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Spectrophotometric Determination of Isoptin (Verapamil Hydrochloride) in Pharmaceutical Preparations

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Abstract

Verapamil Hydrochloride (VH) has been determined spectrophotometrically by using Methyl Orange (MO). In our previous researches MO was used for determination of Mexiletine Hydrochloride [1]. The method was based on complexation between (MO and VH). After shaking and diluting the complex solution with D.W, the pH was adjusted with NaOH and HCl to pH 4. The colored complex formed between VH and the reagents were transferred into separating funnels and extracted using 4.5 ml CH_2Cl_2 and were shaken for (4 minutes). The extracted organic layer was used for the preparation of the calibration curves for spectrophotometric measurements of VH at 437nm. 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, $1.75*10^5$, 0.009253, 0.08 and 0.993 for (VH-MO) respectively. The method was used with reasonable accuracy and precision for the determination of (VH) in synthetic samples of tablets, capsules and ampoules.

Keywords: spectrophotometric determination of Verapamil hydrochloride, methyl orange.

Introduction

Verapamil Hydrochloride 5-[N-(3, 4-dimethoxy-phenethyl)-N-methyl-amino]-2-(3,4-dimethoxyphenyl)-2-isopropylvaleronitrile hydrochloride, or (Isoptin) [2], have the chemical structure shown in Fig.1:

Verapamil was introduced in 1962 as a coronary vasodilator & it is the prototype of the Ca^{2+} antagonists used in cardiovascular diseases. Verapamil major effect is on the slow Ca channel. The inhibition of the action potential inhibits one limb of the reentry circuit believed to underlie most paroxysmal Superaventricular tachycardia that uses the AV node as a reentry point. It is categorized as a class IV antiarrhythmic drug .Hemodynamically; verapamil causes a change in the preload. After load contractility, heart rate, & coronary blood flow .The drug reduces systemic vascular resistance & mean blood pressure, with minor effect on cardiac output [2].

The oldest chromatography method in this review was Gas-Liquid Chromatography Using Nitrogen-Phosphorous Detection applied for determination of Verapamil in Human Plasma [3], which was appeared in (1984). Later, many chromatographic methods for the determinations of this drug and its metabolites have been reported [4-8] attempting different modifications in the method to increase sensitivity, reducing steps of analysis, or other improvements, with the limit of linearity ranged between [2 $ng\,L^{-1}$ – 1000 $ng\,L^{-1}$].

Extensive search in the literature has showed many spectrophotometric methods for the determination of VH [9-13]. Among them, how to use Spectrofluorometric and visible spectrophotometry, with different types of optimizations to obey beers law, and limit of linearity ranged between [0–340 $\mu g\,ml^{-1}$].

Preliminary practical tests on many reagents revealed that methyl orange which has the chemical structure shown in Fig.2, was suitable reagent [14] to form colored complexes with the drug VH and were exploited for their quantitative determination in capsules, ampoules 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. Verapamil hydrochloride ampoule [Knoll] which was (5mg/2ml), was taken as a stock solution, other concentrations were prepared by usual dilution. Methyl orange 0.05% aqueous MO. Phthalate buffer (pH=4) was prepared by mixing 50ml of 0.1M (potassium hydrogen phthalate), with 49.9 ml of D.W and 0.1 ml of 0.1M HCl [15-16], and pH was adjusted with a pH meter.

The recommended procedures

A volume of 500 µL 0.1% (MO) reagent was added to a certain amount of VH standard or samples containing between (0.08-0.8 $\mu g ml^{-1}$) VH. The mixture was shaken for (2 min.) and diluted to about 24ml in volumetric flasks using D.W. The pH was adjusted by adding 1 ml phthalate buffer (pH 4) to the MO mixture and finally completed to 25ml. The resulting complex formed between VH and the reagent were transferred into separating funnels (100ml capacity) and extracted with 4.5 ml CH_2Cl_2 in two portions to wash out the volumetric flasks for quantitative transfer of the solution and was shaken for 4 minutes. After separation, the organic layer was used for the preparation of the calibration curves using spectrophotometric measurements of VH-MO complex at 437nm. The blanks were carried out in exactly the same way throughout the whole procedure.

