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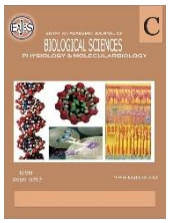

Spectrophotometric Determination of Sulfanilamide Using Oxidative Coupling Reaction With the Presence of The Peroxidase Enzyme

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ABSTRACT

The current study aims to create a new spectroscopic method for measuring sulphanilamide in large quantities and in pharmaceutical form. The proposed method involves using hydrogen peroxide as an oxidizing agent to oxidize sulphanilamide and P-aminophenol. Then, with the help of the peroxidase enzyme, an oxidative coupling reaction takes place between the two oxidation process byproducts to produce a brightly colored molecule with an absorbance value of 511 nm. To get the highest color intensity possible, reaction conditions are tuned. The absorbance was discovered to rise linearly with sulphanilamide content. The rules adhere to the beer base's (2-36 g/ml) range. It was discovered that the correlation coefficients were 0.9984. According to calculations, the sandal's sensitivity is 0.035 g/cm2.These approaches' analysis and recovery investigations have been statistically validated. This technique is used to create pharmaceutical substances. Relative standard deviation ratio is 0.135. The accuracy was assessed using recovery experiments, and the findings revealed that the values were 102.66, 100.56, and 100.75. The methods employed are precise, simple, and repeatable.

INTRODUCTION

Chemically known as 4-amino benzene sulfonamide, With molecular formula C6H8N2O2S and a molecular weight of 172.205 g.mol^{-1}; The basic composition of the drug is shown in **Figure (1).** White or off-white crystals or fine powder A medicinal compound used to prevent some bacterial infections. It is frequently used as a Topical cream or powder to treat superficial infections, as well as pills to treat internal infections. located in the Class of sulfonamide antibacterial drugs that are used to treat a variety of diseases, including strep throat, vaginal infections, urinary tract infections, and some staph infections. The recommended treatment will either be a cream or a tablet, depending on the type of illness (Ravikumar, Pandiarajan *et al.,* 2013). For the detection of sulfanilamide in medication formulations and water samples, a number of techniques have been published in the literature, including spectrophotometric (Schebeliski, Lima *et al.,* 2018), (Beitollahi, Tajik, *et al.*,2022).HPLC(Herrera, Hernandez, *et al.*,2013),(Waleed, Khaleel, *et al* ., 2013),(Mostafa, Shaaban, *et al.,* 2022), flow injection (Sadeghi, Oliaei, *et al.,*. (2021), and ion-selective electrodes (Safronova, Parshina, et al,.2020). On the other hand, numerous articles have been detailed on the detection of furosemide in pharmaceutical formulations and biological materials using spectrophotometric (Said, Rageh, *et al.,* 2018),(Naveed, Qamar, *et al.,* 2014), potentiometric (Zhang, Cui *et al.,* 2022).

Özbek, Berkel *et al* ., 2022, voltametric (Patil, Malode *et al.*, 2022)., HPLC (Kher, Ram *et al.*, 2013), (Darweesh, S. A, 2017), (Patel, Dholakiya, *et al* ., 2020)., GC (Chen, Yang *et al* ., 2021), TLC(Wesley,Mattocks *et al* ., 1982), flow injection (Semaan, de Sousa *et al* ., 2005), and fluorometric (Liu, Wang *et al* ., 2013), (Mattioli, Cervini *et al* ., 2020) methods. Figure (1) shows the drug's basic composition.

