finally completed to 25ml, or with NaOH and HCl in case of XO to pH 5.5 and completed to 10ml. The resulting complex formed between MH and the reagents were transferred into separating funnels (100ml capacity) and extracted using 5.5ml CH_2Cl_2 in two portions to wash out the volumetric flasks for quantitative transfer of the solution in both cases and were shaken for 30 – 60s. After separation, the organic or aqueous layers were used for drawing calibration curves for spectrophotometric measurements of MH at 429nm and 438nm in cases of MO and XO respectively. The blanks were carried out in exactly the same way throughout the whole procedure.

Results and Discussion
Absorption Spectra:

The absorption spectra of the ion-pairs (MH-MO) and (MH-XO) against blank and of the reagents MO and XO against D.W, are shown in Figures (1a, b) showing λ- max of 429 nm and 438 nm. respectively. A clear spectrum of the MH-MO with no observed shoulder is seen in Fig. 1a, with a hypsochromic shift of about 154 nm from that of the reagent MO alone. The spectrum of the complex showed a second small peak which is due to the excess of the reagent. The spectra also show some background of the reagent in the region of the complex which will have a negative effect on the sensitivity of the method. This great shift of (λ-max.) is also an indication of the reaction taken place between MO and MH.

The spectra of the complex with MH as (MH-XO) and XO alone are shown in (Fig.1b). The spectra are different from those of MO and (MH-MO), first, no shift between the λ-max. of the reagent XO and its complex with MH is observed while the other is the appearance of some shoulders due to many steps of dissociation of XO.

The complex; or an ion-pair formation between the two, has caused an increasing intensity of the spectrum. This is certainly unfavoured analytical phenomenon; since no high sensitivity could be expected with this system. However, the two reagents were expected to show promising results therefore, studies were continued for optimization of the conditions.
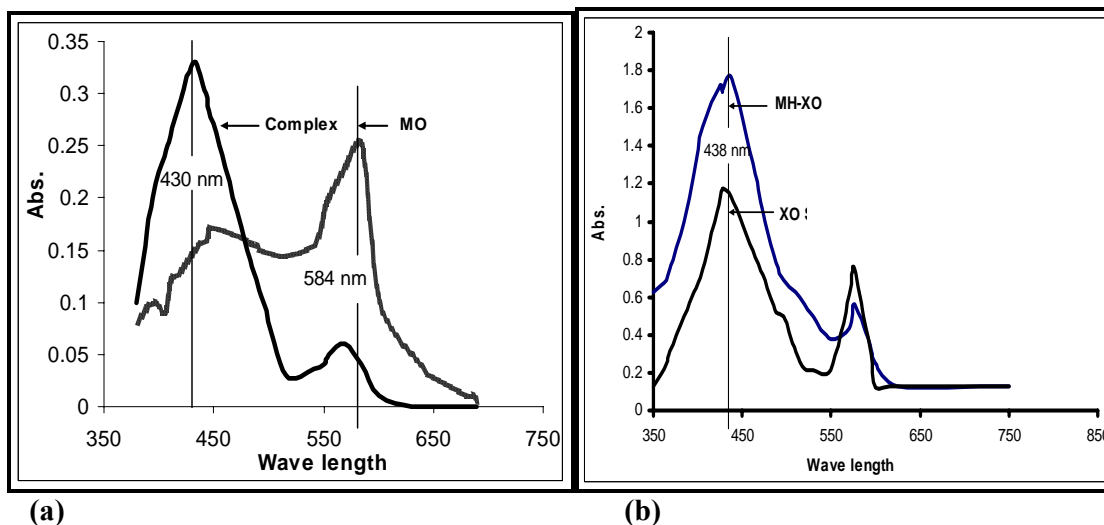
  (a)   (b)

Figure 1: The spectra of (a) MO alone against D.W. and (MH+MO) complex against blank (b) XO alone against D.W. and (MH+XO) complex against blank.

