Spectrophotometric determination of Thiamine.HCl in pharmaceutical preparations using Prussian blue reaction

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Abstract

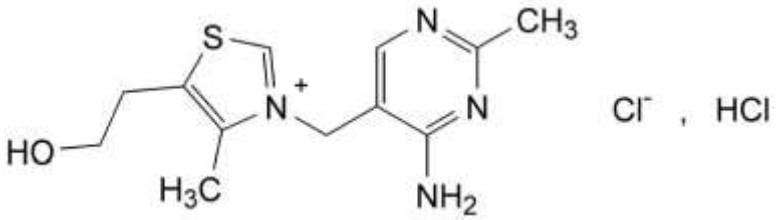
A simple, sensitive , rapid and accurate spectrophotometric method was developed for the determination of Thiamine.HCl in pharmaceutical preparations and in pure form . The method is based on the reduced Fe(III) salt by Thiamine.HCl to form Fe(II) salt which subsequently react with potassium ferric cyanide forming a soluble Prussian blue dye which has a maximum absorption at λmax 747nm linear calibration graph was in the range of (0.2–14) $\mu g.ml^{-1}$ with molar absorptivity of ($2.42\times10^{3} L.mol^{-1}.cm^{-1}$) ,a sandall sensitivity of (139.38×10^{-6} $\mu g.cm^{-2}$) , correlation coefficient of 0.999,detaction limit (0.106 $\mu g.ml^{-1}$) and the relative standard deviation of RSD%(0.4763). The method was applied successfully for the determination of Thiamine,HCl in pharmaceutical preparations. Recovery was in the range of (97.8–104.4)%. The proposed method can be carried out at 40°c temperature with no need for solvent extraction step or pH control.

الخُلاصة

تم تطوير طريقة بسيطة وحساسة وسريعة لتقدير الفيتامين B1 الثيامين هيدروكلورايد بصورة نقية وفي المستحضرات الصيدلانية وبدقة عالية وتعتمد الطريقة على تفاعل الفيتامين مع نترات ألحديديك المائية ليعطي الحديد الثنائي الذي بدورة يتفاعل مع مادة بوتاسيوم سداسي سيانيد ألحديديك مكونا صبغة ذائبة زرقاء اللون (زرقة البر وسيان) والتي تمتص عند الطول ألموجي 747 نانوميتر وقد أظهرت النتائج مدى خطية بين (0.2–14) مكغم\مل ومعامل امتصاص مولاري مقداره (2.42×10^{3}) لتر.مول$^{-1}$.سم$^{-1}$ ودالة ساندل مقدارها (139.38×10^{-6}) مكغم.سم$^{-2}$ وبمعامل ارتباط (0.9999) ومعدل الانحراف القياسي النسبي (–0.617 0.362) وحد كشف (0.106) مكغم\مل واستعادية (95.34–104.4) %.وقد طبقت الطريقة بنجاح لتقدير الثيامين هيدروكلورايد في المستحضرات الصيدلانية

Introduction

Thiamine.HCl is a water-soluble vitamin of the B complex (vitamin B1), whose phosphate derivatives are involved in many cellular processes. Its structure contains a pyrimidine ring and a thiazole ring linked by a methylene bridge. [1]



(Structures of Thiamine Hydrochloride)

The coenzyme, thiamine pyrophosphate or cocarboxylase is intimately connected with the energy releasing reactions in carbohydrate metabolism [2].

Various methods has been reported for the determination of Thiamine.HCl.There are include chromatographic[3,4,5,6,7], spectrophotometric[8,9,10,11], Electrochemical[12], Flow Injection[13].

The method of formation of Prussian blue complex is used to determined of many drug such as Amoxicilline[14] , cephalosporine antibiotics[15] , tinidazol[16], nifedipine[17], folic acid[18], adrenaline[19] , Diclofenac sodium[20] , Metoclopramide.HCl[21] and Rantiden.HCl[22] .

Experimental

Apparatus:

All spectral and absorbance measurement were carried out in a Double beam UV-Vis spectrophotometer-shimadzu-1800. Equipped with a 1cm quarts cell.

