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RESEARCH ARTICLE

The Spectrophotometric Determination of Thiamine Hydrochloride Drug by Coupling with Diazotized Procainamide

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Abstract

For the determination of thiamine hydrochloride (THC) in both pure and formulated forms, a simple, sensitive and accurate spectrophotometric method was described. The technique is based on diazotization of the primary amine group of Procainamide with sodium nitrite and hydrochloric acid followed by combining with thiamine hydrochloride to create Light red azo dye showing peak absorption at (502) nm. Beer's law has followed the concentration range (2.5-40) µg.mL^{-1},with (0.1490) µg.mL^{-1} detection limit. The molar absorptivity and Sand ell's sensitivity were detected to be respectively (7285.0320) L.mol^{-1}.cm^{-1}and (0.0463) µg.cm^{-2}. The method for determining in thiamine hydrochloride pharmaceutical preparation has been applied successfully.

Keywords: *Thiamine hydrochloride, Spectrophotometric determination, Procainamide, Diazotization and coupling.*

Introduction

Vitamin B1 (thiamine hydrochloride, THC) (B1 or aneurine) [1, 2], referred to as thiamine-vitamin, is a sulfur-containing vitamin B1 was isolated and described in 1920, therefore it is called B1 because it was the first organic compound to be identified and found as a vitamin [3]. A water-soluble vitamin, methanol, and glycerol and practically insoluble in acetone, ether, chloroform, and benzene[4, 5]. Shows a significant biological function in the human body's carbohydrate metabolic process. Accurate estimates of vitamin B1 level are therefore very essential in the clinical environment as well as in food [6, 7].Thiamins are used by all living organisms, but only by fungi, bacteria, and plants were synthesized. People need adenosine triphosphate (ATP), which is used for power by all cells of the body [8] (Fig.1).

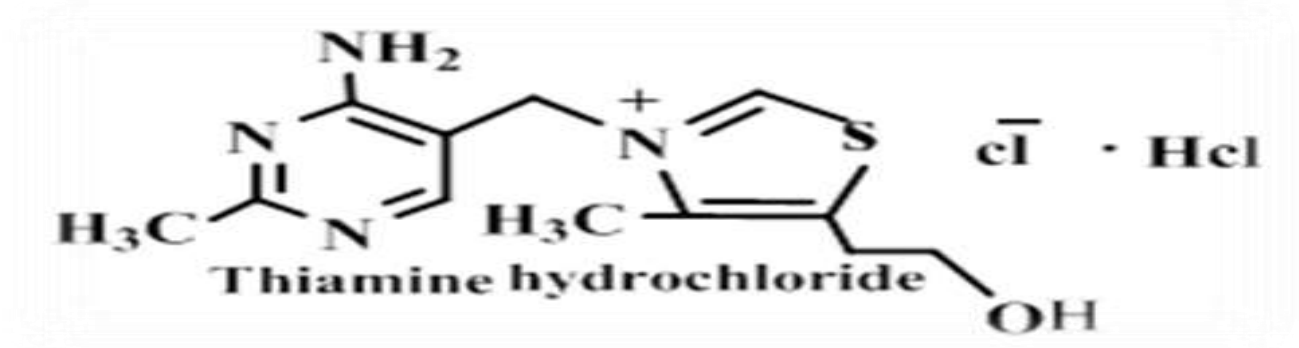


Figure 1: Thiamine hydrochloride Mw = 337.27 g / mol C12H17ON4SCl.HCl

Thiamine hydrochloride earlier revisions have distinct techniques for the assessment of thiamine, comprising electrochemical analysis method [9], spectrophotometry [10, 12], high performance liquid chromatography [13] and spectrofluorimetry [14]. The normal flow injection analysis technique (nFIA) includes the injection of a small volume of sample into

a reagent carrier stream which runs through a thin bore tube to a spectrophotometer where the derivative is estimated [15, 16]. The aim of the current work was to offer simple, sensitive, and rapid spectrophotometric method for the determination of THC. Spectrophotometric techniques for the determination of THC have been defined by its response with diazotized procainamide in alkaline media to create a colored yield that can be identified by spectrophotometricall.

Experimental Part

Apparatus

- A Shimadzu UV-Visible spectrophotometer 1800, Kyoto – Japan (UV probe 2.42 software),and all spectrophotometer measurements were carried out on (Cecil 7200 CE) UV-Visible double beam spectrophotometer with 10 mm matched quartz cells.
- Sartorius BL 210 electronic balance for weighing the samples were used.

Materials and Reagents

All Chemicals used are of the highest purity. A provided from different commercial company.

Standard Thiamine Hydrochloride (Industries and Medical Appliance, SDI, Samara, Iraq) Solution (500 µg.mL^{-1}=1.4825E-03)

Standard Thiamine hydrochoride solution (500) µg.mL^{-1} (stock solution) prepared by dissolving (0.0500) g of pure drug in 20 mL of distilled water and the volume was prepared up to the mark in volumetric flask (100 mL). More dilute solutions were prepared daily by appropriate dilution using distilled water.

