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SPECTROPHOTOMETRIC DETERMINATION OF SULFAMETHOXAZOLE USING THE REAGENT (1,3,5-TRIAZINE-2,4,6 TRIAL) TRIS(AZACITIDINE)TRIS(METHYLIDENE)TRI ANILINE BY THE NITRATION AND COUPLING METHOD

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Abstract: A low-cost, straightforward spectroscopic technique was created to measure the medication sulfamethoxazole accurately. The medication sulfamethoxazole was estimated using the nitrification process and the produced organic reagent (1,3,5-triazine-2,4,6 trial)tris(azacitidine)tris(methylidene) tri aniline. When sodium nitrite is added to an acidic media, it forms a red product that is absorbed at 532 nm when paired with the detector. This process is known as coupling. The molar absorbance was measured at (3621.88) litres/mol.cm, the limits of Beer's law were between 1.25 and 19 mcg/ml, the correlation coefficient was 0..9984, and the statistical results revealed the detection limit value of (0.276) µg.ml1-, the quantitative limit of (0.839) µg.ml1-, the relative standard deviation rate of (0.309%), and the Sandel significance value of (0.069) µg.cm2. This approach was effectively used to determine the amount of sulfamethoxazole in pharmaceutical formulations. The valence between the drug and the reagent was 1:1.

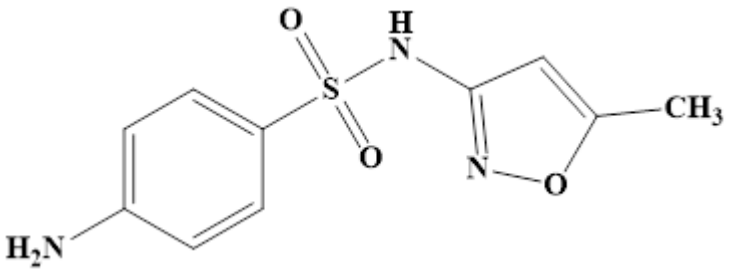
Key words: Sulfamethoxazole, sodium nitrite $NaNO_2$.

1. INTRODUCTION:

Sulfamethoxazole drug:

It is an organic substance that takes the form of an odourless, white, or yellowish powder. It dissolves slowly in water but dissolves easily in acetone, alcohol, dilute alkaline hydroxide solutions, and mineral solutions. Strong oxidising chemicals, heat, and light affect sulfamethoxazole[1]. It has several names

4-Amino-N-(5-methy1-3-sox azoly1)benzenesu1fonamide , N1-(5-methy1-3-isoxazoly1)sulfa nil amide , 5-methy1-3-sulfanilamide isoxazole , 3-sulfanyl amido , 5-methylisoxazole



Its molecular formula is C10H11N3O3S, and its molecular weight is 253.3 g/mol[2]. It melts at a temperature of 167 degrees Celsius [2]. It has a half-life of approximately 11 hours and is similar to

sulfamethoxazole in structure and biological effectiveness[3]. According to its manufacturers, its pharmaceutical formulations come in the forms of tablets, syrup, and oral suspension bearing the following trade names:

Gantanol (rocha) , sinomin (shionogi)

Sulfamethoxazole and trimethoprim produce the cotrimoxazole molecule[4], commonly used in medicines. This compound stops the formation of proteins and nucleic acids necessary for bacterial cells by preventing folic acid synthesis. When it was first manufactured in 1969, urinary tract infections were treated with it[5]. Trimethoprim was combined with sulfamethoxazole to treat Pneumocystis carinii pneumonia in 1989. It is also helpful in treating otitis media and typhoid fever. The drug was also estimated using spectroscopic methods[6][7], chromatographic methods[8][9][10][11], and electrical methods [11].

2. Materials and Methode:

Devices used:

1- Ultraviolet-visible spectrometer
T90UV-VIS Spectrophotometer "double bean from PG Instruments Ltd, with 1cm quartz cells".

2- Sensitive balance

Balance Kern 770 GS/GJ from Sartorius BL 210 S

- **Reagents and chemicals used**

The materials used in this study were prepared in pure form by the company (US, FLUKA, Avonchem UK, SDI, Samrra). Ethanol and distilled water were used as solvents.

