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A Study of Spectrophotometric Determination of Thiamine.Hydrochlorid as Pharmaceutical drug

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Abstract

A simplified, 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 λ_{max} 747nm . Linear calibration graph was in the range of (0.2–14) $\mu\text{g.ml}^{-1}$ with molar absorptivity of $(2.42 \times 10^3 \text{L.mol}^{-1}.\text{cm}^{-1})$,a sandall sensitivity of $(139.38 \times 10^{-6} \mu\text{g.cm}^{-2})$, correlation coefficient of 0.9999 and the relative standard deviation of RSD%(0.617–0.362). 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.

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LIST OF TERMS

ThDP	Thiamine diphosphate
TPP	Thiamine pyrophosphate
TLC	Thin layer chromatography
HPLC	high-performance Liquid chromatography
RP-HPLC	Reversed phase high-performance Liquid chromatography
CL	chemiluminescent
TMP	Thiamine monophosphate
RSD	relative standard deviation
ODS	Octadecylsilane
FeTSPc	iron(III) tetrasulfonatophthalocyanine
GF 254	Green fluorescent 254nm



ONE
Introduction

Introduction

1- Vitamins

Vitamins are organic molecules that are needed in small amounts in the diets of some higher animals. These molecules serve the same roles in nearly all forms of life, but higher animals lost the capacity to synthesize them in the course of evolution. For instance, whereas *E. coli* can thrive on glucose and organic salts. It does not require any vitamin, human beings require at least 12 vitamins in the diet to perform specific biological functions for normal maintenance of optimum growth and health ⁽²⁾. The biosynthetic pathways for vitamins can be complex; thus, it is biologically more efficient to ingest vitamins than to synthesize the enzymes required to construct them from simple molecules. This efficiency comes at the cost of dependence on other organisms for chemicals essential for life; vitamin deficiency can generate diseases in all organisms requiring these molecules^(1,2).

Many vitamins are water-soluble, so boiling causes them to leach out into the water. This is why it is a good idea to use fresh vegetables or include the vitamin-rich broth of canned vegetables when making soup. Steaming vegetables is a better way of cooking them because it preserves their vitamin content. The water-soluble vitamins are more likely than fat-soluble vitamins to be the source of dietary deficiencies since the body does not store them. Vitamins A, D, E, and K are fat soluble and build up

in stored fat—allowing an excess of these vitamins to accumulate in the body can be toxic ⁽³⁾ .

1-1-Classification of vitamins

1-1-1- Fat- soluble vitamins

The four vitamins , namely vitamins (A,E,D,and K) are known as fat or lipid soluble (Table.1-1) .Their availability in diet, absorption and transport is associated with fat .they are soluble in fats and oils and also the fat solvents. Fat soluble vitamins can be stored in liver and adipose tissue⁽²⁾ .

Table.(1-1): Fat-soluble vitamins

Vitamin	Sources	Functions	Effects of Deficiency	Effects of Excess
A	Leafy green and yellow vegetables, liver, egg yolk	Component of eye pigment	Night-blindness, scaly skin, skin sores, and blindness	Drowsiness,headache , hair loss, abdominal pain, and bone pain
D	Milk, egg yolk	Helps calcium be absorbed, and increases bone growth	Bone deformities	Kidney damage, diarrhea, and vomiting
E	Dark green vegetables, nuts, legumes, whole grains	Required component of many enzymes ,Antioxidant	Neural-tube defects, anemia, and gastrointestinal problems	Fatigue, weakness, nausea, headache, blurred vision, and diarrhea
K	Leafy green vegetables, cabbage, cauliflower	Helps blood clot	Bruising, abnormal clotting, and severe bleeding	Liver damage, and anemia

1-1-2- Water-soluble vitamin

The water-soluble vitamins are heterogeneous group of compounds since they differ chemically from each other (Table.1-2). the only common character shared by the solubility in water .Most of these vitamins are readily excreted in urine and they are not toxic to the body .Water soluble vitamins are not stored in the body in large quantities except B₁₂.Their metabolic stores, unlike fat soluble vitamins , are depleted within week and the deficiency symptoms result .For this reason , water soluble vitamins must be continuously supplied in the diet⁽²⁾.

Table.(1-2): Water-soluble vitamins

Vitamin	Sources	Functions	Effects of Deficiency
Thiamine (B1)	Pork, whole grains, leafy green vegetables	Required component of many enzymes	Water retention and heart failure
Riboflavin (B2)	Milk, whole grains, leafy green vegetables	Required component of many enzymes FADH ₂ ,FAD	Skin lesions
Folic Acid	Dark green vegetables, nuts, legumes (driedbeans, peas, and lentils), whole grains	Required component of many enzymes	Neural-tube defects, anemia, and gastrointestinal problems
Cobalamin B12	Chicken, fish, red meat, dairy	Required component of many enzymes	Anemia and impaired nerve function
Pyridoxine B6	Red meat, poultry, fish, spinach, potatoes, and tomatoes	Required component of many enzymes GOT,GPT	Anemia, nerve disorders, and muscular disorders
Pantothenic Acid(B5))	Meat, vegetables, grains	Required component of many enzymes	Fatigue, numbness, headaches, and nausea
Biotin	Legumes, egg yolk	Required component of many enzymes, carboxylation reaction	Dermatitis, sore tongue, and anemia
Niacin (B3)	Nuts, leafy green vegetables, potatoes	Required component of many enzymes, electron transfer	Skin and nervous system damage
Ascorbic acid C	Citrus fruits, strawberries, tomatoes, broccoli, cabbage, green pepper	Required component of many enzymes	Scurvy and poor wound healing

1-1-2-1- Thiamine B1

Thiamine, or thiamin, sometimes called aneurin, is a water-soluble vitamin of the B complex (vitamin B1), whose phosphate derivatives are involved in many cellular processes. The best characterized form is thiamine pyrophosphate (ThPP), a coenzyme in the catabolism of sugars and amino acids. In yeast, (ThPP) is also required in the first step of alcoholic fermentation. Thiamine is synthesized in bacteria, fungi and plants. Animals must cover all their needs from their food and insufficient intake results in a disease called beriberi affecting the peripheral nervous system (polyneuritis) and/or the cardiovascular system, with fatal outcome if not cured by thiamine administration⁽⁵⁾. In less severe deficiency, nonspecific signs include malaise, weight loss, irritability and confusion⁽⁶⁾.

1-1-2-1- 1-Chemical properties of Thiamine:

Thiamine is the only natural compound with thiazole ring⁽²⁾. Thiamine is a colorless compound with a chemical formula $C_{12}H_{17}N_4OS$. Its structure contains a pyrimidine ring and a thiazole ring linked by a methylene bridge Fig.1. Thiamine is soluble in water, methanol, and glycerol and practically insoluble in acetone, ether,

chloroform, and benzene. It is stable at acidic pH, but is unstable in alkaline solutions^(5,7). Thiamine is unstable to heat, but stable during frozen storage. It is unstable when exposed to ultraviolet light⁽⁷⁾ and gamma irradiation^(8,9). Thiamine reacts strongly in Maillard-type reactions⁽⁵⁾. The alcohol (OH) group of thiamine is esterified with phosphate (2 moles) to form the coenzyme thiamine pyrophosphate (TPP or carboxylase) the pyrophosphate moiety is donated by ATP and the reaction is catalyzed by the enzyme thiamine pyrophosphate transferase⁽²⁾.

