

Spectrophotometric determination of sulphite and thiamin hydrochloride via Xylenol orange dye

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ABSTRACT

a simple, accurate and rapid spectrophotometric method for determination of sulphite and thiamine hydrochloride (B₁) is described. This method used proton transfer reaction between sulphite ion with xylenol orange at pH(7.6) to develop red colour adduct that of maximum absorption at 580 nm. This adduct was used for determination of thiamine hydrochloride by bleaching the red colour product (xylenol orange-sulphite) at the optimum conditions established for the determination of sulphite. Beer's law is obeyed in the concentration range (10-1000) µg/25ml (i.e. 0.4-40) of sulphite and 10-2000, µg/25ml (i.e. 0.4-80) for thiamine hydrochloride at the selected wavelength, the molar absorptivity 2.8×10^4 l.mol⁻¹.cm⁻¹ for SO₃²⁻ and 2.69×10^4 l.mol⁻¹.cm⁻¹ for thiamine hydrochloride, Sandell's index for sensitivity 0.002857 µg .cm⁻² for sulphite and 0.012537 µg .cm⁻² for B₁. Good recoveries of sulphite and thiamine hydrochloride from water samples and pharmaceutical formations are achieved using the proposed method.

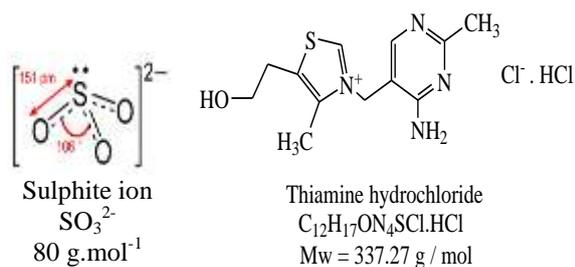
Keywords: Sulphite;Thiamine hydrochloride; Xylenol orange; Proton transfer reaction, pharmaceutical formation, water samples.

INTRODUCTION

Sulphites [1] contain the sulphite ion, as a preservatives are considered as food additives to limit bacterial contamination. Sulphites are often used as a food preservative or enhancer in dried foods, shrimp are treated with sulphites on fishing vessels, in all food products containing at least 10 ppm of sulphite, sulphites are one of the food allergens causing difficult breathing in minutes after eating a food containing sulphites [2]. It is naturally occurring matter in human body as well as in some foods and all wines to some extent. People with aspirin sensitivity and asthmatics are at high risk for sulphites, the potential

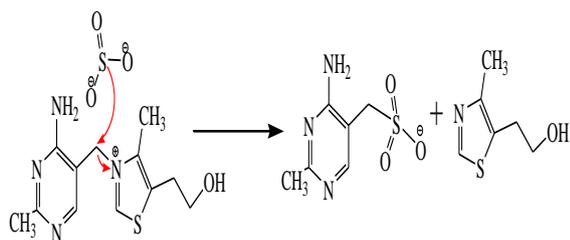
symptoms include swelling of the throat, sneezing, and migraine [3]. Thiamine hydrochloride (B₁ or aneurine) [4], named as the "thio-vitamine" is a sulfur-containing vitamin, it was isolated and characterized in 1920, because it was the first organic compounds recognized and discovered as a vitamin, therefore it is named B₁. It is soluble in water and some organic solvents, and it help for strengthen the immune system also help the human body's ability to withstand stressful conditions so it was called an anti-stress vitamin. Beriberi as a severe chronic disease results from vitamin B₁ deficiency. Beriberi has a serious complications on the nervous

system, and gastrointestinal system [5 & 6]. (Scheme 1).



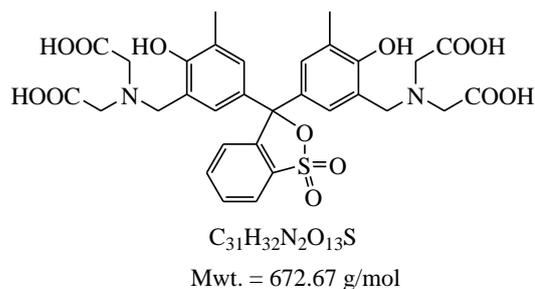
Scheme 1: Sulphite and thiamine hydrochloride structure

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). Many studies done on kind of dogs in deferent ages were dignosed with thiamine deficiency caused by feeding sulphite treated meat [7 & 8] (Scheme 2).



