Egypt. J. Microbiol. **Vol. 57,** pp. 89-99 (2022)

EgyptianJournalofMicrobiology
http://ejm.journals.ekb.eg/

Usage of Trifluoperazine Hydrochloride as Colorimetric Reagent for Quantitative Assay of Sulfamethoxazole Drug and Applications on Different Some Infection Treatment Preparations

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SULFAMETHOXAZOLE (SMS) was determined using a straightforward, quick, and sensitive spectrophotometric approach that was described, the method is centered on the development of a stable water-soluble a product that maximum absorption at 515nm by Trifluoperazine hydrochloride's oxidative coupling reaction (TRF) and (SMS) utilizing nitrate cerium ammonium (CAN) acting as the oxidizing agent in a medium of acidic. Depending on the concentration of SMS, the law of beer was the concentration of range of 2-38g.mL^{-1}and a molar absorptivity of 1.47 x 10^{3}L.mol^{-1}.cm^{-1}, one that is related of -0.026 to +5.5, and in a concentration, Beer's law is applied. a relative standard deviation was 0.136 to 0.319%.the sensitivity of Sandell's was 0.172g.cm^{-1} and the limit of detection (LOD) were 0.645g/mL^{-1} and (LOQ) were 2.15g/mL^{-1} The suggested approach has been effectively used to determine SMS in several.

Keywords: Antibacterial activity, Colorimetric assay, Spectrophotometric method, Sulfamethoxazole, Trifluoperazine hydrochloride.

Introduction

Sulfa medications were among of the first pharmaceuticals to be used in clinical settings (Egbujor et al., 2023) to treat inflammation. Although safer and more potent antibiotics have mostly taken their place, they are still recommended for urinary tract infections. Sulfamethoxazole (SMS) (Amino-N-(5-methylisoxazol-3-yl)-4-benzenesulfonamide) antibacterial agent the chemise formula (C10H11N3O3S). (SMS) is very soluble in water and ethanol. It was a colure crystalline powder has melting pointe it soluble from 172 to 196 C in ethanol and soluble in dilute solution of potassium hydroxide or in dilute (Hassan & Zaki, 1976). Trimethoprim is combined with SMS prepared pills, an intravenous solution, and an oral suspension to create the medication combination known as trimoxazole. There are several methods for determination,(Trujillo-Casarreal et al., 2023), Such as UV spectroscopy, electrolysis, oxidative coupling, and the use of Schiff's base, whether in the substance's pure form, pharmacological formulations, or bodily fluids, thanks to the different domains of analytical chemistry. It is particularly well suited as a topical treatment for chronic inflammation of the stomach and intestines. Antibiotic sulfamethoxazole is used. It eliminates infection-causing germs (Al-Rufaie, 2016). During chemotherapy, this medication may be given to some individuals to avoid pneumonia. Additionally, infections may be treated with this medication. medications and other antibiotics, many bacteria were thought to be the main cause of mortality. Sulfa derivative medications have an inhibitory impact on their growth, which makes them widely employed in medicine.(Ponka, 2006). All of the sulfa derivatives in question have a benzene ring that has an amino group and a sulfate group attached to it. Sulfamethoxazole (SMS), which has the following structure and a molecular weight of 253.3g/mol as shown in Fig. 1, was one of the most significant of these compounds (Hu et al., 2007).

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Received: 15/12/2022; Accepted: 04/04/2023
DOI: 10.21608/EJM.2023.181202.1223

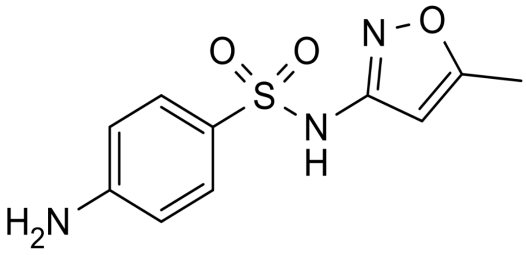


Fig. 1. Sulfamethoxazole chemical structure

One of the sulfonamide antibiotics, that is sulfamethoxazole (SMS), is used frequently to treat bacterial of infection in people, such as prostatitis., infection of the urinary tract individuals bronchitis, that SMS is effective against both gram negative and positive bacteria, which makes it popular in the aquaculture and animal husbandry industries for treating bacterial infections (Ponka, 2006). As prospective access sites, they were highlighted. Also taken into consideration were medications for animals. After being administered to production animals, parent chemicals and metabolites are ejected. They may then enter soil ecosystems by being spread as manure on grassland and arable land or by being left as dung pats on pasture. (Beer et al., 2019). The direct or indirect spectroscopic approaches, which depend on oxidation-reduction, are the most significant of these estimating techniques. The medicine is a group of antibiotic sulfonamides, which is one of the oldest and still commonly utilized sulfonamides, a group of synthetically produced antibiotics launched in 1939 (Christensen, 2021) and used for 60 years in veterinary and human therapy (du Bois et al., 2005)

