

Indirect Spectrophotometric Determination of Thiamine Hydrochloride in Presence of Sulphite Via Chromium-1,5-Diphenylcarbazide Complex

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ABSTRACT

A simple, rapid, accurate and precise spectrophotometric method is proposed for the determination of thiamine hydrochloride (Vitamin B₁) in both pure form and in its pharmaceutical formulations. The method is based on the oxidation-reduction reaction between vitamin B₁ and known amount of chromate CrO₄⁻² in acidic medium of 2N H₂SO₄. Then, the excess of chromate is measured via 1,5-diphenylcarbazide which gives a pinkish-violet, water soluble and stable complex and exhibits maximum absorption at 543 nm, with a molar absorptivity of 1.5×10^4 l. mol⁻¹. cm⁻¹, Sandell's sensitivity index of 0.02248 µg.cm⁻² and a relative standard deviation of ±0.31 to ±0.57%, depending on the concentration level. Beer's law is obeyed in the concentration range from 0.4 to 40 µg. ml⁻¹ of thiamine hydrochloride. The present method has been developed for the determination of thiamine hydrochloride in the presence of sulphite. The proposed method has been applied successfully to the determination of vitamin B₁ in pharmaceutical preparations.

Keywords: Thiamin hydrochloride, sulphite, spectrophotometry, 1,5-diphenylcarbazide.

-5,1-

(B₁)

B₁

(2)

CrO₄⁻²

-

-5,1

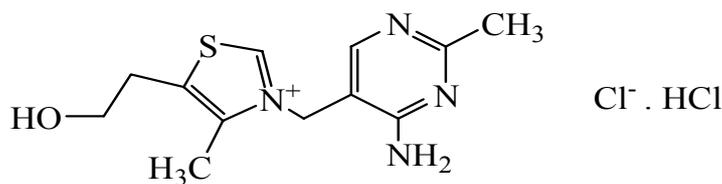
543

2-	0.02248	1-	1-	10 ⁴ × 1.5
		0.57 ±	0.31 ±	
B ₁	1-	40	0.4	
B ₁				
	-5,1			:

INTRODUCTION

Thiamine or thiamin (British Pharmacopeia, 2007), named as the "thio-vitamin" (sulfur-containing vitamin) is a water-soluble vitamin of the B complex, previously known as vitamin B₁ or aneurine. It was isolated and characterized in 1920 and thus was one of the first organic compounds to be recognized and discovered as a vitamin, therefore it is named B₁ (Bettendorff *et al.*, 1996). There are five known natural thiamine phosphate derivatives (Bettendorff *et al.*, 2007): Thiamine monophosphate (ThMP), thiamine diphosphate (ThDP), also sometimes called thiamine pyrophosphate (TPP), thiamine triphosphate (ThTP), recently discovered adenosine thiamine triphosphate (AThTP), and adenosine thiamine diphosphate (AThDP). All living organisms use thiamine in their biochemistry, but it is only synthesized in bacteria, fungi, and plants. Animals must obtain it from their diet and thus, for them, it is an essential nutrient. People need it to form adenosine triphosphate (ATP), which every cell of the body uses for energy (Bettendorff and Wins, 2009).

Thiamine structure contains an aminopyrimidine ring and a thiazole ring with methyl and hydroxyethyl side chains linked by a methylene bridge. It is soluble in water, methanol, and glycerol and practically insoluble in acetone, ether, chloroform and benzene. It is stable in acidic solution and during frozen storage, but it is unstable in alkaline solution, heat, and when it is exposed to ultraviolet light and gamma irradiation (Thornalley, 2005).



Thiamine hydrochloride



$$\text{Mw} = 337.27 \text{ g / mole}$$

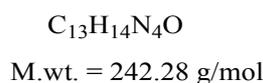
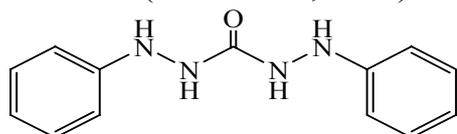
IUPAC name: 2-[3-[(4-Amino-2-methyl-pyrimidin-5-yl)methyl]-4-methyl-thiazol-5-yl] ethanol

Thiamine is sometimes called an "anti-stress" vitamin because it may strengthen the immune system and improve the body's ability to withstand stressful conditions. In mammals deficiency results in Korsakoff's syndrome, optic neuropathy, and a disease

called beriberi that affects the peripheral nervous (polyneuritis) and/or cardiovascular system (Spinazzi *et al.*, 2010). Thiamine deficiency has a potentially fatal outcome if it remains untreated. In less severe cases, nonspecific signs include malaise, weight loss, irritability and confusion (Webb *et al.*, 2007). Sulphites are widely used in foods that include dried fruit and vegetables, wine, soft drinks, juices and meat products, such as sausages and hotdogs to prevent melanosis (black spot) on shrimp and lobster, to "condition" dough bleach food starches and inhibit "browning" in bottled lemon juice and virtually all processed potatoes. In all food products containing at least 10 ppm of sulphite, sulphite will attack thiamine at the methylene bridge in the structure, cleaving the pyrimidine ring from the thiazole ring. The rate of this reaction is increased under acidic conditions also thiamine is degraded by thermolabile thiaminases (present in raw fish and shell fish) (Kappler *et al.*, 2000). Some thiaminases are produced by bacteria. Bacterial thiaminases are cell surface enzymes that must dissociate from the membrane before being activated, the dissociation can occur in ruminants under acidotic conditions (Makarchikov *et al.*, 2003). Rumen bacteria also reduces sulphate to sulphite, therefore high dietary intakes of sulphate can have thiamine-antagonistic activities (Begley *et al.*, 2008).