Scheme 2 - : Sulphite-induced cleavage of thiamine

Xylenol Orange (XO), 3,3'-bis[N,N-bis(carboxymethyl)-aminomethyl]-o-cresolsulfon phthalein, has been known as the most useful sulfonphthalein complex one metallic chromic indicator. It is a textile dye for industrial uses. It has an environmental concern. The current environmental hazards with these dyes and their derivatives revolve around the carcinogenic potential [9]. (Scheme 3).



Scheme 3: Xylenol orange structure

Several spectrophotometric methods are used for determination of sulphite such as quantification of sulphite ions in environmental samples [10], wine samples [11], other method determining of sulphite species in vegetables and dried fruits using a preconcentration process [12], another spectrophotometric methods used phosphate buffer at pH 7 with malachite green [13], or based on visible color changes [14], a flow injection analysis used for determination of sulphite in food and beverages [15], beer [16].

B_1 is determined by spectrophotometric methods in pharmaceutical preparation based on reaction of B_1 with 7-chloro-4-nitrobenzoxadiazole to produce brown color complex measured at 434 nm [17], another used 1,2-naphthoquinone-4-sulphonate measured at 487 nm [18], others spectrophotometric methods using coupling reaction of B_1 with procaine hydrochloride which reacted with sodium nitrate and hydrochloric acid to form a red water-soluble azo dye in basic medium [19], also a flow injection method is used for determination of B_1 with diazotized metoclopramide [20], and a fluorimetric method is also used for the determination of B_1 [21].

In this reserch sulphite and thiamine hydrochloride was determined with xylenol orange using proton transfer reaction, then applied the proposed method to various water samples and pharmaceutical formation respectively.

Material and Methods

Apparatus

Spectral and absorbance measurements are carried out using JASCOV - 630 UV / Vis (Japan) computerized double-beam spectrophotometer with 1-cm matched cells. The measurements of pH carried out by HANA pH meter.

Chemicals

The chemicals used in this research are of analytical grade reagents.

Standard sodium sulphite solution: this 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 [22]. This solution is standardized by iodimetry. Working standard sulphite solution are prepared by suitable dilutions of the standard with distilled water. A standard sulphite solution is stable for at least 3 days.

Thiamine hydrochloride (1000 µg/ml) solution. It was prepared by dissolving 0.1000 g of B₁ in distilled water and diluted to 100 ml using a volumetric flask, this solution was stable for at least 2 days. Working solution of 100 µg / ml B₁ solution was prepared by appropriate dilution of the stock solution with distilled water.

Xylenol orange solution 1×10⁻³ M: This solution was prepared by dissolving 0.0758 gm of xylenol orange (Fluka) in distilled water and the volume was completed to 100 ml in a volumetric flask with distilled water, this solution was stable for at least 3 days.

Procedure for dosage forms

For B₁ tablet (100 µg/ml), a ten tablets (200 mg B₁-HCl/tablet) were weighed, crushed and mixed well. A portion equivalent to 0.01 g was weighed and dissolved in 50 ml of distilled water, stirred and shaken well then filtered using filter paper (Whatman #41), the the final volume is brought to 100 ml in a volumetric flask with distilled water. This solution was then treated as in the recommended procedure.

For capsule (100 µg/ml). At least ten capsules (5 mg B₁-HCl/capsule) of B₁ drug were

weighed. A portion equivalent to 0.01 g was weighed and dissolved in distilled water, mixed and shaken well, filtered using filter paper and diluted with distilled water to 100 ml in a volumetric flask, then it was proceeded as described under recommended procedure.

Results and Discussions

To study the optimum conditions for the determination of sulphite and thiamine hydrochloride, the effect of many parameters on the intensity of the coloured system have been studied then the optimum conditions have been selected.

