MODERN MEDICINE AND PRACTICE
https://inovatus.es/index.php/ejmmp
EUROPEAN JOURNAL OF MODERN MEDICINE AND PRACTICE
Vol. 4 No. 6 (Jun - 2024) EJMMP ISSN: 2795-921X

DETERMINATION OF SULFATHIAZOLES SPECTROPHOTOMETRICALLY BY THE OXIDATIVE COUPLING METHOD USING THE COUPLING REAGENT

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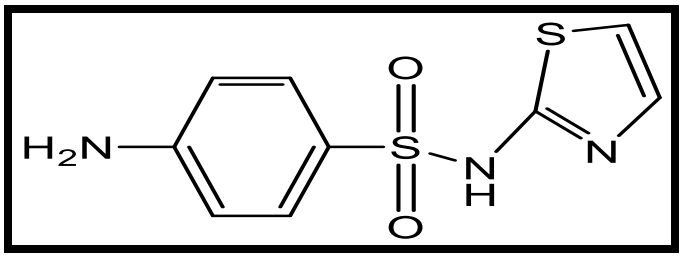
Abstract: This chapter includes the use of a simple and sensitive spectrophotometric method for the determination of sulfathiazoles using N,N-DMPPDAAs a coupling reagent in the presence of potassium periodate as an oxidizing agent to form a violet dye dissolved in water, it gave the highest absorption at the wavelength of 554 nm, the molar absorption value was 4161.699 l/mol.cm, and the Sandel significance value was 0.06134 micrograms/cm2, and the limits of Beer's law were in the range of 3.125-50 micrograms. /ml of pure sulfathiazole in a final volume of 20 ml, the value of the percent recovery rate is 99.65, the value of the relative standard deviation is between 0.0018 - 0.0019%, the value of the detection limit is 0.995 micrograms/ml, and the quantitative limit is 3.319 micrograms/ml. This proposed method was successfully applied to estimate sulfathiazoles. In pharmaceutical preparations, it is in liquid form.

Introduction:

The scientific name of sulfathiazole [1]: isAmino-N-(1,3-thiazolyl)benzenesulfonamide-4chemical composition**:**



Chemical formula C9H9N3O2S2

Molecular weight 255.3 g/mol.[2]

It is an organic sulfur compound used as a short-acting sulfa drug. It is used for regular treatment and to prevent bacterial infections. The drug is used as an anesthetic and to prevent bacterial growth. It was commonly used to treat the urinary tract. As well as resisting infections such as sulfathiazole, it is used as antibiotics in veterinary applications. There are some side effects of the drug: skin rash, itching, allergies, and edema.[3,4]

Many different analytical methods were used to estimate sulfathiazoles, such as Raman chromatographic methods [5,6], spectroscopic methods [7,9], FT-method, flow injection method [10,11], electrochemical method [12,13] and chemiluminescence method. [14], High-performance liquid (HPLC) [15,21] technology. In this research, (GLC) [22] was used for gas and liquid, a simple, accurate and sensitive spectroscopic method to estimate sulfathiazole in its pure form as well as in liquid solutions in (2,4- DNPH) based on oxidative coupling using 2,4 dinitrophenylhydrazine potassium in the basic medium.

Devices used

The devices listed in Table (1) were used.

Table (1) used devices

ت	the device name	Origin
1	Shimadzu UV/VIS 1600 Spectrophotometer	Japan
2	Water bath	Memmert-German
3	Stirrer (BIOSAN MSH 300) Hot Plate with Magnetic	Germany
4	Precisa (XR-205gm)SM-DR	Sweden

Preparing solutions of the materials used:

- **Standard sulfathiazole solution 1000 µg/ml**

This solution was prepared by dissolving 0.1000 g of sulfathiazole in distilled water in a 100 ml volumetric bottle. The volume was completed to the mark with distilled water, and less concentrated solutions were prepared by dilution.

- **Sulfathiazole solution 250 µg/ml**

This solution was prepared by withdrawing 25 ml of the prepared solution at a concentration of 1000 µg/ml and placing it in a 100 ml volumetric bottle, and the volume was completed to the mark with distilled water.

- **N,N-DMPPDA reagent solution 10-2 molar**

The solution was prepared by dissolving 0.209 grams of reagent in a small amount of distilled water, and the volume was completed to the mark with distilled water in a 100 ml volumetric bottle.

- **Potassium periodate solution 1 x 10-2 M**

Prepare this solution by dissolving 0.534 grams of potassium periodate in distilled water, then after dissolving, complete the volume to the mark in a 100 ml volumetric bottle with distilled water.

- **1 M hydrochloric acid solution**

The solution was prepared by withdrawing 16.6 ml of concentrated 12 M HCL and then diluting it with distilled water, the volume was completed to the mark using a 200 ml volumetric vial.

- **Interfering solutions 1000 micrograms/ml**

Solutions of the interferences were prepared by dissolving 0.1000 g of each substance in distilled water and completing the volume with distilled water to 100 ml in a 100 ml volumetric bottle, from which the diluted solutions were prepared.

- **Pharmaceutical solution of sulfathiazole 250 µg/ml**

The preparation is available in liquid form. The preparation contains 40 mg of sulfathiazole. It is prepared by withdrawing 0.625 ml and placed in a 100 ml volumetric vial. The volume is supplemented to the mark

with distilled water, thus obtaining a solution with a concentration of 250 micrograms/ml of the sulfathiazole preparation.

