Spectrophotometric determination of sulfamethoxazole in pure and pharmaceutical formulations

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Abstract

New, easy, simple, and fast spectral method for estimation of sulfamethoxazole (SMZ) in pure and pharmaceutical forms. The proposed method is based on the azotization of the drug compound by sodium nitrite in an acidic medium and then coupling with 2,3dimethyl phenol reagent (DMP) in a basic medium to yield an orange-coloured dye which shows λmax at 402 nm. Different affection of the optimization reaction has been completed, following the classical univariate sequence. The concentration of sulfamethoxazole about (1-15) µg. mL^{-1} with molar absorptivity of (14943.461) $L.mol^{-1}.cm^{-1}$ that obeyed Beer's law. The detection and quantification limits were (0.852, 2.583) µg. mL^{-1} respectively, while the value of Sandell's sensitivity (0.016) $\mu g.cm^{-2}$. The suggested technique was effective and good for the evaluation of sulfamethoxazole in dosage preparation.

Keywords: *Azotization, Spectrophotometric determination, Sulfamethoxazole*.

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التحديد الطيفي للسلفاميثوكسازول في شكله النقي والمستحضرات الصيدلانية

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الخلاصة

طريقة طيفية جديدة وسهلة وبسيطة وسريعة لتقدير سلفاميثوكسازول (SMZ) في شكله النقي والصيدلاني. تعتمد الطريقة المقترحة على ازوتة المركب الدوائي بواسطة نتريت الصوديوم في وسط حمضي ثم الاقتران مع كاشف2،3 ثنائي ميثيل فينول (DMP) في وسط قاعدي لإنتاج صبغة برتقالية اللون تظهر اعلى قمة امتصاص عند 402 نانومتر. تمت دراسة تأثير مختلف الضروف الفضلى ا، باتباع التسلسل الكلاسيكي أحادي المتغير. كان تركيز سلفاميثوكسازول حوالي (1-15) ميكروغرام مل $^{-1}$ المطاوع لقانون بيير بامتصاصية مولية قدرها (14943.461) لتر مول $^{-1}$ سم $^{-1}$ التي تخضع لقانون بير. كانت حدود الكشف والقياس الكمي (0.852,2.583) ميكروغرام مل $^{-1}$ على التوالي ، بينما كانت قيمة حساسية ساندیل (0.016) ميكروغرام سم $^{-2}$ كانت التقنية المقترحة فعالة وجيدة لتقييم سلفاميثوكسازول في تحضير الجرعة.

الكلمات المفتاحية : *الازوتة ، التقدير الطيفي ، سلفاميثوكسازول .*

1. Introduction

Sulfamethoxazole (SMZ) an antibacterial drug that is crystallized as white or yellowish-white coloured powder [1], Figure-1shows the chemical structure of SMZ. Its use with trimethoprim to treat different types of bacterial infections e.g.: eye infections, urinary tract infections, middle ear infections, and respiratory tract infections such as bronchitis, and enteric infections [2]. Sulfamethoxazole used in the prophylaxis of rheumatic fever, nocardiosis, toxoplasmosis, and Pneumocystis carinii pneumonia [3]. Skin reactions and gastrointestinal disturbances (mainly nausea and vomiting) are the most common adverse effects of this drug combination [4]. Spectroscopy is one of the important methods for determining the active substance of most medicinal compound [5-7]. Literature survey revealed that several analytical techniques reported analysing different drugs in pharmaceutical and pure formulas with spectrophotometric methods [8].

They include HPLC [9], micellar electro kinetic chromatography [10], flow injection analysis [11], capillary zone electrophoresis [12], solid-phase extraction [13], voltammetry [14], fluorescence spectrophotometric [15] and NMR methods [16]. In this work, an accurate, sensitive, and simple visible spectrophotometric method was examined for the quantitative estimation of sulfamethoxazole in its pure form and medical preparation.

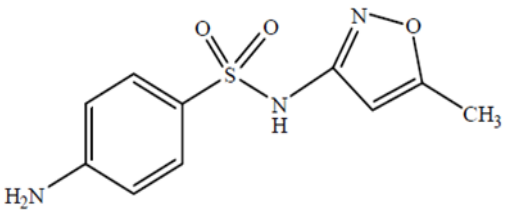

Fig. (1): Chemical structure of sulfamethoxazole

2. Experimental Section

2.1 Materials

Pure form (99.99%) pharmaceutical grade sulfamethoxazole powder was obtained as a gift from State Company for Medical Appliances and Drug Industries Samara-Iraq (SDI). All reagents and chemicals used are of analytical grade

1. Sodium nitrite [0.002 % (m/v)] Sigma Aldrich: has been prepared by weighing 0.002 g of $NaNO_2$ and dissolve in distilled water also complete the volume to the mark in 100 ml volumetric flask.