Effect of Buffer

The pH after addition of 2 ml of xylenol orange was 6.73, therefore, to an aliquot containing 30 µg of sulphite, different amounts (0.1-3.0) ml of 0.1 M of sodium hydroxide and 0.1 M of various kind of acids (hydrochloric acid, sulphuric acid, acetic acid and nitric acid) solutions are added. The intensity of absorption were measured against the reagent blank. Addition of acid or base are avoided because of showing negative results.

The effect of pH was studied and different buffers of pH 6.7 were prepared such as Maleic acid-Tris-NaOH, Na₂HPO₄-NaH₂PO₄, KH₂PO₄-NaOH, and Imidazole-HCl. It was found that these buffers decrease the absorbance [23] (Table 1).

Table 1: Effect of buffer solutions

ml of buffer solution	Absorbance */ ml of buffer added			
	Maleic acid-Tris-NaOH	Na ₂ HPO ₄ -NaH ₂ PO ₄	KH ₂ PO ₄ -NaOH	Imidazole-HCl
1	0.176	0.145	0.188	0.132
2	0.145	0.133	0.179	0.122
3	0.122	0.124	0.153	0.112
4	0.111	0.117	0.126	0.100
5	0.109	0.104	0.105	0.087

Effect of Temperature

Thermostatically controlled water-bath was used for following the colour development at temperature and 0, 10, 20, 30,40 and 50°C .The intervals against reagent blank treated similarly. The experimental results indicate

that the absorbance reached maximum after 3 minutes at 25°C therefore, it was chosen for the subsequent experiments(Fig.1).

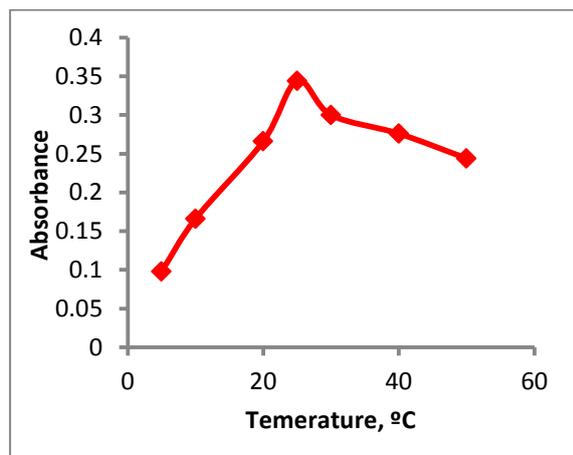


Fig. 1: Effect of temperature

Effect of xylenol orange (X.O) amount

The amount of X.O dye was studied by adding different amount (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0) ml of 1×10^{-3} M X.O dye to solution containing (10, 25, 50, 75, 100 and 150) $\mu\text{g} \cdot \text{ml}^{-1}$ sulphite. The absorbance reached its maximum value when 2 ml of 1×10^{-3} M X.O is used with $r^2 = 0.9991$. Therefore, 2 ml of 1×10^{-3} M X.O was used in all subsequent work.

Effect of surfactants

Various kind of surfactants were studied such as Triton X-100 (non-ionic surfactant), sodium dodecyl sulphate (anionic surfactant), cetyltrimethylammonium bromide and cetylpyridinium chloride (cationic surfactants). The results (Table 2) show that the addition of all kinds of surfactants give no useful effect. Thus, they are avoided in this work.

Table 2: Effect of surfactants

Surfactant	Absorbance / ml of surfactant added			λ_{max}
	1.0	3.0	5.0	
Cetyltrimethylammonium bromide	0.223	0.219	0.189	579
Sodium dodecylsulphate	0.225	0.218	0.187	576
Cetyl pyredinium chloride	0.232	0.221	0.200	580
Triton X-100	0.231	0.209	0.152	581
Without surfactant	0.305			580

Effect of time on the colour development

The effect of time (Fig.2) on the coloured product's absorbance at 580 nm, was studied under the optimal conditions. The results had shown that the coloured complex develops immediately and stabilized after 3 minutes and the absorbance remains constant more than 72 hours.