Several Spectrophotometric methods have been used for the determination of thiamine, such as the determination of vitamin B₁ with 12-tungstophosphoric acid by using resonance rayleigh scattering method (Zhiping *et al.*, 2012), zero-crossing derivative spectrophotometry (Mahmure and Ikbal, 2002), partial least-squares regression (Abera'sturi *et al.*, 2002), parallel factor analysis (Jahanbakhsh and Bahman, 2005), UV-visible spectrophotometry and genetic algorithm based multivariate calibration methods (Ozdemir and Dinc, 2004), horseradish as catalyst in the presence of hydrogen peroxide (Khan *et al.*, 2009), triphenylmethane acid dyes (Shaopu *et al.*, 2002), selective optosensor for UV-photometric (Barrales *et al.*, 1998), UV-photodegradation in a single-line flow-injection assembly (Calatayud and Danet, 1994), multicommuted flow system (Rocha *et al.*, 2003), implementation of flow-through solid phase spectroscopic transduction with photochemically induced fluorescence (Javier *et al.*, 2005). Other methods depend on the inhibitory effect of thiamine on the hemoglobin-catalyzed reaction of H₂O₂ with acid chrome blue K (Yahong and Fengshou, 2010), a flow-injection spectrophotometric method has been also used for the determination of vitamin B₁ (Clezio *et al.*, 1999).

1,5-Diphenylcarbazide (DPC) is an organic compound usually used in analytical chemistry for colorimetric measurements. It exhibits many useful properties and used as an artificial donor during charge separation in photochemical reactions and also photosynthesis electron transport (Sandell, 1959). It is well known that chromate-1,5-diphenylcarbazide chelate shows an intense pinkish-violet colour at pH 0.2. On the other hand, thiamine reduced chromate to chromium (III) then the excess of chromate reacted with 1,5-diphenylcarbazide (Marczenko, 1976).



1,5-Diphenyl carbazide

The purpose of this work is to make use of these facts to develop a simple, sensitive and rapid spectrophotometric method for the determination of thiamine, without requiring

an expensive instrumentation, needless of extraction and temperature control, and the possibility of the application of the proposed method to determination of thiamine in pharmaceutical formulations, also determining B₁ in the presence of sulphite.

EXPERIMENTAL

Apparatus

Spectral and absorbance measurements are carried out using Shimadzu UV-160 UV-Visible computerized double-beam spectrophotometer. In all measurements, 1-cm matched cells are used. The pH measurements are carried out using HANA pH meter.

Chemicals

All chemicals used are of analytical reagent grade.

Thiamine hydrochloride (1000 µg / ml) solution. This solution was prepared by dissolving 0.1000 g of B₁ in distilled water and the volume was completed to 100 ml with distilled water in a volumetric flask. The solution was then transferred to a dark bottle and is stable for at least 2 days. Working solution of 100 µg / ml B₁ solution was prepared by an appropriate dilution of the stock solution with distilled water.

Potassium chromate solution, 8.62×10^{-4} M. This solution is prepared by dissolving 0.1674 g of potassium chromate (Fluka) in 100 ml distilled water in a volumetric flask. The solution was transferred to a dark bottle and it stable for at least one month. Working solution 8.62×10^{-4} M of chromate was prepared by appropriate dilution of the stock solution with distilled water.

1,5-Diphenylcarbazide solution, 1.5×10^{-3} M. This solution is prepared by dissolving 0.0908 g of 1,5-diphenylcarbazide (BDH) in 5 ml of pure acetone, then the volume is completed 250 ml with distilled water in a volumetric flask. This solution is stable for at least 3 days.

Sulphuric acid solution, 2N. This solution is prepared by an appropriate dilution of the concentrated sulphuric acid solution to the mark with distilled water in a 250-ml volumetric flask.

Sodium sulphite solution. An approximately 0.1 N solution is prepared by dissolving a known amount of sodium sulphite anhydrous (Fluka) in distilled water containing 2 ml of 0.01% D(-)-fructose as a stabilizer (Gobbi *et al.*, 1998). This solution is standardized by iodimetry, sulphite solution with (0.01% D(-) fructose) are prepared by suitable dilutions of the standard with distilled water. A 100 ppm sulphite solution is stable for at least 3 days.

Mercuric nitrate solution, 0.01 M. This solution is prepared by dissolving 0.8565 g of 0.01 M mercuric nitrate monohydrate (Fluka) in distilled water then the volume is completed to the mark with distilled water in a 250-ml volumetric flask.

