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SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF THIAMINE HYDROCHLORIDE IN PURE FORM, PHARMACEUTICAL AND BIOLOGICAL FLUIDS

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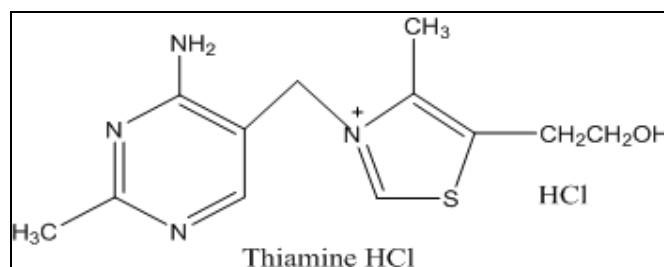
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ABSTRACT: Thiamine hydrochloride, also known as Vitamin B₁, is a water-soluble vitamin which is important biologically and pharmaceutically. A new simple and sensitive spectrophotometric method has been developed for the determination of Thiamine Hydrochloride (Vitamin B₁) in pure form and biological fluids. The method is based on the diazotization reaction of Thiamine Hydrochloride with Procaine HCl which reacted with sodium nitrate and hydrochloric acid to form a red water-soluble azo dye in basic medium, that has a maximum absorption at $\lambda_{\max} = 507$ nm. Beer's law is obeyed over the concentration range (2-60 $\mu\text{g}\cdot\text{ml}^{-1}$), the molar absorptivity of 4.047×10^4 $\text{L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$ and Sandell sensitivity of 7.417×10^{-3} $\mu\text{g}\cdot\text{cm}^{-2}$ and the method have an accuracy and precision between (0.02-0.16) while the relative standard deviation between (0.805-2.049). The structure of dye showed that (1:2) product was formed between the drug and diazotized reagent. The method was applied successfully for the determination of Vitamin B₁ in pharmaceutical preparations and biological samples. The results showed that the concentration of vitamin B₁ were decreased, in both blood and urine samples, with time after taken a therapy.

INTRODUCTION: Thiamine hydrochloride (vitamin B₁), a water-soluble vitamin, is a biologically and pharmaceutically important compound. THC is 3 - [(4-Amino - 2 - methyl pyrimidin-5-yl) methyl]-5-(2-hydroxyethyl) - 4-methylthiazolium chloride hydrochloride, C₁₂H₁₇ClN₄OS, HCl, whereas its chemical structure is ¹ contains an aminopyrimidine ring and a thiazole ring with methyl and hydroxyethyl side chains linked by a methylene bridge.



It is soluble in water, methanol, and glycerol and practically insoluble in acetone, ether, chloroform and benzene. It is stable in acidic solution and during frozen storage, but it is unstable in alkaline solution, heat, and when it is exposed to ultraviolet light and gamma irradiation ².

This vitamin plays a biological role in the metabolic process of the carbohydrate in human body, keeps the normal function of nerve, cardiac muscle and digestive processes, and it is used for

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the treatment of Beriberi and different forms of polyneuritis. Thiamine is also essential to the mental health especially the emotional aspect³. There are a variety of physical conditions and diseases associated with deficiencies in vitamin B₁. Symptoms include pain, numbness and tingling in the extremities, muscle weakness, and a lack of physical coordination, particularly in the larger muscle masses that make up the leg muscles. A deficiency in thiamine can cause enlargement of the heart, and this can lead to congestive heart failure and lung congestion. A severe deficiency in vitamin B₁ can lead to nerve damage, brain damage, and even death. Mental symptoms associated with a serious lack of vitamin B₁ include fatigue, psychosis, and confusion^{4,5}.

Multivitamin pharmaceutical preparations containing mixtures of these substances are very interesting for analysis, and most of them include the water-soluble B-group. The term B-group vitamins usually refers to thiamine, riboflavin, pyridoxine, nicotinic acid, pantothenic acid, biotin, cyanocobalamin and folic acid⁶.

Thiamine is sometimes called an "anti-stress" vitamin because it may strengthen the immune system and improve the body's ability to withstand stressful conditions⁷. It plays an important role in Wernicke-Korsakoff syndrome, a form of amnesia caused by brain damage occurring in long-term alcoholics who rely mainly on alcohol for nutrition⁸.

