Spectrophotometric Determination of Sulfamethoxazole in Pure and Pharmaceutical Preparations Based on Condensation Reaction Method

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Abstract

A new, Simple, sensitive and accurate spectrophotometric methods have been developed for the determination of sulfamethoxazole (SMZ) drug in pure and dosage forms. This method based on the reaction of sulfamethoxazole (SMZ) with 1,2-napthoquinone-4-sulphonic acid (NQS) to form N-alkylamono naphthoquinone by replacement of the sulphonate group of the naphthoquinone sulphonic acid by an amino group. The colored chromogen shows absorption maximum at 460 nm. The optimum conditions of condensation reaction forms were investigated by

(1) univariable method, by optimizing the effect of experimental variables (different bases, reagent concentration, borax concentration and reaction time), (2) central composite design (CCD) including the effect of three experimental factors (reagent concentration, borax concentration, and reaction time). The linearity ranges of sulfamethoxazole are (5-50) $\mu g.mL^{-1}$ at 460 nm with molar absorptivity (6.7878×10^4- 7.0918×10^4) $L.mol^{-1}.cm^{-1}$, Sandell's sensitivity index (0.3755- 0.3571) $\mu g.cm^{-2}$ and detection limit of (0.3755– 0.3594) $\mu g.mL^{-1}$ for each procedure respectively. The results showed there are no interferences of excipients on the determination of the drug. The proposed method has been successfully applied for the determination of sulfamethoxazole in pure and pharmaceutical preparations.

Key words: Sulfamethoxazole, Spectrophotometric determination, dosage forms, 1,2-napthoquinone-4-sulphonic acid (NQS).

ألخلاصة

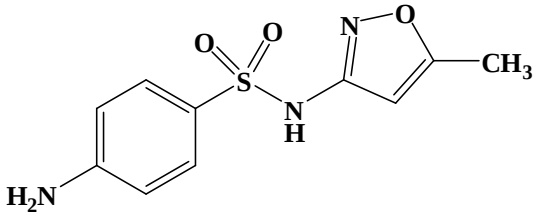
طورت طريقة طيفية جديدة، بسيطة، حساسة ودقيقة، للتقدير ألكمي للسلفاميثوكسازول بشكله ألنقي وفي ألمستحضرات ألصيدلانية. تعتمد ألطريقة على تفاعل ألسلفاميثوكسازول مع 1،2- نفثوكوينون -4- حامض سلفونيك لتكوين ن-الكيل امونونفثوكوينون بأستبدال مجموعة سلفونات من حامض السلفونيك نفثوكوينون بمجموعة أمين يظهر مولد ألون اعلى أمتصاص عند 460 نانوميتر. وقد درست ألظروف ألمثلى بتفاعل ألتكثيف بواسطة (1) طريقة ألمتغيرات ألأحادية بدراسة تأثير ألمتغيرات ألتجريبية على ألظروف ألمثلى للتفاعل (القواعد ألمختلفة، تركيز الكاشف، تركيز ألبوراكس وتأثير زمن ألتفاعل)، (2) تصميم ألتجربة ألمركزي (CCD) بدراسة ثلاثة عوامل تجريبية مؤثرة هي (تركيز ألكاشف، تركيز ألبوراكس، زمن ألتفاعل) مدى ألخطية للسلفاميثوكسازول هو ($5-50\ \mu g.mL^{-1}$) عند 460 نانوميتر مع أمتصاصية مولارية (6.7878×10^4 -7.0918×10^4 $L.mol^{-1}.cm^{-1}$)، دلالة ساندل (0.3571-0.375) $\mu g.cm^{-2}$ وحد ألكشف ($0.3594-0.3755\ \mu g.mL^{-1}$) لكل طريقة على التوالي أظهرت ألطريقة عدم وجود تداخل من ألمضافات على تقدير ألدواء، لقد أمكن تطبيق ألطريقة ألمقترحة بنجاح لتقدير ألسلفاميثوكسازول في شكله ألنقي وفي ألمستحضرات ألصيدلانية.

الكلمات ألمفتاحية: سلفاميثوكسازول، ألتقدير ألطيفي، ألاشكال ألصيدلانية، ، 1،2- نفثوكوينون -4- حامض سلفونيك.