Reagent Solutions

- Sodium nitrite Solution (1%(w/v)): prepared by dissolving (1) g of sodium nitrite (CDH) in (100) mL distilled water.
- Procainamide (0.1%(w/v)): prepared by dissolving(0.1) g of procainamide (sigma co.) in (100) mL distilled water.
- Sulfamic acid (1%(w/v)): prepared by dissolving (1) g of Sulfamic acid in 100 ml distilled water.
- Hydrochloric acid (0.5M): prepared by diluting suitable amount of concentrated hydrochloric acid to (100) mL with distilled water.
- Sodium hydroxide (1 M): prepared by dissolving (4) g of NaOH (CDH) in 100 ml of distilled water.

Pharmaceutical Preparations of Thiamine Hydrochoride

Pharmaceutical preparations were obtained from commercial sources.

- Neurobin Ampoules (Merck, KGaA,Darmstadt,Germany):100mg thiamine hydrochloride.
- Bécozyme Ampoule (france):10mg thiamine hydrochloride.
- KON-B-COMPLEX capsule (KONTAM Pharma-Hongkong):5mg thiamine hydrochloride.
- Samavit tablet (Samara-Iraq (SDI)):100 mg thiamine hydrochloride.

Procedure

Add 0.5 mL of (0.1%) PRA solution to a series of 10 mL calibrated flasks, followed by 0.3 mL of sodium nitrite (1%) and 0.5 mL of (0.5 M) HCl. Each solution was shaken carefully and left to stay in the ice bath for (5) minutes. Then 1 mL of sulfamic acid (1%) was added to remove nitronuim ion and the solutions were allowed to stand for 5 min. Then the increasing volume (0.05-0.8) mL of Thiamine hydrochloride solutions (500 µg.mL^{-1}) was transferred and the solutions were allowed to stand for (3) min and then 1 mL of NaOH (1M). The contents are mixed well and diluted to the mark with distilled water. The absorbances are estimated against the parallel reagent blank at (502) nm using 10-mm quartz cells.

Preparation of Sample Solution

In Tablets

Ten tablets (100mg/tablet) Samavet tablet (Samara-Iraq (SDI)) contents have been weighted and fine grinded. The fine powder of 100 mg was taken from a weight equivalent to 100 mg of tablets and the mean weight value of one tablet was calculated.A powder quantity equivalent to approximately 0.17249 g. was accurately weighed, then approximately 20 mL of distilled water was added. Then transferred to volumetric flask (100 mL) and diluted to the mark using

distilled water to get 1000 µg.mL^{-1}. After complete the volume, the solution was filtered using whitmman (41) mm., this solution was considered as stock solution. The diluted solution (500µg.mL^{-1}) was prepared daily using distilled water.

In Capsules

Ten capsules contents have been opend and the powder was mixed. The fine powder of (5mg /B-COMPLEX capsule (KONTAM Pharma-Hongkong) was taken from a weight equivalent to 5 mg for Capsules and the average weight value of one Capsule was calculated. A powder quantity equivalent to approximately 0.11655 g. was weighed accurately, then approximately 15 mL of distilled water was added. Then transferred to volumetric flask (25 mL), and diluted to the mark with distilled water to get 200 µg.mL^{-1}. All these solutions were filtered,and different concentrations (10,15,20 µg mL^{-1}) were prepared from this solution , and analyzed in three replicate by analytical

In Ampoules

- Each (3) mL of Ampoule solution containing(100mg/Neurobin Ampoules) of thiamine hydrochloride. A volume of (0.75 mL) ampoule solution was transferred to a standard volumetric flask and complete the volume to 50 ml with distilled water to become 500 µg.mL^{-1}.
- Another ampole (Bécozyme Ampoule/france) containing (2) mL of solution 10mg of thiamine hydrochloride. A volume 2 mL of ampoule solution was transferred to a standard volumetric flask and complete the volume to 20 mL with distilled water to become 500 µg.mL^{-1}

Additional suitable solutions of pharmaceutical preparations were prepared up by simple dilution.

Results and Discussion

The method involves the coupling reaction between diazotized procainamide withThiamine hydrochoride in base medium to give a red coloured azo dye .The absorption spectrum of the colored dye is shown in (Figure 2). Typically two stages are requisite to complete the diazotization coupling reaction. The 1st stage is convert the amino compound (Procaineamide) to diazo complex by reaction with nitrous acid ($NaNO_2$/HCl), whereas the 2nd stage is involve a coupling between diazotized reagent and the coupling drug [17]. The red dye product was only made in alkaline medium (sodium hydroxide). According mole ratio and continuous variation data, and the outcomes ratios, the reaction pathway were Suggested to continue as revealed in Scheme (1).