Since 1950, the chemical determination of thiamine has used the derivatisation of thiamine to thiochrom by alkaline K₃[Fe(CN)₆], followed by extraction with 2-methyl-1-propanol. Finally, fluorescence detection was used for quantitation⁹. Although, this procedure is the official U.S.P. method¹⁰, the thiocrome procedure requires a skilled technician since the analytical steps such as oxidation of thiamine, solvent extraction and centrifugation must be performed under subdued light because both thiamine and thiocrome are light sensitive.

Various methods have been reported for determining this drug in pharmaceutical preparations, including high performance liquid chromatography (HPLC)^{11, 12, 13, 14}, ion-exchange

chromatography¹⁵, spectrofluorimetry¹⁶, gas chromatography¹⁷, voltammetric¹⁸, flow injection analysis¹⁹ and spectrophotometric^{20, 21, 22, 23, 24}. The aim of this study was to investigate a new method for the determination of vitamin B₁ in pure form, pharmaceutical preparations and biological fluids.

MATERIALS AND METHODS:

Apparatus: All spectral and absorbance measurements were carried out on a Shimadzu UV-Visible-260 digital double-beam recording spectrophotometer (Tokyo-Japan), using 1-cm quartz cells, balance.

Reagents: All chemicals used were of analytical reagent grade. Thiamine Hydrochloride standard material was provided from the state company for drug industries and medical appliances (SDI) Sammara-Iraq.

1-Thiamine hydrochloride stock solution: (1000 $\mu\text{g}\cdot\text{ml}^{-1}=3.324\times 10^{-3}\text{M}$): a 0.1gm amount of pure Thiamine HCl (SDI) was dissolved in distilled water and complete to 100 ml volumetric flask. Thiamine HCl working solution (500 $\mu\text{g}\cdot\text{ml}^{-1}=1.662\times 10^{-3}\text{M}$), was prepared by diluted 50 ml of the stock solution to 100 ml volumetric flask with distilled water.

2-Procaïne hydrochloride reagent solution ($5\times 10^{-2}\text{M}$): prepared by dissolving 1.3640 gm of pure procaïne HCl (SDI) in distilled water complete the volume to 100 ml volumetric flask, then diluted solutions was prepared by simple dilution.

3-Sodium nitrite solution ($5\times 10^{-2}\text{M}$): prepared by dissolving 0.3450 gm of NaNO₂ (Merck) in distilled water and diluting to the mark of 100 ml volumetric flask, then diluted solutions was prepared by simple dilution.

4-Hydrochloric acid (1M): This was prepared by dilution of the standard concentrated volumetric solution (5 M) with distilled water in volumetric flask by dilute 20 ml of (5M) HCl to 100 ml volumetric flask with distilled water.

5-Sodium hydroxide (1M): This was prepared by dissolving 10 gm of NaOH (Merck) in 250 ml volumetric flask with distilled water.

Pharmaceutical Preparations of Thiamine HCl.

Injection sample: The contents of ten ampoules of Thiamine HCl were mixed. An aliquot corresponding to 100 mg of Thiamine HCl (2 ml) was diluted to 100 ml with distilled water in a volumetric flask to obtain approximately $1000 \mu\text{g ml}^{-1}$ of Thiamine HCl.

Tablets sample: Ten tablets were accurately weighed and finely powdered. An accurately weighed amount equivalent to 100 mg of the pure drug, was transferred into a 100 ml volumetric flask, then dissolved with distilled water and completed to the mark with the same solvent. The flask with its contents was shaken well and filtered.

Biological Fluids Preparations:

a- Blood samples preparations:

There is some steps are automated to prepare blood sample of Vitamin B₁²⁵, they were automated and difficult. In this study two groups of blood samples were used, first group represent the volunteer has been take Vitamin B₁ tablets (Thiamine HCl/100 mg), 10 tablets / 4 days, while the second group represent the volunteer has been take one ampoule of Vitamin B₁ injection (100 mg/2ml).