Procedure and Calibration Graph

To a series of 25-ml calibrated flasks, an increasing volume (0.1-11.3) ml of 100 µg. ml⁻¹ B₁ solution is transferred, followed by 2.5 ml of sulphuric acid solution (2N) and 1 ml of 8.62×10^{-4} M chromate solution, standing for 5 minutes, the 1 ml of 1.5×10^{-3} M DPC reagent solution is added. After diluting the flasks with distilled water, the absorbances are measured at 543 nm against the reagent blank. Beer's law is obeyed over the range of concentration 0.4-40 µg /ml vitamin B₁ (Fig.1). The molar absorptivity being 1.5×10^4 l. mol⁻¹. cm⁻¹, and the Sandall sensitivity is 0.02248 µg. cm⁻².

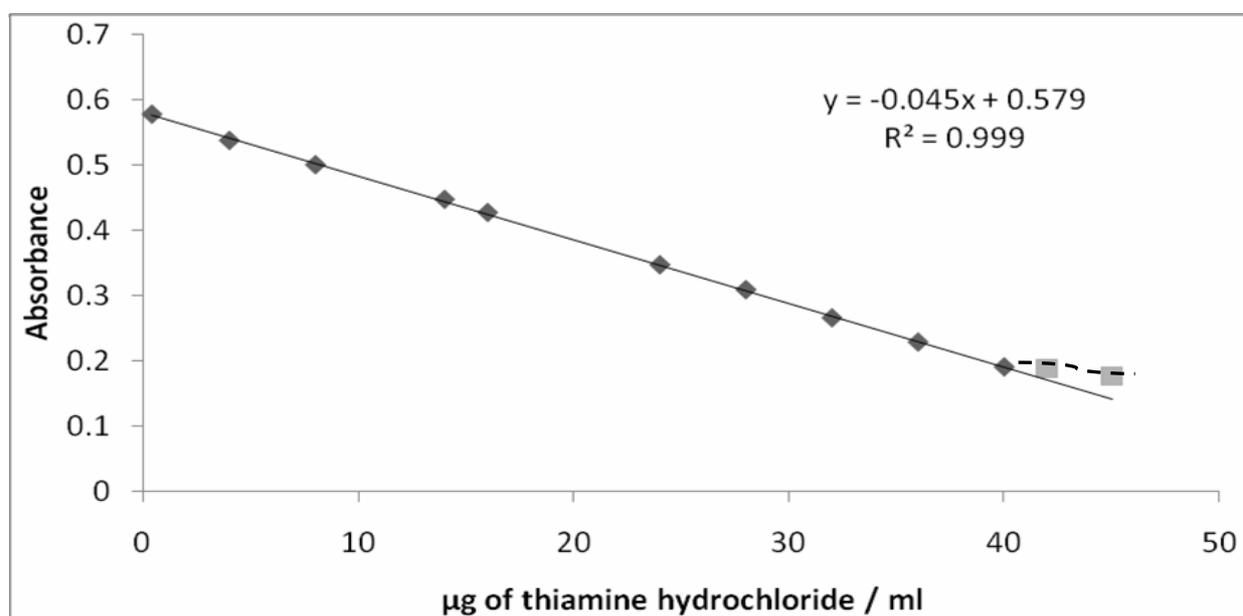


Fig. 1: Calibration graph for vitamin B₁ determination

Procedure for Drugs Forms

For tablet. At least ten tablets (200 mg B₁-HCl/tablet) of the drug (Tetravit) were weighed, powdered and mixed well. A portion equivalent to 0.01 g was weighed and dissolved in 50 ml of distilled water, shaken well, filtered and diluted with water to 100 ml in a volumetric flask. An aliquot of the diluted drug solution was then treated as done in the recommended procedure.

For capsule. At least ten capsules (5 mg B₁-HCl/capsule) of the drug (B-plex) were weighed. A portion equivalent to 0.01 g was weighed and dissolved in 50 ml of distilled water, shaken well, filtered and diluted with water to 100 ml in a volumetric flask, then it was proceeded as described under recommended procedure.

RESULTS AND DISCUSSION

Study of optimum conditions

The effect of various parameters on the oxidation-reduction reaction and the intensity of the coloured complex have been studied and optimum conditions have been selected.

Effect of Sulphuric Acid Amount

In order to choose the optimum amount of sulphuric acid for the reaction of thiamine hydrochloride with chromate, and the formation of a stable coloured complex between chromate and DPC, different amounts (0-5.0) ml of sulphuric acid (2N) are tested. The results shown in Table (1) indicate that 2.5 ml of 2N H₂SO₄ is considered optimum, as it gives the more stable coloured complex (Sandell, 1959). Therefore, it is recommended for subsequent experiments.

Table 1: Effect of sulphuric acid

ml of sulphuric acid (2N)	Absorbance	Final pH	λ_{max}
0.1	0.349	2.52	542.5
0.5	0.358	2.01	542.5
0.7	0.341	1.88	541.5
1.0	0.467	1.77	541.5
1.5	0.533	1.64	541.5
2.0	0.529	1.54	542.5
2.5	0.525	1.41	543.0
3.0	0.532	1.37	542.0
3.5	0.532	1.31	541.0
4.0	0.539	1.27	543.5
5.0	0.560	1.15	544.0

Effect of chromate ion amount

Different amounts of chromate (VI) ion solution $8.62 \times 10^{-4} M$ with different amount (0.1-6) ml of $100 \mu g. ml^{-1}$ thiamine hydrochloride are studied and it was found from the experimental results that 1 ml of chromate which gives higher value of determination coefficient (0.99733862) was optimum and recommended for the subsequent experiments as shown in Table (2).

