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المجلة العراقية لبحوث السوق وحماية المستهلك

SPECTROPHTOTOMETRIC DETERMINATION OF PURE SULFAMETHOXAZOLE IN PHARMACEUTICAL PREPARATIONS BY OXIDATIVE COUPLING REACTION

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ABSTRACT

A new, simple, rapid and sensitive spectrophotometric method for the determination of sulfamethoxazole in both pure form and pharmaceutical preparations has been reported. The adapted technique based on utilization 4-aminobenzene sulfonic acid as a new modern chromogenic through an oxidative coupling reaction with sulfamethoxazole and potassium iodate in basic media to form orange soluble dye product with absorption maxima at 490 nm. Subject to Beer's law in the range 2–32µg mL^{-1}. The values of molarabsorption coefficient (ε) and correlation coefficient were found to be 9.118 × 10^3 and0.9999 respectively whereas the Sandels index was 0. 02778 µg.cm^{-2}.

Keywords: Spectrophotometric, pharmaceutical, sulfamethoxazole, oxidative coupling.

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تقدير الطيفي للسلفاميثوكسازول النقي في المستحضرات الصيدلانية عن طريق تفاعل الاقتران التأكسدي

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الخلاصة

يتضمن هذا الجزء تطوير طريقة طيفية سريعة وحساسة لتقدير كميات ضئيلة من السلفاميثوكسازول (SMZ) في محلول مائي قاعدي، تعتمد هذه الطريقة على الاقتران التأكسدي لل SMZ مع الكاشف 4-امينو بنزين حامض سلفونيك بوجود العامل المؤكسد يودات البوتاسيوم لتكوين ناتج برتقالي اللون ذائب بالماء مستقر، يعطي اعلى امتصاص عند الطول الموجي 490 نانومتر ويخضع لقانون بير بحدود (2-32) مايكروغرام/ مل، وبلغت الامتصاصية المولارية 9.118×10^3 لتر/ مول . سم ، ودلالة ساندل 0.02778 مايكروغرام/ سم^2 وحد الكشف 0.063 مايكروغرام/ مل، والحد الكمي 0.2173 مايكروغرام/ مل، الانحراف القياسي النسبي بين (0.5923-1.4662)% ومعدل الاسترجاعية 100.24%، معامل التقدير 0.9999 وطبقت هذه الطريقة المقترحة بنجاح لتقدير (SMZ) في مستحضراته الدوائية.

الكلمات المفتاحية: المطيافية، الأدوية، السلفاميثوكسازول، اقتران التأكسدي.

INTRODUCTION

Sulfamethoxazoloe (SMZ) is an isoxazole (1,2-oxazole) compound having a methyl substituent at the 5-position and a 4-aminobenzenesulfonamido group at the 3-position. It has a role as an antibacterial agent. IUPAC Name is [4-amino-N-(5-methyl-1,2-oxazol-3-yl)benzenesulfonamide] an antibiotic usually used in the cases of urinary tract, bronchitis and prostatitis **(Ma *et al.*,2007; Kazanjian*et al.*, 2008; Amali*et al.*,2019)**. It has been recorded to be effective against both gram negative and gram positive bacteria, such as ***Listeria momocytogene and E. Coli***. **(Kazanjian*et al.*, 2008)**. After being introduced in the United States in 1960 **(Amali*et al.*,2019)**,it is now mostly used in combination with trimethoprim, abbreviated as (SMZ-TMP) **(Bruntone*t al.*, 2011)**. This combination (formulation) is included registered in WHO model list of essential medicines as a prime choice treatment for urinary tract infection. is also known as: sulfamethalazole, sulfisomezole**(Roth *et al.*, 2018)** and Sulfamethazole. (Figure 1) shows the structural formula of sulfmethaxazole.

