



Spectrophotometric determination of sulphite and thiamin hydrochloride using proton transfer reaction-application to water sample and pharmaceutical formation

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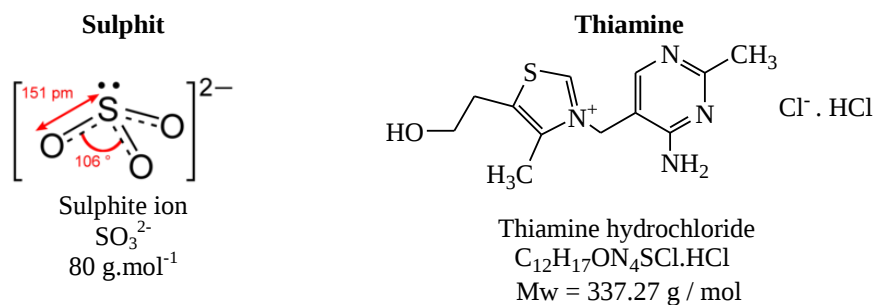
ABSTRACT

In this research, an easy, accurate and rapid spectrophotometric method for determination of sulphite and thiamine hydrochloride is described. This method used proton transfer reaction between sulphite ion with alizarin at (pH 6.73) to develop red colour adduct that bears maximum absorption at 524 nm. This method was developed to determination of thiamine hydrochloride by bleaching the red colour (alizarin-sulphite product) at the optimum conditions established for the determination of sulphite. Beer's law is obeyed in the concentration range (10-1000) $\mu\text{g}/25\text{ml}$ (0.4-40) of sulphite and 10-1500, $\mu\text{g}/25\text{ml}$ (0.4-60) for thiamine hydrochloride at the selected wavelength, the molar absorptivity $2.6 \times 10^4 \text{ l.mol}^{-1}.\text{cm}^{-1}$ for SO_3^{2-} and $3.37 \times 10^4 \text{ l.mol}^{-1}.\text{cm}^{-1}$ for thiamine hydrochloride, Sandell's index for sensitivity $0.00307 \mu\text{g} .\text{cm}^{-2}$ for sulphite and $0.0100 \mu\text{g} .\text{cm}^{-2}$ for B_1 , the precision (RSD) of the method is $\pm 0.23 - \pm 0.51\%$ sulphite and $\pm 0.15 - \pm 0.25$ thiamine hydrochloride. Good recoveries of sulphite and thiamine hydrochloride from water samples and pharmaceutical formation are achieved using the proposed method.

Introduction

Sulphites are compounds that contain the sulphite ion. Sulphites are naturally occurring matter in human body as well as in some foods and all wines to some extent. 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, people with aspirin sensitivity and asthmatics are at high risk for sulphites, the potential symptoms include swelling of the throat, sneezing, and migraine

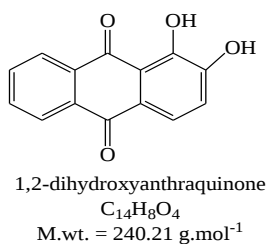
[1]. Thiamine hydrochloride (B_1 or aneurine) [2], named as the "thio-vitamine" is a sulfur-containing vitamin. B_1 was isolated and characterized in 1920, therefore it is named B_1 , because it was the first organic compounds recognized and discovered as a vitamin. B_1 is soluble in water and some organic solvents. All living organisms use thiamine in their biochemistry, but it is only synthesized in bacteria, fungi, and plants. People need it to form adenosine triphosphate (ATP), which every cell of the body uses for energy[3] (Scheme 1).

**Scheme 1: Sulphite and thiamine hydrochloride structure**

B_1 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 B_1 deficiency. Beriberi has a serious complications on the nervous system, and gastrointestinal system [4].

Thiamine in vitamin supplements can be destroyed and cleaved the thiamine molecule by sulphites. Sulphite cleaving the pyrimidine ring from the thiazole ring at the methylene bridge in the thiamine hydrochloride structure, the rate of this reaction was increased by acidic solution. Eating food containing sulphate can have thiamine-antagonistic activities by rumen bacteria which reduced sulphate to sulphite [5,6].

Alizarine is an organic compound known as Mordant red 11. Alizarine or 1,2-dihydroxyanthraquinone. Alizarine was the first natural pigment synthesised in 1869, throughout history alizarine was derived from roots of madder genus' plants and had been used as a prominent red dye [7] (Scheme 2).

