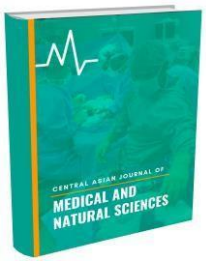

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Article

Spectrophotometric Determination of Sulfamethoxazole in Pure Form and its Pharmaceutical Formulation Using UV-Vis spectrophotometry

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Abstract: A simple and sensitive spectrophotometric method was developed for the determination of Sulfamethoxazole (SMX) in pure and pharmaceutical forms. The method is based on an oxidative coupling reaction between SMX and 2-Naphthol (2-NPH) in an alkaline medium using N-Bromosuccinimide (NBS) as an oxidant, forming a stable red-colored product with maximum absorbance at 500 nm. Reaction conditions were optimized to achieve the highest color intensity and stability. Beer's law was obeyed in the range of 4–20 μg mL^{-1}, with a molar absorptivity of 1.22×10^4 L mol^{-1} cm^{-1} and limits of detection and quantification of 0.1457 μg mL^{-1} and 0.4420 μg mL^{-1}, respectively. The method showed excellent accuracy and precision (recoveries of 99–101%, RSD < 2%) and was successfully applied to Bactrim tablets without interference from excipients. This method is simple, reproducible, and suitable for routine quality control of SMX.

Keywords: Oxidative Coupling Reaction, 2-Naphthol Reagent, N-Bromosuccinimide (NBS), Beer's Law Validation, Analytical Method Development, Pharmaceutical Quality Control, Colorimetric Analysis

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1. Introduction

Sulfamethoxazole (SMX) is a broad-spectrum synthetic antimicrobial agent that belongs to the sulfonamide class, widely employed in the treatment of various bacterial infections such as urinary tract infections, bronchitis, and specific forms of pneumonia. Its mechanism of action is primarily based on the inhibition of bacterial dihydrofolic acid synthesis through competitive antagonism of para-aminobenzoic acid (PABA), thereby interrupting the formation of essential folate cofactors required for DNA replication and cellular growth. Because of its structural similarity to PABA, SMX competes for the active site of the enzyme dihydropteroate synthase, resulting in a bacteriostatic effect against a broad range of Gram-positive and Gram-negative microorganisms[1] Sulfamethoxazole (SMX), a sulfonamide antibiotic commonly combined with trimethoprim, is extensively used to treat bacterial infections of the urinary tract, respiratory system, and gastrointestinal tract. Accurate quantification of SMX in pharmaceutical formulations is essential to ensure drug efficacy and patient safety. Conventional analytical techniques such as high-performance liquid chromatography and capillary electrophoresis provide high accuracy but require expensive instruments and time-consuming sample preparation. Alternatively, spectrophotometric methods employing oxidative coupling reactions offer a simple, rapid, and economical approach for the determination of SMX In this study, an

optimized spectrophotometric method is proposed for the estimation of Sulfamethoxazole based on its oxidative coupling reaction with 2-naphthol in an alkaline medium using N-bromosuccinimide (NBS) as an oxidizing agent[2]. The resulting red-colored product exhibits maximum absorbance at 500 nm[3], enabling accurate and reproducible quantification of SMX in bulk and pharmaceutical dosage forms. a range of advanced methods employing digital and mathematical processing of spectral data have been developed. The success of these techniques largely depends on the extent of spectral interference and the number of components present in the sample[4]. Meanwhile, the pharmaceutical industry has witnessed remarkable advancements in designing combination dosage forms that contain multiple active ingredients. This complexity necessitates the development of precise and sophisticated analytical methodologies capable of accurately separating and quantifying each component. Among these modern techniques, high-performance liquid chromatography holds a pivotal role as a powerful and reliable tool for the separation and analysis of constituents within complex formulations[5][6], to effectively separate and quantify a selected group of decongestants and cough suppressants in their pure forms as well as within multi-component pharmaceutical preparations. After the separation of molecules using HPLC, the next stage is detection, which is most commonly performed using ultraviolet light. Most chemical compounds absorb light at specific wavelengths[7][8The precise quantification of Sulfamethoxazole in pharmaceutical formulations and biological matrices is of critical importance for ensuring dosage accuracy, therapeutic efficacy, and quality assurance. Over the years, several analytical techniques have been developed for this purpose, including spectrophotometry, high-performance liquid chromatography (HPLC), capillary electrophoresis, and electrochemical analysis. Among these, spectrophotometric methods remain particularly advantageous owing to their operational simplicity, low cost, rapidity, and acceptable sensitivity, especially in laboratories with limited instrumental facilities [9]. These values are then used to construct a calibration curve that illustrates the relationship between the molecule's[10] This methodology represents one of the most widely used and precise systems in the bioanalysis of pharmaceutical substances[11].