pH Optimization

A volume of 250μL 1% reagent (either MO or XO) were added to 2ml of 0.216 mg.ml^{-1} MH, shaking for 30 seconds, then diluted to 25ml in case of MO, and to 10ml in case of XO in volumetric flasks. The pH was then adjusted between 2 to 4 and 4 to 9 for both MO and XO respectively, by using 0.1M NaOH or HCl. The rest of the test was then followed according to the procedure. The results reveal that the constant pH ranges for (MH-MO) and (MH-XO) ion-pairs are between 2.5 to 3 and 5.2 to 6 respectively, as shown in Fig.2. The optimum pH chosen for all subsequent experiments were 2.75 for (MH-MO) and 5.5 for (MH-XO).
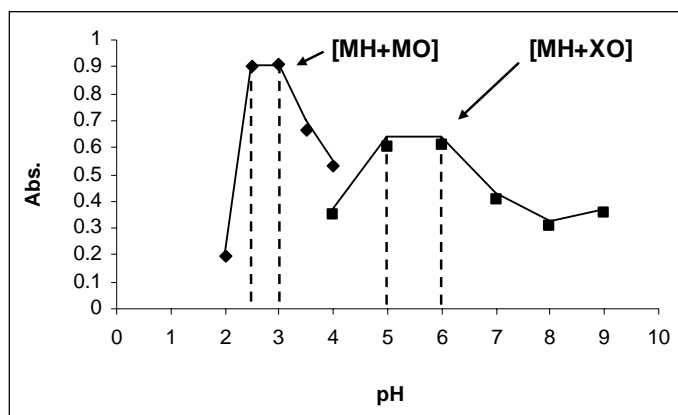

Figure 2:The pH optimization for (MH+MO) and (MH+XO) complexes

Choosing a Suitable Solvent for Extraction:

Many solvents were tested for extracting the ion-pair formed between the reagent (MO and XO) with (MH), and the best choice for both systems was found to be dichloromethane.

Optimum amounts of the Reagents:

Initial experiments showed that 0.1% MO and 0.05% XO were suitable. Experiments were then performed with different volumes of the chosen concentrations to a constant volume 2ml 0.216 mg.ml^{-1} MH. The results shown in Fig.3 indicate that optimum volumes were 1.5ml for MO and 1.2ml for XO .
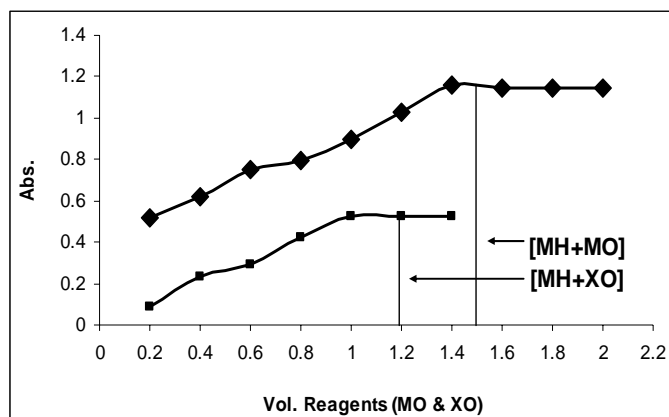

Figure 3:Optimization of the volume of 0.1% MO and 0.05% XO reagents.

Volume optimization of dichloromethane CH$_2$Cl$_2$:

Different volumes of the solvent dichloromethane between 4 to 10 mls and 0.5 to 7 mls were used for extraction of the complexes (MH-MO and MH-XO) and (Fig.4)shows wide ranges between 4 – 8 mls and 5 – 7 mls for both respectively. A volume of 5.5ml CH$_2$Cl$_2$ in both cases was found suitable and also sufficient to complete the analysis.
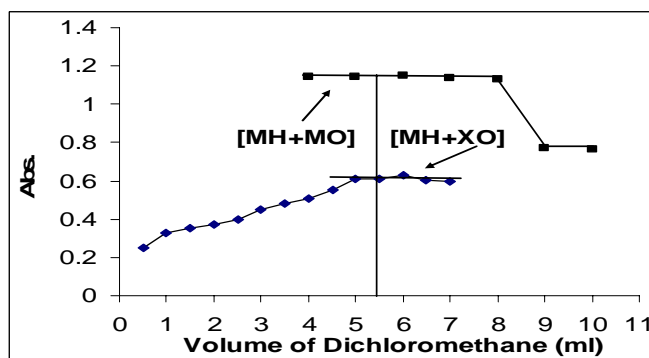

Figure 4: Optimization of CH$_2$Cl$_2$ volume to be added for extraction of the complexes (MH-MO) and (MH-XO)

Using a buffer for pH adjustment:

Phthalate buffer pH = 2.8 [26] was found suitable to adjust pH of the complex (MH-MO). Different volumes of this buffer were added in two ways; either before completing the volumetric flask by D.W to the mark, or until a small volume about 2ml was remaining then the buffer was added and completed to the mark. The results indicated that optimum volume and suitable time of addition were equal to 1ml phthalate buffer followed the addition of D.W until 2ml remaining to be completed with the buffer to the mark.

