مجلة الدراسات التربوية والعلمية - كلية التربية - الجامعة العراقية
العدد الثالث والعشرون - المجلد الخامس - علوم الكيمياء - آيار 2024 م
doi.org/10.52866/esj.2023.05.23.06

Spectrophotometric determination of sulfanilamide in pharmaceutical preparations by Schiff reaction

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ABSTRACT:

To determination of sulfanilamide drug (SNA) developed to estimation the pare pharmaceutical in a fast, easy and sensitive spectrophotometric method. This method relied on coupling the drug (SNA) with reagent Para Dimethylaminobenzaldehyde P-(ADMB) in the presence of hydrochloric acid by a Schiff reaction method to form a yellow colored product soluble in distilled water, noticed the highest absorption at 462 nm and is subject to Beer's law in the range of 3-27 (µg/ml), the molar absorbance value (0.5234×10^4 L/mol.cm), Sandel's significance (0.0328 µg/cm2), the detection limit (0.14 µg/ml) and the quantification limit (0.49µg/ml), R^2 0.9992, recovery value (100.41%), and the relative standard deviation rate ranged between (0.15-0.068) This method was successfully applied to estimate (SNA) in its pharmaceutical preparations in the form of industrial powder.

Key word: Spectrophotometric, Sulfanilamide, Para dimethylaminobenzaldehyde, Schiff reaction.

التقدير الطيفي لعقار السلفانيلاميد في مستحضراته الصيدلانية بواسطة تفاعل شيف

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مستخلص

تضمن البحث تقدير عقار السلفانيلاميد (SNA) بشكله النقي وفي مستحضراته الصيدلانية بطريقة طيفية سريعة ويسيرة وحساسة إذ إعتمدت الطريقة على إقتران العقار (SNA) مع الكاشف4-ADMB بوجود حامض الهيدروكلوريك بطريقة تفاعل شف لتكوين ناتج اصفر اللون ذائب في الماء المقطر حيث اظهرت أعلى امتصاص عند الطول الموجي 462 نانوميتر ويخضع لقانون بير في نطاق 3-27) مايكروغرام/مل (وبلغت قيمة الامتصاصية المولارية (0.5234×10^4 لتر/مول.سم) ودلالة ساندل (0.0328 مايكروغرام/سم^2) وحد الكشف(0.14% مايكروغرام/مل) وحد التقدير الكمي (0.49% مايكروغرام/مل) ومعامل التقدير(0.9992) وقيمة الاسترجاعية (100.41%) وقيمة معدل الانحراف القياسي النسبي تتراوح بين (0.15-0.068%) طبقت هذه الطريقة بنجاح لتقدير (SNA) في مستحضراته الصيدلانية على شكل مسحوق صناعي.

الكلمة المفتاحية: القياس الطيفي، السلفانيلاميد، بارا ثنائي ميثيل أمينوبنزالدهيد, تفاعل شيف.

1. Introduction

Sulfanilamide chemically is 4-amino benzene sulfonamide (Figure1), it is a medicinal compound used to guard against certain bacterial infections. It is frequently used in the form of a topical cream or powder 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. Depending on the type of infection, either a cream or a pill will be prescribed [1].

Sulfanilamide is an antibacterial substance belonging to the sulfonamide group. Chemically it is an organic compound consisting of an aniline derived from the sulfonamide group.Sulfonamides were the first drugs that act selectively on bacteria and that could be used systemically. Today they are used infrequently, in part because of the widespread resistance. The target of the sulfonamides, and the basis for their selectivity, is the enzyme dihydrobutyrate synthase (DHPS) in the folic acid pathway Mammalian cells are dependent on endogenous formation of folic acid and generally lack DHPS. Instead, they have a folate absorption system that most prokaryotes lack., DHPS enzymes show a clear sensitivity to sulfonamides but normal binding to the p-aminobenzoic acid substrate, despite the close structural similarity between the substrate and the inhibitor

Modern antibiotics have replaced sulfanilamide on the battlefield; However, the term “sulfanilamide” is also used to describe a family of molecules that contain these functional groups. Examples include: [2-3]<BSTRACTtionFurosemide, a diuretic, Sulfadiazine, an antibiotic Sulfanilamide was determination for several methods such titration with strong base [4] Solid phase extraction (SPE) [5] HPLC [6-7] liquid chromatography–mass spectrometry [8-9] Derivative Spectrophotometry [10-11] .

