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Spectrophotometric Estimation of Thiamine in Tablet form Application to Content Uniformity Testing

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ABSTRACT

A simple, rapid, accurate and sensitive spectrophotometric method for the determination of thiamine has been developed. The method is based on the direct reaction between thiamine and sodium hydroxide to produce a yellow colored product having maximum absorption at 335 nm. Beer's law was obeyed over the concentration range of 2-20µg/ml,with molar absorptivity of $1.58x10^4$ l/mol.cm. The present method was considered to be simple because it does not need heating, hydrolysis and solvent extraction steps. The ingredients often formulated with thiamine, have been shown not to interfere, and is suitable for the routine determination of thiamine. The proposed method has been successfully applied for the determination of thiamine in pure form and in pharmaceutical preparations(tablets).

Keywords: Thiamine, Spectrophotometry, Pharmaceutical Preparations.

تقدير الثيامين طيفيا في مستحضراته الصيدلانية: تطبيق الطريقة لاختبار الاتساق

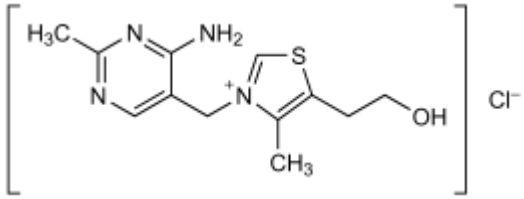
الملخص

تم وصف طريقة طيفية سهلة وسريعة وحساسة لتقدير الثيامين. تعتمد الطريقة على التفاعل المباشر بين الثيامين وهيدروكسيد الصوديوم لتكوين ناتج اصفر اللون له أقصى امتصاص عند طول موجي 335 نانو ميتر. ووجد بان قانون بير يسري على الكميات التي تتراوح بين2- 20مايكرو غرام/ مل بامتصاصية مولارية $1.58x10^4$ لتر\مول. سم 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 (الأقراص).

الكلمات الدالة: ثيامين، طريقة طيفية، مستحضرات صيدلانية.

INTRODUCTION

Thiamine or vitamin B1 (sulfur-containing vitamin) is the first vitamin of the water soluble B complex group category of vitamins(Martindale, 2009).The chemical name is3-(4-Amino-2-methylpyrimidin-5-ylmethyl)-5-(2-hydroxyethyl)-4-methylthiazolium chloride hydrochloride(Fig.1)



$C_{12}H_{17}ClN_4OS.HClM.Wt=337.3$ g/mol

Fig. 1: Chemical structure of thiamine

Thiamine deficiency develops when the dietary intake is inadequate; severe deficiency leads to the development of a syndrome known as beri-beri (Martindale, 2009 and British National Formulary, 2009). In humans, thiamine deficiency (TD) causes brain dysfunctions known as Wernicke's encephalopathy and Wernicke-Korsak off Syndrome (WKS), respectively, and is considered by some authors as the acute and chronic stages of the same disorder. Symptoms observed in WKS include oculomotor disturbance, ataxia and confusion (acute symptoms), and amnesia and confabulation (chronic symptoms) (Gibson *et al*., 2016). Analytical procedures for the determination of thiamine include HPLC (Polliana *et al*., 2017; Basiri, 2016; Japanese Pharmacopoeia, 2016), LC-MS (Gentili *et al*., 2008), spectrofluorimetric (Qiu-ying *et al*., 1999), amperometric (Erol *et al*., 2006), chemometric (Barthus *et al*., 2007), capillary electrophoresis (Bellini *et al*., 2000), and spectrophotometric (Barbara, 2013; Khairi, 2014; Rahman *et al*., 2016). In the view of the need in the industry for routine analysis of thiamine, attempts are being made to develop simple and accurate instrumental methods for quantitative estimation of thiamine. Thus, there is a need for the development of new, simple, sensitive and accurate analytical method for the quantitative estimation of thiamine as an active pharmaceutical ingredient. This paper reports a simple, sensitive, and accurate, spectrophotometric method for the determination of thiamine in pure form,pharmaceutical formulations, and application to content uniformity testing.

