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Spectrophotometric Determination of Sulfanilamide in Pure and Synthetic Sample by New Derivative from Pyrazoline Derived from 2, 4 – Dinitro Phenyl Hydrazine

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Abstract

Simply, sensitive and accurate spectrophotometric methods have been improved for the determination of sulfanilamide (SNA) drug in pure and synthetic sample. This method is based on the reaction of sulfanilamide (SNA) with new organic reagent (BYN) it was prepared by reaction between ethyl acetoacetate with 2, 4-Dinitrophenylhydrazine. The azo dye product shows absorption maximum at 435 nm. The linearity ranges of sulfanilamide are (2 – 20 µg. mL^{-1}) with molar absorptivity (7671.2 L.mol-1.cm^{-1}), Sandell's sensitivity index (0.0224 µg.cm^{-2}), detection limit and Quantification limit (0.1598, 0.4843 µg / ml) respectively. The results showed there are no interferences of excipients on the determination of the drug. The proposed method has been successfully applied for the determination of sulfanilamide in pure and in synthetic sample.

Keyword: Sulfanilamide, Spectrophotometric, Pyrazoline, Azo dye.

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التقدير الطيفي لتقدير عقار السلفانيل أمايد بصورته النقيه وفي نموذج محضر بأستخدام مشتق جديد من البايرازولين المشتق من ثنائي فنيل هيدرازين

جاسم محمد خليل القره غولي، أسماء احمد محمد الراشدي و خالد عبدالعزيز البدراني

قسم الكيمياء – كلية التربية للعلوم الصرفة – جامع تكريت

الخلاصة

يتضمن البحث تطوير طريقة طيفية بسيطة و حساسة لتقدير عقار السلفانيل أمايد في صورته النقية وفي نموذج محضر، الطريقة تستند على تفاعل العقار المؤزت مع كاشف عضوي جديد (BYN) حضر من تفاعل ايثايل أسيتواسيتيت مع ثنائي نايترو فنيل هيدرازين أعطى صبغة أزو ذائبة في الماء سجلت أعلى امتصاص عند الطول الموجي 435 نانوميتر .كانت حدود قانون بير في مدى التراكيز 2-20 مايكروغرام/مل من السلفانيل أمايد والامتصاصية المولارية 7671.2 لتر.مول$^{-1}$سم$^{-1}$ ودلالة ساندل 0.0224 مايكروغرام.سم$^{-2}$، وحد الكشف الكمي والنوعي (0.1598, 0.4843) مايكروغرام/ مل على التوالي. تمّ تطبيق هذه الطريقة بنجاح لتقدير السلفانيل أمايد بصورته النقية وفي نموذج محضر ولم يكن للمتداخلات أي تأثير.

الكلمات المفتاحية: الطريقة الطيفية، السلفانيل أمايد، البايرازولين، صبغة الأزو.

Introduction

Sulfanilamide was the first sulfonamide developed in 1906, although it was not used as an antimicrobial until the late 1930s. Several thousand other substances have since been developed from sulfanilamide which was widely used in the dye-making industry; its patent had since expired and the drug was available to anyone. [1].

Sulfanilamide chemically is 4-amino benzene sulfonamide $C_6H_8N_2O_2S$, 172.205g.mol^{-1} figure 1, it is a medicinal compound that is used to guard against certain bacterial infections. It is frequently used in the form of a topical powder or cream to treat surface infections, as well as

a pill for internal infections. It falls into the category of sulfonamide antibacterial drugs, common infections treated by sulfanilamide include urinary tract infections, vaginal infections, strep throat, and some staph infections. Either a cream or a pill will be prescribed, depending on the type of infection [2-3].

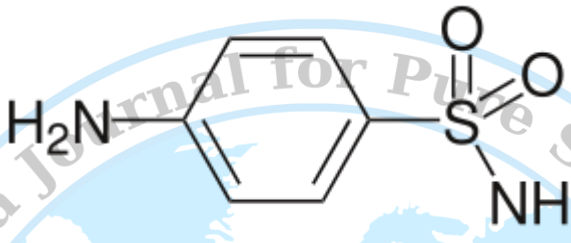

Figure 1: The chemical structure of Sulfanilamide

Different methods such as, Spectrophotometric [4-8], HPLC [9-12], flow injection [13], ion-selective electrodes [14] have been described in the literature for the determination of sulfanilamide in water samples and pharmaceutical preparations.