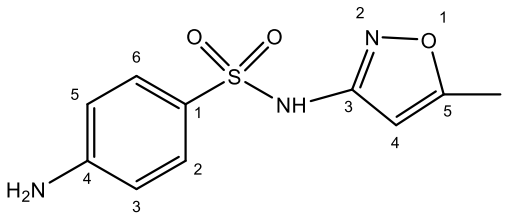

Figure (1): The structural formula ofSulfamethazole**.**

Various analytical methods for determining SMZ have been recorded, including differential calorimetry scanning **(Agafonova*et al.*,2013)**, spectrofluorimetry**(Chen *et al.*,1999)**, potentiometric titration **(Fritz *et al.*, 1952)**, sequential injection chemiluminescence**(Soto *et al.*, 2005; Aintisar*et al.*, 2019)**, capillary zone electrophoresis **(Li*etal.*, 2008)**,reverse phasehighperformance liquid chromatography **(Soto *et al.*, 2005)**, micellarelectrokinetic chromatography **(Berzas Nevado*et al.*,2005)**, spectrophotometry

(Hamzah*et al.*, 2014) and nuclear magnetic resonance **(Salem *et al.*, 2012)**. The system used by the Bratton and Marshall was considered to be the most popular colorimetric method used for sulfa drugs **(Zhou *et al.*, 2009)**.

MATERIALS AND METHODS

Instruments

Absorbance measurements were performed using T92 + Spectrophotometer wavelength at the range (190-900) nm. (Beijing, China).Measurement was performed by a spectrophotometer in an analytical chemistry laboratory. (Table 1)shows the chemicals used.

Table (1): Chemicals.

Chemicals	M.wt (g/mol)	Chemical Formula	Assay (%)
Sulfamethoxazole (SMZ)	253.28	$C_{10}H_{11}N_3O_3S$	99
Iodate Potassium	214	KIO_3	99
4-aminobenzensulfonic acid	173	$C_6H_7NO_3S$	98
Soduim Hydroxide	40	NaOH	98

Reagents

1. All chemicals used in this study were of analytical grades.
2. The stock solution for sulfamethazole (100µg/mL)was prepared by dissolving 0.10g in 5 mL of ethyl alcohol sulfamethoxazol and completing the volume in 100 mL of volumetric flask with distilled water.
3. Sodium hydroxide solution (0.2 M) was prepared by dissolving 0.8 g of NaOH in a specified amount of distilled water in a volumetric flask of 100 mL and then the volume was completed to the mark .
4. Aminobenzensulfonic acid solution (2×10^{-2} M) was prepared by dissolving 0.346 g of 4-aminobenzensulfonic acid in distilled water and then progressing to the point of mark with the same solvent in a 100 mL volumetric flask.
5. Oxidizing agent (KIO_3) (2×10^{-2} M) solution was prepared by dissolving 0.428 g of potassium iodate powder in distilled water filled in a 100 mL volumetric flask to the point of the mark.

Pharmaceutical preparations of sulfamethoxazole

Pharmaceutical preparations from industrial sources were collected as follows:

1.Trimoks Pills of (SDI, Samarra-Iraq): 400 mg sulfamethoxazole and 80 mg trimethoprim for each Pills.
2. Sulprim syrup (Jerusalem Pharmacy products Co.Ltd-palestine):200 mg sulfamethoxazole and 40 mg trimethoprim for each 1 mL ofsyrup (100 mL).

It was prepared by taking 1 mL of the Sulprim and diluting it with a small amount of distilled water and filtering and thecomplement filtrate with distilled water to the mark to obtain a concentration of 100 µg / mL.

The principle of the method is based on the coupling of the reagent (4-aminobenzene sulfonic acid) with (Sulfamethoxazole) in the presence of potassium iodate as oxidizing agent in basic medium.

Preliminary study

We put (1 mL, 2×10^{-2} M) of reagent 4-aminobenzensulfonic acid and add an oxidizing agent potassium iodate(1 mL, 2×10^{-2} M) to 2 mL Sulfamethoxazole (100µg/ mL) a orange product was formed. The intensity of the color increased in the presence of 1 mL (0.2 M) of NaOH solution. Absorbance measurements were performed after diluting the solution with

distilled water in a 25 mL volumetric flask. The maximum absorbance value was measured against an blank solution at a wavelength of 490 nm.