EXPERIMENTAL

Apparatus

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

Reagents

All chemicals used were of analytical or pharmaceutical grade .Standard materials and pharmaceutical preparations (Pharmaceutical grade thiamine and tablets100 mg were kindlysupplied as a gift sample from state company of drug industries and medical appliance(NDI) Nineveh- Iraq,

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

This solution was preparedbydissolving 0.01g ofthiaminein 100 ml of distilled waterin calibrated flask.

Sodium Hydroxide Solution (5 N).

Recommended Procedure

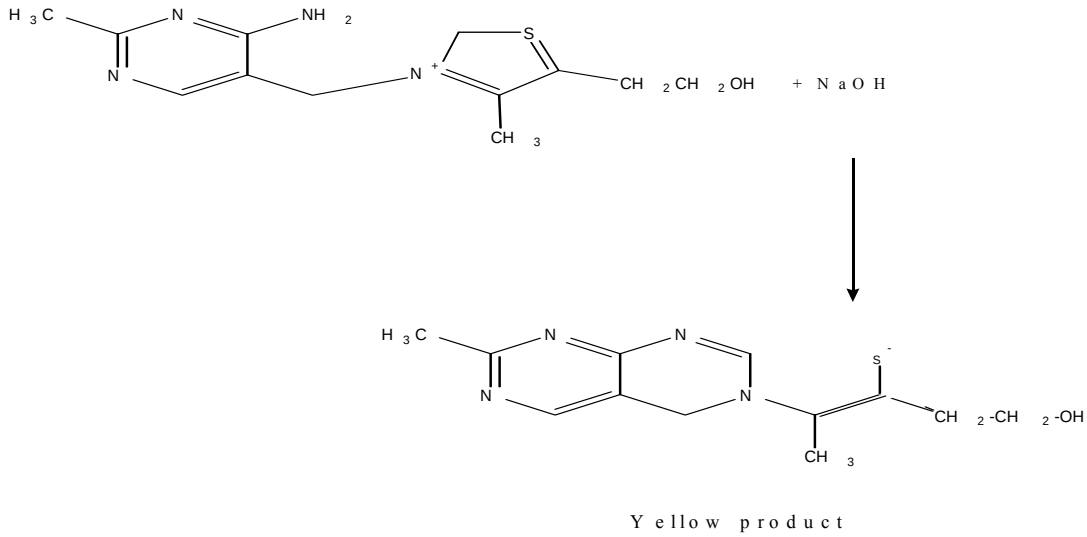
Aliquots of standard solution of thiamine (50-500 µg) were transferred into a series of 25ml volumetric flasks and1 ml of 5N NaOH solution was added. The contents were diluted to the mark with distilled water. The absorbances were measured at 335 nm against a reagent blank.

Procedures for Pharmaceutical Preparations (tablets)

To minimize a possible variation in the composition of the tablet, the mixed content of 10 tablets were weighed and grounded, then, the powder equivalent to 100 mg of thiamine was accurately weighed and transferred into a 100 ml calibrated flask, 60 ml of distilled water was added and the solution was shaken for 20 min. Then the volume was made up to the mark with distilled water, mixed well, and filtered using a whatman No.42 filter paper. 10 ml of this solution was diluted to a 100 ml with distilled water in a calibrated flask. 3 ml of this solution was treated as mentioned under recommended procedure.

RESULTS AND DISCUSSION

Developmentof spectrophotometric methods for the determination of drugs has been increased considerably in recent years because of their importance in pharmaceutical analysis (Nief *et al*., 2013). A new method has been developed for the spectrophotometric determination of thiamine. The method was based on the formation of a pale yellow coloredproduct; the suggested reaction isas shown below. (Attih *et al*., 2015).