Venous blood samples (5 ml) were drawn at different time points (0.5, 2, 4, 6, 24) hours after the last day of administration and injection, then transferred immediately to a clean dry plain tube. The blood sample was allowed to clot for at least 10-15 min at room temperature and then centrifuged for 10 min at 3000 Xg. Serum was collected and stored at -20°C for later determination, 0.5 ml of the serum were used in subsequent experiments.

b- Urine samples preparations:

Two groups of urine samples were used first group represent the volunteer has been take Vitamin B₁ tablets (Thiamine HCl/100 mg), 10 tablets / 4days, while the second group represent the volunteer has been take one ampoule of Vitamin B₁ injection (100 mg/2ml). Both samples were drawn at different time points (2, 4, 6, 8, 24) hours²⁶ after the last day of administration and injection. The urine samples were centrifuged for 20 min at 3000 Xg. urine was stored at -20°C for later determination, 0.5 ml of the urine were used in subsequent experiments.

Analytical Procedure for Calibration:

Into a series of 10 ml volumetric flask, transfer 1 ml of procaine HCl ($2.5 \times 10^{-2} \text{M}$) and 1 ml of NaNO₂ ($2.5 \times 10^{-2} \text{M}$) followed by 1 ml of 0.1 M HCl shake well then added increasing volumes of standard stock solution ($500 \mu\text{g. ml}^{-1} = 1.662 \times 10^{-3} \text{M}$) containing (0.1-2.0 ml) of Thiamine HCl to cover the range of the calibration graph ($50\text{-}1000 \mu\text{g}$ in a final volume of 10 ml) i.e; $5\text{-}100 \mu\text{g.ml}^{-1}$, to this solution added (1 ml) of NaOH (1M), the solution was shaking thoroughly and the contents was diluted to the mark with distilled water and shake well, after 15 min the absorbance of the azo dye was measured at 507 nm against a reagent blank containing the same materials except drug. For the optimization of conditions and in all subsequent experiments, a solution of $500 \mu\text{g.ml}^{-1}$ of the drug in a final volume of 10 ml was used.

RESULTS AND DISCUSSION:

Preliminary Studies:

Throughout the preliminary study on the diazotization reaction of Thiamine HCl, with diazotized Procaine HCl in basic medium a red water-soluble azo dye was obtained with a maximum absorbance at 507 nm (**Fig. 1**). The absorbance of the azo dye solution measured versus reagent blank which has a negligible absorbance at this wavelength.

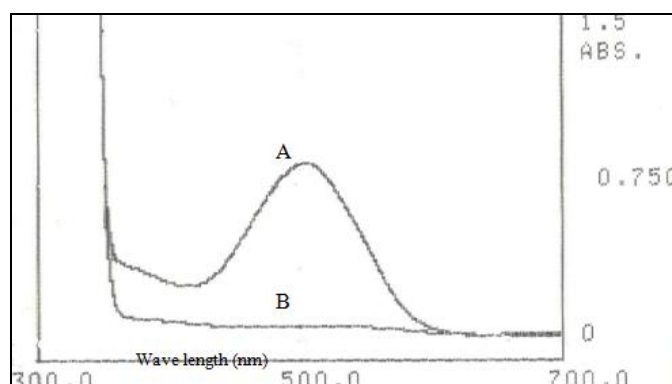


FIG.1: ABSORBANCE SPECTRA OF THE AZO DYE ($500 \mu\text{g.ml}^{-1}$ of THIAMINE HCl), AGAINST BLANK (A) AND BLANK AGAINST DISTILLED WATER (B).

Optimization of the Experimental Conditions:

The effect of various parameters on the color development was studied to establish the optimum conditions for the determination of Thiamine HCl.

In the subsequent experiments, 1 ml of Thiamine HCl solution ($500 \mu\text{g.ml}^{-1} = 1.662 \times 10^{-3} \text{M}$) with diazotized procaine HCl and NaOH was taken in to 10 ml final volume and the absorbance of the series

of solutions were measured by varying one and fixing the other parameters at 507 nm. versus reagent blank.

1- Effect of acid:

In practice, the addition of acid to the diazonium reaction was necessary for the formation of diazonium salt between the amine group (Procaine HCl) and sodium nitrite, which couples with amino reagent (Thiamine HCl) to give an azo dye therefore, various acids were studied (acetic acid, hydrochloric acid, nitric acid, phosphoric acid and sulfuric acid), hydrochloric acid seems to be the most suitable acid through the high absorbance under the reaction condition. When various concentrations of Hydrochloric acid (0.1-3.0M) were added to the solution of 1 ml of Procaine HCl (2.5×10^{-2} M) with equal amount of sodium nitrite, 1 ml of Thiamine HCl and 1 ml of 1M NaOH. The concentration of 1M seems to be the suitable concentration, and was considered to be optimum as shown in (Fig. 2).