The other methods of analytical for (SMS) used for determination compound like aerometric titration (Arvand & Alirezanejad, 2011), ion-selective electrode (ISE) potentiometric (Pnei, 1981), potentiometric titration (Shoukry et al., 1995), Monatshefte fur Chemise (Sinan & Al-Uzri, 2011), differential pulse polarographic (Al-Rufaie, 2016), differential scanning calorimetric thin layer chromatography (TLC) (Agbaba et al., 1996; Elahi et al., 2021), reverse phase high performance liquid chromatographic (RP.HPLC) (Ristuccia, 1987; Schulz & Schmoldt, 2003; Román et al., 2004), capillary zone electrophoresis (CZE) (Berzas Nevado et al., 2001), sequential injection (SI) (Mesquita & Rangel, 2009), chemiluminescence (Paseková et al., 2001), flame atomic absorption spectrophotometry (AAS) (Taha et al., 2016), fourier transform Raman spectroscopy (Chase, 1987), spectrofluorimetry (Sabry, 2006) and nuclear magnetic resonance [NMR] (Sinan & Al-Uzri, 2011).

The suggested technique focuses on the oxidative coupling reaction, which was initially utilized with comparatively lower detection limits, high sensitivity, with a wider dynamic range to create color content between the tested TRF reagent and the drug. These methods' flexibility to be employed at room temperature without the requirement for extraction is a key feature.

Materials and Methods

Apparatus

- Digital recording spectrometer with a double-beam UV visible 160 wavelength (Japan)-was utilized for both those absorbance as well as spectral assessments.

- Analytical balance (Sartorius BL210S)

Material and reagents

The chemicals that were utilized with a large degree of pureness in the protocol as well as did not need to be cleansed were compelled with the following solution:

Sulfamethoxazole SMS 500ppm: Simple material was synthesized from Samara-Iraq (SDI) (State of the Drug Industries as well as Medical Appliances Company). The 500ppm standard concentration solution of SMS was dissolved by 0.05g of bulk material 100mL of it was diluted of deionized waterdistilled water in a volumetric flask after being dissolved in 10mL of ethanol to enhance solubility. The standard approach leads to perpetuate working concentrations (Thani et al., 2022).

Nitric acid solution 1M: It was compelled by introduction concentrated nitric acid in 1.786mL form in 20mL volumetric flask, well mixed and distilled water finished the volume.

Reagents

All of the substances utilized in this experiment were analytical reagents, and SMS standard material was supplied by the general medical supply and pharmacy facility in Samarra, Iraq.

Trifluoperazine hydrochloride (TRF) solution, 1.5 x10^{-2}M

To make this solution, 0.1442g of TRF were dissolved in 20mL of distilled water.

Ammonium ceric sulphate (ACS) solution, 4 x10^{-3} M

0.0438 g of (ACS) were dissolved in 20ml of distilled water to create this solution.

Samples of pharmaceutical preparations SMS drug

The different types of SMS pharmaceutical preparations that containing SMS are using in this study as applications. The oral suspension of solution forms was used. Table 1 shows the oral suspension of solution forms which utilized in the paper and their companies.

Suggested procedure

Of a series in 20 ml volatile flasks, equalitarian volumes of standard solutions from solution SMS of a concentration from 2-38ppm sequentially introduced one at a time in the latest volume, accompanied by an addition of 0.5mL from 1M nitric acid with 0.5mL of TRF and added 3.5ml of ACS, then we left the solutions for 15 min at room temperature, and finally we added 1 ml of SMS in the 10 At 515nm, the absorbance was calculated progressively against the blank reagent while also building a calibration graph (Negoiu et al., 2010).