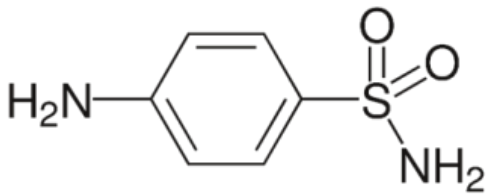


Fig.1: Chemical Structure of Sulfanilamide

The purpose of this study is to develop and validate a simple, sensitive and specific spectrophotometer Method for the determination of sulfanilamide in pharmaceutical preparations

2.Experimental Part

2.1. Instrumentation Used:

Spectrophotometric measurements were made using UV-visible double beam a type (T92+Spectrophotometr, China), with using (1cm) quartz cells.

2.2. Materials:

The substances used in this study were all standard (Fluka, bdh,SDI), and throughout the test, methanol and distilled water were used as solvents to preparing solutions.

Sulfanilamide standard solution (1000 µg.ml-1)

It was prepared by dissolving 0.1000 g of Sulfanilamide powder in an amount of methanol and then completed the volume 100 ml volumetric flask, and Concentration 250µg.ml-1 was prepared by taking 25 ml of the standard solution (1000 µg.ml-1) and in a volumetric flask of 100 ml and filled the volume to the mark with the distilled water.

Pharmaceutical solutions 250 µg/ml

Fresh due to the difficulty of providing the pharmaceutical preparation,mix solution by prepared with a weight of 0.025 g of sulfanilamide powder with 0.005 g of each interfering substance (glucose Sucrose, lactose, starch, vanillin (mix the mixture well and weigh 0.025 g) It is dissolved in 10 ml of ethanol, then filtered, and the volume is filled with distilled water in a volumetric bottle of 100 ml, thus we obtain a sulfanilamide preparation with a concentration250 µg/ml[12]

0.01M Para-Dimethylaminobenzaldehyde reagent solution

It was prepared by dissolving 0.149 g of reagent powder molecular weight (149.19 g/mol) in a small amount of distilled water and complete to 100 ml

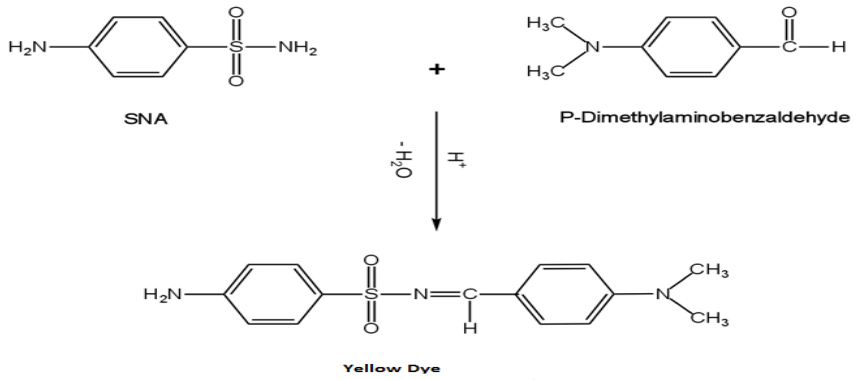
Approximtely1M Hydrochloric acid solution

A solution of hydrochloric acid was prepared by 1 M from 11.8 M (8.47) ml complete to 100 ml (D.W).

