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# Spectrophotometric Determination of Thiamin Hydrochloride in Pharmaceutical Preparations by Azo-Coupling Reactions with Sulphacetamide Sodium Reagent.

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**A B S T R A C T**

A simple, accurate and sensitive spectrophotometric method for the determination of Thiamin Hydrochloride (THH) in aqueous solution has been proposed . The method is based on coupling of thiamine hydrochloride with diazotized sulphacetamide sodium (SUD) in alkaline medium and in presence of cetyltrimthylammumonium bromide (CTAB) to form a pink water- soluble dye that is stable and has maximum absorption at 512nm. The molar absorptivity and Sandells sensitivity values of the formed dye were $1.36\times10^4$ L.mol$^{-1}$.cm$^{-1}$, 0.02479 µg.cm$^{-1}$ respectively. Beer's law is obeyed in the concentration range (0.5-40µg.mL$^{-1}$). The linear regression coefficients values were a=0.0404, b=-0.0137, and r2=0.9985 calculated for the general equation of the calibration curve (y=ax+b). The limit of detection (LOD) and limit of quantification (LOQ) were found to be (0.135 and 0.452µg.mL$^{-1}$) respectively. The method has been successfully applied to the determination of thiamine hydrochloride in pharmaceutical formulations as tablets & injections.





## التقدير الطيفي لهيدروكلوريد الثيامين في المستحضرات الصيدلانية بواسطة الاقتران الآزوي مع كاشف سلفاسيتاميد الصوديوم

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**قسم الكيمياء ، كلية العلوم، جامعة الموصل، موصل، العراق**

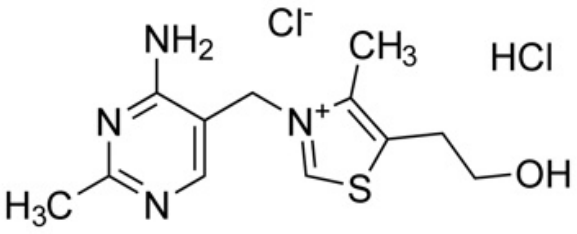
**الخلاصة**

تم اقتراح طريقة طيفية سهلة، دقيقة وحساسة لتقدير الثيامين هيدروكلوريد (THH) في الوسط المائي. تتضمن الطريقة تفاعل ازوتة كاشف السلفاسيتاميد الصوديوم ومن ثم اقترانه مع الثيامين هيدروكلوريد في الوسط القاعدي وبوجود عامل الشد السطحي ستريمونيوم بروميد(CTAB) لتكوين صبغة ذائبة ذات لون وردي واعطى امتصاص عند اعلى طول موجي 512 nm وكانت الامتصاصية المولارية ودلالة ساندل هي $1.36\times10^4$ لتر.مول$^{-1}$.سم$^{-1}$ و 0.02479 مايكروغرام.سم$^{-1}$ على التوالي . وكان مدى التركيز 0.5 - 40 مايكروغرام .مللتر$^{-1}$، وكانت قيم معاملات الانحدار الخطي a=0.0404, b=-0.0137, r2=0.9985، اما حد الكشف وحد التقدير الكمي فكانت القيم 0.135 و 0.452 على التوالي .

**الكلمات المفتاحية**: هيدروكلوريد الثيامين، اقتران ازوي، كاشف سلفاسيتاميد الصوديوم، الطيفي.

## 1. Introduction

Thiamine hydrochloride (THH), or vitamin B1, occurs as white crystals or crystalline powder that usually has a slight characteristic odor. Freely soluble in water. The chemical name of thiamine hydrochloride is thiazolium, 3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-5-(2-hydroxyethyl)-4-methylchloride, monohydrochloride and it has the following structural formula:




$C_{12}H_{17}ClN_4OS$ •HCl - M.W. 337.27

Thiamin is naturally present in some foods, added to some food products, and available as a dietary supplement. This vitamin plays a critical role in energy metabolism and, therefore, in the growth, development, and function of cells [1]. Food sources of thiamine include whole grains, meat, and fish[2]. Lack of thiamine causes the deficiency disease called beriberi. Many methods for the determination of thiamine have been proposed and developed, spectrophotometric[3-8], FIA-spectrophotometric[9-10], fluorescence[11], Chromatographic [12-13], or Electrochemical methods [14-15].

