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Colorimetric Assay of Thiamine Hydrochloride in Pharmaceutical Preparations

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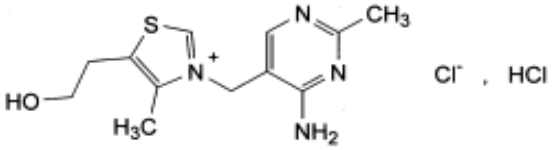
Abstract:

A simple, rapid, accurate and sensitive spectrophotometric method for the determination of thiaminehydrochloride has been developed. The method is based on the formation of the Schiff's base between the primary amino group present in thiamine hydrochloride and aldehyde group present in the vanillin reagent to produce a yellow colored complex having maximum absorption at 390 nm. Beer's law has obeaid over the concentration range of 2-28µg/mL, with molar absorptivity of $0.96x10^4$L/mol.cm. The average recovery which is a measure of accuracy is 100±1.3% and the relative standard deviation (RSD) is less than1.5 .The present method is considered to be simple because it does not need heating, hydrolysis and solvent extraction steps. The ingredients often formulated with thiamine and have been shown not to interfere, and is suitable for the routine determination of thiamine hydrochloride. The proposed method has been successfully applied for the determination of thiamine hydrochloride in pure form and in pharmaceutical preparations.

Key words: Pharmaceutical, Preparations Spectrophotometric, Thiamine hydrochloride.

Introduction:

Thiamine hydrochloride or vitamin B1 (sulfur-containing vitamin) is the first vitamin of the water soluble B complex group category of vitamins.The chemical name is3-(4-Amino-2-methylpyrimidin-5-ylmethyl)-5-(2-hydroxyethyl)-4-methylthiazolium chloride hydrochloride $C_{12}H_{17}ClN_4OS,HCl$ (Fig. 1)(1,2).



$C_{12}H_{17}ClN_4OS,HCl$ =337.3

Figure 1. Chemical Structure of Thiamine hydrochloride (1,2).

Thiamine hydrochloride deficiency develops when the dietary intake is inadequate; severe deficiency leads to the development of a syndrome known as beri-beri(1,2). Only a few HPLC(3-4), Spectrofluorimetric(5), Amperometric(6), Chemo metric (7), Capillary electrophoreses (8), Ion pair Liquid Chromatography(9) and Spectrophotometric(10-13).

In the view of the need in the industry for routine analysis of thiamine hydrochloride, attempts are being made to develop simple and accurate instrumental methods for quantitative estimation of thiamine hydrochloride. This paper reports a simple, accurate, and precise spectrophotometric method for the determination of thiamine hydrochloride in pure form, pharmaceutical formulations, and application to content uniformity testing,

Materials and Methods:

Apparatus

Spectra-scan 50 UV- visible (double beam) spectrophotometer with 1.0 cm quartz cells was used for absorption measurements.

Reagents

All chemical used were of analytical or pharmaceutical grade. Standard materials and pharmaceutical preparations (Pharmaceutical grade thiamine hydrochloride and tablets 100 mg were kindly supplied as a gift sample from State Company of Drug Industries and Medical Appliance(NDI) Nineveh- Iraq, and thiamine hydrochloride ampoule 100mg (were provided from the State Company of Drug Industries and Medical Appliance (SDI) Samara- Iraq .)

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Thiamine Hydrochloride Standard Solution: 100µg/ml ($2.965x10^{-4}$M)

This solution was prepared by dissolving 0.01 gm. of thiamine hydrochloride in 100 mL of distilled water in a calibrated flask.

Vanillin Solution: 0.1%

This solution was prepared by dissolving 0.1 gm. of vanillin in amount of ethanol and completed to 100 mL in volumetric flask.

Sodium Hydroxide Solution: (1N).

This solution was prepared by dissolving 4gm of sodium hydroxide in 100 mL of distilled water in a calibrated flask.

Recommended Procedure

An aliquots of standard solution of thiamine hydrochloride (50-700 µg) were transferred into a series of 25mL volumetric flasks, 2 mL of 1N sodium hydroxide, and 1 mL of vanillin solution were added. The contents were diluted to the mark with distilled water. The absorbencies were measured at 390 nm against a reagent blank.