2. Materials and Methods

Experimental part

Instruments

Apparatus used in the study are shown in Table (1).

Table 1. Apparatus used.

Apparatus Name	Apparatus Type	Origin
UV-Visible Spectrophotometric	T92+Spectrophotometer double beam	China
Sensitive balance	Sartorius BL210 SAG	Germany
Heater	HPL-248	China
Computer	DEL, Windows 7, uv probe 2.34	China
PH Meter	Jenway 3310	China

Reagents and chemical materials

All chemicals used were of high purity as shown in Table (2).

Table 2. Reagents and materials used.

Materials Names	Chemical Formula	Mwt g/mol	Purity%	Company
Sulfamethoxazole	$C_{10}H_{11}N_3O_3S$	253.28	99	
pyridoxine (Vitamin B6)	$C_8H_{11}NO_3$	169.18	99	

Procaine	$C_{13}H_{20}N_2O_2$	236.31	98	
Metol	$C_{14}H_{20}N_2O_6S$	172.19	99	Fluka
2,4-Dinitrophenyl hydrazine	$C_7H_6O_3$	198.14	99	Fluka
4-Bromoaniline	$BrC_6H_4NH_2$	172.02	98	Fluka
2-Naphthol	$C_{10}H_8O$	144.17	99	Fluka
Sulfanilic acid	$C_6H_7NO_3S$	173.19	98	Fluka
4-Aminoantipyrine	$C_{11}H_{13}N_3O$	203.24	99	BDH
4-hydroxy aniline	C_6H_7NO	109.13	99.9	BDH
4-nitro aniline	$C_6H_6N_2O_2$	138.12	99	BDH
m-Aminopheno	C_6H_7NO	109.13	99.9	BDH
2,4-Diaminopyridine	$C_5H_7N_3$	109.13	98	BDH
Ammonium peroxydisulfate	$(NH_4)_2S_2O_8$	228.21	98	BDH
N-Bromosuccinimide	$C_4H_4BrNO_2$	177.98	99.9	BDH
Potassium persulfate	$K_2S_2O_8$	270.33	99.9	Fluka
potassium iodate	KIO_3	214.001	99.9	Fluka
Potassium periodate	KIO4	230.0	99.9	Fluka
Hydrochloric acid	HCl	36.46	37	BDH
Sodium hydroxide	NaOH	40	99	BDH
Potassium hydroxide	KOH	56.106	99	BDH
Sodium carbonate	Na_2CO_3	105.98	99.9	BDH
Sodium nitrite	$NaNO_2$	68.99	99	BDH

Preparation of solution

A. Standard Sulfamethoxazole (SMX) solution 250 μg/ml

It was prepared by defrosting 0.0250g from SMX in distilled water and completed with DW in a volumetric flask of 100 ml. The Standard SMX solution was stable for approximately ten days.

B. Naphthol (2-NPH) 5X10-3 M

It was prepared by defrosting 0.072 g of 2-Naphthol in DW and completed with DW in a flask of 100ml.

C. N-Bromosuccinimide solution(NBS) 3X10-3 M

It was prepared by defrosting 0.0534 g of NBS in 3.0 ml of acetone; then the volume was completed to 100 ml with DW.

D. Sodium carbonate solution, approximate 1X10-2 M

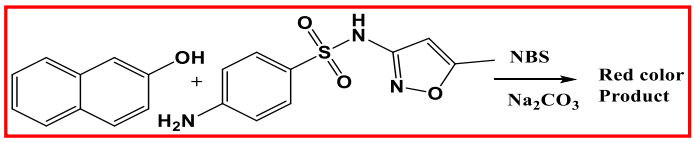
It was prepared by defrosting 0.1059 g of Na2CO3 in 100 ml of DW.

