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SPECTROPHOTOMETRIC ESTIMATION OF THIAMINE HYDROCHLORIDE BY SCHIFF'S BASE COMPOSITION USING 2-CHLOROACETOPHENONE REAGENT

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Abstract: A simple, fast and sensitive method for estimating of Thiamine hydrochloride in pharmaceuticals has been proposed. The method is based on the reaction of 2-chloroacetophenone in sodium hydroxide to form a Schiff's base, the highest absorption of the product was 0.767 at Laboratory temperature at 480 nm. Beer's Law is obeyed in a concentrations range of (4 – 66 µg/ml) with a molar absorptivity 9.205×10^4 L\mol.cm, the limit of detections (LOD) and limitb of quantification were found to be 0.011 and 0.032 µg/ml. The method has been successfully applied in the estimation of thiamine in pharmaceuticals without interference by known substances that are used as additives to the drug. The results of the method were consistent with the adopted standard method.
Keywords: Thiamine hydrochloride, 2-chloroacetophenone, Schiff's base.

1. Introduction:

Thiamine Hydrochloride is a colorless pharmaceutical compound scientific name of 3-[(4-amino-2-methylpyrimidine-5yl),methyl]-5-(2hydroxyethyl)-4-methylthiazoliumchlorid HCl, and trade name[(1)] of surbex and the molecular formula of the drug $C_{12}H_{17}ClN_4OS$,HCL with a molecular weight of 337 g/mol is soluble in water and cholesterol and slightly soluble in alcohol[(2)] . They have used many and varied techniques to estimate the Thiamine hydrochloride drug, such as chromatographic methods [(3,4,5,6)], spectral methods [(7,8,9,10)] and electrolysis [(11)], and that mechanical coin like all B vitamins, vitamin B1 is soluble in water and is absorbed directly into the blood from the gastrointestinal tract. Once absorbed into the circulatory system, thiamine can circulate freely without carrier molecules in plasma and red blood cells until it is finally excreted in the urine. While in the body, it can be stored in the liver, but for a maximum of eighteen days. It can cross the blood-brain barrier and once absorbed into the blood, the enzyme thiamine diphosphotanesferase converts thiamine from its provitamin form to its active form, thymine pyrophosphate which is an enzymatic helper used in energy metabolism [(12)].

2. Aim of the research: The research aim to find a simple, fast and economical way to estimate the Thiamine hydrochloride drug using a reagent that gives a colored output when associated with this drug, as well as to find out the equivalence of the drug with the reagent by adopting the molar ratio method and the method of continuous changes (job method), and the success of the proposed method in estimating the drug in the

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pharmaceutical preparation.

3. Apparatus: UV- VIS Spectrophotometer. T92+ UV Spectrophotometer "PG INSTRUMMENTS with1cm PLastic cells". UV-VIS Spectrophotometer "Single beam from Genesis UV10. UV-VIS Spectrophotometer "double beam from Shimadzu (model UV-1800 2- Balance Kern 770GS/GJ from Sartorius BL210S. Semi-Micro Analytical Balances. PH meter

4. Materials:

Material	Company	Molecular formula	Molecular weight (g/mol)	Purity %
Thiamine HCl	SDI Samarra Iraq	$C_{12}H_{17}ClN_4OS$	300.81	99.9
2-Chloroacetophenone	Merck	C_8H_7ClO	154.59	97
Ethanol	Scharlau	C_2H_5OH	46.068	99.9
Sodium hydroxide	GCC	NaOH	40	98
Ammonium hydroxide	GCC	NH_4OH	35.05	25
Potassium hydroxide	GCC	KOH	56.105	98
Sodium Lauryl Sulphate	GCC	$NaC_{12}H_{25}SO_4$	288.38	99
Lactose Monohydrate	GCC	$C_{12}H_{22}O_{11}H_2O$	360.31	99
Cellulose	BDH	$(C_6H_{10}O_5)_n$	162.1406	99
Magnesium stearate	BDH	$(C_{18}H_{35}O_2)Mg$	591.27	96

5. Preparation of solutions:

- Standard Thiamine hydrochloride solution at a concentration of 1000 µg/ml

It was prepared with a weight of 0.1000 gm and dissolved in dimethyl sulfoxide with heating in a water bath to 75 degrees Celsius and completing the volume to the mark in a 100 ml volumetric flask, and the other solutions were then prepared by dilution.