3.Results and discussion

3.1. The general principle of the method

The principle of the method is based on the use of a solution of the reagent Para-Dimethylaminobenzaldehyde at a concentration of 10^{-2} M and adding it to the drug (sulfanilamide) in the presence of hydrochloric acid at a concentration of 1 M. A yellow-colored product is formed, which is shown in the equation below: -



3.2. Preliminary study

It was observed when adding 3 ml of reagent solution at a concentration of 10^{-2} molar to 1.5 ml of a sulfanilamide solution at a concentration 250 µg/ml and then adding to it 1 ml of hydrochloric acid at a concentration of 1 M. A yellow solution will be formed. The absorption spectrum of the colored product (after diluting it with distilled water in a volumetric bottle of 25 ml to the mark) was measured against the blank solution and it was found that it gives the highest absorption at the wavelength of 462 nm, while the blank solution did not give any absorption in this region as shown in the fig. (2)

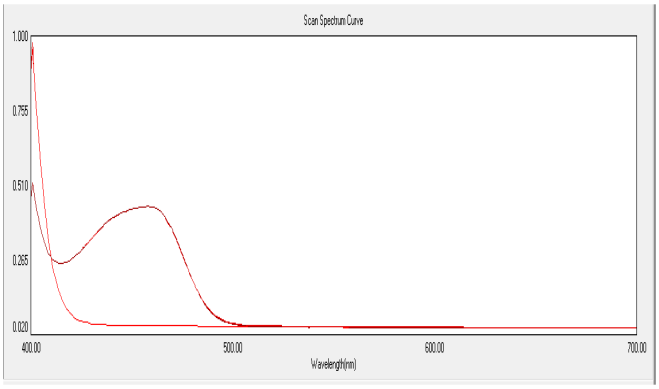


Fig2 : Absorption spectrum of sulfanilamide

3.3. Optimum conditions

Subsequent experiments were carried out using 1.5 ml of a sulfanilamide solution with a concentration of 250 µg/ml in a final volume of 25 ml, and the absorbance of the solutions was measured at the wavelength of 462 nm against blank solution.

3.4. Choose the best acid

The following acids were prepared (HCl, H_2SO_4, HNO_3 and CH_3COOH)) at concentrations of 1 M to each 1.5 ml of a sulfanilamide solution of a concentration of 250 µg / ml was added with 3 ml of the reagent solution (paradimethylaminobenzaldehyde) and then 1 ml of one of these acids was added to it the volume is then completed D.W, and the results are recorded in Table 1.

Table1: Choosing the best acid

Type of acid	Absorbance
HCl	**0.465**
H_2SO_4	0.402
HNO_3	0.322
CH_3COOH	0.241

It was noted from the above table that hydrochloric acid gave the highest absorption of the colored product at the wavelength of 462 nm compared to the other used acids, so it was used as the best acid and was adopted in subsequent experiments

3.5.The amount of acid used in the Schiff reaction

For every 1.5 ml of 250 µg/ml sulfanilamide solution, 3 ml of reagent solution (paradimethylaminobenzaldehyde) was added, then (0.5- 3) ml of hydrochloric acid was added to get a Schiff base in a 25 ml volumetric flask, after which the volume was completed. To the distilled water and the results are recorded in Table2.

Table2: effect the volume of HCL

Volume add of (1M) HCl	Absorbance
0.5	0.411
1	**0.463**
1.5	0.355
2	0.319
2.5	0.294
3	0.254

It was noted from the above table that (1) ml of acid gave the highest absorption of the colored product at the wavelength of 462 nm compared to the other used volume, so it was used as the best size and was adopted in subsequent experiments.

3.6. effect of the amount of reagent 10^{-2} M

The effect of the quantity of the used reagent was studied, as different volumes of the reagent with a concentration of (10^{-2}) M were used, ranging between (4-0.5) ml the results are recorded in Table3.