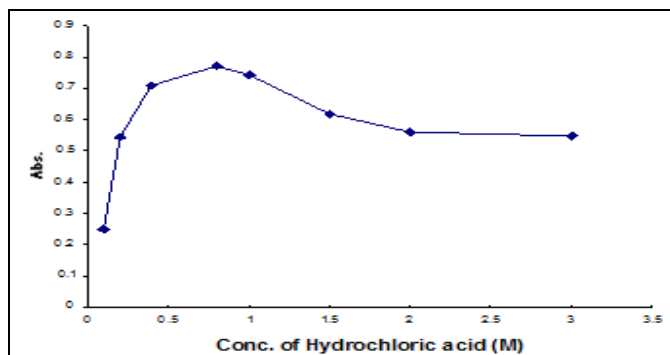


FIG. 2: EFFECT OF CONCENTRATION OF HYDROCHLORIC ACID (M)

The effect of different volumes of 1M hydrochloric acid (0.1-5.0 ml) was studied, and 1 ml of hydrochloric acid was found optimum as shown in (Fig. 3).

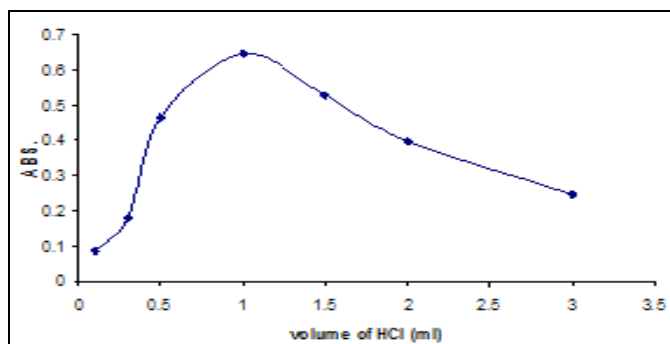


FIG. 3: EFFECT OF VOLUME OF 1M HYDROCHLORIC ACID

2. Effect of reagent concentration: When various concentrations of diazotized Procaine HCl solutions (0.1-4 ml) were added to affixed amount of drug in basic medium to develop the color of reaction to its full intensity. One ml volume of diazotized Procaine HCl was gave the optimum value as shown in (Fig. 4).

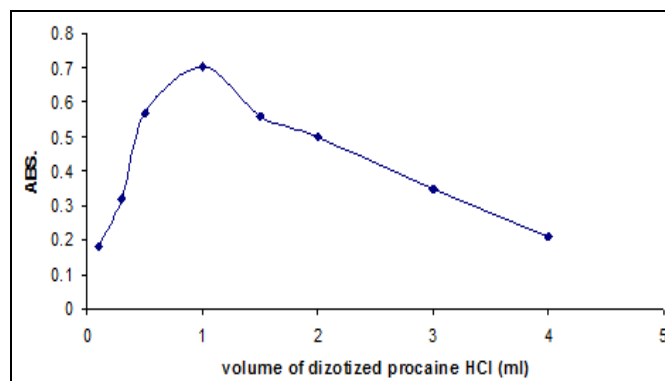


FIG. 4: EFFECT OF VOLUME OF DIAZOTIZED PROCAINE HCl

3-Effect of order of addition:

To optimum results, the order of addition of reagents should be followed as: diazotized procaine HCl followed by Thiamine HCl solution and then NaOH was taken in to 10 ml final volume otherwise a loss in color intensity and stability was observed.

4- Effect of temperature:

The effect of temperature on the diazotization and coupling reaction show that the absorbance of the azo dye (which contains 1 ml of $500 \mu\text{g}\cdot\text{ml}^{-1} = 1.664 \times 10^{-3}$ M Thiamine HCl, 1 ml of diazotized 2.5×10^{-2} M procaine HCl, 1 ml of NaOH), remains constant at room temperature (25 C°) for more than 90 min, and decrease at higher than 45 C° , and low temperature ($0-5 \text{ C}^\circ$).

