African Journal of Advanced Pure and Applied Sciences (AJAPAS)

Online ISSN: 2957-644X

Volume 2, Issue 3, July-September 2023, Page No: 127-139

Website: *https://aaasjournals.com/index.php/ajapas/index*

||Arab Impact factor 2022: 0.87|| SJIFactor 2023: 5.689|| ISI 2022-2023: 0.557

Spectrophotometric Estimation of Sulfasalazine Via Oxidative Coupling Reaction Using 8-amino -2-naphthlene sulfonic acid and Potassium Periodate

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Received: June 05, 2023	Accepted: July 19, 2023	Published: July 28, 2023

Abstract:

A simple, fast, and sensitive spectrophotometric technique has been proposed for the determination of sulfasalazine via oxidative coupling with 8_amino_2_naphthalene sulfonic acid reagent in the presence of potassium periodate as an oxidizing agent in the basic medium at room temperature to give stable yellow color dye with maximum absorption 457 nm. beer's low is obeyed over the concentration range of 0.25-20 µg/ml of sulfasalazine in a final volume with the molar absorptivity of 2.9202×10^4 $L.mol^{-1}.cm^{-1}$ and Sandell,s sensitivity index 0.0136 $\mu g/cm^2$. The proposed method was successfully s applied for the determination of sulfasalazine in pharmaceutical preparation (Tablets).

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Keywords: Sulfasalazine, Oxidative, 8-Amino-2-Nephthalene, Sulfonic Acid, Coupling

Cite this article as: A. E. Ahmed, K. M. Omar, "Spectrophotometric Estimation of Sulfasalazine Via Oxidative Coupling Reaction Using 8-amino -2-naphthlene sulfonic acid and Potassium Periodate", *African Journal of Advanced Pure and Applied Sciences (AJAPAS)*, vol. 2, no. 3, pp. 127 –139, July-September 2023.

الملخص:

تم تطوير طريقة طيفية سهلة وسريعة وحساسة لتقدير السلفاسلازين، إذ اعتمدت الطريقة على تفاعل الاقتران التأكسدي بين السلفا سلازين الكاشف **8**-امينو -**2** - نفثالين حامض السلفونيك بوجود بيريودات البوتاسيوم كعامل مؤكسد، وتم التفاعل بوسط قاعدي باستخدام هيدروكسيد الصوديوم وبدرجة حرارة الغرفة وأعطى الناتج الملون أعلى امتصاص عند الطول الموجي 457 نانومتر ،يتبع قانون بير في المدى **20-0.25** مايكروغرام / مللتر ،وقد بلغت قيمة معامل الامتصاص المولاري **$2.9203 *10^4$** لتر مول$^{-1}$ سم $^{-1}$ ودلالة ساندل **0.136** مايكروغرام/ سم^2 وتم تطبيق الطريقة بنجاح على المستحضر الصيدلاني (اقراص).

الكلمات المفتاحية: سلفاسلازين، أكسدة، **8**-امينو-**2**-نفثالين، حامض السلفونيك، اقتران.

Introduction

Sulfasalazine is prepare from diazotizing sulfapyridine and coupling diazotized intermediate with salicalic acid [1], It is sulf drug [2], It is anti -inflammatory drug that mainly used for the treatment of rheumatoid arthritis science discovery in the 1930 [3] and crohn's disease and ulcerative colitis [4,5]. Various analytical methods are used developed for determination of SSZ such as HPLC [6,8], NMR [9], smartphone-based colometric [10],kinetic spectrophotometric method[11], In this research accurate, sensitive and simple spectrophotometric method is

determination of SSZ in pure form based on oxidative coupling using 8-amino-2-naphthalen sulfonic acid (ANS)in the presence of potassium periodate as oxidizing agent in basic medium..

Material and methods

Experiment part

Apparatus

A double beam UV-Visible spectrophotometer (JASCO V-630) with 1.0 cm quartz cells were employed for the absorption spectrum and absorbance measurement, professional Benchto pH meter TRANSE BP 300l was used for pH measurements.

The used chemicals and reagents:

All the used chemicals and reagents were of high purity.

Preparation of solution

Pure sulfasalazine solution (100µg/mL)

This solution was prepared by dissolving 0.01 g of pure sulfasalazine in 0.5 ml of sodium hydroxide solution (1 M), the volume was completed with distilled water using a volumetric vial of 100 ml. A solution was prepared with a concentration of (50 µg / ml) 1.255×10^{-2} M and kept in a dark-colored vial and stable for at least a week.

Reagent solution 8-amino-2-naphthalene sulfonic acid (1×10^{-2})mol/l

The solution was prepared by dissolving 0.2232 g of 8-amino-2-naphthalene sulfonic acid (Fluka) in 0.200 ml of sodium hydroxide solution 1 M. and then completing the volume with distilled water using a 100 ml volumetric bottle.