1. Introduction

Sulfamethoxazole (SMZ) is a member of the sulfonamide family of antibacterial and chemically name is 4-Amino-N-(5-methyl-3-isoxazolyl)-benzene sulfonamide with molecular formula ($C_{10}H_{11}N_3O_3S$), and molecular weight of 253.279 $g.mol^{-1}$, the basic structure of the drug is shown in Scheme (1). White and yellowish white colored, crystallized powder, its use has been limited by the development of resistance and it is now used mainly as a mixture with trimethoprim (Wormser, 1982; European Pharmacopoeia.,2005).

Mixture of sulfamethoxazole and trimethoprim which is known as co-trimoxazole is used to treat a wide variety of bacterial infections e.g.: middle ear infections, genito-urinary tract infections, respiratory-tract infections such as bronchitis, and enteric infections. Its main uses now are in Pneumocystis carinii pneumonia, toxoplasmosis, and nocardiosis. Gastrointestinal disturbances (mainly nausea and vomiting) and skin reactions are the most common adverse effects for this drug combination (Sohrabi *et al.,* 2010; Dinça *et al.,* 2011).



Scheme (1): The chemical composition of sulfamethoxazole.

Literature survey indicated that few analytical methods have been reported for analysis sulfamethoxazole. They include some spectrophotometric method (Givianrad and Mohagheghian, 2012; Upadhyay *et al.,* 2012; Abdulsatar, 2009; Raja *et al.,* 2009; Nagaraja *et al.,* 2007), HPLC (Herrera *et al.,* 2013; Mahmoud *et al.,* 2013; Pamreddy *et al.,* 2013; Asadi and Gharbani, 2013; Pietron *et al.,* 2013; Hererra *et al.,* 2013; Shaaban and Go´ recki, 2011; Liu *et al.,* 2013;), flow injection analysis (Icardo *et al.,* 2003), micellar electro kinetic capillary chromatography (MEKC) (Injac *et al.,* 2009). The aim of the present study is to suggest a simple and sensitive spectrophotometric procedures for the determination of sulfamethoxazole in pharmaceutical formulations. The methods are based on the reaction between sulfamethoxazole and NQS reagent to form a colored condensation reaction compound. In addition, the reaction conditions were studied univariatly one-factor-a time and multivaraitly by experimental design approaches in order to optimize the analytical response.

2. Experimental

2-1: Instruments

Cecil 7200 CE double beam UV-visible spectrophotometer possessing a fixed slit width (1.8 nm) with quartz cells of 10 mm path length connected to a P. IV computer loaded.

2-2: Materials and reagents

All reagents were of analytical grade. Sulfamethoxazole was obtained from State Company for Drug Industries and Medical Appliance (SDI) Samarra-Iraq, tablets, and syrup were purchased from a local market.

2-3: Preparation of standard stock solutions

Solution of 1000 $\mu g.mL^{-1}$ sulfamethoxazole was prepared by dissolving accurate weighted 0.100 g of pure drug in 10 mL of 0.4 M HCl and further diluted to the mark in volumetric flask 100 mL with distilled water and stored in a cool (<25 ^{0}C) and dark place, working solution were prepared fresh daily by subsequent dilutions. Sodium 1,2-Naphthoquinone-4-sulphonate (NQS) solution 0.5% (m/v) in D.W. was prepared fresh daily. Sodium hydroxide 0.01 M prepared by dissolving 0.20 gm of pure substance in 100 mL distilled water, Sodium tetraborate decahydrate (Borax) 0.03 M prepared by dissolving 0.57207 g in 25.0 mL double distilled water and diluting to the mark in a 50 mL volumetric flask.

2-4: Solution for the analysis of sulfamethoxazole in pharmaceutical preparations

2-4-1: In Tablets

The contents of 10 tablets were grinded and mixed well. A certain amount of the fine powder was accurately weighted to give an equivalent to 800 mg for tablets and the mean

value of the weight of one tablet was calculated. An amount of the powder equivalent to about 0.0632 g. was accurately weighted, then about 10 mL of 0.4 M HCl was added. Then transferred into 100 mL volumetric flask, and the solution was shaken swirled, leaved to stand for 5 mints and diluted to the mark in a volumetric flask 100 mL with distilled water to get 500 µg.mL^{-1}. The solution was filtered by using Whatman filter paper No.41 to avoid any suspended or un-dissolved material before use, and the first portion of the filtrate was rejected. Working solutions were freshly prepared by subsequent dilutions with distilled water, and analyzed by the recommended procedure.