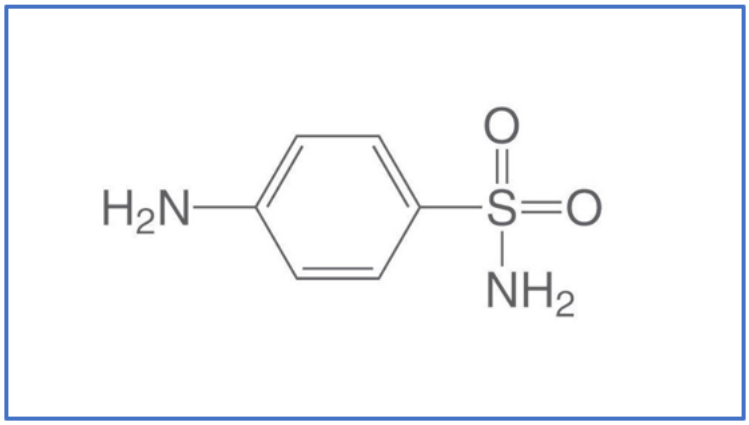

Fig. 1: Chemical structure for Sulphanylamide

MATERIALS AND METHODS

Apparatus Two spectrophotometers both from Shimadzu Company (UV-2900 PC Type and UV-1650 PC type) were used to follow the UV-Vis spectra. The samples were incubated using a Water bath with Type of GFL 1083 (GFL, Germany). The pH value of the used solutions was adjusted using a pH meter (WTW, China). An electronic balance was used in order to weigh all samples.

Materials:

Pure Sulphanylamide drug ($C_6H_8N_2O_2S$) was purchased from SDI (Samarra, Iraq) with purity100%. P-amino phenol ($HOC6H4NH_2$) was purchased from BDH with a purity of 80%. Peroxidase enzyme was purchased from Elabscience with purity100%. Hydrogen peroxide (H_2O_2) was purchased from Merck with a purity of 30%. Dipotassium hydrogen phosphate (K2HPO4) was purchased from BDH with a purity of 100%. Potassium hydrogen phosphate (KHPO4) was purchased from BDH with a purity of 100%.

Preparation of Solutions:

A precise 0,05 g of sulphanilamide was weighed and diluted in ethanol to create a capacity of 100 ml volumetric flask for the standard solution of sulphanilamide (500). A 100 ml volumetric flask was created by weighing and dissolving 2g of P-amino phenol and 2% (w/v) 2-amino phenol in ethanol. Just before experiments, hydrogen peroxide was generated by dissolving 0.1 ml of 30 percent hydrogen peroxide in 200 ml of distilled water to make 0.01M. Buffer (7.0 pH) Potassium Phosphate also dissolves 0.53 gm KH2PO4 and 1.06 gm K2HPO4 in distilled water, and then re-diluent to 100 ml. Buffers should be kept cold and re-equilibrated at 25 °C. Peroxidase was prepared by dissolving 0.223 g of peroxidase in 100 ml of distilled water. Keep chilled and reheat frequently.

RESULTS AND DISCUSSION

The maximum wavelength of absorbance was measured prior to creating the calibration curve while operating under the ideal conditions attained in the preceding tests. Without including the drug solution (hydrogen peroxide, peroxidase enzyme, buffer solution, and reagnt in the length range), the absorbance of the mixture against the form solution was measured in the same manner. The calibration curve was constructed using the wavelength between (190-800) nm, where the colored product displayed the

maximum absorbance at 511nm, while the blank solution exhibited a modest absorption at this wavelength, as shown in Figure 2.

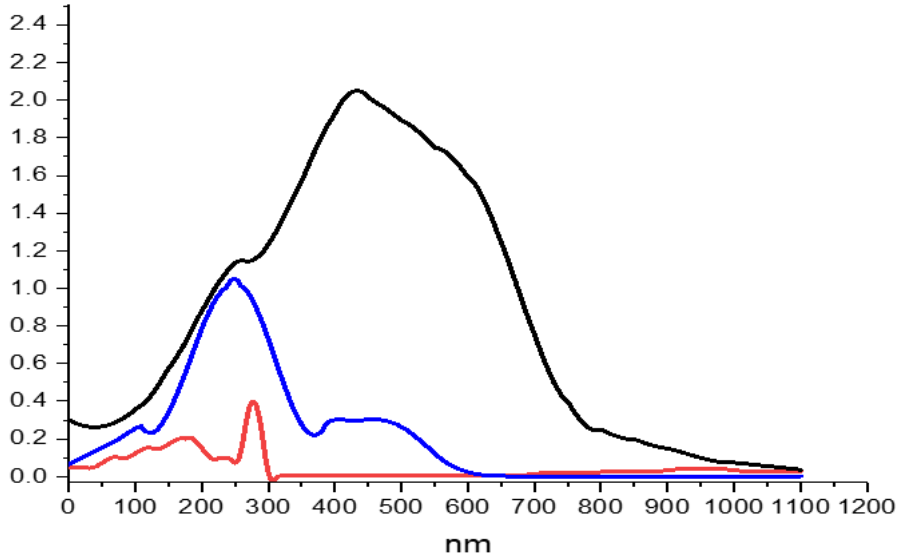

Fig. 2: Absorption Spectrum of the Sample (A), Blank (B), and sulphanilamide Pure (C)