Table 2: Effect of chromate (VI) ion

ml of $8.62 \times 10^{-4} M$ chromate solution	Absorbance / μg thiamine hydrochloride in 25 ml								r^2
	10	30	50	70	100	200	400	600	
0.5	0.577	0.552	0.528	0.519	0.484	0.453	0.378	0.299	0.97040045
1.0	0.591	0.582	0.565	0.553	0.541	0.491	0.411	0.311	0.99733862
1.5	0.628	0.609	0.591	0.57	0.561	0.533	0.481	0.442	0.94197102

Effect of time on the reduction of chromate (VI) ion

A study of time effect on the reduction of chromate ion by thiamine hydrochloride has been investigated. The results shown in (Fig. 2), indicated that 5 min. reaction time was optimum because it gives lower absorbance of the colored complex and this time was recommended for the subsequent experiments.

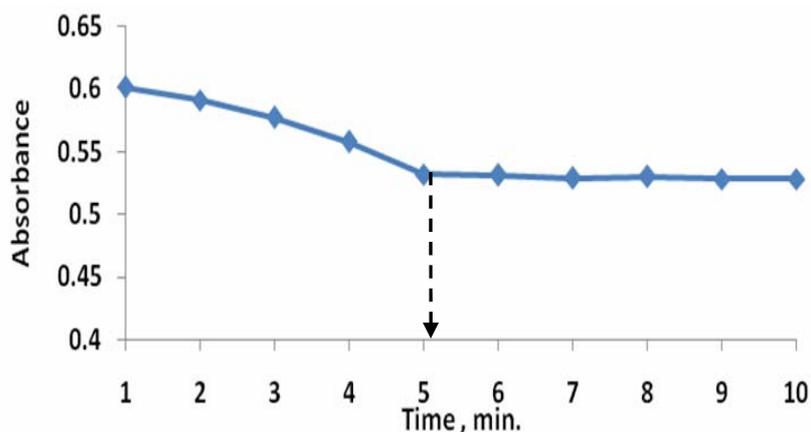


Fig. 2: Effect of time on the reducing chromate by B₁

Effect of 1,5-diphenylcarbazine amount

The effect of the amount of DPC reagent on maximum formation of the coloured complex is investigated. It was found from the experimental results that 1 ml of DPC reagent 1.5×10^{-3} M was optimum (determination coefficient=0.992464969), and recommended for the subsequent experiments as shown in Table (3).

Table 3: Effect of 1,5-diphenylcarbazine amount

ml of 1.5×10^{-3} M DPC solution	Absorbance / μg thiamine hydrochloride in 25 ml								r^2
	10	30	50	70	100	200	400	600	
0.5	0.593	0.57	0.527	0.513	0.492	0.455	0.359	0.306	0.94668874
1.0	0.602	0.593	0.569	0.551	0.544	0.490	0.408	0.313	0.992464969
1.5	0.611	0.604	0.583	0.568	0.551	0.517	0.492	0.448	0.921857171

Effect of order of addition

The different orders of addition were studied. The results shown in Table (4) indicate that the first order was optimum because it gives lowest absorbance value, therefore it is recommended for the subsequent experiments.

Table 4: Effect of order of addition

Reaction components	Order number	Absorbance
B ₁ + H + Cr + DPC	I	0.528
B ₁ + DPC + Cr + H	II	0.541
B ₁ + Cr + H + DPC	III	0.547
B ₁ + DPC + H + Cr	IV	0.578
B ₁ + H + DPC + Cr	V	0.588
B ₁ + Cr + DPC + H	VI	0.616

B₁=Vitamin B₁, Cr=Chromate, H=Sulphuric acid, DPC=1,5-Diphenylcarbazine.

Development time and stability period

To test the effect of time on the absorbance of the coloured complex for different amounts of thiamine hydrochloride at the wavelength of maximum absorption at 543 nm, under the optimum experimental conditions, the absorbances were measured at different intervals of time. The experimental results shown in (Fig. 3) indicated that the coloured complex develops immediately and maximally after 5 min. and the absorbance remains constant for at least 2 hrs.

