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Azo Coupling Reaction for Spectrophotometric Determination of Sulfanilamide Using β -Naphthol as a Reagent

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ABSTRACT

This research is based on one simple and good method for determining sulfanilamide in its pure state and in pharmaceutical preparations. Spectrophotometric determination of Sulfanilamide using the azo coupling reaction in an aqueous solution and several standard solutions were prepared. The optimal conditions were also studied. The effects of the type of acid and its volume, the influence of the base type and its volume, the effect of reagent volume (100 µg/ml), the effect of reaction time on the color stability of the product, the effect of adding sequence, the solvent effect, and the effect of temperature on the formation and stability of the colored product. The product for the Sulfanilamide drug is auburn at a wavelength of 489 nm; the method obeyed Beer's law in a range of concentrations ranging from 1–12 µg/ml; and the molar absorptivity was 28840.76 L/mol.cm. Sandal's sensitivity (0.00091 µg/cm), detection limit (0.2472 µg/ml), correlation coefficient value 0.9984, and RSD% value 0.01365, By applying the method to a pharmaceutical preparation containing Sulfanilamide, the recovery value ranged between 102.1306%.

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1. INTRODUCTION

One of the most commonly used antibiotics worldwide is sulfonamide [1]. It is an organic molecule composed of aniline that is produced from the sulfonamide group chemically. It is mostly used as an antibiotic for sulfanilamide and to treat vaginal infections [2]. β-naphthol is an organic compound belonging to the class of aromatic alcohols [3]. It is a colorless (or sometimes yellow) crystal compound that can become black when exposed to light. Its melting point is 121 to 123 °C, and its boiling point is 285 °C. It is soluble in simple alcohol, ethers, and chloroform. [4]. Diazonium salts are generally prepared by treating aromatic amine with nitrous acid and increasing mineral acid [5].

Aromatic and fate amines can form diazonium salts and are stable. High at low temperatures, but the most stable compounds are aromatic as part of the benzene ring that possesses resonance. Nitrification is usually done at a very low temperature, below zero degrees Celsius. Numerous researchers have calculated sulfanilamide in aquatic systems[6].

2. Experimental

2.1 Material: All chemicals were used through this work and purchased from SDI, Merck, and BDH Companies.

2.2 Devices used: UV-visible spectrophotometric Shimadzu, 800, Japan. Water bath - Velp Scientific made in Europe. Center fudge - Geemy Company plc-03, Taiwan. Electrical balance - Kern&SOHN GmbH, China.

2.3 Preparation of standard solution:

1. A standard solution of sulfanilamide (1000 µg/mL) was prepared by dissolving (0.1 g) in a volumetric flask (100 ml) and filling the volume to the mark with distilled water.

2. The standard solution of the reagent (β-Naphthol) at a concentration of 1000 µg/mL was prepared by dissolving (0.1 g) in 20 ml of ethanol. After dissolving, it was added in a volumetric flask (capacity 100 mL) and completed to the mark with distilled water.

3. Sodium nitrite (1% w/v) was prepared by dissolving (1 g) in water in a volumetric flask of capacity (100 mL). Fill the volume with distilled water up to mark.

4. Preparation of sulfamic acid (1% w/v) by adding 1 gm of sulfamic acid to water in a volumetric flask of 100 mL capacity, then supplement with distilled water to mark

2.4 Spectrophotometric determination of Sulfanilamide using azo coupling reaction in aqueous solution

In the initial experiments, the product of the nitrification reaction was prepared by adding 1 mL of 100 µg/mL of sulfanilamide in a volumetric flask of 10 ml capacity in an ice bath with 1 mL of hydrochloric acid at a concentration of 1 M and 1 ml. 1%) of sodium nitrite with shaking for 10 minutes, then adding 1 mL of 1%) sulfamic acid to remove the excess sodium nitrite. After five minutes of shaking in an ice bath, add 1 mL of 100 µg/ml of the reagent β-Naphthol directly, followed by the addition of 1 mL of 1 M potassium hydroxide for the coupling reaction, then leave in an ice bath for 20 minutes, then we complete the volume with distilled water to the mark. To produce a colored complex that differs from the blank solution prepared with the same additives but without the drug. Several factors that affect the absorption of the resulting azo formulation were studied to obtain the best sensitivity and detection limit for determining the drug, and these conditions were analyzed with a wavelength according to the drug used [7]. The wavelength of the colored complex Sulfanilamide was 489 nm. Sulfanilamide gives the greatest absorption at a temperature of 30 °C, as when the temperature rises, the absorption begins to decrease, which is attributed to the disintegration of the product, and this can be seen from the strength of the color**.**