Reagents:

All chemicals used were of analytical-reagent grade .

- drugs obtained were in a pure form was provided from Samarra Drug Industries, SDI-Samarra-Iraq.

-stok solutions (1mg/ml) of Thiamine. HCl (vitamin B1) were prepared by dissolving 0.1000 gm Thiamine HCl in deionized water and diluting to the mark in a100 ml volumetric flask . Working solutions were prepared by diluting the standard solution in deionized water.

- Hydrous ferric nitrate (0.1M) stock solution was prepared by dissolving 4.0384 gm of $Fe(NO_3)_3.9H_2O$ in sufficient deionized water containing 1ml of nitric acid and the solution made up to the mark in 100 ml volumetric flask with deionized water.

- Potassium hexacyanoferrate(III) (0.1M) stock solution was prepared by dissolving 3.2900 gm of $K_3[Fe(CN)_6]$ in deionized water and diluting to the mark in 100 ml volumetric flask .

Recommended Procedure:

In to a series of 25 ml volumetric flask , transfer increasing volume of Thiamine.HCl solution (100 $\mu g.ml^{-1}$) to cover the range of calibration curve (0.2–14 $\mu g.ml^{-1}$), added 0.19 ml (0.1M) of $Fe(NO_3)_3.9H_2O$ and shake well .Added 0.6 ml (0.1M) of $K_3[Fe(CN)_6]$, dilute the solution to the mark with distilled water, and allow the reaction to stand for 20 min in water bath at 40ºC. measure the absorbance at 747 nm against a reagent blank prepared in the same way but containing no Thiamine.HCl.

Procedure for Pharmaceutical Preparations:

- Vitamin B1 Tablets:

provided from (SDI) Samarra-Iraq . 10 tablets were grinded well and a certain portion of the final powder was accurately weighted to give an equivalent to about 10 mg of vitamin B1 was dissolved in deionized water . The prepared solution transferred to 100 ml volumetric flask and made up to the mark with deionized water forming a solution of 100 µg/ml concentration . The solution was filtered by using a whatmann filter paper No.42 to avoid any suspended particles.

Results and Discussion:

Absorption spectra:

It was found preliminary that the reaction of Thiamine .HCl (vitamin B1) with Ferric Nitrate and Potassium hexacyanoferrate produced highly coloured prussian blue soluble dye that has a maximum absorption at λ_{max} (747nm) Fig (1) . The above reaction can be utilized for the determination of Thiamin.HCl using spectrophotometric method .Initial studies were directed toward optimization of the experimental conditions, in order to establish the most Favorable parameters for the determination of Thiamine.HCl .

