

**A Simple and Sensitive Spectrophotometric Method for the Determination of Trace Amounts of Sulfadiazine in Pharmaceutical Drugs Sample**

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**Abstract**

This study includes a simple and sensitive spectrophotometric method for the estimation of trace amounts of sulfadiazine in aqueous solution utilizing oxidative coupling reaction of sulfadiazine with pyrocatechol in the existence of potassium periodate ( $KIO_4$ ) in acidic medium to yield a water-soluble dye which appear a maximum absorbance at 515 nm. The linearity is in the concentration range 0.2 - 20  $\mu\text{g/ml}$  of SDZ, the molar absorptivity  $1.2765 \times 10^4$  liter.  $\text{mol}^{-1} \cdot \text{cm}^{-1}$  and the relative error ranges from 1.20 - 1.36 % depending on the concentration level of SDZ. The study was successfully applied to estimate the sulfadiazine in pharmaceutical drugs sample in the form of injections and burn cream.

**Keywords:** Sulfadiazine, Pyrocatechol, Spectrophotometry.

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### طريقة طيفية بسيطة وحساسة لتقدير كميات ضئيلة من السلفاديازين في عينة العقاقير الصيدلانية

اسراء طالب حميدي

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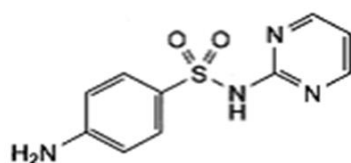
#### الخلاصة

تتضمن هذه الدراسة طريقة طيفية بسيطة وحساسة لتقدير كميات ضئيلة من السلفاديازين في الوسط المائي، باستخدام تفاعل الازدواج التأكسدي للسلفاديازين مع البايروكاتيكول في وجود بيرويدات البوتاسيوم ( $KIO_4$ ) في الوسط الحامضي لإنتاج صبغة دائبة في الماء تظهر أقصى امتصاص في 515 نانومتر. الخطية في مدى التركيز 2.0 - 20 مايكروغرام /مل من SDZ وبلغت قيمة الامتصاصية المولارية  $1.2765 \times 10^4$  لتر. مول<sup>-1</sup>. سم<sup>-1</sup> والخطأ النسبي يتراوح 1.20 - 1.36 % اعتمادا على مستوى تركيز SDZ. وقد طبقت الدراسة بنجاح لتقدير سلفاديازين في مستحضراته الصيدلانية في شكل حقن ومرهم للحروق .

**الكلمات المفتاحية:** السلفاديازين، البايروكاتيكول، المطيافية الضوئية.

#### Introduction

Sulfadiazine (4-amino-N-pyrimidin-2-ylbenzenesulphonamide) [1] Scheme1 is an antibiotic of sulfonamides group. This medication is used to treat and prevent a wide variety of infections. It works by stopping the growth of bacteria and other organisms [2]. It is used in a treatment for cramps, treat urinary tract, in clinical medicine, and also used against bacteria consisting of folic acid. Side effects for the drug is nausea, vomiting, diarrhea, loss of appetite, or headache [3].



$C_{10}H_{10}N_4O_2S$

M.Wt: 250.3 g/mol

**Scheme 1:** Chemical Structure of Sulfadiazine

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Several analytical techniques published in the literature for the estimate of sulfadiazine are UV–spectrophotometry<sup>(4-12)</sup>, HPLC<sup>(13-17)</sup>, Electrochemical method<sup>(18,19)</sup>, and Flow injection-chemiluminescence method<sup>(20,21)</sup>. This study includes a simple and sensitive spectrophotometric method for the estimation of trace amounts of sulfadiazine in aqueous solution utilizing oxidative coupling reaction of sulfadiazine with pyrocatechol in the existence of potassium periodate (KIO<sub>4</sub>) in acidic medium.

### Experimental

#### **Instrumentation**

Shimadzu UV/VIS 160 spectrophotometer with quartz cells (1 cm) were used for all spectral measurements. PH meter Jenway 3310 was used along with sensitive balance Sartorius BL210 S.