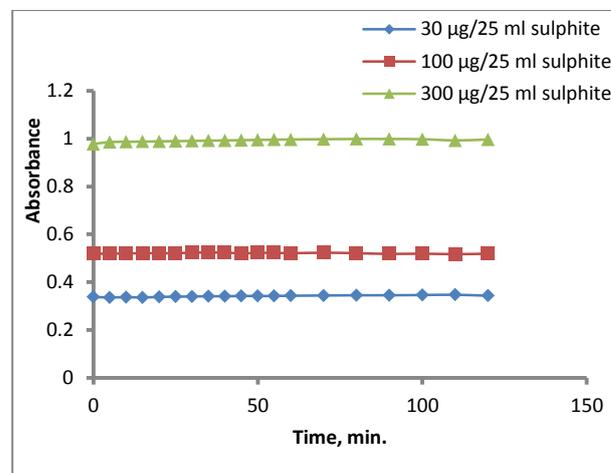


Fig. 2: Stability of the coloured product

Calibration graph and recommended procedure

A procedure of calibration graph (Fig.3) carried out by using a series of calibrated flasks (25 ml) containing an increasing amount (10, 30, 50, 100, 200, 300, 500, 700, 1000) $\mu\text{g}/25\text{ml}$ of sulphite solution (100 $\mu\text{g}/25\text{ml}$) and 2 ml of 1.0×10^{-3} M X.O reagent solution then standing for 3 minutes and dilution with distilled water to the mark, the absorbance are measured at 580 nm against the reagent blank. The range of Beer's law was 0.4 - 40.0 $\mu\text{g}/\text{ml}$ sulphite, the molar absorptivity was 2.8×10^4 $\text{l} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$, and the Sandall sensitivity was 0.002857 $\mu\text{g} \cdot \text{cm}^{-2}$.

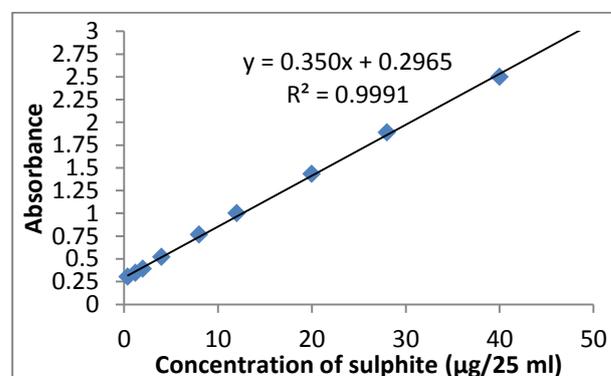


Fig. 3: Calibration graph for sulphite determination

The calibration graph for B₁ is constructed (Fig.4). Different amount of (0.1-20) ml of 100 µg .ml⁻¹ B₁ solution were added to the coloured product (SO₃²⁻X.O) (500 µg /25ml of SO₃²⁻+2 ml of 1×10⁻³ M X.O) then diluted to the mark with distilled water, also measured at 580 nm against reagent blank. The range of Beer's law was 0.4-80 µg /ml vitamin B₁. The molar absorptivity is 2.69×10⁴ l.mol⁻¹.cm⁻¹, and the Sandall sensitivity was 0.012537 µg.cm⁻² (Fig.4).

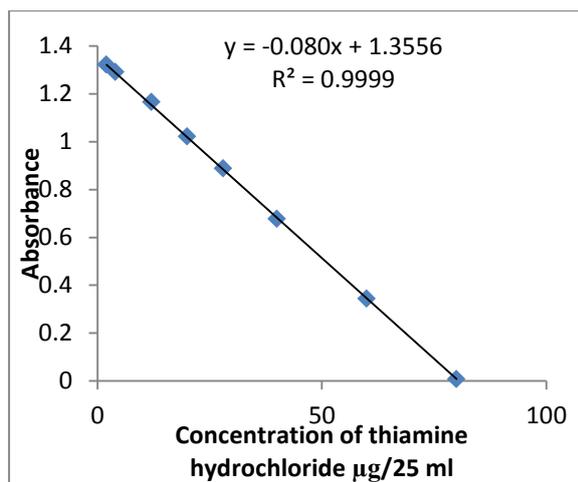


Fig. 4: Calibration graph for thiamine hydrochloride

Final absorption spectrum:

When sulphite and thiamine hydrochloride in an aqueous solution was treated according to the recommended procedure, the absorption spectra shows a maximum absorption at 580 nm. The reagent blank shows low absorption at this wavelength (Fig.5 and 6).