Assay procedure for Sulfamethoxazole and trimethoprim in oral suspension dose forms. Drug preparations, we take 0.25mL of oral suspension and dilute it to 20mL of distilled water after performing a filtration process for the solution. Figure 2. Appears The spectrum absorbance for 500ppm SMS treated per the suggested method and compared to (A) colored solution and (B) pure medicines. SMS (C) all-natural components, no medications.

Biological activity

Bacterial isolates

The following microbial pathogenic isolates of multidrug resistance (MDR): *Staphylococcus aureus* and *Enterococcus faecalis*, six Gram +ve bacteria, were identified, together with *Proteus mirabilis* and *Enterobacter cloacae*, two Gram +ve bacteria. clinical samples such as a wound, burns, additionally diabetic ulcer. The isolates were identified using phenotypic and biochemical experiments, and they were afterwards recently verified using the portable Vitek-2 instrument. Both bacterial isolates were procured. Automated bacterial detection tool for GP and GN cards d in glycerol-added BHI broth at (-20°C) (15 percent). Prior to usage, the isolates were sterilized at 37°C for 24h while also being sub-cultivated on BHIA (Samie et al., 2010).

Results and Discussion

To determine the ideal circumstances, the impact of several variables on the development of color was investigated. The results showed that the interaction between SMS and (TRF) in the by using spectrophotometric analysis, the presence of an oxidizing agent (ACS) results in a strongly colored product that may be used as an appropriate test technique for SMS in various Inflammation of urinary tract preparations. The violet product absorbs most light at a wavelength of 515nm (Fig. 2).

TABLE 1. The oral suspension of solution forms is utilized in the paper and their companies

The solution preparation	Company applied
Piotrim solution (each 5ml of the piotrim suspension contains 200mg of sulfamethoxazole and 40mg trimethoprim)	Manufactured by pioneer co. for pharmaceutical industries-Iraq
Neotrim suspension (each 5ml contains 200mg of sulfamethoxazole and 40mg of trimethoprim	Al-gadeed pharmaceutical Industries co. Amman – Jordan
Septrin tablets (each tablet containes400mg of sulfamethoxazole BP and 80mg trimethoprim BP	Aspen Bad oldesloe GmbH, Industriestrasse32-36 D-23843Bad Oldesloe, Germany
Metheprim tablets (each table contains400mg of sulfamethoxazole and 80mg trimethoprim)	SDI- IRAQ