On the other hand, many publications described for use sulfanilamide in the determination of many substance such Cardanol [15] Anacardic Acid [16]. Chalcones are natural products that can also be obtained synthetically using a relatively simple synthesis procedure.

The general method applied to synthesize chalcones is the Claisen-Schmidt reaction. An important feature of chalcones is the ability to act as an intermediate for the synthesis of biologically active heterocyclic compounds such as, cyclohexenone, pyrazoline, pyridine, pyrimidine, and isoxazoline derivatives [17 and 18].

The aim of the present work is to provide an optimized spectrophotometric method to produce azo dye by reaction between SNA drug and new derivative from pyrazolin derived from di nitro phenyl hydrazine.

Experimental

Instruments

T90 UV-Visible Spectrophotometer PG Instrumental Ltd, UK with 10 mm quartz cell was used for all spectrophotometric quantities, and Sartorius Balance 210S kern was used to perform all weight measurements.

Reagents and Materials

All the chemicals and solvents used were of Aldrich and Fluka products and were used without further purification and Sulfanilamide typical material was provided from State Company for Drug Industries and Medical Appliance (SDI) Samarra – Iraq. Distilled water was used to prepare all solutions. A 1000 µg/ml (M) SNA of solution was prepared by dissolving the (0.1 g) of in few drops of concentrated HCl and added distilled water to the mark in volumetric flask 100 mL, stored in dark and used, for at least one month, as stock solution. More dilute working solutions of the drug were prepared by serial dilutions with distilled water. solutions 1M of each of sodium hydroxide, Potassium hydroxide, sodium carbonate, hydrochloric acid nitric acid, sulfuric acid was prepared and used. A10.35x10^{-2}M of sodium nitrite was prepared by dissolving 0.06gm pure substance in 0.5mL of 1M HCl and diluted to the mark in volumetric flask 25mL.

A10^{-2}M of BYN (organic part –Ex. 1) was prepared by dissolving 0.0126gm substance in 10 ml of 1M NaOH and diluted to the mark in volumetric flask 10mL.

Organic Part

Materials and Methods

Chemicals and Reagent

2,4-Dinitrophenylhydrazine**,** Various aldehyde, Ethanol, NaOH, Ethyl acetoacetate, Sodium acetate

Experimental

Melting points are uncorrected and were recorded in an open capillary tube on Stuart melting point apparatus.

Infrared spectra have been recorded on a Shimadzu FTIR-8100 spectrophotometer using KBr discs –, ^{1}H-NMR Spectra have been measured on an MH_Z spectrometer using (DMSO-d_6) as solvent and ^{13}C-NMR Spectra have been measured, in the university of Seljuq \ Turkey, on an MH_Z spectrometer using (DMSO-d_6) as solvent.

1-Preparation of 2 - (2,4-dinitrophenyl)-5-methyl-2,4-dihydro-3H-pyrazol-3-one (G_1) [19] (BYN)

In a conical flask puts (0.01mol) from ethyl acetoacetate and added gradually (0.01mol) from 2,4-Dinitrophenylhydrazine dissolved in (40mL) from ethanol with stirring at a temperature of less than (60 °C), leaves the mixture for(80 min) at temperature of no exceeding (60 °C), The mixture cools or leaves in the freezer to give compound.

Discussion

The synthesis of Pyrazoline were performed as Shown in scheme 1. Pyrazoline (G1) was prepare from reaction of 2, 4-Dinitrophenylhydrazine with ethyl acetoacetate, the IR spectra of compound (G1) showed characteristic (C=O) stretching at (1724cm-1) and (C=C) stretching frequencies at (1589 cm-1), and band at (1554 cm-1) for (C=C) group and band at (3062 cm-1) for (Ar-H) group and a band at (1604cm-1) for (C=N) group figure 2.

^{1}H NMR Spectrum of compound (G_1 **)**, figure 3 Showed the following signals :a singlet signal at (δ=2.13ppm) due to a protons of (3H-CH_3) group , multi signal at (δ=7.84-8.88ppm) due to a protons of phenyl, and signal at (3.68ppm) for (2H,CH_2)