2-4-2: In Syrup

Each 5.0 mL of the syrup contains (200 mg of sulfamethoxazole with 40 mg of trimethoprim). An accurately measured volume (1.25 mL) was transferred into a 100 mL volumetric flask, then added 10 mL of 0.4 M HCl swirled, leaved to stand for 5.0 mints and diluted to the mark with distilled water to get 500 µg.mL^{-1} SMZ solutions. The solution was filtered by using Whatman filter paper No.41 to avoid any suspended or un-dissolved material before use, and the first portion of the filtrate was rejected. Working solutions were freshly prepared by subsequent dilutions with distilled water, and analyzed by the recommended procedure.

2-5: Determination of SMZ Drug in pharmaceutical preparation by Standard Additions Method (SAM)

1- Preparation of SMZ stock solution 1000 µg.mL^{-1} according to the method of preparation in the previously mentioned.
2- Prepare solution of commercial pharmaceutical preparation (syrup or tablets) concentration of 500 µg.mL^{-1} according to the method of preparation in the previously mentioned.
3- Preparation of (7) solutions in 10 mL volumetric flask for measurements by adding 0.1, 0.2, 0.3 mL of solution commercial pharmaceutical preparation (syrup or tablets) from (500 µg.mL^{-1}) commercial pharmaceutical preparation, and added (0, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30 mL) of (1000 µg.mL^{-1}) standard solution of SMZ drug. A volume of 1.0 mL of 0.05 M borax solution were added to each flask, followed by 1.0 mL of 0.90 % (m/v) NQS solutions were added, then the mixture was shaken gently until the appearance of orange color. Leave to stand for 5.717 min., and the contents were diluted up to the mark with distilled water. The absorbance of each solution was measured at 460 nm against the reagent blank.

2-6: General recommended procedure

2-6-1: Under univariate conditions

Aliquots of the standard solution (1000 µg.mL^{-1}) containing (50-500) µg of sulfamethoxazole were transferred into a series of 10 mL volumetric flasks. A volume of 1.0 mL of 0.03 M borax solution were added to each flask, followed by 1.0 mL of 0.75 % (m/v) NQS solution were added, then the mixture was shaken gently until the appearance of orange color. Leave to stand for 3.0 min., and the contents were diluted up to the mark with distilled water. The absorbance of each solution was measured at 460 nm against the reagent blank.

2-6-2: Under multivariate conditions

Aliquots of the standard solution (1000 µg.mL^{-1}) containing (50-500) µg of sulfamethoxazole were transferred into a series of 10 mL volumetric flasks. A volume of 1.0 mL of 0.05 M borax solution were added to each flask, followed by 1.0 mL of 0.90 % (m/v) NQS solution were added, then the mixture was shaken gently until the appearance of orange color. Leave to stand for 5.717 min., and the contents were diluted up to the mark with distilled water. The absorbance of each solution was measured at 460 nm against the reagent blank.

3. Results and discussion

When the solution of sulfamethoxazole was mixed with NQS in alkaline medium at room temperature, intense coloration was developed, showing a broad band in the region of 420-600 nm. It was found that the product is orange colored exhibiting a λ_{max} at 460 nm, against reagent blank (Figure 1), and the λ_{max} of derivative chromogenic reagent (sodium 1,2-naphthoquinone-4-sulfonic) is at 430 nm., which indicates the formation as sulfamethoxazole possesses amino groups, it involves in yielding colored produced by nucleophillic displacement of the sulfonic acid group of 1,2-naphthoquinone-4-sulfonic acid in alkaline conditions. The intensity of this band was increased with increasing concentration of sulfamethoxazole.