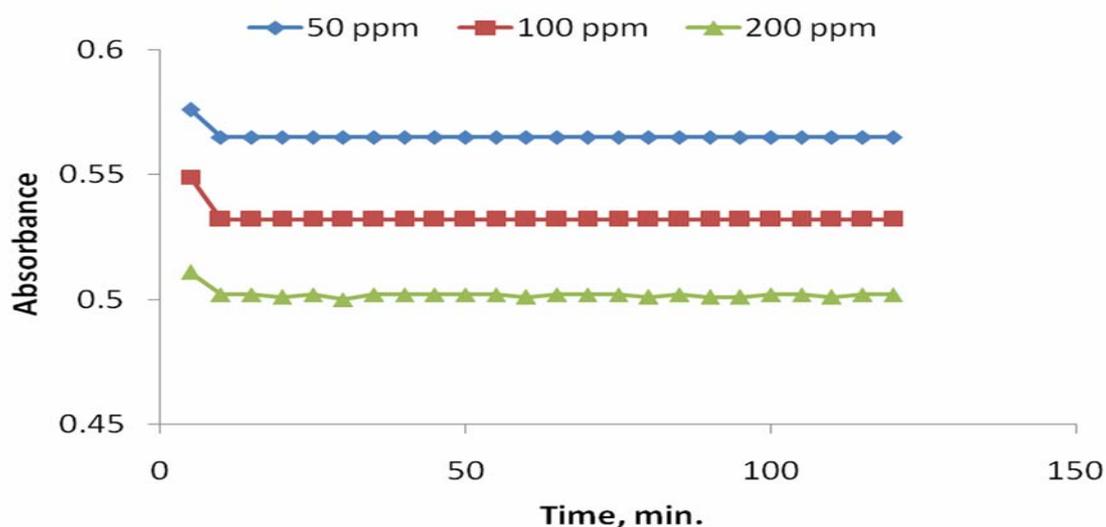


Fig. 3: Effect of time on the coloured complex.

Absorption Spectra

When vitamin B₁ is treated according to the recommended procedure, the absorption spectra is shown in (Fig. 4). The sample solution shows a maximum absorption at 542.5 nm, characteristic of the chromate-DPC complex, in contrast to the reagent blank which shows a slight absorption at this wavelength, emphasising the need for measurements to be performed against the reagent blank.

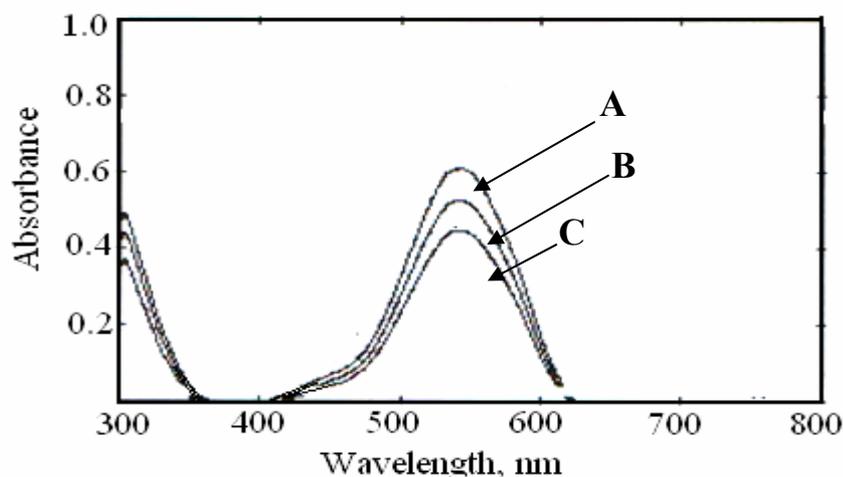


Fig. 4: Absorption spectra of (A) optimum amount of chromate against reagent blank, (B) 100 µg of B₁ / 25 ml measured against reagent blank, (C) 350 µg of B₁ / 25 ml measured against reagent blank.

Accuracy and Precision

To check the accuracy and precision of the calibration curve, thiamine hydrochloride is determined at four concentrations. The results shown in Table (5) indicate that these are reliable.

Table 5 : Accuracy and precision

Amount of thiamine hydrochloride taken, $\mu\text{g}/25\text{ml}$	Recovery*, %	Relative standard deviation*, %
50	99.86	± 0.34
100	100.08	± 0.57
200	100.19	± 0.31
400	99.19	± 0.32

* Average of five determinations.

Nature of the reaction between B₁ and chromate.

Job's method has been used in the determination of the reaction ratio of B₁ with chromate. The obtained result showed that the ratio of B₁ to chromate is 1:2 as in (Fig. 5).