The Best Concentration:

Effect of Buffer:

Each enzyme exhibits a specific pH range where they are most active. The structure of the enzyme, substrate, or cofactors, as well as their ionic state, are only a few of the elements that may contribute to this pH perfect. The effect of buffer volume was investigated by varying the volume of the buffer (0.5–6ml) since the buffer's absorbency peaked at 2ml and decreased thereafter. Figure 3, illustrates how phosphate buffering, with a pH of 7, affects the absorbance of colored product when using sulphanilamide

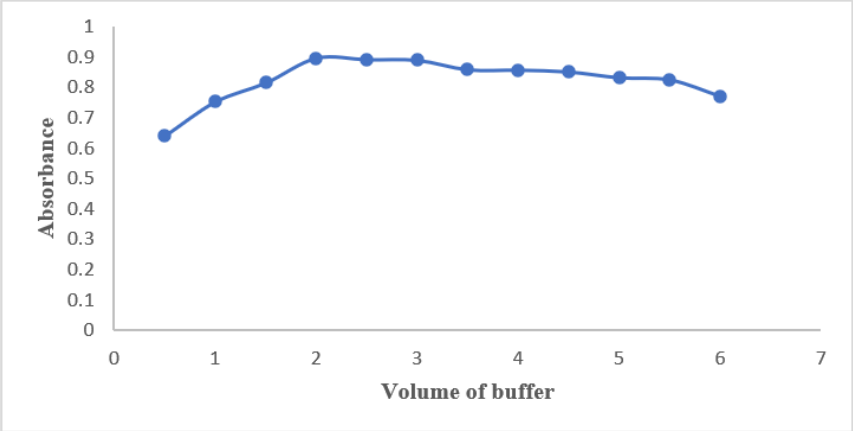

Fig. 3: Effect of buffer of sulphanilamide.

Effect of Temperature:

The suggested method was tested at various temperatures, and it was discovered that in the range of 0 - 40, the temperature remains constant, possibly because the colored product dissolves as the temperature rises, while the colored solution had the highest absorption at a temperature of 5, which was chosen as the reaction's ideal temperature. The effects of temperature for sulphanilamide on the absorbance of colored product are depicted in Figure 4.

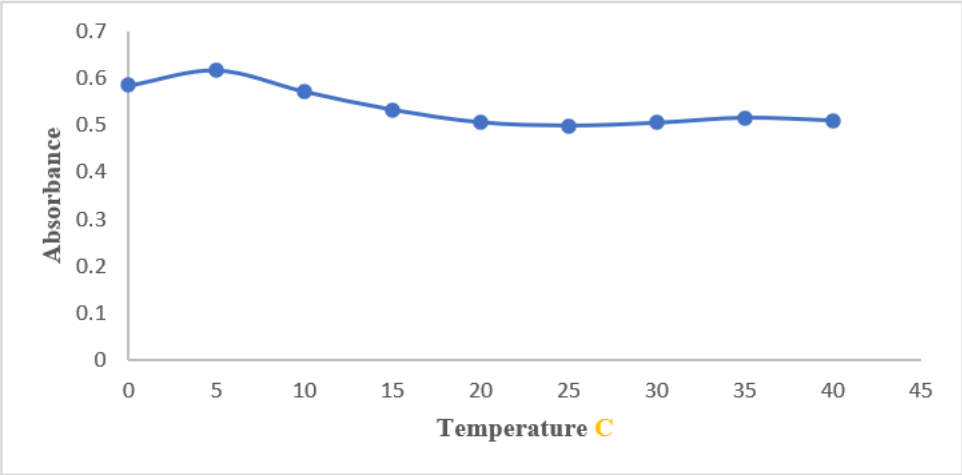

Fig. 4: Effect of temperature of Sulphanilamide.

