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Spectrophotometric Determination of Amiodarone Hydrochloride in Pharmaceutical Preparations

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Abstract

Amiodarone hydrochloride (AH) has been determined spectrophotometrically Using methyl orange (MO). In our previous researches MO was used for determination of Mexiletine Hydrochloride [1]. The method based on complexation between MO and AH. After shaking and diluting the complex solution with D.W, the pH was adjusted with NaOH and HCl to pH 3. The colored complex formed between AH and the reagent were transferred into separating funnels and extracted using 5.5ml CH_2Cl_2 and were shaken for (5 minutes). The extracted organic layer was used for preparation of the calibration curves for spectrophotometric measurements of AH at 434nm. 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 ($\mu g.ml^{-1}$) and r^2 were, 5.74 $*10^5$, 0.04 and 0.982 for (AH-MO) respectively. The method was used with reasonable accuracy and precision for the determination of (AH) in synthetic samples of tablets, capsules and ampoules.

Keywords: spectrophotometric determination of amiodarone hydrochloride, methyl orange.

Introduction

Amiodarone 2-butyl-3-benzofuranyl-4-[2-(diethyl amino) ethoxy] -3-5diiodophenyl ketone or (Cordarone), have the chemical structure as shown in (Fig.1):

It was introduced as an antiaginal agent. It has very pronounced class III action and is especially effective in maintaining sinus rhythm in patients who have been treated by direct current shock for a trial fibrillation. Like class III antiarrhythmic drug, amiodarone lengthens the effective refractory period by prolonging the action potential duration in all myocardial tissues. Amiodarone is eliminated very slowly from the body, with a half-life of about 25 to 30 days after oral doses [2].

The oldest chromatography method in this review was used for amiodarone metabolites in biological fluids [3] using Liquid-Solid Extraction and HPLC techniques, appeared in (1988). Later, many chromatographic methods for the determinations of this drug and its metabolites have been reported [4-9] attempting different modifications in the method to increase sensitivity, reducing steps of analysis, or other improvements, with the limit of linearity ranged between [0.05 mg ml^{-1} – 20 mg ml^{-1}].

During the literature review found that there was a method for determination of amiodarone using Amiodarone-selective membrane electrode [10], they applied a liquid membrane electrode based on an amiodarone-dipicrylamine ion-pair complex

Extensive search in the literature has showed many spectrophotometric methods for the determination of MH [11-14]. Among them, how used derivative UV spectrophotometry, and visible spectrophotometry, with different type of optimizations to obey beers law.

Preliminary practical tests on many reagents revealed that methyl orange which the chemical structure as shown in the (Fig.2), was suitable reagents which have the following chemical structure [15] to form colored complexes with the drug AH and were exploited for its quantitative determination in capsules, ampoules, serum, and tablets.

Experimental

Apparatus

All measurements were taken with TU-1800S UV-Vis spectrophotometer, with (1cm) path length quartz cells. The pH of the solutions was adjusted by using Hanna pH-meter with combined glass electrode (910600) Orion Comb pH, Water bath Thermostat Shaker (GFL 1083) was used during the extraction steps and Micro pipettes (variable and fixed).

Chemicals, reagents, and drugs

Both Analar 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. Amiodarone hydrochloride ampoule (150mg/3ml), [sanofi ~ synthelabo], was taken as a stock solution; other concentrations were prepared by usual dilution. Methyl orange 0.005% aqueous MO. Phthalate buffer (pH=3) was prepared by mixing 50ml of 0.1M (potassium hydrogen phthalate), with 22.3 ml of 0.1M HCl [16, 17], and pH was adjusted with a pH meter.

The recommended procedures

A volume of 0.2ml 0.1% MO reagent was added to a certain amount of AH standard or samples containing between (0.04-0.22 $\mu g ml^{-1}$) AH. The mixture was shaking for (30 sec.) and diluted to 23ml in volumetric flasks using D.W. The pH was adjusted by adding 0.5ml phthalate buffer (pH 3) to the MO mixture and finally completed to 25ml. The resulting complex formed between AH and the reagent were transferred into separating funnels (100ml capacity) and extracted with 5.5ml CH_2Cl_2 in two portions to wash out the volumetric flasks for quantitative transfer of the solution and was shaken for 5 minutes. After separation, the organic layer was used for preparation of the calibration curves using spectrophotometric measurements of AH at 434nm. The blanks were carried out in exactly the same way throughout the whole procedure.