Results and Discussion

Preliminary work

The absorption spectra of the complex (VH-MO) against blank with λ-max at 437nm, and blank against D.W (according to the recommended procedure) are shown in Fig.3. 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.

The complex; or an ion-pair formation between the VH and MO has shown 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 500 µL of 0.1% MO was added to 1 ml of 0.1$mg.ml^{-1}$ VH, shaking for 2 min., then diluted to about 24ml in volumetric flasks using D.W. The pH was then adjusted

between 2 to 6, 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 (VH-MO) complex is 4, as shown in Fig.4.

2. Type of buffer used (pH-adjustment)

For pH adjustment of (VH-MO) system different buffers were tried, such as; acetic acid-sodium acetate and citric acid-sodium hydroxide, but Phthalate buffer of pH = 4, was found suitable to adjust pH of the complex (VH-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 were equal to 1 ml phthalate buffer with no difference in order of addition before or after 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 VH [Carbon tetrachloride, Benzene, Ethyl acetate, Dioxane, Acetone, chloroform] and dichloromethane was found to be the best.

4. Amounts of the Reagent:

Preliminary test shows that 500 µL of 0.1% MO was suitable. Experiments were then performed with different volumes of the different concentrations to a constant volume 1 ml 0.1 $mgml^{-1}$ VH. The results shown in Fig.5 indicate that optimum volumes were 1.2 ml for 0.05% MO.

5. Effect of dichloromethane CH_2Cl_2 volume:

Different volumes of dichloromethane between (4 to 10 ml) were used for extraction. The results are shown in Fig.6, the optimum volume was found between 4 – 5 mls of dichloromethane. A volume of 4.5 ml CH_2Cl_2 was found suitable and also sufficient for complete extraction.

6. Stability of the complexes:

The stability of the complex formed between (VH—MO) was followed by measuring absorbance against time. As shown in (Fig.7). It was found that the complex (VH—MO) was stable for a period of 35 minutes, after separation and only 5 minutes were needed to reach the true absorbance, and through this time there was time for shaking and for completeting complexation that was shown in the (Fig.8). Absorbance has, then increased after that which may be due to the vaporization of the solvent.

Stoichiometry of the [VH-MO] complex:

The stoichiometry of the drug and MO complexing reagent was examined by the mole ratio method at wave length of 437nm:

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

Structure suggestion:

It was not possible to put forward a reasonable structure for the stoichiometry of VH: 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 VH and MO thus:-

$$2\ VH + MO \text{ ---------}\blacktriangleright MO(VH)_2$$

Calibration curves:

The calibration curves obtained according to the recommended procedure were drawn for VH-MO complex. The linear range was 0.08 – 0.8 $\mu g.ml^{-1}$ of VH with ($r^2 = 0.993$) for VH-MO complex shown in Fig.10.

Determination of Verapamil hydrochloride [Isoptin] in synthetic sample solutions:

The recovery test was performed for different concentrations of VH with MO reagent; the results obtained are shown in table 2.

Determination of Verapamil hydrochloride in tablets:

Two tablets were powdered and mixed thoroughly. An amount equivalent to 160 mg of verapamil hydrochloride was weighed accurately and extracted with chloroform to eliminate any interference from excipients. It was filtered through Whatmann No. 42 filter paper and the residue was washed well with chloroform for complete recovery of the drug. The chloroform was evaporated to dryness and the drug was dissolved in doubly distilled water and diluted to 1000mL with distilled water, It was further diluted (1 to 10) [12].

Three different volumes (0.2, 0.5 and 1) mls of the sample of VH were determined by MO according to the recommended procedure. The results obtained 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 ($\mathcal{X}$) 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 conclusions 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 (0.2, 0.5 and 1) mls suggests the existence of both constant and proportional systematic errors.