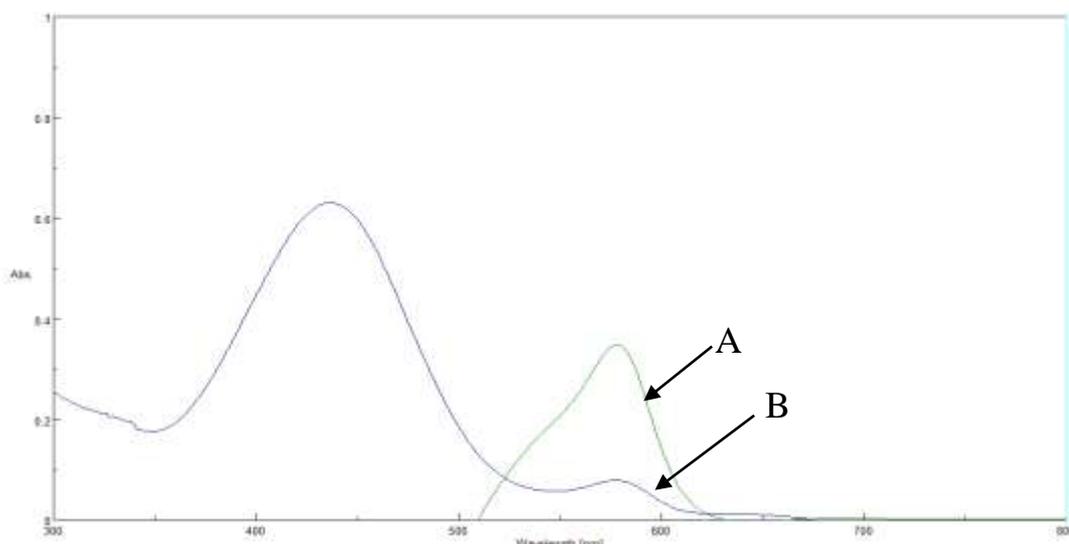


Fig. 5. Absorption spectra of 30 µg of SO₃⁻² /25 ml measured against (A) reagent blank, (B) blank measured against distilled water