RESULTS AND DISCUSSION

Study of the optimal reaction conditions

Experiments were performed using 2 mL of Sulfamethoxazole 100 µg/ mL in a final volume of 25 mL and the absorbance values of the solutions was measured at 490 nm wavelength versus a blank solution.

Effect of the best coupling reagent

Series of experiments were held (performed) by mixing 1mL of each of the reagent solutions shown below in (Table 2) with 1 mL of (2×10^{-2}M) of potassium iodate as oxidizing agent solution with2 mL of (100µg/ mL) sulfamethoxazolesolution in the presence of 1 mL NaOH solution 0.2 M and the absorbance values were recorded at 490 nm wavelength.

Table (2): Effect of the best coupling reagent.

Reagent 2×10^{-2} M	Variable	Absorbance
P-nitro aniline	SB	0.15
	BW	0.07
O-aminophenol	SB	0.17
	BW	0.08
P-aminophenol	SB	0.18
	BW	0.097
Resorcinol	SB	0.163
	BW	0.09
4-aminobenzen sulfonic acid	SB	0.520
	BW	0.091

S= Sample , B= Blank , W= Water

Oxidizing agent optimization:

1 mL, 2×10^{-2} M of series of oxidizing agents were added to 1 mL, 4-amino benzene sulfonic reagent solution,2 mL of (100µg/ mL) sulfamethoxazole solution and 1 mL, 0.2 M NaOH solution in 25 mL volume flask followed by absorption measurements. For each sample,the blank solution was fixed at wavelength (800-400) nm. The best oxidizing agent was noted to be KIO_3 for giving the maximum absorption value of the colored product at the wavelength 490 nm. The results are shown in (Table 3).

Table (3): Test from best oxidizing agent.

Oxidizing agent2×10^{-2} M	Absorbance		ƛ max(nm)
	Blank	Sample	
Potassium Iodate	0.091	0.524	490
Potassium per Sulphate	0.040	0.290	427
Ammonium per Sulphate	0.042	0.192	466
Ammonium ferric Sulphate	0.039	0.018	518

Effect of the coupling reagent quantity

Various aliquots of the conjugating reagent (4-aminobenzenesulfonic acid) solutions (0.3-2.1) mL with concentration equal to (2×10^{-2} M) were mixed with (1 mL, 2×10^{-2}M) KIO_3, (1mL, 100µ g/ mL) sulfamethoxazole solution and (1 mL, 0.2 M) NaOH solution. (Table 4) shows that the maximum absorption value was obtained using 1 mL of the reagent 4-amino benzesulfonic acid solution.

Table (4): Effect of the coupling reagent quantity used.

mL of Reagent2×10^{-2} M	Absorbance	
	BW	SB
0.3	0.075	0.352
0.6	0.080	0.447
0.8	0.092	0.514
1.0	0.088	0.522
1.3	0.108	0.510
1.5	0.113	0.483
1.8	0.081	0.425
2.1	0.097	0.315

Effect the base used for coupling

One mL of different types of bases (strong and weak) has been used; optimum results wererecorded in the case of NaOH as shown in the (Table 5).

Table (5): Effect of the base used in the coupling.

Base Solution Used 0.2 M	NaOH	KOH	Ca(OH)	NH_4OH
Absorbance	0.519	0.467	0.376	0.189

Effect of the amount of base used

Various quantities of the used base (NaOH) have been added that have been selected to find the optimal amount that gives the highest absorption of the formed product. Maximum absorption was recorded in the case of (1 mL) with pH=11.86. This volume was adopted in the subsequent experiments and all the results are shown in the (Table 6).