- **Chemical solutions and reagents**

✓ Sulfamethoxazole standard solution 1000 mg/ml (3.33×10^{-3}) molar

In a 100 ml volumetric vial, dissolve 0.1000 g of sulfamethoxazole powder. Add distilled water to the flask to bring the volume to the desired level

✓ Sulfamethoxazole standard solution 250 mg/ml, (8.33×10^{-4}) molar

To make this solution, 25 milliliters of standard solution (1,000 µg per milliliter) were diluted in a 100-milliliter volumetric flask, and the remaining volume was filled with distilled water.

✓ Reagent solution(1×10^{-2}) Molar

To prepare this solution, dissolve 0.435 g of reagent powder in a certain volume of distilled water. Then, fill a 100 ml volumetric bottle with the remaining distilled water to the mark.

✓ Working solution sodium nitrite $NaNO_2$ (1×10^{-2}) molar

The solution was prepared by dissolving 0.0345 g of it in a certain volume of distilled water, then adding more distilled water to fill a 50 ml volumetric bottle.

✓ Hydrochloric acid solution (1 molar) at a concentration of approx.

It is made by filling a 100 ml volumetric bottle with pure water and 8.28 ml hydrochloric acid solution (12.77 M). Distilled water was used to finish the volume to the appropriate level.

✓ A solution of sulfuric acid (1 molar) at a concentration of approx.

To prepare, fill a volumetric bottle with 100 ml of distilled water, add 5.42 ml of hydrochloric acid solution (18.44 M), and then cover the bottle with more distilled water.

✓ Nitric acid solution (1 molar) with a concentration of approx

It was diluted using a 100 ml volumetric bottle and 6.3 ml of concentrated acid (15.78 M).

✓ Potassium hydroxyl solution with a concentration of (1) molar, with a concentration of approx.

Potassium hydroxide (5.6 g) was dissolved in a 100 mL volumetric bottle to form a solution.

✓ Ammonium hydroxide solution with a concentration of (1) molar, with a concentration of approx.

7.7 mL of concentrated basic solution (6.49 M) was used to form the NH4OH solution, which was then diluted with 50 mL of distilled water.

✓ Sodium hydroxide solution with a concentration of (1) molar, with a concentration of approx.

4.0 g of sodium hydroxide was dissolved in 100 ml of a volumetric bottle to form a solution.

✓ Solutions of additives (starch - glucose - lactose) 1000 mcg/ml

In a 100 ml volumetric vial, 0.1 g of these solutions was dissolved.

✓ Pharmaceutical solutions (250 mcg/ml)

Metheprim tablets are produced in Samarra, Iraq, by the General Company for Pharmaceuticals and Medical Supplies. Sulfamethoxazole (400 mg) is present in each tablet. To prepare this solution, grind ten tablets well, extract a weight of sulfamethoxazole (0.1000 g), and then dissolve it in water. The residue is rinsed repeatedly after filtering the solution, just as I would dissolve the standard solution. Next, the filtrate was poured into a 100 ml volumetric bottle, and the volume was filled to the brim with distilled water. Then, pour a quarter of this mixture into a bottle. Volumetric capacity (100 ml), then add distilled water to the remaining volume to fill it up to the mark to obtain a solution with a concentration of (250 µg/ml)

3. Results and discussion

➢ The general principle of the method

The nitrifying product creates a colourless intermediate when administered as a solution of sulfamethoxazole in the primary medium. This reaction occurs quickly after adding a portion of the nitrifying agent and the acid to the reagent solution.

➢ Preliminary study

A colorless intermediate product is created when 1 milliliter of reagent solution is combined with 1 milliliter of an iodising agent solution, sodium nitrite, in hydrochloric acid. Shake and wait for 5 minutes. Next, 1 mL of sulfamethoxazole drug solution was added in the presence of essential NaOH medium. The solution is then left for 5 minutes or until the reaction is complete and stabilized. A red colour product has been created. After filling a 25 ml volumetric vial with distilled water to the mark, the absorbance of the colored product relative to its mock solution was measured. It was discovered that it gives maximum absorption at the wavelength of 532 nm. At the same time, the Sori solution did not provide any absorption in this area.

Studying the optimal conditions for producing the product

In the following tests, 1 mL of a 250 µg/mL sulfamethoxazole solution was used in a final volume of 25 mL. The absorbance of the solution was measured at 532 nm compared to the mock solution.