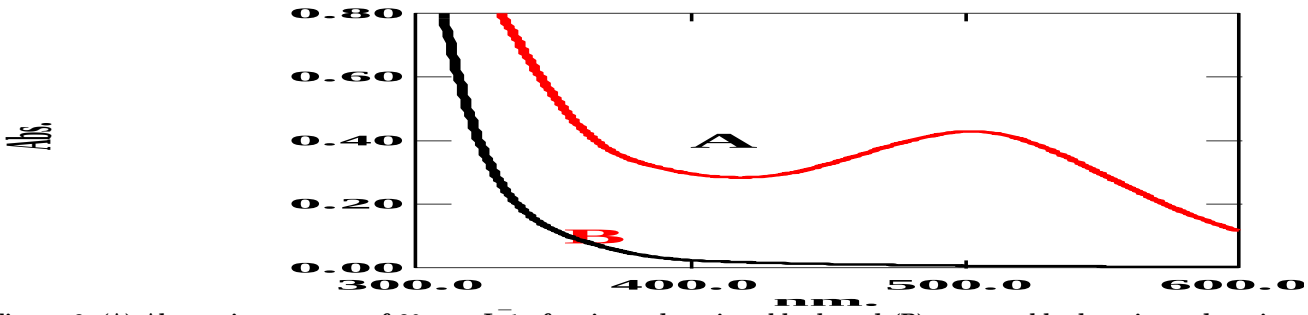
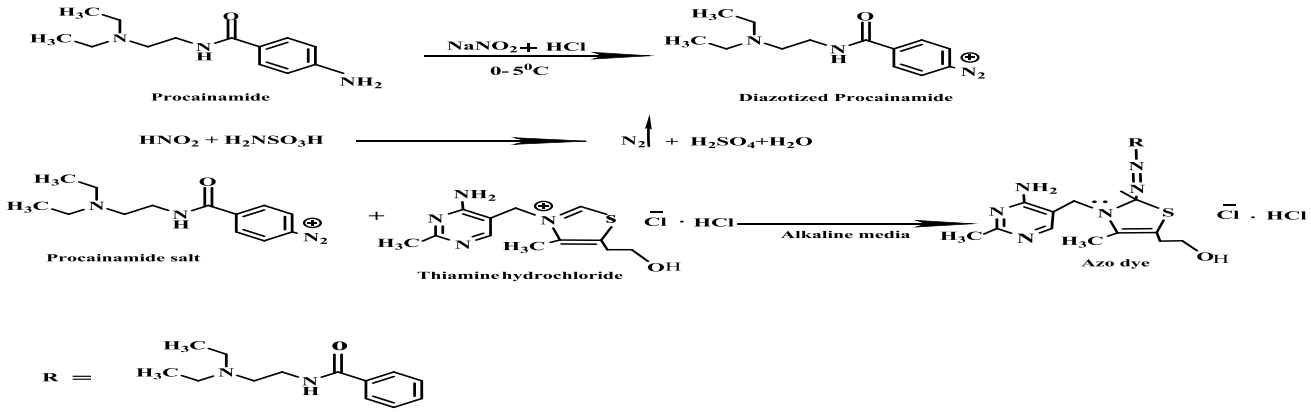


Figure 2: (A) Absorption spectra of 20 µg.mL^{-1} of estimated against blank and (B) reagent blank estimated against distilled water



Scheme 1: The proposed steps of the formation of the colored azo-dye

Study of the Optimum Reaction Conditions

The influence of numerous factors on the color development of azo dye was deliberate to acquire the optimal conditions to determine the THC. The optimization of all following experiments were achieved with (20 µg.mL^{-1}) in an ice-bath to increase the stability of the azo dye.

Effect of Reagent Volume

Different volumes of reagent (Procainamide (0.1%) was used in the rang of (0.1-2 mL) with fixing the volumes of HCl and NaOH. The maximum absorbance intensity was found with 0.5 ml of PRA (Figure 3).

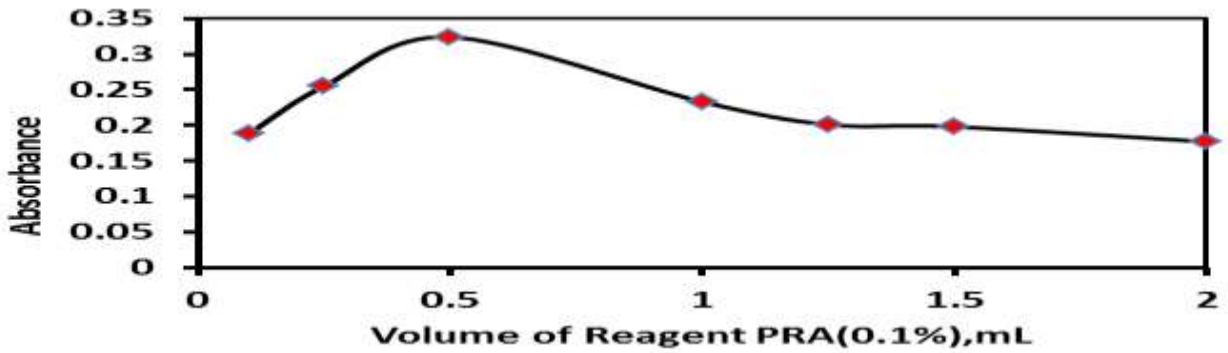


Figure 3: Effect of volume (0.1%) reagent, mL

Effect of Nitrite Volume

Figure (4) shows that the maximum absorbance reading at 502 nm is obtained by adding 0.3 mL of 1% sodium nitrite. Higher volumes of sodium nitrite gave a low absorbance. This may be attributed to the fact that the dizonium salt will be unstable because of the surplus amount of nitrous acid produced in the medium at addition of excess sodium nitrite mentioned [18].