5- Effect of time on the stability of the dye:

The stability of the dye was studied for 90 min. following the mixing of the reagents (which contains 1 ml of procaine HCl and 1 ml of $500 \mu\text{g}\cdot\text{ml}^{-1} = 1.662 \times 10^{-3}$ M Thiamine HCl, followed by 1 ml of NaOH. The absorbance of the dye became intense and sharp after 10 -15 mins, and remained stable for at least 90 min. as shown in (Fig.5).

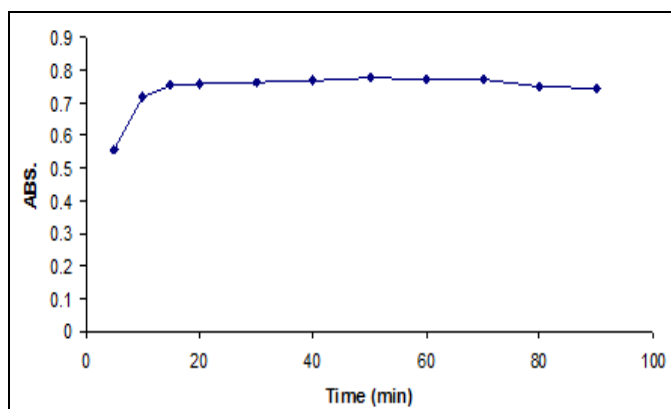


FIG.5: EFFECT OF TIME

Calibration Graph: Employing the conditions described under procedure, a linear calibration graph (Fig.6) for Thiamine HCl was obtained, and Beer's law was obeyed over the concentration range of graph (50-1000 μg in a final volume of 10 ml), or (5-100 $\mu\text{g}\cdot\text{ml}^{-1}$) with a correlation coefficient of 0.9956 and an intercept of 0.0189. The conditional molar absorptivity of the red dye formed with reference to Thiamine HCl was found to be

$4.047 \times 10^4 \text{ L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$ and a Sandell sensitivity of $7.4173 \times 10^{-3} \mu\text{g}\cdot\text{cm}^{-2}$.

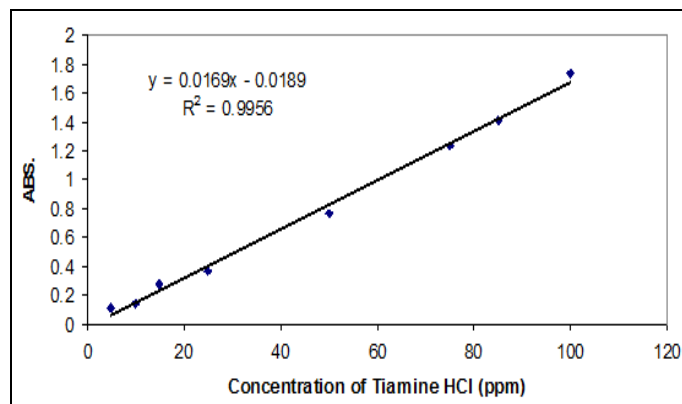


FIG.6: CALIBRATION GRAPH FOR THIAMINE HCl

Accuracy and Precision: To determine the accuracy and precision of the method, Thiamine HCl was determined in three different concentrations. The results shown in Table 1, indicate that a satisfactory precision and accuracy could be obtained with the proposed method.

TABLE 1: ACCURACY AND PRECISION OF THE PROPOSED METHOD

Concentration of Thiamine HCl. $\mu\text{g}\cdot\text{ml}^{-1}$		Error %	Recovery %	Relative standard deviation (R.S.D) %
Present	Found*			
10.00	10.016	+0.16	100.16	2.049
25.00	25.036	+0.14	100.14	1.632
75.00	75.016	+0.02	100.02	0.805

* for three determinations.