Results and discussion

Preliminary study:

The sulfathiazole solution was mixed with the reagent solution N,N-DMPPDA in the presence of the oxidizing agent potassium periodate in an acidic medium, where a violet-colored compound was formed, as shown in Figure (1). It gave the highest absorption at a wavelength of 554 nm, while the mock solution showed a lower absorption. At the wavelength above, therefore, the optimal conditions for the coupling reaction were studied to obtain the best possible results in order to develop a sensitive and simple spectroscopic method for the determination of sulfathiazole.

Figure (1) The product formed from the drug sulfathiazole

Study of control of experimental conditions

Subsequent experiments were conducted using 2.5 ml of 0.01 M potassium periodate oxidizing agent solution, 2 ml of 0.01 N,N-DMPPDA reagent solution, 1 ml of 1 M hydrochloric acid solution, and 3 ml of sulfathiazole solution with 250 µg/ml in volume. A final amount of 20 ml was measured, and the absorbance of the solutions was measured against the mock solution at a wavelength of 554 nm.

Effect of oxidizing agent

Several experiments were conducted to find the best oxidizing agent to form the colored product. That is, several solutions of oxidizing agents were used, including (potassium periodate - sodium periodate - potassium chromate - ferric potassium cyanide - bromo succinimide) at a concentration of 0.01 molar each and in a volume of 1 ml added to 3 ml of dapsone at a concentration 250 µg/ml, then 1 ml of N,N-DMPPDA reagent at a concentration of 0.01 M was added into a 20 ml volumetric vial, and the volume was completed to the mark with distilled water. After that, the absorption of each model was measured against its mock solution in a wavelength range between 200-800 nm. It was found that the best oxidizing agent was potassium periodate because it gave the highest absorption at the wavelength of 554 nm. This oxidizing agent was used in subsequent experiments.

Effect of the size of the oxidizing agent

The effect of the size of the oxidizing agent, which is potassium periodate (KIO4) at a concentration of 0.01 molar, was studied by adding different volumes of (0.5-4) ml to volumetric bottles with a capacity of 20 ml containing 3 ml of a sulfathiazole solution with a concentration of 250 micrograms/ml, and then adding 2 ml of the solution. The reagent N,N-DMPPDA was prepared at a concentration of 0.01 molar, and the volume was supplemented with distilled water to the mark and the results were as shown in Figure (2).

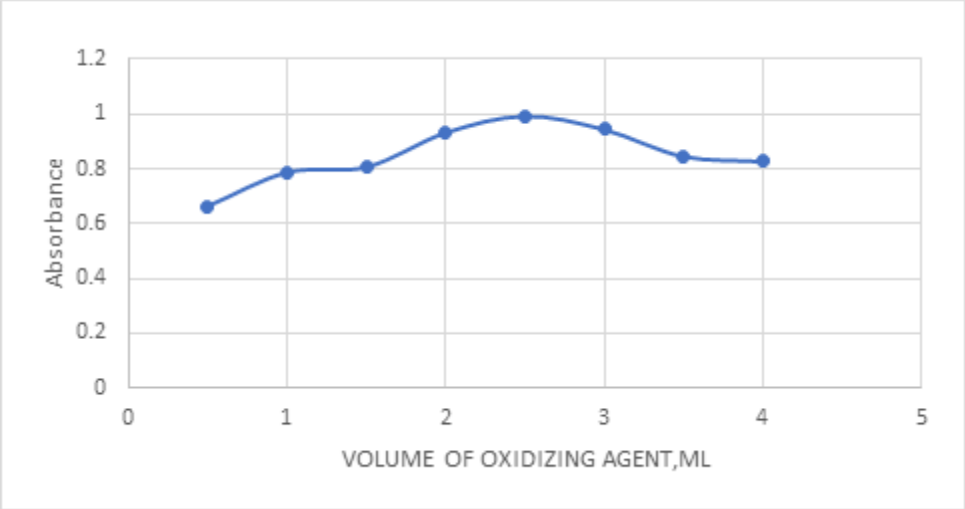


Figure (2) Effect of the size of the oxidizing agent on the absorption of the colored product

Coupling detector effect

He used several chemical compounds that can be used as coupling reagents, such as (N,N-DMPPDA, 4,2-dinitrophenylhydrazine, 2,4-dichloroaniline, 4-bromoaniline, N,N¬-dimethyl para-phenylene diamine dihydrochloride). At a concentration of 0.01 molar and a volume of 1 ml in the determination of sulfathiazole, it was added to 3 ml of sulfathiazole at a concentration of 250 micrograms/ml, then 1 ml of the oxidizing agent potassium periodate was added at a concentration of 0.01 molar, and then the absorbance of each sample was measured against its mock solution in a wavelength range between 800 -200 nm, and N,N-DMPPDA was chosen as the best coupling agent because it gave the highest absorption at the wavelength of 554 nm for the colored product.