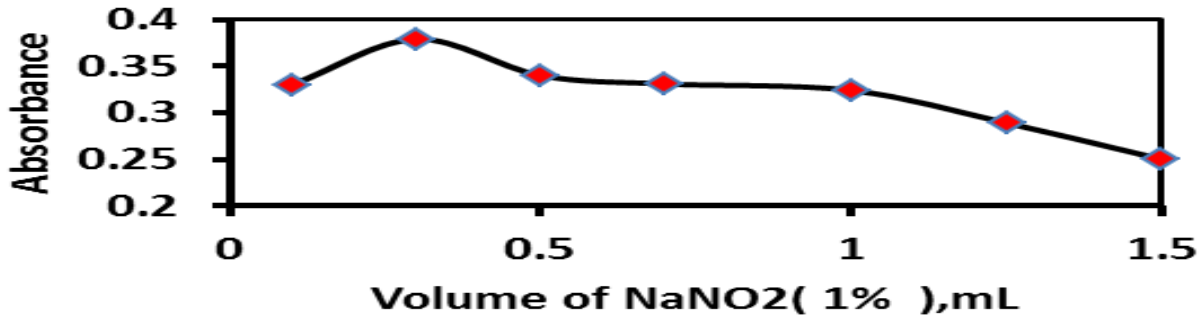


Figure 4: Influence of volume of (1%) $NaNo_2$, mL

Effect of Acid

Acidic medium is very necessary for complete the diazotization reaction. For that purpose the influence of diverse prepared acids solutions 0.5 mL of (0.5M) were tested for example; nitric acid, hydrochloric acid, sulfuric acid and acetic acid. The HCl gave a greater absorbance than other acids, hence HCl was the best appropriate acidic medium and was used in all subsequent experiments,Table (1).

Table 1: Effect of different acids on diazotization reaction

Type of acid	Absorbance
H2SO4	0.350
CH3COOH	0.286
HNO3	0.317
HCl	0.379

Accordingly, the influence of diverse volumes of HCl (0.5 M) was optimized on the maximum absorbance by change the volumes of HCl between (0.1-1.5) mL and fixing the optimized. The highest absorbance was obtained 0.5 mL of acid and was selected for addition used. Figure (5).

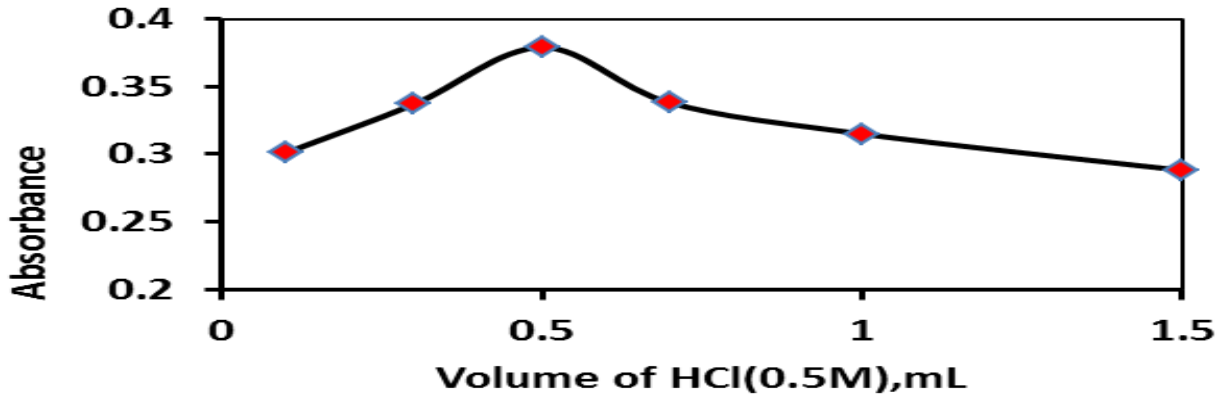


Figure 5: Influence of the volume of HCl (0.5M),mL

Effect of Diazotization Reaction Time

Table (2) shows that the optimum time for the diazotization of (PRA) was maxiumum when the diazotization reaction was let to stand for 5 minutes,where longer times led to extremely low absorbance values. This implies that long time destroy the diazotized product.

Table 2: The influence of diazotization reaction time

Time (minute)	Absorbance
0	0.300
3	0.321
5	0.379
10	0.342
15	0.302
20	0.289

Effect of Sulfamic Acid Volume and Time

The amount of sodium nitrite remaining from the dizoatization is not desirable as it works to neutralize the coupling factor which reduces its effectiveness towards the coupling process [19]. In addition, it works to increase the absorption of the blank solution most nitrozo compounds are predominantly yellow [20]. Therefore, the sulfamic acid was used to remove the remaining nitrite by reducing it to inert nitrogen gas [21]. It was found that the volume is 1 mL of 1% sulfamic acid solution and at (5) minutes time was enough to destroy the sodium nitrite and giving the best results from a higher absorption side to the solution of the model and less absorption of the solution blank.