3. Results and Discussion

3.1 Spectrophotometric determination of Sulfanilamide using azo coupling reaction in an aqueous solution

Using the coupling reaction of the azo dye in the aqueous solution, the absorption spectrum of sulfanilamide was recorded for the materials under investigation, while solutions containing 100 µg/mL of sulfanilamide were generated with the water solvent. The colored complex solution produced a high absorbance of (1.342) at the wavelength (489) nm when these solutions were scanned using visible and ultraviolet spectroscopy within a wavelength range between 200 and 800) nm for the drug. The recorded wavelengths were then used as the greatest wavelength for all subsequent measurements of materials, as shown in Figure 1 [8].

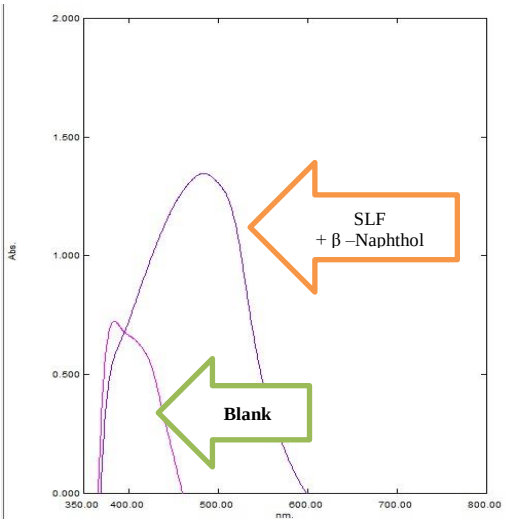


Figure (1)Absorption spectrum of Sulfanilamide drug(100)µg/mL in conjunction with β-naphthol(100)µg/mL detector versus Blank solution in aqueous solution

3.2 Study the optimal conditions for the reaction:
Several tests have been carried out to investigate the impact of various parameters on the spectral evaluation of the medication utilized in this investigation.

3.2.1 Effect of the type of acid:

A series of experiments were used in this study to prepare acid solutions at a concentration of 0.5 M. The results obtained are summarized in Table 1, which unambiguously demonstrate that hydrochloric acid at a concentration of 0.5 M is the optimal acid for sulfanilamide because it gave the highest absorbance (0.492) [9].

Table (1): Absorption data for the Effect of the acid type

1 ml from 0.5M different acids	HCl	H_2SO_4	HNO_3	H_3PO_4	CH_3COOH
Absorbance at λmax = 489 nm	0.492	0.318	0.401	0.452	0.414

3.2.1.1 The effect of acid volume:

To conduct this investigation, many trials were set up using varying quantities of hydrochloric acid at varied concentrations (0.5 M).The results are compiled in Table (2), which unequivocally demonstrates that 0.6 ml of hydrochloric acid at a concentration of 0.5 M is the ideal acid volume for colored azo dye. It illustrates how the absorbance rises with increasing acid volume and then abruptly falls as a result of the principal amine (drug) becoming. inactive as it produced the maximum absorbtion (0.553). Subsequent trials using 0.6 ml of hydrochloric acid and sulfanilamide showed that this was the optimal volume for maximum absorption [10].

Table (2) The effect of acid volume on the absorbance of the colored product of SLF

V of 0.5M acids	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
Ab at λ max = 489	0.526	0.528	0.532	0.539	0.546	0.553	0.541	0.436	0.427	0.512

3.2.2 The influence of the base type:

Table 3 represents the effect of different alkaline solutions (0.5 M) that were studied. It was found that KOH was the most suitable alkaline medium for maximum absorbance (0.657) and was used in all subsequent experiments [11].