For the pH adjustment of (MH-XO) system different buffers were tried, such as; acetate, citrate and phosphate buffers, but none of them was suitable. Results showed that red colors for both the blank and the analyte solutions of same absorbance were produced. Therefore, pH adjustment in this case was performed with 0.1M HCl or NaOH.

From this study it was found better that MH was mixed with XO both having pH=5.5, and then the volume was completed to 10 ml with D.W of pH=5.5 also. These results show that each component that participated in the reaction between MH and XO must have pH = 5.5 before entering the ion-pair formation. This precaution in both cases may be due to the narrow pH-range of measurements as was shown in Fig.2.

Stability of the complexes:

The stability of the complexes formed between (MH & both MO&XO) was followed by measuring absorbance against time. As shown in (Fig.5a and b). It was found that the complex (MH –MO)was stable for a period of 35 minutes, after separation and only 5minutes were needed to reach the true absorbance. While the complex (MH with XO)is stable during the time range between (15-35) minutes. Absorbance has, then decreased after that due to the dissociation of the complexes.

(a)   (b)
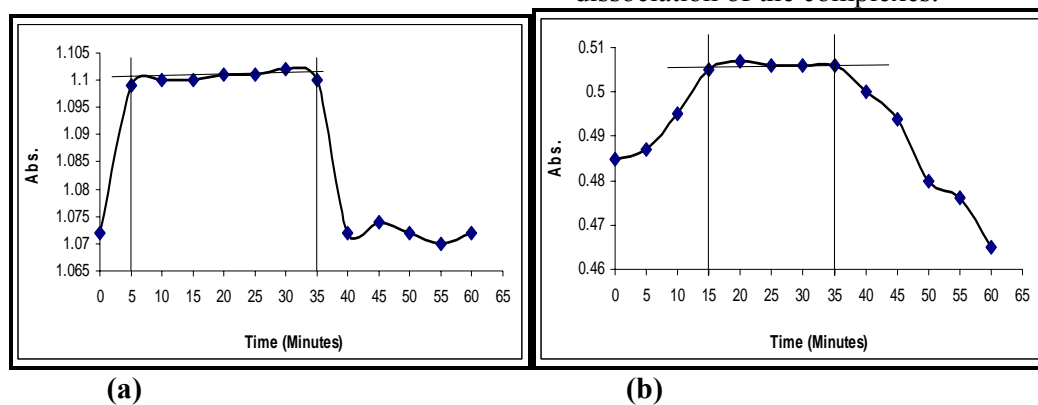
Figure 5: Stability of the complexes after separation (a) (MH -MO) and (b) (MH -XO)

Selectivity of the methods:
Study of interferences:

Table 1 shows the result of the study of interfering effects of the most possible ions present in significant quantities in blood, urine and also

those additives usually used with capsules and ampoules, on the determination of MH in those samples. The study was performed on 4 ppm, and 5 ppm of MH in cases of MO and XO respectively, which can cause not

more than 5% error at their maximum levels and referred to it as a tolerance level. The cations chosen were (Na+, K+, $Ca^{2+,}$ $Mg^{2+,}$ and Fe^{3+}) in the forms of (Cl^-, HPO_4^{2-}, HCO_3^-, and NO_3^-). Generally the effect of the interferences were rather strong, ranged between 1 – 16 fold tolerance level in case of MO and 2 – 20 fold with XO. It was also found that the effect is mostly due to the cations. This is confirmed by examination of the first five cations in the table (Na^+, K^+, $Ca^{2+,}$ $Mg^{2+,}$ and Fe^{3+}) all in chloride form showing that Na^+ has higher tolerance level than K^+ and Ca^{2+} more than Mg^{2+} ,although they have the same Cl^- and NO_3^- more effective than Cl^- . The tolerance levels of these ions with both reagents (MO and XO) were of little variation in value and direction (i.e. + or -). Most of the interferences were of negative direction, apparently both cations and anions have close interfering effects which made the overall also effective either (+) or (-).