2. 2,3 dimethyl phenol (DMP) [0.01 % (m/v)]: has been prepared by weighing 0.01 g of DMP, dissolve in 25 mL methanol then complete the volume by purifying water in a 100 mL volumetric flask.

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3. Hydrochloric acid [1M]: from concentrated HCl transfer 8.75 mL then complete the volume of 100 mL volumetric flask to the mark by purifying water.

4. Sodium hydroxide [1M]: dissolving 4 g of NaOH in distilled water then complete the volume using purifying water in a 100 mL volumetric flask. Chemicals used are of analytical grade.

2.2 Preparation of stock SMZ solution (100 µg.mL^{-1})

Weighed 0.01 g pure substance of SMZ, dissolved in 10 mL methanol to prepare the standard SMZ solution and complete the volume to the mark in 100 ml volumetric flask with distilled water. Serial dilution is newly prepared for working solutions.

2.3 Instrumentation

All spectroscopic measurements were performed and absorption spectra were recorded, on a Shimadzo 1800, Kyoto,Chapan UV-Vis dual-beam spectrophotometer with an identical 1 cm quartz cell.

2.4 Procedure

This procedure is recommended to determine SMZ via the proposed methods. From a standard solution of (100 µg.mL^{-1}), equivalents (1-15) µg.mL^{-1}sulfamethoxazole were transferred to a series of 10 mL volumetric flasks. Before cooling in an ice bath, 0.5 mL of a 0.002% sodium nitrite solution (m/v) and 0.5 mL of 1 M HCl were added to each flask. The prepared solutions were coupling with DMP by adding 1.7 mL of 0.01% followed by the addition of about 0.5 mL NaOH of 1 M. Solutions were complete to the mark with purifying water and the absorbance of the resulting dye was measured at 402 nm against a reference solution.

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2.5 Estimation Sulfamethoxazole in tablets medical formulation

Medicinal tablets were selected to determine the active ingredient by applying the proposed method. The equivalent (800 and 400) mg of tablets were weighed, and take the average weight of one tablet, added about 10 mL of methanol to completely dissolve, transferred to a volumetric flask of 100 mL and diluted with distilled water to the mark, the resulting solution is filtered by filter paper of number 41 to get rid of suspended and undissolved materials. Transferred 1 mL of the prepared solution to a volumetric bottle of 100 mL then completed with purifying water to the exact mark to get 80 μg. mL^{-1} and completing the suggested method according to the conditions obtained by univariate optimization.

3. Results and Discussion

3.1 The absorption spectrum and reaction pathway

As nitrous acid is added to a solution of acidified primary aromatic amine in an ice bath, the solution forms diazonium salt. Under the optimum conditions, diazonium salts combine with phenolic aromatic compound to produce azo compound, a reaction known as the diazo coupling reaction [16]. The suggested approach entails coupling the diazotized SMZ with 2,3dimethyl phenol in a basic medium to yield an azo dye with orange color (scheme -2) that has a maximum absorbance of 402 nm (figure -2)

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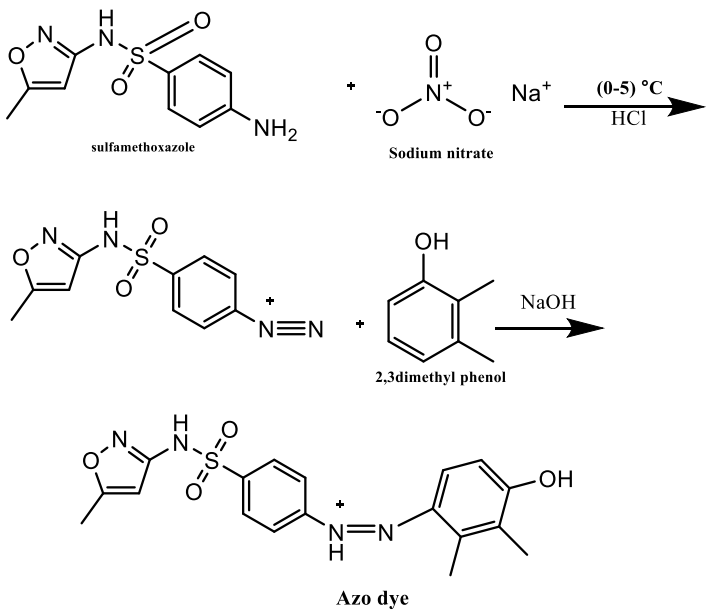