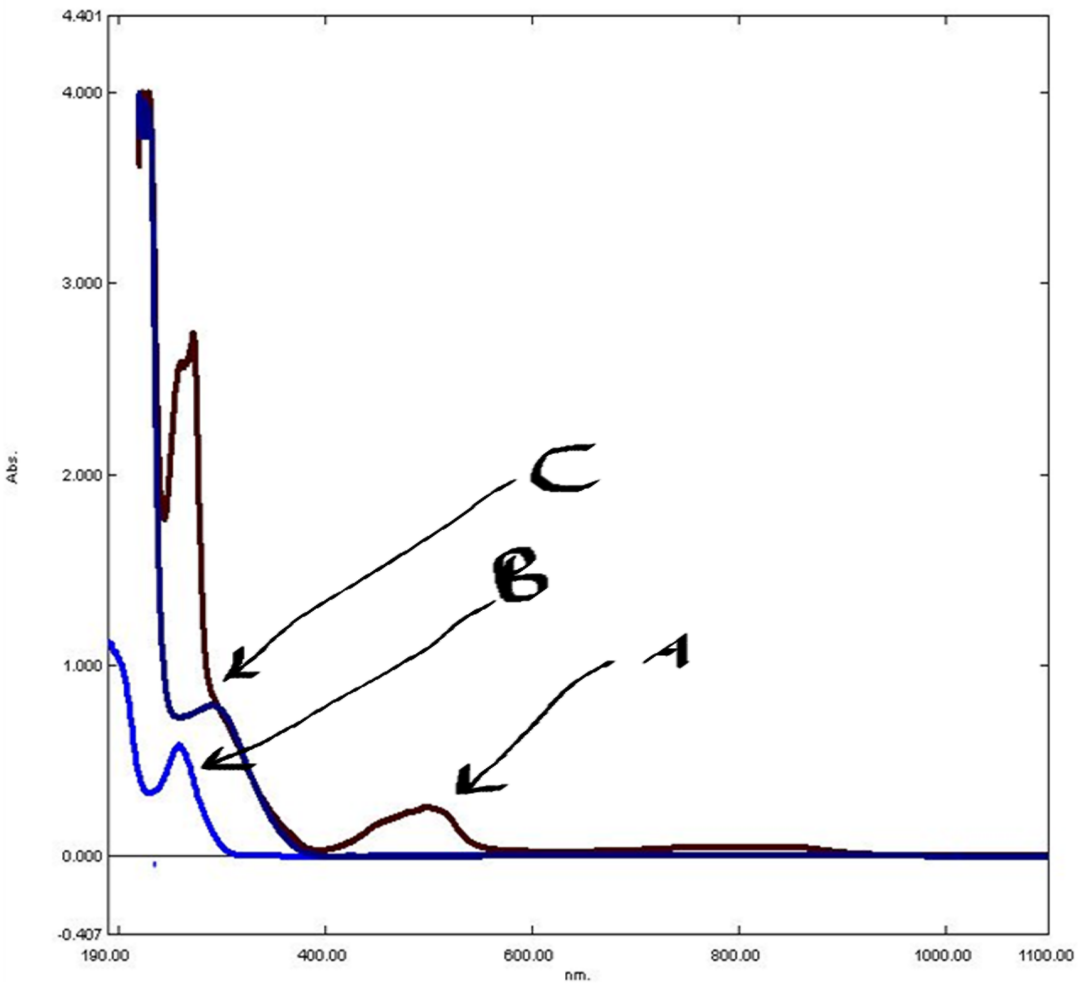


Fig. 2. The spectrum absorbance for 500ppm SMS treated per the suggested method and compared to (A) colored solution and (B) pure medicines. SMS (C) all-natural components, no medications. for optimization, 20mL of final volume containing 500ppm of SMS (20ppm) was employed

Acidic impact

As a result, a few acids, including HCl, CH_3COOH, H_2SO_4, and HNO_3, are examined at 1M as concentrations; the results show every one of the examined acids obtained of the absorbance of the products colored with nitric acid being the best acid that achieves the greatest absorption. The ideal volume was 0.5mL for the acid, which produces a large absorption, that results in a product with a high absorbance (Blazheyevskiy et al.,2022). Figure 3 shows the volume of reagent effect on the reaction.

Effect of reagent concentration

To research the impact of reagent concentration on the absorbance of trifluoperazine hydrochloride (TRF). It was made using 1mL of 500ppm Sulfamethoxazole SMS drug, which was transferred into a series of volumetric flasks with a volume of 25mL. Varying volumes for the reagent 1.5x 10-2M were taken, ranging from 0.1 to 2.2mL, and the volumes were completed to (25mL) by distilled water. For the formation, colored solution was added, and the perfect volume was 0.5 (TRF), which produces a highly absorption, that was utilized in the next experiences and appearing in Fig. 4 (Negoiu et al., 2010).

Effect of reaction time

After responding to the medication SMS with a solution of TRF and ceric acid and stabilizing it for 15min, the product's color intensity was at its highest. In this way, the proposed technique demonstrated that a 15-minute progress window was chosen as the best. Within 24 hours, the hue had stable. This may be seen in Fig 5.

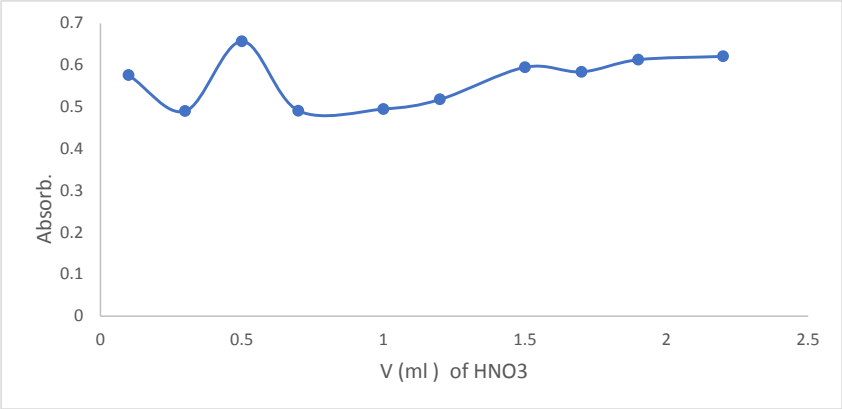


Fig. 3. The volume influence of acid (1M)