Table (3): Effect of the type of base on the absorbance of the colored products of (SLF)

1ml from 0.5M base	NaOH	KOH	NH_4OH	Na_2CO_3	K_2CO_3	$NaHCO_3$
Ab at λ max = 489	0.512	0.657	0.536	0.484	0.411	0.479

3.2.2.1 The effect of the ideal volume of (0.5 M) of (KOH):

Potassium hydroxide concentrations of 0.5 M were used to prepare volumes ranging from 1 to 10 milliliters of the base. The data obtained were reported in Table 4, which demonstrate that, given the maximum absorption (0.780), 0.3 mL of potassium hydroxide base at a concentration of 0.5 M is the optimal base volume for the medication sulfanilamide.

Table (4): Effect of base size on the absorbance of the stained product of Sulfanilamide

V of 0.5M bases	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
Ab at λ max = 489	0.764	0.766	0.780	0.764	0.733	0.696	0.682	0.673	0.668	0.657

3.2.3 The effect of the optimal volume of 1% of sodium nitrite:

The effect of variable volumes of the solution's sodium nitrite (1%) was examined on the maximum absorbance of the formed product. Table 5 demonstrates that 0.5 mL is the optimal volume to obtain the maximum absorbance[12].

Table (5): Effect of 1% volume of sodium nitrite on the absorbance of the colored product of Sulfanilamide

V of 1% Sodium Nitrite	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
Ab at λ max = 489	0.812	0.828	0.851	0.863	0.876	0.835	0.814	0.797	0.789	0.780

3.2.4 Effect of 1% volume of sulfamic acid

Sulfamic acid was extracted in different quantities from the solution at a concentration of 1%. The data are shown in Table 6,, which unequivocally demonstrates that 0.3 mL of sulfamic acid at a concentration of 1% is the optimal volume for obtaining the maximum absorbance for colored products (0.928).

Table (6): Effect of 1% volume of sulfamic acid on Sulfanilamide

V of 1% Sulfamic acid	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
Ab at λ max = 489	0.916	0.920	0.928	0.901	0.898	0.895	0.891	0.886	0.881	0.876

3.2.5 Effect of reagent volume (100 µg ml-1):

Sulfanilamide was added to varying quantities of β-Naphthol reagent at a concentration of 100 µg mL-1. The outcomes are shown in Table 7, which unambiguously demonstrate that 0.5 mL is the optimal volume of β-Naphthol reagent for sulfanilamide, as it produced the greatest absorbance (0.996). Reagent volume increases, causing absorption to rise, but when the volume is increased, absorption falls because the volume is unsuitable for conjugation with the drug. [13, 14].

Table (7): The effect of the reagent volume at a concentration (100 µg ml^{-1}) on the absorbance of Sulfanilamide

V of (100 µg ml^{-1}) Reagent	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
Ab at λ max = 489	0.894	0.916	0.935	0.973	0.996	0.984	0.945	0.937	0.930	0.92

3.2.6 The effect of reaction time on the color stability of the product:

The reaction time of the colored product (5–65) minutes was investigated, and high absorbance was obtained when the color was developed at 50 minutes (1.273). [15]

Table (8): Effect of reaction time on the stability of the colored product of MCH

Time (min)	5	10	15	20	25	30	35	40	45	50	55	60
Ab at λ max = 489	0.897	0.943	0.982	0.998	1.1 4 3	1.166	1.204	1.215	1.221	1.273	1.182	1.135

3.2.7 Effect of adding sequence

Different additive sequences were studied. A high absorbance was obtained at sequence (1); the results are displayed in Table 9 [16].

Table (9): Shows the Effect of the addition sequence

No.	1	2	3	4	5	6
Addition	D+H+N+S+R+B	R+H+N+S+D+B	D+H+N+B+R+S	D+B+R+N+H+S	R+B+D+H+N+S	R+H+N+B+D+S
λmax=489	1.273	0.421	0.875	0.181	0.143	0.217

D: (Sulfanilamide), H: (hydrochloric acid), S: (sulfamic acid), N: (Sodium nitrite), R: (β -Naphthol), B: (Potassium hydroxide).