Scheme -1 Suggested path way of formation azo dyes

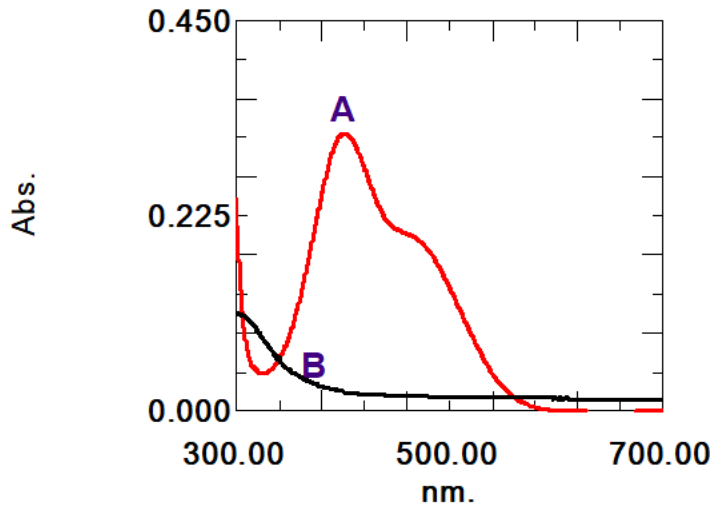

Fig. (2): Absorption spectra for 5 µg.mL^{-1}sulfamethoxazol with reagent (A) against blank solution (B).

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3.2 Optimize the conditions for the estimation process

Various parameters to estimate 5 µg.mL^{-1} (effect of diazotization reaction time, sodium nitrite concentration, acidity on diazotization, 2,3dimethyl phenol concentration, and coupling reaction time) were first optimized, to develop a colour intensity, improvement procedure was done by changing the parameters all once upon a time and controlling all other defined factors.

3.3 Effect of Diazotization Reaction Time

The optimum diazotization reaction time was determined at ~ 0-5 ºC by recording the absorbance for the resulting azo dye for the period of (0-40) min. It was found that a coloured product with maximum absorbance at 402 nm takes place within 10 minutes, (figure -3), shows the results.

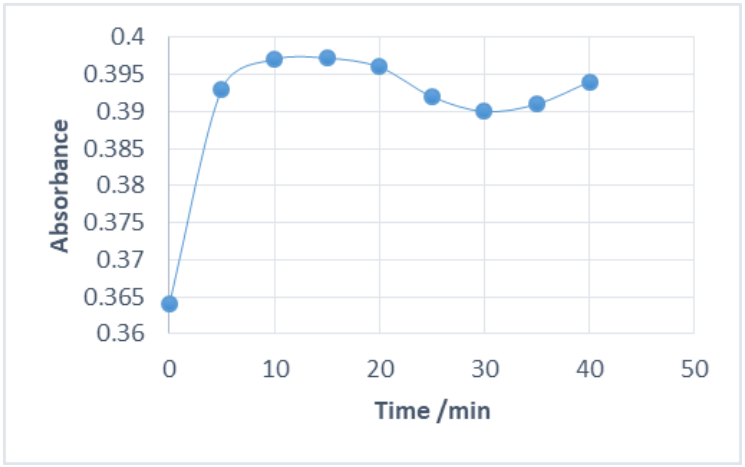

Fig. (3): Effect of azotization time on determination of 5 µg.mL^{-1} SMZ.

3.4 The concentration of Sodium Nitrite Effect

The effect of $NaNO_2$ concentration was studied by measuring the resulting absorbance at 402 nm in the range of (0.1-2) mL (Figure -4). It was found that 0.5 mL of a 0.002% sodium nitrite solution is necessary for constant and maximum colour

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intensity for azo dyes complex, low absorbance values were observed with higher concentrations.