The difference between ($\mathcal{X} - \mu$) and [$t.s / \sqrt{N}$] was not significant at 95% C.L. in all cases (0.2 to 1) mls of VH sample, indicating the non existence, or presence 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 VH determination by MO complexing reagent was performed on three synthetic samples containing VH 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 (VH-MO) complex ranged between 0.75 – 1.75% showing reasonable precision even at lower concentrations of VH. 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 VH 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 VH using two methods [12] and are summarized in (Table 4), the table 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 with no significant deference between the methods compared.

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

Adding the buffer before dilution.	Adding the buffer after dilution.
0.5 ml ⟶ Abs.= 0.137	0.5 ml ⟶ Abs.= 0.135
1 ml ⟶ Abs.= 1.378	1.ml ⟶ Abs.= 1.380
1.5 ml ⟶ Abs.= 1.168	1.5 ml ⟶ Abs.= 1.169
2 ml ⟶ Abs.= 1.12	2 ml ⟶ Abs.= 1.12

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

[VH-MO] complex.			
VH Present ppm	VH Found ppm	R%	E%
0.4	0.413	103.25	3.25
0.4	0.413	103.25	3.25
0.4	0.404	101	1.00

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

Conc. Of VH ppm found in Tablets by MO Using the Eqn. Y = 0.3568X + 0.0077			
Vol.(ml) Of the sample taken	VH ppm	R%	E%
0.2	0.1288	100.6487	0.6487
0.5	0.3324	103.9039	3.9039
1	0.6454	100.8530	0.8530

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 %	Detection Limit (µg/ml)	r2
calibration curve for (VH+MO)complex	1.75 * 10^5	0.08	100.6487 103.9039 100.8530		0.75 – 1.75	D.L.=3S.D 0.009253	0.993
Reference method (Two Methods A&B)	A = 1.18 * 10^3	A = 12.50	A	100.30 100.21 100.30	A = 0.28 – 0.23	A = 0.77	A = 0.9999
	B = 9.82 * 10^3	B = 2.00	B	99.96 99.93 99.96	B = 0.15 – 0.27	B = 0.4	B = 0.9998

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

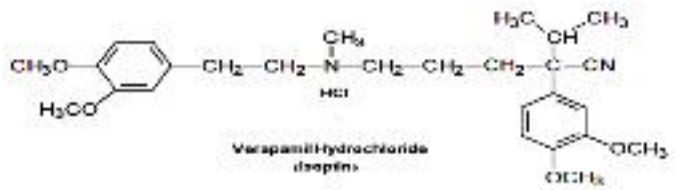
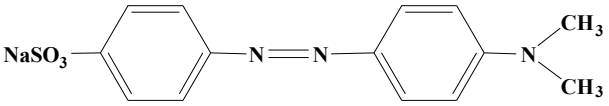
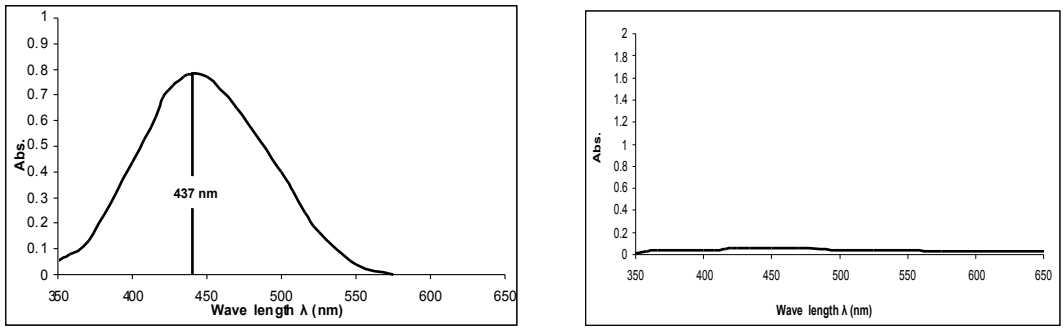