E. Solution of SMX tablet formulation 250 μg/mL

Ten tablets (Bactrim every tablet contains 400 mg SMX) were weighed accurately. After grinding and mixing well, the weight was equal to 3.057g; after that, a weight of 0.019 g of this crushed, equivalent to 0.025g of the drug, and dissolved in DW, the solution was mixed fine, and filtered to 100ml (volumetric-flask). Then, the volume was completed to the sign with DW.

General procedure

The principle of the method is the coupling of the reagent with the SMX drug in the presence of the oxidizing agent in a basic medium where a solution of red color is formed and gives the λmax at 500 nm against the blank solution. Scheme (1) shows the proposed mechanism to produce the red colored dye.



Scheme 1. The proposed mechanism to produce the red colored dye.

3. Results and Discussion

Optimization of the experimental conditions

The effect of diverse variables on the absorbance intensity of the color dye formed from the reaction of SMX with 2-NPH was investigated, and the optimum conditions have been selected as follows:

Selection the best coupling reagent

Different types of coupling reagent have been inspected to select the best reagent that gives the highest color intensity, the results are shown in Table (3), indicate that 2-NPH reagent gives the highest color intensity and a good color contrast in comparison with another reagent. So, this reagent is chosen in ulterior experimental.

Table 3. Selection the best coupling reagent.

Reagent($5x10^{-3}$M)	Variable	max,nmλ	Absorbance
2-Naphthol	SB	500	0.518
	BW	490	0.075
Metol	SB	482	0.375
	BW	387	0.081
2,4-Dinitrophenyl hydrazine	SB	472	0.412
	BW	362	0.062
4-Bromoaniline	SB	421	0.385
	BW	322	0.061

*Where S = The dye, B = Blank, S B: Absorption spectrum SMX. solution vs. Blank

BW: Absorption of blank vs. D.W

Effect of the volume of reagent

A study was carried out to establish the typical amount of reagent solution (2-NPH), which gives the maximum absorption of the colored product by adding diverse volumes (0.5-2.0 mL) of 2-NPH reagent (5x10-3M) to the vials containing 1.0 ml (SMX, 250µg/mL), 2.0 mL (NBS, 3x10-3M), and 1.0 ml (Na2CO3), the volume was completed to 25 mL. Table (4) shows that 1.0 mL of 2-NPH solution is the optimal volume, giving the highest absorbance of the formed product.

Table 4. Effect of the amount of reagent.

mL of Reagent($5X10^{-3}$) M	Absorbance	
	SB	BW
0.5	0.460	0.079
0.8	0.485	0.080
1.0	0.516	0.075
1.5	0.490	0.074
1.8	0.466	0.071
2.0	0.436	0.065

Selection of the best oxidizing agent

Different types of oxidizing agent have been inspected to select the best agent that gives the highest color intensity, the results are shown in Table (5), indicate that the best oxidizing agent is NBS, where it gave the highest absorbance of the colored product therefore it was used in subsequent experiments.

Table 5. Selection the best oxidizing reagent.

Oxidizing agent $3x10^{-2}$	Absorbance		λmax
	Sample	Blank	
N-Bromosuccinimide	0.517	0.076	500
Potassium persulfate	0.395	0.090	490
potassium iodate	0.379	0.051	508
Potassium periodate	0.402	0.071	481

The impact of the amount of NBS

A study was carried out to establish the typical quantity of NBS (3x10-3M) by adding diverse volumes (1.0-2.5ml) of NBS to flasks containing 1.0 ml of SMX solution, and 1.0 ml of 2-NPH reagent solution in the presence of 1.0 ml (sodium carbonate). The volume was completed to 25 ml with DW and measured at wavelength 500 nm. The results are shown in Table (6), indicate that the best volume of oxidizing agent is 2.0, where it gave the highest absorbance of the colored product therefore it was used in subsequent experiments.