➢ Effect of acid type

Strong acids (H_2SO_4), HCl, and HNO_3 were used in this research at a concentration of 1 M each. The optimal acid that produces the highest absorption value was chosen, and the results are shown in Table No. (2-2)

Table (2-2): The effect of acid type on the absorption values of the product

Acids	Abs.
HCl	0.632
H_2SO_4	0.251
HNO_3	0.318

The volume that produced maximum absorption was determined by gradually increasing the volume (0.1–1 mL) of HCl at a concentration of 1 M. The results are shown in Figure (1-2). It was found that the ideal additional volume of acid is (0.5 ml).

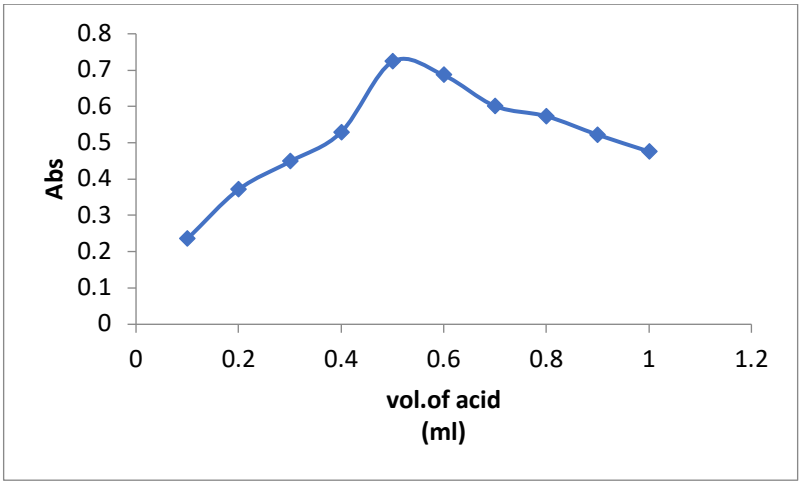


Figure (1-2) Effect of acid size

➢ The effect of base type

The bases NH_4OH, KOH, and NaOH were used at concentrations of 1 M each, plus a similar added volume of 1 mL, to determine which base formed the product with the best absorption. It has been shown that sodium hydroxide (NaOH), as shown in Table (3-2), is the ideal base for use.

Table (3-2): The effect of the type of base on the absorption value of the product

Bases	Abs.
NH_4OH	0.286
NaOH	0.703
KOH	0.558

➢ Effect of base size of sodium hydroxide

The volume achieving the highest absorption was determined by gradually increasing the volume (0.1–1.5 mL) of NaOH at a concentration of 1.0 M. The results are shown in Figure (2-2), and it was discovered that the ideal additional volume is (1 ml).

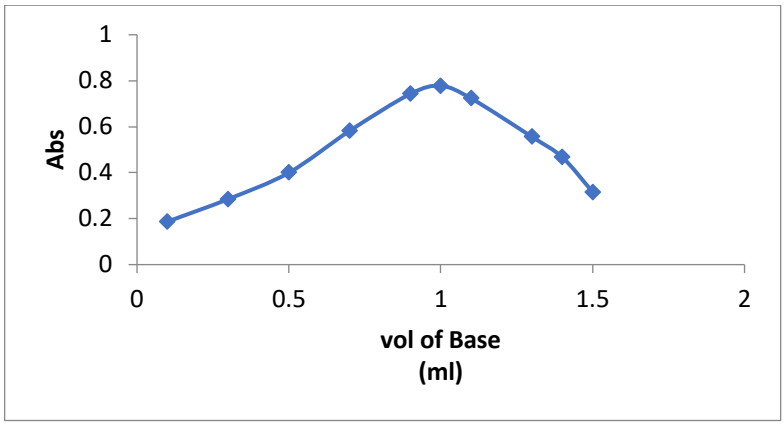


Figure (2-2): Effect of base size

➢ The effect of the size of the fueling agent

As shown in Figure (2-3), increasing volumes (0.1-1.5 ml) of the ionising agent NaNO2 were introduced at a concentration of 1 x 10-2 to ascertain the extent of its effect on the absorption of the product. It was discovered that the optimal volume added to it was (0.8 ml).