Results and Discussion

Preliminary work

The absorption spectrum of the complex (AH-MO) against blank is shown in Figure (3) showing λ- max 434nm. A clear spectrum of the AH-MO with no observed shoulder and blank spectrum are seen in Figure (3). The spectrum also shows some background of the reagent in the region of the complex which will have a negative effect on the sensitivity of the method.

The complex; or an ion-pair formation between the AH and MO, has show an increasing intensity of the spectrum. This is certainly a negative analytical phenomenon; since no high sensitivity could be expected with this system. However, the reagent was expected to show promising results therefore, studies were continued for optimization of the conditions.

Optimization

1. pH Optimization

A volume of 0.2 of 0.1% MO was added to 0.5ml of $1mg.ml^{-1}$ AH, shaking for 30 seconds, then diluted to 25ml in volumetric flasks. The pH was then adjusted between 1.5 to 5, by using 0.1M NaOH or 0.1M HCl. The rest of the test was then followed according to the procedure. The results reveal that the optimum pH for (AH-MO) complex is 3, shown in (Fig.4).

2. Type of buffer used (pH-adjustment)

For the pH adjustment of (AH-O) system different buffers were tried, such as; acetic acid-sodium acetate and citric acid-sodium hydroxide, but Phthalate buffer pH = 3 [28, 27] was found suitable to adjust pH of the complex (AH-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 0.5ml phthalate buffer added buffer before completing the volumetric flask by D.W (after mixing), and the result is shown in table (1).

3. Choosing a Suitable Solvent for Extraction

Many solvents were tested for extracting the complex formed between the MO reagents with AH and the best solvent among many organic solvents like [Carbon tetrachloride, chloroform, Dichlorobenzene, Acetophenone) was found is dichloromethane.

4. Optimum amounts of the Reagents

Preliminary test shows that 0.1% MO was suitable. Experiments were then performed with different volumes of the different concentrations to a constant volume 0.5ml 1mgml^{-1} AH. The results shown in (Fig.5) represent the optimum amount of MO.

5. Volume optimization of dichloromethane CH_2Cl_2

Different volumes of the solvent dichloromethane between 4 to 10 mls was used for extraction of the (AH-MO) complex as shown on (Fig.6) shows the ranges between 4 – 6 mls the complex. A volume of 5.5ml CH_2Cl_2 was found suitable and also sufficient to complete the analysis.

6. Stability of the complexes

The stability of the complex formed between (AH—MO) was followed by measuring absorbance against time. As shown in (Fig.7). It was found that the complex (AH—MO) was stable for a period of 35 minutes, after separation and only 10 minutes were needed to reach the true absorbance, and through this time there was time for shaking and for complete complexation that shown in the (Fig.8). Absorbance has, then increased after that due to the vaporization of the solvent.

Stoichiometry of the [AH-MO] complex

The stoichiometry of the drug and MO complexing reagent was examined by the mole ratio method at wave length of 434nm:

The result of mole ratio method was obtained in two ways first by adding constant amount of MH to a series of different amounts of MO solutions, and second by adding constant amount of MO to a series of different amounts of AH solutions. Fig.(9a and 9b) show the results of mole ratio method. It was found that the ratio of (AH-to-MO) was about (2 to 1).

Structure suggestion

It was not possible to put forward a reasonable structure for the stoichiometry of AH: MO of 2:1. According to their chemical structure, it was thought that a 1:1 or 1:2 ratios would be reasonable. This will make a salt-like formation between the AH and MO thus:-

$$2\,AH + MO \text{ ---------►} MO(AH)_2$$

Calibration curves:

The calibration curves obtained according to the recommended procedure were drawn for both (AH + MO) complex. The linear range was 0.04 – 0.24 µg.ml^{-1} of AH with ($r^2 = 0.982$) for (AH + MO) complex as shown in (Fig. 10).

Determination of Amiodarone hydrochloride in synthetic sample solutions:

The recovery test was performed for different concentration of AH with MO reagent, results shown in (table 2).

Determination of mexiletine hydrochloride in tablets:

Amiodarone tablets were powdered, mixed thoroughly and weighed accurately to an equivalent to 200mg of AH. The mixture was stirred well with dichloromethane and filtered through a piece of Whatmann No. 42 filter paper. The residue was washed with dichloromethane for complete recovery of the drug. The filtrate was diluted to known volume of standard flask.