Structure of the Dye: Under the reaction conditions, the reaction was carried out as described in Fig. 7. The aromatic amino group present in Procaine HCl is diazotized with nitrous acid (NaNO_2/HCl) and diazonium salt thus formed is coupled with THC at room temperature. The stoichiometry of the reaction was studied using equimolar concentrations ($1.662 \times 10^{-3} \text{ M}$) of the drug and diazotized reagent under the recommended optimum conditions and applying Job's method²⁷. Moreover a mole ratio²⁸ method was also applied using increasing volume (0.1 to 3.00 ml) of $1.662 \times 10^{-3} \text{ M}$ of diazotized reagent to a fixed volume (1ml) of THC of the same concentration. The obtained results in (Fig. 7) and (Fig. 8) showed that a 1:2 (THC: DPH) product was formed between the drug and diazotized reagent at 507 nm, therefore the free amino group of THC is reacted with diazonium salt and the other diazonium molecule is reacted through the thiazole ring²⁹.

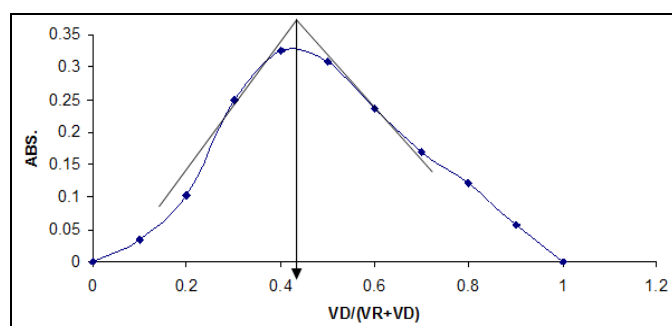


FIG. 7: CONTINUOUS VARIATION PLOT

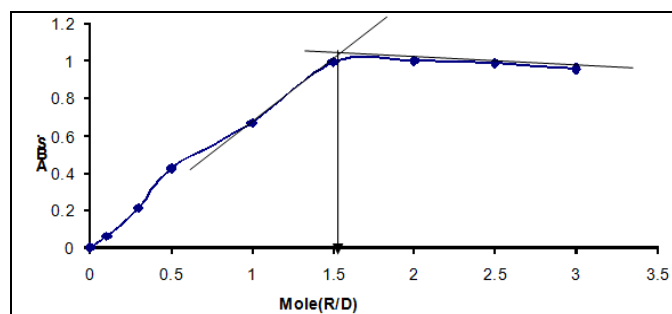
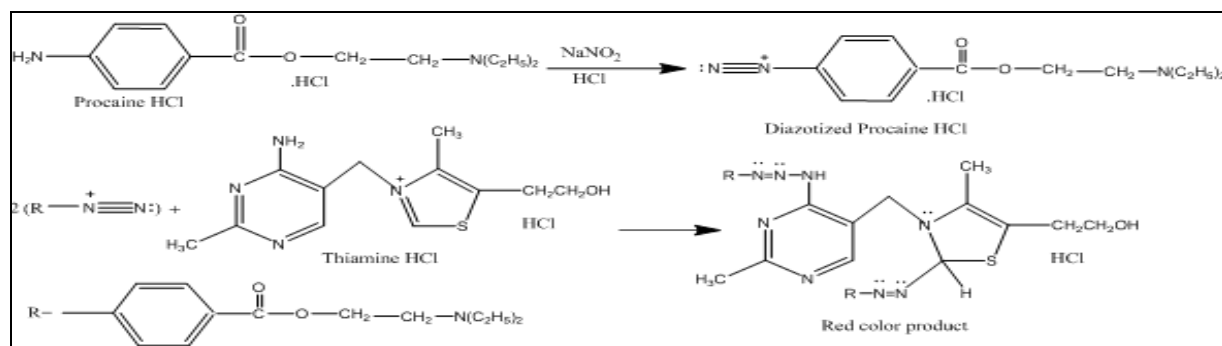


FIG. 8: MOLE RATIO PLOT

A reaction subsequent based on the above results is shown in **Scheme 1**.



SCHEME 1: REACTION SEQUENCE

Stability Constant of Reaction Product:

The conditional or apparent stability constant²⁷ of the 1:2 (THC: DPH) product was evaluated as follows: An equimolar concentration (3.324×10^{-4} M) of both drug and reagent were prepared. Two sets of solutions were prepared, first set of solutions were formulated to contain a twofold excess amount of the reagent (DPH) and the second set was formulated to contain tenfold excess of reagent.