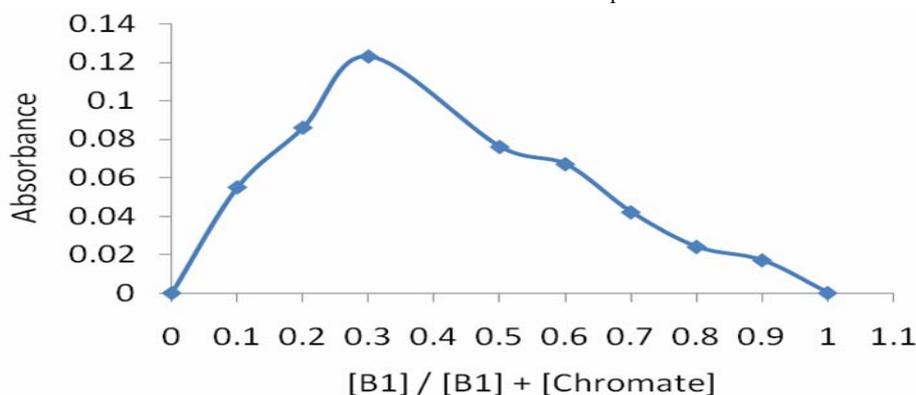
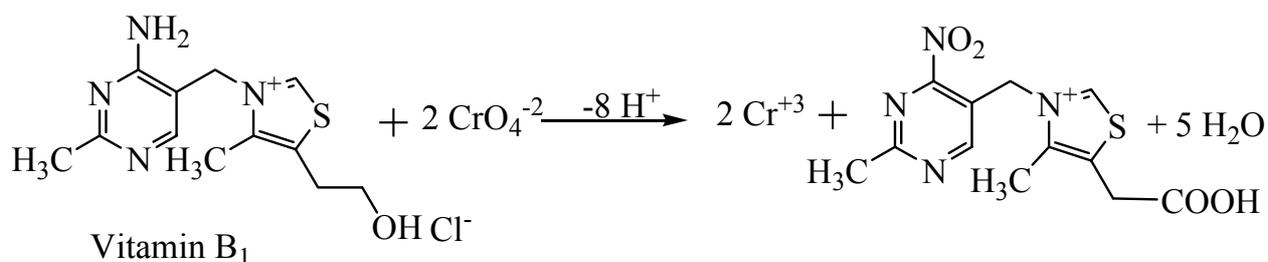


Fig. 5: Job's plot for B₁ - chromate

As a result the following reaction is suggested between vitamin B₁ and chromate:



Nature of chromate- DPC complex.

The stoichiometry of the reaction is investigated using the Job's method under the optimized conditions. The result in (Fig. 6) showed that the ratio of chromate to DPC is 1:2.

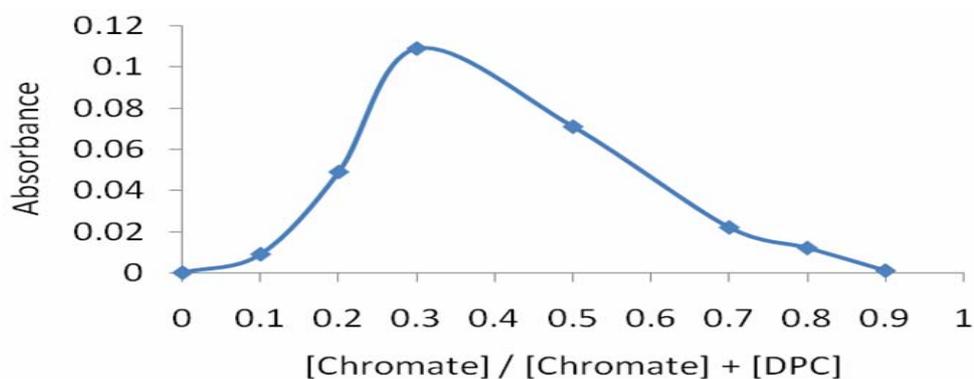


Fig. 6: Job's plot for Chromate - DPC.

Effect of Interferences

In order to test the efficiency and selectivity of the proposed method, the effect of some foreign substances (e.g., acacia, glucose, lactose, sulphite, menthol and starch) that are usually present in dosage forms were studied by adding different amounts of foreign substances to 100 μg B₁ / 25 ml. It was observed that the studied foreign species did not interfere in the present method except in the presence of $\text{SO}_3^{=}$, which interfere seriously as in Table (6).

Table 6: Effect of interferences on the determination of 100 μg B₁

Interferences	Recovery(%) of 100 μg B ₁ / μg of interference added		
	100	500	1000
Acacia	100.38	99.62	99.44
Glucose	99.62	100.75	99.25
Lactose	100.19	100.38	99.06
Menthol	99.81	101.32	100.75
Starch	99.62	100.19	100.56
Sulphite	62.22	47.93	34.59

Determination of B₁ in Presence of Sulphite

As sulphite (SO_3^{2-}) was clearly interfered by attacking thiamine at the methylene bridge in the structure, cleaving the pyrimidine ring from the thiazole ring, the proposed method has been developed for determining thiamine hydrochloride in presence of sulphite. This method depends on masking sulphite by mercuric ion (Hg^{2+}) as a masking agent (Williams, 1979) using the following experiments:

To a series of 25-ml calibrated flasks, 1 ml of 100 μg .ml⁻¹ B₁ solution are transferred, followed by 2.5 ml of sulphuric acid solution (2N) and (50 -500) $\mu\text{g}/\text{ml}$ of

sulphite SO_3^{-2} , then an increasing volume (0.5-1.5) ml of 0.01 M mercuric nitrate solution have been added, shaken well then, standing for 5 minutes. Finally, 1 ml of 8.63×10^{-4} M chromate solution and 1 ml of 1.5×10^{-3} M DPC reagent solution have been added. After the dilution of the flasks with distilled water, the absorbance is measured at 543 nm against the reagent blank as shown in Table (7).