Table 3: effect of the amount of reagent

Volume of (ml) reagent	Absorbance
0.5	0.274
1	0.309
1.5	0.359
2	0.402
2.5	0.436
3	**0.460**
3.5	0.439
4	0.420

It was noticed from the results shown in the above table that the volume of 3 ml of the reagent solution with a concentration of (10^{-2}) M gave the highest absorption at the wavelength of 462 nm, so it was adopted in the subsequent experiments

3.7. temperature effect

The effect of temperature on the absorption of the formed colored product was studied using temperatures rang from (15-60) °C the results are listed in Table 4.

Table 4 : Effect of temperature

Temp. °C	15	20	25	30	35	40	45	50	55	60
Absorbance	0.460	0.462	**0.466**	0.461	0.457	0.442	0.419	0.398	0.372	0.322

3.8. The effect of the sequence of additions

The effect of changing the sequence of adding reaction materials on the absorption of the colored product was studied the results are listed in Table 5 .

Table 5: the sequence of additions

Order number	Order of addition	Absorbance SB
1	A + D + R	0.421
2	R + A + D	0.440
3	D + R + A	0.462
4	D + A + R	0.402
5	R + D + A	0.430

sulfanilamide drug solution (D), reagent solution (R), HCL (A)

It was noted from the above table that the addition sequence (3) achieves the highest absorption of the colored product, so it was adopted in the subsequent experiments

3.9. Effect of time and stability on the absorption

3ml of the reagent solution (paradimethylaminobenzaldehyde) was added to 1.5 ml of a sulfanilamide solution with a concentration of (250) µg / ml, then (1) ml of acid was added to it to get a yellow solution, then left for a period of (50-5) minutes before dilution with water The distilled was in a volumetric vial of 25 ml measured at wavelength 462 nm the results are shown in Table 6 .

Table 6: Effect the time after completion of the reaction

Time minutes	5min	10min	15min	20min	25min	30min	35min	40min	45min	50min
Absorbance	0.464	0.462	0.460	0.459	0.444	0.423	0.407	0.396	0.395	0.393

It was noted from the above table that 15-20 minutes is sufficient to the reaction .

3.10. final absorption spectrum

The final absorption spectrum was measured after reaching the optimal conditions, which is adding 3 ml of the reagent solution (Para-Dimethylaminobenzaldehyde) to 1.5 ml of a (sulfanilamide) solution with a concentration of 250 µg / ml, then adding to it 1 ml of (hydrochloric acid), The absorption spectrum of the colored product (after diluting it with distilled water in a volumetric flask 25 ml) was measured against the blank solution and it was found that it gives the highest absorption at 462 nm, as in the fig3 .

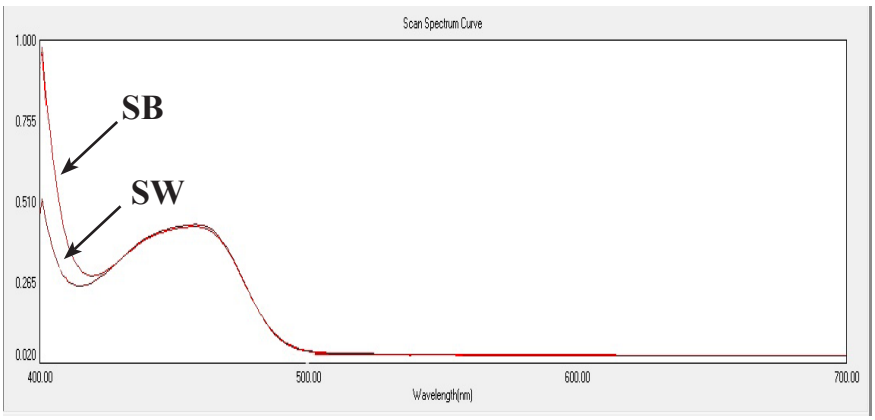


Fig3: absorption spectrum of sulfanilamide
SW the absorption spectrum of a sulfanilamide solution against distilled water
SB the absorption spectrum of the colored product against blank

3.11. calibration curve

After fixing the optimal conditions for the method, which are shown in Table 7 below .