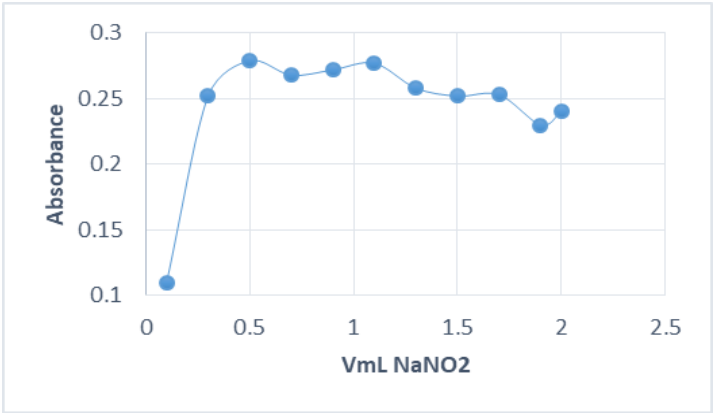

Fig. (4): Concentration of Sodium Nitrite Effect on determination of 5 µg.mL^{-1} SMZ

3.5 The effect of acidity on the production of colored compound

The effect of hydrochloric acid concentration on the diazotization reaction was investigated over a 0.1-1.3 mL range. When added about 0.5 mL of 1 M HCl, the absorption rate reached a maximum and remained stable, during which the absorption of the reaction started to decrease, therefore; 0.5 mL of 1 M was selected as the optimal value, as showed in (Figure - 5).

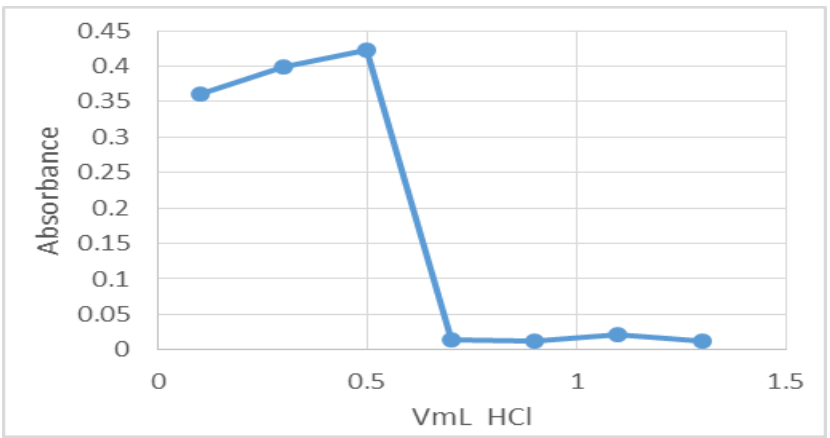

Fig. (5): Acidity effect

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3.6. Effect of 2,3dimethyl phenol Concentration

The concentration of 2,3- dimethyl phenol ranged from 0.1- 1.9 mL solutions were examined to obtain the highest colour intensity of the azo dye as shown in (Figure -6). The result showed that 1.7 mL of 0.01 % DMP gave better and stable intensity, the absorbance remained unchanged above this concentration.

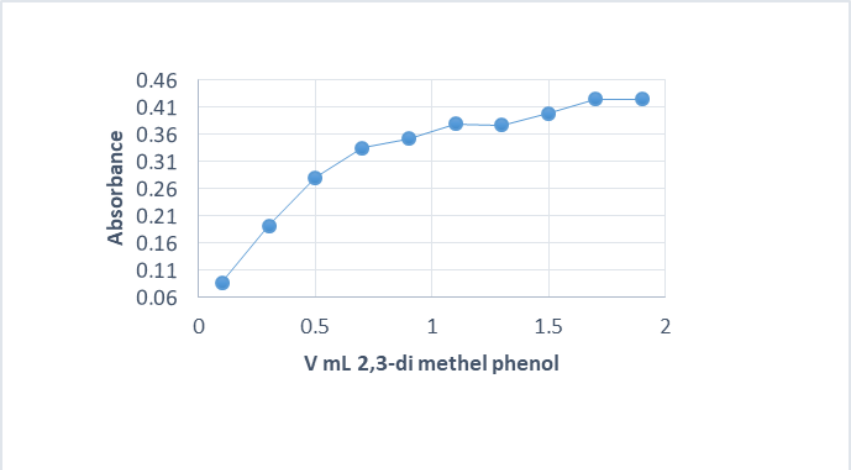

Fig. (6): Effect of 2,3dimethyl phenol Concentration.