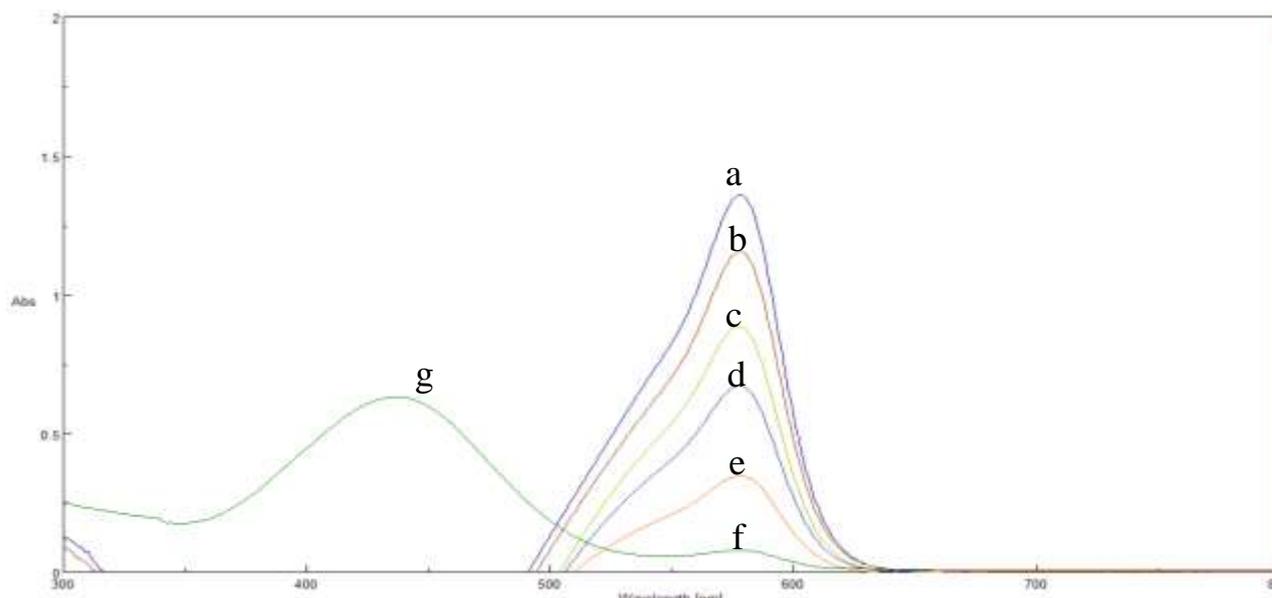


Fig. 6. Absorption spectra of (a=100, b=200, c=400, d=600, e=800 and f=1000) μg of B_1 /25 ml measured against reagent blank, (g) reagent blank against distilled water

Accuracy and precision

Under the experimental conditions which included in the recommended procedure, the precision and recovery of the method are calculated for 50,100, and 300 μg of sulphite, and or 100, 300, 500 μg of B_1 . The recovery and relative standard deviation are shown in table 3, indicating the method has good accuracy and precision.

Table 3: Accuracy and precision

Amount of SO_3^{2-} taken, $\mu\text{g}/25$ ml	Amount of SO_3^{2-} found, μg	Recovery*, %		Relative standard deviation*, %
		(200 mg /tablet) NDI-Iraq	(5 mg capsul) SDI-Iraq	
20	20.19	99.53		0.003329
100	99.34	100.51		0.006345
300	299.01	100.07		0.002182
Amount taken of B_1 , μg	Amount of B_1 , found, μg	Recovery(%) of B_1 *		Relative standard deviation*, %
		(200 mg /tablet) NDI-Iraq	(5 mg capsul) SDI-Iraq	
100	100.13	100.15	100.09	0.001053
300	299.18	100.23	99.97	0.000978
500	500.11	100.31	99.96	0.001116

* Average of five determinations.

Nature of the reaction between SO_3^{2-} and xylenol orange.

The reaction ratio of sulphite with X.O dye was studied by using Job's method of the continuous variations. The obtained results (Fig.7) showed that the molar ratio of sulphite to X.O was 1:1 .

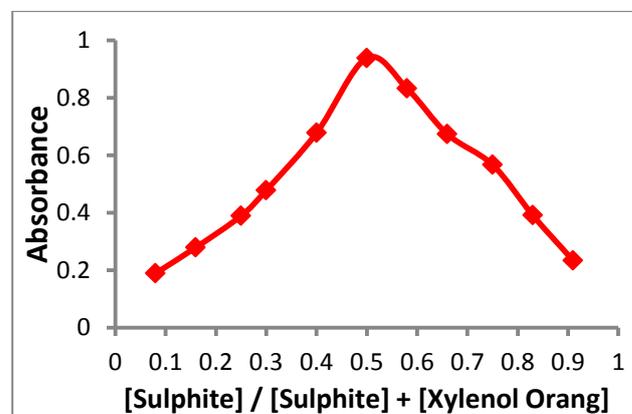
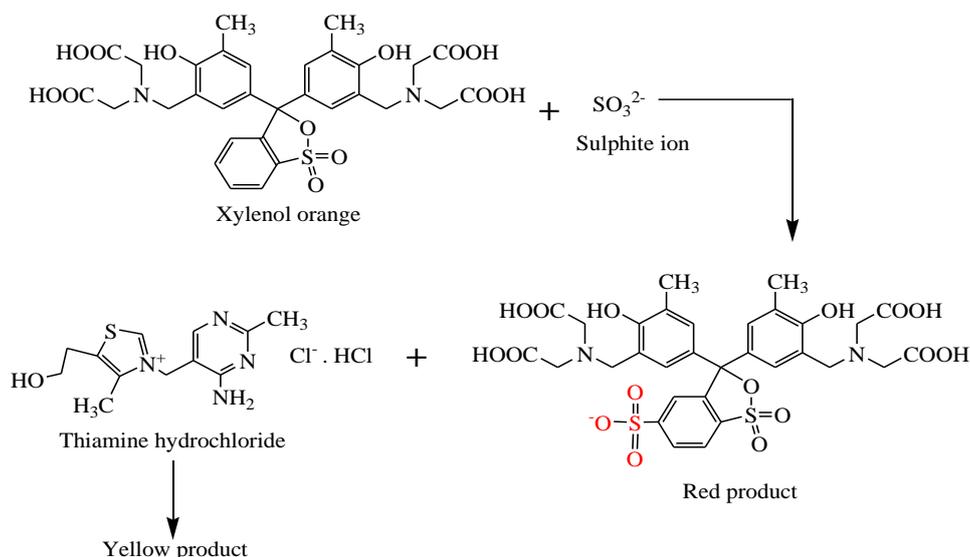