Table 6. Effect of the volume of oxidizing agent.

mL of NBS $3x10^{-3}$ M	Absorbance	
	SB	BW
1.0	0.487	0.077
1.5	0.500	0.078
2.0	0.516	0.077
2.3	0.493	0.077
2.5	0.479	0.072

The impact of the base type solution

The coupling reaction between the reagent 2-NPH and the SMX occurs in an alkaline medium, so the effect of various bases was investigated to determine which produced the higher absorbance. From the results in Table (7), it is clear that sodium carbonate gives the highest absorbance. Therefore, it was utilized in trailing experiments.

Table 7. Effect of carbonate type on absorbance.

Base solution	Na_2CO_3	NaOH	KOH
Absorbance	0.515	0.412	0.362

The impact of the amount of sodium carbonate solution

A study was carried out to establish the typical amount of base solution by adding diverse volumes (0.5-2.0ml) of sodium carbonate; it was found that 1.0 ml of sodium carbonate gives the highest (Abs) at pH=10.6. So, the volume was adopted in the later experiments. The outcomes are shown in Table (8).

Table 8. The impact of the amount of sodium carbonate solution.

ml of Na_2CO_3 ($1x10^{-2}$)	Absorbance		
	SB	BW	PH
0.5	0.473	0.075	8.2
0.8	0.495	0.076	9.7
1.0	0.519	0.078	10.6
1.3	0.488	0.078	11.4
1.5	0.469	0.077	11.7
2.0	0.454	0.076	12.1

Effect order of addition

The order of addition was inspected to choose the best sequence that gives maximum absorption of the colored product. The sequences shown in Table (9) were selected, and it was found from the recorded results that the order No. 2, NBS (O), SMX Solution (E), sodium carbonate solution (B), and 2-NPH solution (R) the best addition sequence. So, it was chosen in subsequent experiments.

Table 9. Effect of sequences of addition.

Order number	Order Of addition	Absorbance
1	O + R + E + B	0.490
2	O + E+ B + R	0.517
3	E + R + O + B	0.488
4	E + O + R + B	0.496
5	R + B + E + O	0.470
6	O + B + E + R	0.420

(E) Drug solution, (R) Reagent solution, (O) Oxidizing solution, (B) Base solution

Effect of Time

The time required to complete the oxidative coupling reaction for SMX drug was studied when the solutions were left; after adding The reagent solution, oxidizing agent solution, and the base solution for different periods, then the process was carried out dilution, the absorption intensity of the solution was measured against its blank solution, Table (10) indicate that the best time required to complete the coupling process was 5.0 minutes.

Table 10. Effect of Time on Absorbance of Colored Product.

Time/min	2	3	5	10	15	20
Absorbance	0.486	0.500	0.517	0.517	0.517	0.516

The impact of temperature

The impact of temperature in the range (5.0-40°C)on the absorbance of the formed dye has been examined, it was found that a temperature (25°C) gave the finest absorbance. Therefore, the later experiments were carried out at this temperature, the outcomes are shown in Table (11).

Table 11. Effect of Temperature.

Temperature°C	Abs.
5.0	0.462

10	0.479
15	0.491
20	0.503
25	0.516
30	0.505
35	0.488
40	0.472

The impact of time on the steadily of the formed dye

The stability of the formed dye was probed by examining the effect of time on the absorbance of concentrations (10μg/mL) of SMX according to the procedure of the proposed method. The results in Table (12), show that the red color dye is stable for 50 minutes.

Table 12. The impact of time on the steadily of the formed dye.

Time(min)	Absorbance (10μg/ml)
2	0.488
3	0.502
5	0.518
10	0.518
15	0.518
20	0.517
25	0.517
30	0.516
35	0.516
40	0.515
45	0.515
50	0.513
60	0.486

The eventual absorption spectrum

The eventual absorption spectrum was measured by using 1.0 ml of SMZ (250μg/ml), 1.0 ml of 2-Naphthol (5x10-3M), and 2.0 ml of NBS (3x10-3M) in the presence of sodium carbonate solution (1x10-2) at a temperature of 25°C and the solution was left for 5.0 minutes to complete the reaction. In a volumetric flask, the volume was completed to 25 ml; the absorption was measured against the blank solution; it was found to give a higher Abs at (500 nm), while its blank gave a little absorption at the same wavelength. The results are shown in Figure (1).