Table (6): Effect of quantity of the base used.

mLof NaOH0.2 M	Absorbance		pH
	BW	SB	
0.2	0.108	0.351	11.72
0.4	0.109	0.405	11.77
0.6	0.098	0.471	11.83
0.8	0.103	0.516	11.84
1	0.104	0.525	11.86
1.2	0.102	0.502	11.87
1.4	0.095	0.484	11.89
1.6	0.082	0.425	11.91
1.8	0.071	0.368	11.93
2	0.103	0.216	11.94

Sequence of addition

Sulfamethoxazole (D), reagent solution 4-aminobenzen sulfonic(R), potassium iodate solution (O), and sodium hydroxide (B) alkaline solution were added to each other in different sequence keeping the same volume and concentration thereof. Maximum absorption was recorded in the sequence mode of addition (IV) as shown in (Table 7).

Table (7): Effect of order sequence of addition.

Order Number	Order of addition	Absorbance	
		BW	SB
I	D+O+B+R	0.041	0.438
II	B+D+R+O	0.020	0.227
III	O+R+B+D	0.052	0.348
IV	R+O+D+B	0.097	0.523

Effect of temperature

The temperature of mixture and the colored product formed was varied at the range 10-70°C. Maximum and stable absorption of the formed colored product solution was observed at the temperature range 20-30°C. 25°C was chosen to be the optimal temperature for the reaction mixture. The details of the results are shown in the (Table 8).

Table (8): Effect of the temperature.

Temperature (°C)	15	20	25	30	35	40	50	60	70
Absorbance	0.42	0.49	0.523	0.51	0.44	0.35	0.28	0.15	0.08

Stability of the reaction product:

The stability of the reaction product was studied throughout observing the values of absorption of the formed colored solution at duration of time 5-60min using 2 mL, 100µg/mL of the sulfamethoxazole solution and 2 mL of the drug. Results exhibited in (Table 9) shows that 60 min was very sufficient time period to hold the measurements.

Table No (9): Effect of time on absorption of the formed product.

µg /mL of SMZ	Absorbance/ min. Standing time								
	5	10	15	20	25	30	45	60	75
2	0.520	0.521	0.521	0.522	0.521	0.521	0.522	0.519	0.320

Ultimate absorption spectrum

The ultimate absorption spectrum shown in (Figure 2) was achieved on adopting the optimum conditions: 25 mL of aqueous solution comprising of (1mL, 2×10^{-2} M) 4-amino benzesulfonic acid, (2mL, 100 µg/ mL) sulfamethoxazole, (1mL, 2×10^{-3}M) KIO_3, (1mL, 0.2M) NaOH. Maximum absorption was recorded against blank solution for orange product solution at the wavelength 490 nm. The emergence of a peak of 490 nm indicates electronic transitions $n \rightarrow \pi*$, $\pi \rightarrow \pi*$

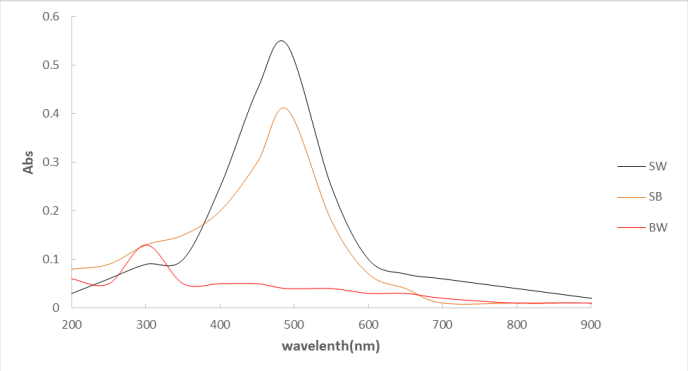

Figure (2): Ultimate normal spectrum.

Approved working method and calibration curve

Subsequent to the determination of the optimum conditions, standard calibration curve was obtained byapplicationthe following procedure of mixing:

Series of aliquots 0.5-8 mL of 100 µg/mL Sulfamethoxazole were added to a number of 25 mL volumetric bottles each containing (1 mL, 2×10^{-2} M) 4-aminobenzenesulfonic reagent, (1 mL, 2×10^{-2} M) of the oxidizing agent potassium iodate, and (1 mL, 0.2 M) of a (NaOH) base solution. The volume of the formed mixture was completed by distilled water to the mark point. Absorption measurements were performed for each solution against the official solution at the wavelength of 490 nm.