Study the effect of the amount of coupling reagent

The effect of the size of the coupling reagent was studied. 3 ml of the drug sulfathiazole at a concentration of 250 micrograms/ml was added in 20 ml volumetric bottles, and then 2.5 ml of the oxidizing agent potassium periodate KIO4 was added at a concentration of 0.01 molar, after which different volumes of the reagent were added. N,N-DMPPDA started from (0.5-4) ml at a concentration of 0.01 molar, and the volume was supplemented with distilled water to the mark, and the absorption was measured against the mock solution, where it was found that the volume of 2 ml gave the highest absorption, as shown in Figure .(3)

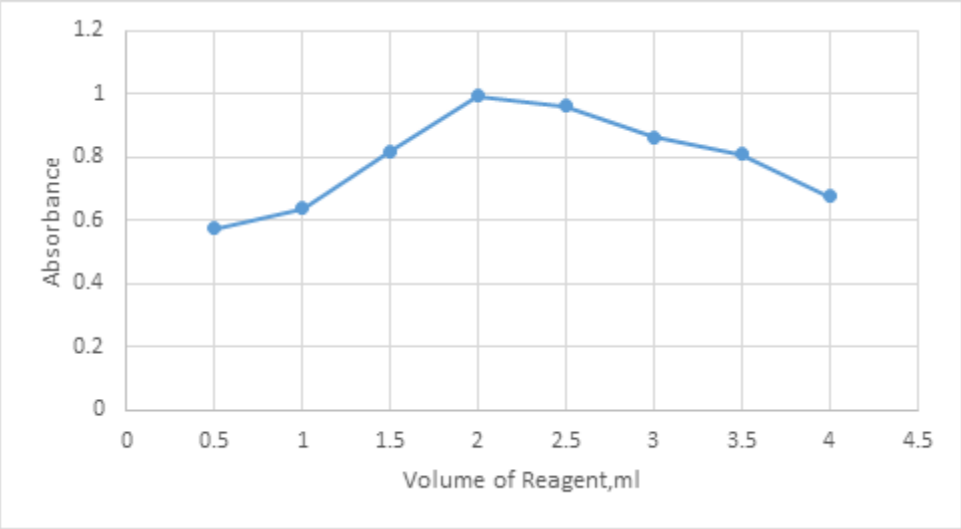


Figure 3: Effect of reagent size on absorbance of the colored product

Study the effect of acid size

The effect of acid volume on absorption was also studied, where different volumes (0.5-4) ml of 1 M hydrochloric acid solution were added to a series of 20 ml volumetric bottles, each containing 3 ml of sulfathiazole at a concentration of 250 µg/ml and 2.5 ml. One ml of potassium periodate at a concentration of 0.01 M. The bottles were then left for a period of time to complete the oxidation process. Then 2 ml of the reagent N,N-DMPPDA at a concentration of 0.01 M were added. Then these solutions were diluted with distilled water to the mark, and the absorption at the wavelength was measured. 554 nm compared to its mock solution, as it was found through absorption that it gave the highest absorption at a volume of 1 ml, so it was adopted in subsequent experiments as shown in Figure**.(4)**

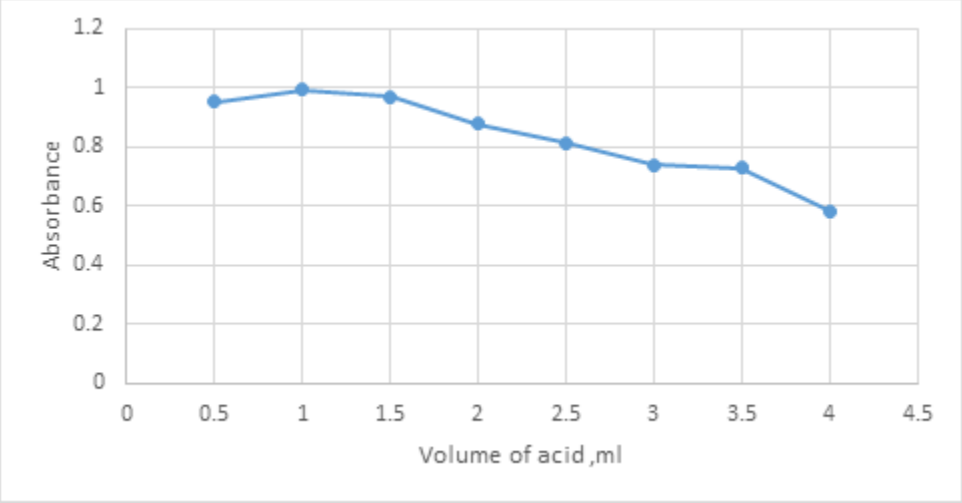


Figure (4) Effect of acid size on the absorption of the colored product

Study the effect of oxidation time

The time required for the oxidation of sulfathiazole by the oxidizing agent potassium periodate was studied by taking a series of volumetric bottles with a capacity of 20 ml, each containing 3 ml of a sulfathiazole solution with a concentration of 250 µg/ml and adding to it 2.5 ml of a solution of potassium periodate with a concentration of 0.01 molar, and the solutions were left for a period of time. Then 2 ml of N,N-DMPPDA reagent solution was added at a concentration of 0.01 molar, then it was diluted with distilled water in a 20

ml volumetric bottle, and the absorbance of the solutions was measured at a wavelength of 554 nm against their mock solutions and the results are shown in Figure (5). .