## 2. Experimental

### 2.1. Apparatus

All absorption measurement were done by a double beam shimadzu with 1.0 cm matched glass cell and the PH measurement were conducted by UV-Vis spectrophotometer.

### 2.2. Reagents And Materials

All reagents and chemical materials used were in high degree of purity.

#### 2.2.1. Thiamine Hydrochloride Solution (100 µg.mL$^{-1}$)

This solution was prepared by dissolving 0.0 1 g of pure substance in enough amount of water and the volume was completed to 100 mL with distilled water in a volumetric flask. The solution was then transferred to a dark bottle and it is stable for at least 5 days.

#### 2.2.2. Potassium Hydroxide (1M)

This solution is prepared by dissolving 5.61 g of potassium Hydroxide in (100 ml) distilled water in a volumetric flask.

### 2.2.3. Diazotized Sulphacetamide Sodium (SUD) Reagent ($4\times10^{-3}$M)

The diazotized SUD ($4\times10^{-3}$M) solution was prepared by dissolving (0.1016 g) of SUD in 60mL distilled water then 3mL of concentrated hydrochloric acid was added finally the mixture was transferred to a 100 mL volumetric flask and cooled at (0-5)$^{o}$ C in an ice-bath. Then a 0.0276 g of nitrite was added and the mixture was stirred vigorously, after 5 minutes the solution was made up to 100 mL with cold distilled water and was kept in a dark bottle in the refrigerator which stay stable for one week.

### 2.2.4. Tablets Solution (100 µg.mL$^{-1}$)

Weight and mix the contents of Ten tablets (each one contains 0.3942 g thiamin-HCl) ), an accurately weighed amount of powder equivalent to 0.01g thiamin-HCl was dissolved in 100 ml distilled water in a volumetric flask. The solution was filtered by using a Whatman No.1 to avoid any suspended particles.

### 2.2.5. Ampoules Solution (100 µg.mL$^{-1}$)

Each ampoule (JIANGSU KANGBAO Ltd, China) solution containing (100mg/2mL) of thiamine hydrochloride. transferred one ampoule to 100 mL standard volumetric flask and complete the volume to (100 mL) with distilled water. Then transferred 10 ml from the result solution to 100 mL standard volumetric flask and complete the volume to (100 mL) with distilled water.

### 2.3. Procedure And Calibration Graph

The aqueous solution 0.05–4.0 mL containing thiamine hydrochloride (100 µg.mL$^{-1}$) was transferred into a series of 10 mL calibrated flasks. To each flask 0.5mL of diazotized SUD solution ($4\times10^{-3}$M), 1 mL of potassium hydroxide solution (1M) and 2 mL of CTAB ($1\times10^{-3}$M) solutions were added then the volume was completed to the mark with distilled water. The absorbance was measured at 512 nm against a reagent blank which was prepared in a similar way but without the addition of thiamine hydrochloride. The calibration curve as shown in Figure (1).

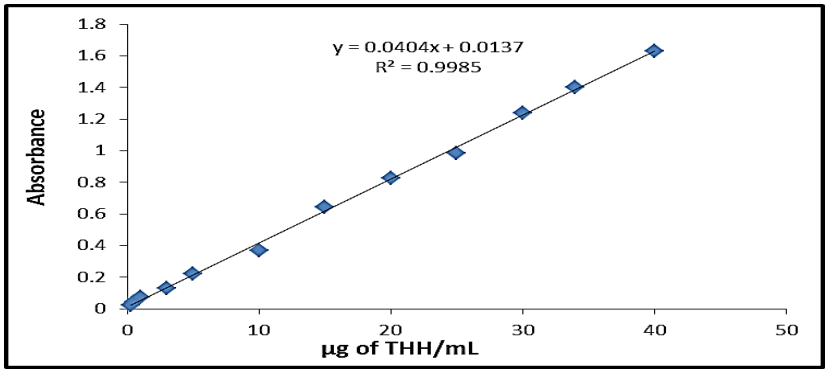
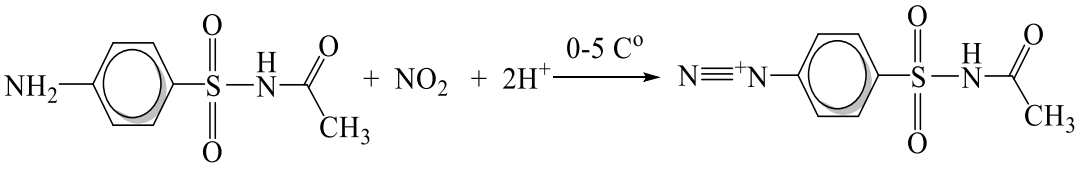