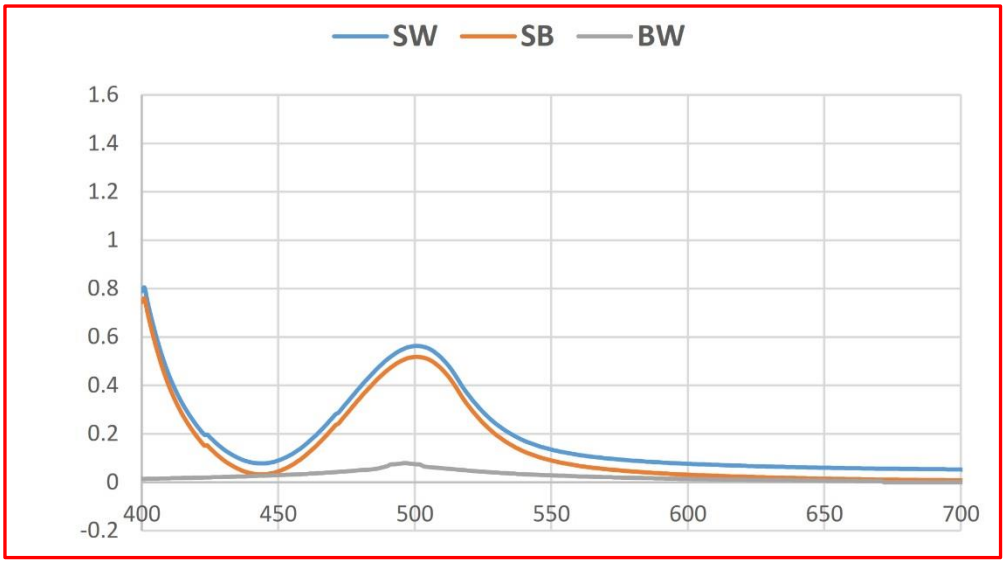


Figure 1. The eventual absorption spectrum for the determination of SMX (10µg/mL) against distilled water (SW), SMX against blank solution (SB), and Blank solution against distilled water (BW).

Table 13. The optimum conditions of the developed method.

Optimum conditions	Parameter
λ max	500 nm
Amount of 2-Naphthol reagent ($5x10^{-3}$)	1.0 mL
Amount of NBS oxidizing agent ($3x10^{-3}$)	2.0 mL
Amount of sodium carbonate ($1x10^{-2}$)	1.0 mL
Temperature	25°C
Solvent	Distilled water

The calibration graph

After selecting the optimized experimental conditions shown in Table (13), SMX solution (4-20 µg/mL) was rushed to a series of flasks(25 mL), thereafter 1.0 mL of 2-NPH reagent, 2.0 mL of NBS oxidizing agent solution, and 1.0 mL of sodium carbonate, the solutions have been left for 5.0 minutes to complete the reaction, and then the volumes were completed to the sign with DW. The absorbance of the solutions was measured against the blank solution at a wavelength (500 nm). Figures (2) and (3)show that the calibration curve is linear over the concentration range (4-20 µg/mL), while higher concentration shows a negative deflection from Beer's law. The molar absorption coefficient was 1.2219 x104 L.mole-1.cm-1, and the Sandel sensitivity value was 0.0207 µg/cm2.

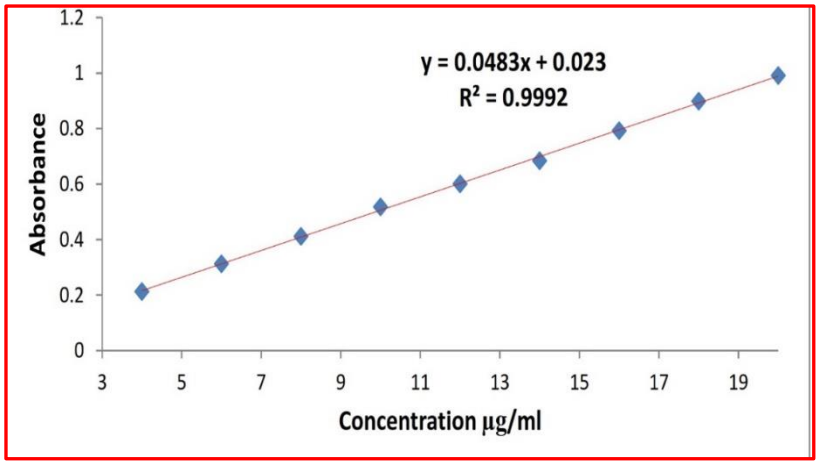


Figure 2. The calibration graph for the SMX drug.