Table 7: Determination of $100 \mu\text{g}\cdot\text{ml}^{-1}$ of thiamine hydrochloride in presence of sulphite

ml of 0.01 M Hg^{2+} solution	Absorbance */ μg of Sulphite present in 25 ml								
	50	75	100	150	200	250	300	400	500
0.5	0.487	0.477	0.480	0.479	0.476	0.489	0.478	0.481	0.471
1.0	0.538	0.534	0.529	0.535	0.529	0.534	0.537	0.533	0.527
1.5	0.612	0.612	0.611	0.613	0.622	0.619	0.621	0.624	0.632

* Absorbance without SO_3^{-2} = 0.531

The results given in Table (7) indicate that a complete removal of sulphite (50 -500 μg) from B_1 is achieved using 1 ml of (0.01 M) mercuric nitrate solution as it gave the nearest absorbance in comparison to the B_1 solution alone.

Application of the Method

The proposed method was successfully applied to the determination of B_1 in its pharmaceutical preparations (tablet and capsule). The results which are shown in Table (8) indicate that a good recovery was obtained.

Table 8: Analytical applications

B_1 amount, μg	Recovery(%) of B_1 *			
	(200 mg/ tablet) Iraq	NDI-	(5mg/capsule) Iraq	SDI-
50	100.57		99.71	
100	99.96		99.89	
250	100.41		100.66	

* Average of five determinations.

The calculated value of t-test (Christian, 2004), did not exceed the theoretical values at the 95% confidence level for five degrees of freedom when the proposed method has been compared with literature method (Jahanbakhsh and Bahman, 2005) as shown in Table (9).

Table 9: The results of t-test analysis

Drug	Pharmaceutical preparation	t-test
B ₁ (NDI-Iraq)	Tablet	0.475

Comparison of the Methods

Table (10) shows the comparison between some of analytical variables for the present method with that of other literature spectrophotometric methods.

Table 10: Comparison of the methods

Analytical parameters	Present method	Literature method (Zhiping <i>et al.</i> , 2012)	Literature method (Mahmure and Ikbal, 2002)
Method	Chromate-DPC	12-Tungstophosphoric acid	Second derivative spectrophotometry
pH	1.41	0-1.0	-----
Kind of acid	2 N H ₂ SO ₄	-----	0.1 N HCl
λ_{\max} (nm)	543	335	228.9
Reaction time (min)	5	-----	-----
Stability period (minutes)	120	-----	-----
Beer's law range (ppm)	0.4-40	0.25-1.75	Up to 20
Molar absorptivity (l.mol ⁻¹ .cm ⁻¹)	1.5×10 ⁴	-----	-----
R.S.D. (%)	±0.31-±0.57	1.1-3.4	-----
Colour of the product	Pink-violet	-----	-----
Application of the method	Pharmaceutical preparations	Urine samples and B ₁ tablets	Synthetic mixture and commercial pharmaceutical preparations

The results indicate that the proposed method is sensitive and can be applied successfully to the determination of B₁ in pharmaceutical preparations.

CONCLUSION

A simple, sensitive, selective and inexpensive spectrophotometric method for the determination of B₁ has been carried out by the rapid reduction of known amount of chromate CrO₄⁻² in the presence of B₁ in acidic medium of sulphuric acid. Then the excess of chromate is measured via 1,5-diphenylcarbazide which gives a pink-violet, water-soluble and stable complex, which exhibit minimum absorption at 543 nm. Beer's law is obeyed in the concentration range of 10-1000 µg/25 ml vitamin B₁ with a molar absorptivity of 1.5×10⁴ l. mol⁻¹. cm⁻¹, Sandell's sensitivity index of 0.02248 µg. cm⁻² and a relative standard deviation of ± 0.23% to ± 0.57% depending on the concentration level. The present method has been developed for the determination of thiamine hydrochloride in the presence of sulphite. The proposed method has been applied successfully to the determination of vitamin B₁ in pharmaceutical preparations.