**Fig. 1:** Calibration graph for thiamine hydrochloride according to the proposed method

## 3. Results and Discussion

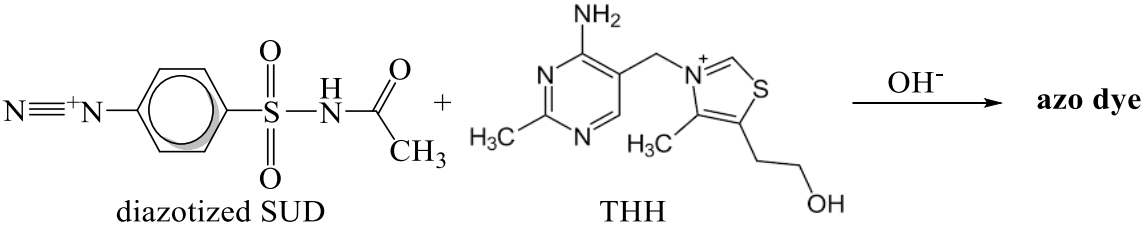
### 3.1. Principle of The Method

The proposed method depends on two steps: The first step include formation of diazotized SUD as following:




SUD diazotized SUD

the second step is the coupling of diazotized SUD with thiamine hydrochloride in basic medium to form a colored azo-dye that gives the highest absorption value at 512 nm, as shown in the following equation:




diazotized SUD THH

### 3.2. Study of The Optimum Reaction Conditions

The effect of various variables on the color development was studied to establish the optimum conditions for the determination of THH by coupling with diazotized SUD reagent using 1 mL (100 µg) of drug solution in final volume of 10 mL.

### 3.3. Effect of Reagent Amount

The effect of reagent amount on absorbance of formed azo-dye was investigated by adding different amounts of reagent and the drug. Results in Table 1, indicate that 0.5 mL of reagent gave highest values of absorbance and determination coefficient value, so it was selected for the subsequent experiments.

**Table 1:** Effect of reagent amount on absorbance

| mL of reagent (4mM) | Absorbance for of µg THH /mL | | | | | |
|---|---|---|---|---|---|---|
| | 5 | 10 | 15 | 20 | 25 | $R^2$ |
| 0.25 | 0.159 | 0.292 | 0.606 | 0.74 | 1.029 | 0.9831 |
| 0.5 | 0.155 | 0.331 | 0.607 | 0.719 | 1.028 | 0.9842 |
| 1.0 | 0.147 | 0.263 | 0.481 | 0.588 | 0.872 | 0.9759 |
| 1.5 | 0.098 | 0.179 | 0.33 | 0.429 | 0.733 | 0.9392 |
| 2.0 | 0.041 | 0.067 | 0.141 | 0.154 | 0.201 | 0.9628 |
| 2.5 | 0.009 | 0.024 | 0.046 | 0.064 | 0.219 | 0.7393 |

### 3.4. Effect Of Base

In studding of several types of weak and strong bases such as (KOH, NaOH, $Na_2CO_3$, $NaHCO_3$) and at a concentration of (1 M) it found that the use of a solution of (1 mL) of KOH gives the best results, so it was used for this use in subsequent experiments.

**Table 2:** Effect of type and number of bases on absorbance

| Base used (1M) | Absorbance/mL of Base used | | | | | |
|---|---|---|---|---|---|---|
| | Variable | 0.5 | 1 | 1.5 | 2 | 2.5 |
| NaOH | A | 0.201 | 0.249 | 0.273 | 0.241 | 0.243 |
| | λ | 492 | 492 | 494 | 492 | 494 |
| | pH | 12.40 | 12.73 | 12.98 | 13.00 | 13.08 |
| KOH | A | 0.322 | 0.345 | 0.335 | 0.342 | 0.344 |
| | λ | 496 | 496 | 494 | 496 | 495 |
| | pH | 12.30 | 12.57 | 12.85 | 12.62 | 12.89 |
| $Na_2CO_3$ | A | 0.095 | 0.148 | 0.108 | 0.096 | 0.075 |
| | λ | 300 | 342 | 346 | 344 | 316 |
| | pH | 6.64 | 6.89 | 7.43 | 7.70 | 7.83 |
| $NaHCO_3$ | A | No color contrast | | | | |

### 3.5. Effect Of Surfactants

In order to determine extent effect of surfactants on the absorption and stability of the formed dye formed, a (3 mL) of different types of surfactant were added to the medium of reaction. The obtained results listed in table 3.