Table 1 also shows the effect of the compounds [urea, starch, glucose, fructose, and sucrose], which are either present in blood or urine or as an additive to pharmaceutical preparations. It was found that very small interferences were observed, in which their tolerance levels ranged between 4000 – 25000 folds. It was also found that interferences of the cations [Na^+, K^+, Ca^{2+}, and Mg^{2+}] were more effective in the (NO_3^-) forms.

For removing cation interferences, the principle of blank compensation was tried by preparing, what the authors named, a suppressing solution (S.S.).

The basic principle of this idea is that, those interferences which are expected to be present in the samples are also added to the calibration standards and to the blank. The net result is subtraction of their effects from the samples. This solution will be added only when the blood and urine samples are analyzed. Ampoules are usually pure, and Table 1 showed that the additives, if present in capsules, will not be effective because of their high tolerance levels.

Table 1: Tolerance levels of interferences on 4 ppm, and 5 ppm of MH in case of MO and XO respectively, which cause not more than 5% error at their maximum levels. N.B: Tolerance level = [Interference] / [Analyte].

Cations	Forms (salts) of cations	Tolerance level (folds) in case of MO	Tolerance level (folds) in case of XO
Na^+	NaCl	+1	+1
K^+	KCl	-0.5	+0.4
Ca^{2+}	$CaCl_2.6H_2O$	-0.75	-0.6
Mg^{2+}	$MgCl_2.6H_2O$	-0.75	-0.4
Fe^{3+}	$FeCl_3$	-0.5	-0.8
Na^+	NaHCO3	+4	-4
K^+	$KHCO_3$	-2	-3.5
Na^+	Na_2HPO_4	+1.25	-1.2
K^+	K_2HPO_4	-0.5	-0.6
Ca^{2+}	$CaHPO_4.2H_2O$	-1.25	-1
Na^+	NaH_2PO_4	+1.25	+1.2
K^+	$KH_2PO_4.2H2O$	-1.25	-1
Ca^{2+}	$Ca(H_2PO_4)_2$	-0.5	-0.6
Na^+	$NaNO_3$	+0.5	-0.5
K^+	KNO_3	-0.25	-0.4
Ca^{2+}	$Ca(NO_3)_2.4H_2O$	+0.5	-0.4
Mg^{2+}	$Mg(NO_3)_2.6H_2O$	+0.5	-0.4
Fe^{3+}	$Fe(NO_3)_3.9H_2O$	-0.75	-0.4
Urea	$(NH_2)_2CO$	No effect in the range 1 to 5000	No effect in the range 1 to 5000
Glucose	-------	No effect in the range 1 to 1000	No effect in the range 1 to 800
Fructose	-------	No effect in the range 1 to 2500	No effect in the range 1 to 2000
Sucrose	--------	No effect in the range 1 to 2500	No effect in the range 1 to 2000
Starch	---------	No effect	No effect

Extensive experiments showed that 2.5ml. of these ions at the following concentrations gave reasonable results for both(MH+MO) and (MH+XO) complexes. These concentrations were (2900ppm Na^+, 1250ppm Fe^{3+}, 375ppm K^+, 92ppm Ca^{2+} and 1ppm Mg^{2+}) all in nitrate forms.

Calibration Curves :

The calibration curves carried out according to the recommended procedure were drawn for both (MH + MO) and (MH + XO) ion pairs in the presence of 2.5ml (S.S.). They were found linear in the range of 4 – 20