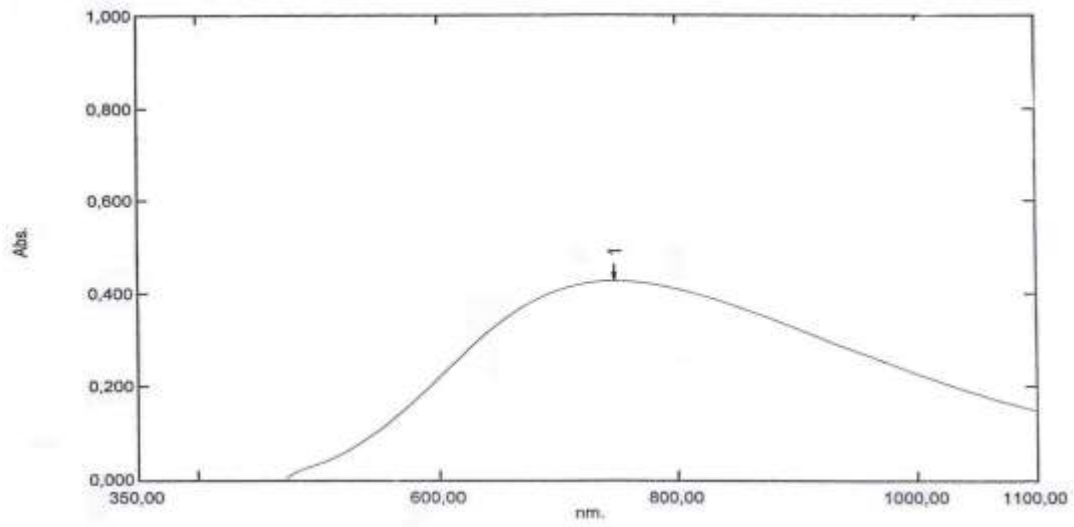


Fig (1): Absorption spectra of (6µg.ml^{-1}) of Thiamine.HCl treated with $Fe(NO_3)_3.9H_2O$(7.5×10^{-4} M) , $K_3Fe(CN)_6$ (2.5×10^{-3} M) at room temperature and measured against blank solution .

Optimization of the Experimental Condition:

The influence of various reaction variables such as concentration of reactants, order of addition , time and temperature were investigated.

Effect of Iron (III)Nitrate Concentration:

The effects of different concentration of Iron (III)Nitrate in the range of (1×10^{-4} –7.5×10^{-3}M) were investigated .A Concentration of (7.5×10^{-4} M) give the highest absorption Fig. (2) and thus was chosen for further use.

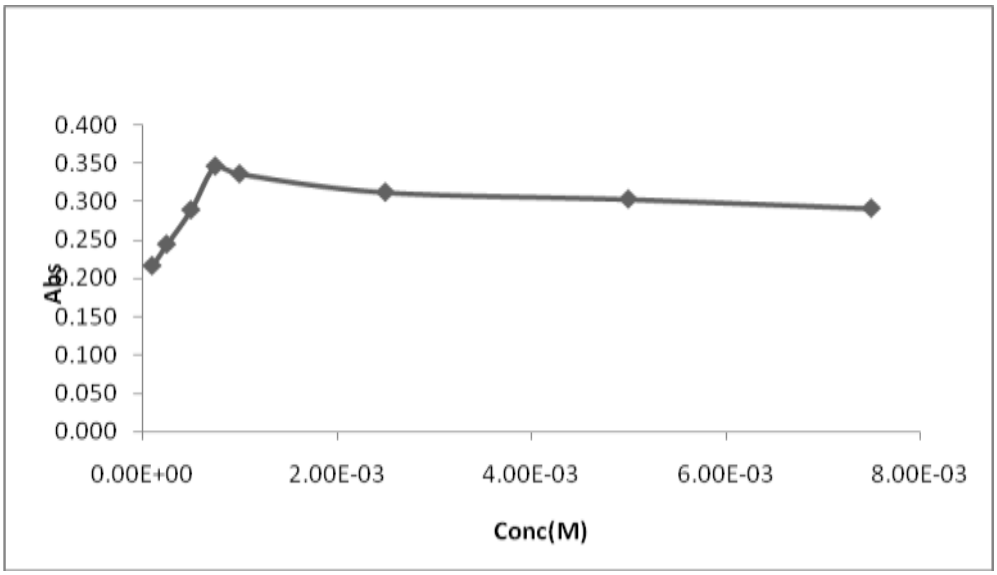


Fig. (2) :Effect of Iron (III)Nitrate Concentration

Effect of$K_3Fe(CN)_6$ Concentration:

The effect of_potassium Hexacyanoferrate(III) Concentration in the range of(2.5×10^{-4}–1×10^{-2} M) was similarly studied. A (2.5×10^{-3} M) of $K_3Fe(CN)_6$ solution gave the best results. The results obtained are shown in Fig (3).