**Table 3:** Effect of Surfactants

| *Order | Abs./mL of CPC ($1\times10^{-3}$M) | | | Abs./mL of SDS ($1\times10^{-3}$M) | | | Abs./mL of CTAB ($1\times10^{-3}$M) | | | Abs./mL of Tween(1%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 mL | 2 mL | 3 mL | 1 mL | 2 mL | 3 mL | 1 mL | 2 mL | 3 mL | 1 mL | 2 mL | 3 mL |
| I | 0.196 | 0.136 | 0.156 | 0.128 | 0.188 | 0.182 | 0.299 | 0.376 | 0.310 | 0.196 | 0.159 | 0.159 |
| II | 0.203 | 0.136 | 0.136 | 0.126 | 0.208 | 0.201 | 0.285 | 0.368 | 0.327 | 0.218 | 0.108 | 0.109 |
| III | 0.161 | 0.117 | 0.108 | 0.188 | 0.195 | 0.238 | 0.294 | 0.362 | 0.294 | 0.185 | 0.145 | Turbid |

*I= sample(S) + Surfactant(Sur.) + Reagent(R) + Base(B), II= S+R+Sur.+B, III=S+R+B+Sur

Results in Table (3) indicate that order (I) gave the highest absorption and stability to the azo dye formed in the aqueous solution, so it was adopted in subsequent experiments.

### 3.6. Reaction Time and Stability of The Azo- Dye Formed

The stability of the resulting dye was studied after establishing the optimal reaction condition. Table (4) shows that the dye is formed immediately after the addition of reaction components are remains stable at least for (30 min) which is enough period for several measurements.

**Table 4:** Effect of time on the absorbance

| Standing time (min.) | Absorbance for 100µg of THH |
|---|---|
| 5 | 0.373 |
| 10 | 0.367 |
| 15 | 0.365 |
| 20 | 0.363 |
| 25 | 0.360 |
| 30 | 0.358 |
| 35 | 0.354 |
| 40 | 0.349 |
| 45 | 0.346 |
| 50 | 0.344 |
| 55 | 0.339 |
| 60 | 0.337 |

### 3.7. Final Absorption Spectra

An absorption spectrum of the formed colored dye by coupling of THH with diazotized SUD in the presence of CTAB and in basic medium, against its corresponding reagent blank shows a maximum absorbance at (512 nm).

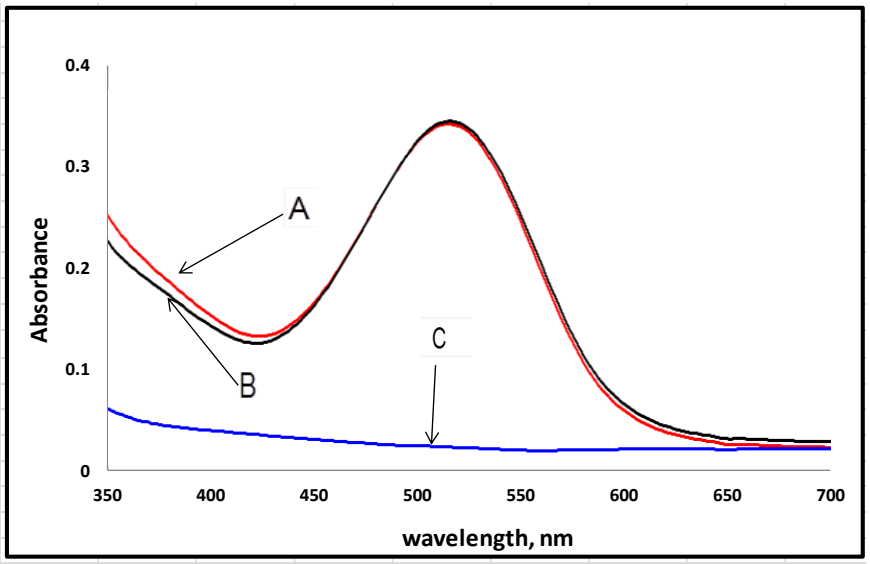



**Fig. 3**: Absorption spectra of 100µg of THH/10 mL treated according to the recommended procedure and measured against: (A) distilled water, (B) Blank, and (C) blank measured against distilled water.