#### **Materials and Chemical Reagents**

All materials and chemical reagents used at a high level of purity and they have been prepared as follows:

##### **1- Sulfadiazine standard solution (1000 $\mu$ g.ml<sup>-1</sup>):**

It was attended by dissolving 0.1000 g of powder sulfadiazine in 1 ml of concentrated hydrochloric acid and then completes the volume to 100 ml at distilled water. 15 ml of sulfadiazine solution is diluted to 100 ml at distilled water, to get a solution at a concentration of 150  $\mu$ g.ml<sup>-1</sup>.

##### **2- Pyrocatechol solution (3.5 $\times 10^{-3}$ M):**

It was attended by dissolving 0.0385 g of pyrocatechol in 100 ml of distilled water utilizing a volumetric flask.

##### **3- potassium periodate solution (5 $\times 10^{-3}$ M):**

A 0.1150 g of KIO<sub>4</sub> was dissolved in distill water and the volume was completed to 100 ml in a volumetric flask using distilled water.

##### **4- Hydrochloric acid solution (approximate, 0.2M):**

It was prepared by diluting 1.7 ml of 11.8 M hydrochloric acid to 100 ml at distilled water in a volumetric flask.

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**5- Preparation of sulfadiazine samples (150  $\mu\text{g}\cdot\text{ml}^{-1}$ )**

- **bio prime liquid injections** ( Bioagripharm GmbH-germany), each 1.0 ml contains 60 mg of sulfadiazine, and attend this solution by take the equivalent of 0.1000 of sulfadiazine and dissolve the same way that dissolve in the standard solution, and completed its volume to the mark of distilled water to get a solution with a concentration 1000  $\mu\text{g}/\text{ml}$ . 150  $\mu\text{g}/\text{ml}$  of solution was attended by dilution of 15 ml of the above solution to 100 ml by distilled water.

- **Flaumizin Burn Cream** (Domina company of pharmaceuticals industries - Damascus, Syria). Each 100 g containing 1.0000 g sulfadiazine- silver, and attended this solution by take the equivalent of 0.1000 g of a sulfadiazine- silver (after mixing two cans of ointment, each can 50 g) and dissolved with 50 ml of diethyl ether, using suitable separating funnel to extraction by three batches of distilled water (25 ml each batch). After collecting aqueous layer containing sulfadiazine- silver, filtered and diluted to 100 ml at distilled water, then took 25 ml of this solution and dilute to 100 ml with distilled water to get a solution at concentration 150  $\mu\text{g}\cdot\text{ml}^{-1}$ .

**Study of the optimum Conditions**

Subsequent experiments was performed using 2 ml of sulfadiazine solution (150  $\mu\text{g}\cdot\text{ml}^{-1}$ ) in a final volume (25 ml) and the different variables which effect on the absorption intensity of the color product were studied and the optimal conditions were chosen.

**Effect of acid**

To know the influence of diverse acids and choosing the best acid, the study was performed using various amounts ranging of 0.3 - 3.0 ml of (strong and weak) acids (0.2 M), Table (1) illustrate that 1.5 ml (0.2 M) hydrochloric acid (pH= 2.3) gave highest sensitivity and this amount was chosen in later studies.

**Table 1:** Effect of Acid on the Absorbance

Acid (0.2M)	ml of Acid / Abs.							
	0.3	0.5	0.75	1	1.5	2	2.5	3
HCl	0.257	0.381	0.433	0.496	0.579	0.538	0.472	0.409
HNO <sub>3</sub>	0.158	0.217	0.288	0.325	0.367	0.359	0.308	0.312
H <sub>2</sub> SO <sub>4</sub>	0.194	0.243	0.279	0.332	0.358	0.319	0.259	0.213
CH <sub>3</sub> COOH	0.048	0.096	0.121	0.163	0.144	0.129	0.055	0.037

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### Effect of oxidizing agent concentration

The effect of different amounts of potassium periodate was studied in the range (0.2-4.0 ml) of ( $\text{KIO}_4$ ) ( $5 \times 10^{-3}$  M). A 2.0 ml of  $5 \times 10^{-3}$  M  $\text{KIO}_4$  solution is found sufficient to development the color intensity, therefore it was considered an optimal amount. Fig (1)