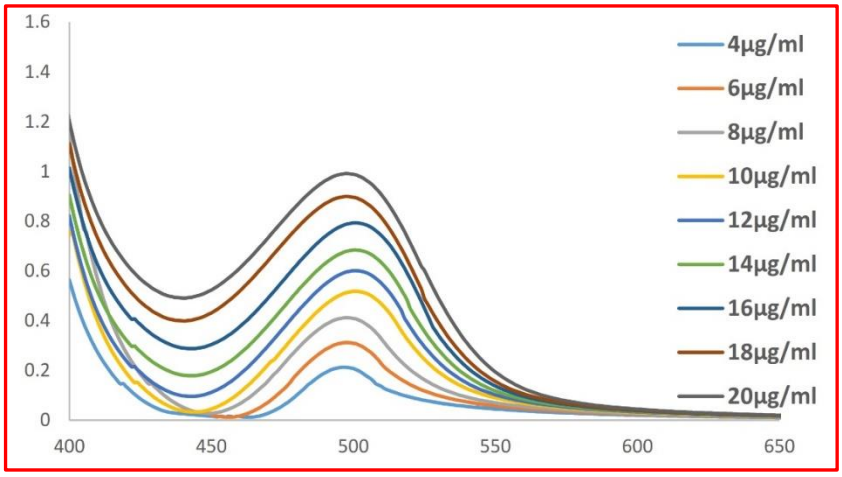


Figure 3. Calibration curve spectra for determination of SMX by oxidative coupling with 2-Naphthol.

The precision and accuracy

The precision and accuracy of the method were examined by metering the recovery%, and precision through the relative standard deviation (RSD%) and the relative error (RE%) For three different concentrations (4.0,10, and 15) µg/mL (measuring the absorbance of six determinations) at wavelength 500 nm for each concentration and taking the average for it. The findings in Table (14), showed that the technique for determining SMX had acceptable precision and accuracy.

Table 14. The results of precision and accuracy.

Amount of SMX µg/ml		*RE%	*Recovery%	*RSD%
Taken	Measured			
4	4.04	1.0	101.0	0.852
10	10.06	0.60	100.60	2.936
15	15.03	0.20	100.20	1.513

*Average of six determinations

Limit of detection (LOD) and Limit of quantitation (LOQ)

The (LOD) was calculated by measuring the absorption of the blank solution [12] at optimized condition (six determinations) at wavelength 500 nm by using the following equations:(2-1,2-2) LOD= 3.3 SD/b, LOQ= 10 SD/b [13][14] Where (SD) is the standard deflection and (b) is the slope of the calibration graph. The outcomes are shown in Table (15).

Table 15. LOD and LOQ values.

Absorbance of blank *	Standard deflection (SD)	Slope (b)	LOD	LOQ
0.076	0.002135	0.0483	0.1457µg/ml	0.4420 µg/ml

* Six determinations

The nature of the formed product

Job's method and molar ratio method were applied to know the nature of the formed product (stoichiometry of drug with the reagent) [15]. In both methods, the concentration of each of the standard SMX solution and 2-Naphthol reagent solution were equal to 9.87 × 10-4M.

The continuous variation method (Job's method)

In a sequence of flasks (25ml), diverse volumes of the SMZ drug ranging from (1-9ml) and diverse volumes (9-1mL) of 2-Naphthol reagent solution were mixed, 2.0 mL (NBS), and 1.0 mL (sodium carbonate solution) was added, and then completed to the mark with DW. The (Abs) was measured at 500 nm against the blank solution. The results in Figure (4), showed that the proportion is 1:1.