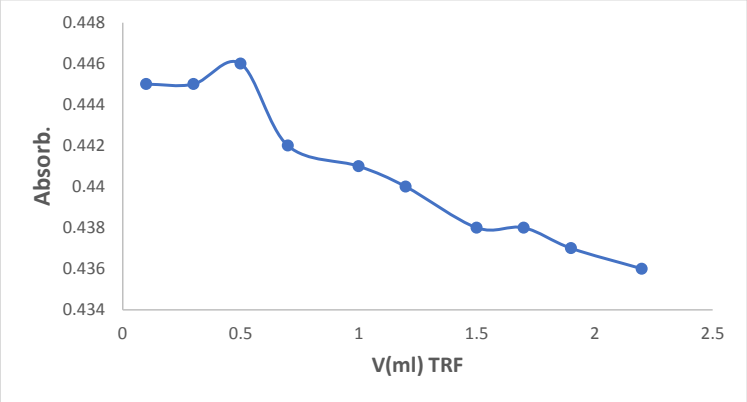


Fig. 4. The volume of reagent effect on the reaction

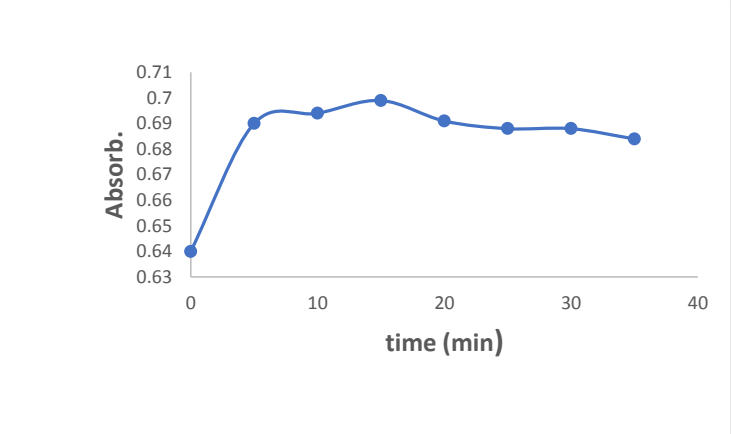


Fig. 5. The time effect of reaction

Calibration graph

The calibration diagram for SMS identification is shown in Fig. 6 under controlled conditions. The graph in the concentration range of 2-38 ppm is linear with a correlation coefficient of 0.9984, a slope of 0.0058, and an intercept of 0.3913. According to calculations, the yellow product's molar absorbance is 1.47 103L mol^{-1} cm^{-1} and its Sandall's sensitivity is 0.0172g cm^{-1}. 0.645g ml^{-1} and 2.15g mL^{-1}, respectively, were the LOD and LOQ. The Average of three concertation of the methods of recovery are 101.89 and RSD are 0.217. Table 2. 3 appears the analytical data for the studied procedure.

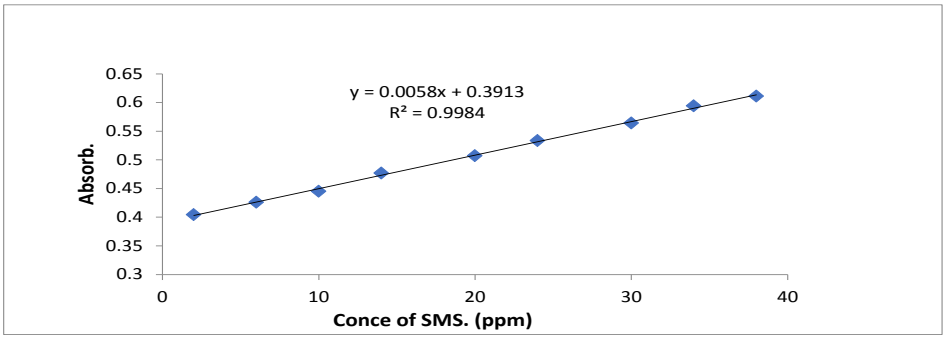


Fig. 6. The SMS calibration curves

TABLE 2. Analytical data for the studied procedure

Parameter	Value
λ max, nm	515
R2, the correlation coefficient	0.9984
(b) Slope	0.0058
Molecular of the absorptivity ($L.mol^{-1}.cm^{-1}$)	$1.47x10^3$
Limitations of Beer Law (ppm)	2 - 38
Sensitivity of Sandell ($g.cm^{-1}$)	0.172
Intercept (a)	0.3913
The quantification limit (LOQ) (ppm)	0.645
The detection limit (LOD) (ppm)	2.15