### 3.8. Nature of The Dye Formed

In order to know the reaction ratio between the Diazotized SUD reagent and thiamine hydrochloride, the two solutions were prepared at a concentration of ($3.3x10^{-3}$)mole/L for each, and application of (Job's Method) [16], as shown in figure (5).

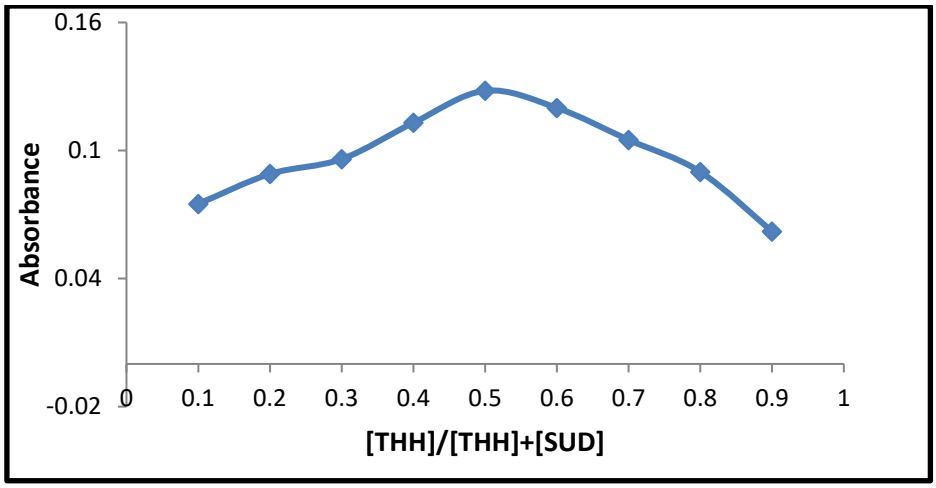
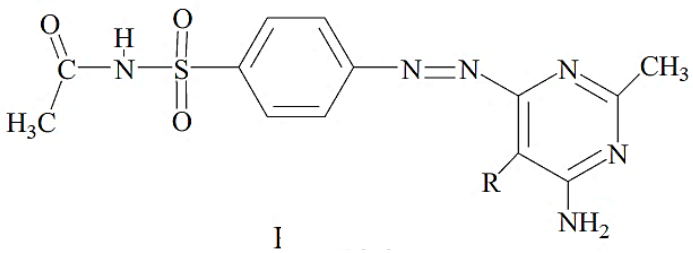



**Fig. 4:** Job's method for the formed azo- dye

So, the suggested formula of the produced azo-dye will be as following:




I

**R=$C_7H_{12}ONSCl.HCl$** **Pink colored Azo-**

### 3.9. Application of The Method

The proposed method was applied to determine Thiamine-HCl in its pharmaceutical preparations (tablet and injection), and the results are shown in table (5).

**Table 5:** Analytical applications of the proposed method.

| Pharmaceutical preparation | Taken amount of THH, µg | Found amount of THH, µg | Recover % | Relative error % | RSD% | t-test |
|---|---|---|---|---|---|---|
| B1 Tablets 100mg of THH/tab. USA | 100 | 99.8 | 99.8 | -0.16 | 0.11 | 1.68 |
| | 200 | 197 | 98.5 | -1.5 | 1.4 | 1.79 |
| $B_1$ Injection 100 mg THH/$mL^2$ China | 100 | 99.2 | 99.2 | -0.8 | 1.6 | 1.84 |
| | 200 | 198.2 | 99.1 | -0.9 | 2.2 | 1.92 |

*Average of five determinations

The results obtained are in the agreement with certified values compared with standard addition method (Fig. 5) and (Table 6).

### 3.10. Evaluation of The Suggested Procedure

The standard additive method [17] was successfully applied to verify the selectivity of the proposed procedure using two amounts from work solution of each available pharmaceutical preparations, and the results shown in (Fig. 5) and (table 6) show that the current method can be successfully applied to determine the presence of the rest of the components of the preparation.