**Scheme 2: Alizarine structure**

Several spectrophotometric methods are used for determination of sulphite such as quantification of sulphite ions in environmental samples [8], wine samples [9], other method based on a discoloring reaction between sulphite and flower anthocyanins in alkaline medium [10], another spectrophotometric methods used phosphate buffer at pH 7 with malachite green [11], or based on the bleaching of brilliant green dye by sulphite [12]. A flow injection analysis used for determination of sulphite in food and beverages [13], also a flow-injection determination of sulphite in white wines involving gas diffusion through a concentric tubular membrane [14].

B_1 can be determined with 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 [15], another used 1,2-naphthoquin-4-sulphonate measured at 487 nm [16], others spectrophotometric methods using coupling reaction with diazotized sulfanilic acid [17], or diazotization 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 [18], or using prussian blue reaction [19], also a flow injection method used for determination of B_1 with diazotized metoclopramide [20], and a fluorimetric method can be used for the determination of B_1 [21].

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

Experimental

Apparatus

Spectral and absorbance measurements are carried out using shimadzu UV-160, UV-Visible 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 reagent.

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 $\mu\text{g/ml}$) solution. It was prepared by dissolving 0.1000 g of B_1 in distilled water and the final volume bring to 100 ml using a volumetric flask, this solution was stable for at least 2 days. Working solution of 100 $\mu\text{g/ml}$ B_1 solution was prepared by appropriate dilution of the stock solution with distilled water.

Alizarine solution $3 \times 10^{-3} \text{ M}$: This solution was prepared by dissolving 0.0720 gm of alizarine (BDH) 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 (100 µg/ml) solution.

For B₁ tablet, 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, stirring and shaken well then filtered using filter paper, the final volume bring to 100 ml in a volumetric flask with distilled water. This solution was then treated as done in a recommended procedure.

For capsule. 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 discussion

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 of the solution (sulphite ion and alizarine solution) was measured before this study which found to be (6.73) then the effect of pH was studied by adding different amounts (0.1-3.0) ml of 0.1M of sodium hydroxide and 0.1 M of hydrochloric acid solutions to an aliquot containing 20 µg of sulphite. The intensity of absorption were measured against the reagent blank (Fig.1), while effect of adding of acid or base are omitted because of giving no useful results.

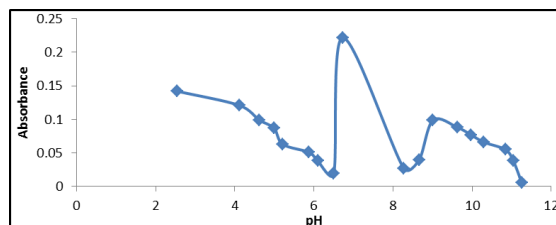


Fig. 1: Effect of pH

The pH after addition of 2 ml of alizarine was 6.73, therefore different buffers[23] of pH 6.73 were prepared such as sodium acetate-acetic acid, phosphate buffer, KH_2PO_4 -NaOH, and Imidazole-hydrochloric acid. It was found that these buffers decrease the absorbance (Table 1).

Table 1: Effect of buffer solutions

ml of buffer solution	Absorbance */ ml of buffer added				
	sodium acetate-acetic acid	phosphate buffer	KH_2PO_4 -NaOH	Imidazole-HCl	KH_2PO_4 -NaOH
1	0.071	0.093	0.091	0.126	0.095
2	0.062	0.071	0.081	0.104	0.071
3	0.049	0.053	0.063	0.063	0.062
4	0.030	0.041	0.044	0.044	0.048
5	0.018	0.026	0.017	0.017	0.031

Reaction time and temperature

The effect of reaction time studied when the colour development was folloed at room temperature and 0, 10, 20, 30,40 and 50°C in thermostatically controlled water-bath so the intervals against reagent blank treated similarly. The experimental results indicate that the absorbance reached maximum after 2 minutes at room temperature and remains constant more than 2 hours therefore, room temperature and 2 minutes reaction time were chosen.