3.2.8 Solvent effect:

A variety of solvents were used, including acetone, 1-propanol, ethanol, methanol, and acetonitrile. The data are displayed in Table (10), which unequivocally demonstrate that water is the ideal solvent for the azo-dye product (1.273). Due to its affordability and ease of acquisition, water solvent is regarded as one of the best solvents. It is also regarded as one of the solvents that are safe and environmentally friendly [17].

Table (10): Effect of the solvent on the absorption of the colored product of Sulfanilamide

No.	1	2	3	4	5	6
Solvent	Water	Ethanol	Methanol	Acetonitrile	1-Propanol	Acetone
λmax=489	1.273	0.934	0911	0.643	0.952	0.865

3.2.9 The effect of temperature on the formation and stability of the colored product:

This investigation was carried out at various temperatures and through a series of trials. The data were compiled in Table (11), which unequivocally demonstrate high absorbance was obtained when the color was developed at a temperature of 30 °C. The strength of the color indicates that absorption starts to diminish as temperature rises, which is thought to be caused by the product dissociating [18].

Table (11): Effect of temperature on the absorption of Sulfanilamide

Temp.C^{O}	5	10	15	20	25	30	35	40	50	60
Abs of SLF	0.931	1.083	1.182	1.232	1.268	1.281	1.272	1.209	1.163	1.104

3.3 The nature of the resulting product

3.3.1 Method of Continuous Variation (Job's Method)SLF(4×10-4) β-naphthol(4×10-4)

Figure (2) shows that a 1:1 azo dye was formed between SMZ (D) and β-naphthol (R) [19].

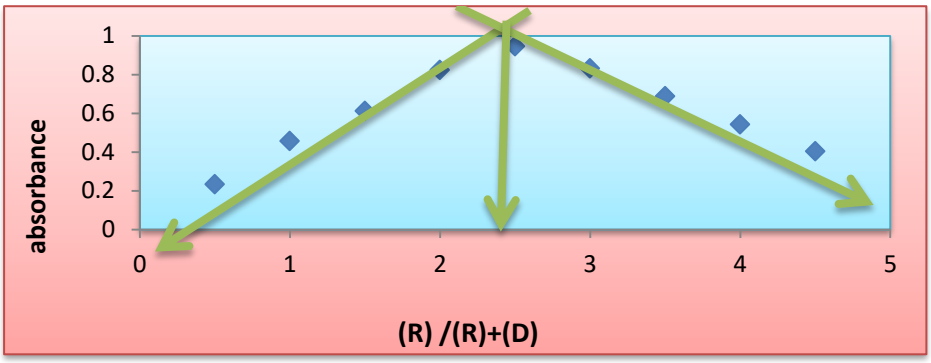


Figure (2): The method of continuous changes (JOB) for Sulfanilamide

3.3.2 Molarity method:

The findings were plotted and shown in Figure 3, indicating the existence of a 1:1 ratio of SMZ (D) and β-naphthol (R) [20].