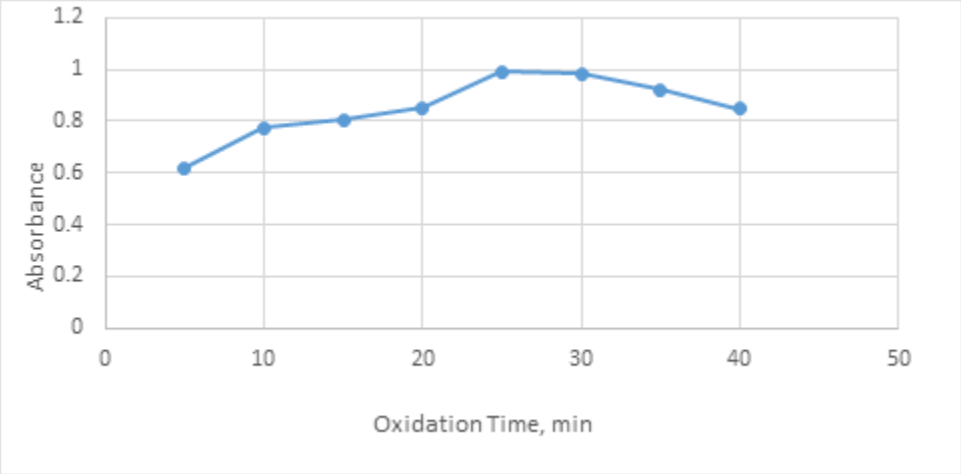


Figure (5) Effect of oxidation time on absorption

It is noted from Figure (5) that the time required to complete the oxidation process is (3-20) minutes, and the time of 25 minutes was adopted in subsequent experimentsن

Study the effect of temperature on the absorption of colored products

The effect of temperature on the absorption of the formed colored product and on its stability was studied using temperatures (-545°C). This was done using the ideal conditions obtained from previous experiments, and as shown in Figure (6), it appears that the optimal temperature is (25 -35°C. Absorption decreases as the temperature increases, so (30°C) was used in subsequent experiments.

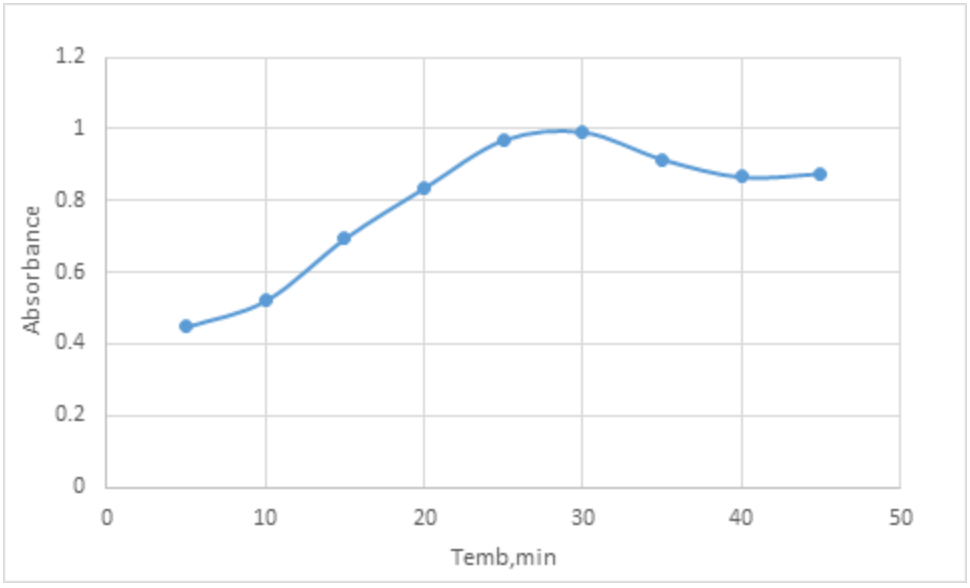


Figure (6) Effect of temperature on absorption

Study the effect of settling time

The stability of the resulting product was tracked using the optimal conditions obtained from previous experiments, where a volume of 3 ml of sulfathiazole solution with a concentration of 250 µg/ml was taken, after which 2.5 ml of the oxidizing agent potassium periodate with a concentration of 0.01 M was added and

the bottles were left for 25 minutes. To complete the oxidation process, then 2 ml of N,N-DMPPDA reagent solution with a concentration of 0.01 molar was added, and the volume was supplemented with distilled water to the mark in volumetric bottles with a capacity of 20 ml, after which the absorption of the colored solutions was measured after a certain time in minutes at a wavelength of 554 nm against their solutions. The mock results are as shown in Table.(2)

Table (2) Stability of the resulting output

Time	Absorbance
0.0	0.819
5	0.832
10	0.852
15	0.860
20	0.985
25	0.984
30	0.988
35	0.993
40	0.992
45	0.992
50	0.990
55	0.991
60	0.990

- It is noted from the results in Table (2) that the stability of the product is stable after 20 minutes and for a period of 60 minutes, which is a sufficient period to conduct measurements.
- Sequence of additions
- The effect of changing the sequence of additions to the solutions used in the reaction was studied because the sequence of addition has an effect on the color intensity of the resulting compound. Thus, a number of experiments were conducted with different addition sequences, noting that all concentrations and volumes of the materials used are the same in all cases. It is noted from the results obtained in Table (3) that the first arrangement gives the highest absorption and thus it was used in subsequent experiments.Sulfathiazole (D)
- KIO_4 (O)
- N,N-DMPPDA (R)
- HCl (A)

Table 3: Effect of addition sequence on absorption of colored product

No	Order of additions	
1	D + O + R+A	0.993
2	D+ R+O+A	0.785
3	R +D+ O+A	0.462
4	O+ D+R+A	0.455

Effect of solvent type

After adding all the reaction components according to the optimal values in the previous experiments, different solvents were used to complete the volumes to the mark in 20 ml volumetric bottles, and the results are as shown in Table (4).