Potassium periodate solution (1×10^{-2}) mol/l

This solution was prepared by dissolving 0.23 g of potassium periodate in 100 mL of distilled water in a volumetric vial.

Sodium hydroxide solution(1M)

This solution was prepared by diluting 10 ml of an ampoule of 10 mol/l 100 ml (Fluka) with distilled water in a volumetric vial of 100 ml and kept in a plastic bottle.

Tablet solutions:

This solution was prepared by weighing 10 tablets of the pharmaceutical preparation (Salazopyrine) from two different origins (Sweden, and Turkey). Each tablet contains 500 mg of sulfasalazine, grinding and mixing them well, the equivalent of 0.01 g of sulfasalazine was weighed and dissolved in 0.5 ml sodium hydroxide solution (1 M), then 50 ml of distilled water was added to it with the proper stirring, then the volume was completed 100 ml by using distilled water, after that the solution was filtered by filtering paper no 1, then it diluted in an appropriate amount to prepare 50 µg/ml (1.255×10^{-4} M).

Results and discussion

Preliminary study

An initial experiment was carried out by adding 1.0 ml (50 µg/ml) of sulfasalazine to a series of 10 ml of volumetric vials, to each1.0 ml of potassium periodate was added as an oxidizing agent and 1.0 mL of different coupling reagents were added to each of the vials and waited for some time (3-5 minutes), then 1.0 ml of sodium hydroxide solution (1 M) was added, as the product was formed immediately after adding the base at room temperature the volume was filled with distilled water to the mark, and the absorption spectrum of the colored product was measured against its blank solution, where the reagent 8-amino-2-naphthalene sulfonic acid gave the highest absorption at a wavelength of 457 nm

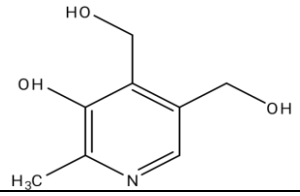
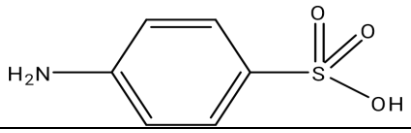
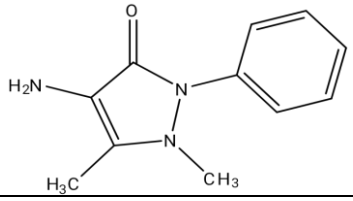
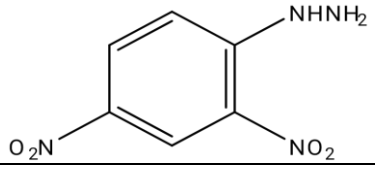
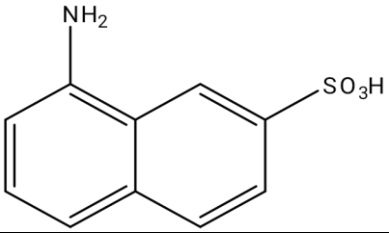
Setting the optimal conditions for the reaction

Subsequent experiments were carried out in 10 ml of volumetric vials using 50 µg/ml of sulfasalazine and measuring the absorbance of the colored product against its mock solution at 457 nm.

select of coupling reagent

Several chemical reagents that can be used as coupling factors in oxidative coupling reactions were used to estimate sulfasalazine, and the results are shown in Table (1) The results in Table (1) show that 8-amino-2-naphthalene sulfonic acid gave the highest absorption of the colored product, so it was chosen in subsequent experiments.

Tablet (1) selection of coupling reagent

Coupling agent 1×10^{-2}M,ml	Structure	Absorbance	λ_{max} (nm)
Pyridoxine		0.3528	456
Sulfanilic acid		0.3603	456
4- amino antipyrine		0.3861	457
2,4di nitro hydrazine		0.3891	457
8 amino-2-naphthalen sulfanic acid		0.3966	457

Effect an oxidizing agent

To find the best oxidizing agent, several oxidizing agents were studied such as (potassium persulfate , potassium iodate, potassium periodate, N-bromosuccinimide, and N-chlorosuccinimide) at a concentration of 0.01 M for each oxident in a volumetric flask of 10 ml containing 1.0 ml of sulfasalazine solution and 1.0 ml of 8-amino-2-naphthalene sulfonic acid solution with 1.5 ml (1 M) of sodium hydroxide at room temperature, after that the volume was completed with distilled water up to the mark. The absorption intensity of each solution was measured against its blank solution, and the results are listed in Table (2).