Table 7: the optimum conditions

Optimum Conditions	Volume of ml
HCL	1 ml
P–Dimethylaminobenzaldehyde	3 ml
Temp.°C	25 °C

Increasing concentrations of (27-3) µg/ml of sulfanilamide solution ranged between (0.3 – 2.7) ml of sulfanilamide solution of 250 µg/ml were added to a series of volumetric flask of 25 ml, then 3 ml of reagent solution was added to it (Para dimethylaminobenzaldehyde), then 1 ml of hydrochloric acid was added and the volume was completed to the distilled water absorbance of the solutions against the blank solution was measured at 462 nm, as in Fig4. The molar absorptive value was 0.5234 x 10^4 liters.mol-1.cm-1 and Sandel's Sensitive value was 0.0328 µg/cm^2.

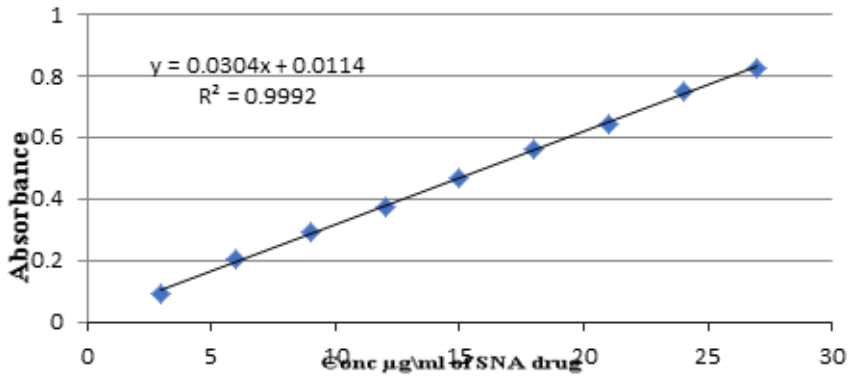


Fig 4: Calibration curve of sulfanilamide

3.12. Accuracy and precision

Optimum conditions were used in the calibration curve to test the accuracy and precision of the method, where three readings were taken for three different concentrations of sulfanilamide solution within the limits of Beer's law in the calibration curve relative standard deviation (RSD) it was found that the method has good accuracy (100.20% recall rate). It has good agreement, its mean and the relative standard deviation were calculated mathematically as follows:

RE % = AT/T×100

So RE% = Percentage Relative Error

AT = The difference between the analytical (practical) and the real value.

T = real value

The recovery value is calculated from the following law

Recovery % =RE % + 100

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

$$RSD = \frac{\sigma}{\bar{X}} \times 100$$

σ = standard deviation

$\bar{X}$ = reading rate

The results are recorded in Table 8.

Table 8: Accuracy and precision

Conc of SNA µg / ml	Conc of SNA µg /ml Found	RE,%	RSD*, %	Recovery, *%	Average of Recovery,*%
9	9.19	2.11	0.15	102.11	100.20
15	14.92	-0.53	0.094	99.46	
21	20.80	0.95-	0.068	99.04	

3.13. the detection limit and the quantification limit value

To find the value of the limit of detection (LOD) and the value of the limit of quantification (LOQ), the absorbances of ten blank solutions were measured at the wavelength of 462 nm against distilled water, by applying the mathematical relationship (Valcarcel 2000), where it was found that the value of the limit of detection (LOD) and the value of the limit of quantification (LOQ) The detection was equal to 0.14 µg/ml and the quantification limit was equal to 0.49 µg/ml

3.14. Stoichiometry of Reaction

To find out the nature of the yellow color product formed and the ratio of the drug's bond with the reagent, the two methods (Job's method) and the molar ratio were applied. In both methods, the concentration of each of the sulfanilamide solution and the solution of the organic reagent is ($2x\ 10^{-3}$) M,

In the Job method, in a series of 25 ml volumetric flask, different volumes of the drug solution were placed, ranging from (0.5 - 2.5 ml), and supplements of these volumes were added to a volume of 3 ml of the reagent solution with a concentration of (2×10^{-3}) M, and after dilution to a limit distilled water, and the absorption of these solutions was measured at the wavelength of 462 nm. Fig5 shows that the ratio is 1:1.