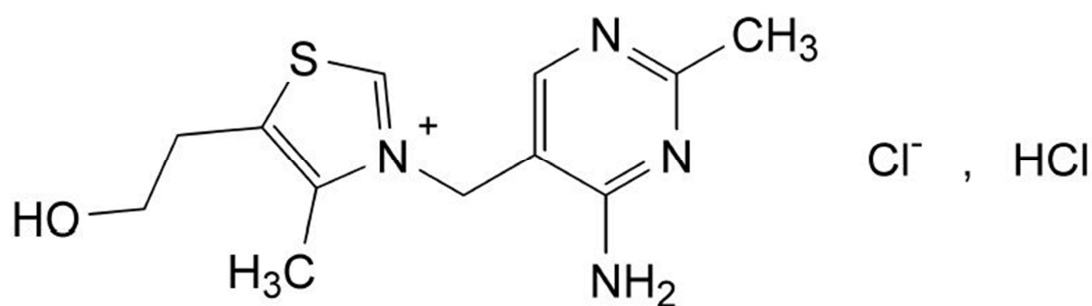


Fig.1. Structure of Thiamine Hydrochloride

1-1-2-1- 2-Sources of Thiamine :

Thiamine is found in a wide variety of foods at low concentrations. Yeast and pork are the most highly concentrated sources of thiamine. Cereal grains, however, are generally the most important dietary sources of thiamine, by virtue of their ubiquity. Of these, whole grains contain more thiamine than refined grains, as thiamine is found mostly in the outer layers of the grain and in the germ (which are removed during the refining process). For example, 100 gm of whole wheat flour contains 0.55 mg of thiamine, while 100 gm of white flour only contains 0.06 mg of thiamine . Some other foods rich in thiamine are oatmeal, flax and Sunflower seeds, brown rice, whole grain rye, asparagus, kale, cauliflower, potatoes, oranges, liver (beef, pork and chicken) and eggs ⁽⁶⁾.

1-1-2-1- 3-Thiamine Deficiency

The disease beri-beri has been known for thousands of years in China, but large epidemic only appeared at the end of the nineteenth century, with the advent of the mechanical milling of rice. It is caused by over-reliance on a single staple food, polished rice, and is thus relieved by increasing the variety in the diet. Thiamine (and indeed many other micronutrients) are present in the germ and aleurone layers of the rice

grain and these are removed by the polishing process. Hence a diet dominated by polished rice provides large amounts of carbohydrate but not enough thiamin to metabolize it. This situation is relatively rare nowadays, but thiamin deficiency is becoming increasingly common amongst derelict alcoholics in all countries. Here it is due to a monotonous diet of alcoholic beverages, which contain little thiamin, combined with alcohol-induced damage to the intestine, affecting absorption, and to the liver, affecting phosphorylation of thiamin. Several distinct forms of beri-beri have been described, although there are also patients who show features of more than one form. Dry beri-beri is the chronic form, and is characterized by a progressive peripheral neuropathy. This leads to muscle wasting, which is the most prominent sign. Symptoms include a burning sensation, muscle cramps and stiffness in the legs, then numbness which starts in the fingers and toes and gradually spreads centrally. Loss of motor function follows loss of sensation, and this in turn leads to muscle wasting. When this affects the respiratory muscles the patient becomes unable to cough effectively, and is thus highly vulnerable to respiratory infection, leading to pneumonia and death. Wet beri-beri is recognized as the acute form. Peripheral oedema is the most prominent sign, and is usually accompanied by anorexia. Death may occur through heart failure, because of an inability to clear the fluid load. Acute cardiac beri-beri (shoshin) is characterized

by cardiac hypertrophy and weakness in the absence of neuropathy or significant oedema. The main signs are breathlessness and palpitations. Acute infantile beri-beri causes anorexia, tenderness, oedema, then tachycardia and tachypnoea and ultimately death from heart failure. A large proportion of the victims of the large-scale outbreaks of beri-beri in the late nineteenth century were infants. Typically they were being breastfed by a mother with marginal thiamin status who may have shown no signs of deficiency herself, but her breast milk thiamin content would be low and the baby's high metabolic rate makes its thiamin requirement per unit energy intake rather higher than the adult's. Thiamin deficiency has also been identified as the cause of the Wernicke-Korsakoff syndrome. Wernicke's encephalopathy involves nystagmus (rapid, jerky eye movements), confusion, muscle weakness and ataxia, giddiness and anorexia. It can be rapidly reversed by thiamin injections, but may progress to an irreversible stage⁽⁴⁾.

1-1-2-1- 4-Biochemical function of Thiamine :

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

1- the enzyme **pyruvate dehydrogenase** catalyses (oxidation decarboxylation) the irreversible conversion of pyruvate to acetyl CoA .this reaction is dependent on (TPP) .

2- **α -Ketoglutarate dehydrogenase** is an enzyme of the citric acid cycle . this enzyme is comparable with pyruvate dehydrogenase and requires (TPP)

3- **Transketolase** is dependent on (TPP). This is an enzyme of the hexose monophosphate shunt (HMPshunt) . this pathway is concerned with the production of ribose and NADPH, respectively required for nucleic acid and lipid synthesis .

4- The **branched chain α - keto acid dehydrogenase** (decarboxylase) catalyses the oxidative decarboxylation of branched chain amino acids (valine, leucine, and isoleucine)To the respective keto acids .this enzyme also requires (TPP)

5- (TPP) plays an important role in the transmission of nerve impulse. It is believed that (TPP) is required for acetylcholine synthesis and the ion translocation of neural tissue.

1-1-2-1- 5-Determination Methods of Thiamine .HCl

Chromatographic techniques are among the most widely used techniques in the separation, identification and assay of thiamine.

Paper chromatographic method have been developed for the determination of thiamine using EtOAc-Py-H₂O (50:35:15 v/v)⁽¹⁰⁾ and a mixture of 95% EtOH - 10% NaCl solution. The spots are visualised by spraying with diazotized *p*-amino- acetophenone and NaOH solution ⁽¹¹⁾.

Methods of performing qualitative and quantitative analysis of multivitamin preparations containing thiamine by two dimensional chromatography have been developed. Thiamine is eluted with pH 2.0 buffer and determined at 247 nm ⁽¹²⁾.

A modified method for the chromatographic determination of thiamine as thiochrome involves elution of the spots with 0.01 N NaOH and 2 - BuOH followed by fluorimetric measurement ⁽¹³⁾.

Thiamine in multivitamin preparations has been determined by circular chromatography after separation with BuOH - Me₂CO - EtOH - H₂O (50 : 10 : 10 : 50, v/v) as solvent. The bands are eluted with water or ethanol and the content determined by spectrophotometric measurement ⁽¹⁴⁾.

The determination of thiamine in multivitamin preparations has been carried out on a chromatographic paper impregnated with phosphocitrate buffer of pH 3.5 using BuOH saturated with water as solvent. The spots are eluted with water and the absorbance measured at 270 nm ⁽¹⁵⁾.

Thiamine, riboflavin and vitamin PP in multivitamin dragées have been determined by spectrophotometric assay after chromatographic separation with an accuracy of $\pm 10\%$ ⁽¹⁶⁾.

Quantitative analysis of thiamine in tablets containing vitamin B1, B6 and B12 has been carried out by TLC on silica gel GF₂₅₄ and determination by fluorescence quenching with a densitometer ⁽¹⁷⁾.

Similar methods for the determination of thiamine in pharmaceutical mixtures using direct densitometry on silica gel G plates after separation with EtOH - water (2 : 1, v/v) as solvent system have been developed ⁽¹⁸⁾.

Thiamine in pharmaceutical preparations has been determined spectrophotometrically at 246 nm by separation from its degradation products and other ingredients on silica gel G plates using CHCl₃ - EtOH - H₂O(50 : 25 : 1, v/v) after extracting the spots with 2M HCl ⁽¹⁹⁾.

Thiamine, pyridoxine and cyanocobalamin in pharmaceutical preparations have been determined by TLC in situ by reflectance

measurements The method is selective and sensitive for pharmaceutical determinations ⁽²⁰⁾.