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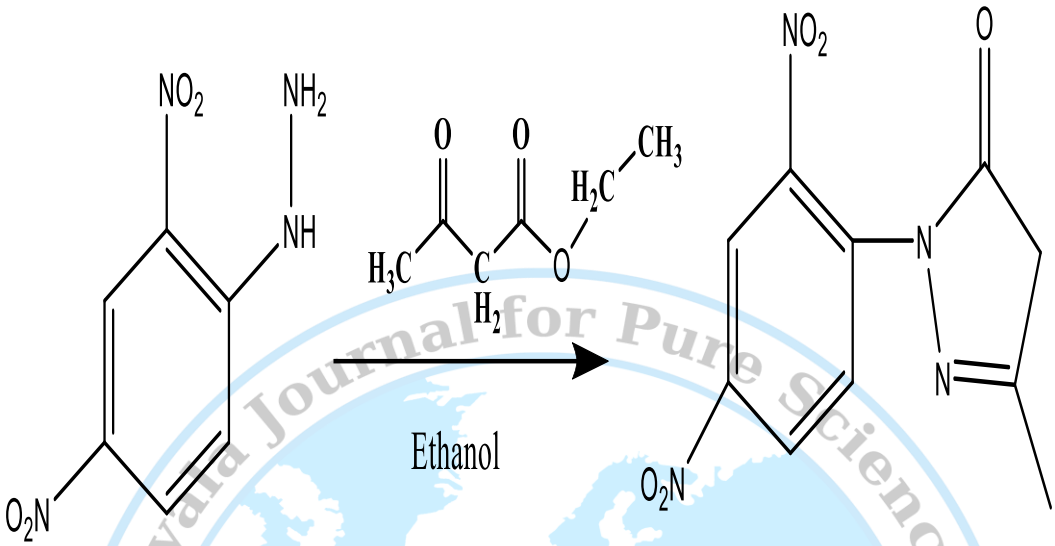

Scheme 1: Synthesis of Pyrazoline derivatives (G_1)