TABLE 3. The researched method's accuracy and precision

SMS concen. (ppm)		Error%	Recovery%	RSD%
True	Found			
14	14.78	5.5	105.50	0.196
20	20.04	0.2	100.20	0.319
38	37.99	-0.026	99.97	0.136

Stoichiometry and the mechanism

The evaluation of stoichiometry for the reaction between the observed SMS as well as the reagent indicated and, in all situations, the drug/TRF ratio was 1:1 (i.e., 1mole of the drug and 1mole of the reagent reacted). The result from this reaction was a novel ligand that interacted with to create new complex absorption at 515nmm. For this reaction between the created ligand as well as, the molar ratio as well as the continuous variation method also discovered the ratio to be 1:1. Depending on these findings it was presumed that the SMS reactions with (TRF), as well as, proceeded along the direction provided (Al-Abbasi et al., 2011). Figure 7 shows the molar ratio of SMS potential reaction mechanism for the figuration of SMS medicine complexes with TRF. appears in Scheme 1.

Effect of excipients substances

The excipients under examination were Starch, Talc, Lactose, Sucrose, Calcium chloride, Sodium Disulfate, PVP, Sodium Sulfite, lactose, Benzoic acid, Trimethoprim as well as Fructose, which it is founding with SMS in the cream›s dosage forms, there are not effect on the measurements. The study's solution included SMS and each of the ingredients were brought individually were assessed under the same conditions at concentrations 10 times greater than those of SMS. operation on the drug's caliper graph 1mL at 500ppm of the solution and 1mL from each 5000ppm excipient was brought for interference research and mark dilution. an interference level was deemed acceptable if the error was not larger of ± 2 percent compared to the predicted no interference was witnessed in an assessment for SMS in the existence of the learned ingredients (Median of three evaluations) (Table 4) (Ahmed et al., 2018). Table 4 is indicating that the method is not suffering any interference from common excipients and other substances.

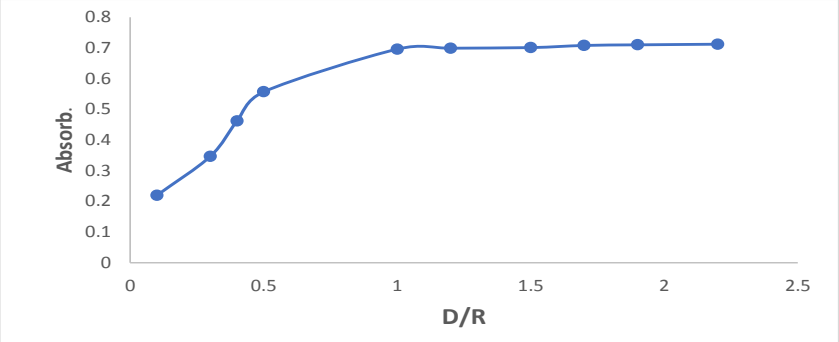
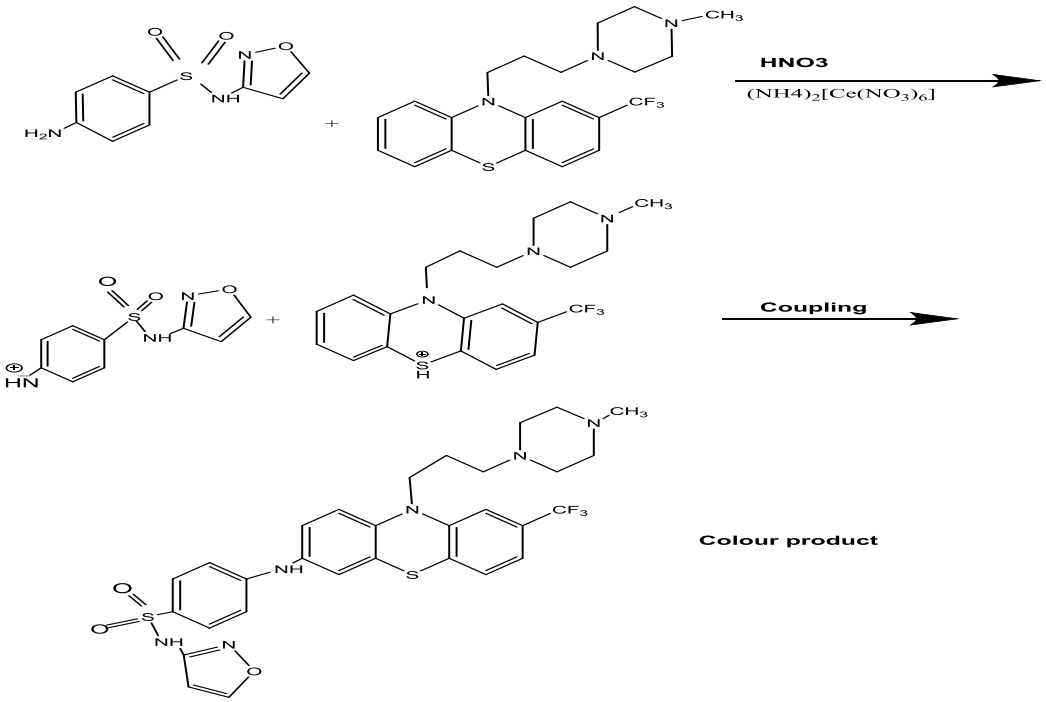