TLC - densitometry has been used for the determination of thiamine in the presence of its hydrolytic and oxidation products in ampoules at various temperatures ⁽²¹⁾.

The quantitative chromatographic analysis of thiamine using high-performance TLC (HPTLC) silica gel plate and two different mobile phases has been elaborated. After chromatographic separation, thiamine is derivitized by treating with potassium hexacyanoferrate (III) - NaOH as reagent to produce thiochrome with a limit of detection of 500 pg per chromatogram zone. With this reagent thiamine can be determined free of systematic errors, in pharmaceutical preparations ⁽²²⁾.

An over-pressure layer chromatographic method with photo densitometric detection for the simultaneous determination of thiamine and other water - soluble vitamins in multivitamins preparations has been developed. The method uses HPTLC plates with silica gel layers and BuOH - pyridine - H₂O (50 : 35 : 15, v/v) mixture as mobile phase. The quantitation is carried out without derivitization. This method is fast, accurate, specific and suitable for routine quality control use ⁽²³⁾.

A HPLC technique using reversed phase media (C18) with ion-pair reagents was developed to simultaneously separate the common B-vitamins using UV detection. The ion pairing reagent (5mM sodium salt of octanesulfonic acid) in the mobile phase (methanol : water (30:70) and 0.1% TEA), Using UV 254 nm. The detection limit for B1 is 0.17 µg/ml. The coefficient of variation for all analyses were less than 2% ⁽²⁴⁾.

RP-HPLC Determination of vitamins B1, B3, B6, folic acid and B12 in multivitamin tablets. methanol-5mM heptanesulphonic acid sodium salt 0.1% triethylamine TEA(25:75 V/V); pH 2.8 as the mobile phase. The column effluents were monitored at 290 nm for B1, B3, B6 and folic acid. (RSD. from 0.5% to 4.1 %) ⁽²⁵⁾.

Thiamine and riboflavin were determined in the sea urchin, *Paracentrotus lividus*, by reversed-phase liquid chromatography with fluorescence detection and a mobile phase consisting of 72% 0.005 M NH₄OAc and 28% MeOH. Limits of detection were 9 pg/ml and 80 pg/ml for thiamine and riboflavin, respectively. Good results were obtained with respect to repeatability (RSD% < 2.4) and recovery (> 91.3%) ⁽²⁶⁾.

The analytical procedure of thiamine quantification in premixtures and compound feeding stuffs by HPLC method. The measurement was done by a fluorescence detector. The recovery rate for thiamine added in the

form of hydrochloride was 102.3% in premixes and 98.9% in compound feedingstuffs. extinction wavelength $\lambda=365$ nm and emission wavelength $\lambda=435$ nm was used for the analysis⁽²⁷⁾.

The water-soluble vitamins were analyzed by HPLC on a Discovery C-18 150 mm \times 4.6 mm column with 0.1 mol L⁻¹ KH₂PO₄ (pH 7.0)–methanol, 90:10, as mobile phase (0.7 mL min⁻¹) in isocratic mode. The detection limits ranged from 0.1 to 0.5 mg L⁻¹. The accuracy of the method was tested by measuring average recovery; values ranged between 96.51 and 99.40%⁽²⁸⁾.

Spectrofluorimetric method is proposed for the determination of thiamine by using mimetic enzyme iron(III) tetrasulfonatophthalocyanine (FeTSPc) as a catalyst for the oxidation reaction between thiamine and hydrogen peroxide. It is based on the oxidation of thiamine in alkaline medium to give an intensively fluorescent compound, which has an excitation wavelength of 375 nm and an emission wavelength of 440 nm⁽²⁹⁾.

Glassy carbon electrode (GCE) modified with poly (4(2pyridylazo)-resorcinol) (PAR) film was used for the Determination of thiamine. Thiamine was accumulated at the PAR modified GCE surface under open circuit condition. Thiamine gives sensitive oxidation peaks at 160 mV upon using the differential pulse voltammetry (DPV) technique.

Thiamine gives linear response over the range of 0.030 mM to 1.1mM. The lower detection limits were found to be 0.008mM for thiamine⁽³⁰⁾.

Cyclic flow injection system with a sensitive chemiluminescent (CL) detection was developed for a determination of thiamine. The CL reaction is initiated by oxidation of thiamine in an alkaline potassium ferricyanide solution and then produces thiochrome of an emitter. The **CL** was also enhanced until an available level for the determination by the energy transfer to a dye-sensitizer uranine. The limit of determination: $2.0 \times 10^{-5} \text{M}$ ⁽³¹⁾.

Thiamine monophosphate and thiamine mixtures have been separated by adsorption on a weak cation exchanger, and elution with a concave gradient of increasing concentration of HCO_3NH_4 a, pH 4.0 and determined a. 273 nm For 10 measurements the standard deviations are about 0.15% for cocarboxylase < 1.6% for thiamine monophosphate and about 2.3% for thiamine⁽³²⁾.

Ion-pair chromatography with a reversed-phase C18 cartridge in a radial compression system and a pH 3.6 mobile phase of MeOH- H_2O (85:15,v/v) containing 0.005 M heptane sulphonic acid and 0.5% Et3N has been used to separate and determine the water soluble vitamins at 280 nm. Quantitation in multivitamin-mineral preparations is excellent with recoveries of 98.2-102.0%⁽³³⁾. The method has been

applied to the simultaneous assay of B-vitamins in liquid multivitamin formulations ⁽³⁴⁾.

Liquid chromatographic methods have been developed for the assay of vitamins B, B2, B6, PP and antipyrine in tablets, syrups and injectables by separation on a Hibar-Lichrosorb RP column. The eluents used are:

a) Distilled water containing 10^{-3} M tetraammonium bromide (TEAB) and 1% HOAc for (B-complex tablets).

b) Distilled water containing 10^{-3} M TEAB and 0.25% HOAc for (B-complex syrups and injectables). The methods are used for the analytical control of B-complex dosage forms in industry ⁽³⁵⁾.

Thiamine and thiamine phosphates have been determined by liquid chromatography after oxidation to thiochrome using a mobile phase containing Bu_4NBr , EDTA and a borate buffer. Thiamine, thiamine monophosphate (TMP) and thiamine pyrophosphate (TPP) are observed as 3 distinct peaks and the minimum detectable amount is 10 fmol ⁽³⁶⁾.

Thiamine phosphate in human plasma has been determined by HPLC after dephosphorylation and oxidation to thiochrome. The detection limit is 3 ng/mL and recovery is 98%. Calibration curves are linear in the range 0-500 ng/mL ⁽³⁷⁾.

A sensitive HPLC method for the determination of total thiamine in whole blood has been developed. After enzymatic hydrolysis of thiamine phosphate esters and oxidation to thiochrome the detection is carried out fluorimetrically. The within-assay and between-assay RSD values are 4.2 and 4.4% respectively. The mean between-assay recovery of thiamine diphosphate added to blood samples is 99.9% ⁽³⁸⁾ .

A sensitive assay method for thiamine in human plasma has been developed which is based on precolumn oxidation of thiamine to thiochrome followed by HPLC separation and fluorescence detection. The minimum amount detectable is 5 fmol. Inter-and intra-assay RSD% values are 8.3 and 6.3% respectively at a stock plasma concentration of 10.8 nmol/L. The recovery of 5 nmol/L added thiamine is 102 and that of 30 nmol/L is 94%. The method has been used to follow the pharmacokinetics of thiamine ⁽³⁹⁾ .

A reversed phase HPLC method has been developed to study the losses of total thiamine in milk during pasteurization at 75, 82 and 89°C and direct and indirect ultra-high temperature treatment. The method involves acid hydrolysis of thiamine phosphate, oxidation to thiochrome and fluorimetric determination (λ_{ex} 368 nm, λ_{em} 410nm) following reversed-phase HPLC separation through Hypersil ODS. A detection limit of 20, $\mu\text{g/kg}$ and a reproducibility of 10% are reported⁽⁴⁰⁾ .