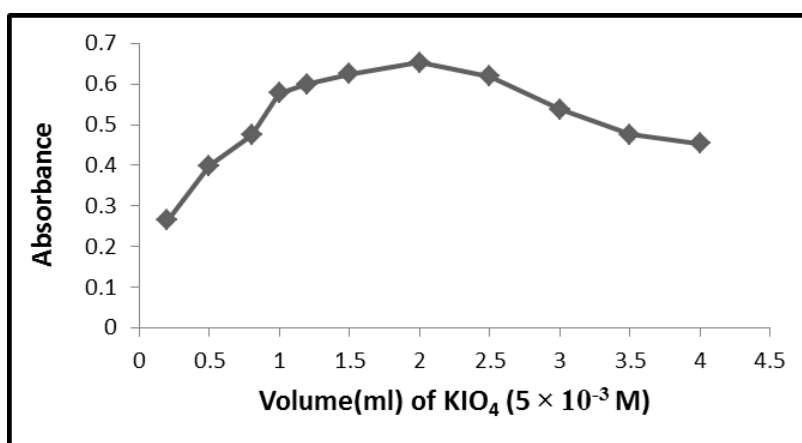


Figure 1: Effect of  $\text{KIO}_4$  Amount on the Absorbance

### Effect of pyrocatechol amount

Different amounts (0.2-4.0 ml) of pyrocatechol reagent ( $3.5 \times 10^{-3}$  M) were examined. Fig. (2) refers to that using 1.8 ml of pyrocatechol reagent resulted to the higher value of formed product absorbance at 515 nm, this amount was selected for later studies.

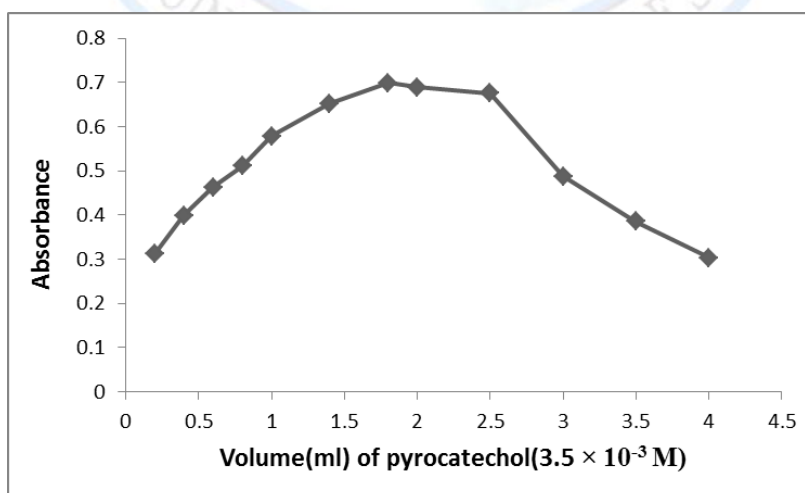


Figure 2: Effect of Pyrocatechol Reagent Amount on the Absorbance

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### Effect of time on the absorbance

The effect of the time on the absorbance was tested. It was noted that the red color for the formed product form from the beginning as shown in Fig. (3). The absorption increases with time and the red color prove after 15 min and remain stable for more than 60 min.