$\mu g.ml^{-1}$ of MH with ($r^2 = 0.9935$) for (MH + MO) ion pair as shown in (Fig.8a), and 5 - 20 $\mu g.ml^{-1}$ of MH with ($r^2 = 0.9936$) in case of (MH + XO) as shown in Fig. 8b. Lower and higher concentrations from these ranges lead to deviation from linearity.
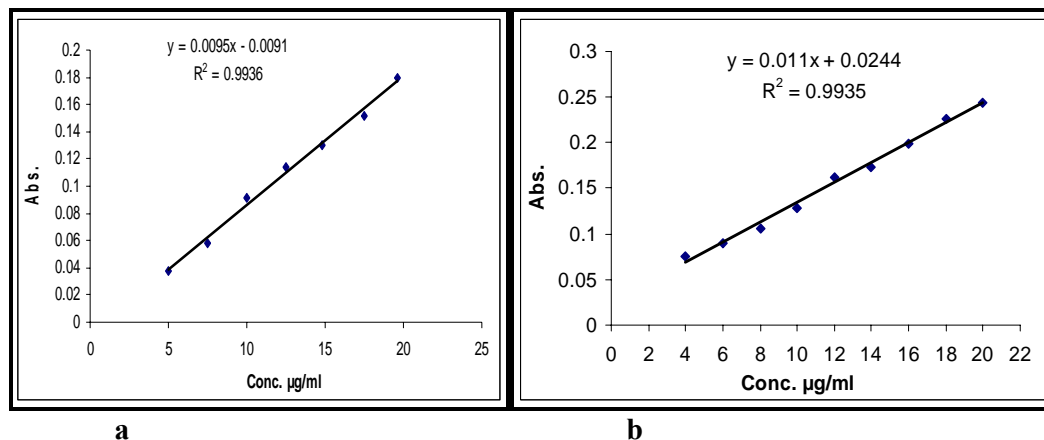

a   b

Figure 8: Calibration curves, (a) for (MH+MO) and (b) for (MH+XO) complexes in the presences of suppressing solution

Determination of mexiletine hydrochloride in synthetic sample solutions:

The accuracy based on the recovery of known concentration of MH for both reagents (MO and XO), with and without suppressing solution is shown in Table 2. The reagent (XO) gave reasonable accuracy with and without

(S.S.) so that the error was always less than 3%. On the other hand, the reagent (MO) gave lower accuracy when no (S.S.) is used while the error becomes similar to those obtained with (XO) when (S.S.) was used. This will indicate that interferences are more effective in case of (MO) and that (XO) has better selectivity.

Table 2: The accuracy of MH determination in synthetic samples using MO in the presence of suppressing solution

MO Reagent + No Suppressing Soln.				MO Reagent + Suppressing Soln.		
MH Present ($\mu g/ml$)	MH Found ($\mu g/ml$)	R%	E%	MH Present ($\mu g/ml$)	R%	E%
4.00	3.79	94.75	-5.25	3.96	99.0	-1.0
4.00	3.79	94.75	-5.25	4.05	101.25	1.25
4.00	3.85	96.25	-3.75	4.05	101.25	1.25
XO Reagent + No Suppressing Soln.				XO Reagent + Suppressing Soln.		
10.00	10.09	100.9	0.9	10.22	102.2	2.2
10.00	10.27	102.7	2.7	10.11	101.1	1.1
10.00	10.27	102.7	2.7	10.22	102.2	2.2

Determination of mexiletine hydrochloride in capsules:

In preparation of capsules, MH has been removed from the additives to make a solution. Therefore MH could be determined by normal calibration curves in both cases, without a need to the (S.S.). Three different volumes (0.25, 0.5, and 0.75 mls) of the sample of MH were determined by MO, and another three (0.15, 0.20, and 0.25mls) of the same sample by XO according to the recommended procedure. The results are shown in tables 3.

To test for the existence of a systematic error in the results shown in tables 3, the actual difference between (x) and (μ) was compared by t-test with the term [t.S / √N] at 95% confidence limit DOF = 2.

Table 3: Results of different volumes of MH sample (capsules) determined by MO and XO reagents.

Concn. Of MH μg.ml⁻¹ found in Capsules by MO using the Eqn. y= 0.0194 x + 0.0074				Concn. Of MH μg.ml⁻¹ found in Capsules by XO using the Eqn. y = 0.0108 x - 0.015			
Vol.(ml) of the sample taken	MH μg.ml⁻¹	R%	E%	Vol.(ml) of the sample taken	MH μg.ml⁻¹	R%	E%
0.25	6.27	94.43	-5.57	0.15	9.82	98.60	-1.40
0.50	13.07	98.42	-1.58	0.20	12.57	94.65	-5.35
0.75	19.15	96.10	-3.90	0.25	16.21	97.65	-2.35

From the results of t-test for both MO and XO reagents the following conclusions were made:

The unidirection of the errors (-) suggest the existence of a systematic error. This may be due to the extraction steps. Non dependence of these errors on sample size between 0.25 to 0.75 mls suggests the existence of both constant and proportional systematic errors.