A fully automated HPLC method for the determination of thiamine, riboflavin, nicotinamide and pyridoxine in multivitamin-mineral capsules and tablets has been developed with UV (280 nm) detection. The column used is 5 μ m silica bonded to dimethyloctylchlorosilane and the mobile phase is composed of THF, sodium heptane- sulphonate, KH_2PO_4 , H_3PO_4 and aqueous Et_3N solution. The recoveries are 97.6-105.2% and RSD values are 2.0-2.4% ⁽⁴¹⁾ .

Thiamine, riboflavin, pyridoxine, cyanocobalamin and ascorbic acid in capsules (5-10 ng each) have been determined by ion-pair reversed-phase HPLC on Lichrosorb RP-48 column using MeOH- H_2O -conc. H_3PO_4 -octanesulphonic acid as the eluent. The RSD values are 1.1-3.2% ⁽⁴²⁾ .

Direct spectrophotometric method for the determination of ascorbic acid and thiamine - HCl, each in the presence of its degradation products have been proposed. The methods are based on the use of UV derivative spectrophotometric measurements : the first derivative at 215 nm for ascorbic acid and second derivative at 254 nm for thiamine. The relative standard deviations for the assay are 0.52% (mean recovery 101.5%) and 1.07% (mean recovery 99.4%) respectively. The method has been successfully applied to monitor the stability of the two vitamins ⁽⁴³⁾ .

A mixture of thiamine, riboflavin, pyridoxine, vitamin PP and Ca pantothenate in injections has been determined by first derivative spectrophotometry. The method is based on the use of a diode array spectrophotometer and the diode processing by microcomputers ⁽⁴⁴⁾ .

Kalman filter - UV spectrophotometry has been applied to the simultaneous determination of thiamine, riboflavin, pyridoxine and nicotinamide in compound pharmaceutical preparations. The method is simple and accurate with recoveries of 96 - 104% ⁽⁴⁵⁾ .

Thiamine in pharmaceuticals can be determined spectrophotometrically by treatment with phosphomolybdic acid and measurement of absorbance at 660 nm. Beer's law is obeyed in the concentration range of 10 - 60 mg/ml. The ratio of the reagent to the drug is 1 : 3 ⁽⁴⁶⁾ .

A spectrophotometric determination of thiamine using an induced iodine - azide reaction to generate iodide has been developed. The method is based on the determination of a decrease of iodine by measuring the absorbance at 350 nm and subsequent calculation of thiamine content ⁽⁴⁷⁾ .

Thiamine in single dosage forms or in B-complex preparations can be determined colorimetrically at 490 nm by the formation of a complex

with sodium Phenylhydrazine - 4 - sulphonate in alkaline solutions. Beer's law is obeyed in the concentration range of 1 - 16 $\mu\text{g/ml}$.

The recoveries are (99.7 - 100.6%) and relative standard deviation of the method is 0.39%. Common excipients do not interfere in the method⁽⁴⁸⁾.

A colorimetric method for the determination of thiamine in pharmaceutical preparations is based on the measurement of absorbance at 375 nm of a colored product formed by the reaction between sulphide ions generated from the S of thiazole moiety with Pb (II) - EDTA solution. It can determine the vitamin at a concentration of 0.5% with a standard deviation of 3%⁽⁴⁹⁾.

A flow injection-fluorimetric method for the determination of thiamine in pharmaceuticals with a RSD% of 1.8% has been reported⁽⁵⁰⁾.

A kinetic-potentiometric method has been proposed for the determination of thiamine.HCl by reaction with N-bromosuccinimide and monitoring the rate of production of bromide with a bromide-selective electrode. The method is simple, reliable and relatively free from interference from common excipients and coexisting vitamins in tablets and injections⁽⁵¹⁾.

A simple and selective argentometric titration method has been developed for the determination of thiamine in pharmaceutical preparations. It is based on direct Potentiometric titration in alkaline medium in which a chemical transformation takes place creating two acidic groups, the protons of which are replaced by Ag ions. No interference is caused by other vitamins, and inactive excipients normally present in multivitamin preparations ⁽⁵²⁾ .

The Polarographie reduction of thiamine at the dropping Hg electrode has been studied as a function of pH using acetate, phosphate and ammonical buffer solutions. The results show the possibility of following chemical-reactions : the transformation of thiamine in its yellow and thiol forms by measuring the height of the corresponding reduction waves of thiamine and its yellow form, and the height of the oxidation wave of the thiol form ⁽⁵³⁾ .

A study of the Polarographic behavior of thiamine and pyridoxine at the dipping Hg electrode shows well-defined irreversible wave in the aqueous and non-aqueous media, i.e. water mixed with DMSO, DMF, EtOH, and propylene carbonate, using KNO₃ and Li₂SO₄ as supporting electrolytes ⁽⁵⁴⁾ .

Thiamine, riboflavin, pyridoxine, nicotinamide and ascorbic acid have been determined in a fast and simple method employing linear, sweep voltammetry, at carbon paste electrode. Fe^{2+} and ascorbic acid interfere with the assay of thiamine and can be removed by ion-exchange Chromatography. The vitamins can be determined in pharmaceutical preparations with standard deviation of $\leq 2\%$ ⁽⁵⁵⁾.

The method of formation of Prussian blue complex is used to determine many drugs such as Amoxicillin ⁽⁵⁶⁾, cephalosporine antibiotics ⁽⁵⁷⁾, tinidazole ⁽⁵⁸⁾, nifedipine ⁽⁵⁹⁾, folic acid ⁽⁶⁰⁾, adrenaline ⁽⁶¹⁾, Diclofenac sodium ⁽⁶²⁾, Metoclopramide.HCl ⁽⁶³⁾ and Rantiden ⁽⁶⁴⁾.

1-2 Aim of the present work:

The aim of the present work was to develop a simple, selective, suitable, sensitive, accurate and new spectrophotometric method for the determination of Thiamine.HCl (vitamin B1) manufactured in Iraq at Samarra Drug Industry (SDI) in pharmaceutical preparations such as tablets.



Two
Experimental Part

2- Experimental Part

2-1- Apparatus

- Double beam UV-Vis spectrophotometer-shimadzu-1800.

Equipped with a 1cm quartz cell.

- Single beam UV-Vis spectrophotometer-Spectroflex-6100.

Equipped with a 1cm quartz cell.

- Sensitive balance Sartorius B210S

- Heating-Cooling Water Bath-Haak .

- Stop watch .

- Digital pH-Meter .

2-2- Reagents and Chemical Materials Used:

All reagent and chemical materials used were analytical reagent

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

material	formula	Purity	state	Source
Thiamin hydrochlorid	$C_{12}H_{17}N_4OS.HCl$	99%	soled	SDI
Hydrous ferric nitrat	$Fe(NO_3)_3.9H_2O$	99%	soled	BDH
Potassium hexacyanoferrat	$K_3[Fe(CN)_6]$	99.9%	soled	BDH
Nitric acid	HNO_3	(67-72)%	liquid	Fluka

2-3- Preperation of Solutions.

1- Thiamine. HCl (vitamin B1) stock solution(1mg/ml) was prepared by dissolving 0.1000 gm Thiamine.HCl in 100 ml of deionized water. Required concentration were prepared by dilution of the corresponding stock solution with deionized water.

2- Hydrous ferric nitrat (0.1M) stock solution was prepared by dissolving 4.0384 gm of $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ in deionized water contaning 1ml of nitric acid and the solution made up to 100 ml deionized water.