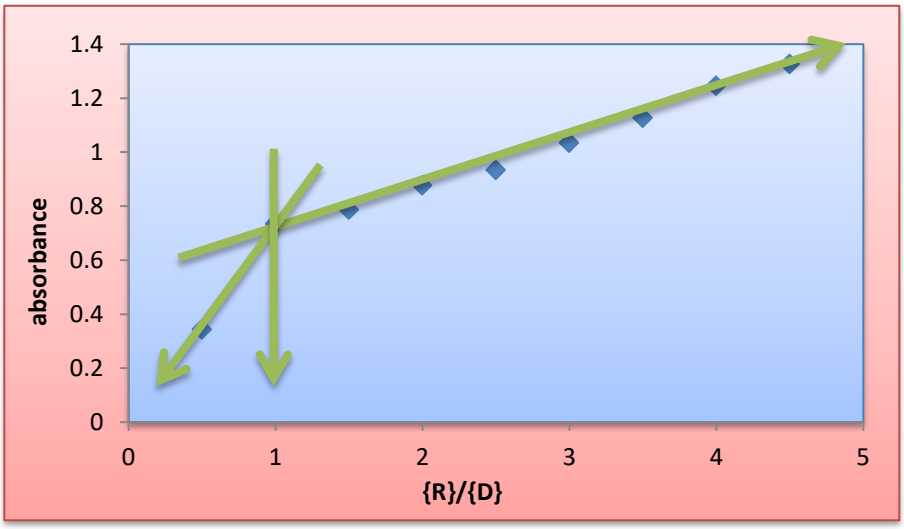
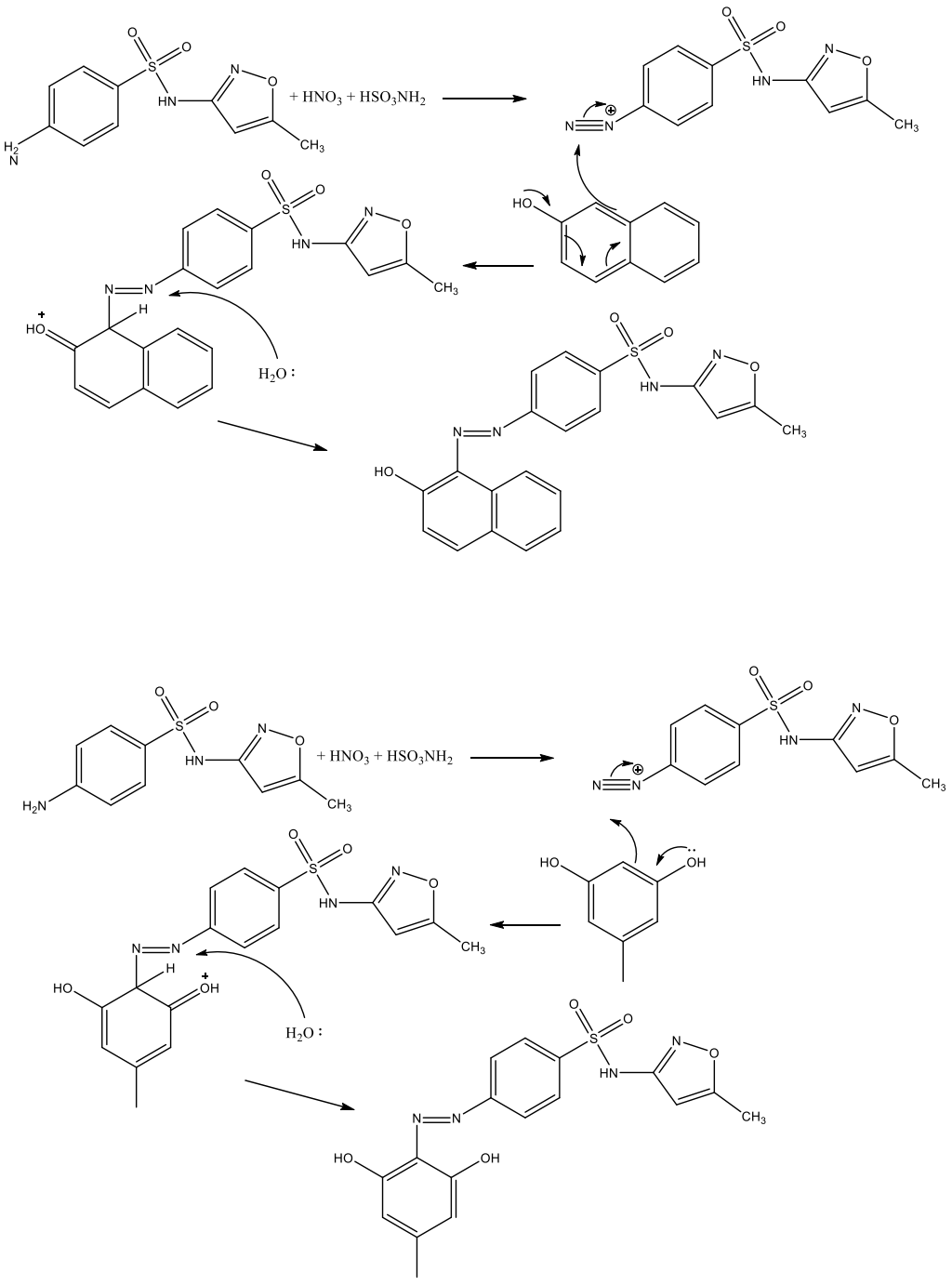