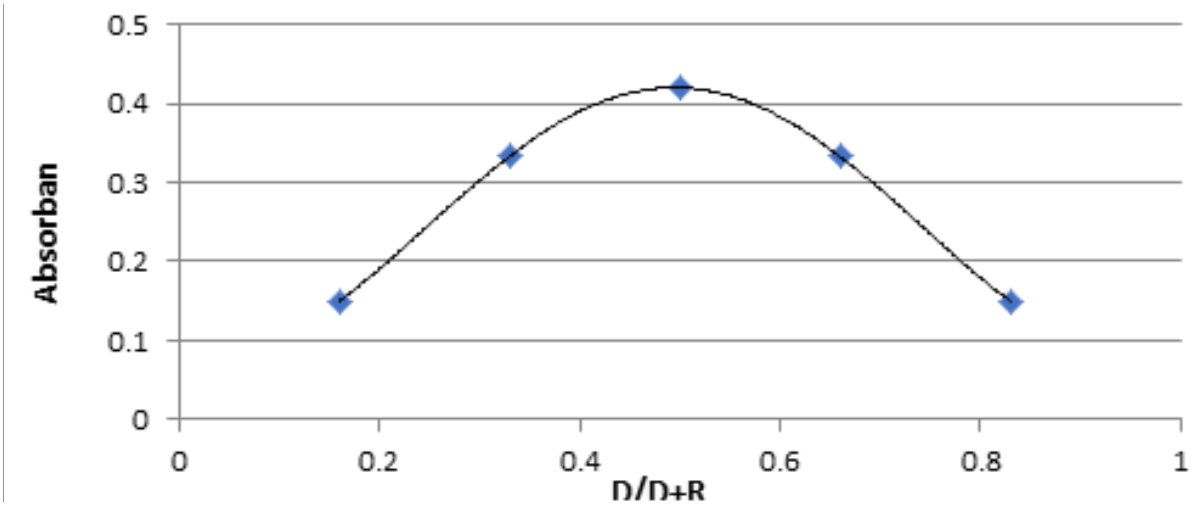


Fig5: Job's method for the determination of sulfanilamide with reagent

As for the molar ratio method, 1.5 ml of the drug was placed in a series of 25 ml volumetric bottles, and volumes of the reagent ranging from 0.2-1.4 ml were added to it, then 1 ml of hydrochloric acid was added, yellow color appeared and the volume was completed to distilled water, then the absorption of this was measured. The solutions at the wavelength of 462 nm compared to the blank solution for each of them, it was found that the molar ratio agrees with the method 1:1 as in Fig 6 .