3-potassium hexacyanoferrate(III) (0.1M) stock solution was prepared by dissolving 3.2900 gm of $\text{K}_3[\text{Fe}(\text{CN})_6]$ in 100 ml of deionized water.

2-4- Solution of Pharmaceutical Preparations containing Thiamine .HCl

- 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 and 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 $\mu\text{g}/\text{ml}$ concentration . The solution was filtered by using a whatmann filter paper No.42 to avoid any suspended particles.

2-5- Recommended Procedure :

In a series of 25 ml volumetric flask , transfer of increasing volume of Thiamine.HCl solution ($100 \mu\text{g}.\text{ml}^{-1}$) in(100ml) to cover the range of calibration curve ($0.2\text{--}14 \mu\text{g}.\text{ml}^{-1}$), added 0.2ml (0.1M) of $\text{Fe}(\text{NO}_3)_3.9\text{H}_2\text{O}$ and shake the solution. Added 0.6ml (0.1M) of $\text{K}_3\text{Fe}(\text{CN})_6$, dilute the solution to the mark with distilled water, and allow the reaction to stand for 20 min in water bath at 40°C . measure the absorption at 747 nm against the blank solution prepared in the same way but without Thiamine.HCl



Three
Results and
Discussion

3-1-Results and Discussion:

3-1-1- Preliminary Investigations :

It was found preliminary that the reaction of thiamin .HCl (vitamine B1) with Ferric Nitrate and Potassium hexacyanoferrate produced highly coloured prussian blue soluble dye that has a maximum absorption at λ_{\max} (747nm) Fig (3-1) . 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 Thiamin.HCl . The influence of various reaction variables such as concentration of reactants, sequence of addition , time and temperature were investigated. These studies were started with initial parameters given in Table(3-1)

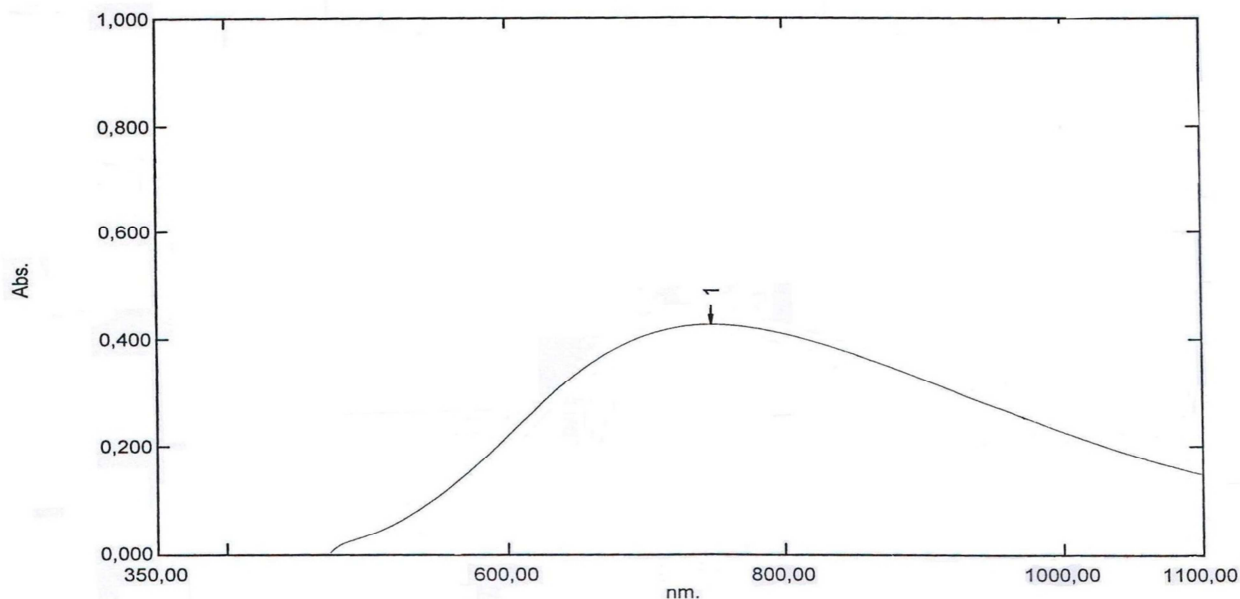


Fig (3-1): Absorption spectra of ($6\mu\text{g} \cdot \text{ml}^{-1}$) of Thiamine.HCl treated as described under procedure and measured against blank solution .

Table (3-1): Initial Chemical and Physical conditions

Preliminary parameter	value
Conc. Of $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$	$1 \times 10^{-4} \text{ M}$
Conc. Of $\text{K}_3\text{Fe}(\text{CN})_6$	$1 \times 10^{-3} \text{ M}$
Conc. Of Thiamin.HCl	$6 \mu\text{g} \cdot \text{ml}^{-1}$
Time of reaction	20 min
Temperature of reaction	30°C
Wave length	747 nm

3-1-2-Optimization of the Experimental Condition :

3-1-2-1-Effect of Iron (III)Nitrate Concentration.

The first step to form a coloured product depends on the reduction of Iron (III) to Iron (II), using initial experimental parameters in Table (3-1). The effects of different concentration of Iron (III)Nitrate in the range of ($1 \times 10^{-4} - 7.5 \times 10^{-3} \text{ M}$) were investigated .A Concentration of ($7.5 \times 10^{-4} \text{ M}$) give the highest absorption Fig. (3-2) , Table(3-2) and thus was chosen for further use.

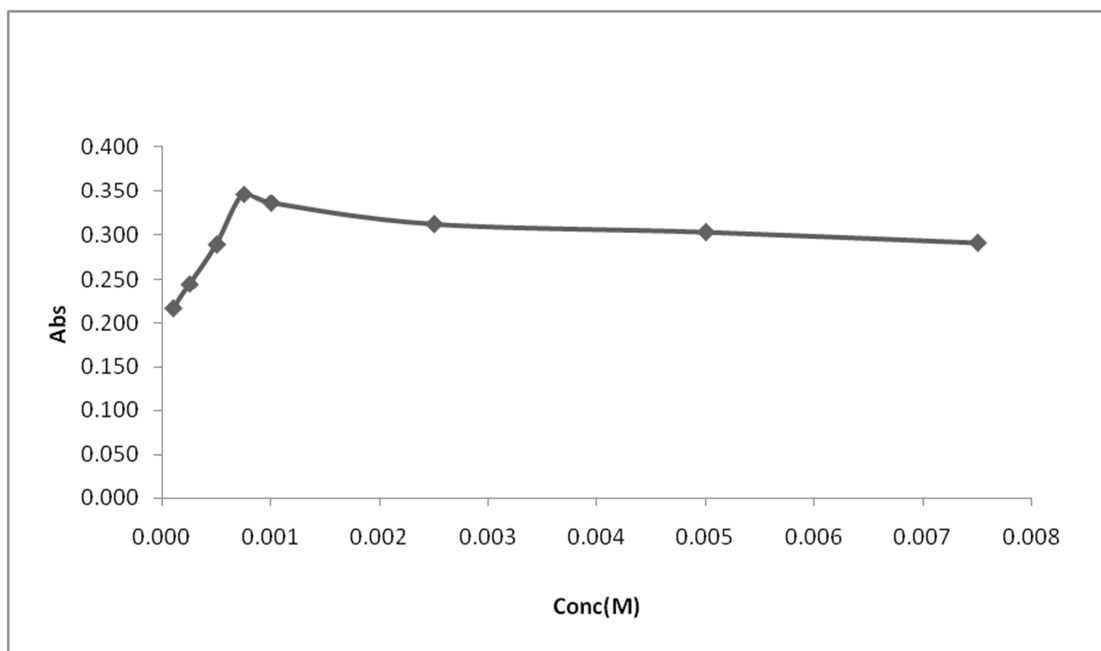


Fig. (3-2) Effect of Iron (III)Nitrate Concentration on the absorption of the coloured product.