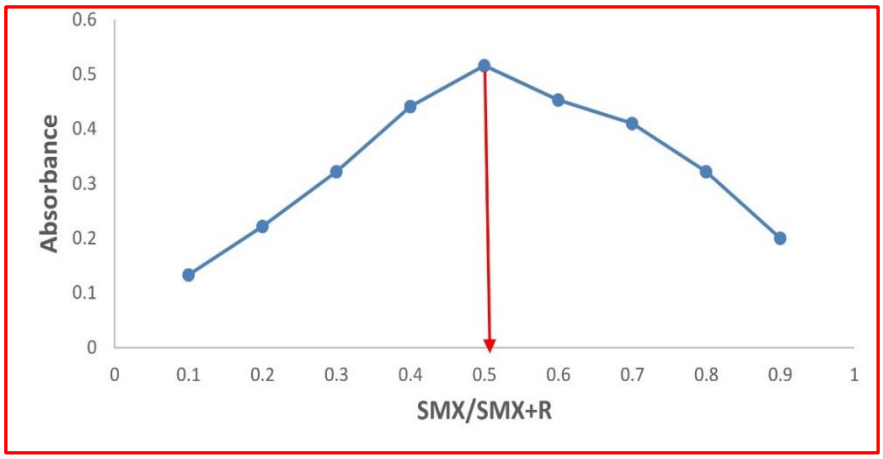


Figure 4. Job's method for the product formed by oxidative coupling.

Molar ratio method

In a series of volumetric flasks containing 1.0 mL of the standard drug solution (9.78×10-4M), diverse volumes (0.3-2.5) mL of 2-Naphthol reagent solution in the same concentration of SMZ then 1.0 mL of NBS (3×10-3 M), and 1.0 mL (1×10-2 M) of sodium carbonate solution were added. The volumes were completed to 25ml with DW, and the absorbance was measured at 500 nm against the blank solution. The results in Figure (5) agree with the Job's method results.

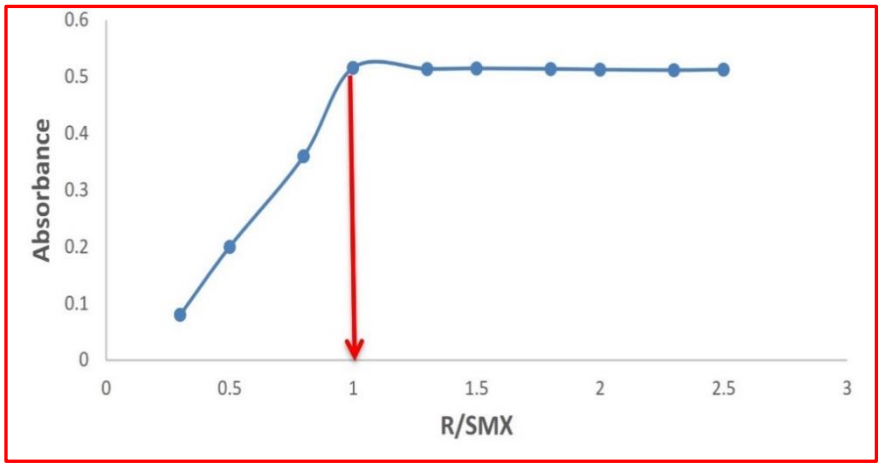
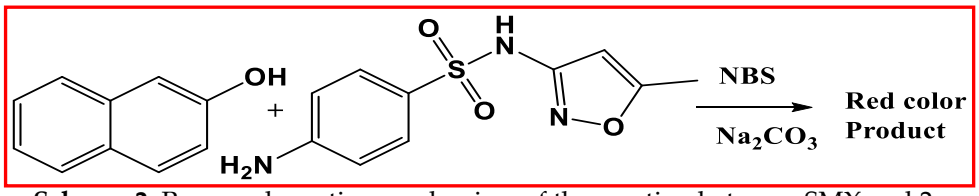


Figure 5. Molar ratio method for the product formed.

(Scheme 2) shows the suggested mechanisms for the red dye formed by the reaction of SMZ with the 2-Naphthol.



Scheme 2. Proposed reaction mechanism of the reaction between SMX and 2-NAPH.

Applications

The methods were applied to a pharmaceutical formulation containing SMX drug, which is the pharmaceutical formulation (Bactrim) in the form of tablets and each tablet contained (400 mg) of the SMX.