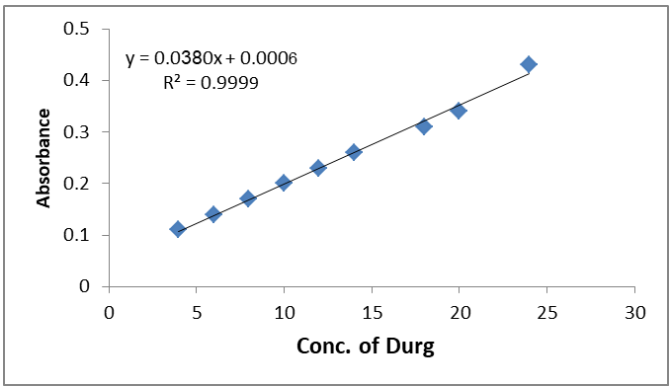

Figure (3): Calibration curve.

The standard curve obtained which follows Lambert-Beer's law in the limits of concentrations (2-32)µg /mL. The linear regression was utilized to calculate the equation constants that are: Molar absorption coefficient ε, 9.118×10^{3},correlation coefficient 0.9999, The Sandelis0. 02778 $\mu g.cm^{-2}$

Accuracy andprecision

The accuracy of the method represented by relative error (E%) and recovery(Rec%) was calculated to estimate sulfamethoxazole and the compatibility of the method represented by relative standard deviation (RSD%) (measuring three different concentrations) of Sulfamethoxazole 100 µg/ mL, as it appears from the results obtained. (Table10) it demonstrates that the technique has good accuracy andprecision.

Table (10): The accuracy and precision of the method *

Conc. Of SMZ Present (µg/mL)	Conc. of SMZ Measured(µg/mL)	RE (%)	Recovery(%)	Average of Recovery (%)	RSD(%)
9	8.88	-1.33	99.18	100.24	0.5923
16	15.97	-0.187	100.5		0.6615
32	32.54	1.687	101.02		1.4662

*Each value averages five readings

Detection Limit

The detection limit was calculated for at the wavelength of 490 nm, by measuring the absorption of the lowest concentration (2 µg/ mL) taken from the calibration curve depicted from (for) seven readings under the same conditions. Results exhibited in (Table 11).

$$\text{D. L} = 3 \times S \times \text{Conc.} / \bar{x}$$

$$\text{Q. L} = 10 \times S \times \text{Conc.} / \bar{x}$$

Table (11): Detection Limit

Conc.(µg/mL)	$\bar{X}$	S	D.L.(µg/mL)	Q.L.(µg/mL)
2	0.122	0.001326	0.063	0.2173

Then

Conc.: The lowest concentration of L in the calibration curve.

$\bar{X}$: The absorption rate for a series of measurements of no less than seven values.

S: Standard deviation.

D.L.: Detection limit.

Q.L.: Quantitative limit.

The nature of the formed product

To identify the nature of the formed product and the ratio of the drug's attachment to the detector, both continuous changes method (the Jop method) and molar ratio method **(Niknia, 2018)** have been applied. Different volumes of drug solution ranging from 1-9 mL were placed into series of 25 mL volumetric flasks containing decreasing volumes of the reagent 9-1 mL. The rest of the additives were in the optimal sizes according to the adopted method of work in this study, followed by filling the bottles to the mark with distilled water. According to absorption measurements of the solutions at 490 nm against their blank solutions the following (Figure 4) was depicted for JOP method. 1:1 ratio found to be the optimal mixing ratio.