Table (4) Effect of solvent type

Solvent	λ_{max}(nm)	Absorbance
Water	554	0.993
Ethanol	559	0.839
THF	523	0.089
Methanol	471	0.528
Chloroform	Break up	Break up

Final absorption spectrum

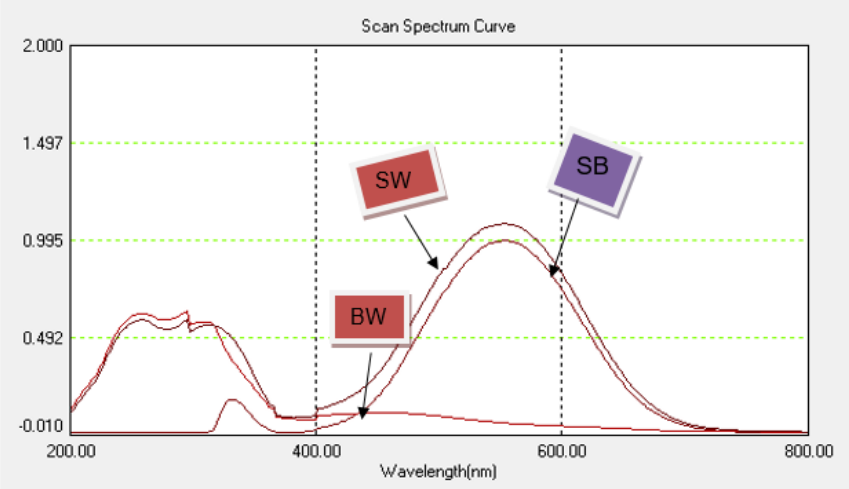
Based on the results of previous experiments, the optimal conditions for the determination of dapsone were summarized as shown in Table.(5)

Table (5): Summary of optimal conditions for sulfathiazole determination

Experimental Conditions	he value
λ_{max} (nm)	554
Amount (ml) of 1 $\times 10^{-2}$ M Potassium periodate	2.5
Amount (ml) of 1 $\times 10^{-2}$ M N,N-DMPPDA	2
Oxidation time	25 min
Solvent	Water

The wavelength of the highest absorption under the optimal conditions for sulfathiazole estimation was confirmed by measuring the absorption spectrum of the resulting solution, as shown in Figure (7). It was shown from the drawing that the wavelength obtained for the highest absorption was 554 nm, according to what was found in the initial tests.

Figure (7) Final absorption spectrum of the colored product



SB: represents the absorption spectrum of the colored product solution versus the mock solution.

SW: Absorption spectrum of the colored product solution versus distilled water.

BW: represents the absorption spectrum of the photo solution versus distilled water.

How to work and prepare the calibration curve

After establishing the optimal conditions for the determination of sulfathiazoles, the standard curve was prepared as follows:

Increasing volumes (0.25-4 ml) of a sulfathiazole solution with a concentration of 250 µg/ml were added to a series of 20 ml volumetric bottles, and 2.5 ml of a 0.01 M potassium periodate oxidizing agent solution was added to them. These solutions were left for 25 minutes to complete the oxidation reaction. Then, 2 ml of N,N-DMPPDA reagent solution was added at a concentration of 0.01 molar, and the volume was then completed with distilled water to the mark, where the absorbance of all solutions was measured at a wavelength of 554 nm against its mock solution, and Figure (8) represents the standard curve that It follows Beer's law for a range of concentrations between (3.125 - 50) micrograms/ml of sulfathiazole, and the molar absorbance value reached 4161.699 L.mol-1.cm-1 and the Sandel value was 0.06134 micrograms/cm**2.**