Direct method

In this method, it was taken three different concentrations (4, 10, and 15 µg/mL) of the pharmaceutical formulation were conveyed to 25 mL of the volumetric flasks, the solutions transaction as in the construction of a calibration graph, and the absorbance was measured at wavelength 500 nm (six determinations), relative error(RE%), recovery(Rec%), and relative standard deviation were calculated. The outcomes are shown in Table (16).

Table 16. The results of the direct method for the identify of SMX in pharmacological compositions.

Conc. µg/mL Taken	Conc. µg/ml Measured	*RE%	*Rec%	*RSD%
4	3.95	-1.25	98.75	0.560
10	10.11	1.10	101.1	0.360
15	14.77	-1.53	98.47	0.285

*Average of Six determinations

Standard addition method

Standard addition was applied to demonstrate that the method was devoid of interferences. The addition of constant volumes (0.4,1.0 mL), which is equivalent to (4.0,

and 10 µg/mL) of pharmaceutical solution in two series of six volumetric flasks of 25 mL, then adding increasing volumes (0.2 - 1.0 mL) of SMX solutions, and then the absorption was measured against the blank solution at the wavelength 500 nm. The findings are listed in Table (17), and Figure (6).

Table 17. Standard addition method.

Conc. µg/ml Taken	Conc. µg/ml Measured	*RE%	*Recovery %
4	3.88	-3.0	97.0
10	9.94	-0.6	99.4

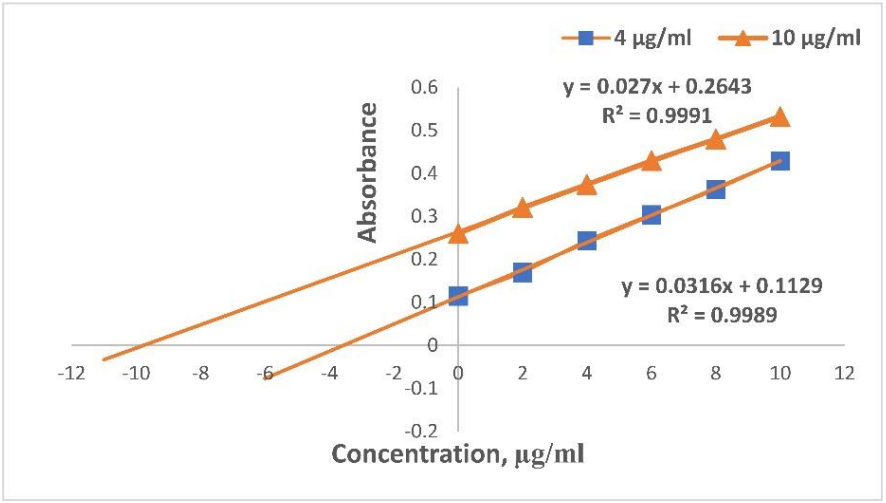


Figure 6. Standard addition curve for determination of SMX in pharmaceutical formulation tablets.

4. Conclusion

In this study, a simple, accurate, and sensitive spectrophotometric method was successfully developed for the determination of Sulfamethoxazole (SMX) in pure and pharmaceutical forms. The method is based on an oxidative coupling reaction between SMX and 2-Naphthol in an alkaline medium using N-Bromosuccinimide (NBS) as an oxidizing agent, forming a stable red-colored product with a maximum absorbance at 500 nm. The reaction conditions were systematically optimized to achieve maximum color intensity and stability, resulting in a linear response within the concentration range of 4–20 µg mL^{-1} that follows Beer's law. The calculated molar absorptivity (1.22 × 10^4 L mol^{-1} cm^{-1}), low limits of detection (0.1457 µg mL^{-1}) and quantification (0.4420 µg mL^{-1}), and high recovery values (99–101%) confirm the high sensitivity and accuracy of the proposed method. Moreover, the procedure exhibited excellent reproducibility with RSD values below 2% and was successfully applied to commercial Bactrim tablets without interference from excipients. Therefore, this method can be reliably recommended for routine quality control analysis of Sulfamethoxazole in pharmaceutical preparations, offering a cost-effective and time-saving alternative to more complex instrumental techniques.

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