Table (3-2) Effect of Iron (III)Nitrate Concentration

Conc. $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ (M)	Abs.
1.00×10^{-4}	0.216
2.50×10^{-4}	0.244
5.00×10^{-4}	0.289
7.50×10^{-4}	0.346
1.00×10^{-3}	0.336
2.50×10^{-3}	0.312
5.00×10^{-3}	0.303
7.50×10^{-3}	0.291

3-1-2-2-Effect of potassium Hexacyanoferrate(III) Concentration

Using the optimized Iron (III)Nitrate Concentration at (7.5×10^{-4} M), and keeping all other parameters constant as in Table (3-1), the effect of potassium Hexacyanoferrate(III) Concentration was similarly studied. (2.5×10^{-3} M) of $K_3Fe(CN)_6$ solution gave the best results. The results obtained are shown in Fig (3-3) and Table (3-3).

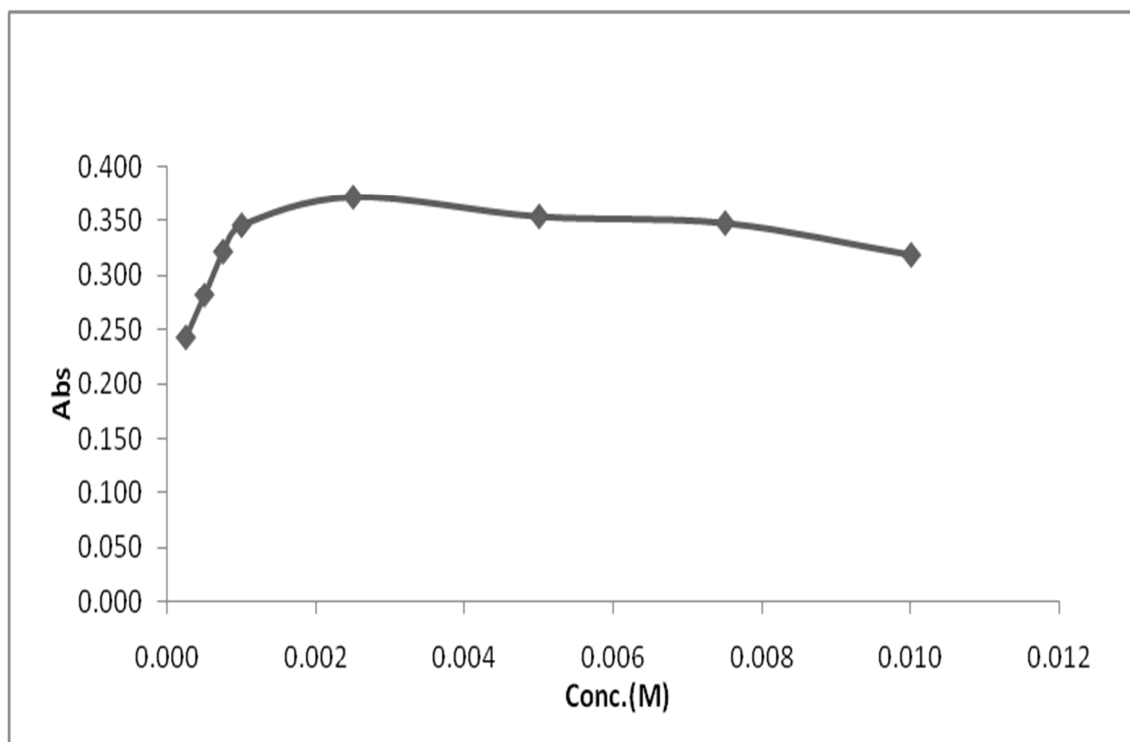


Fig (3-3) Effect of potassium Hexacyanoferrate(III) $K_3Fe(CN)_6$ Concentration on the absorption of coloured product.

Table (3-3) Effect of potassium Hexacyanoferrate(III) Concentration

Conc. $K_3Fe(CN)_6$ (M)	Abs.
2.50×10^{-4}	0.243
5.00×10^{-4}	0.282
7.50×10^{-4}	0.322
1.00×10^{-3}	0.346
2.50×10^{-3}	0.372
5.00×10^{-3}	0.354
7.50×10^{-3}	0.348
1.00×10^{-2}	0.319

3-1-2-3-Sequence of addition :

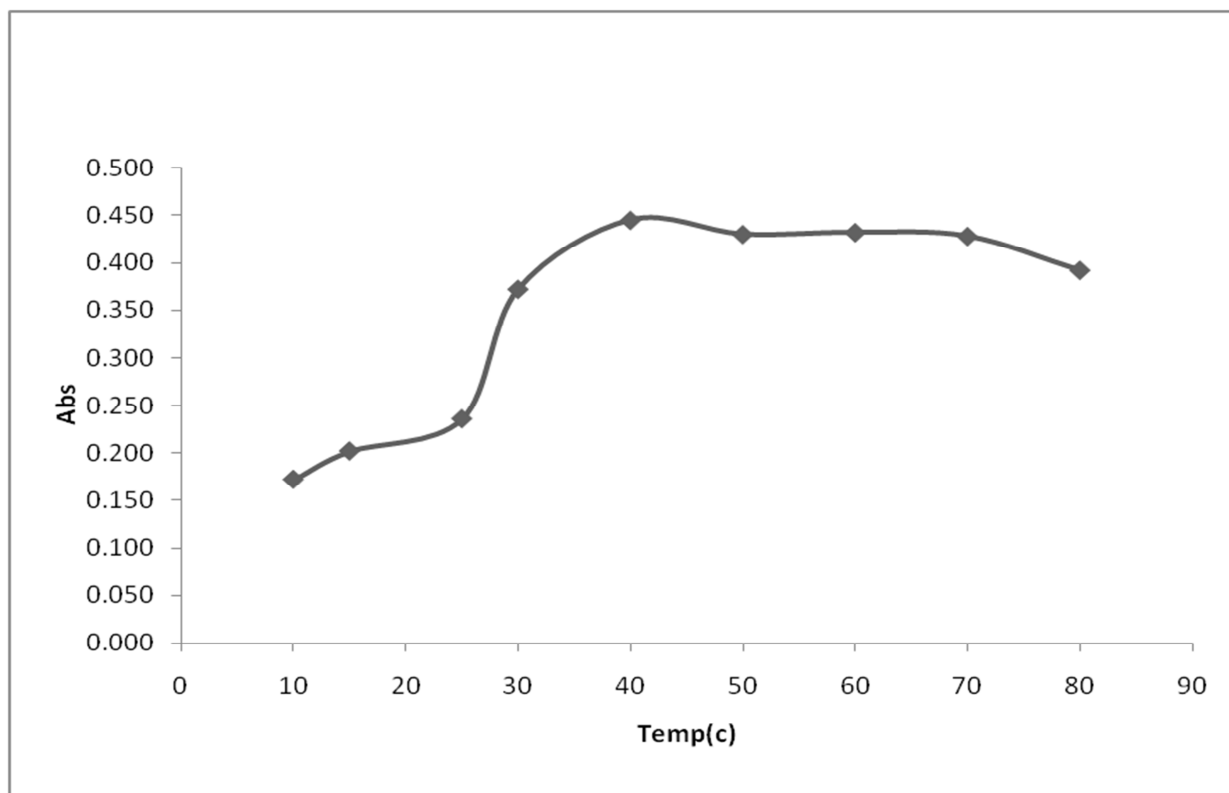
The effect of Sequence of addition on the absorbance of Prussian blue color dye was studied . Table .(3-4) , shows the Sequence of addition could be followed , Drug : $Fe(NO_3)_3 \cdot 9H_2O$: $K_3Fe(CN)_6$. Due to gave the highest absorption .