Tablet (2) effect an oxidizing agent

Oxidizing agent (1×10^{-2}M)	**Absorbance**	**λ_{max}(nm)**
Potassium periodate	**0.3954**	**457**
N-chlorosuccinimide	0.2896	450
N-Bromosuccinimide	0.1270	424
Potassium per disulfate	0.3483	458
Potassium iodate	0.3534	456

The results in Table (2) show that the best absorption was obtained by using the oxidizing agent potassium pe riodate, so it was chosen for the subsequent trials

The effect of amount coupling reagent quantity

The effect of the amount of reagent 8-amino-2-naphthalene sulfonic acid on the absorbance value was studied by adding different volumes of the reagent (0.1-1.2) ml to a fixed concentration of sulfasalazine solution (50 µg / ml) and adding 0.5 ml of potassium periodate and 1.5 ml of sodium hydroxide 1M) into volumetric flask of 10 mL, after waiting 5 minutes the volume was completed up to the mark by using distilled water. The results in Table (3) show that using a volume of 0.5 ml of the reagent 8-amino-2-naphthalene sulfonic acid gave the highest absorption, so it was chosen in subsequent experiments.

Table (3) The effect of the amount of coupling reagent

Coupling agent (1×10^{-2} M),ml	**Absorbance**
0.1	0.3971
0.2	0.3995
0.4	0.3905
0.5	**0.4086**
0.7	0.4063
1.0	0.4001
1.2	0.4029

Effect of the base type and pH:

The effect of adding different types of bases (sodium hydroxide, potassium hydroxide, sodium bicarbonate, and sodium carbonate) was studied to show their effect on the absorption value, by adding 1.0 ml at a concentration (1M) to the reaction components which contain 1.0 ml of sulfasalazine (50 µg/ml) and 0.5 ml of the oxidizing agent potassium periodate and 1 ml of 8-amino-2-naphthalene sulfonic acid, the volume was compiled to the mark with distilled water and the absorption intensity of the formed product was measured at wavelength 457 nm against its blank solution. The results are listed in the table (4). The results in table for shows that the reaction needs a strong alkaline medium and since sodium hydroxide gave the highest absorption of the formed product, it was chosen in the subsequent experiments.

Table (4) The effect of different types of base and pH

Type of base used(1M)	**Absorbance**	**pH**
NaOH	**0.3990**	**11.46**
KOH	0.3871	12.30
$NaHCO_3$	0.0488	9.26
Na_2CO_3	0.0484	4.98
NH_4OH	0.1026	9.96
without	0.2952	4.98

Effect of the amount of sodium hydroxide

The results in Table (5) shows that a volume of 1.2 ml of sodium hydroxide at a concentration (1M) gave the highest absorption, so it was adopted for the subsequent experiments.

Table (5) The effect of the amount of sodium hydroxide

ml of 1M NaOH	Absorbance
0.5	0.3812
0.7	0.3843
1.0	0.3826
1.2	**0.3926**
1.5	0.3988

Effect of adding sequence

To choose the best addition sequence of the reactants, several addition sequences were studied as listed in Table (6). The results in Table (6) explain that all addition sequences gave close absorption values, the sequence (I) which was adopted in previous experiments, was select for subsequent experiment.

Table (6) The effect of adding sequence on absorbance

Order of number	Reaction component	Absorbance
I	**S+OX+R+B**	**0.4022**
II	S+R +OX +B	0.3966
III	R+OX +S +B	0.3930
IV	S+B +OX +R	0.3979

The effect of oxidation time

The effect of oxidation time to complete the reaction was studied using a set of 10 ml volumetric flask ,which containing 1.0 ml of sulfasalazine solution at a concentration of 50 µg/ml , 0.5 ml of potassium periodate (0.01M), and 0.5 ml of 8-amino-2-naphthalene sulfonic acid solution (0.01M), the solutions were left for different periods (3-25) minutes to complete the oxidation process, then they were diluted with distilled water up to the mark, and the absorbance was measured at a wavelength of 457 nm against it blank solution.The results are illustrated in Table (7).

Table (7) the effect of the time on the oxidation process

Time, min.	3	5	10	15	20	25
Absorbance	0.3790	0.3920	0.3906	0.3946	0.3906	0.3960

The results in Table (7) shows that the required time to complete the oxidation process is (5) minutes, so it was selected in the subsequent experiments.

Effect of temperature on absorbance

The effect of different temperatures (10-50 °C) on absorbance of the colored product was studied according to the method under the conditions that were approved in the previous experiments. The results in Table (8) shows that the value of product absorbance decreases at high and low temperatures and that room temperature is the best.

Table (8) the effect of temperature on absorbance

Temp ˚C	0	10	(25+2C˚)	40	50
Absorbance	0.2092	0.3909	0.3986	0.3661	0.3736

Surfactant effect study

The effect of adding different types of surface agents on absorbance value was studied by taking different volumes of these materials and adding them to the reaction mixture.

The results in Table (9) explain that the addition of surface agents led to a decrease in the absorbance value, so it was excluded from the subsequent experiments.