Procedures for Pharmaceutical Preparations (Tablets):

To minimize a possible variation in the composition of the tablet, the mixed content of 10 tablets(100mg/tab) were weighed and grounded, then the powder equivalent to 100 mg of thiamine hydrochloride was accurately weighed and transferred into a 100mL calibrated flask ,amount of distilled water was added and the solution was shaken for 20 minutes. Then the volume was made up to 100mL with distilled water, mixed well, and filtered using a whatman No.42 filter paper. 10mL of this solution was diluted to a 100mL with distilled water in a calibrated flask. 3mL of this solution was treated as mentioned under recommended procedure.

Injection

An ampoule containing 100mg of thiamine hydrochloride which were provided from the state company of drug industries and medical appliance (SDI) Samara- Iraq.The content of 5 ampoules were mixed well in 250ml dried beaker. A aliquots equivalent to 100 mg of thiamine hydrochloride was transferred into 100 mL volumetric flask and diluted up to the mark with distilled water, 10mL of this solution was diluted to 100mL with distilled water and a aliquot of this solution was treated as described above for analytical procedure.

Results and Discussion:

Spectrophotometric methods development for the determination of drugs has been increased considerably in recent years because of their importance in pharmaceutical analysis(14, 15). A new method has been developed for the spectrophotometric determination of thiamine hydrochloride. The method was based on the formation of the Schiff's base between the primary amino group present in thiamine hydrochloride and the aldehyde group present in vanillin against the corresponding reagent blank as shown below(Fig.2).

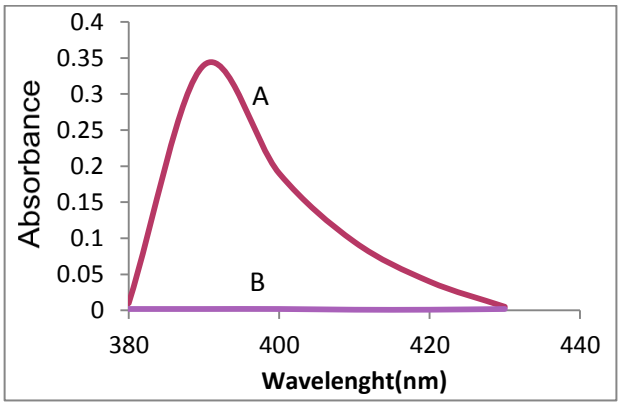


Figure 2. Absorption spectra of (A) - 12µg/ml of thiaminehydrochloride with vanillin against reagent blank. (B) -reagent blank against water.

Study of the Optimum Reaction Conditions

The effect of various parameters on the absorption of the product formed was studied and the reaction conditions are optimized.

Effect of Sodium Hydroxide Concentration

The amount of sodium hydroxide solution for maximum color intensity was examined. Themaximum constant color intensity was reached when using 2 mL of 1 N sodium hydroxide solution. This amount was selected for subsequent experiments (Fig 3)

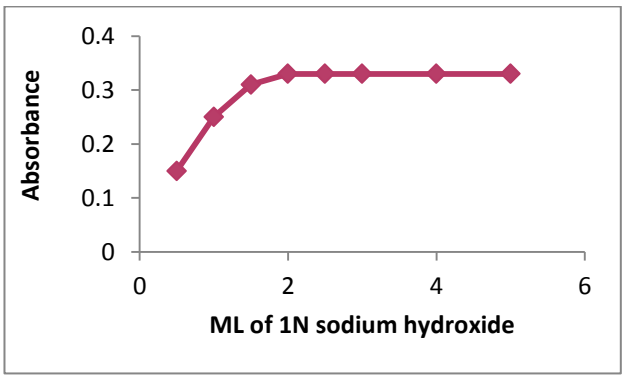


Figure 3. Effect of the amount of 1N sodium hydroxide

Effect of Vanillin Reagent

The effect of the amount of vanillin solution amounts on the absorbance was investigated. A maximum and constant absorbance was found with 1 mL (Fig. 4)

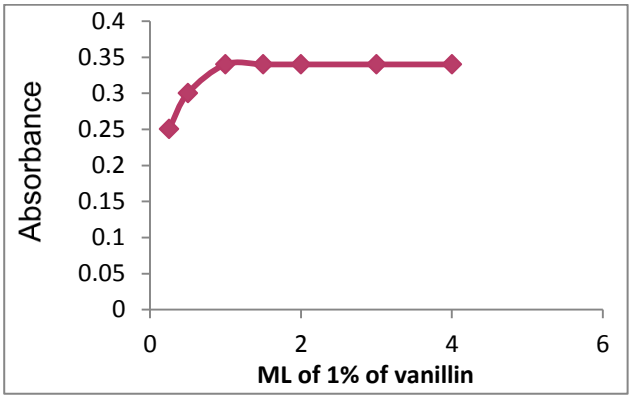


Figure 4. Effect of the amount of 1% Vanillin Solution

Effect of Reaction Time

The time for complete color formation occurred immediately and remained stable for at least 24hours.