Table .(3-4) Effect of Sequence of addition

Sequence of addition	Absorption at $\lambda_{max}(747nm)$
Drug : $Fe(NO_3)_3 \cdot 9H_2O$: $K_3Fe(CN)_6$	0.375
Drug : $K_3Fe(CN)_6$: $Fe(NO_3)_3 \cdot 9H_2O$	0.255
$K_3Fe(CN)_6$: $Fe(NO_3)_3 \cdot 9H_2O$: Drug	0.262
$K_3Fe(CN)_6$: Drug : $Fe(NO_3)_3 \cdot 9H_2O$	0.221
$Fe(NO_3)_3 \cdot 9H_2O$: Drug : $K_3Fe(CN)_6$	0.336
$Fe(NO_3)_3 \cdot 9H_2O$: $K_3Fe(CN)_6$: Drug	0.281

3-1-2-4-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). While a decreased in absorption was observed when the solution was placed in an ice bath. Therefore ,it is recommended that the reaction should be carried out at 40°C as shown in Fig(3-4)and Table (3-5).



Fig(3-4):Effect of Temperature on the intensity of the coloured product.

Table (3-5) Effect of Temperature data

Temp. °C	Abs.
10	0.172
15	0.202
25	0.236
30	0.372
40	0.445
50	0.430
60	0.432
70	0.428
80	0.392

3-1-2-5-Effect of Time:

The color intensity reached a maximum absorption after Thiamine.HCl has been reacted with Iron (III) Nitrate and $K_3Fe(CN)_6$ at 20 min. Therefore 20 min development time was chosen for further use. The results obtained are shown in Fig(3-5) and table (3-6).

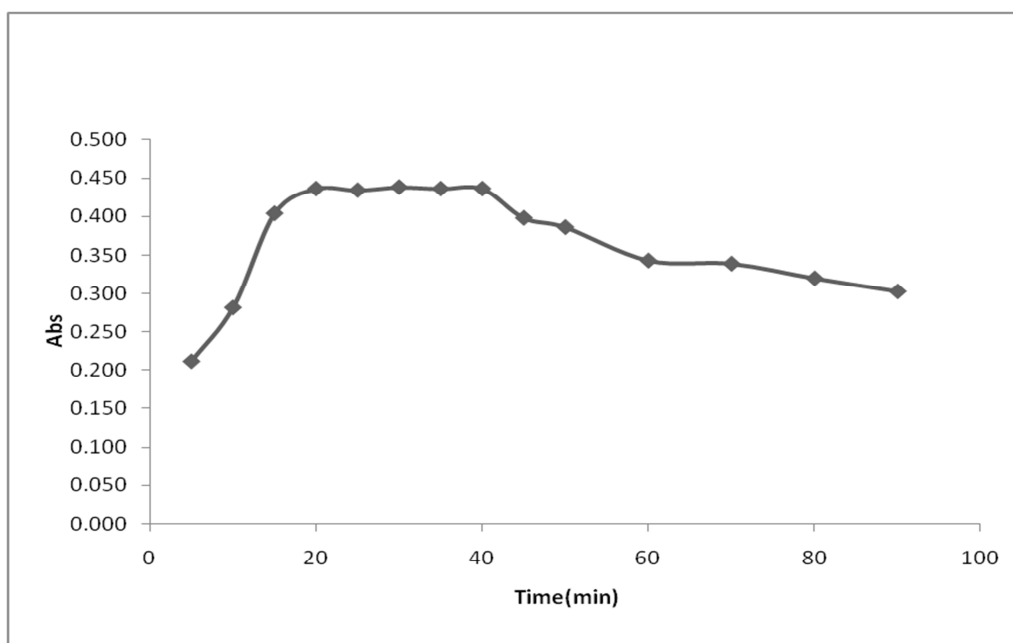


Fig.(3-5)Effect of the reaction time on the intensity of the coloured product.

Table (3-6) Effect of the reaction time

Time.min	Abs.
5	0.212
10	0.282
15	0.404
20	0.436
25	0.434
30	0.438
35	0.436
40	0.436
45	0.398
50	0.386
60	0.343
70	0.339
80	0.320
90	0.303

3-1-3-Recommended Analytical conditions :

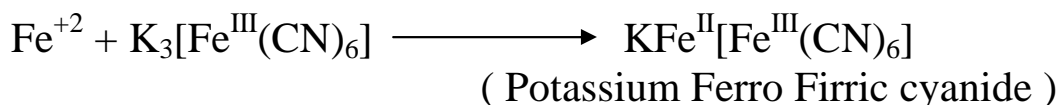
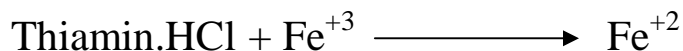
According to the results obtained previously the optimum conditions for the determination of Thiamin.HCl using spectrophotometric determination method are given in Table (3-7)

Table (3-7):optimum condition for the determination of Thiamin.HCl

Parameter	Value
Conc. Of $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$	$7.5 \times 10^{-4} \text{ M}$
Conc. Of $\text{K}_3\text{Fe}(\text{CN})_6$	$2.5 \times 10^{-3} \text{ M}$
Conc. Of Thiamine.HCl	$6 \mu\text{g} \cdot \text{ml}^{-1}$
Time of reaction	20 min
Temperature of reaction	40°C
Wave length λ_{Max}	747nm

3-1-4-Mechanism of Reaction:

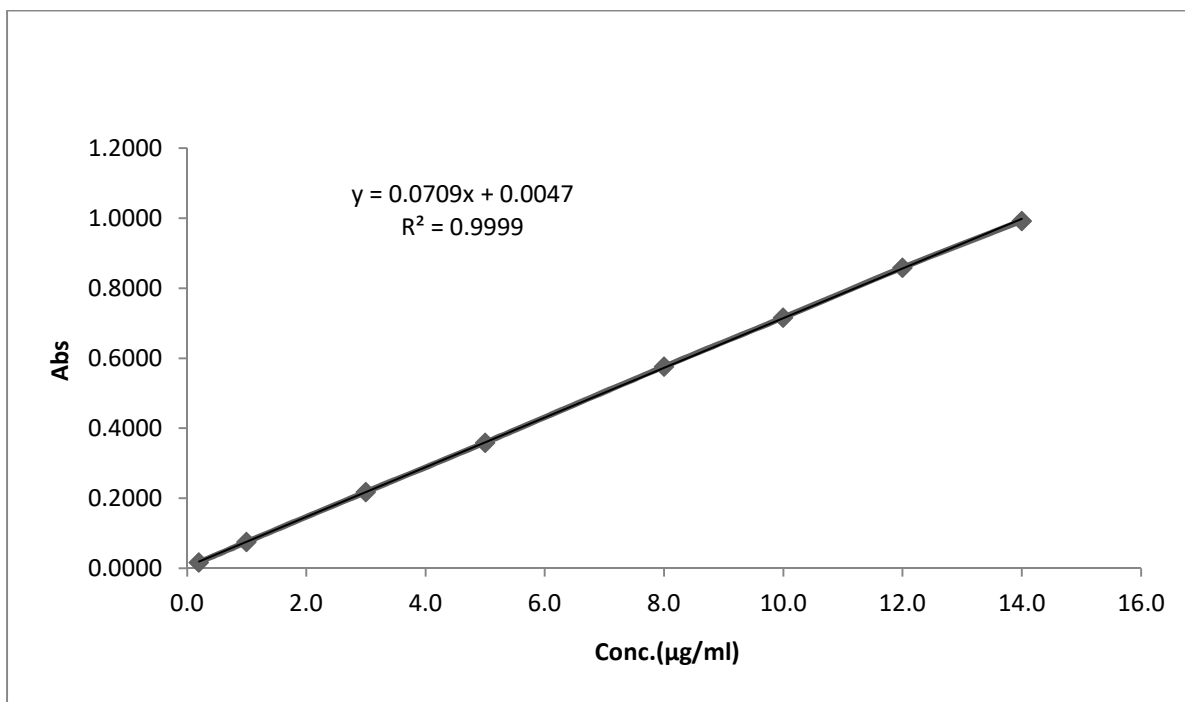
Thiamine.HCl reduce iron(III) ion in aqueous medium to form iron(II) ion , which subsequently chelate with potassium hexacyano - ferrate(III) forming a soluble Prussian blue ⁽⁶⁵⁾. This substance so-called Turn bulls blue result from the interaction of 1:1 molar proportion of Fe(II) and $\text{K}_3[\text{Fe}(\text{CN})_6]$, which has the approximate composition ⁽⁶⁶⁾ $\text{KFe}^{\text{II}}[\text{Fe}^{\text{III}}(\text{CN})_6] \cdot \text{XH}_2\text{O}$ as in the Following equation ;



The intense colour is due to charge transfer ⁽⁶⁷⁾ from Fe^{II} to Fe^{III} .