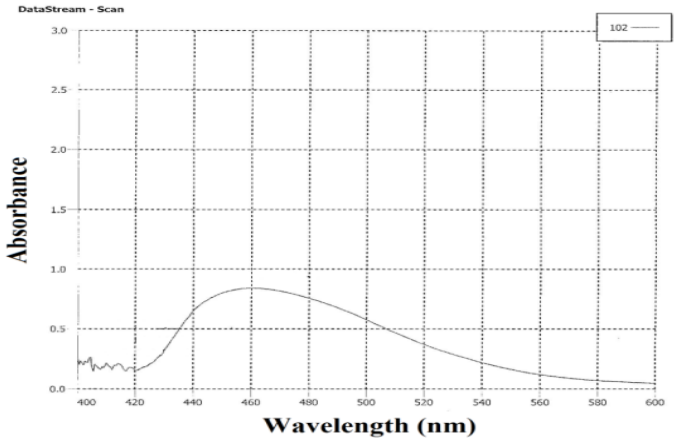


Figure (1): Absorption spectrum of the reaction products of sulfamethoxazole with NQS against blank.

In order to optimize the conditions, a number of parameters namely reagent concentration, borax concentration, and time. The optimum conditions were established univariatly by changing one variable and observing its effect on the absorbance of the colored product (table 1) and (Figure 2).

Table (1): Effect of different bases on condensation reaction and effect of coupling reaction time.

Alkaline solution (0.01M)	Absorbance	Time (min.)	Absorbance
NaOH	0.285	1	0.510
$Na_2B_4O_7.10H_2O$	0.311	2	0.531
NH_4OH	0.246	3	0.545
KOH	0.238	5	0.537
Na_2CO_3	0.163	8	0.539
-	-	10	0.533
-	-	60	0.538

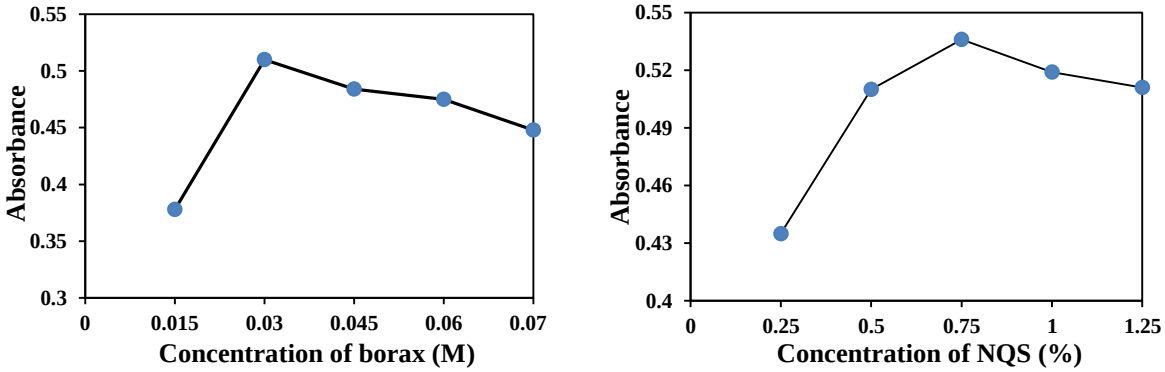


Figure (2): Effect of 1.0 mL of each of borax and reagent concentration on the color development of dye on the determination of 20.0 µg.mL^{-1} SMZ.

On the other hand, experimental design methodology and central composite design model, were used to multivariate optimization of three of factors that could have an important effect on the reaction. The factors of interest were reagent concentration, borax concentration and time. To study these factors, 1 mL of (0.25-1.25 %) m/v NQS and 1.0 mL of (0.015-0.075 M) borax concentration and coupling of reaction time (1-10 min.). Table 2 shows the equivalent matrix of the central composite design as well as the absorbance data. The experiment corresponding to the central point executed in four replicates. All experiments were carried out with solution of sulfamethoxazole set at (30 µg.mL^{-1}).

Table (2): Matrix and results from of the central composite design.

Exp. No.	Reagent Conc. (% m/v)	Borax Conc. (M)	Reaction Time (min.)
1	0.750	0.045	10.0
2	1.250	0.045	5.5
3	0.750	0.045	5.5
4	0.750	0.045	5.5
5	1.250	0.075	10.0
6	0.250	0.045	5.5
7	0.750	0.075	5.5
8	0.750	0.045	1.0
9	0.750	0.045	5.5
10	0.750	0.045	5.5
11	0.250	0.075	1.0
12	1.250	0.075	1.0
13	0.250	0.015	10.0
14	0.750	0.045	5.5
15	0.250	0.015	1.0
16	1.250	0.015	10.0
17	0.750	0.045	5.5
18	0.250	0.075	10.0
19	0.750	0.015	5.5
20	1.250	0.015	1.0