Effect of Order of Addition

To test the effect of order of the addition of the reagents on the absorbance of the product, different orders were tested. The selected order was thiamine hydrochloride solution, NaOHsolution followed by vanillin solution ,this order gives the highest absorbance value. Under the experimental conditions described, Beer's law is obeyed over the concentration range 2.0- 28µg/mL (Fig. 5).

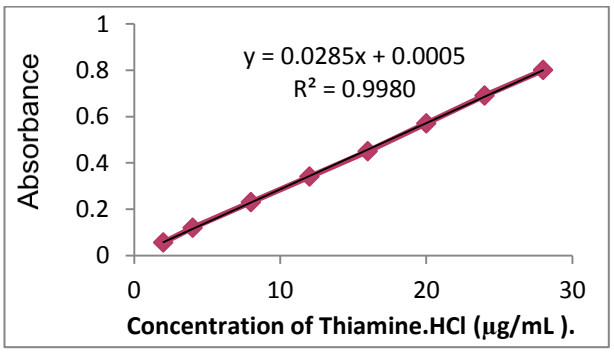


Figure 5. Calibration curve of Thiamine hydrochloride

The optical characteristics such as absorption maxima, Beer's law limits, Molar absorptive and Sandal's sensitivity for this method are presented in Table 1.

The accuracy and precision of the method were established by analyzing the pure drug solution at three different levels. The average recovery which is a measure of accuracy is 100±1.3% revealing high accuracy of the method. The relative standard deviation (RSD), which is an indicator of precision, is less than 1.5% the results are complied in Table 1.

Table 1. Optical Characteristics and Statistical Data for Regression Equation of The Proposed Method.

Parameters	Value
λ max (nm)	390
Beer's law limits, (µg/ml)	2-28
Molar absorptive, (l/mol.cm)	$0.96x10^4$
Sandal's sensitivity, (ng\cm^2)	35
Correlation coefficient (r)	0.998
Regression equation (y= ax +b)	Y= 0.0285X+0.0005
Intercept (b)	0.0005
Slope (a)	0.0285
Recovery, (%)	100 ±1.3
Relative standard deviation, (%)	< 1.5

Effect of Interferences

In order to assess the possible applications of the proposed method, the effect of substance that often accompany with thiamine hydrochloride in (tablets) were studied by adding various amounts of substances to 10 µg of thiamine hydrochloride. An attractive feature of the method is its relative freedom from interference by the usual diluents and excipients in amounts for in excess of their normal occurrence in pharmaceutical preparations. The results are given in Table 2.

Table 2. Determination of 10µg / ml of Thiamine Hydrochloride In The Presence of Excipients

Interfering substances	Amount added(mg of interfering)	Amount of drug found*,µg	Recovery, %
Corn starch	40	10.08	100.8
Microcrystalline cellulose	20	9.98	99.8
Lactose	30	9.96	99.6
Magnesium stearate	40	10.09	100.9
Polyethylene glycol	20	10.05	100.5

*Average of six determinations.

Composition of the Colored Product

The stoichiometry of the reaction between thiamine hydrochloride and vanillin was investigated using mole ratio methods of equimolar solution ($2.965x10^{-4}$M),(Fig. 6). The result obtained shows that 1:1 Thiamine–vanillin at 390 nm.

The suggested reaction and structure of the product might be written as:

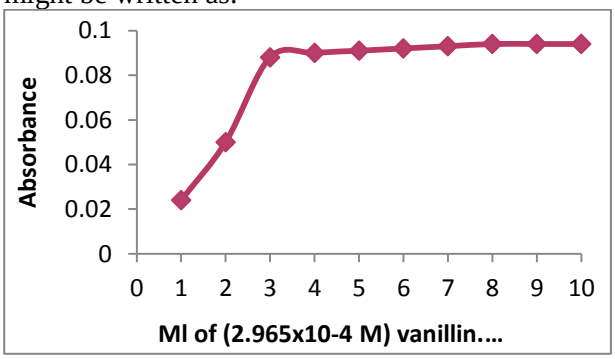
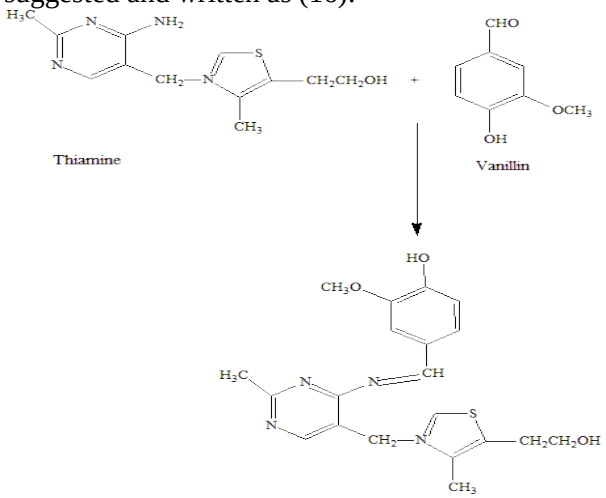