$[K = (1-\alpha)/4\alpha^3 C^2]$ and $\alpha = A_m - A_s / A_m$, A_m and A_s = absorbance of the solution containing³⁰.

Accordingly to the previous equations it was found that α is equal to 0.605 ($A_m = 0.354$ and $A_s = 0.145$, (average of three determinations) for 3.324×10^{-4} M of THC and the stability constant of the dye product was $0.404 \times 10^6 \text{ L}^2 \cdot \text{mol}^{-2}$.

Pharmaceutical Applications:

Two types of syrup containing Thiamine HCl have been analyzed using the proposed procedure 1ml of $500 \mu\text{g} \cdot \text{ml}^{-1}$ for Vitamin B₁ injection and 1ml of $50 \mu\text{g} \cdot \text{ml}^{-1}$ for Vitamin B₁ tablets and they gave the results shown in **Table 2**.

TABLE 2: APPLICATION OF THE PROPOSED METHOD OF THIAMINE HCl IN PHARMACEUTICAL PREPARATIONS

Drug sample	Concentration of Thiamine HCl $\mu\text{g} \cdot \text{ml}^{-1}$		Error%	Recovery %	R.S.D %
	Present	Found*			
Vitamin B ₁ (Tablet 100mg)	50.00	51.157	+2.314	102.314	1.618
Vitamin B ₁ (Injection 100mg/2mL)	50.00	50.670	+0.013	100.013	0.993

* for three determinations

Biological Samples:

Blood samples: The precipitation of protein using 10 % trichloroacetic acid (TCA 10%) was not necessary in this study, because there is no difference between the absorption with or without TCA addition. Two types of drugs containing Vitamin B₁ (Thiamine HCl/100 mg) have been take

as a tablets and injection. The samples of blood were treatment as above and analyzed using the proposed procedure and they gave the results shown in **Fig.9** and **Table 3**. Our findings show that the concentration of Vitamin B₁ decreased with time.

TABLE 3: APPLICATION OF THE PROPOSED METHOD ON VITAMIN B₁ IN BLOOD SAMPLE

Blood sample Conc. ($\mu\text{g} \cdot \text{ml}^{-1}$) 0.5 ml of sample	Time (hrs.)				
	0.5	2	4	6	24
Vitamin B ₁ (Tablet 100mg)	79.343	71.887	71.414	67.508	52.893
Vitamin B ₁ (Injection 100mg/2ml)	82.065	73.485	70.881	66.739	59.520

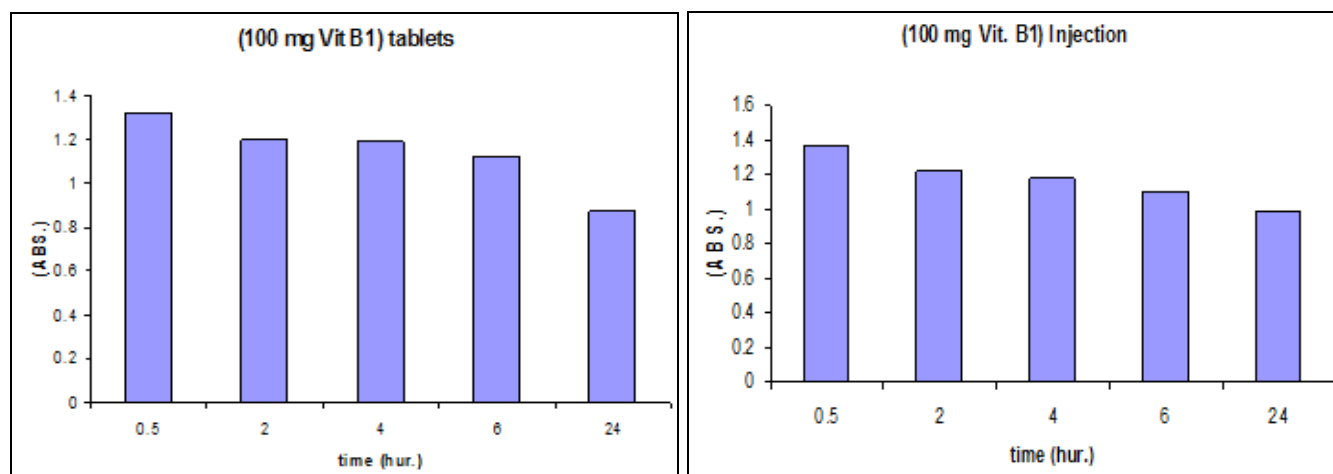


FIG.9: EFFECT OF TIME ON ABSORBANCE OF TWO BLOOD SAMPLES CONTAINING VITAMIN B₁.