Effect of Incubation Time:

While maintaining the other parameters constant, the impact of incubation time on the creation of maximum color was examined. The reaction mixture's contents were kept at laboratory temperature for up to 45 minutes using all of the indicated ways. When the reaction temperature is 10 5C, absorbance is measured at various standing time breaks. It was discovered that the maximum absorption occurs at five minutes. The above-mentioned incubation times are chosen for additional research. Figure 5, illustrates how sulphanilamide's incubation period affects the color product's absorbance.

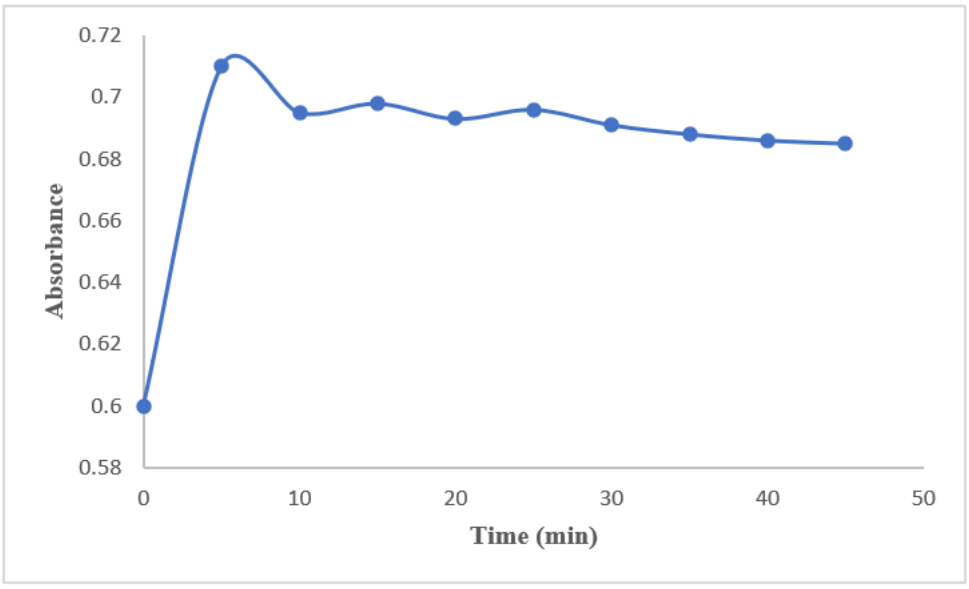

Fig. 5: Effect of incubation time.

Effect of Enzyme Peroxidase:

The best absorbance was observed at volume 0.7, and then a decrease in the absorbance values occurred, which is due to the saturation of the enzyme sites with the substrate. The effect of changing the size of the enzyme was studied by taking a range from (0.1 - 2.2ml) and measuring the absorbance. Figure 6, illustrates how sulphanilamide and enzyme peroxidase affect the absorbance of colored product.