The difference between ($\overline{x}$ - μ) and [t.s / √N] was not significant at 95% confidence limit (C.L.) in case of 0.25ml and 0.5ml sample when MO was used and both 0.15ml and 0.25ml in case of XO, indicating the non existence, or presence of a very small systematic error.

In all other cases the error was significant at 95% C.L. and not significant at 99% C.L. These again suggest the existence of small systematic error which is mainly due to the extraction steps.

If the recovery tests were considered only as it is the case with research works usually seen in the literature, the value of (R %) in both tables are quite reasonable giving sufficient validity to the methods.

Determination of MH in human serum and urine:

Patients using this drug could not be found in Sulaimani area to obtain real samples, instead, they were synthesized by addition of certain amounts of MH to specimens of serum and urine taken from normal persons, not taking MH or other drugs. MH was then determined by the recommended procedures in two ways, before and after deprotonization. This, of course, is similar to the determination of unmetabolized MH

residue in serum or excreted from the body with urine.

Table 4 represents the application of the reagents MO and XO for the determination of MH added to three different samples of serum and urine before and after deprotinization. The aims of these experiments were to establish the effect of deprotinization as well as the use of (S.S.) on the performance of the two methods, by using t-test. The difference between the means of any two pairs of the results $(\overline{X}_1-\overline{X}_2)$ of Table 4, was compared with the value obtained from the term $[t.S_{pool}x(\sqrt{N_1+N_2} / N_1N_2)]$ at 95% confidence limit(C.L.), DOF = 10. This test was performed after assurance that there was no significant differences between the Variances(S^2)

of the corresponding pairs using F-test as shown in the Table.

The t-test showed that the difference between the means before and after deprotinization was not significant at 95% (C.L.).for the determination of MH using MO or XO reagents in the absence and presence of (S.S.) indicating no effect of deprotonization on their performances.

The other important comparison in Table 4, has showed that the difference between the two means before and after the addition of (S.S.) was significant when MO was used and not significant when XO was used at 95% C.L.DOF 10. These results reveal that the addition of (S.S.) in case of MO is necessary to remove interferences while this is not required in case of XO showing higher selectivity.

Table 4: Determination of MH added before and after deprotinization (DP) for different serum and urine samples by MO and XO reagents, with and without S.S

One ml of prepared samples	Concn. (µg/ml) by [MO Method]				Concn. (µg/ml) by [XO Method]			
	Before DP		After DP		Before DP		After DP	
	No S.S	With S.S	No S.S	With S.S	No S.S	With S.S	No S.S	With S.S
S1 serum	3.74	4.14	3.89	3.96	8.43	8.9	10.18	9.90
S2 serum	3.74	3.96	3.84	4.05	8.61	8.65	10	10.22
S3 serum	3.70	3.87	3.70	4.05	8.7	8.33	10.27	10.00
S1 urine	3.84	4.05	3.84	3.96	8.52	8.65	10.09	10.11
S2 urine	3.7	3.78	3.74	3.96	8.7	8.53	10.09	9.80
S3 urine	3.84	3.96	3.74	4.14	8.61	8.33	10.27	10.11
F-Calculated	3.894< 5.05		1.040< 5.05		3.894< 5.05		2.009< 5.05	
F-(5,5)Table	This value for all was 5.05 at 95% Confidence Limit. Thus the differences between the Variances (S^2) are not Significant at 95% C.L							

Recovery tests in biological fluids with and without (S.S.):

The recovery tests were performed for the determination of MH in serum and urine before deprotinization. A certain volume of MH was added to all samples so that the true value was

Table 5: The accuracy of the recovery of a known amount of MH (4.0 μg/ml) added to samples of serum and urine before deprotinization and determined by MO or XO reagents.