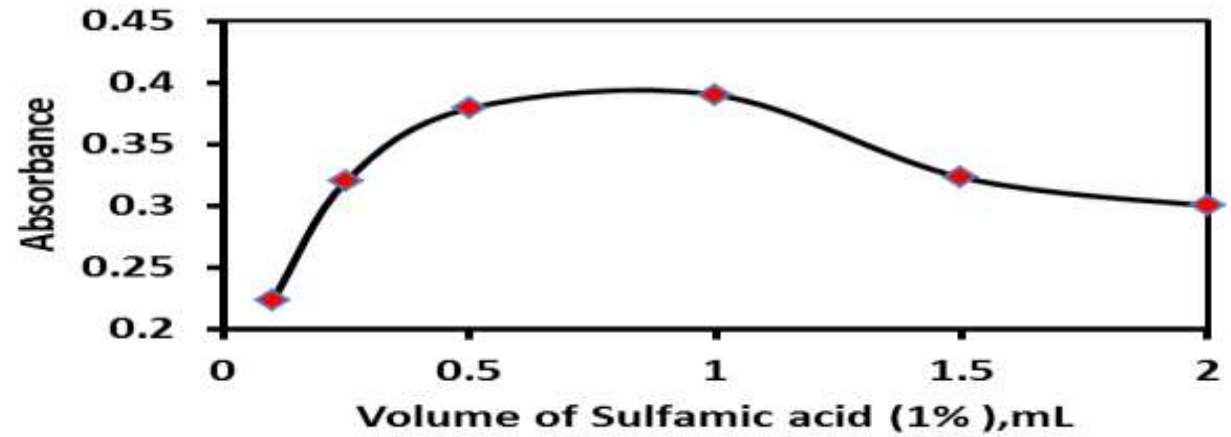


Figure 6: Effect of volume of (1%) sulfamic acid, mL

Table 3: The effect of time to destroy the sodium nitrite

Time (minute)	Absorbance
0	0.293
3	0.366
5	0.390
10	0.376
15	0.297
20	0.260

Effect of Temperature

The aryl and alkyl diazonium ions stability influenced by temperature [22]. Consequently, the effect of temperature on the colour intensity on the resulting product was studied. It Was found the coloured product was stable in an ice-bath at (0-5)°C, but at higher temperatures the absorbance decrease when the calibrated flask was placed in a water –bath at (45)°C or at room temperature (25) °C. Table(4).

Table 4: The effect of temperatures

Temperature, °C	0-5	25	45
Absorbance	0.390	0.338	0.201

Effect of the Reaction Time between the Drug and the Diazonium Salt

Table (5) shows the optimum reaction time between the drug and diazonium salt was determine by the color development. Thorough color intensity was attained after 3 min.

Table 5: Effect of the reaction time between the drug and the diazonium salt

Time (min)	Absorbance
0	0.399
3	0.411
5	0.390
10	0.378
15	0.369
20	0.356

Effect of the Base

The coupling reaction between diazonium ion and THC provides colour in base medium only as primary investigations indirected that. Consequently, diverse bases solutions(1M) such as sodium or potassium hydroxide, and ammonium hydroxide, table (6) it was clear that the sodium hydroxide was the appropriate alkaline medium for a greatest absorbance and it was used in all next experiments.

Table 6: Influence of different bases on the development of dye on the estimation of 20 µg.mL^{-1} THC

Type of bases	Absorbance
NaOH	0.429
KOH	0.420
NH4OH	0.008

The influence of NaOH volumes on color intensity also has been examined and it was initiated that 1mL of NaOH solution shows greatest absorbance Figure (7). So, this volume was designated for addition work.

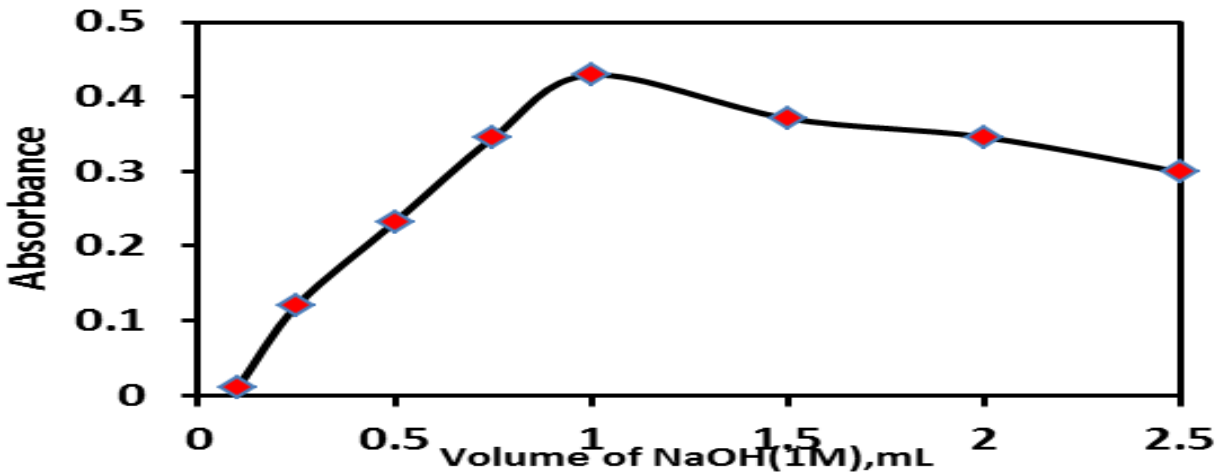


Figure 7: Effect of volume (1M) NaOH, mL

Effect of Time Before Dilution

The time required for complete diazotization reaction was found to be (0) minutes. Therefore the substances were diluted with distilled water to the mark and mixed well. Table (7). Letting the reaction for longer time intervals may favor the dissociation of the azo dye and the loss in colour intensity.