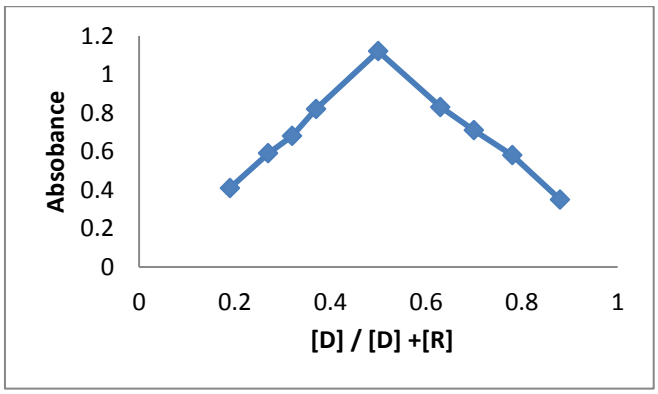

Figure (4): The JOP method.

According to this method mixture comprising of Sulfamethoxazole with the 4-amino benzensulfonic reagent in the presence of the oxidizing agent potassium iodate, and 1 mL of a NaOH-base solution 0.2M was estimated. To ensure that the interaction ratio between Sulfamethoxazole and the reagent 4-aminobenzen sulfonic is 1:1, the molar ratio method was used and as follows: 2 mL of the drug solution were placed in a series of 25 mL volumetric bottles containing different volumes of reagent solution 0.2-2 mL with the remaining additives at the optimum sizes, and diluted with distilled water to the point of the mark. Absorption measurements of these solutions at the wavelength of 490 nm against the formed solution for each of them were held. Molar ratio was found to be consistent with the method of continuous changes. (Figure 5) shows that the ratio is 1:1.

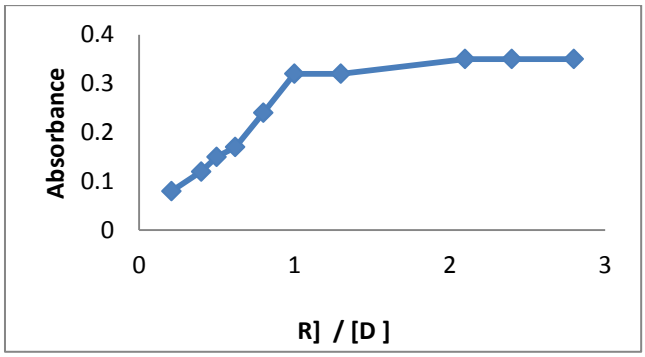

Figure (5): Shows that the molar ratioof Sulfamethoxazole.

The reaction equation is as (Figure 6).

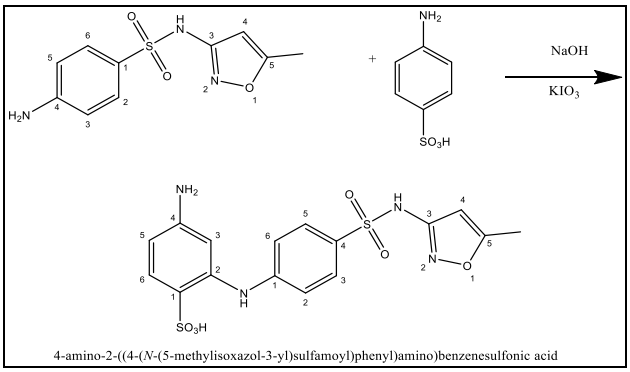

Figure (6): Reaction scheme**.**

Applications

This method can be applied to the following pharmaceutical preparations containing: Sulprim syrups (Jerusalem Pharmaceuticals Co.Ltd-palestine):200 mg sulfamethoxazole and 40 mg trimethoprim for each 5 mL of suspension 100 mL.

The direct method

Three different concentrations were taken from the solution of each product (Suspensions) whose preparation is indicated in its preparation in the paragraph, namely 10,20, 30µg/mL. The solutions were treated following the same steps in preparing the titration curve and measuring the absorbance for them at the wavelength of 490 nm against the blank solution. An average of five readings was calculated for each, as well as the calculation of retrospective and RSD according to the results shown in (Table 12).

Table (12): The direct method.