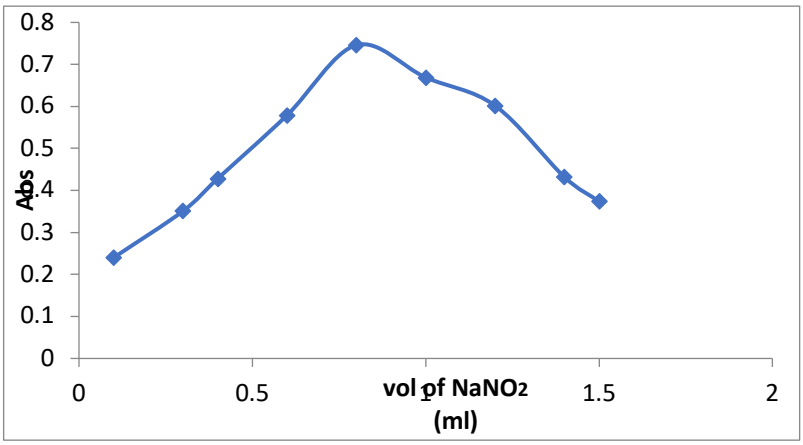


Figure (3-2): Effect of the size of the nitrifying agent

➢ Effect of detector size

Figure (2-4) shows the effect of increasing the volume of the reagent (0.1-1.5) at a concentration of 1 x 10-2 molar on the absorption of the product. Notably, (0.5 ml) is the ideal volume for adding the reagent.

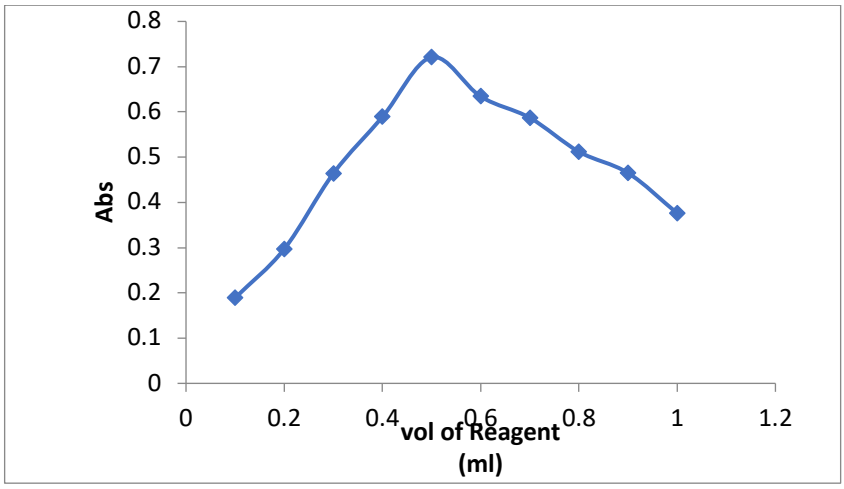


Figure (4-2): Effect of detector size

➢ The effect of the sequence of additions

It investigated how additions to solutions are arranged and how they affect the intensity of the final colour. Therefore, several experiments were performed using different addition sequences in 25 ml bottles and subsequent experiments with the same standards and ideal quantities, according to Table (2-4). The absorption maximum is in sequence 5, and has been used in subsequent studies.

Table (4-2): Effect of the sequence of additions on absorption values

No.	Addition sequence	Abs
1	D + R + A + N + B	0.413
2	R + N + D + A + B	0.497
3	D + A + N + R + B	0.573
4	D + N + A + R + B	0.624
5	R + N + A + D + B	0.762

So: (D) the drug sulfamethoxazole, (R) the reagent, (N) the ionizing agent sodium nitrite, (A) hydrochloric acid, and (B) the base sodium hydroxide.

➢ Study of the time of nitrogen

In order to study the effect of the amount of time required to complete the nitrite and conjugation process, a series of 25 ml volumetric bottles were used. These bottles contained 0.5 ml of reagent and were supplemented with 0.8 ml of sodium nitrite, 0.5 ml of hydrochloric acid, 1 ml of sulfamethoxazole, and 1 ml of sodium hydroxide. The solutions were then allowed to stand for varying periods before being diluted with distilled water to a final volume of 25 ml. The absorbance of the solutions was measured at a wavelength of 532 nm compared to their mock solutions, and the results are shown in Table (2-5). It was found that a time of 5 minutes is sufficient to complete the nitrogenation process.