Alizarine amount

The effect of alizarine amount was studied by adding different amount (0.5, 1.0, 1.5, 2.0, 2.5, 3.0) ml of 3×10^{-3} M alizarine to solution containing (10, 25, 50,

75, 100, 150, 200, 300) $\mu\text{g} \cdot \text{ml}^{-1}$ sulphite, it was obtained that the absorbance reached maximum on using 2 ml of 3×10^{-3} M alizarine with $r^2 = 0.999204$. Therefore, 2 ml of 3×10^{-3} M alizarine 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). It was obtained from the results in (Table 2) that the addition of all kinds of surfactants gave no useful effect. Therefore, omitted in this study.

Table 2: Effect of surfactant

Surfactant	Absorbance / ml of surfactant added			
	1.0	3.0	5.0	λ_{max}
Cetyltrimethylammonium bromide	0.092	0.021	0.003	529
Sodium dodecylsulphate	0.133	0.102	0.093	533
Cetyl pyridinium chloride	0.081	0.051	0.010	526
Triton X-100	0.112	0.097	0.054	518
Without surfactant	0.203			524

Effect of time on the colour development

The effect of time on the absorbance of the produced complex at the wavelength of maximum absorption at 524 nm, was studied under the optimal conditions,.

The experimental results had shown that the coloured complex develops immediatley and stabilized after 3 minutes and the absorbance remains nearly constant more than 2 hours.

Calibration curve

A procedure of calibration curve carried out by using a series of calibrated flasks (25 ml) containing an increasing volume (0.1-10) ml of $100 \mu\text{g} \cdot \text{ml}^{-1}$ sulphite solution and 2 ml of $3.0 \times 10^{-3} \text{ M}$ alizarine reagent solution then standing for 3 minutes and dilution with distilled water to the mark, the absorbance are measured at 524 nm against the reagent blank. The range of Beer's law was 10-1000 $\mu\text{g} / 25\text{ml}$ sulphite i.e (0.4-40) ppm (Fig.2). The molar absorptivity was $2.6 \times 10^4 \text{ l} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$, and the Sandall sensitivity was $0.00307 \mu\text{g} \cdot \text{cm}^{-2}$.

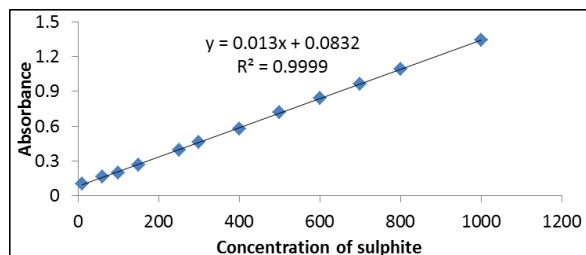


Fig. 2: Calibration curve for sulphite determination
Then different amount of (0.1-15) ml of $100 \mu\text{g} \cdot \text{ml}^{-1}$ B_1 solution were added to the coloured product (SO_3^{2-} -alizarine) ($500 \mu\text{g} / 25\text{ml}$ of SO_3^{2-} + 2 ml of alizarine $3 \times 10^{-3} \text{ M}$) then diluted to the mark with distilled water, also measured at 524 nm against reagent blank. The range of Beer's law was (10-1500) $\mu\text{g} / 25\text{ml}$ vitamin B_1 i.e (0.4-60) ppm (Fig.3). The molar absorptivity being $3.37 \times 10^4 \text{ l} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$, and the Sandall sensitivity was $0.0100 \mu\text{g} \cdot \text{cm}^{-2}$.

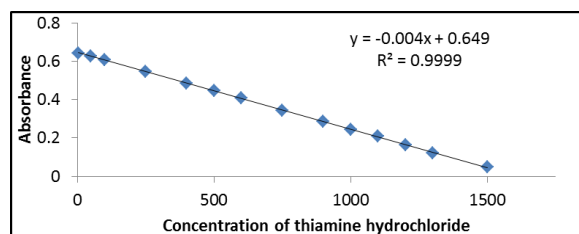


Fig. 3: Calibration curve for Thiamine hydrochloride determination

Final absorption spectrum: When sulphite and thiamine hydrochloride in an aqueous solution was treated according to the recommended procedure, the absorption spectrum showed a maximum absorption at 524 nm. The reagent blank shows low absorption at above wavelength (Fig.4 and 5).