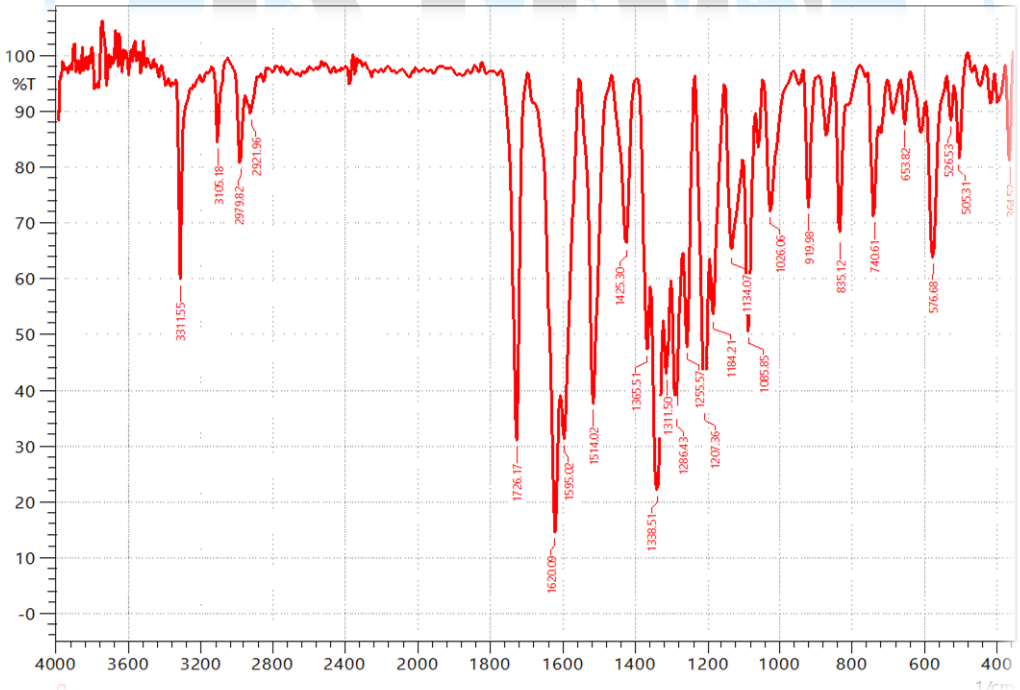

Figure 2: IR spectrm of G_1

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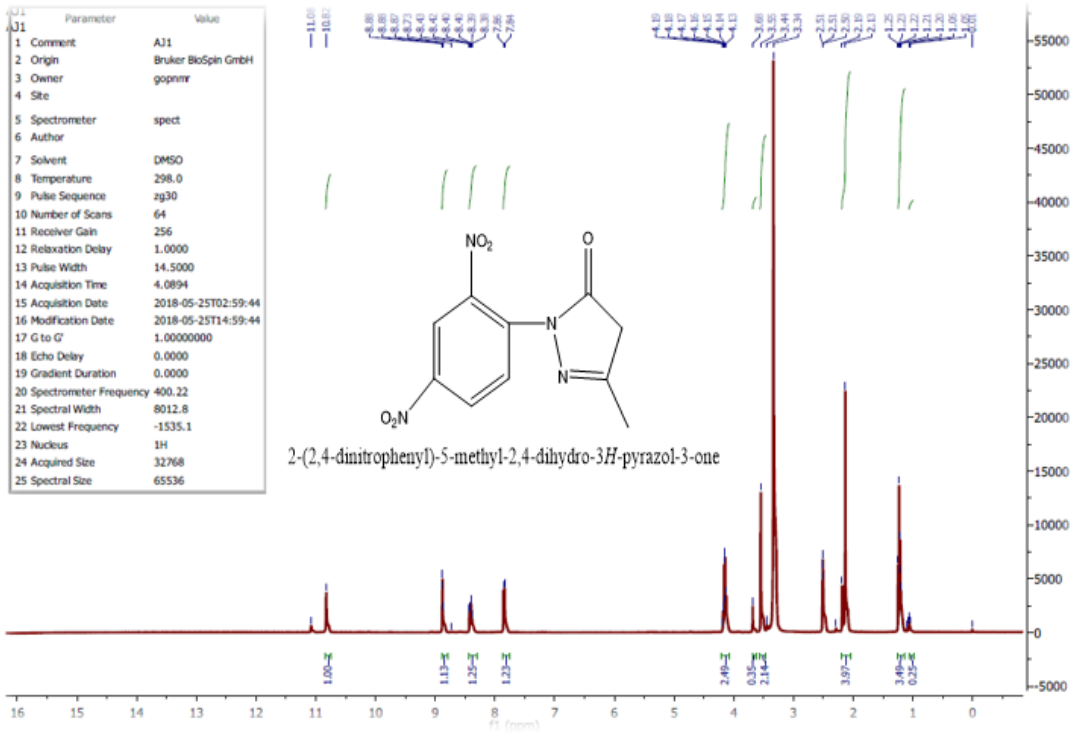

Figure 3: HNMR Spectrm of G_1

Interference Solutions of 1000 ppm

These solutions are prepared by dissolving 0.1gm of (glucose, fructose, lactose, sucrose and vanillin) in suitable solvent (water or ethanol) and then completing the volume to 100 ml with distilled water.

General Procedure for Determination of Sulfanilamide SNA by using Diazotization Coupling Reaction

1ml from 500 ppm of SNA drug transferred to 25 ml volumetric flask then 2 ml of 10.35×10^{-2} M of sodium nitrite is added and cooled mixture in ice bath 0 – 5 degrees for 10-15 min and then add 0.5ml of the BYN 10^{-2} M The mixture is then diluted to produce orange-brown solution and the value of absorbance is measured at 435 nm.

Preparation of Sulfanilamide Drug Sample

0.005 gm of interfering substance mixture (lactose, glucose, sucrose, fructose and vanillin) was add to 0.025 gm of the bulk drug. Dissolved 0.0125 gm of the above mixture in few drops of concentrated HCl and diluted to the mark with distilled water in volumetric flask 100ml to obtain 100 µg / ml.

Results and Discussion

When SNA was treated by BYN according to the recommended procedure, showing abroad band in the region of (350-550 nm) the recorded absorption spectrum of the formed reaction product against reagent blank shows an extreme absorption at 435 nm, while, the blank has not any significant absorbance in this region, as it is shown the figure 4.