Table 7: The effect of time on diazotization reaction

TIME (minute)	*Absorbance*
0	0.430
3	0.368
5	0.290
10	0.206
15	0.194
20	0.176

Effect of Time on the Stability of the Complex Composed

The resulting colored product of the suggested method was found to be formed quickly and directly, Figure (8) shows the influence of time on THC yield was examined by consenting standing for variable times. The results indicated that the complex remains stable at least for 60 min.

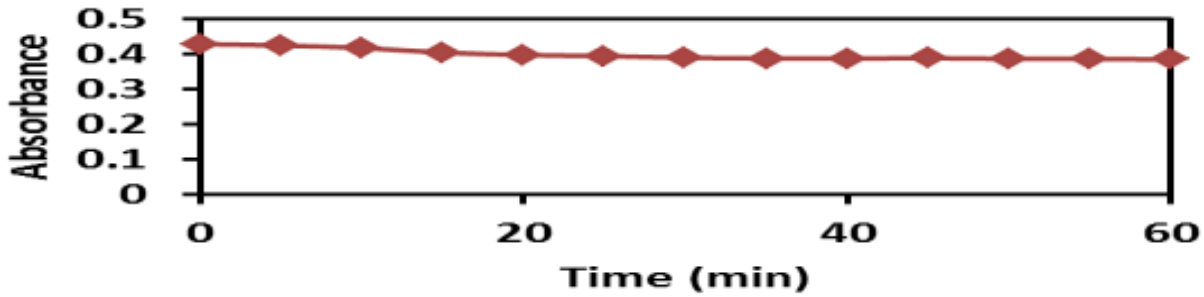


Figure 8: Effect of time on the stability of PRO-THC complex

Effect of Reagent Mixing Order

The order of reagent addition is very essential, so diverse orders of addition of reagent were tested and it was detected that the order of addition of reagent by mixing PRA with HCl then sodium nitrite, sulfumic acid, THC and soduim hydroxide gave the maximum absorbance and was deliberated in all future works.

Table 8: The effect order of addition

Addition order	*Absorbance*
(R+NaNO2+HCL)+Sulfamicacid+D+B	0.415
(NaNO2+R+HCL)+Sulfamicacid+D+B	0.420
(R+HCL+NaNO2)+Sulfamicacid+D+B	0.429
(R+HCL+NaNO2)+Sulfamicacid+B+D	0.384
(R+HCL+NaNO2)+D+Sulfamicacid+B	0.426
(R+HCL+NaNO2)+B+D+Sulfamicacid	0.188
(R+HCL+NaNO2)+B+Sulfamicacid+D	0.150

D:Drug, R:Reagent, B:Base

Calibration Curve

At the optimized conditions, a linear calibration graph was obtained from 2.5 to 40 ($\mu g.mL^{-1}$) of THC with correlation coefficient of 0.9998 and intercept of (0.0016). The detection limit was (0.1490) $\mu g.mL^{-1}$ as shown in Figure (9).

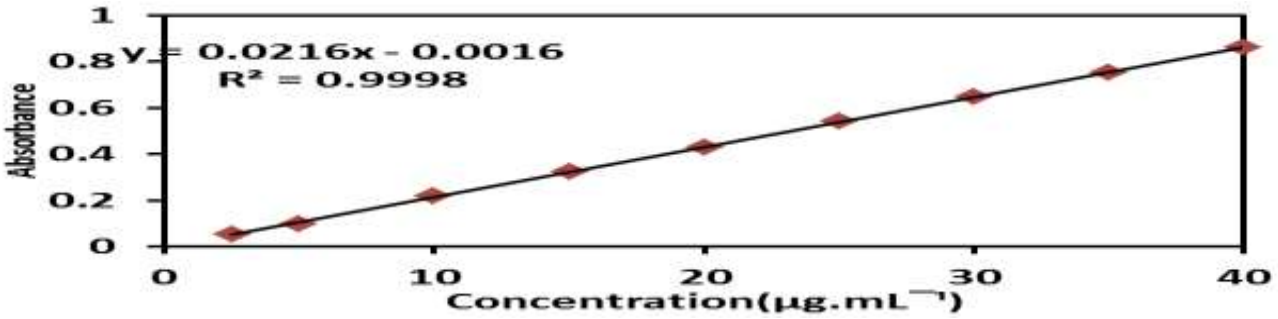


Figure 9: The calibration graph of THC

Nature of Product

Continuous variation and mole ratio methods [23]. The stoicheiometry of the reaction between equimolar solutions of THC and the reagent was examined using Job's method and mole ratio method, and the compound was designed in the ratio of 1:1 as demonstrated in Figures (10 and 11).