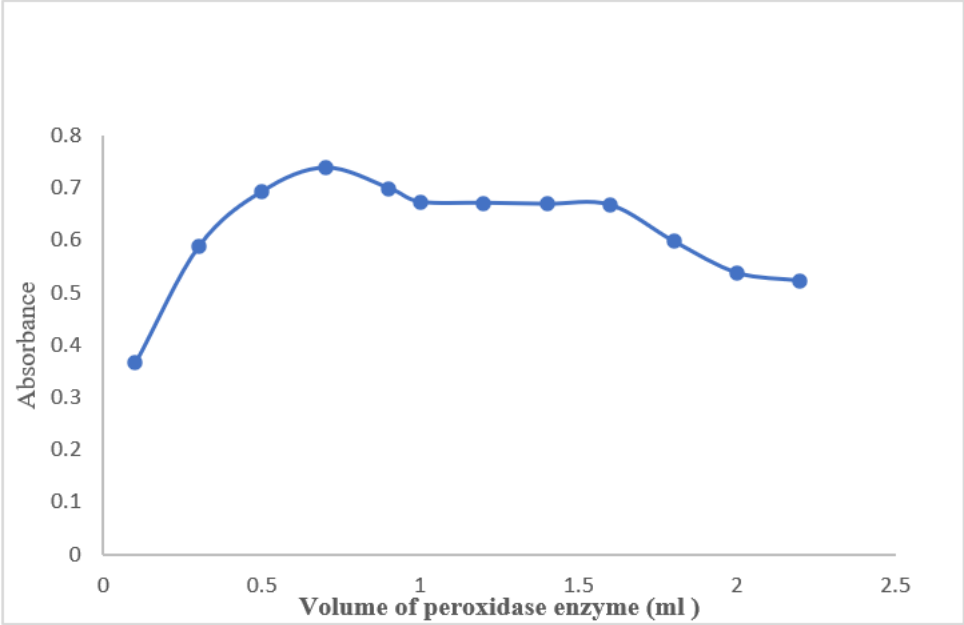

Fig. 6: Effect of Enzyme peroxidase.

Hydrogen Peroxide Effect:

The volume of hydrogen peroxide 0.01 M and the reaction rate (0.1-3.0 ml) were estimated for the spectrum of reactions. The rate of reaction accelerated with increasing hydrogen peroxide concentration and stabilized at 1 ml. Beyond this volume, the starting rate remains steady. As a result, it was advised to use a similar volume of 0.01 M hydrogen peroxide for all of the determination processes. The effects of hydrogen peroxide for sulphanilamide on the absorbance of colored product are depicted in Figure 7.

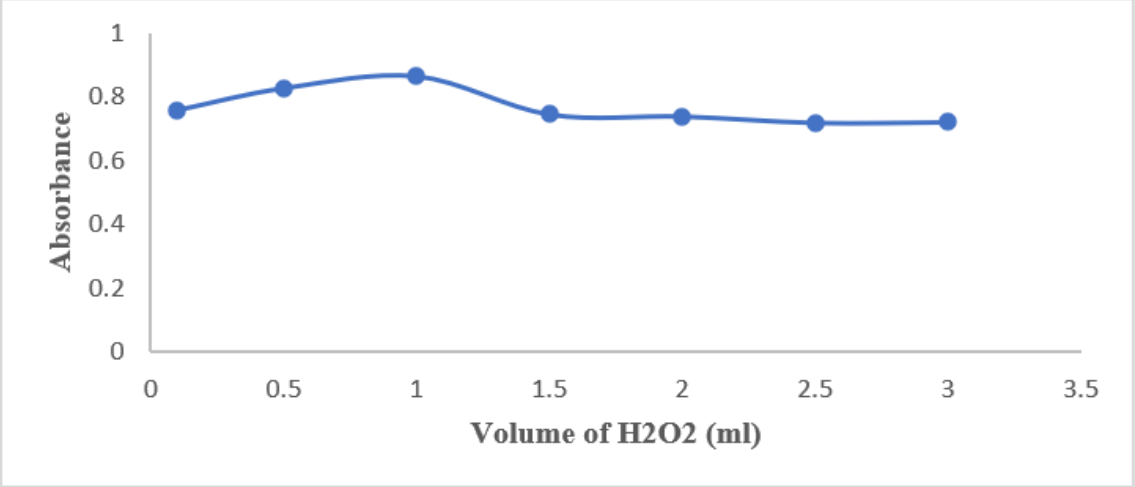

Fig. 7: Effect of hydrogen peroxide.

Effect of Reagent (P-amino phenol):

Variable volumes (0.1 - 4.5 ml) of 1% 2-AP were used to test the volume effect of 2% P-AP on product color enhancement. A total of 2.5 ml of 2% P-AP was used to attain the highest absorbance. There is no change in absorbance over this volume. Figure 8, illustrates how sulphanilamide's reagent (P-amino phenol) affects the absorbance of colored product.