Table (5-2): Study of nitrogen time

Time (min)	directly	5	10	15	20	25	30	35
Abs	0.718	0.721	0.720	0.721	0.721	0.721	0.720	0.719

➢ The effect of time on the stability of the resulting product

The stability of the final product is crucial in determining how long it will remain stable. Table (2-6) and the image show the results of tracking the interaction over time under ideal conditions.

Table (6-2): The effect of time on the stability of the coloured product

Time(min)	directly	5	15	25	35	45	55	65	75
Abs	0.716	0.721	0.720	0.720	0.719	0.718	0.717	0.710	0.705

From the table, it was noted that the absorption values at a wavelength are stable over time, as the absorption value of the product is constant for 55 minutes, and this period is sufficient to perform measurements.

➢ Final absorption spectrum

Once ideal conditions were reached, 0.5 ml of reagent at a concentration of 1×10-2 was added, then (0.8 ml) of the isotropic agent NaNO2 at a concentration of 1×10-2 M, and then (0.5 ml) of hydrochloric acid at a concentration of 1 M was added and left for five minutes. Next, (1 mL) of sulfamethoxazole at a concentration of 250 µg/mL, and finally (1 mL) of NaOH were added. After adding distilled water to fill a 25 ml volumetric bottle to the full mark, the solution was allowed to stand for five minutes to terminate and stabilize the reaction. The final absorption spectrum of the developed reddish-colored product was then compared to its mock solution. It was foundto gives the highest absorption at the wavelength (532) nm, while its mock solution does not provide any absorption in this region, as shown in Figure (5-2).

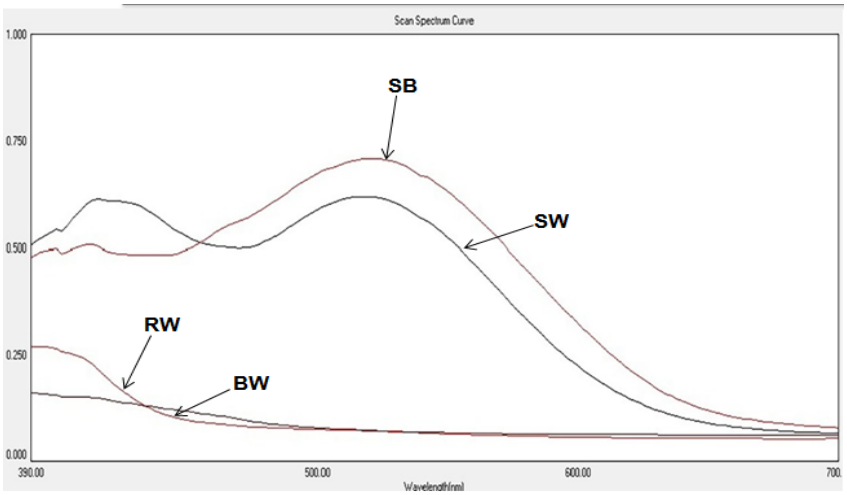


Figure (5-2): Final absorption spectrum for sulfamethoxazole determination
SW = represents the absorption spectrum of the complex versus distilled water
SB= represents the absorption spectrum of the complex versus the formal solution
BW= represents the absorption spectrum of the photo solution versus distilled water
RW= represents the absorption spectrum of reagent solution versus distilled water

➢ Calibration curve

A series of solutions with increasing final concentrations were prepared, starting with volumes of 0.125-1.9 µg/ml containing 1.25-19 µg/ml of sulfamethoxazole (0.5 ml) of the reagent solution at a concentration of 1 x 10-2 M, (0.8 ml) of the ionizing agent NaNO2 at a concentration of 1 x 10-2 M, and (0.5 ml) of HCl with a concentration of 1 M thereafter. Five minutes of sporadic shaking later, add 0.125–1.9 ml of sulfamethoxazole at a concentration of 250 µg/ml. Then, add 1 ml of sodium hydroxide at a concentration of

1 molar. After that, let the solution sit for 5 minutes to finish and stabilize the reaction. Next, add distilled water to volumetric bottles containing 25 ml to bring the volume up to the correct level. Finally, compare the absorbance of each solution at 532 nm to the mock solution. The standard curve for a range of sulfamethoxazole concentrations between (-1.25 and 19 micrograms/ml) is shown in Figure (6-2). The molar absorbance was 3621.88 L.mol-1.cm-1, and the correlation coefficient for the estimate was 0.9984. The micrograms for the Sandel coefficient are (0.069). cm-2.