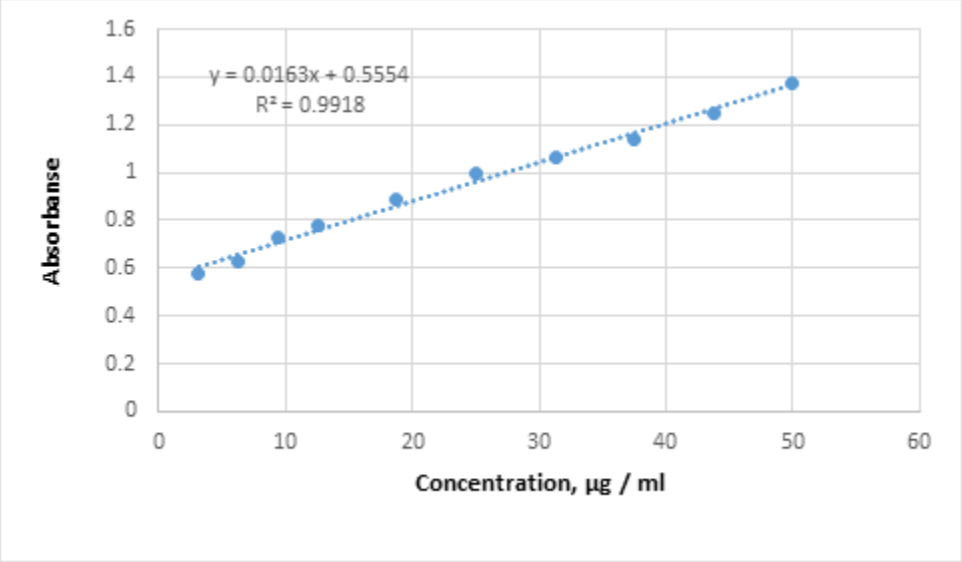


Figure (8) shows the calibration curve for the sulfathiazole solution

Compatibility and accuracy of the proposed method

The compatibility and accuracy of the proposed method for estimating sulfathiazole shown in the method of working under optimal conditions was calculated. This was done by calculating the recall and relative standard deviation for three concentrations of sulfathiazole 6.25, 12.5, and 43.75 micrograms/ml, and this was done by taking an average of six readings for each of them. The value of the recall rate is 65%99, and the relative standard deviation does not exceed 0.0018-0.0019%, meaning that the method has satisfactory agreement and high accuracy. Both the recall, its rate, and the relative standard deviation were calculated mathematically as follows[23]

RE% =xi-x/x×100

RE = relative error

= xi practical value

= x real value

The recovery value is calculated from the following law

Recovery % =RE % + 100

As for calculating the percentage value of the relative standard deviation, the following law is applied:

S = standard deviation

= reading rate

The results are shown in Table.(6)

Table (6) Method accuracy and compatibility

Amount of Promethazine taken µg /ml	RE, %	Recovery, %	Average recovery,%	RSD, %
6.25	-1.1152	98.88	99.65	**0.7063**
12.5	0.3623	100.36		**0.6521**
43.75	-0.2962	99.7		**0.2814**

Detection limit and quantity limit

The absorbances of ten mock solutions were measured at a wavelength of 548 nm against distilled water to find the value of both the limit of detection (LOD) and the limit of quantification (LOQ). It was found that the value of the limit of detection equals 0.995 µg/ml and the value of the limit of quantification equals 3.319 µg/ml. .

Table 7: Detection limit and quantity limit

(Xi) Absorbance of blank	$(Xi - \bar{X})$	$(Xi - \bar{X})^2$
0.1548	-0.00703	0.0000494
0.1597	-0.00213	0.0000045
0.1537	-0.00813	0.0000660
0.1611	-0.00073	0.0000005
0.1603	-0.00153	0.0000023
0.1624	0.00057	0.0000003
0.1601	-0.00173	0.0000029
0.1689	0.00707	0.0000499
0.1695	0.00767	0.0000588
0.1672	0.00537	0.0000288
∑ Xi=1.6183		∑ =0.0002634

X=∑Xi/n =2.1898/10 =0.16183

After that, the standard deviation (S) is calculated through the following mathematical relationship:

$$S=\sqrt{\frac{\sum(Xi - X)2}{n-1}} = \sqrt{\frac{0.0002634}{9}} = 5.41 \times 10^{-3}$$

By applying the following two mathematical relationships, the values of (LOD) and (LOQ) were found

$C_{LOD} = \frac{3S}{B}$ $C_{LOQ} = \frac{10S}{B}$

Where B represents the slope of the standard curve and the results are shown in the following calculations:

$$\textbf{CLOD} = \frac{3 \times 5.41 \times 10^{-3}}{0.0163} = 0.995$$

$$\textbf{CLOQ} = \frac{10 \times 5.41 \times 10^{-3}}{0.0163} = 3.319$$

The nature of the resulting product[24]

To know the nature of the product formed and the rate of binding of the drug with the reagent, both the continuous changes method (Job's method) and the molar ratio method were applied. In both methods, the concentration of both the sulfathiazole solution and the reagent solution N,N-DMPPDA is 0.01 molar, where in (Job's method) I put Different volumes of the drug solution ranged from (1-9) ml in 20 ml volumetric bottles. Supplements of these volumes were added to 10 ml of the reagent solution, then the rest of the additions were added in the optimal volumes and according to the method of work, then they were diluted with distilled water to the mark. The absorption of these solutions was measured at a wavelength of 554 nm against their mock solutions, and Figure (9) shows that the ratio is 1:1 between the sulfathiazole and the reagent.