Fig. 7. The mole ratio of SMS



SMS + TRF ----------▶ Color product

Scheme 1. Potential reaction mechanism for the figuration of SMS medicine complexes with TRF by finding the nitric acid and ceric ammonium nitrate

TABLE 4. The impact of excipients substances on the assessment of medicine

Interference	Conc. of SMS found (ppm)	% Error	% Recovery
Calcium chloride	19.777	-1.115	98.88
Sodium disulfite	19.948	-0.26	99.74
Starch	19.931	-0.345	99.65
Talc	19.81	-0.95	99.05
Lactose	19.84	-0.80	99.20
Fructose	19.79	-1.05	98.95
Sodium Sulfite	19.78	-1.10	98.90
PVP	19.96	-0.20	99.80
Benzoic acid	19.92	-0.40	99.60
Sucrose	19.83	-0.85	99.15
Trimethoprim	19.959	-0.205	99.79

Average recovery % and average RSD% of the present methods and standard methods

Tables 5, 6 show application of the study on the different samples the assessment of SMS in oral suspension and tablets dose forms was using the strategy researched as well as compared with the strategy. Figure 8 appears the biological activity of the product compound on the different types of bacteria by study comparison of pure sulfamethoxazole antibacterial activity (inhibition region, mm) toward multidrug resistance bacteria.

TABLE 5. Assessment of SMS in oral suspension and tablets dose forms was using the strategy researched as well as compared with the strategy

NO.	Composition	Present method		Standard method		Values (t), (F)
		% Recovery	% RSD	% Recovery	%RSD	
1-	Pure SMS	101.89	0.217	100.35	0.69	(F) calculated values =1.413 Theoretical values = 6.39 (t) calculated values =0.882 Theoretical values = 2.31
2-	Piotrim	99.90	0.71	99.30	0.56	
3-	Sptrim	100.13	0.59	101.06	0.58	
4-	Metheprim	101.54	0.75	98.70	0.57	
5-	Neotrim	99.29	0.84	99.53	0.74	

TABLE 6. Antibacterial activity of pure Sulfamethoxazole (inhibition region, mm) including three separate Sulfamethoxazole Oral suspension toward multidrug resistance bacteria

Compounds	*S. aureus*	*E. faecalis*	*E. cloacae*	*P. mirabilis*
Pure SMS	20	22	19	21
Piotrim	25	17	18	14
Neotrim	22	20	19	16
Septrin	26	23	20	17
Metheprim	25	22	18	18