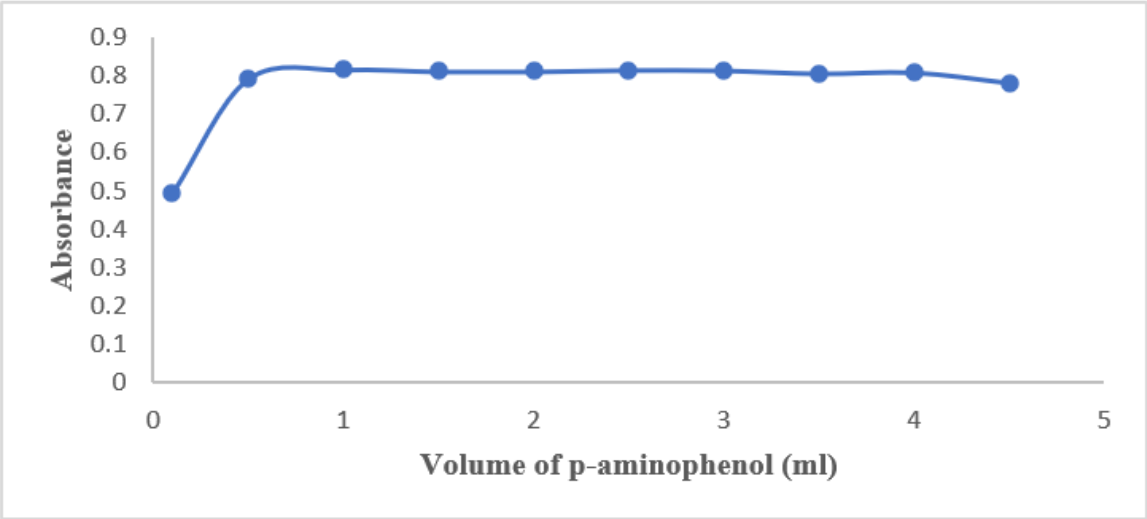

Fig. 8: Effect of reagent P-aminophenol.

Sequence of Addition:

To ascertain whether the absorbance of the colored products was impacted by the different orders in which the reactants were applied, a number of tests were carried out. Using buffer, P-AP, and H2O2 / HRP, we may determine the desired addition or arrangement of the reactants to determine Sulphanilamide. Table 1**,** shows the effects of the order in which compounds are added on the product's ability to absorb color. R stands for reagent, D for drug, B for buffer solution, and E for enzyme.

Table 1: Sequence of addition of Sulphanilamide

No.	order of addition	Absorbance
1.	D+P+E+B+R	1.408
2.	D+R+P+E+B	1.402
3.	P+R+D+E+B	1.403
4.	P+D+R+E+B	1.396
5.	R+D +P+E+B	1.400
6.	B+R+D +P+E	1.395
7.	B+D+R+P+E	1.394
8.	E+P+D+R+B	1.399

Calibration Curve:

The best conditions were used to prepare a series of solutions containing various volumes of sulphanilamide (0.1-2.0 ml) per concentration (500 g/m) in a volumetric flask of 25 ml with the addition of 1 ml of hydrogen peroxide, o.7 ml of enzyme, 2 ml of buffer, and finally the reagent 2.5 ml at a temperature of 10 ° C. After 5 minutes, the solutions were measured, and it is discovered that the purple product generated has an absorptivity of. The sulphanilamide calibration curve is shown in Figure 9.