In preparation of tablets, AH has been removed from the additives to make a solution. Therefore AH could be determined by calibration curve. Three different volumes (0.5, 1 and

1.5) mls of the sample of AH were determined by MO 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 ($\bar{x}$) and (μ) was compared by t-test with the term [t.S / $\sqrt{N}$] at 95% confidence limit DOF = 2.

From the results of t-test MO reagent the following conclusion were made:

The direction of the errors (-) suggest the existence of a systematic error. This may be due to the extraction steps. Nearly non dependence of these errors on sample size between (0.5 to 1.5)mls suggests the existence of both constant and proportional systematic errors.

The difference between ($\bar{x}$ - μ) and [t.s / $\sqrt{N}$] was not significant at 95% C.L. in all cases (0.5 to 1.5)mls of AH sample, indicating the non existence, or present of a very 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 workers, the value of (R %) in tables are quite reasonable.

Precision and Accuracy:

The precision of AH determination by MO complexing reagent was performed on three synthetic samples containing AH in the range of the calibration curve, and their absorbance 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 (AH-MO) complex ranged between 0.39 – 0.92% showing reasonable precision even at lower concentrations of AH. 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 AH in the tablets.

Sensitivity of the methods:

The results of the proposed method were statistically compared with those obtained by the spectrophotometric method for determination of AH using two methods [13] and are summarized in (Table 4), the table show 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 with no significant deference between the methods compared.

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Table (1): Result of adding different volume phthalate buffer before and after adding D.W to the mixture of AH and MO.

Adding the buffer before completing the volumetric flask by D.W (after mixing).	**Adding the buffer after mixing and after adding D.W until about 2ml were remaining to complete the volume.**
0.25 ml ⟶ Abs.= 0.58	0.25 ml ⟶ Abs.= 0.44
0.5 ml ⟶ Abs.= 0.807	0.5 ml ⟶ Abs.= 0.51
0.75 ml ⟶ Abs.= 0.736	0.75 ml ⟶ Abs.= 0.52
1 ml ⟶ Abs.= 0.723	1.ml ⟶ Abs.= 0.49
1.25 ml ⟶ Abs.= 0.68	1.25 ml ⟶ Abs.= 0.48
1.5 ml ⟶ Abs.= 0.523	1.5 ml ⟶ Abs.= 0.12

Table(2): The accuracy of AH determination in synthetic samples using MO

[AH-MO] complex.			
AH Present ppm	**AH Found ppm**	**R%**	**E%**
0.2	0.191	95.5	-4.5
0.2	0.191	95.5	-4.5
0.2	0.189	94.5	-5.5

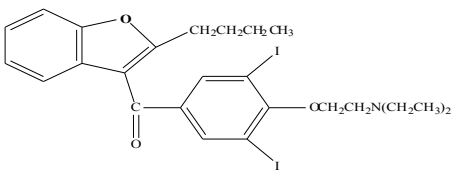
Table (3): Results of different volumes of AH sample (tablets) determined by MO reagent

Conc. Of AH ppm found in Tablets by MO Using the Eqn. Y = 0.8764X + 0.0006			
Vol.(ml) Of the sample taken	**AH ppm**	**R%**	**E%**
0.5	0.0758	94.75	-5.25
1	0.1613	100.81	0.81
1.5	0.23	95.83	-4.17

Table (4): Sensitivity of the proposed method

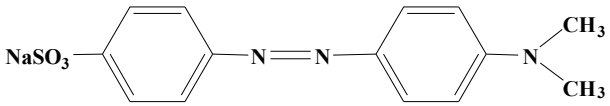
Type of calibration	ε ($L.mol^{-1}.cm^{-1}$)	Limits of linearity (µg/ml)	Recovery %		R.S.D	D.L.= 3S.D (µg/ml)	r2
calibration curve for (AH+MO)complex	$5.74*10^5$	0.04	94.75 100.81 95.83		0.39 - 0.92	0.00253	0.982
Reference method (Two Methods A&B)	A= $1.42*10^3$ B= $7.50*10^3$	A= 10 B= 2	A	100.11 100.06 100.07	A= 0.11 - 0.91	A= 1.091	0.9999
			B	100.10 100.17 100.06	B= 0.16 - 1.41	B= 0.161	

a. Molar absorptivity ε (L.mol-1.cm-1), b. Limits of linearity, c. (Recovery % and R.S.D) of different volumes of AH sample (tablets) using proposed method, d D.L with correlation coefficient



Amiodarone
(Cordarone)

Fig.(1) Amiodarone Chemical Structure



Methyl Orange
pK1 = 3.76
pH transition interval = 3.1 (Red) – 4.4 (Yellow)