Fig 7: Job's plot for SO_3^{2-} – X.O.

Hence the coloured product may have the following suggested mechanism (Scheme 4):



Scheme 4: Suggested mechanism of sulphite-X.O.

Effect of foreign ions

The effect of many foreign ions on the determination of 10 µg sulphite per 25 ml of solution or 100 µg/25 ml B₁ is examined under the experimental conditions used. The results obtained are summarized in table 4 and table 5.

Table 4: Effect of foreign ions

Foreign ion	Tolerance, µg	Foreign ion	Tolerance, µg
SO ₄ ²⁻	500	Cd ²⁺	1000
NO ₂ ⁻	750	Pb ²⁺	1000
NO ₃ ⁻	1000	Be ²⁺	500
CO ₃ ²⁻	1000	Cr ³⁺	1000
S ₂ O ₃ ²⁻	750	Ti ⁺	750
PO ₄ ³⁻	500	Si ⁺	1000
Ca ²⁺	1000	Ni ²⁺	1000
Mg ²⁺	1000	Mn ²⁺	1000
Li ⁺	1000	Cu ²⁺	1000
Fe ³⁺	5	Co ²⁺	750
Fe ²⁺	500	Hg ²⁺	1000

The results were shown in (Table 4) indicate that the method seemed to be selective except towards ferric ion which interfere seriously.

Table 5: 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	100.07	100.15	100.62
Glucose	99.92	100.23	100.77
Lactose	100.69	100.85	100.92
Menthol	100.54	100.46	100.61
Starch	100.15	100.38	100.31

Application of the method:

The proposed method was successfully applied for the determination of sulphite and thiamine hydrochloride in various water samples and pharmaceutical preparations. A good recovery was obtained from the results showed in Table (6 & 7). The performance of the proposed method was assessed by calculating the student t-test [24] with the literature method [13& 25]. The results in Table (8) show that the calculated "t" values for five degrees of freedom (N₁+N₂-2=5) at the 95% confidence level do not exceed the theoretical level t value (2.571), indicating that there is no significant difference between the proposed method and the reported method.

Table 6: Determination of sulphite in water samples

ml of water sample	Sulphite added, µg	Recovery (%)*			
		Tap water	Well water*	Natural spring water	Sea water**
1	50	100.29	100.27	99.71	99.13
3	50	99.71	99.71	99.41	98.76
5	50	99.42	99.42	100.29	98.77
7	50	100.87	100.86	99.70	100.59
1	100	98.84	98.82	98.80	99.71
3	100	98.83	98.86	98.85	100.38
5	100	99.13	99.11	99.13	99.61
7	100	98.56	98.54	98.51	100.19
1	300	98.27	98.22	98.27	99.71
3	300	100.28	100.21	100.20	100.39
5	300	99.72	99.70	99.91	99.75
7	300	99.41	99.43	99.82	99.72

* Average of five determinations.

Water samples applied from * al-shoura village in Mosul, ** white middle sea in Turkey.

Table 7: Determination of thiamine hydrochlorid

Amount of B ₁ , µg	Recovery(%) of B ₁ *	
	(200 mg / tablet) NDI-Iraq	(5 mg capsul)SDI-Iraq
50	100.81	100.48
100	100.78	100.78
200	100.98	100.89

* Average of five determinations.