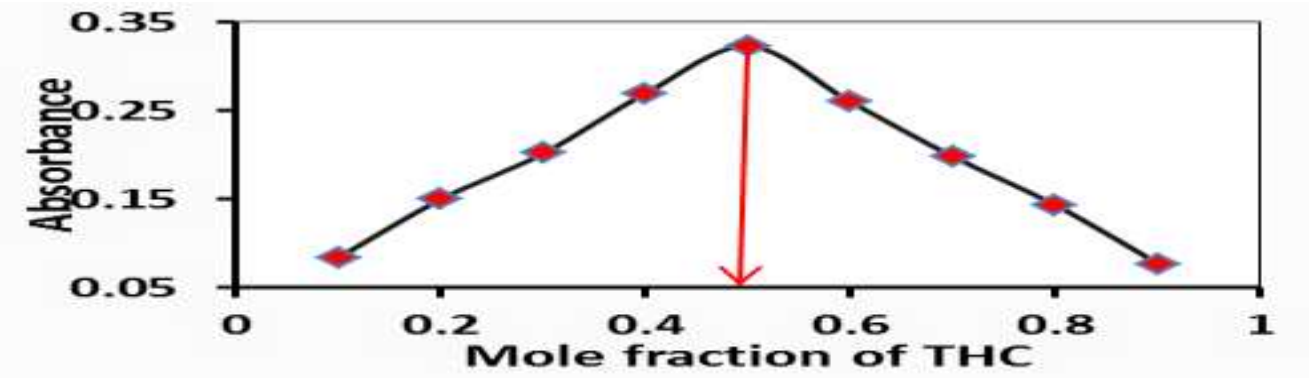


Figure 10: Job's method of continuous variation

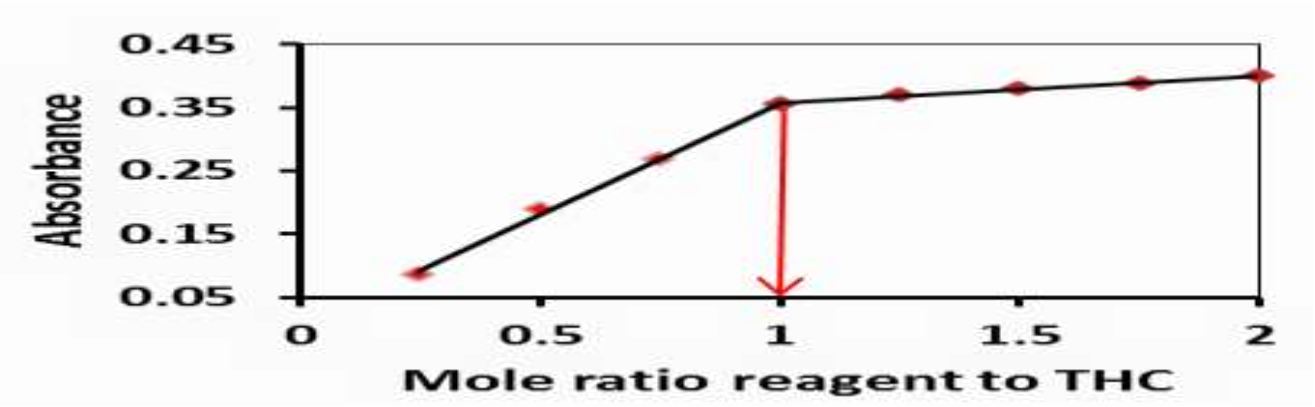


Figure 11: Mole ratio method

Analytical Data

Analytical values of statistical dealings for the are summarized calibration graph Table (9) demonstrates the optical and statistical characteristics of the optimization method.

Table 9: Analytical values of statistical dealings

Parameter	*Value*
Optical characteristics	
1.λmax (nm)	502
2.Molar absorptivity,ε(L.mol^{-1}.cm^{-1})	7285.0320
3.Sandell 's sensitivity, S (µg.cm^{-2})	0.0463
Regression analysis	
Slope(mL.µg^{-1})	0.0216
Intercept	0.0016
Correlation coefficient(r)	0.9998
Validation parameters	
Beer's Law Limit(Linearity,µg.mL^{-1})	2.5-40
Limit of Detection(µg.ml^{-1})	0.1490
Limit of Quantitation(µg.ml^{-1})	0.4518

Accuracy and Precision

Table (10) indicated good accuracy with reasonable precision of the proposed method. Thiamine hydrochloride was determined at three different concentrations in are briefed.

Table 10: Accuracy and precision of suggested method

Conc.,µg.ml^{-1}		*E%** $E\%=\frac{x-x^{\circ}}{x^{\circ}}x100$	*Rec.%** *Rec.%=100+E%*	*RSD%** $RSD\%=\frac{S}{X}x100$
Present	*Found*			
10	10.2592	2.5925	102.5926	0.4512
20	19.9814	-0.0925	99.9074	0.2316
30	30.0740	0.2469	100.2469	0.1539

*Average of three determinations,X=value,x°=true value

Interferences Study

In order to measure the analytical potential of the suggested method, the influence of certain excipients, glucose, sucrose, lactose,and acacia, was investigated by determination 20 µg.mL^{-1} of THC in the existence of a compound (1000 µg.mL^{-1}) above. The resultings are shown in Table (11). The suggested technique showed excellent tolerance for interference.