The experimental data showed an excellent fitting using a linear-quadratic second-order polynomial main effects model (r = 0.9998) according to the given equation:

$$\text{Abs.} = 0.3028 + 0.6783\ X1 + 9.4782\ X2 + 0.0059\ X3 - 0.3807\ (X1)^2 - 95.7576\ (X2)^2 - 0.0006\ (X3)^2$$

The results showed that the optimum values of the studied parameters are: reagent concentration solution is 1.0 mL of (0.9 % m/v), borax concentration is 1.0 mL of

(0.05 M), and reaction time is (5.717 min.). A three-dimensional response surface graph which represent the absorbance values obtained experimentally from central composite design is given in Figure(3).

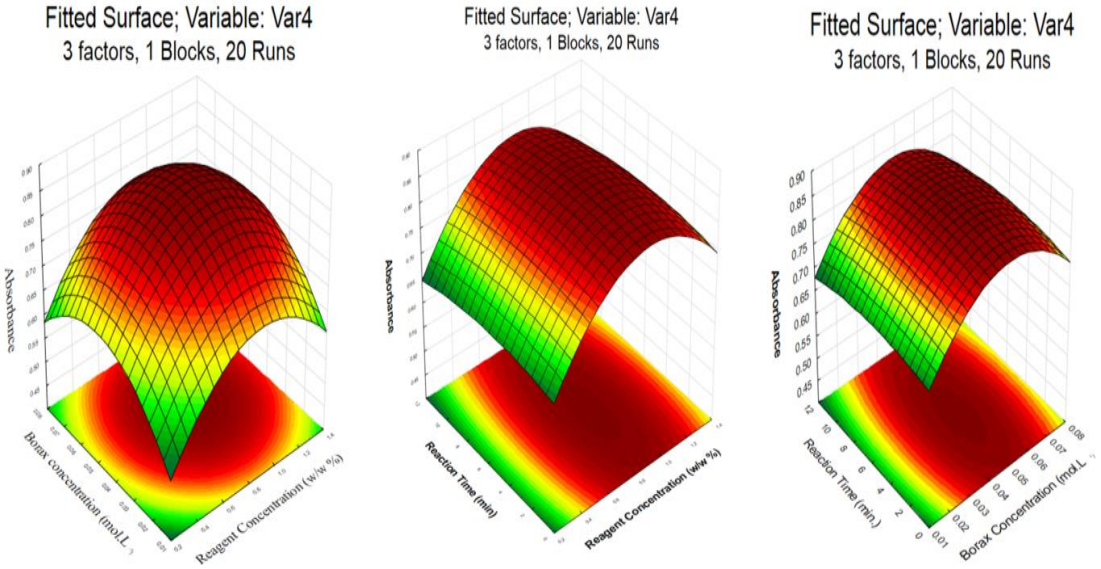
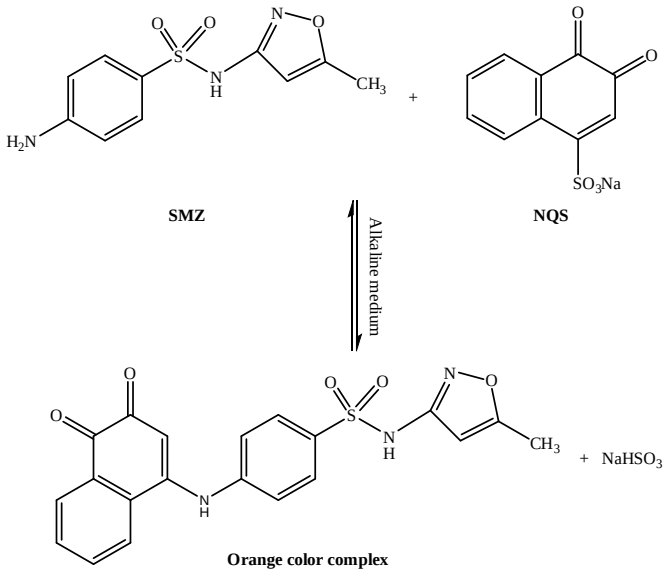


Figure (3): Response surface of quadratic model for absorbance values as a function of reagent concentration, borax concentration, and reaction time.