Figure 6. Mole Ratio of Thiamine and Vanillin.

The reaction and structure of the product might be suggested and written as (16):



Yellow Schiff's base compound

Analytical application

The proposed method was satisfactorily applied to the determination of thiamine hydrochloride in its pharmaceutical preparations (tablets), the results of the assay of the pharmaceutical preparations revels that there is close agreement between the results obtained by the proposed method and the label claim Table 3.

Table3: Determination of Thiamine Hydrochloride in Pharmaceutical Formulations

Pharmaceutical formulations	Label amount (mg)	Found by proposed method *mg	Recovery%	RSD (%)
Tablet: Samavit B1 (NDI)	100mg/tab	99.90	99.90	1.35
Ampoule: Samavit B1 (SDI)	100mg/ampoule	99.88	99.88	1.4

*Mean value of ten determinations.

Application of The Proposed Method to Content Uniformity(17)

Content uniformity or the uniformity of dosage unit was defined as the degree of uniformity in the amount of active substance among dosage units. The risk assessment strategy underlying content uniformity testing is the assumption that some pre-specified limits exist where safety and efficacy outcomes may change if content uniformity fails. The proposed method proved to be suitable for the content uniformity test, where a great number of assays on individual tablets are required. Data presented in table indicate that the proposed method cans accurately and precisely quantitative thiamine hydrochloride in its commercially available tablets. The mean percentage (with RSD) of the labeled claim found in ten tablets was 100.92(1.45%) which fall within the content uniformity limits specified by the US Pharmacopoeia (18), the results were shown in Table 4.

Table 4. Content Uniformity Testing of Thiamine Hydrochloride Tablets Using the Proposed Method

Parameter	% of the label claim
Tablet.1	102.2
Tablet.2	101.4
Tablet.3	102.1
Tablet.4	101.6
Tablet.5	99.4
Tablet.6	98.6
Tablet.7	101.9
Tablet.8	98.6
Tablet.9	102,1
Tablet.10	101.3
Mean(X)	100. 92
%RSD	1.45
Max. allowed unit value(18)	±15%

Conclusion:

The developed method is found to be sensitive, accurate, simple, precise, economical, and can be used for routine quality control analysis of thiamine hydrochloride in pure form and pharmaceutical formulations.

Acknowledgments

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Conflicts of Interest: None.

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تقدير الثيامين هيدروكلوريك لونيا في مستحضراته الصيدلانية

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الخلاصة:

تم وصف طريقة طيفية سهلة وسريعة وحساسة لتقدير الثايمين. تعتمد الطريقة على تكوين قاعده شيف بين مجموعه الامين للثيامين هيدروكلوريك ومجموعه الالديهايدللفانلين لتكوين ناتج اصفر اللون له أقصى امتصاص عند طول موجي 390 نانو ميتر. ووجد بان قانون بير يسري على الكميات التي تتراوح بين2-28 مايكرو غرام\ مل بامتصاصية مولارية$0.96x10^4$ لتر\مول. سم . أن ودقة الطريقة 100±1.3%ومعامل الانحراف القياسي للطريقة النسبي اقل من1. 5 % مما يدل على ضبط ا وتعد الطريقة الحالية بسيطة كونها لا تحتاج الى تسخين او تحلل مائي او استخلاص مذيبي حيث ان المواد الداخلة في تحضير المستحضرات المحللة لا تتداخل مع الثيامين مما جعلها طريقة ناجحة للتحليل الروتيني للثيامين بشكله النقي وفي مستحضراته الصيدلانية.

الكلمات المفتاحية : ثايمين هايدروكلورايد، طيفية، مستحضرات صيدلانية .