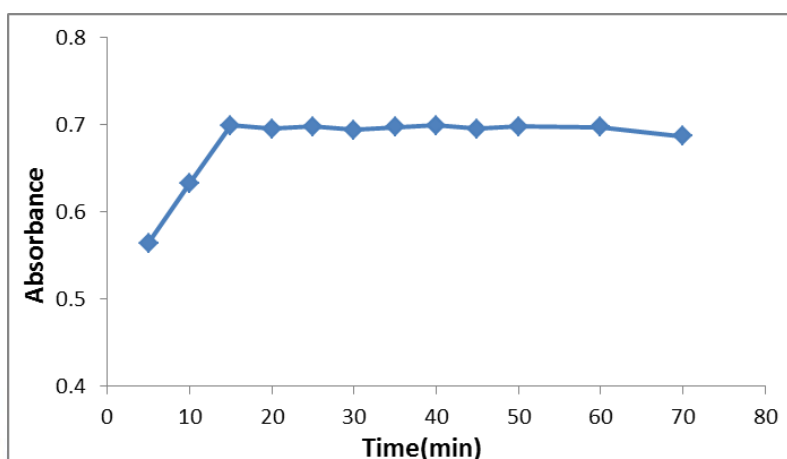


Figure 3: Effect of Time on the Absorbance

### Effect of Temperature on the Absorbance

The color intensity for formed product were examined at various temperatures. Fig.4 refers that the 25°C give the best value of absorbance for this reaction, this means that the room temperature was used in next experiences.

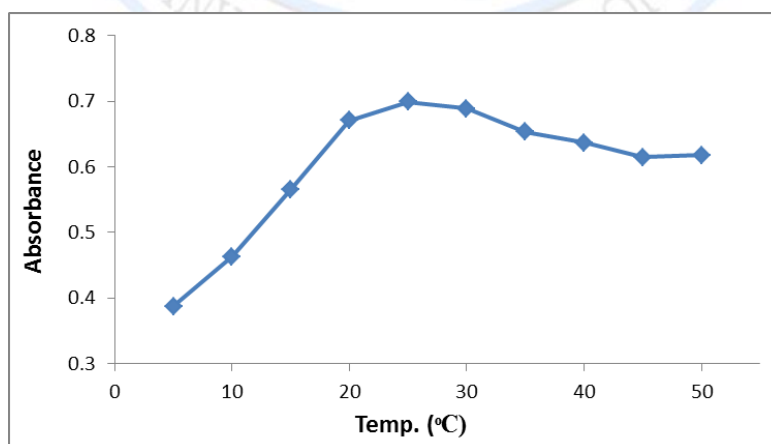
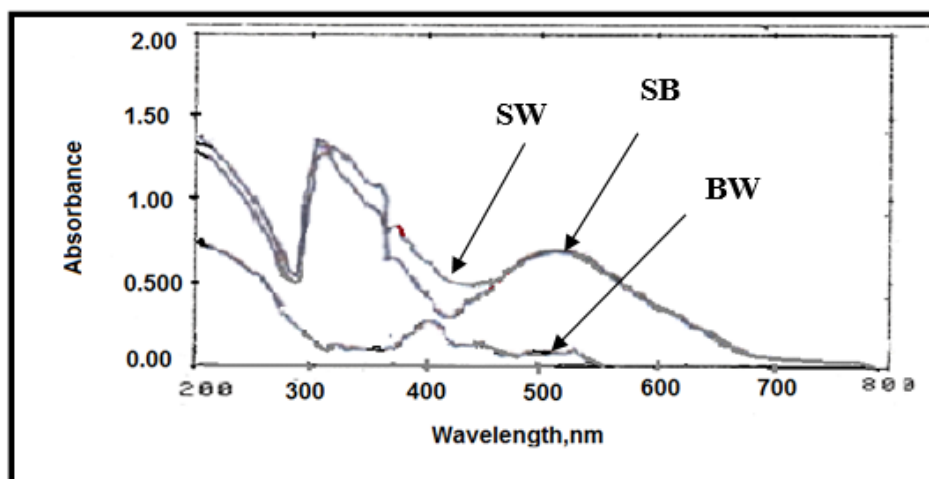


Figure 4: Effect of Temperature on the Absorbance

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Based on the optimal conditions of the experiment, Fig.5 explains a maximum absorption for colored product at 515 nm versus the blank reagent.



**Figure 5:** Absorption Spectrum for Colored Product Measured Versus blank (SB), distilled water (SW) and blank versus distilled water (BW).