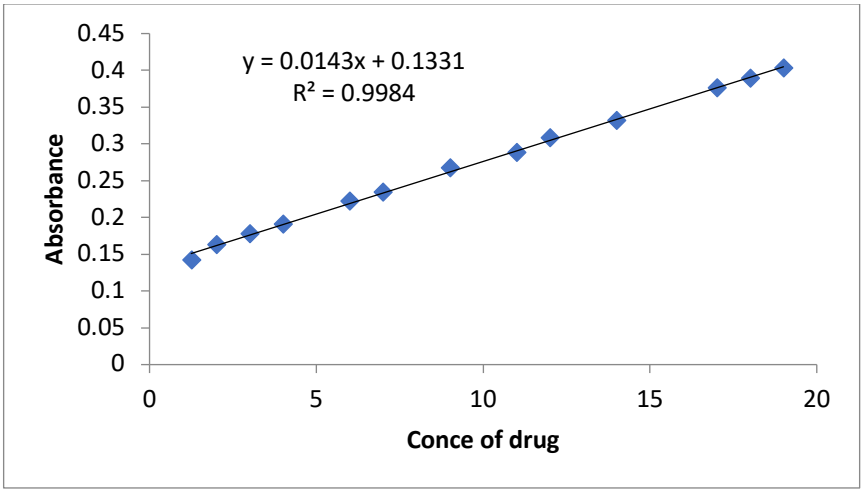


Figure (6-2): Calibration curve for sulfamethoxazole determination

➢ Accuracy and compatibility of the proposed method

Three distinct drug concentrations were measured under the ideal working conditions described in the working technique, and the results were used to calculate the accuracy and consistency of the proposed method for the determination of sulfamethoxazole. The results obtained and shown in Table (7-2) show that the method has good accuracy and agreement.

Table (7-2): Accuracy and compatibility of the proposed method

Cons. **(ppm)**	**RE**%	%RSD
6	3.61	0.54
11	1.52	0.41
18	0.58	0.31

* (Each value is the average of 6 readings)

The value of the limit of detection (L.O.D) and limit of quantity (L.O.Q) for this method was also calculated from the subsequent relationship, where it was found that the limit of detection = 0.276 micrograms/ml and the limit of quantity = 0.839 micrograms/ml.

➢ Equality of the resulting output

Two continuous variation methods (Job's method and molar ratio method) were used to find the equivalence of the resulting substance and the rate of association of the drug with the reagent sulfamethoxazole. The drug and reagent concentrations are the same in both procedures. It is (1 x 10-3) molar. In the Job method, different drug sizes ranging from (0.5 - 4.5) ml were placed in a series of volumetric bottles with a capacity of (25 ml). Then decreasing volumes of the reagent were added, starting from (4.5 - 0.5) ml, to which (0.8 ml) of the denitrifying agent sodium nitrite was added, in a 25 ml volumetric vial containing (0.5 ml) of

hydrochloric acid and (1 ml) of sodium hydroxide. After diluting it with distilled water to the appropriate level, the wavelength at which absorption was recorded was 532 nm, as shown in Figure (7-2).

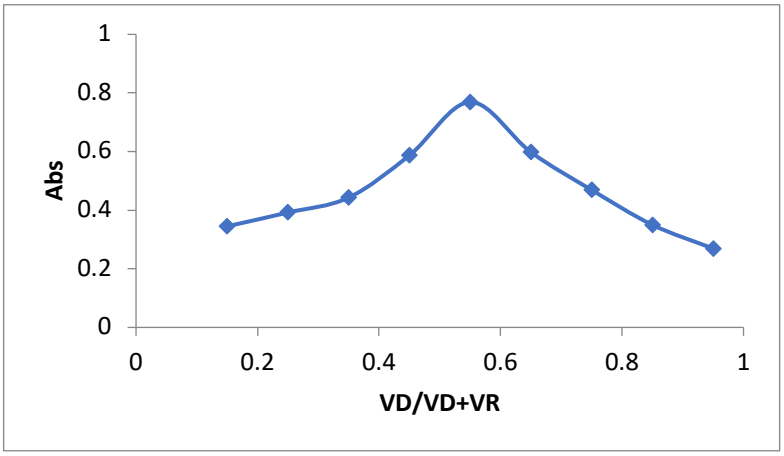


Figure (7-2): Continuous changes method

As for the molar ratio method, it included filling a series of volumetric bottles (25 ml) to the brim with distilled water after adding a fixed volume of the drug (1 ml) at a concentration of (1 x 10-3) molar to increase the reagent volumes starting from (0.3-3.3 ml). With the same focus. The absorbance of these solutions was then measured at the wavelength (532 nm) against the photo solution for each of them, and the results are shown in Figure (8-2).