- Reagent solution of 2-chloroacetophenone at a concentration of 0.01 molar

Prepared by weighing 0.1540 gm of 2-chloroacetophenone and dissolving it in ethanol and completing the volume to the mark in a 100 ml volumetric flask.

- Sodium hydroxide solution with a concentration of 1.0 molar

The preparation was carried out by weighing 4 gm of sodium hydroxide and dissolving it in distilled water and completing the volume to the mark in a volumetric flask of 100 ml.

- Potassium hydroxide solution with a concentration of 1.0 molar

The preparation was carried out by weighing 5.6 gm of potassium hydroxide and dissolving it in distilled water and completing the volume to the mark in a 100 ml volumetric flask.

- Ammonium hydroxide solution with a concentration of 1.0 molar

It is prepared by taking 7.7 ml of concentrated NH_4OH base solution (6.49 molar) and diluting it with distilled water to the limit of the mark in a 50 ml volumetric flask.

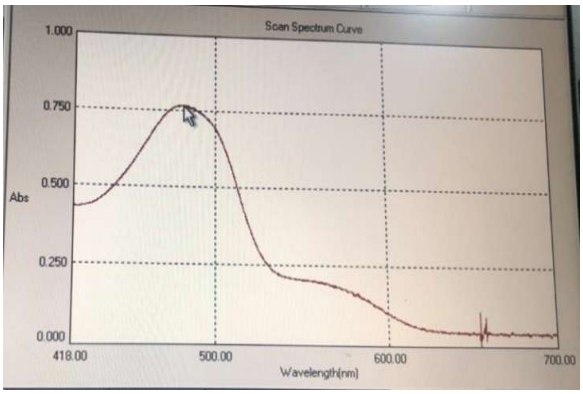
-Solutions (Sodium lauryl sulfate - Cellulose-Magnesium stearate-Lactose monohydrate) 1000 µg/ml

It was prepared by weighing 0.1000 gm of it and dissolving it in distilled water, except for cellulose, it was

dissolved with ethanol and completing the volume to the mark in a 100 ml volumetric flask.

6. Working steps: By conducting several preliminary experiments, the optimal conditions for the formation of the Schiff's base were reached by adding 2 ml of Thiamine hydrochloride solution at a concentration of 500 µg/ml, after which 0.5 ml of Sodium hydroxide was added at a concentration of 1.0 molar, then followed by the addition of 2 ml of 2-chloroacetophenone reagent, then the volume was completed with ethanol to the mark in a 10 ml volumetric flask against its nanometers, while his optical solution did not give any absorption in this region.

7. Results and discussion: The absorption spectra are shown in figure (1), which represents the absorption spectrum of the complex versus blank to form the product with the highest absorption at 480 nm, and figure (2) which represents the absorption spectrum of the blank solution versus ethanol.



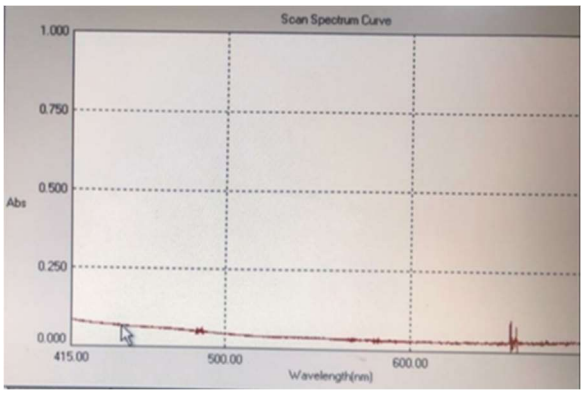


Figure (1): Absorption spectrum of complex against blank. Figure (2): Absorption spectrum of the blank against ethanol

8. Optimal conditions for the formation of product:

8-1 Effect of the base volume: By adding increasing volumes of Sodium hydroxide solution whose concentration is 1.0 molar, to determine the extent of its effect on the absorption value of the product, and as shown in figure (3), it was found that the best added volume is 0.5 ml.