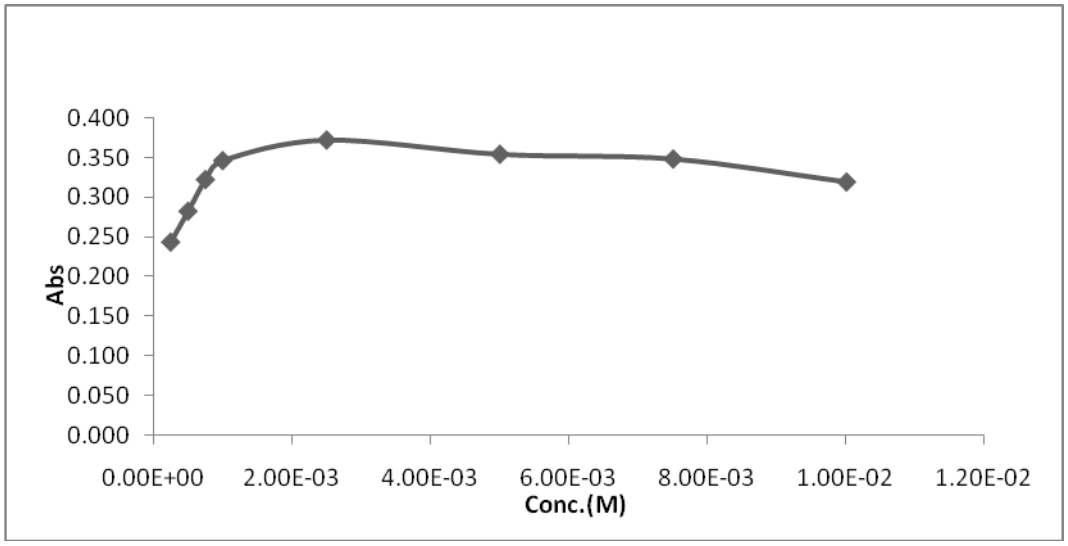


Fig. (3) :Effect of $K_3Fe(CN)_6$ Concentration

Order of addition:

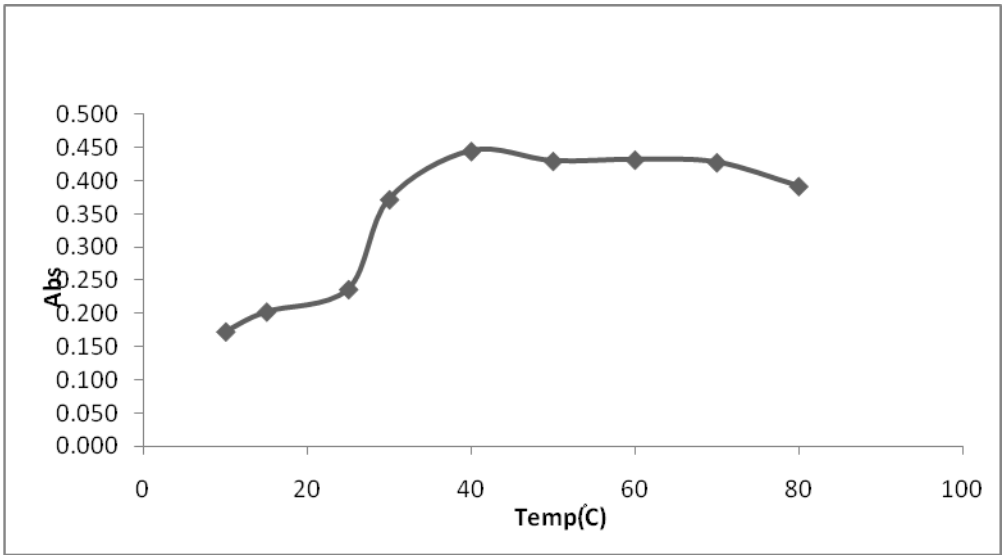
The effect of order of addition on the absorption of Prussian blue color dye was studied . Table .(1) , shows the order of addition could be followed , Drug : $Fe(NO_3)_3.9H_2O$: $K_3Fe(CN)_6$. Due to gave the highest absorption .