Type of Calibn.		For MO reagent			For XO reagent		
		S_1 serum	S_2 serum	S_3 serum	S_1 serum	S_2 serum	S_3 serum
Normal Calibn.	R%	94	98	96.8	93.63	96.63	95.8
	E%	-6	-2	-3.2	6.37	6.37	4.2
Calibn. in the presence of S.S	R%	94.5	94.5	90	92.84	95.23	94
	E%	-5.5	-5.5	-10	-7.16	-4.77	-6
		S_1 urine	S_2 urine	S_3 urine	S_1 urine	S_2 urine	S_3 urine
Normal Calibn.	R%	91.5	95.25	95.25	95.8	96.82	93.63
	E%	-8.5	-4.75	-4.75	-4.2	-3.18	-6.36
Calibn. in the presence of S.S	R%	96.75	92.5	90	95.23	92.84	95.23
	E%	-3.25	-7.5	-10	-4.77	-7.16	-4.77

Calculated to be 4μg.ml^{-1} and 8.8μg.ml^{-1} in both cases of MO and XO respectively. The purpose was to test for the accuracy of the method and Table 5 shows that although few high errors reaching 10% are observed, but most of the results are lower. The unidirection of the error also indicate the presence of a systematic error, which may be due not only to the extraction steps but also to the deprotinization procedure. But as a whole it is indicated that the method can be used for the determination of MH in serum of those who take this drug for treatment.

Precision and Accuracy:

The precision of MH determination by both reagents MO, and XO was performed on three synthetic samples containing between 5 – 18 (μg/ml) MH, and their absorbance values were measured 10 times for the same unknown, showing the precision of measurements. The precision was also found on 10 times repeating of the whole operation on the same sample. This will show the precision of the operation.   The relative standard deviation for the (MH-MO) ion-pair ranged between 1 – 3.6% while that for (MH-XO) ion-pair ranged between 0.7 – 4.1% showing reasonable precision even at lower concentrations of MH.

The accuracy shown in the previous sections also revealed reasonable accuracy giving sufficient validity for the application of both reagents to be used for the determination of MH in blood, Urine and pharmaceutical preparations.

Sensitivity of the methods:

Tables 6 shows the results concerning sensitivity of the methods, values of molar

absorptivity (ε), slopes of the calibration curves (m), limits of linearity and detection limit (D.L= 3 S.D). The results indicate reasonable sensitivity of the methods and that MO is more sensitive for the determination of MH than XO. This is quite reasonable considering the difference between the spectra of both complexes shows in Fig.1. The Table also indicates that the suppressing solution has lowered sensitivity, therefore, it should only be used when it is necessary.

Table 6: Sensitivity of the methods.

Type of calibration	ε (L.mol^{-1}.cm^{-1})	Limits of linearity (μg/ml)	D.L.=3SD ppm	r^2
Normal calibration curve for (MH+MO) ion-pair	4.2×10^3	4	0.32	0.9961
Normal calibration curve for (MH+XO) ion-pair	2.3×10^3	5	1.35	0.9961
Calibration curve in the presence of (S.S.) for (MH+MO) ion-pair	2.4×10^3	4	0.32	0.9935
Calibration curve in the presence of (S.S.) for (MH+XO) ion-pair	2.0×10^3	5	1.35	0.9936

A Comparison of the Data of the Present Work with Those of Reference 24:

Attempts to apply the published method [24] for comparison with the present method has

failed, since it did not give any results, therefore only the data obtained in that paper were used, as shown in table 7. Although the paper [24] presents data of better results, but there are a number of drawbacks in it. The calibration presented has only six points, two of them in the erratic position (1 and 1.2) so they are well apart. While in the present work (7-9) points well close together all in the acceptable region. The table also shows that, the published work has neither included interference studies nor has applied the method to blood and urine samples, as it is the case in the present work. From all these, the present work is superior and more reliable.

Table 7: Comparison of the Data of Present Work With Those of Reference 24

The Method	Linear Range μg.ml^{-1}		Point Nos. in good region	r^2	Interference Study	Accuracy E% or Recovery %	Precision S.D.%	Applied to
Ref.24	1.08-10.08		4	0.9998	Nil.	100.06 ± 1-02		Capsules only
Present Method	MO	4-20	7	0.9961	23 Species	1.6 – 5.6	1 – 3.6	Blood, Urine and Capsules
	XO	5-20	9	0.9961		1.4 – 5.3	0.7 - 4	

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