3.7. Study the effect of coupling time and stability

The ideal time, stability of the color compound was determined at room temperature. The reaction mixture was left to stand for various periods, and it was shown that a 10 minute was needed for color development; the color has been stable for at least 1 hour.

3.8. Analytical Data and Calibration Curves

The study showed the linearity of the drug compound concentrations with absorption at a specified range of concentrations. Beer's law (a) was applied in the range of (1-15) µg. mL^{-1} of SMZ, a plot of residual indicated random error and absence of systematic error type (b). The results can be seen in (Figure -7). The molar

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absorptivity, regression equation, limit of detection (LOD), and limit of quantification of the (LOQ), listed in (Table 1).

A **B**

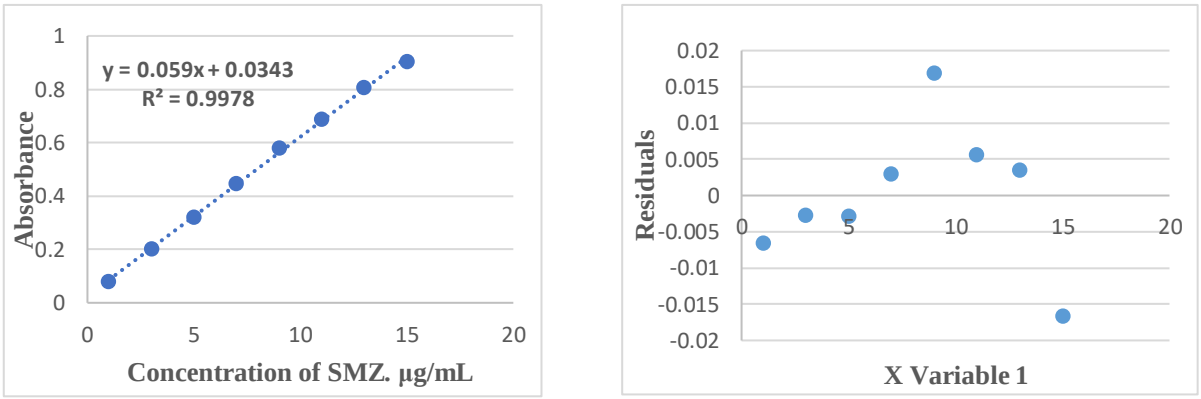

Fig. (7): A: demonstrate linear rang, B: residual plot.

Table (1): Analytical characterization of the proposed method

Parameter	Value
λmax (nm)	402
Colour	Orange
Linear range (µg. mL^{-1})	1-15
Molar absorptivity($L.mole^{-1}.cm^{-1}$)	14943.461
Sandell's sensitivity ($g.cm^{-2}$)	0.016
LOD(µg. mL^{-1})	0.852
LOQ(µg. mL^{-1})	2.583

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4. Stoichiometry study

Jobs and the mole ratio method have been used in the determination of stoichiometry of the coloured product. sulfamethoxazole and 3,5-DMPH solution of 2×10^{-2}M were prepared and mixed in a various molar ratio in 10 mL volumetric flasks with the suggested procedure, the maximum intensity was recorded at 402 nm. The graph of the results obtained as in (Figures -8) gave maximum at mole ratio X_{max}=0.5, X_{max} =1 in Jobs and mole ratio methods (a, b) respectively, showed that 1:1 sulfamethoxazole to 3, 5-dimethyl phenol ratio is obtained.