Urine samples: Two types of drugs containing Vitamin B₁ (Thaimine HCl/100 mg) have been take as a tablets and injection, and the samples of urine was treatment as above and analyzed using the

proposed procedure and they gave the results shown in **Fig. 10** and **Table 4**. Our findings show that the concentration of Vitamin B₁ decreased with time.

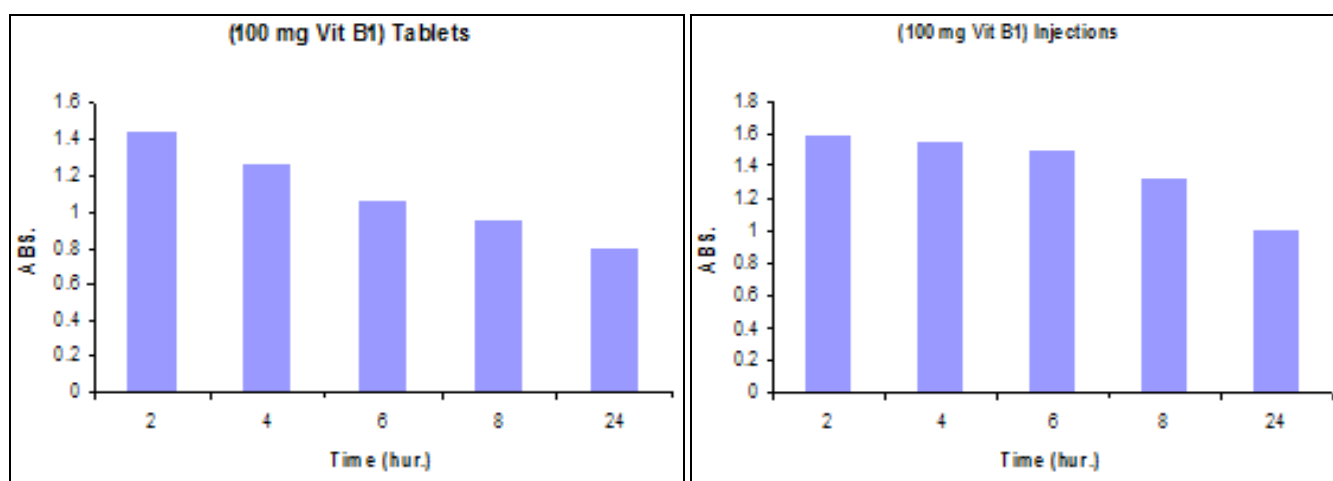


FIG.10: EFFECT OF TIME ON ABSORBANCE OF TWO URINE SAMPLES CONTAINING VITAMINE B₁.

TABLE 4: APPLICATION OF THE PROPOSED METHOD ON VITAMIN B₁ IN URINE SAMPLES

Urine sample Conc.($\mu\text{g}.\text{ml}^{-1}$) 0.5 ml of sample	Time (hrs.)				
	2	4	6	8	24
Vitamin B ₁ (Tablet 100mg)	86.739	73.378	63.781	57.272	47.982
Vitamin B ₁ (Injection 100mg/2ml)	94.905	92.751	89.689	79.639	60.822

CONCLUSION: A simple, spectrophotometric method has been proposed for the determination of trace amount of Thiamine HCl in aqueous solution and pharmaceutical preparations based on the diazotization reaction and coupling with Procaine HCl at room temperature, for the determination of vitamin B₁ in blood and urine sample. The results shows that the concentration of vitamin B₁ were decreased with time after taken a therapy and that

typical with the results of pharmacopeia of vitamin B₁ in human body.

The proposed method has some advantages like the fast determination of the drug on its pure form and in pharmaceutical preparations also it did not require temperature control, solvent extraction and expensive reagents and solvents.

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CONFLICT OF INTRESTS: The authors declare that they have no conflict of interests.

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