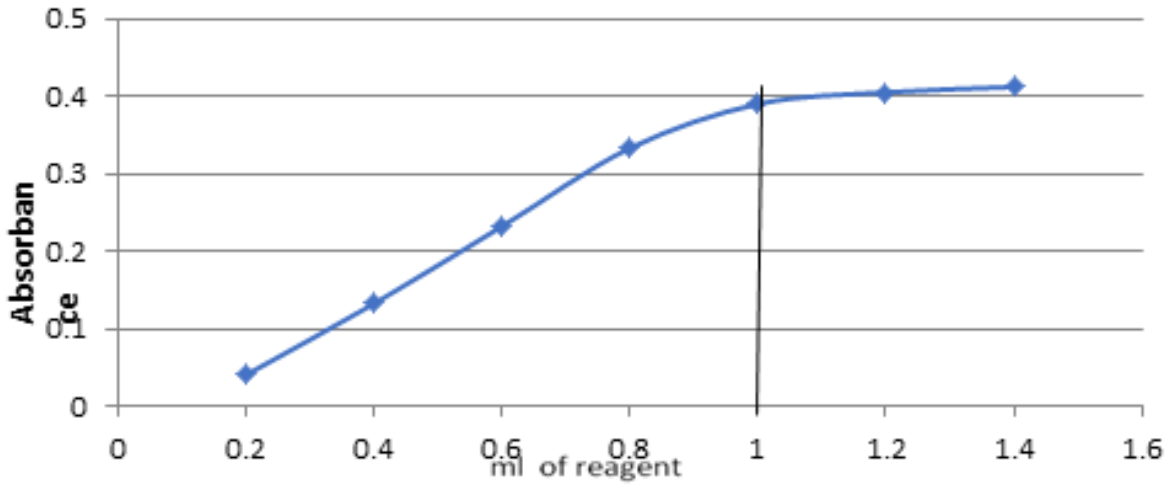
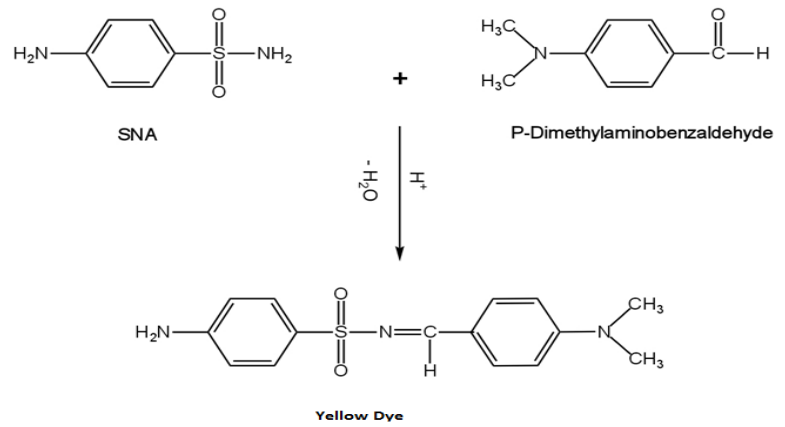


Fig6: molar ratio method of sulfanilamide

Therefore, the proposed reaction equation is as follows :



4. Applications

This method could be applied to synthetic pharmaceutical preparations containing sulfanilamide.

4.1. the direct method

Three different concentrations of the preparation solution (250 µg / ml) were taken, they are 9, 15 and 21 µg / ml. The solutions were treated with the same steps followed when preparing the calibration curve, and the absorption was measured for it at 462 nm compared to the blank solution, and the average of five measurements was calculated for each concentration the results are recorded in Table 9 .

Table 9: The direct method

Conc of SNA µg/ml	Conc of SNA Found µg/ml	Recovery	Average of Recovery,*%
9	9.06	100.66	100.41
15	15.11	100.73	
21	20.97	99.85	

The results of the above table showed the success of the proposed method for the determination of sulfanilamide in the pharmaceutical preparation containing it. The value of the recovery rate was 100.41% in the industrial preparation.

5. Comparing the method with other methods

The analytical variables of the current method for the determination of sulfanilamide were compared with other spectrophotometric determination methods using Schiff reaction and Table 10 shows the results of that comparison.

Table 10: a comparison of the Determination of sulfanilamide with other methods

Analytical parameter	Literature [13] method	Present Method
Reagent	NQS	P–DMAB
Beers law range µg.ml^{-1}	5-30	3 - 27
Molar absorptivity (l.mol^{-1}.cm^{-1})	6.9568 X10^4	0.5234 X10^4
Sandells Sensitivity µg /cm^{-2}	2.4753	0.0328
λ_{max} (nm)	455	462
Recovery(%)	100.66	100.41
LOD	0.546	0.14
LOQ	1.564	0.49
Colour of the dye	Orange	Yellow

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