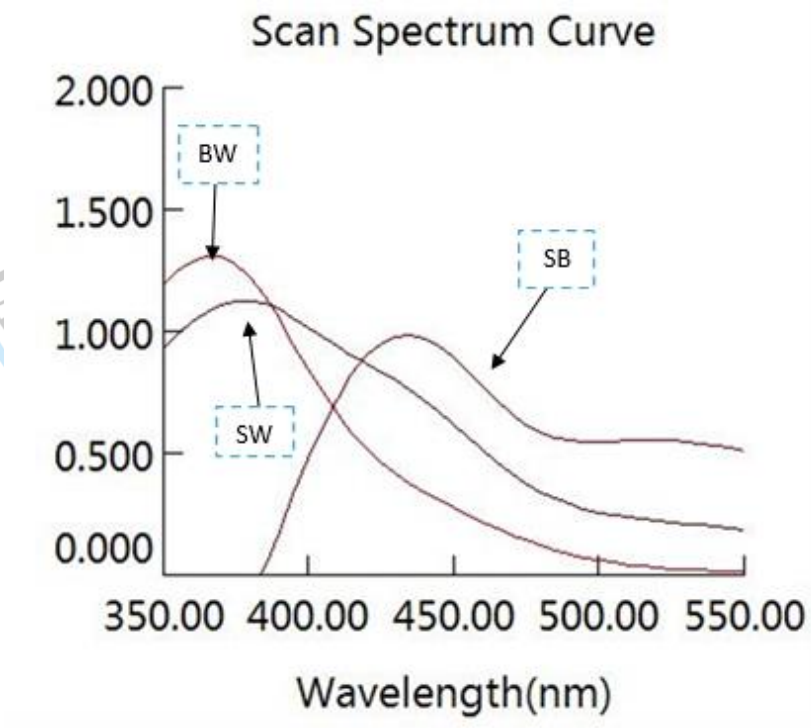

Figure 4: SB: Absorption Spectrum of SNA Solution Versus Blank.
SW: Absorption Spectrum of SNA solution Versus Distilled Water.
BW: Absorption of Blank Versus Distilled Water.

Optimization of Reaction Conditions

The effect of different parameters on the absorption intensity of the azo dye formed was studied and the reaction conditions are optimized.

Effect of Type of Acid to Prepare Sodium Nitrite Solution

Different types of 1M acids (HCl, H_2SO_4 and HNO_3) are investigated to dissolved sodium nitrite to obtain suitable acidic solution use of diazotization reaction.

Table1: Effect of Different Acids

Type of acid	Absorbance			Mean Absorbance
	A1	A2	A3	
HCl	0.842	0.847	0.848	0.846
H_2SO_4	0.171	0.162	0.147	0.160
HNO_3	0.257	0.242	0.234	0.244

It is found that 1M HCl acid give the maximum absorbance table 1 there for used in all subsequent experiments.

Effect of Volume of Hydrochloric Acid

The effect of different volumes of 1M HCl solution on the colored product was studied it was illustrating table 2.

Table 2: Effect of Volume of HCl in Absorbance intensity

Volume add of 1M HCl	A1	A2	A3	Mean Absorbance
0.5	0.837	0.84	0.843	0.84
1	0.488	0.491	0.494	0.491
1.5	0.429	0.429	0.435	0.431
2	0.275	0.271	0.252	0.266
2.5	0.103	0.076	0.073	0.084
3	0.091	0.092	0.087	0.081
3.5	0.155	0.150	0.151	0.077

It is found that 0.5 ml of 1M HCl is acceptable for completing reaction during 15 min figure 5.

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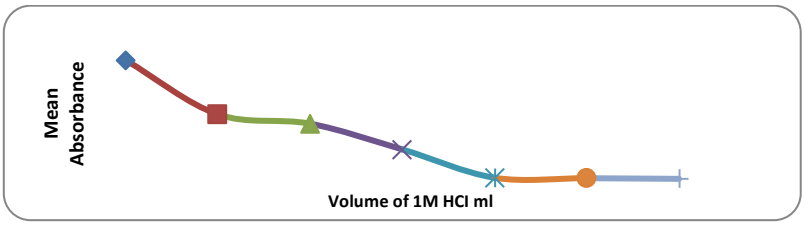

Figure: Effect of Volume of HCl in Absorbance intensity

Effect of Type of Base to Prepared Organic Reagent Solution

The effect of different alkaline solution 1 M such as (sodium hydroxide, ammonium hydroxide, potassium hydroxide and sodium carbonate) was studied it found 1 M of sodium hydroxide the best to prepare organic reagent and obtain stability of solution color table 3.

Table 3: Effect of alkaline solution

Type of base	A1	A2	A3	Mean Absorbance
NaOH	**0.832**	**0.838**	**0.842**	**0.837**
KOH	0.619	0.632	0.636	0.629
Na_2CO_3	No soluble	No soluble	No soluble	
NH_4OH	No soluble	No soluble	No soluble	

Effect of the volume organic reagent (BYN)

The effect of different volumes of BYN solution on the absorbance of the colored product was studied keeping other conditions constant.