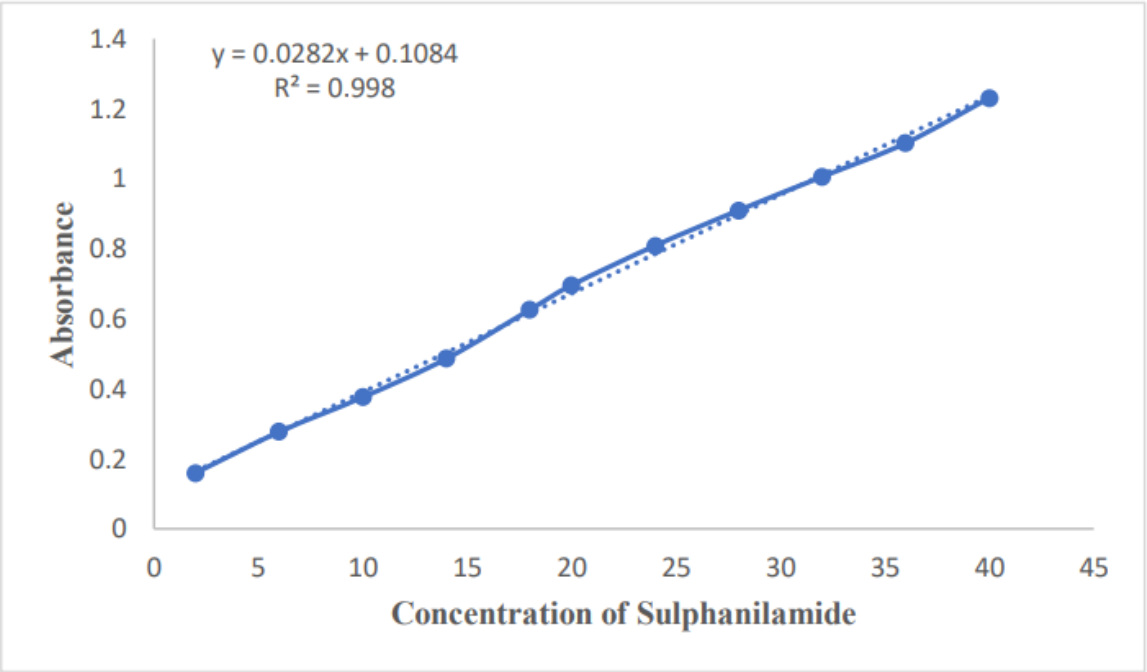

Fig. 9: Calibration Curve of Sulphanilamide.

Table 2: Analytical Parameter for Determining (Thy.)

Parameters	Values
beer's law limit (µg/mL)	(2-36)
Molar Absorptivity (L / mol.cm)	4.96×10^3
Sandell's sensitivity (µg/cm2(	0.035
Limit of Detection (LOD))µg/mL	0.1493
Limit of Quantitation (LOQ) µg/mL	0.498
R.S.D%	0.268
Correlation Coefficient	0.9984
Slope	0.0288
Intercept	0.0385

The Stoichiometry of Sulphanilamide:

In order to identify the compositional ratio of the colored product between the drug and reagent, the two methods of the mole ratio process as well as continuous variation(Job's method) were applied, as the first method was performed by adding increasing volumes of the drug (0.5-4.5ml) to decreasing volumes of the reagent (4.5-0.5ml) at a concentration of 0.001 M for each of The reagent and drug so that the total volume of both the reagent and the drug is 5 ml, and by diluting to end volume of 25 ml, following the same steps used in building the standard calibration curve and the results obtained are plotted as shown in the(figure10), The second method was performed by adding increasing volumes of the reagent (0.1-2ml) to a fixed volume of the drug (1 ml) and at a concentration of 0.001 M for both the reagent and the drug in a final volume of 25 ml with the same steps followed by building the standard calibration curve and plotting the results obtained as In(Figure11) And based on the results obtained from Figures 10 and 11, it was found that the ratio of drug to reagent binding is 1:1 .