Fig. (1): Verapamil Hydrochloride Chemical Structure



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

Fig. (2): Methyl Orange Chemical Structure



A B

Fig. (3): (A) The spectrum of the (VH+MO) complex against blank, and (B) blank against D.W spectrum

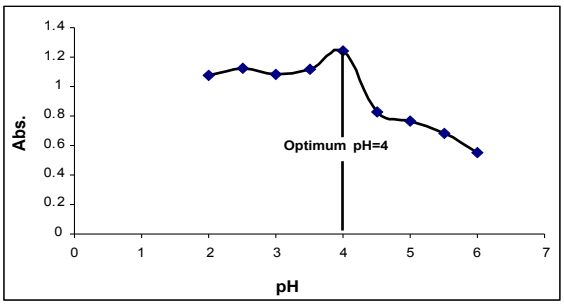


Fig. (4): The pH optimization for (VH+MO) complex

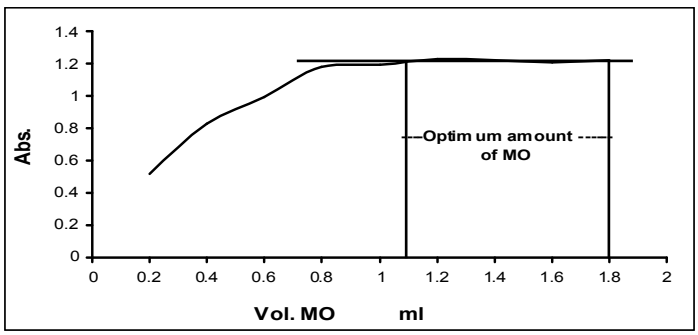


Fig. (5): Optimization of volume of 0.05% MO

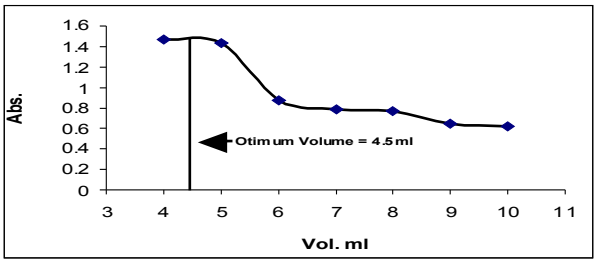


Fig. (6): Optimization of CH_2Cl_2 volume to be added for extraction the (VH-MO) complex