Table 4: Effect of the volume organic reagent (BYN)

Volume of solution	A1	A2	A3	Mean Absorbance
0.2	0.055	0.057	0.059	0.057
0.4	0.114	0.114	0.117	0.115
0.6	0.430	0.491	0.521	0.481
0.8	0.623	0.666	0.711	0.666
1	0.841	0.844	0.850	0.845
1.2	0.781	0.782	0.786	0.783
1.4	0.622	0.626	0.630	0.626
1.6	0.579	0.583	0.584	0.582

It was found that a maximum absorbance and stable color is established with 1ml of 10^{-2}M for BYN Thus it was chosen in all subsequent experiments as shown in figure 6.

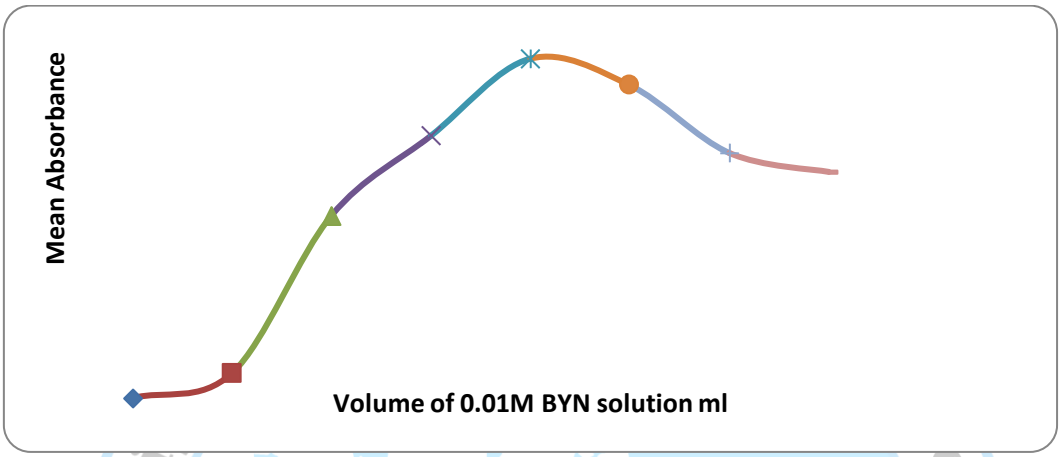

Figure 6: Effect of Volume of Organic Reagent (BYN) Solution

Effect of Volume of Sodium Nitrite Solution

The effect of different volumes (0.2- 2.6) ml of sodium nitrite solution on the absorbance of the colored of Azo dye product.

Table 5: Effect of Sodium Nitrite Solution

Volume $NaNO_2$	conc $NaNO_2$	A_1	A_2	A_3	Mean Absorbance
0.2	0.000856	0.580	0.600	0.650	0.610
0.4	0.00171	0.654	0.655	0.659	0.656
0.6	0.00256	0.671	0.673	0.678	0.674
0.8	0.00342	0.698	0.701	0.707	0.702
1	0.00428	0.729	0.734	0.736	0.733
1.2	0.00513	0.749	0.751	0.756	0.752
1.4	0.00599	0.768	0.771	0.773	0.771
1.6	0.00684	0.779	0.783	0.784	0.782
1.8	0.00770	0.798	0.806	0.813	0.806
2	0.00856	0.839	0.837	0.841	0.838
2.2	0.00991	0.544	0.548	0.551	0.549
2.4	0.0102	0.348	0.351	0.356	0.352
2.6	0.011	0.251	0.256	0.258	0.255

It was found that a maximum absorbance and stable color is established with 2 ml of sodium nitrite solution. There for it was chosen in all subsequent experiments as shown in figure7