### Construction of Calibration Curve

Optimal conditions that are installed in the proposed method shown in the procedure. Fig. (6) shows that the linear of standard calibration curve followed the Beer-Lambert law on the concentration range from (2.0 – 20)  $\mu\text{g}\cdot\text{ml}^{-1}$  and a negative deviation occurs when the concentration higher of 20  $\mu\text{g}\cdot\text{ml}^{-1}$ . The value of the molar absorption coefficient of the product formed is  $1.2765 \times 10^4 \text{ liter}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$ , correlation coefficient is 0.9984 and the Sandell's sensitivity is  $0.0196 \mu\text{g}\cdot\text{cm}^{-2}$ , this indicates that the standard curve gave good linear specifications.

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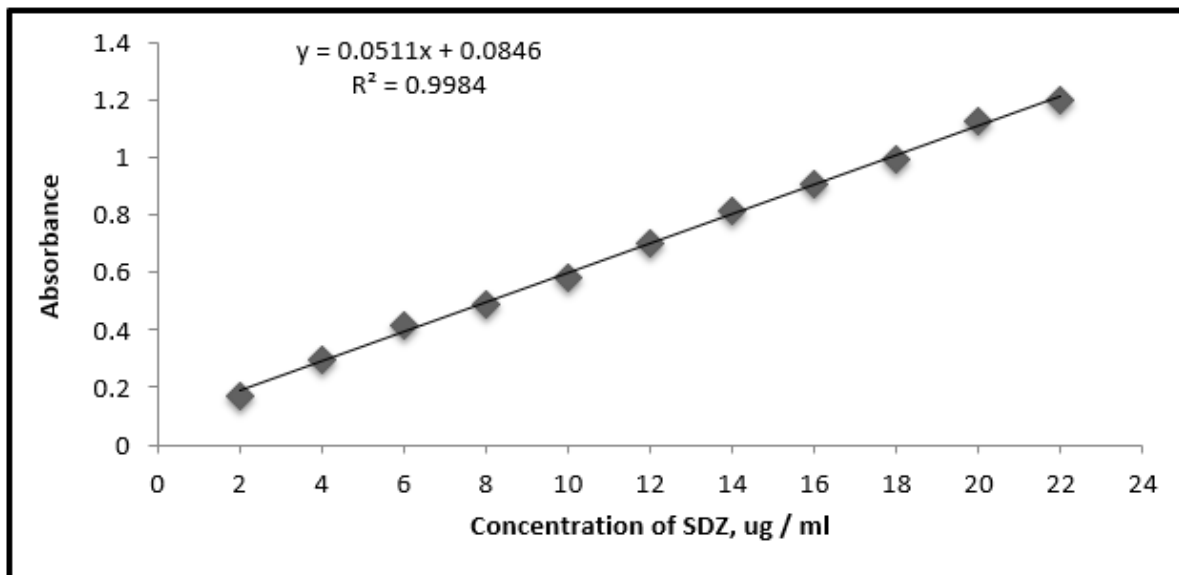


Figure 6: Standard Calibration Curve for SDZ with Pyrocatechol.

### Accuracy of the Method and their Precision

Accuracy of the method and their precision were calculated under supported optimal conditions at three different amounts of sulfadiazine (4, 6, 8)  $\mu\text{g}\cdot\text{ml}^{-1}$  by taking the average six readings for each of them. Table (2) shows that the suggested method is on a high accuracy and a satisfactory precision.

Table 2: Results of Accuracy and Precision.

Conc. of SDZ (ppm)	RSD %	Recovery* %	Average recovery %	RE %
4	1.245	98.65	98.70	-0.765
6	1.897	100.12		0.534
8	1.564	97.34		0.398