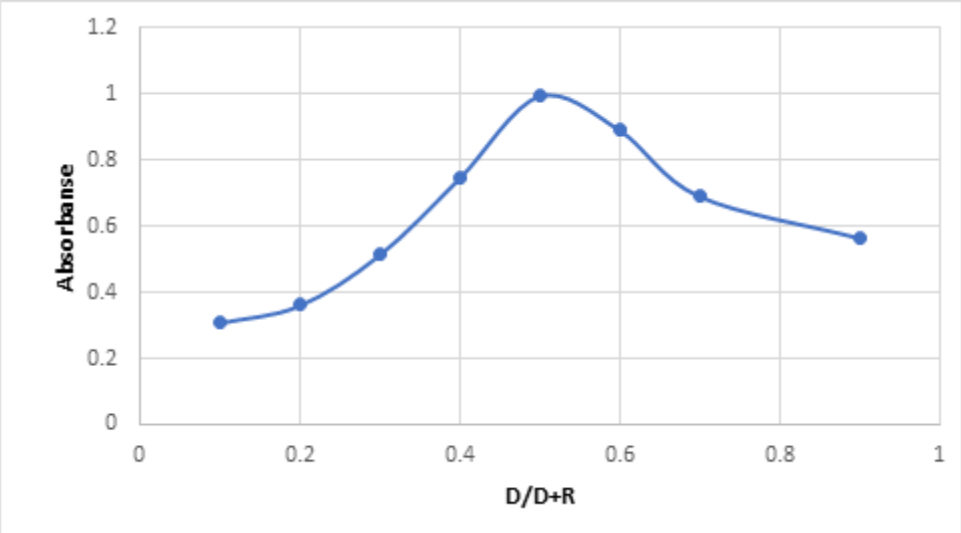


Figure (9) Job method

It was confirmed that the reaction ratio between the sulfathiazole and the reagent was 1:1, using the molar ratio method (72), where 2 ml of the sulfathiazole drug solution was placed in a series of volumetric bottles with a capacity of 20 ml, and the reagent solution was added to it in different volumes (4-0.25 ml), then the The rest of the additions were completed in the optimal sizes, after which they were diluted with distilled water to the mark, and the absorption of these solutions was measured at a wavelength of 554 nanometers against their mock solutions, as the molar ratio agrees with the method of changes, and Figure (10) confirms that the ratio is 1:1. Among sulfathiazoles: N,N-DMPPDA.

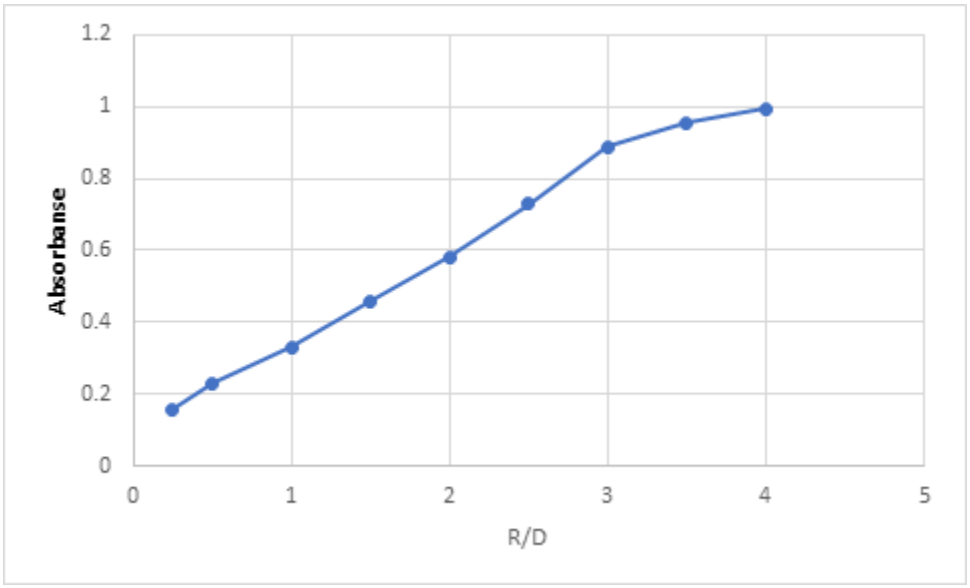
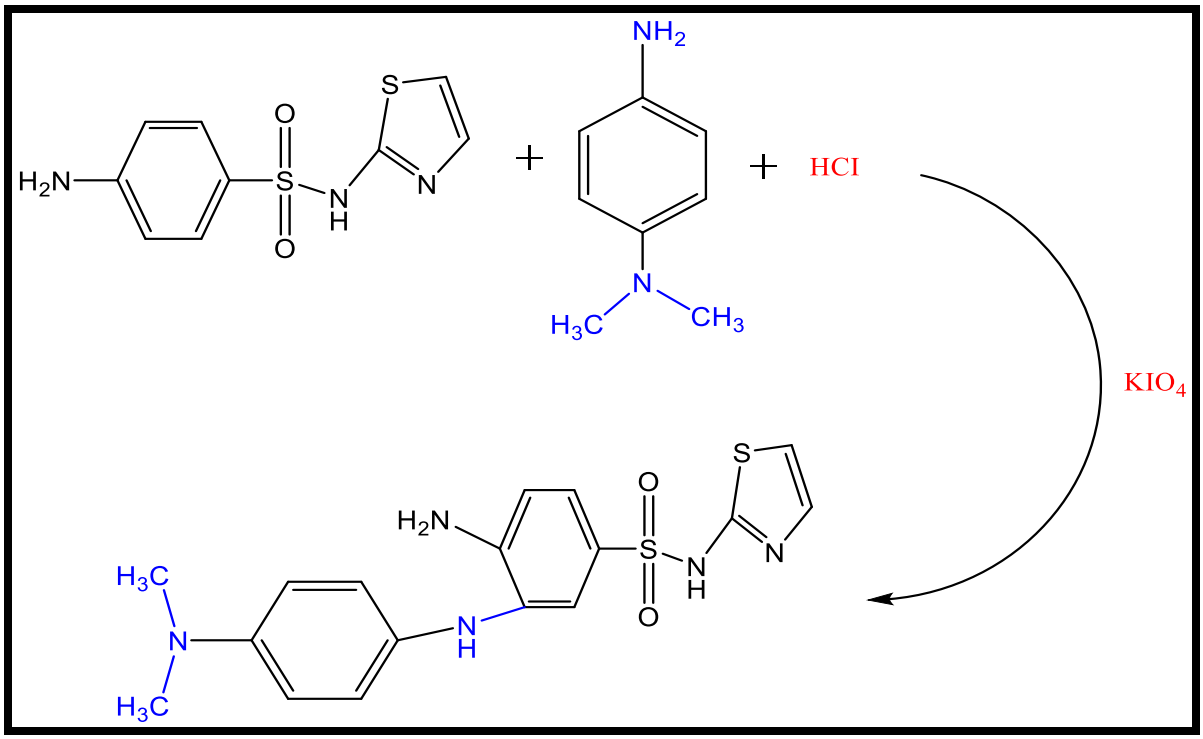