The product having maximum absorption at 335 nm against the corresponding reagent blank as shown below Fig. (2).

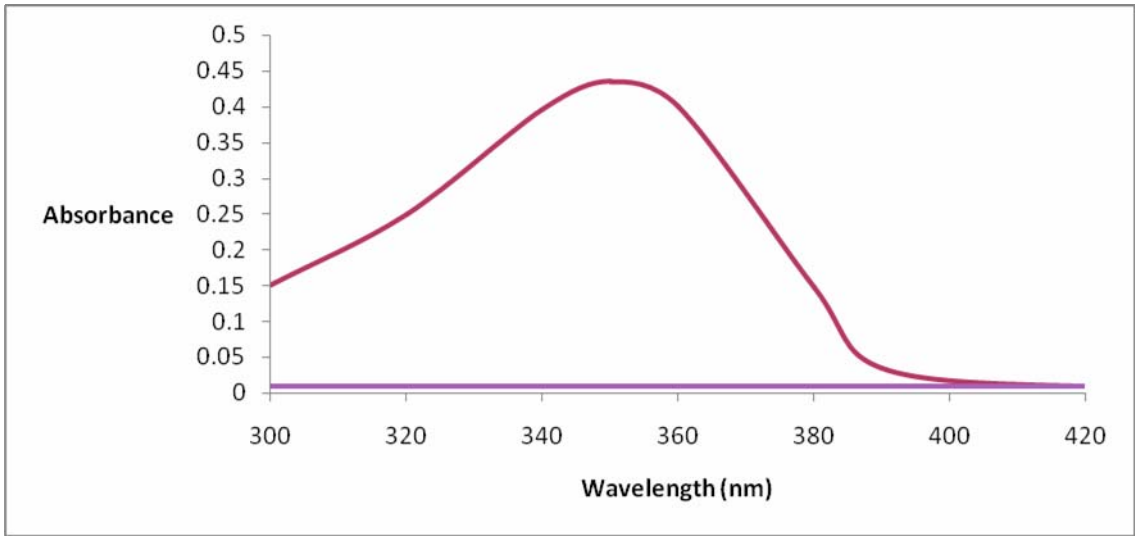


Fig. 2: Absorption spectra of 12µg/ml of thiamine with NaOH against reagent blank.

The optimum conditions were established by varying one parameter at a time and keeping the others fixed and observing the effect produced on the absorbance of colored species and incorporated in the procedure. The reaction was found to be quantitative in basic medium, 1ml of 5N NaOH is considered optimum and selected for the subsequent investigation, and the results obtained indicated that complete color formation occurred immediately and not affected by temperature. Therefore, room temperature was selected as suitable temperature. The absorbance remained constant for at least 6 hours, and 5 min, was selected as a suitable time. Under the experimental conditions described, Beer's law is obeyed over the concentration range 2- 20µg/ml Fig. (3). Linear regression equation: Y=0.047X-0.033 (r = 0.998, n = 6). Where Y is the absorbance and X is the concentration in µg\ml. The apparent molar absorptivity was 1.58×10^{4}l/mol.cmand Sandell's sensitivity was 46.84 ng.cm^{-2}.