\* Average of six readings

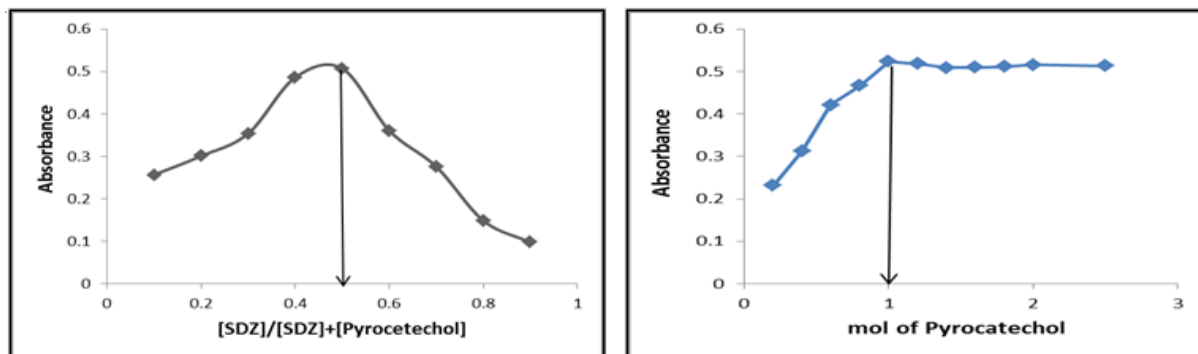
### Stoichiometry and mechanism of the reaction product

continuous variations (Job's method) and mole ratio methods used to find a relationship between the sulfadiazine and pyrocatechol, the concentration of each of the sulfadiazine solution and reagent solution in both methods is  $5.99 \times 10^{-4}\text{M}$ . Both methods show that the ratio of sulfadiazine to pyrocatechol is 1:1(Fig.7).

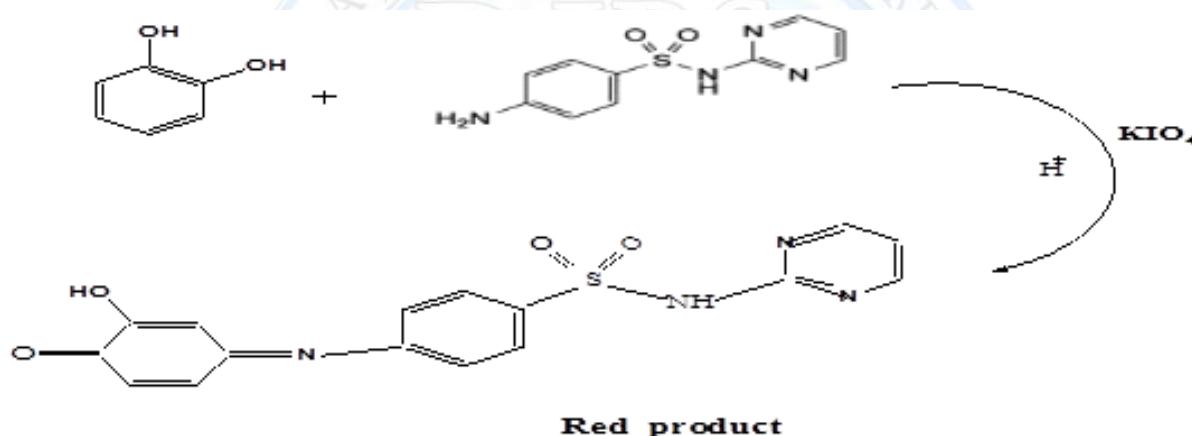


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**Figure 7:** Job's method (A) and mole ratio method (B) of formed product SDZ – Pyrocatechol  
 The suggested reaction mechanism between SDZ and pyrocatechol is presented in Scheme 2.



**Scheme 2:** the proposed structure of the complex

### Examination of interferences

For test the selectivity of the suggested method, the effect of number of foreign materials (e.g. glucose, lactose, dextrose, acacia and starch) that usually existent in dosage forms have been examined by adding various volumes (2.5, 5.0, 7.5 ml) of foreign materials (1000  $\mu\text{g/ml}$ ) to 2 ml of SDZ (150  $\mu\text{g/ml}$ ) gave a final concentration of (100, 200, 300  $\mu\text{g/ml}$ ). The results appear that the examined foreign species did not overlap in the suggested method.