Accordingly, the reaction mechanism between sulfamethoxazole and NQS is suggested on the basis of results mentioned Scheme (2).



Scheme (2): The proposed mechanism of the reaction between sulfamethoxazole and NQS.

3-1: Quantification

With univariate and multivariate experimental design conditions, the intensity of absorption at 460 nm was found to be a function of the concentration of the sulfamethoxazole. In both cases studied, Beer's law plots were linear in two concentration ranges of the drug. The molar absorptivity values were 6.7878×10^4 $L.mol^{-1}.cm^{-1}$ when calibration curve was constructed under those experimental conditions obtained univariatly. While two linear regression equations were attained for the proposed procedures under multivariate experimental conditions with values of molar absorptivity of 7.0918×10^4 $L.mol^{-1}.cm^{-1}$. Table (3) shows other quantitative and statistical parameters for the determination of sulfamethoxazole.

Table (3): Quantitative parameters for the reaction of the studied sulfamethoxazole with NQS.

Parameter	Univariate conditions	Multivariate conditions
λmax (nm)	460.0	
Color	Orange	
Linearity range (μg.mL^{-1})	5.0-50.0	
Regression equation	Y=0.0268[SMZ. μg.mL^{-1}]+0.0152	Y=0.0280[SMZ. μg.mL^{-1}]+0.0165
Calibration sensitivity (mL. μg $^{-1}$.cm $^{-1}$)	0.0268	0.0280
Correlation coefficient (r)%	99.98	99.98
Correlation of linearity (r^2)%	99.97	99.97
Molar absorptivity (L. mol $^{-1}$.cm $^{-1}$)	ε =6.7878×10^4	ε =7.0918×10^4
Sandell's sensitivity (μg.cm^{-2})	3.7314	3.5714
Detection limit (μg.mL^{-1})	0.3755	0.3594
Quantification limit (μg.mL^{-1})	1.1380	1.0893

3-2: Precession and accuracy of method

The precision and the accuracy of the proposed method was checked under univariate and multivariate central composite conditions by calculating the relative standard deviation percent and relative error percent for five replicates at four different concentration levels of the drug. Table (4) shows the method precise and accurate results.

Table (4): Evaluation of accuracy and precision for the determination of SMZ by proposed method.

	Conc. of SMZ (μg.mL^{-1})		Relative Error %	C.V %
	Taken	Found*		
For univariate	10.0	10.022	0.220	1.377
	20.0	19.746	-1.270	1.026
	30.0	30.119	0.396	0.692
For DOE	10.0	10.039	0.388	1.718
	20.0	19.953	-0.234	0.710
	30.0	30.139	0.462	0.528

*Average of five determinations.

3-3: Interference Study

In pharmaceutical analysis, it is important to test the selectivity towards the excipients added to the pharmaceutical preparations. Commonly encountered excipients such as (vanillin, glucose, lactose, starch, sucrose) did not interfere in the determination of SMZ and did not effect on the reaction between the SMZ and NQS. 20.0 μg.mL^{-1} of SMZ was analyzed and design of experiment method was used for analyzing (Table 5).

Table (5): Percent recovery for 20.0 μg.mL^{-1} of sulfamethoxazole in the presence of different concentration of Excipients.

Excipients	Concentration μg.mL^{-1}	Sulfamethoxazole Conc. Taken (20 μg.mL^{-1})	
		Conc. Found μg.mL^{-1}	Recovery %
Vanillin	1000	20.102	100.51
Glucose		20.132	100.66
Lactose		19.874	99.37
Starch		20.184	100.92
Sucrose		20.151	100.75

3-4: Application in Pharmaceutical preparation by Standard Additions Method (SAM)

Standard additions technique was followed to check the validity of the proposed method has given good recoveries of the drug in pharmaceutical preparations suggesting a noninterference from pharmaceutical preparations. Hence, this method can be recommended for adoption in routine analysis of SMZ in quality control laboratories.
(Table 6) shows the result of recovery and coefficient of variation (C.V) for the standard additions method. Figure 4 to 7 show plot of determination of SMZ in syrup and tablet by standard additions method.

Table (6): Application of the proposed method to the SMZ concentration measurements in pharmaceutical preparation by (SAM).