Table .(1) Effect of order of addition

Order of addition	Absorbance at λ_{max}(747nm)
Drug : $Fe(NO_3)_3.9H_2O$: $K_3Fe(CN)_6$	0.375
Drug : $K_3Fe(CN)_6$: $Fe(NO_3)_3.9H_2O$	0.255
$K_3Fe(CN)_6$: $Fe(NO_3)_3.9H_2O$: Drug	0.262
$K_3Fe(CN)_6$: Drug : $Fe(NO_3)_3.9H_2O$	0.221
$Fe(NO_3)_3.9H_2O$: Drug : $K_3Fe(CN)_6$	0.336
$Fe(NO_3)_3.9H_2O$: $K_3Fe(CN)_6$: Drug	0.281

Effect of Temperature:

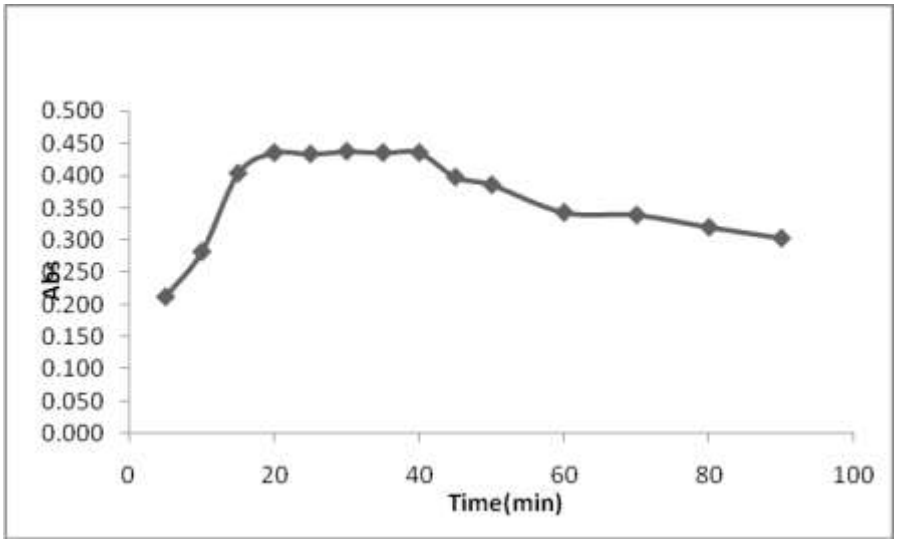
The effect of Temperature on the color intensity of the product was studied in practice the highest absorption was obtained when the colored product was developed when the calibration flask was placed in an water bath(40°C). as shown in Fig(4)



Fig(4): Effect of Temperature

Effect of Time:

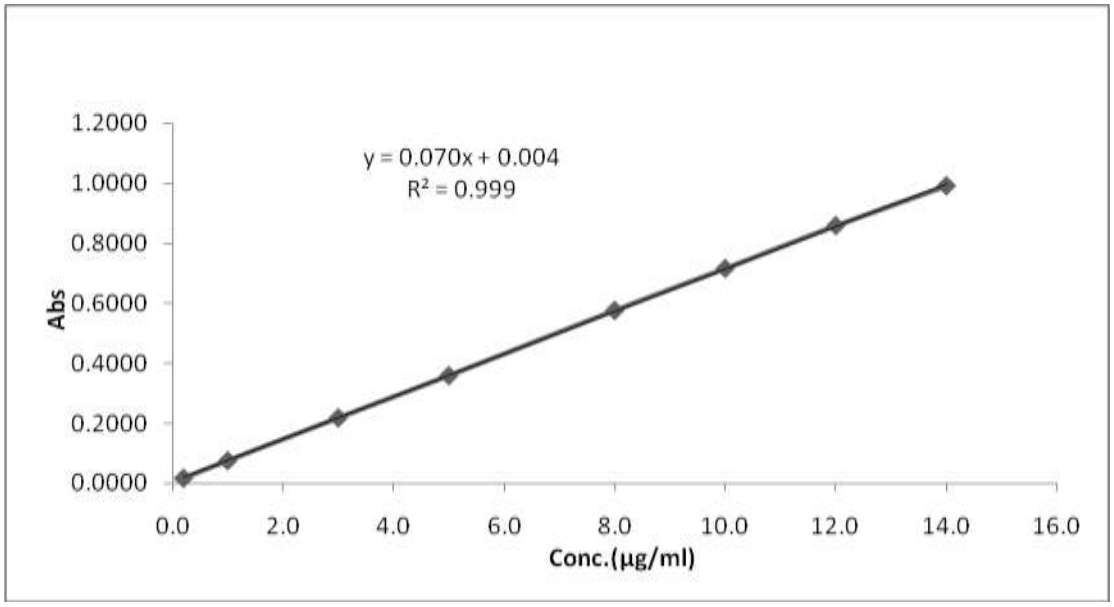
The color intensity reached a maximum absorption after Thiamin.HCl has been reacted with Iron (III)Nitrate and $K_3Fe(CN)_6$ at 20 min. Therefore 20min development time was chosen for further use. The results obtained are shown in Fig(5)