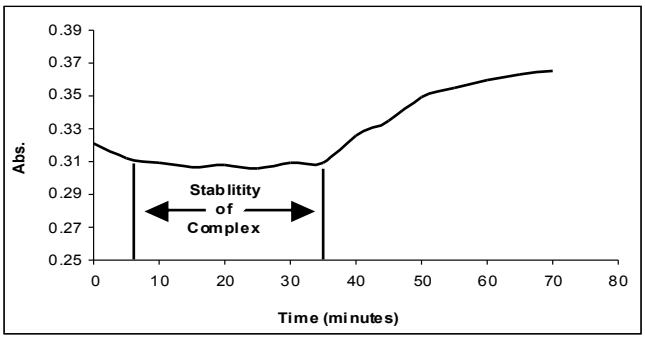


Fig.(7): Stability of the (VH –MO) complex after separation

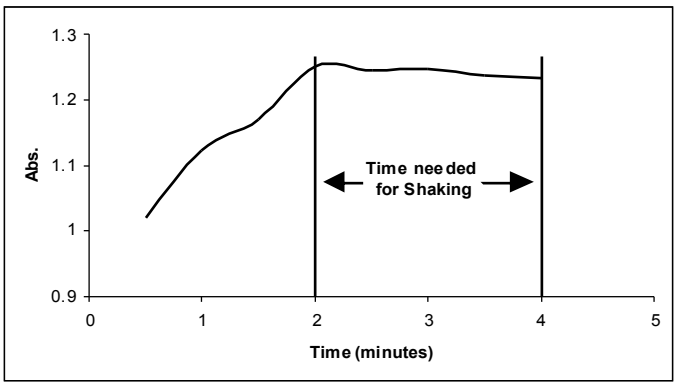
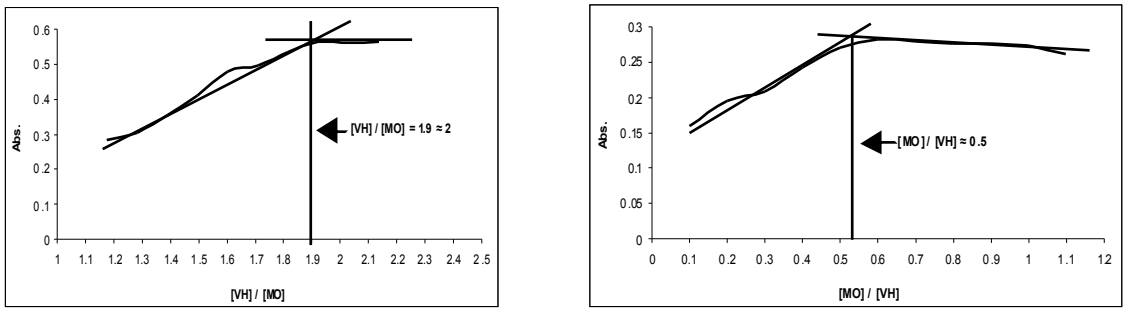


Fig.(8): Suitable time for shaking the (VH –MO) complex after separation



(a) (b)

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

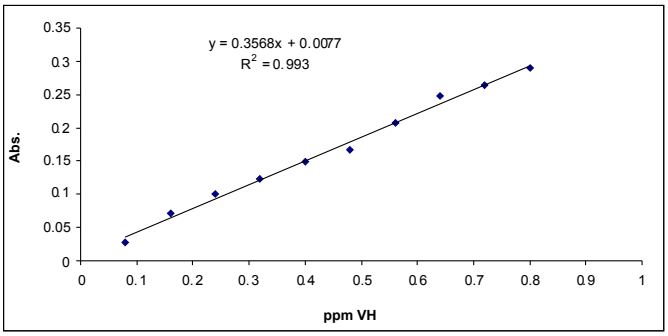


Fig.(10): Calibration curve for (VH-MO) complex

التقدير الطيفي للايزوبتين (هايدروكلوريد الفيراباميل) في المستحضرات الصيدلانية

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الخلاصة

تم تقدير هايدروكلوريد الفيراباميل VH طيفياً باستعمال المثيل البرتقاليَ MO، اذ ان في بحوثِنا السابقةِ استعمل كاشف المثيل البرتقاليَ MO لتقدير هايدروكلوريد المكسيليتين MH. الطريقة مستندة الى تكوين معقد ملون بين MO مع VH. بعد الرج وتَخفيف محَلول المعقّدِ بالماء المقطر D.W، ثبت الـ pH على (4) بالـ NaOH وHCl. المركّب المُلَوَّن التكونَ بين VH والكاشف يحول إلى قمع فَصْل ويستخلص باستعمال 4.5 مليلتر من ثنائي كلوريد المثيل CH_2Cl_2 ثم الرج مدة (4 دقائقِ). الطبقة العضوية المستخلصة إستعملتْ لتحضيرِ منحنى المعايرة للتقدير الطيفي لـ VH في الطول الموجي (437 nm). ان الـ Blank نُفّذت بالضبط الطريقِة الكاملة نفسها في الجوانب كافة. ان الـ Molar absorptivity (ε $L.mol^{-1}.cm^{-1}$) ، حد الكشف detection limit، المدى الخطي limit of linearity ($\mu g.ml^{-1}$) و(r^2) عبارة عن ($1.75*10^5$)، (0.009253)، (0.008) و (0.993) على التوالي. الطريقة إستعملتْ بدقةِ ونجاح في تقدير (VH) في العيناتِ الصناعيةِ على الأقراصِ والكبسولاتِ والحقن الدوائية.