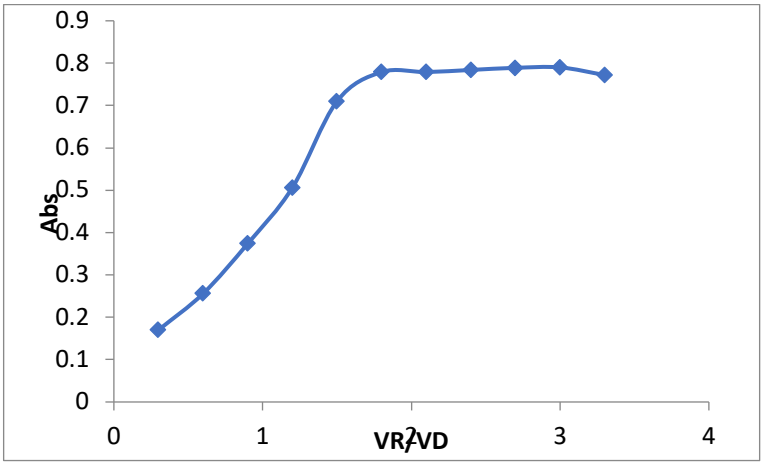


Figure (8-2): The product molar ratio method

Both methods confirmed that the drug-reagent binding ratio was 1:1

Effect of additives

The effect of adding higher amounts of additives to pharmaceutical formulations of sulfamethoxazole in tablet form and 25 ml volumetric vial was studied. By adding two quantities of each substance, the results shown in Table (2-8) show that these compounds do not interact with the reagent sulfamethoxazole, which confirms the feasibility of using this method in pharmaceutical preparations.

Table (8-2): Effect of additives

RE%	Added focus (ppm)	RE%	Added focus (ppm)	Additives
3.84-	160	0.52	80	Starch
1.79-	160	1.22-	80	Lactose
2.94-	160	3.83-	80	Glucose

- **Applications**

The proposed method was used to calculate the amount of sulfamethoxazole in pharmaceutical preparations in the form of tablets. To do this, ten tablets were finely ground, and a weight of 0.1000 g of sulfamethoxazole was extracted and dissolved in the same way as the standard solution. The mixture was then filtered, and the precipitate was washed repeatedly afterward. In other words, he filled a 100 ml volumetric bottle with the filter and added distilled water to fill the remaining space. He measured 25 ml of this solution and added a sufficient amount of distilled water to fill a volumetric bottle containing 100 ml to obtain a solution with a concentration of 250 micrograms. Three different concentrations were taken to estimate the sulfamethoxazole in the pharmaceutical preparation, and the results are shown in Table (9-2).

Table (9-2): Application of the proposed method for drug estimation in pharmaceutical preparations

Pharmaceutical preparation	taken (ppm)	The result (ppm)	Retrospective Rec.%	the manufacturing company
Plasil	6	6.07	101.28	**India**
	11	11.25	102.29	
	18	17.68	98.25	

4. Conclusion:

Nitrogenization and coupling reaction was designed as a straightforward and sensitive spectroscopic analytical method for the determination of sulfamethoxazole. Since the resulting intermediate product is colorless and immediately turns red when combined with the drug in a basic solution, the approach is based on isodization of the reagent by sodium nitrite in an acidic medium, the wavelength at which the colored product absorbs most is 532 nm. Within a range of concentrations (1.25-19 µg/mL), it obeyed Beer's rule. The molar absorption value was 3621.88 l/mol.cm, the detection limit was 0.276 µg/mL, the estimation factor was 0.9984, and the relative standard deviation did not exceed 0.309%. The Sandel significance was 0.069 µg/cm2. This technique has been found to provide good compatibility and accuracy. The process is straightforward, the reaction ends, stabilizes after five minutes, and remains constant for over fifty-five minutes. The proposed technique has been effectively used to measure sulfamethoxazole in pharmaceutical preparations.

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