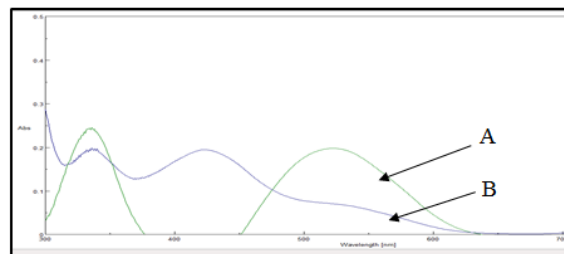


Fig. 4. Absorption spectra of $20 \mu\text{g}$ of $\text{SO}_3^{2-} / 25 \text{ ml}$ measured against (A) reagent blank, (B) blank measured against distilled water

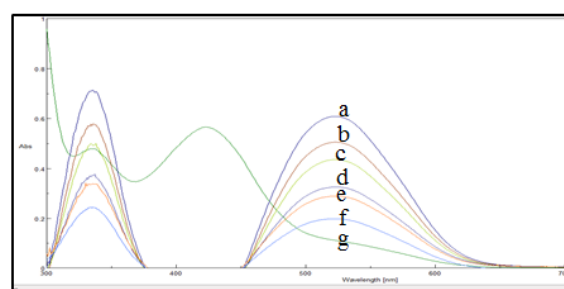


Fig. 5. Absorption spectra of (a=100, b=200, c=400, d=600, e=800 and f=1000) μg of $\text{B}_1 / 25 \text{ ml}$ measured against reagent blank, (g) reagent blank against distilled water

Accuracy and precision

Under the experimental conditions which included in the recommended procedure, the precision and recovery of the method have been checked for 20,100, and 300 μg of sulphite, also the method have been checked for 100, 300 and 500 of thiamine hydrochloride, the recovery and relative standard deviation are shown in table 3, indicating the method has good accuracy and precision.

Table 3: Accuracy and precision

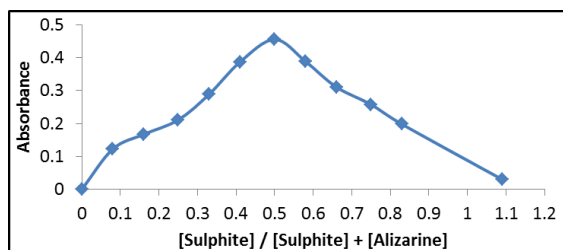
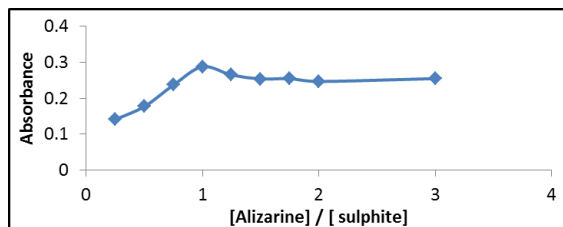
Amount of SO_3^{2-} taken, $\mu\text{g}/25 \text{ ml}$	Recovery*, %		Relative standard deviation*, %
20	99.50		± 0.49
100	100.29		± 0.23
300	100.46		± 0.51
Amount of B_1 , μg	Recovery(%) of B_1 *		Relative standard deviation*, %
	(200 mg /tablet) NDI-Iraq	(5 mg capsul) SDI-Iraq	
100	99.91	100.13	± 0.15
300	100.03	100.24	± 0.17
500	100.12	99.93	± 0.25

* Average of five determinations.

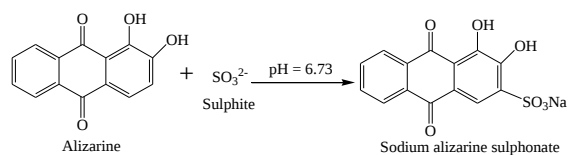
Nature of the reaction between SO_3^{2-} and alizarine.

the reaction ratio of sulphite with alizarine reagent was studied by using Job's method of the continuous variations and mole ratio method. The obtained

results (Fig.6 & 7) showed that the molar ratio of sulphite to alizarine was found to be 1:1.

Fig 6: Job's plot for SO_3^{2-} – Alizarine.Fig 7. Mole ratio plot for SO_3^{2-} – Alizarine.

Hence the coloured product may have the following suggested mechanism (Scheme 3):



Scheme 3: Suggeste mechanism of sulphite-alizarine

Effect of foreign ions

The effect of many foreign ions on the determination of 20 μg sulphite per 25 ml of solution is examined under the experimental conditions used. Also the effect of interferences is examined on the determination of thiamin hydrochloride (100 $\mu\text{g}/25\text{ml}$). The results obtained are summarized in table 4 & 5.