Figure (10) Molar ratio method

Therefore, the proposed reaction equation is as follows:



Effect of interferences:

For the purpose of examining the selectivity of the method and its applicability to pharmaceutical preparations, the effect of the agents was studied by adding different volumes of each of these agents, 2.5, 5, and 7.5 ml at a concentration of 1000 µg.ml-1, to a series of 20 ml volumetric bottles containing 3 ml of sulfathiazole solution. At a concentration of 250 µg/ml and 2.5 ml of the oxidizing agent at a concentration of 1 x 10-2 molarity, and adding 2 ml of the reagent N,N-DMPPDA, the solutions were left for 25 minutes to complete the reaction, after which they were diluted with distilled water to the mark, then the absorption of all Solutions at a wavelength of 554 nm versus their mock solutions. By calculating the recoverability of

each addition, it was found that there is no effect of the interferences used on absorption, which makes it possible to apply the method to pharmaceutical preparations and the results are shown in Table**.(4-8)**

Table (8-4) Effect of interferences on absorption

Foreign Compound	Recovery (%) of 250 µg Dapson / µg and Foreign Compound Added		
	125	250	375
Glucose	97.67	99.95	100.06
Lactose	98.99	99.99	98.92
Sucrose	101.09	100.03	99.41
Maltose	100.01	99.78	101.15

Applied part

A method can be applied to the pharmaceutical product containing sulfathiazole, which is liquid.

Estimation of the sulfathiazole in the drug liquid 40 mg in the direct way:

The estrangement of the salvityzol in this way is to take different pilgrims (1-3-3.5) millimeters of the drug solution 40 mg In three volumes of 20 ml, it was added to each two 2.5 ml of the oxidized worker Periodate potassium with a concentration of 0.01 Mullari and adding 2 ml of the N-N-DMPPDA with a concentration of 0.01 Mullari and absorption measure Al -Muji 554 Nanometer, after which the retrospective and RSD account and the results are shown as in Table.(8)

Table (8) Estimation of the Salviciazole in the pharmaceutical tablets (40 mg) in the direct way

Amount of Sulfathiazole taken µg /ml	RE, %	Recovery*, %	Average recovery,%	RSD, %
12.5	0.5190	99.68	100.16	1.195
37.5	0.5382	101.87		**0.**362
43.75	-0.4995	98.95		**0.**4308

The results described in Table (8) confirmed the success of the proposed method of estimating the salvityzol in the pharmaceutical tablets that were studied.

Comparing the method with other methods

The proposed method of estimated al -Salfathiazole has been compared to another spectrum, as shown in Tab**le. (9)**

Table (9) Comparing the proposed method with other methods

Analytical Parmeters	Present method	Literatue method[25]
λ_{max} (nm)	554	485
Temperature(ºC) 5	Room temperature temperature	**Room temperature**
Solvent	Water	**Water**
Color	Purple	**Pink-redish**
Beers Law rang(ppm	3.125-50	**2.5-25**
£(L.mol^{-1}.cm^{-1})	4161.699	**26500**

RSD,%	0.0018-0.0019	**1.595-2.817**
LOD µg/ml	0.995	**0.20ppm**
LOQ µg/ml	3.319	-------
Sandells sensitivity (μgcm^{-2})	0.0724	**0.0095**
Reagent	N,N-DMPPDA	**2,4-dinitrophenyl hydrazine**

Conclusions:

A new spectral method has been developed to estimate the highly sensitive sulfathiazole, which is directly appreciated by Salviciazole, in the manner of oxidative conjunction with the detector N, N-DDPPDA with a concentration of 0.01 Mullari with the oxidizing worker Periodate Potassium with a concentration of 0.01 Mullari, and the interaction is made in an acidic medium where it was shown. The result is a melted purple color in the water and stable and gave the highest absorption at a wavelength of 554 nm. Mall -1. Cm -1, Sandle sign 0.06134 microgram cm 2-, and the relative standard deviation does not exceed 0.0018 -0.0019 and a detection of 0.995 microgram/ ml, and the amount of 3.319 microgram/ ml and this proposed method successfully applied to estimate the salvysiazole in its pharmaceutical products and is liquid.

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