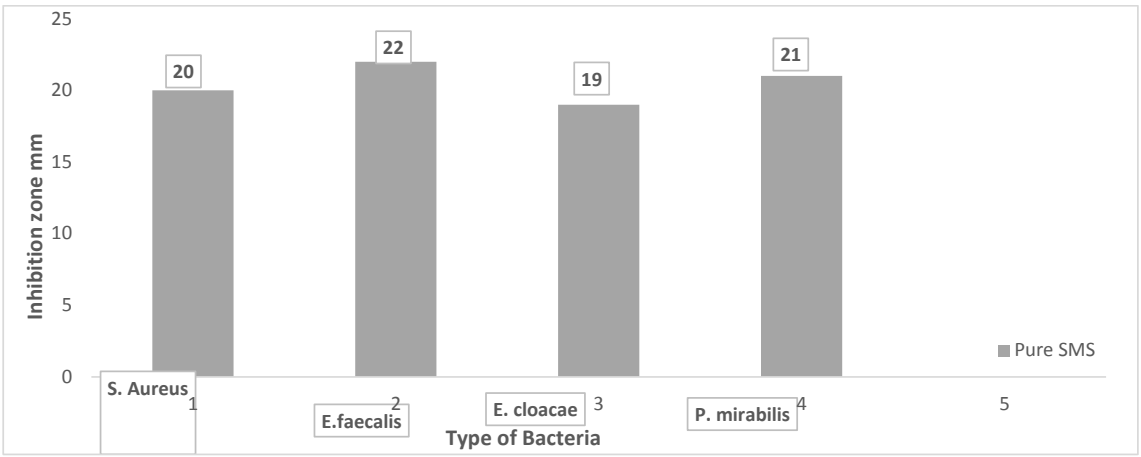


Fig. 8. Comparison of pure sulfamethoxazole antibacterial activity (inhibition region, mm) toward multidrug resistance bacteria

Conclusion

At the time, the spectrophotometric technique was Low sulfamethoxazole concentrations can be determined using a rapid, simple, sensitive, and accurate approach. For the detection of minute quantities of sulfamethoxazole (SMS) in water, a quick, accurate spectrophotometry of the method based on the conjugation reaction at trifluoperazine, and sensitive zine hydrochloride (TRF), in the presence in nitric acid, and an oxidizing agent of ceric ammonium nitrite was developed. The suggested procedure took fifteen minutes to complete at room temperature without the need for a solvent extraction step. It was effectively used to detect stomach infections caused by sulfamethoxazole.

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استخدام هيدروكلوريد Trifluoperazine ككاشف قياس لوني للمعايرة الكمية لعقار سلفاميثاكزازول والتطبيقات على بعض مستحضرات معالجة العدوى المختلفة

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تم تطوير طريقة طيفية سريعة وحساسة لتقدير دواء السلفاميثاكسازول في محلوله النقي وفي مستحضراته الصيدلانية، تعتمد الطريقة على الاقتران التأكسدي مع مركب كاشف منhydrochloride Trifluoperazine في وجود محلول حامضي وعامل مؤكسد إنتاج محلول ملون يظهر اعلى امتصاص عند nm.515 حيث تم دراسة الظروف المثلى للتفاعل باختيار افضل حجم من محلول الكاشف TRFهوmL0.5 وافضل حامض هو النتريك بحجم افضل 0.5mL وافضل عامل مؤكسد هو السيريوم بحجم افضل 3.5mL وتسلسل الإضافة الأمثل هو (حامض النتريك، كاشفTRF، دواء SMS، عامل مؤكسد (ويتم تخفيفه بالماء المقطر إلى 25mL وتكون درجة الحرارة المثلى 25ºC وافضل وقت لحصول التفاعل وتكوين منتج ملون هو 15 min. بعد دراسة الظروف المثلى تم قياس منحني المعايرة وتم حساب المعلومات التالية والتأكد من مطابقتها لقانون بير فب نطاق التركيز 2-38µg.mL وعلى النحو الاتي و كان معامل الامتصاص المولاري 1.47×10^3 L.mol^{-1}.cm^{-1}, وحساسية سيندل 0.172 g.cm^{-1} معامل الارتباط المولي 0.9984 1-وحد القياس الكمي LOQ كانت 2.15 g/ml^{-1} وكان حد الكشف LOD هو 0.645 g/ml^{-1} والانحراف المعياري RSDهو 0.217%, الاسترداد 99-105.5%. واظهرت النتائج مقاومتها للبكتريا بعد اخذ الفعالية البيولوجية للمحلول المحضر وتمت مقارنة الطريقة المقاسة مع الطريقة القياسية ووجدناها اقل من القيم النظرية الاخرى.