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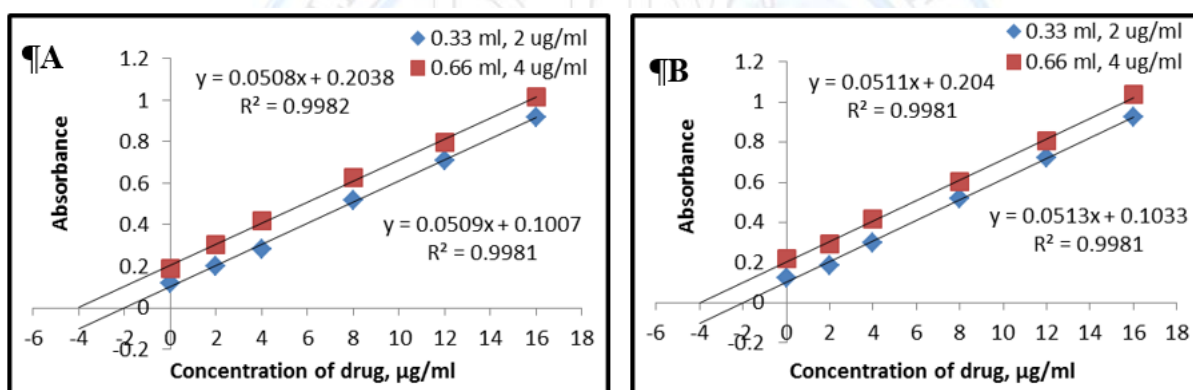
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**Table 6:** Effect of Interferences

Foreign compound	Recovery (%) of 12 µg/ml of SDZ per µg/ml foreign compound added		
	100	200	300
Glucose	99.45	100.04	99.27
lactose	100.15	100.67	98.73
dextrose	97.28	99.43	99.59
acacia	99.15	98.84	100.06
Starch	99.55	98.13	100.26

**Applications of the analytical study for determination of SDZ**

Analytical study was successfully applied to estimate the sulfadiazine in pharmaceutical drugs sample in the form of injections and burn cream using standard additions method.



**Figure 7:** Graph of standard additions of SDZ using in (A) bio prime liquid injections (B) Flaumizin Burn Cream

**Table 7:** Results of Standard Additions Method

pharmaceutical	SDZ present µg/ml	SDZ measured µg/ml	Recovery*, %
bio prime liquid injections	2.000	1.978	98.90
	4.000	4.012	100.30
Flaumizin Burn Cream.	2.000	2.014	100.70
	4.000	3.992	99.80

Results of the analysis in the previous table proved that standard additions method agree completely with the proposed method for evaluate SDZ in the pharmaceutical drugs which indicates that good method.

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**Comparison method with literature methods**

Table (8) shows the comparison of the proposed method for determining sulfadiazine with other literature spectrophotometric methods.

**Table 8:** Comparison of the Proposed Method with the Literature

Analytical parameter	Literature <sup>(4)</sup> method	Literature <sup>(5)</sup> method	proposed method
Reagent	2,5-dimethoxy aniline	$\gamma$ -Resorsolic acid	pyrocatechol
linear range $\mu\text{g/ml}$	0.1 – 5	0.4 - 12	0.2 – 20
Solvent	Ethanol	Water	Water
$\lambda_{\text{max}}$ (nm)	478	458	515
pH	Acidic medium	12.35	2.3
Recovery (%)	99.70 – 98.89		97.34 – 100.12
RSD (%)	- 1.197 0.734	0.27-1.21	- 1.897 1.245
Molar absorptivity ( $\text{L mol}^{-1}\text{cm}^{-1}$ )	$8.26 \times 10^4$	$4.38 \times 10^4$	$1.2765 \times 10^4$
Sandel Index $\mu\text{g/cm}^2$	0.003	-----	0.0196
Correlation coefficient, $r^2$	0.9956	0.9998	0.9984
Temperature	RT	RT	RT
Colour of the dye	orange	Yellow	Red
Pharmaceutical preparation	burn cream	Burn cream ,tablet	injections , burn cream

The results shown in the above table indicate that the proposed method is good sensitive and accurate does not require the use of organic solvents.

**Conclusions**

Results of the analysis showed that the proposed method for the estimation of trace amounts of sulfadiazine in aqueous solution is good sensitivity and stability of formed product. This method do not require the use of organic solvents may be expensive and unavailable and does not require the process of extracting and could be applied in estimating the sulfadiazine in pharmaceutical drugs sample in the form of injections and burn cream.

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