Table 11: Percent recovery for 20µg.mL^{-1} of THC in the presence of excipient

Excipient (1000µg.mL^{-1})	*Conc.of THC,µg.ml^{-1}*		Erel.,*%	*Rec.%*
	Persent	*Found*		
Sucrose	20	19.9351	-0.3240	99.6759
Glucose	20	19.8888	-0.5555	99.4444
Lactose	20	19.9814	-0.0925	99.9074
Acacia	20	19.9351	-0.3240	99.6759

Erel. = Relative error

Pharmaceutical Application

This method was applied for the determination of THC in its pharmaceutical preparations (capsules and Ampoules).

Direct Method

Tablet (containing 100 mg THC / tablet), capsule (containing 5 mgTHC / capsule) and ampoule (containing 100 mgTHC / ampoule) real samples with known THC content.This method was applied the samples, Table (12) shows the efficiency and success of the method developed to determine THC in its pharmaceutical formulation.

Table 12: The application of proposed method for determinationof THC in pharmaceutical preparation

Pharmaceutical Preparation	*Conc.of THC,µg.ml^{-1}*		*Erel..%*	*Rec%*	*RSD%*
	Present	*Found**			
Neurobin (Ampoules 100mg) Merk KGaA, Darmstadt, Germany	10	10.3209	3.2098	103.2099	0.6851
	15	15.1358	0.9053	100.9053	0.3531
	20	19.9814	-0.0925	99.9074	0.2316
KON-B-COMPLEX(capsule 5mg) KONTAM Pharma-Hongkong	10	10.2592	2.5925	102.5926	0.4512
	15	15.1358	0.9053	100.9053	0.3531
	20	19.9351	-0.3240	99.6759	0.2322
Samavit (Tablet 100mg) (Samara-Iraq (SDI))	10	10.3518	3.5185	103.5185	0.4472
	15	15.0895	0.5967	100.5967	0.1771
	20	19.9506	-0.2469	99.7530	0.1339

*Average of three determination

Standard Additions Method

The technique of standard additions is used to determine (10 mg THC Bécozyme Ampoule (france) pharmaceuticals to demonstrate that the technique created is free of interference. Various amounts (0.2, 0.3ml) of a pharmaceutical formulation solution (500 µg / ml) were transmitted to six volumetric flasks (10 ml) for each quantity, followed by an increase in quantities (0.1-0.5 ml) of (500 µg / ml) of standard THC solution, leaving the sixth flask without addition. The solution has been handled as in the calibration curve building. The absorbances was evaluated at 502 nm (Figure12) and the findings shown in Table (13) show that the standard additions technique is compatible with the direct method, indicates that the technique is satisfactory and free of interference with the acceptable range of error.

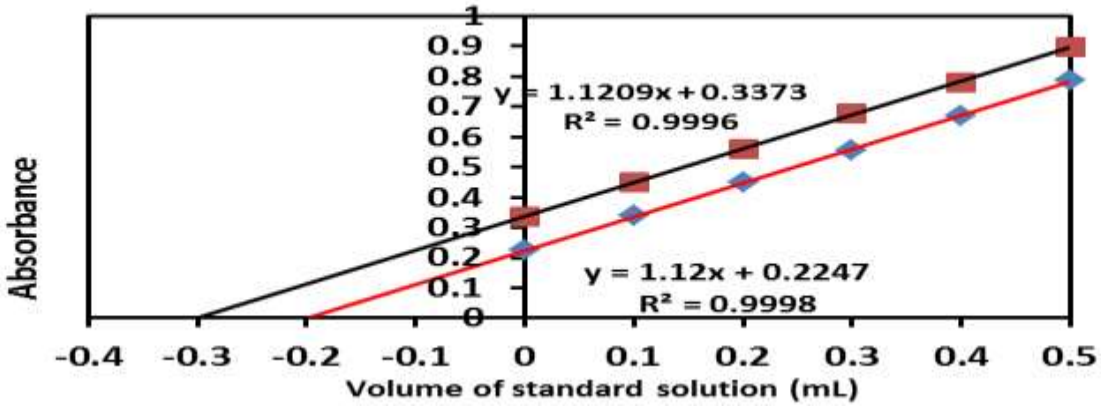


Figure 12: Determination of THC in Bécozyme (Ampoule 10 mg) france by standard addition method

Table 13: Application of the suggested technique in pharmaceutical preparation for THC concentration measurements by (SAM)

Pharmaceutical Preparation		*Conc.of THC, µg.ml^{-1}*		*Erel.%*	*Rec %*
		Present	Found		
Bécozyme (Ampoule 10mg) france	0.2 mL	500	496.1666	-0.7666	99.2333
	0.3 mL	500	498.7500	-0.2500	99.7500

Conclusions

In Soduim hydroxide, thiamine hydrochloride coupling with dizotization salt primary amine group was found to be a simple, sensitive, accurate and economic spectrophotometric method for quantitative determination of thiamine hydrochloride in pure form and pharmaceutical formulations. Different variables affecting the complet of the reaction were optimized. The proposed method offers good linearity and precision.

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