Figure (3): Curve of the SLF drug molar ratios method



Scheme (1): Proposed mechanism for the formation of the colored product of Sulfanilamide

3.4 Calibration curve for Sulfanilamide complexed with β-naphthol:

The following were placed in multiple 10-milliliter volumetric vials: (0.6 milliliters of hydrochloric acid, (0.5 milliliters of sodium nitrite, (0.3 milliliters of sulfamic acid, and (0.5 milliliters of β-naphthol), (0.3 milliliters of potassium hydroxide), and variable volumes of (SLF) drug ranging from (0.1–1.2) ml with concentrations (1–12 µg/ml). After using distilled water to the appropriate level, the absorbance is measured at the highest wavelength in comparison to the blank solution. The calibration curve for the drug (SLF) in the (1–12) µg mL–1 concentration range, which complies with Beer's law, is depicted in Figure 15. This substance has a molar absorption coefficient of 190005.48 L/mol and a sensitivity of 0.00091 µg/cm2 [21-22].

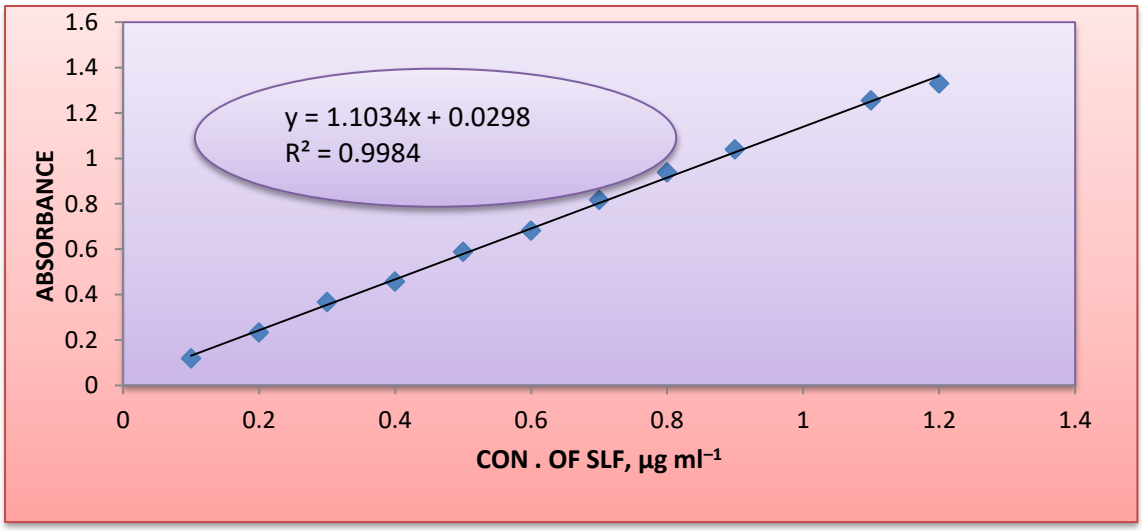


Figure (4): Calibration curve for (SLF) drug

3.5 Interference effect:

Each of the interfering substances—lactose, starch, talc, glucose, calcium phosphate, calcium carbonate, cobalt chloride, and gum Arabic—was employed in one milliliter (1 ml) and at a concentration of 1000 mg/ml in combination with one milliliter (100 ppm) of each of the two medications to determine the effects of the substances on the drug. After the additive is supplied in its appropriate quantities, the remaining amount is diluted in a 10-ml volumetric vial with distilled water. Next, the absorbance of sulfanilamide azo-dye is measured at 489 nm. Table 12 presents the results for sulfanilamide azo-dye. The table's data demonstrate none of these substances interfered with sulfanilamide in pharmaceutical preparation interactions [23-24].