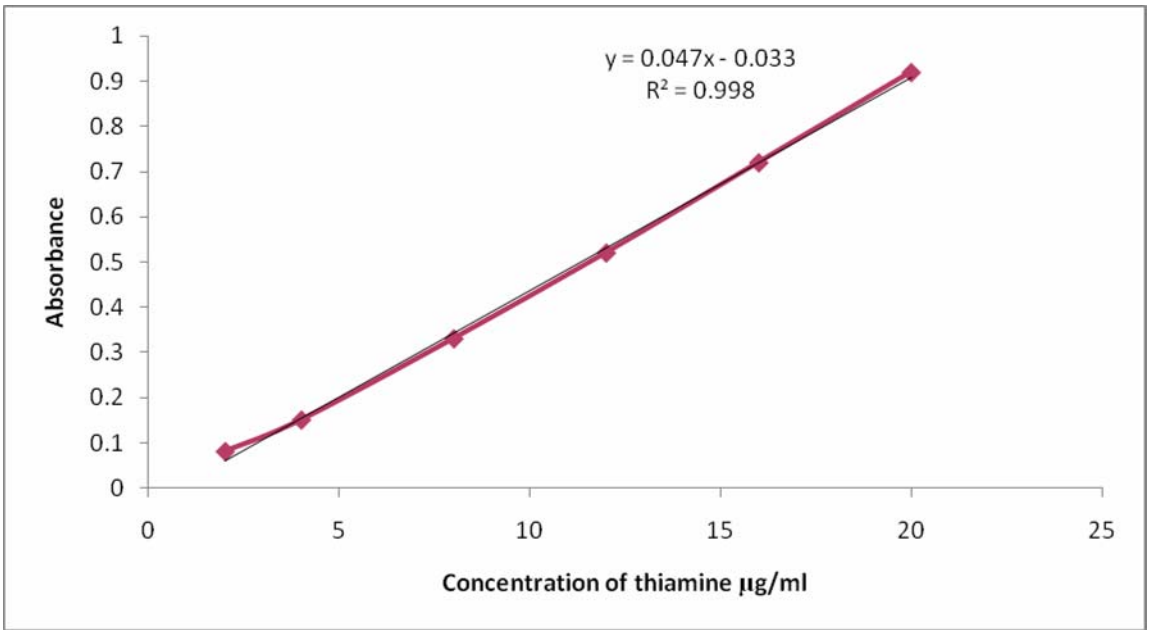


Fig. 3: Calibration curve of thiamine

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

Accuracy and Precision

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±0.55% 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 compiled in (Table 1).

Table 1: Optical characteristics and statistical data of the proposed method

Parameters	Value
λ max (nm)	335
Beer's law limits, (µg.ml^{-1})	2-20
Molar absorptivity, (l.mol^{-1}.cm^{-1})	1.58x10^{4}
Sandell's sensitivity, (ng\cm^{2})	46.84
Correlation coefficient (R^2)	0.998
Regression equation (y= a + bx)	Y= 0.047X-0.033
Intercept (a) Slope (b)	-0.033 0.047
Recovery, (%)	100 ±0.55
Relative standard deviation, (%)	< 1.5

Interference Studies

In order to assess the possible applications of the proposed method, the effect of substance that often accompanies thiamine in (tablets), were studied by adding various amounts of substances to 10 µg of thiamine. 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 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.

Analytical Application

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

Table 3: Determination of thiamine in pharmaceutical formulation

Pharmaceutical formulation	Label amount (mg)	Found by proposed method *mg	Recovery %
Tablet: Samavit B1 (NDI)	100mg/tab	99.95	99.95

*Mean value of ten determinations.

Application of the Proposed Method to Content Uniformity (Ahmad, 2014)

Content uniformity or the Uniformity of dosage unit was defined as the degree ofuniformity 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 4indicate that the proposed method cans accurately and precisely quantitative thiamine in its commercially available tablets. The mean percentage (with RSD) of the labeled claim found in ten tablets was 100.1(0.1176%) which falls within the content uniformity limits specified by the United State Pharmacopeia 33-NF28USP 33(USP, 2010).

Table 4: Content uniformity testing of thiamine tablets using theproposed method

Parameter	% of the label claim
Table No.1	100.13
Table No.2	99.97
Table No.3	100.20
Table No.4	100.20
Table No.5	99.96
Table No.6	100.10
Table No.7	99.98
Table No.8	100.20
Table No.9	100,21
Table N0.10	99.98
Mean(X)	100.093
%RSD	0.1176
Max. allowed unit value(USP,2010)	±15%

CONCLUSION

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

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