3-1-5-Calibration Graph:

Under the optimum conditions, shown in Table (3-8), a linear calibration graph for the determination of Thiamine.HCl was obtained over the concentration range of $(0.2 - 14)\mu\text{g.ml}^{-1}$. Analytical data for the calibration plot of Thiamine.HCl is summarized in Table (3-9). The linear regression equation for the range of $(0.2-14) \mu\text{g.ml}^{-1}$ Thiamine.HCl is $Y = 0.0709x + 0.0047$. and the linear calibration graph is shown in Table(3-8) and Fig.(3-6).



Fig(3-6): calibration graph for the determination of Thiamine.HCl

Table (3-8) calibration graph data

Conc. Of Thiamine.HCl $\mu\text{g} \cdot \text{ml}^{-1}$	Abs.
0.2	0.0168
1	0.075
3	0.218
5	0.359
8	0.576
10	0.716
12	0.859
14	0.992

Table (3-9):Analytical data for the determination of Thiamine.HCl

Analytical data	Value
Detection limit(D.L)	$0.106 \mu\text{g} \cdot \text{ml}^{-1}$
Sensitivity	$0.0709 \text{ abs unit of } 1 \mu\text{g} \cdot \text{ml}^{-1}$
Correlation Coefficient	0.9999
Linear range	$(0.2-14) \mu\text{g} \cdot \text{ml}^{-1}$
RSD%	0.4763
Recovery%	99.18
ϵ	$2.42 \times 10^3 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$
Sandells sensitivity	$139.38 \times 10^{-6} \mu\text{g} \cdot \text{cm}^{-2}$

3-1-6-Accuracy and precision of the proposed method :

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 $\mu\text{g.ml}^{-1}$. The results obtained are shown in Table (3-10).

Table (3-10): Accuracy and precision of the proposed method

Conc.Of vitamin B1 ($\mu\text{g.ml}^{-1}$)		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.

3-1-7-Application of the proposed method for the determination of Thiamine.HCl in pharmaceutical preparations.

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 (3-11).

Table (3-11): Application of the proposed method for the determination of Thiamine.HCl in pharmaceutical preparations

Drug sample vitamin B1 (10mg) SID	Conc.B1 $\mu\text{g.ml}^{-1}$		Proposed method			Standard ⁽⁶⁸⁾ method
	Taken	Found	RSD*%	Error*%	Recovery*%	Recovery*%
	3.000	2.860	0.617	-4.66	95.34	104.0
	5.000	5.220	0.450	4.40	104.4	
	10.000	9.780	0.362	-2.20	97.80	

*Average of five determinations

3-1-8-Statistical Analysis

The results obtained by the proposed method were compared successfully with the result of standard methods using **F**- and **T**-test ⁽⁶⁹⁾.

F- test

$$F = \frac{\sigma_1^2}{\sigma_2^2} \text{-----(3-1)}$$

σ_1 = standered deviation of the First method

σ_2 = standered deviation of the second method

$$\sigma_1 > \sigma_2$$

$$\sigma^2 = \sum \frac{(X_1 - \bar{X})^2}{(N-1)} \text{-----(3-2)}$$

X_1 , \bar{X} = analytical value and average of the analytical values respectively.

N = degree of freedom.

From the results in Table (3-11)

$$F_{\text{exp}} = \frac{\sigma_1^2}{\sigma_2^2} = \frac{0.846}{0.532} = 1.590$$

By comparing the F- calculated value with tabulated F-value=5.05 with a confidence limit of 95%, the results indicated that there was no significant difference between the precision of two methods.

T-test

t-test can be applied as follows:

$$\pm t = \frac{(\bar{X}_{i2} - \bar{X}_{i1})}{\sigma \sqrt{(n_1 n_2) / (n_1 + n_2)}} \text{ ----- (3-3)}$$

\bar{X}_{i1} and \bar{X}_{i2} average recovery of standered method and the proposed method.

$$\sigma = \sqrt{\sum (X_{i1} - \bar{X}_{i1})^2 + (X_{i2} - \bar{X}_{i2})^2 / (n - 2)} \text{ ----- (3-4)}$$

$$\sigma = \sqrt{\frac{0.846 + 0.532}{8}} = 0.415$$

$$\pm t = \frac{(104 - 104.4)}{0.415 \sqrt{\frac{(5*5)}{(5+5)}}}$$

$$\pm t = 0.609$$

By comparing the t-calculated value with the tabulated value $t=2.776$ given in the references with a confidence limit of 95%

of results indicate that there was no significant difference between the accuracy of two methods.

3-1-9- Comparison with other methods:

The proposed method was compared with a number of published spectrophotometrical methods, which were found in the literature. The proposed method is characterized by simplicity , accuracy ,sensitivity and do not needs farther steps such as solvent extraction , PH control and expensive reagent. Table (3-12) show a comparison of the proposed method with the other methods published in the literature .

Table (3-12) comparison of analytical data of the proposed method with a number of other spectrophotometric methods.

Reaction for determination	λ_{\max}	Linearity	Recovery %	Ref
phosphomolybdic acid	660 nm	10 - 60 mg/ml	-----	(46)
sodium Phenylhydrazine - 4 - sulphate	490 nm	1-16 $\mu\text{g/ml}$	99.7%	(47)
Direct determination	254 nm	-----	99.4%	(42)
oxidation reaction between thiamine and hydrogen peroxide	440nm.	1×10^{-8} - 1×10^{-4} mol.L ⁻¹	98.1%	(60)
Oxidation reaction between thiamine and Ferric Nitrate and Potassium hexacyanoferrate	747nm	0.2-14 $\mu\text{g/ml}$	97.8–104.4	proposed method

3-1-10-Conclusion:

- 1-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.
- 2- A sensitive and accurate spectrophotometric method was developed for the determination of Thiamine.HCl in pharmaceutical preparations.
- 3-The method was applied successfully for the determination of Thiamine.HCl in pharmaceutical preparations.
- 4- The method does not need to solvent extraction step or pH control.
- 5- The method is more economic because of using a low cost reagents.



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الخلاصة

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 الحديد المائية ليعطي الحديد الثنائي الذي بدوره يتفاعل مع مادة بوتاسيوم سداسي سيانيد الحديد مكوّن صبغة ذائبة زرقاء اللون (زرقة البر وسيان) والتي تمتص عند الطول الموجي 747 نانومتر وقد أظهرت النتائج والحسابات الإحصائية 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) والانحراف المعياري النسبي (0.362–0.617%) وحد كشف (0.106) مكغم/مل واستعادية (95.34–104.4%). وقد طبقت الطريقة بنجاح لتقدير الثيامين هيدروكلورايد في المستحضرات الصيدلانية



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