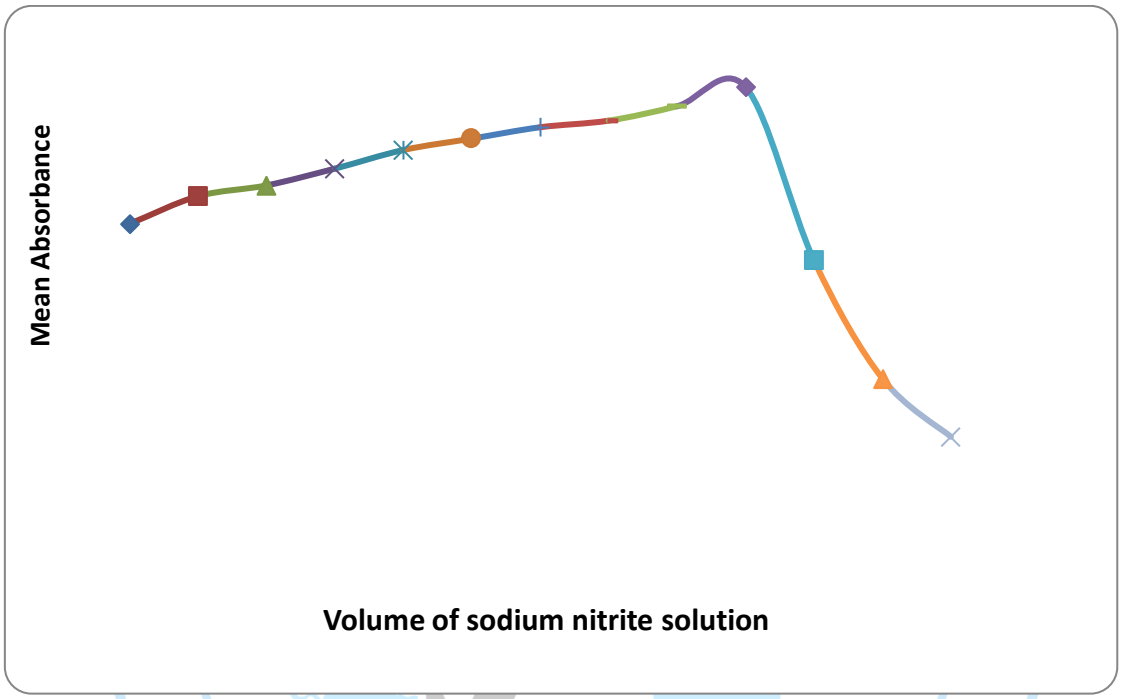

Figure 7: Effect of Volume of Sodium Nitrite Solution

Procedure for Construction of Calibration Curve

In a series of volumetric flasks (25 ml) 2 ml of Sodium nitrite solution add to. concentrations of 2-20 µg / ml of Sulfanilamide solution (0.1 - 1 ml of 500 µg / ml) solution, mix in an ice bath for a period of time (10 - 15) minutes and temperatures ranging from (0 - 5) C then add 1 ml of the BYN with a concentration of 5×10^{-3} molar and then left solutions for 30 minutes and then complete the volume to the mark with distilled water. The spectra and the absorption of solutions versus the blank were measured at 435 nm and figure 8. The calibration curve drawn by using the mean absorbance of product solution, gave a correlation coefficient of 0.9992. The value of the molar absorbance was calculated at 7671.2 $L.mol^{-1}.cm^{-1}$

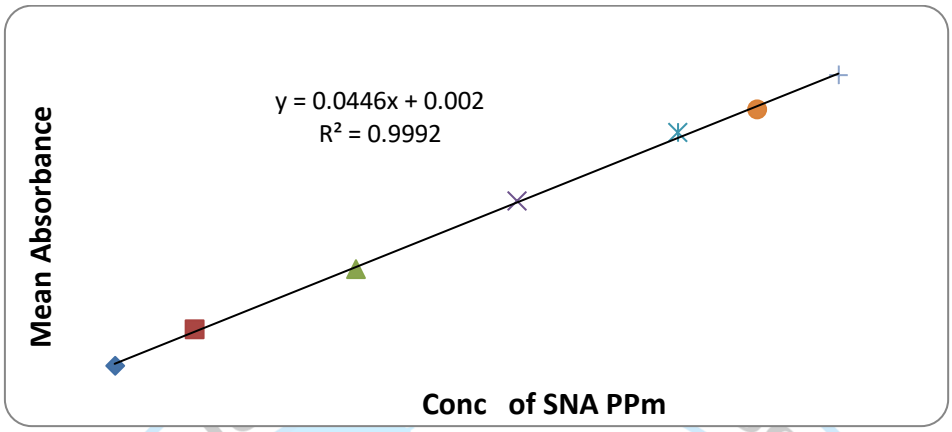

Figure 8: Calibration Curve for Determination SNA By Using Diazotization Coupling Reaction

Accuracy and Precision

Accuracy and precision were studied by measuring absorption (n=3) at 435 nm for three different concentrations of the drug within the limits of Beer's law, the average recovery (100.55 %) and the relative standard deviation (3.74×10^{-3}%) indicate that the method is of high accuracy and precision. The results are shown in table 8.