Fig.(2) Methyl Orange Chemical Structure

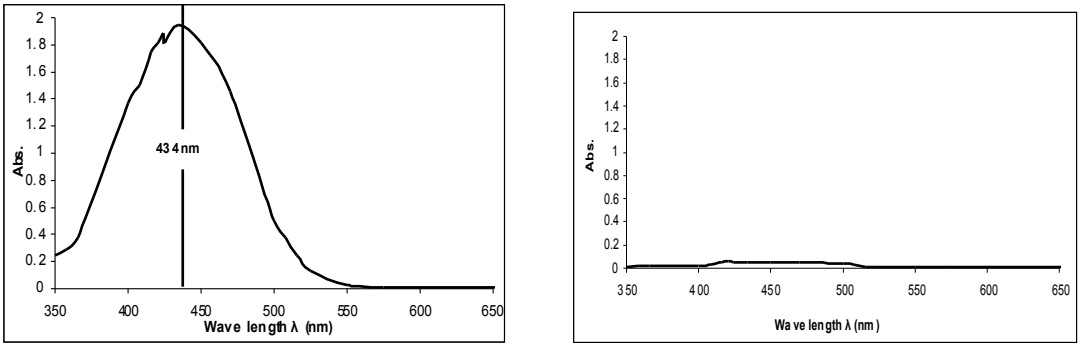


Fig. (3) The spectrum of the (AH+MO) complex, and blank spectrum

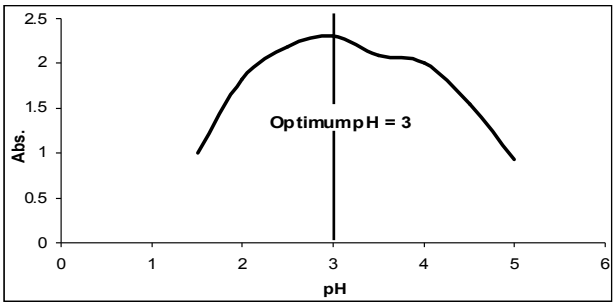


Fig. (4) The pH optimization for (MH+MO) complex

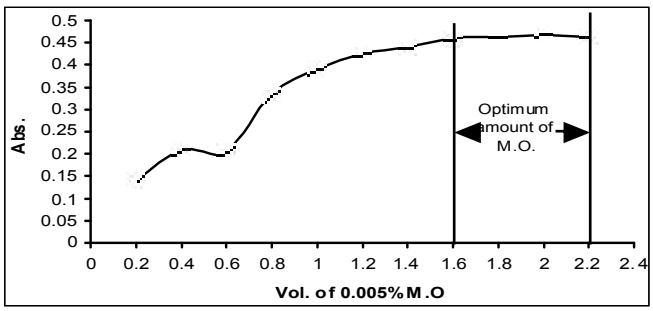


Fig. (5) Optimization of volume of 0.1% MO

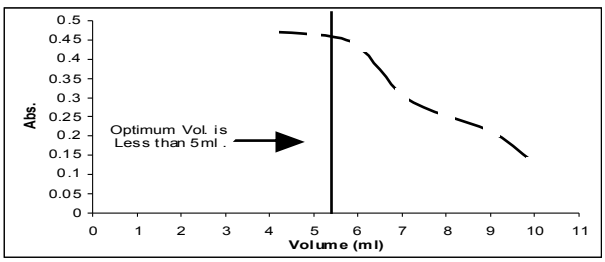


Fig. (6): Optimization of CH_2Cl_2 volume to be added for extraction the (AH-MO) complex