Table 4: Effect of foreign ions

Foreign ion	Form added	Relative error (%)			
		50	150	300	500
I^-	KI	-1.82	-2.56	-6.15	-9.33
Cl^-	NaCl	+2.33	+20.54	+40.21	+73.33
F^-	NaF	+25.51	+37.33	+61.66	+95.76
Br^-	KBr	-1.78	-2.55	-4.15	-5.25
$\text{CO}_3^{=}$	Na_2CO_3	+5.67	+8.43	+20.32	+33.17
$\text{SO}_4^{=}$	Na_2SO_4	-0.29	-0.43	-0.59	-0.69
$\text{S}_2\text{O}_3^{=}$	$\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$	-4.82	-2.55	-0.03	-0.82
$\text{PO}_4^{=}$	$\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$	-0.47	-0.58	-0.27	-0.63
$\text{C}_2\text{O}_4^{=}$	$\text{Na}_2\text{C}_2\text{O}_4$	-1.08	-1.15	-1.38	-1.47
CH_3COO^-	CH_3COONa	-0.87	-0.91	-0.86	-0.89
La^{+3}	$\text{La}_2(\text{CO}_3)_3$	-0.32	-0.21	-0.10	-0.42
Bi^{+3}	$\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$	-0.09	-0.27	-0.31	-0.90
Ba^{+2}	$\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$	-0.66	-0.62	-0.52	-0.78
NH_4^+	NH_4Cl	-0.49	-0.91	-0.52	-0.56
Fe^{+3}	$\text{Fe}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$	+0.10	+3.82	+11.29	+23.20
Fe^{+2}	$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	+0.91	+2.82	+5.29	+7.20
Mg^{+2}	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	-0.26	-0.27	-0.63	-0.34
Hg^{+2}	$\text{Hg}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$	-0.98	-0.79	-0.41	-1.22
Zr^{+4}	$\text{ZrOCl}_2 \cdot 5\text{H}_2\text{O}$	-1.19	-0.79	-0.52	-1.23

The results were shown in (Table 4) indicated that the method seemed to be selective except towards Cl^- , F^-

and $\text{CO}_3^{=}$ which interfere seriously, also ferric ion in high concentrations was interfered.

Table 5: Effect of interferences on the determination of 100 μg B_1

Interferences	Recovery (%) of 100 μg B_1 / μg of interfere added		
	100	500	1000
Acacia	100.21	100.09	100.12
Glucose	100.12	99.97	100.31
Lactose	100.33	100.13	100.40
Menthol	100.42	100.36	100.17
Starch	100.11	99.98	99.98

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 respectively. A good recovery was obtained from the results showed in Table (6 & 7).

Table 6: Determination of sulphite in water samples

ml of water sample	Sulphite added, μg	Recovery (%)			
		Tap water	Well water from al-shoura village in Mosul	Natural spring water	Sea water from white middle sea in Turkey
1	5	101.23	101.06	99.97	100.22
3	5	101.33	101.14	100.03	101.46
5	5	100.94	101.22	100.12	101.91
7	5	101.53	101.43	100.06	100.87
1	50	102.42	101.53	99.98	100.43
3	50	102.13	101.28	99.96	100.22
5	50	102.55	101.62	99.98	102.51
7	50	101.43	101.33	100.08	102.63
1	100	100.93	100.94	100.12	101.31
3	100	100.94	100.53	100.11	101.83
5	100	102.53	100.21	100.23	102.44
7	100	102.41	101.63	100.28	102.58

Table 7: Determination of thiamine hydrochloride

Amount of B_1 , μg	Recovery(%) of B_1^*	
	(200 mg /tablet) NDI-Iraq	(5 mg capsul) SDI-Iraq
50	101.33	100.94
100	100.62	100.72
200	100.85	101.07

The value of t-test was calculated [24]. It did not exceed the theoretical values for five degrees of freedom at the 95% confidence level when the proposed method has been compared with literature method [25] as shown in Table 8 & 9.

Table 8: The result of t-test analysis.

Drug	Pharmaceutical preparation	t-test
B_1 (NDI-Iraq)	Tablet	0.4284

Table 9: The result of t-test analysis.

Sample	Tap water	Natural spring water
The value of t-test	1.0137	0.7213

Comparison of the methods

Table 10, shows a comparison between the proposed method and the other literature spectrophotometric methods through some measured analytical parameters.

Table 10: Comparison of the methods.