Fig(5) :Effect of Time

Calibration Graph:

Under the optimum conditions, a linear calibration graph for the determination of Thiamine.HCl was obtained over the concentration range of (0.2 – 14)µg.ml^{-1}. The linear regression equation for the range of(0.2–14) µg.ml^{-1} Thiamine.HCl is$Y= 0.0709x+0.0047$ and correlation coefficient of 0.999 the linear calibration graph is shown in Fig(6).



Fig(6): calibration graph for the determination of Thiamine.HCl

Mechanism of Reaction:

Thiamine.HCl reduce iron(III) ion in eqeous medium to form iron(II) ion , wich subsequently chelate with potassium hexacyano -ferrate(III) forming a soluble Prussian blue [23]. This substance so-called Turn bulls blue result from the interaction of 1:1 molar proportion of Fe(II) and $K_3[Fe(CN)_6]$,wich has the approximate composition[24] $KFe^{II}[Fe^{III}(CN)_6].XH_2O$ as in the Following equation ;

$$\text{Thiamin.HCl} + Fe^{+3} \longrightarrow Fe^{+2}$$

$$Fe^{+2} + K_3[Fe^{III}(CN)_6] \longrightarrow KFe^{II}[Fe^{III}(CN)_6]$$

(Potassium Ferro Firric cyanide)

The intense colour is due to charge transfer[25] from Fe^{II} to Fe^{III} .

Accuracy and precision:

The accuracy of the proposed method tested by determining the recoveries of different amount of Thiamine.HCl and the precision of the method was investigated by determining the relative standard deviation of five determinations at three concentration level of Thiamine.HCl 3,5and 10 $\mu g.ml^{-1}$. The results obtained are shown in Table (2).

Table (2): Accuracy and precision of the method

Conc.Of vitamin($\mu g.ml^{-1}$)		**RSD*%**	**Recovery*%**	**Error*%**
Taken	Found			
3.000	3.130	0.550	104.330	4.330
5.000	5.088	0.413	101.760	1.760
10.000	9.850	0.217	98.500	-1.500

*Average of five determinations

Analytical Application:

The application of the proposed method for the assay of the pharmaceutical tablets was investigated using Tablets from SID (10mg) containing Thiamine.HCl. A good precision and recovery were obtained according to the results obtained in Table (3).

Table (3): Application of the proposed method for the determination of Thiamine.HCl in pharmaceutical preparations

Drug sample vitamin B1 (10µg) SID	Conc.B1 µg.ml^{-1}		Proposed method			Standard [26] method
	Taken	Found	R.S.D*%	Error*%	Recovery*%	Recovery*%
	3.000	2.860	0.617	-4.66	95.34	103.2
	5.000	5.220	0.450	4.40	104.4	
	10.000	9.780	0.362	-2.20	97.80	

*Average of five determinations

Conclusion:

A rapid , simple , sensitive and accurate spectrophotometric method was developed for the determination of Thiamine.HCl in pharmaceutical preparations. The method is based on the reaction of Thiamine.HCl with Fe(III) ion to produce Fe(II) ion which is upon further reaction potassium hexacyanoferrate(III) to produce a soluble Prussian blue dye. It has several advantages, do not needs farther steps such as solvent extraction , PH control and expensive reagent.

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