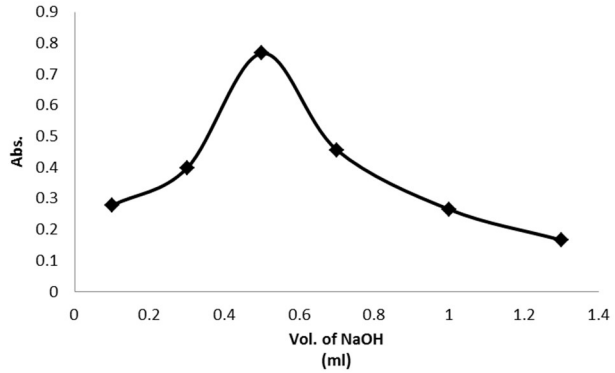


Figure (3): Effect of NaOH Volume on the absorption values of the product

8-2 Effect of the use different bases

When different bases were used (Sodium hydroxide, Potassium hydroxide, Ammonium hydroxide) and their

concentration was 1.0 molar and add a volume of 0.5 ml, to find out which bases used give the best absorption when the product is formed, as shown in table (1).

Table (1): Effect of the use different bases on product

Absorption	Base
0.587	KOH
0.131	NH_4OH
0.766	NaOH

From the table it is shown that the use of Sodium hydroxide gives the highest absorption value.

8-3 Effect of reagent volume

By adding variable volumes of the reagent with an initial concentration of 0.01 molar, it was found that the absorption is best when adding a volume of 2 ml, as shown in figure (4).

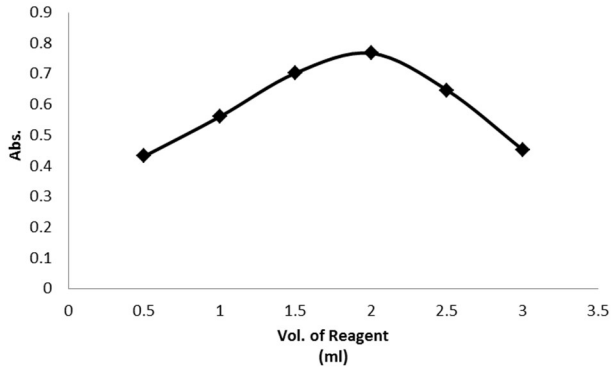


Figure (4): Effect of reagent volume on the absorption values of the product

8-4 Effect of time on the stability of the product

Through the importance of the stability of the product formed by the interaction of the drug with the reagent in a base medium to identify the period of time that the formed product can remain constant, and through the experiments conducted, it was found that the stability of the absorption values to about 60 minutes, and this is enough time to make the required measurements, according to what is shown in table (2).

Table (2) : Effect of time on the stability of the product

Absorption value	Time (min.)
0.764	0.00
0.765	10
0.766	20
0.767	30
0.767	40
0.767	50
0.765	60
0.760	70

0.756	80

8-5 The effect of additives on the resulting product

When adding different volumes of additives to the resulting product, it turned out that it does not affect the absorption value, as two different concentrations were added to each of the additives in a 25 ml volumetric vial, and it turned out that this method can be applied to pharmaceuticals, as shown in the table (3).

Table (3): the effect of additives

RE%	Added concentration (µg/ml)	RE%	Added concentration (µg/ml)	Additives
-0.72	160	1.55	80	Lactose monohydrate
1.94	160	0.42	80	Magnesium stearate
2.75	160	1.63	80	Sodium lauryl sulfate
3.06	160	2.44	80	Cellulose

8-6 Calibration curve

Figure (5), shows that the linearity of the calibration curve under optimal conditions was within the concentrations (4-66) µg/ml, but at higher concentrations the curve deviates from linearity, i.e. (non-compliance) with beer's law.

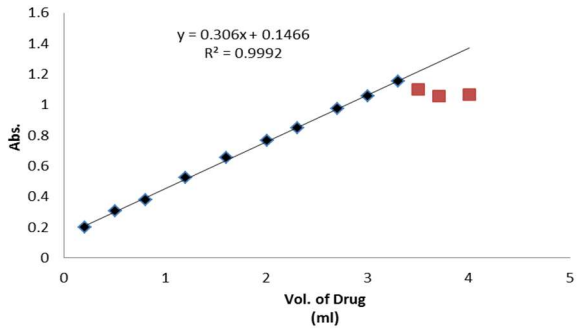


Figure (5): calibration curve for Thiamine HCl – 2-chloroacetophenone product

8-7 Correlation ratio: To set the ratio of the Binding of Thiamine hydrochloride with the reagent 2-chloroacetophenone in the spectroscopically formed product, this is done by two methods, the molar ratio and the method of continuous changes (job's method), where in the first method increasing volumes (0.3-2.7) ml of the reagent with a concentration of 0.01 molar were added to a fixed volume of 1.0 ml of Thiamine hydrochloride with an initial concentration of 0.01 molar in a volumetric vial of 10 ml, with the same steps and the same conditions the results shown in figure (6).