Table 8: The value of "t" test of natural waters and pharmaceutical preparations.

Water samples	Recovery* (%)		"t" exp.
	Present method	Reported method (Shu-Juan and Li-Li, 2007)	
Tap water	100.79	100.74	0.7482
Well water	99.82	99.70	1.1742
Pharmaceutical preparations	Recovery* (%)		"t" exp.
	Present method	Reported method (Shekho et al., 2013)	
(200 mg / tablet) NDI-Iraq	100.80	100.73	0.5671
(5 mg capsul) SDI-Iraq	100.97	100.60	0.6086

* Average of five determinations.

Comparison of the methods

Table 9, shows the comparison between the proposed method and the other literature spectrophotometric methods through some measured analytical parameters.

Table 9: Comparison of the methods.

Analytical parameters	Present method	Literature method	
		Sulphite ion [13]	B ₁ [25]
Method	Xylenol orange	Malachite green	Chromate-DPC
pH	7.6	7.0	1.41
λ _{max} (nm)	580	615	543
Buffer solution	-----	Phosphate buffer	2 N H ₂ SO ₄
Reaction time (min)	Immediately	-----	5
Stability period (minutes)	Mor than 2 hrs	-----	120
Beer's law range (ppm)	0.4-40 SO ₃ ²⁻ 0.4-80 B ₁	0~ 0.6mg/L	0.4-40
Molar	2.8×10 ⁴ SO ₃ ²⁻	-----	1.5×10 ⁴

absorptivity (l.mol ⁻¹ .cm ⁻¹)	2.69×10 ⁴ B ₁		
Colour of the product	Win -red	color-fading	Pink-violet
Application of the method	Various water samples for SO ₃ ²⁻ and pharmaceutical preparations for B ₁	Food	Pharmaceutical preparations

The results in (Table 9) indicate that the method is sensitive and can be applied for the determination of sulphite and thiamine hydrochloride in different samples.

CONCLUSION

The proposed method permits rapid, precise and accurate determination of sulphite and thiamine hydrochloride with an application to various water samples and pharmaceutical preparations respectively. The short analysis time and low cost are the main advantages of this method for routine analysis in quality control.

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التقدير الطيفي للكبريتيت وهيدروكلوريد الثيامين باستخدام صبغة الزايلينول البرتقالي

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الخلاصة

يتضمن البحث طريقة طيفية بسيطة وسريعة لتقدير الكبريتيت وهيدروكلوريد الثيامين. تعتمد الطريقة المقترحة على تفاعل الكبريتيت مع صبغة الزايلينول البرتقالي عند دالة حامضية 6.7 لتكوين ناتج أحمر اللون يعطي أقصى امتصاص عند الطول الموجي 580 نانومتر. تم تطوير هذه الطريقة لتقدير فيتامين B₁ على أساس قصر لون الناتج الاحمر (الكبريتيت-صبغة الزايلينول البرتقالي) في الظروف المثلى لتقدير الكبريتيت. تتبع الطريقة قانون بير في نطاق التركيز (10-1000) مايكروغرام/25 مل (0.4-40) مايكروغرام.مل⁻¹ من الكبريتيت و(10-2000) مايكروغرام/25 مل (0.4-80) مايكروغرام.مل⁻¹ لهيدروكلوريد الثيامين عند الطول الموجي المختار ومعامل الامتصاص المولاري 2.8×10⁴ لتر.مول⁻¹.سم⁻¹ للكبريتيت و 2.69×10⁴ لتر.مول⁻¹.سم⁻¹ لهيدروكلوريد الثيامين اما دلالة ساندل للحساسية فقد كانت 0.002857 مايكروغرام.سم⁻² للكبريتيت و 0.012537 مايكروغرام.سم⁻² لهيدروكلوريد الثيامين وكانت استرجاعية الطريقة جيدة للكبريتيت وهيدروكلوريد الثيامين في النماذج المائية والمستحضرات الصيدلانية على التوالي.

الكلمات المفتاحية: الكبريتيت، هيدروكلوريد الثيامين، صبغة الزايلينول البرتقالي، تفاعل انتقال البروتون.