REFERENCES

- Aberásturi, F.J.; Jiménez, A.I.; Arias, J.J.; Jiménez, F. (2002). Simultaneous spectrophotometric determination of folic acid, pyridoxine, riboflavin, and thiamine by partial least-squares regression. *Anal. Lett.*, **35**(10), 1677-1691.
- Barrales, O.; Córdova, M.L.; Díaz, M. (1998). A selective optosensor for UV spectrophotometric determination of thiamine in the presence of other vitamins B. *Anal. Chem. Acta.*, **376**(2), 227-233
- Begley, T.P.; Chatterjee, A.; Hanes, J.W.; Hazra, A.; Ealick, S.E. (2008). Cofactor biosynthesis—still yielding fascinating new biological chemistry. *Curr. Opin. Chem. Biol.*, **12**(2), 118-125.
- Bettendorff, L.; Mastrogiacomo, F.; Kish, S.J.; Grisar, T. (1996). Thiamine, thiamine phosphates and their metabolizing enzymes in human brain. *J. Neurochem.*, **66** (1), 250-258.
- Bettendorff, L.; Wins, P. (2009). Thiamine diphosphate in biological chemistry: new aspects of thiamine metabolism, especially triphosphate derivatives acting other than as cofactors. *FEBS J.*, **276**(11), 2917-2925.
- Bettendorff, L.; Wirtzfeld, B.; Makarchikov, AF, Mazzucchelli, G.; Frédérich, M.; Gigliobianco, T.; Gangolf, M.; De Pauw, E.; Angenot, L.; Wins, P. (2007). Discovery of a natural thiamine adenine nucleotide. *Nature Chem. Biol.*, **3**(4), 211-212.
- British Pharmacopoeia, CDROM, (2007). 3rd ed., System Simulation Ltd, Stationary Office, London.
- Calatayud, J.; Dănet, A. (1994). FIA-spectrophotometric determination of thiamine after UV-irradiation. *Talanta*, **41**(12), 2147-2151.
- Christian, G.D. (2004). "Analytical Chemistry". 6th ed. John Wiley and Sons Inc., New York, pp. 83-99.
- Clezio, A.; Pereira, A.V.; Costa-Neto C.O.; Fatibello-Filho, O. (1999). Flow-injection spectrophotometric determination of vitamin B₁ (thiamine) in multivitamin preparations. *Lab. Robotics Autom.*, **11**(1), 45-50.
- Gobbi, G.; Zappia, G.; Sabbioni, C. (1998). Sulphite quantification on damaged stones and mortars. *Atmos. Environ.*, **32**(4), 783-789.
- Jahanbakhsh, G.; Bahman, A. (2005). Simultaneous spectrophotometric determination of group B vitamins using parallel factor analysis: PARAFAC. *J. Chinese Chem. Soc.*, **52**, 1123-1129.
- Javier, L.; María, L.F.; Antonio, M. (2005). Implementation of flow-through solid phase spectroscopic transduction with photochemically induced fluorescence: determination of thiamine. *Anal. Chem. Acta*, **535**(1-2), 161-168.
- Kappler, U.; Bennett, B.; Rethier, J.; Schwarz, G.; Deutzmann, R.; Mcewan, A.; Dahl, C. (2000). Sulphite: cytochrome c oxidoreductase from *Thiobacillus novellas*. *J. Biol. Chem.*, **275**(18), 13202-13212.
- Khan, M.A.; Jin, S.O.; Lee, S.H.; Chung, H.Y. (2009). Spectrofluorimetric determination of vitamin B₁ using horseradish peroxidase as catalyst in the presence of hydrogen peroxide. *Luminescence*, **24**(2), 73-78.
- Mahmure, O.; Ikbali, K. (2002). Determination of ternary mixtures of vitamins (B₁, B₆, B₁₂) by zero-crossing derivative spectrophotometry. *Turk. J. Chem.*, **26**, 385-391.

- Makarchikov, A.F.; Lakaye, B.; Gulyai, I.E.; Czerniecki, J.; Coumans, B.; Wins, P.; Grisar, T.; Bettendorff, L. (2003). Thiamine triphosphate and thiamine triphosphatase activities: from bacteria to mammals. *Cell Mol. Life Sci.*, **60**(7), 1477–1488.
- Marczenko, Z.; (1976). "Spectrophotometric Determination of Elements". John Wiley and Sons, Inc., pp. 213-219.
- Ozdemir, D.; Dinc, E. (2004). Determination of thiamine HCl and pyridoxine HCl in pharmaceutical preparations using UV-visible spectrophotometry and genetic algorithm based multivariate calibration methods. *Chem. Pharm. Bul. l (Tokyo)*, **52**(7), 810-817.
- Rocha, F.; Filho, O.; Reis, B. (2003). A multicommuted flow system for sequential spectrophotometric determination of hydrosoluble vitamins in pharmaceutical preparations. *Talanta*, **59**, 191-200.
- Sandell, E.B. (1959). "Colorimetric Determination of Trace of Metals". 3rd ed. Interscience Publishers Inc. New York, pp. 178-400.
- Shaopu, L. ; Zhuyuan, Z.; Qin, L.; Hongqun, L.; Wenxu, Z. (2002). Spectrophotometric determination of vitamin B₁ in a pharmaceutical formulation using triphenylmethane acid dyes. *J. Pharm. Bio. Anal.*, **30**(3), 685–694
- Spinazzi, M.; Angelini, C.; Patrini, C. (2010). Subacute sensory ataxia and optic neuropathy with thiamine deficiency, *Nat. Rev. Neurol.*, **6**, 288-93.
- Thornalley, P.J. (2005). The potential role of thiamine (vitamin B(1)) in diabetic complications. *Curr Diabetes Rev.*, **1**(3), 287–98.
- Webb, M.E.; Marquet, A.; Mendel, R.R.; Rebeille, F.; Smith, A.G. (2007). Elucidating biosynthetic pathways for vitamins and cofactors. *Nat. Prod. Rep.*, **24**(5), 988–1008.
- Williams, W.J. (1979). "Handbook of Anion Determination". Butterworth and Co (publisher), pp. 588-590,601-603.
- Yahong, C.; Fengshou, T. (2010). Enzymatic catalytic spectrophotometric determination of thiamine in food. *Food Anal. Methods*, **3**(1), 7-11.
- Zhiping, C. ; Shaopu, L.; Zhongfang, L.; Xiaoli, H. (2012). Determination of vitamin B₁ with 12-tungstophosphoric acid by resonance Rayleigh scattering method. *Anal. Methods*, **4**, 434-438.