Table (12): Effect of Interactions on the Absorption of Sulfanilamide

No.	100ppm interference	Abs.	Recovery %	Erel%
1	Lactose	0.653	96.884	-3.2159
2	Starch	0.662	92.284	-1.1812
3	Arabic Gum	0.664	98.516	-1.506
4	Glucose	0.671	99.554	-0.447
5	Talc	0.667	98.961	-1.049
6	$Ca_3(PO_4)_2$	0.625	92.729	-7.84
7	SLF	1.342	102.1306	2.130555
8	Trimethoprim	0.661	98.071	-1.966
9	$COCl_2$	0.632	93.768	-6.645
10	$CaCO_3$	0.660	97.922	-2.121
11	Without interference	0.0.675	100.14	0.148

3.6 Detection limit and quantitative limit for drugs:

The detection and quantitative detection limits were calculated by taking ten iterations of the blank solution [25]. As shown in Table (13).

Table (13): Calculating the detection limit and the quantitative limit for Sulfanilamide

Parameters	$\bar{x}B$	$SB = [(Xi-\bar{x})^2/n-1]^{1/2}$	$LOD = \bar{x}B+3\ SB$	$LOQ = \bar{x}B + 10\ SB$
Sulfanilamide	0.213	0.0116	0.2472	0.327

3.7 Colored output stability constant.

The value of the stability constant of the product in water under the described experimental conditions is high as shown in table (14).[26]

Table (14): Stability constant data for the colored product of (SLF)

V $4x10^{-4}$M of SLF /ml	Final con. SLF /M	As*	Am*	α	K(L..Mol^{-1})	Mean of K (L.Mol^{-1})
0.3	1.2×10^{-5}	0.358	0.360	0.0056	9.94×10^{6}	
0.5	2×10^{-5}	0.579	0.582	0.0052	1.2435×10^{7}	7.872925×10^{6}
0.7	2.8×10^{-5}	0.798	0.802	0.0050	1.243775×10^{6}	

3.8 Accuracy and precision testing:

Chinese company VEYONG pharmaceutical lotion containing 10 mg of sulfanylamide is used every 1 g, and the sample is prepared. The results shown in Table 16 are confirmed. The proposed method's success in determining sulfanilamide in the used preparation.

Table (16): Data for determining Sulfanilamide in the pharmaceutical preparation (VEYONG)

Amount of SLF /$\mu g\ mL^{-1}$	*Found	Recovery %	Average Recovery %	Erel%	Average Erel%	RSD%
12	11.589	96.5817		-3.4183		0.0051
9	8.516	94.6244	90.2724	-5.3756	9.7277	0.0171
6	5.387	89.7900		-10.2100		0.0006
3	2.402	80.0933		-19.9067		0.0024

Table (17): Statistical results of the proposed spectral method for drug estimation (SLF)

Parameter	Sulfanilamide with β -Naphthol
Colour of Product	DARK RED
λ max	489 nm
Regression equation	y = 1.1034 X + 0.0298
Standard deviation of regression	0.0116
Correlation coefficient (r)	0.9984
C.L for slop (b±tS_b) at 99%	1.1034± 0.057321
C.L for Intercept (b±tS_a) at 99%	0.014878 ± 0.035239
Concentration range (µg ml^{-1})	(1-12) µg ml^{-1}
Limit of Detection (µg ml^{-1})	0.2472
Limit of Quantitative (µg ml^{-1})	0.327
Sandals Sensitivity (µg ml^{-1})	0.00091
Molar absorbance (L.mol^{-1}.$cm^{-1)}$	190005.48
Composition of product	1:1
Recovery %	102.1306
RSD% n=5	0.01365
C.L for con.12(µg ml^{-1})	12.0892±0.17019
C.L for con.9(µg ml^{-1})	9.1148 ±0.14380
C.L for con.6(µg ml^{-1})	6.0930 ±0.11906
C.L for con.3(µg ml^{-1})	3.1486±0.0.19842

4. Conclusions

A newly developed analytical method was shown for determining pharmaceutical preparations using the nitrification reactions of the used Sulfanilamide, where encouraging, real and effective results appeared as new reagents were used to estimate the drugs used in this study through the azo conjugation reaction and study their optimal conditions. This method gave good and fast results, and it was an economical, more sensitive, and selective method in drug estimation. The possibility of using this method to estimate drugs in the pure state and their pharmaceutical preparations and use them in very small quantities.

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