Con.of (SMZ) (Syrup)	Con.of (SMZ) Measured	RE(%)	Recovery(%)	Average of Recovery (%)	RSD(%)
10	10.14	1.4	101.4	100.405	0.1163
20	20.01	0.05	100.05		0.3519
30	29.93	-0.233	99.766		0.5685

The results exhibited in the above table show that the proposed method was successful in estimating the pharmaceutical preparations that contain them. The value of the recovery rate was 100.405% for syrups.

Standard additions method

The standard addition method was used to demonstrate the efficiency of the proposed method, as the method includes the addition of constant quantities (1.3, 2.0 mL) of prepared pharmaceutical solutions at a concentration of 100µg/mL in two series of volumetric bottles of 25mL in capacity and adding increasing volume 1, 1.5,2.5 and 3.5 mLof a standard solution of 100 µg/mLconcentration and leaving one of the bottles without addition. The absorbance of the solutions was measured against the blank solution at the 490 wavelength. The results are shown in (Table 13) and (Figure 7).

Table (13): Standard additions method.

Pharmaceutical preparation	Amount taken (µg/mL)	Amount measured	Recovery (%)
Sulprimsuspensions	5	4.9	98.33
	9.8	9.7	98.86
Pharmaceutical preparation	Amount taken (µg/mL)	Amount measured	Recovery(%)
Sulprimsuspensions	5.2	5.6	107.69
	8	8.37	103.75

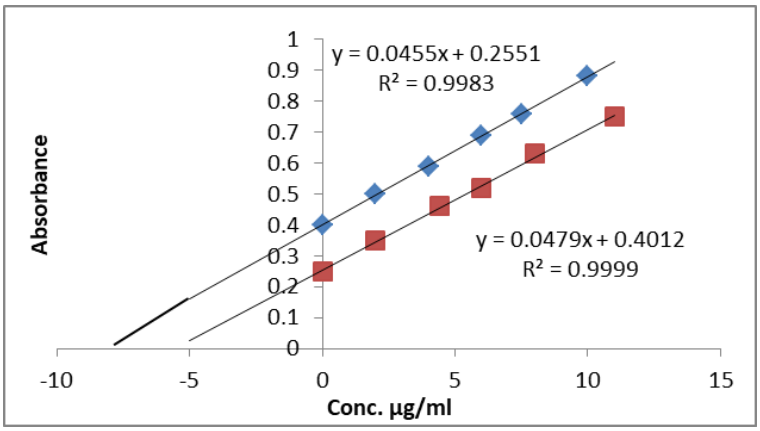

Figure (7): Direct method.

From the results shown in the (Table 13), it is shown that the standard addition method is well in agreement with the direct method, within the range of acceptance of the error, which indicates that it is the satisfactory method.

Statistical evaluation of the results of the proposed method

A comparison was made between the proposed analytical method and the standard method to find out the accuracy and validity of the analytical application of the proposed method by applying the following two tests. The obtained result was less than the tabular value for F, t. F=4.36, t=2.84 (which is required). While the tabular value for F, t was F=7.25, t=4.776 at confidence limit 95% and for four degrees of freedom. Consequently, these values demonstrate the success of the proposed method asshown in (Table 13).

Table (13): Statistical evaluation.

Preparation	Normal value	Recovery (%)± RSD	
Sulprimsuspensions	200/40mg	Proposed method	Literature method
		100.08±0.56	103.56±1.57
		t= 2.84	t= 4.776
		F= 4.36	F=7.25

CONCLUSIONS

Many reagents were used as an oxidative coupling for this drug. Many methods were used to determine this drug and its properties. This paper proposes an oxidative coupling reaction ofsulfamethoxazole with 4-aminobenzenesulfonic acid with potassium iodate in basic media to form an orange soluble dye product has been used. According to the findings, the current method is suitable for the routine analysis of this drug in its pharmaceutical preparations and its pure form. This method has alsobeen characterized by linearity, accuracy, and high compatibility, and the procedure does not require special conditions, like temperature or pH limit. The technique was very simple, fast, cheap and fairly selective than some of the colorimetric methods published.

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