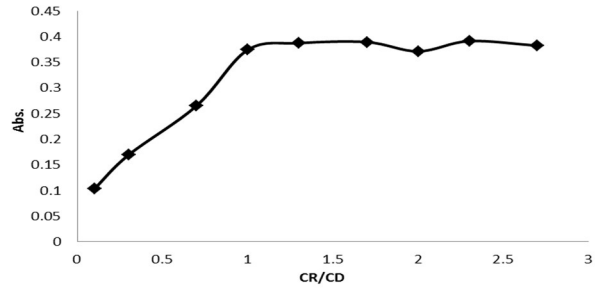


Figure (6): Molar ratio for the product

The second method (job's method), used decreasing volumes (4.5-0.5) ml of the reagent at a concentration of 0.01 molar and adding increasing volumes of the drug (0.5-4.5) at a concentration of 0.01 molar, so that the sum of the added volumes of the drug and the reagent is equal to 5 ml, and using pre-installed conditions and steps, the results shown in Figure (7) were obtained.

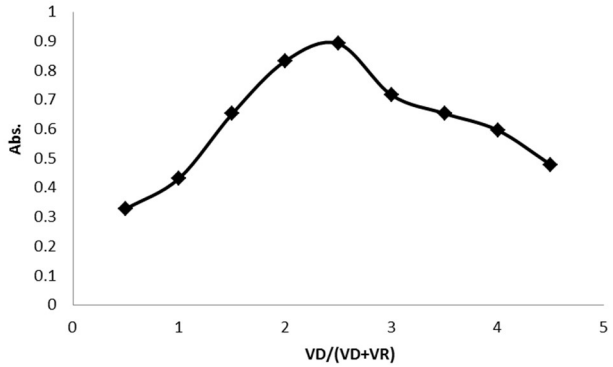
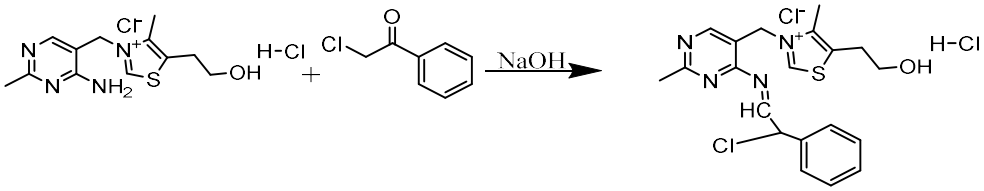


Figure (7): Job's method for the product

Both methods confirmed that the equivalence ratio between the drug and the reagent (1:1), where the drug binds to the reagent by the amine group, as the proposed reaction can be based on a condensation reaction between thiamine hydrochloride and 2-chloroacetophenone in a base medium to form a yellow-colored product (Schiff's base) [13], as shown in the equation below:



9. Applications

The proposed method was applied to estimate Thiamine hydrochloride in the pharmaceutical preparation in the form of tablets, as 10 tablets were weighed and weighed 11.2813 gm, and then it was grinded well until it turned into a fine powder and 0.2256 gm was taken from it and dissolved in dimethyl sulfoxide and the volume was completed to the mark in a 100 ml volumetric vial, then it was filtered and three volumes were taken from the filterer to estimate Thiamine hydrochloride in the preparation [14-37], the results are shown in table (4).

Table (4): Application of the proposed method

Pharmaceutical preparation	Taken (µg/ml)	Obtained In the proposed method (µg/ml)	% Rec.	The manufacturer
BENEDAY	40	39.65	99.13	(Neutec ilac San. Tic. A.S.) Turkey
	80	80.05	100.06	
	115	114.24	99.34	

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