Table 6: Results of accuracy and precision

Conc of SNA µg / ml	Conc of SNA Observed*	Recovery, *%	RSD*, %
4	4.10	102.5	2.16×10^{-3}
12	12.08	100.66	3.74×10^{-3}
18	17.73	98.5	5.35×10^{-3}

n=3

Interference Study

In pharmaceutical analysis, it is important to test the selectivity towards the excipients added to the pharmaceutical preparations such as (vanillin, glucose, lactose, starch, sucrose) did not interfere in the determination of SNA and did not effect on the reaction between the SNA and

BYN. (12µg.mL-1) of SNA was analyzed and design of experiment method was used for analyzing table 7.

Table 7: Percent recovery for (12µg.mL-1) of sulfanilamide in the presence of different concentration of Excipients

Excipients	*Concentration* (µg.mL-1)	Sulfanilamide *Conc. Taken* (12 µg. mL-1)	
		Conc. Found* µg.mL-1	Recovery* %
Sucrose	1000	12.06	100.5
Vanillin		12.08	100.66
Glucose		12.04	100.33
Lactose		11.97	99.75
Starch		11.95	99.59

n = 3

Applications

Direct Method

In this method, different volumes (2, 3, 4 ml) of a pharmaceutical formulation solution (500 µg/ml) were transferred to 25 ml volumetric flasks and the resulting concentrations (8.12.16 µg/ml) and were treated as in construction of calibration curve. The absorbance was measured at 435 nm for three times. RE was calculated and the results are shown in table 8.

Table (8): Determination of SNA in pharmaceutical formulation

Conc of SNA µg/ml	Conc of SNA Observed*	RE
8	7.80	-2.5
12	12.04	0.33
16	16.34	2.125

***n=3**

Table 8 shows the efficiency and success of the developed method for the determination of SNA in its pharmaceutical formulation.

Standard Additions Method

To prove that the developed method is free from interferences, method of standard additions is applied for determining of SNA in its pharmaceuticals. Different volume (3 ml) of a pharmaceutical formulation solution (100 µg/ml) were transferred to four volumetric flasks (25 ml) for each volume, then increasing volumes (0.5-1.5 ml) of 500 µg / ml of SNA standard solution were added with leaving the four flasks without addition. The solution was treated as in construction of calibration curve. The absorbance was measured at 435 nm figure 9.

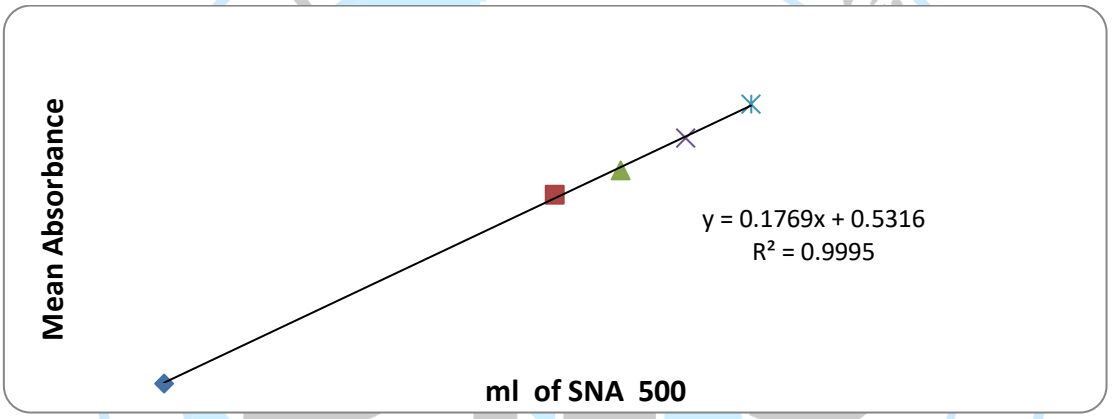

Figure 9: Standard additions curve for the determination of SNA in injection

Conclusions

The results obtained confirm that the proposed method is simple of good sensitivity for the determination of Sulfanilamide SNA. The method is based on the reaction of sulfanilamide (SNA) with new organic reagent (BYN) it was prepare by reaction between ethyl acetoacetate with 2, 4-Dinitrophenylhydrazine.

The azo dye product shows absorption maximum at 435 nm. This method not use of organic solvents, or solvent extraction and it can be applied successfully for determination of SNA in pharmaceuticals formulation.

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