Sample	Conc. taken (µg.mL$^{-1)}$	Conc.* found (µg.mL^{-1})	Recovery %	C.V*%
Bactrim tablet (Roche-France)	500.00	504.74	100.95	0.093
Bactrim syrup (Roche-France)	500.00	504.70	100.94	0.109
Methoprim tablet (NDI-Iraq)	500.00	510.68	102.136	0.243
Cotrim syrup (Asia-Syria)	500.00	506.40	101.28	0.100

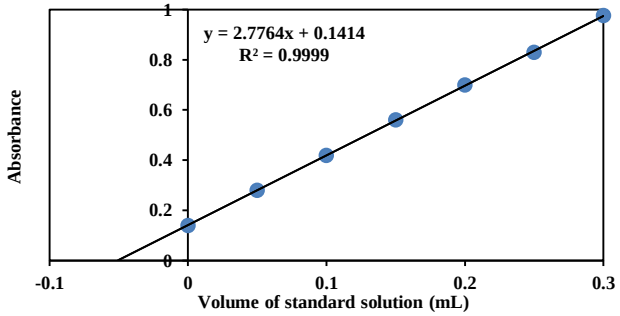


Figure (4): Determination of SMZ in Pharmaceutical preparation (Bactrim syrup) sample by standard additions method, (level one 0.1 mL from 500 µg.mL^{-1})

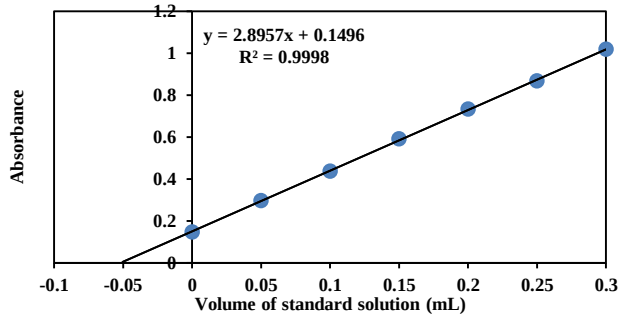


Figure (5): Determination of SMZ in Pharmaceutical preparation (Bactrim tablet) sample by standard additions method, (level one 0.1 mL from 500 µg.mL^{-1})

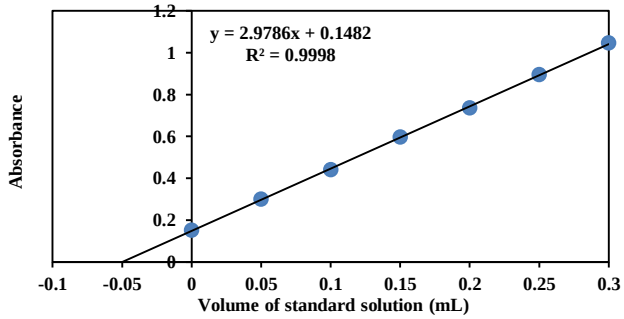


Figure (6): Determination of SMZ in Pharmaceutical preparation (Cotrim syrup) sample by standard additions method, (level one 0.1 mL from 500 µg.mL^{-1})

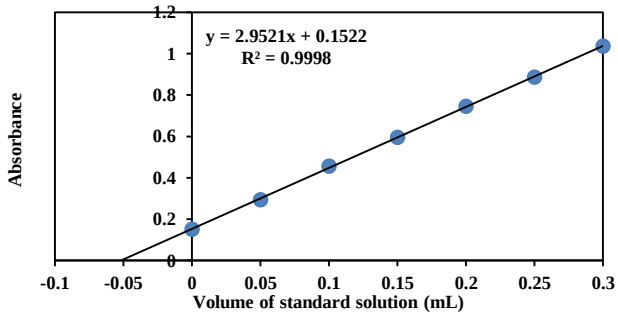


Figure (7): Determination of SMZ in Pharmaceutical preparation (Methoprim tablet) sample by standard additions method, (level one 0.1 mL from 500 µg.mL^{-1})

4. Conclusions

A simple, rapid, and inexpensive spectrophotometric method for determination of sulfamethoxazole is proposed, which provide gain of sensitivity without the need of additional step as extraction or heating. The method involve mild reaction conditions and gives precise and accurate results. Its usefulness for the cited drug determination in pharmaceutical formulations was demonstrated, suggesting its use as an attractive alternative to many other previously reported methods for analysis of sulfamethoxazole.

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