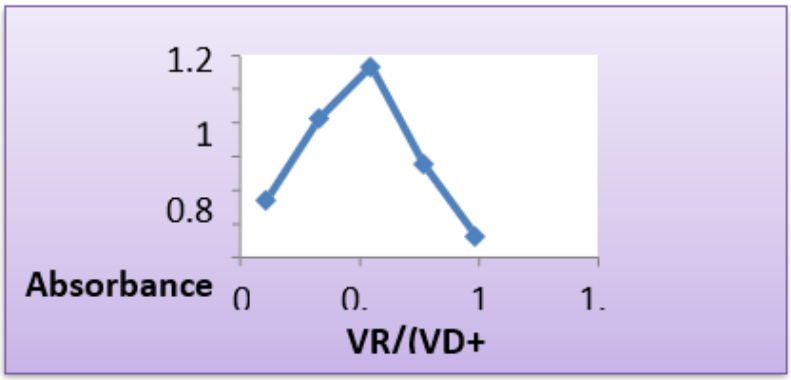

Fig. 10: Job's method

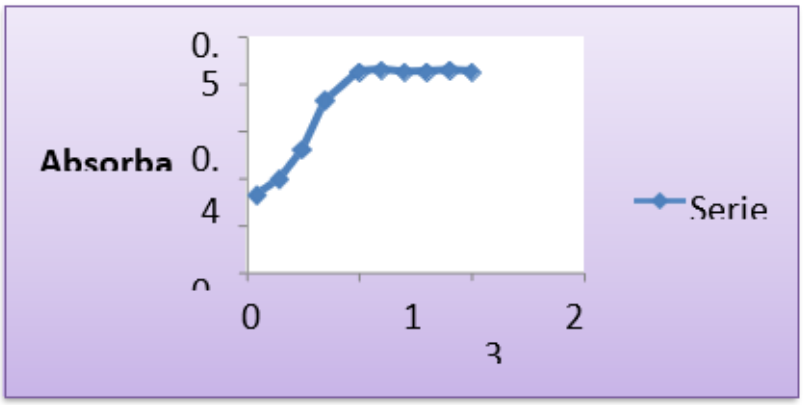

Fig. 11: mole ratio of Sulphanilamide

Precision and Accuracy:

The precision and accuracy of the proposed methods were evaluated using three replicates at levels of 10, 20, and 30 ppm Sulphanilamide

E% = [(x - x°) / x°] × 100 ……

The calculated amount X is equal to the genuine amount X°.

Rec. % = 100 ± E% .....(2)

$SD = \frac{\sqrt{(Xi-X)2}}{n-1}$……. (3)

The standard deviation is SD.

$RSD\% = \frac{SD}{X} x100\%$.....(4)

Table 3: Value Precision and Accuracy for Sulphanilamide Product Compound

	Concentration of Sulphanilamide (ppm)				
No.	**True amount**	**Calculatedamount**	**Relative Error%**	**Recovery%**	**R.S.D%**
1.	**6**	**6.16**	**- 2.66**	**102.66**	**0.405**
2.	**18**	**18.10**	**-0.56**	**100.56**	**0.077**
3.	**24**	**24.18**	**-0.75**	**100.75**	**0.323**

Interferences Effect:

The effect of the interactions was examined by spectrally estimating the anticipated medicinal compounds and separately adding the substances from the solutions studied and the concentration of the substances in order to ensure the selectivity of the aimed process for the purpose of applying it in routine studies on various samples, in particular on pharmaceutical preparations including Sulphanilamide. Using the same procedures as those used to create the standard titration curve (Sulphanilamide 1 ml at 500 ppm, hydrogen peroxide 1 ml, peroxidase enzyme 0.7 ml, buffer solution 2 ml, reagent P-AP, and finally 1 ml of the prepared interference at 5000 ppm), and by

adhering to the best practices used in the calibration curve and by final dilution of 25 ml with distilled water). As the interfering effects were suitable if the error rate was not more than (2%) when compared to the amounts in the absence of interference, the solution's absorbance was estimated, along with the error rate and percentage recovery. The existence of such compounds that have no impact on the Sulphanilamide determination methods is evident in the results. Each value is a three-analysis average.

Table 4: The Effect of the Presence of Interferences at a concentration of 5000ppm.

Interference	**% Error**	**% Recovery**
Starch	-1.5	98.5
Benzoic acid	-2	98
Sodium salicylate	-2	98
Fructose	-2	98
Ascorbic acid	1.49	100.5
Calcium chloride	0.5	100.5
Talc	2	102
Urea	0.9	100.9
Cellulose	0.6	100.6
Sodium disulfate	1.45	101.45
Spartum	1.2	101.2
Cholesterol	1.75	101.75
Sucrose	2	102
Lactose	1.45	101.45
Sodium chloride	0.9	100.9

Table 6: Circumstances for the proposed method's improvement.

Factor	**Investigation condition**	**Condition in procedure**
λmax (nm)	0-1200	511
Effect of buffer(ml)	1-6	2
Effect of temperature (^{0}C)	0-40	5
Incubation time(min.)	0-45	5
Effect of Enzyme (ml)	(0.1-2.2)	0.7
Effect of volume of 0.01M H_2O_2 (ml)	0.1-3.0	1
2% P-AP (ml)	0.1-4.5	2

Conclusions:

There has been devised an efficient, exact, sensitive, and verified enzymatic technique for the determination of Sulphanilamide. The statistical characteristics and data from the recovery research provided a clear illustration of the system's reproducibility and accuracy. The large-scale production of Sulphanilamide in prescription dose forms necessitates routine quality control.

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