Analytical parameters	Present method	Literature method	
		Sulphite ion [25]	B_1 [26]
Method	Alizarine	Fe(II)-1,10-Phenanthroline	Chromate-DPC
pH	6.73	3.00	1.41
Buffer	-----	Glycine-HCl	2 N H_2SO_4
λ_{max} (nm)	524	510	543
Reaction time (min)	Immediately	Immediately	5
Stability period (minutes)	120	120	120
Beer's law range (ppm)	0.4-40 SO_3^{2-} 0.4-60 B_1	0.04-4.0	0.4-40
Molar absorptivity ($\text{L.mol}^{-1}.\text{cm}^{-1}$)	2.6×10^4 SO_3^{2-} 3.37×10^4 B_1	4.56×10^4	1.5×10^4
R.S.D. (%)	$\pm 0.23 - \pm 0.51$ $\pm 0.15 - \pm 0.25$	$\pm 0.52 - \pm 1.31$	$\pm 0.31 - \pm 0.57$
Colour of the product	Red	Orange –red	Pink-violet
Application of the method	Pharmaceutical preparations for B_1 and water samples for SO_3^{2-}	Water samples	Pharmaceutical preparations

The results in (Table 10) are indicated that the method is sensitive and has an application part

Conclusion

The proposed method is simple, sensitive, inexpensive and does not require temperature control or solvent extraction step. Sulphite in different water samples has been carried out by the rapid reaction of sulphite with alizarine at pH 6.73 to form a red water soluble and stable product, which exhibit maximum absorption at 524 nm. Beer's law were obeyed in the concentration range of (0.4-40) ppm of sulphite with a molar absorptivity of $2.6 \times 10^4 \text{ L.mol}^{-1}.\text{cm}^{-1}$, Sandell sensitivity index of $0.00307 \mu\text{g}.\text{cm}^{-2}$ respectively, and

relative standard deviation of $\pm 0.23 - \pm 0.51$, the proposed method is developed for determination of B_1 by bleaching red colour of sulphite-alizarine product, Beer's law were obeyed in the concentration range of (0.4-60) ppm of sulphite with a molar absorptivity of $3.37 \times 10^4 \text{ L.mol}^{-1}.\text{cm}^{-1}$, Sandell sensitivity index of $0.0100 \mu\text{g}.\text{cm}^{-2}$ respectively, and relative standard deviation of $\pm 0.15 - \pm 0.25$ depending on the concentration level. Good recoveries of sulphite and B_1 from various water samples and pharmaceutical preparation are achieved using the proposed method.

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التقدير الطيفي للكبريتيت وهيدروكلوريد الثيامين باستخدام تفاعل انتقال البروتون - التطبيق على نماذج مائية ومستحضرات صيدلانية

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الملخص

يتضمن البحث طريقة طيفية بسيطة وسريعة لتقدير الكبريتيت وهيدروكلوريد الثيامين. تعتمد الطريقة المقترحة على تفاعل الكبريتيت كاشف الاليزارين عند دالة حامضية 6.73 لتكوين ناتج أحمر اللون الذي يعطي أقصى امتصاص عند الطول الموجي 524 نانومتر. تم تطوير هذه الطريقة لتقدير فيتامين B₁ على أساس قصر لون الناتج الأحمر (كبريتيت -اليزارين) في الظروف المثلى لتقدير الكبريتيت. وتتبع الطريقة قانون بير في نطاق التركيز (10-1000) ميكروغرام / 25 مل (0.4-40) من الكبريتيت و 10-1500، ميكروغرام / 25 مل (0.4-60) لهيدروكلوريد الثيامين عند الطول الموجي المختار ومعامل الامتصاص المولاري 2.6×10^4 لتر. مول⁻¹. سم⁻¹ للكبريتيت و 3.37×10^4 لتر. مول⁻¹. سم⁻¹ لهيدروكلوريد الثيامين اما دلالة ساندل للحساسية فقد كانت 0.00307 للكبريتيت و 0.0100 لهيدروكلوريد الثيامين والانحراف القياسي النسبي تراوح بين $0.23 \pm - 0.51$ للكبريتيت و $0.15 \pm - 0.25$ لهيدروكلوريد الثيامين وكانت استرجاعية الطريقة جيدة للكبريتيت وهيدروكلوريد الثيامين في النماذج المائية والمستحضرات الصيدلانية على التوالي.