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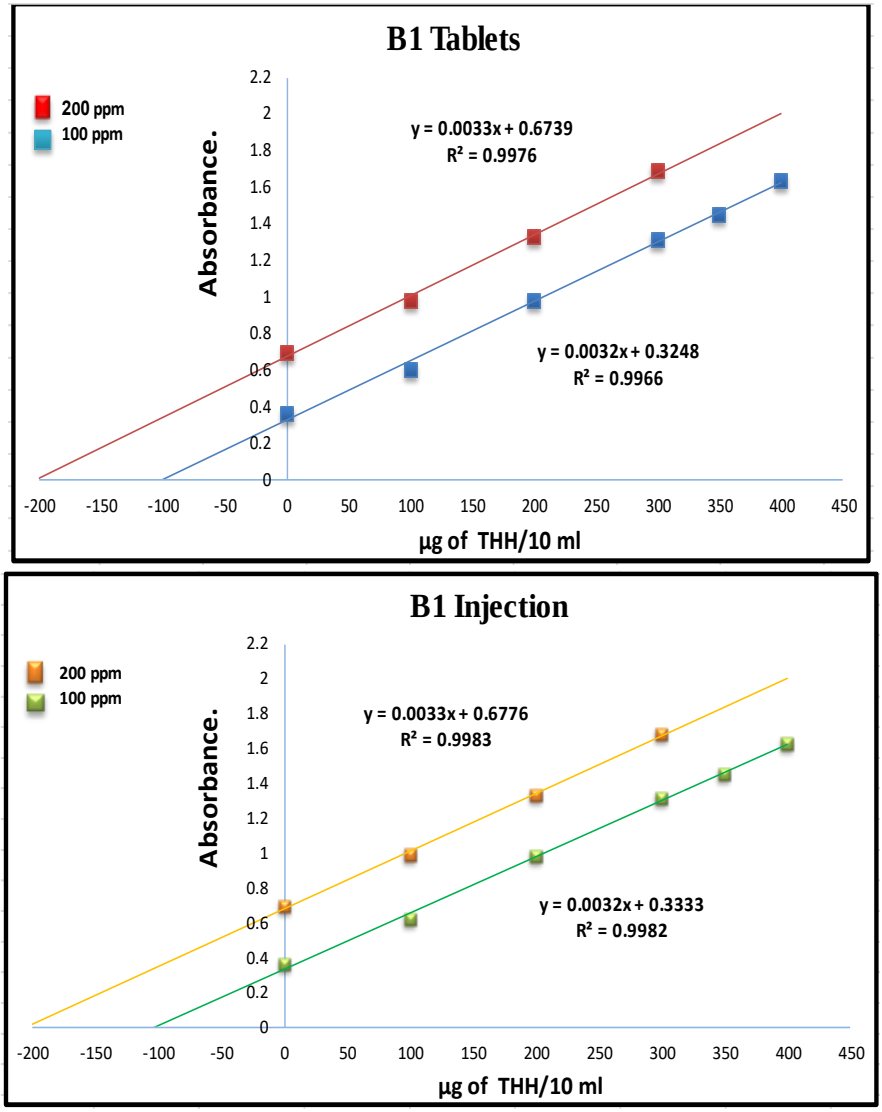


**Fig. 5**: Calibration graphs of standard addition methods for analysis of ESM in pharmaceutical preparations

**Table 6:** The results of standard addition method

| Pharmaceutical Preparation | Amount taken, µg/10mL` | Recovery % | |
|---|---|---|---|
| | | Present method | Standard addition method |
| B1 Tablets 100mg of THH/tab. USA | 100 | 99.8 | 100.8 |
| | 200 | 98.5 | 101.2 |
| $B_1$ Injection 100 mg THH/mL[2] China | 100 | 99.2 | 103.5 |
| | 200 | 99.1 | 102.6 |

*Average of five determinations

## 4. Conclusion

An easy, fast, and sensitive spectroscopic method was proposed for the determination of the pharmaceutical compound thiamine hydrochloride. The method was based on diazotized sulphacetamide sodium and then coupling with thiamine hydrochloride in a basic medium in the presence of the surfactant CTAB at room temperature to form a colored azo dye dissolved in water. The proposed method was successfully applied in pharmaceutical preparations, and when the results of the proposed method were compared with the results of the standard addition, the results were acceptable with a permissible error rate.

## 5. Acknowledgements

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