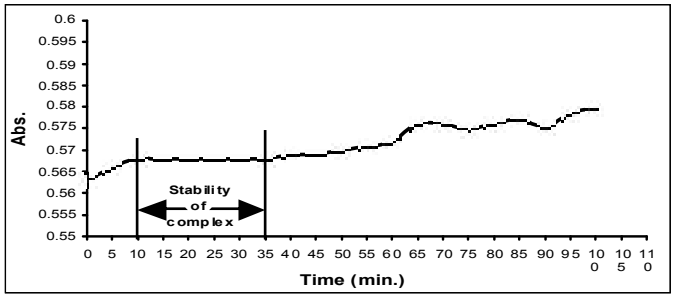


Fig.(7) Stability of the (AH –MO) complex after separation

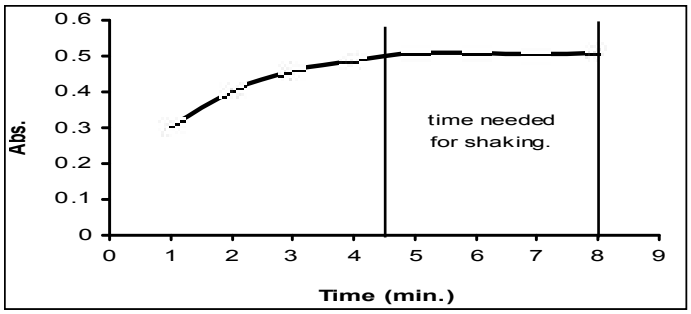
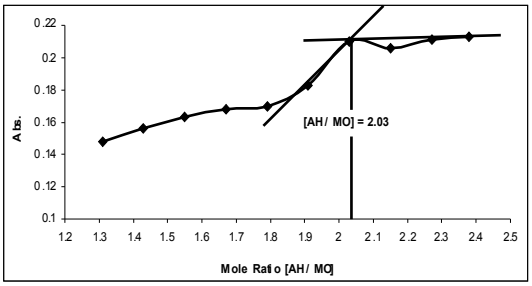
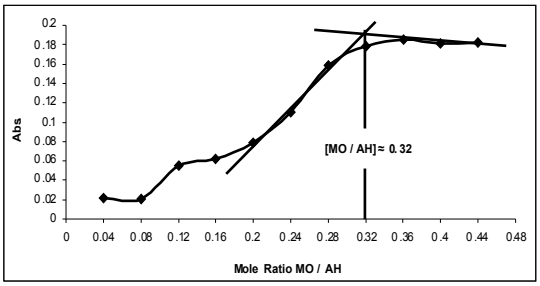


Fig.(8) Suitable time for shaking the (AH –MO) complex after separation



(a)



(b)

Fig.(9a and b) Determination of the stoichiometry of the (AH to MO) by mole ratio

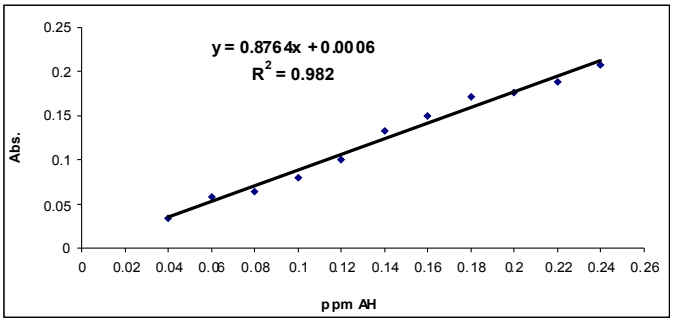


Fig.(10) Calibration curve for (AH-MO) complex

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التقدير الطيفي لهايدروكلوريد الاميوداورون في المستحضرات الصيدلانية

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الخلاصة

قدر هايدروكلوريد الاميوداورون AH طيفياً باستعمال المثيل البرتقاليَ MO. اذ ان في بحوثِنا السابقةِ تم استعمال كاشف المثيل البرتقاليَ MO لتقدير هايدروكلوريد المكسيليتين MH. الطريقة مستندة على تكوين معقد ملون بين MO مع AH. بعد الرج وتَخفيف محَلول المعقّدِ بالماء المقطر D.W، ثبّت الـ pH على (3) بالـ NaOH وHCl. المركّب المُلَوَّن التكونَ بين AH والكاشف يحول إلى قمع الفَصْل ويستخلص باستعمال 5.5 مليلتر من ثنائي كلوريد المثيلَ CH_2Cl_2 ثم الرج مدة (5 دقائقِ). الطبقة العضوية المستخاصة استعملتْ لتحضيرِ منحنى المعايرة للتقدير الطيفي لـ AH في الطول الموجي (434 nm). ان الـ Blank نُفّذت بالضبط بالطريقِة الكاملة نفسها في الجوانب كافة. ان الـ Molar absorptivity (ε L.mol^{-1}.cm^{-1}) ، و حد الكشف detection limit، المدى الخطي (μg.ml^{-1}) limit of linearity و(r^2) عبارة عن (5.74 *10^5) ، (0.04) و (0.982) على التوالي. الطريقة استعملتْ بالدقةِ والضبط المعقولين لتقدير (AH) في العيناتِ الصناعيةِ مِنْ الأقراصِ والكبسولاتِ والأنبوباتِ