A B

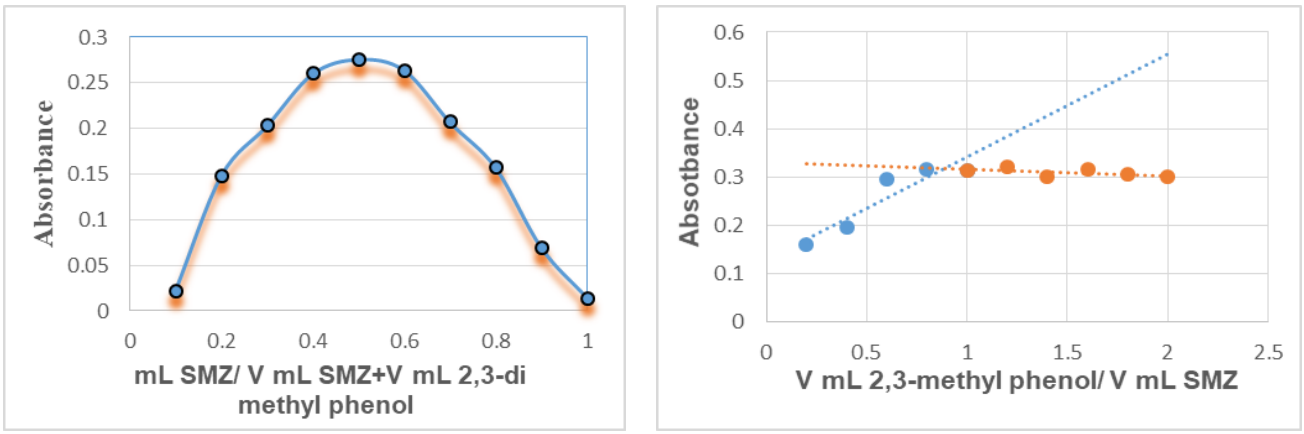

Fig. (8): Stoichiometric study with continuous variation (A) and mole ratio (B)

5. Reproducibility and Accuracy

To calculate the relative error to know the method's accuracy, three various concentrations of the drug compound were determined for five replicate analyses. The reproducibility of results was selected by measuring the standard deviation for the same drug sample solution (Table 2). The low SD and error values at the range less than 3.947 mean that the suggested procedure is accurate and precise.

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Table (2): Accuracy and precision represented as relative error and standard deviation

Conc.*mg/L taken	Conc.*mg/L Found	RE%	SD
1	1.001	0.169	0.187
3	2.801	1.801	0.010
5	4.947	3.947	0.585

*****_Replicate five times_

6. Interference Study

To study the selectivity of the suggested method to the drug compound, the influence of several typical excipients; sucrose, glucose, lactose, starch, which often accompany drugs, were investigated by determining 3.0 $\mu g.mL^{-1}$ of SMZ in a presence of the above compounds. The results presented in (Table 3), indicate no interferences were found from any of the excipients studied in the determination of SMZ.

Table (3): Effect of interferences with 3.0 $\mu g.mL^{-1}$ SMZ

interferences	Vml	Found	SD	%Recovery
Glucose	0.5	2.930	0.030	97.666
	1	2.944	0.002	98.133
	1.5	2.605	0.005	86.833
Maltose	0.5	2.412	0.014	80.400
	1	2.605	0.010	86.833
	1.5	2.819	0.001	93.966

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Sucrose	0.5	3.023	0.001	100.770
	1	2.977	0.002	99.233
	1.5	3.006	0.003	100.200
Lactose	0.5	2.848	0.001	94.934
	1	2.735	0.008	91.167
	1.5	2.859	0.001	95.300
Acacia	0.5	3.192	0.013	106.400
	1	3.040	0.0036	101.333
	1.5	3.051	0.0153	101.700
Starch	0.5	2.932	0.0005	97.733
	1	3.090	0.0138	103.000
	1.5	3.045	0.0145	101.500

7. Application

To examine the success of the suggested method in estimation SMZ, the method was applied to a different manufacturing source containing SMZ, the result was listed in Table 4.

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Table (4): Data recovery obtained by applying the current method in drugs formulation

Pharmaceutical	Labelled amount (SMZ+trim) Mg	Conc. taken* mg/L	Conc. Found mg/L	Rrecovery %	C.V %
Septrin, tablet, Aspen , Germany	400+80	3.00	3.040	101.33	0.115
		5.00	5.034	100.68	0.514
		7.00	7.000	100.00	0.369
Bactrimel, tablet, Roche Germany	800 +160	3.00	2.759	91.96	0.277
		5.00	4.898	97.96	1.440
		7.00	6.898	98.54	0.141
Piotrim, tablet, Pioneer, Iraq	400+ 80	3.00	2.920	97.33	0.052
		5.00	5.022	100.44	1.404
		7.00	7.016	100.22	0.418
Trimethbrim, tablet, Samara, Iraq	400+80	3.00	2.921	97.36	2.344
		5.00	5.277	105.22	1.962
		7.00	6.859	97.99	1.115

*Three replicate (trim: trimethbrim)

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8. Conclusion

The diazotization reaction of the primary amine group, come after by reaction with 2,3-dimethyl phenol in an acidic medium, has been appearing to be a quick, sensitive, precise, and cost-effective spectroscopic process for quantifying (SMZ) in pure form and pharmaceuticals. The classic univariate approach was used to optimize the different variables influencing reaction completion. The suggested approach appeared to be accurate and compatible**.**

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