Table (9) Surfactants effect on absorbance

Surfactants	**Absorbance/ ml of surfactants added**		
	0.5	1.0	2.0
CPC (1 x 10^{-3}M)	97.9	96.8	96.6
SDS (1 x 10^{-3}M)	98.12	95.7	96.67
CTAB (1 x 10^{-3}M)	100.9	98.2	97.7
Triton-X - 100(1.0%)	101.13	99.6	99.5
Without	0.4150		

Studying the effect of organic solvents compared to water

The effect of several organic solvents on the absorbance of the product were studied by adding 1 ml of sulfasalazine, 0.5 ml of potassium periodate, 0.5 m of 8-amino-2-naphthalene sulfonic acid, then1.2 ml of sodium hydroxide was added, and after waiting five minutes, the volume was diluted with organic solvents an absorbance , the was measured at maximum wavelength of each for one , as Table (10) Fig (1) and which show that ethanol, DMSO and acetone gave the highest value of absorbance comparing to water, but due to the good sensitivity of water and its availability and being safe compared to the other organic solvents, so it was keep using in the subsequent experiments.

Table (10) The Effect of several of solvents on absorption

Solvent	**(nm)λ_{Max}**	**Absorbance**	ε , $L/mol.cm$
Ethanol	454	0.4638	36956.175
Methanol	Turbid	---------	---------
Acetone	460	0.5385	42908.366
Water	**457**	**0.4008**	**31936.067**
Propanol	458	0.3718	29625.498
Acetic acid	360	0.4162	33163.346
DMSO	476	0.5135	40916.334

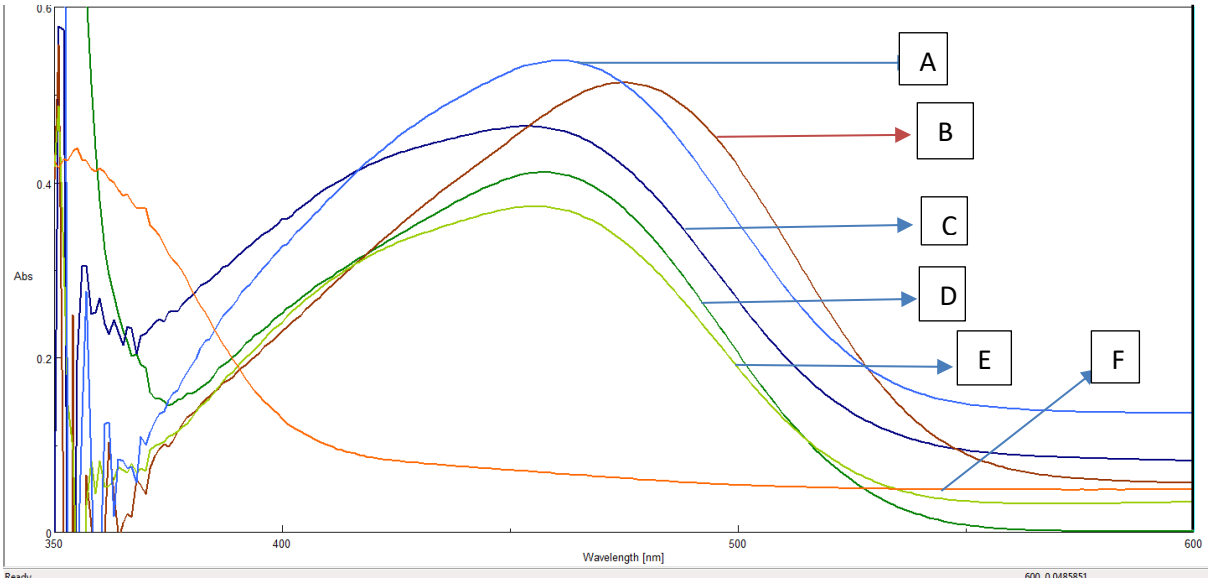


Figure (1) The effect of some solvents on the absorption spectrum

A. Acetone - B. DMSO - C. Ethanol – D. Water -E. Propanol -F. Acetic acid

Stability time for the formed product

The stability of the product that formed from the oxidative coupling reaction was studied by taking two different concentrations of sulfasalazine and applying the method under optimal conditions, the absorbance was measured at a wavelength of 457 nm against the blank solution in different periods. The results are shown in Table (11):

Table (11) the stability time

Time (min)	Absorbance of SZZ µg /ml	
	25	50
After addition	0.2107	0.4019
5	0.2108	0.4030
10	0.2110	0.4000
15	0.2112	0.4005
20	0.2112	0.4005
25	0.2113	0.4021
30	0.2113	0.4010
35	0.2110	0.4030
40	0.2113	0.4015
45	0.2113	0.4016
50	0.2109	0.4030
55	0.2110	0.4044
60	0.2112	0.40139

The results in Table (11) show that the resulting complex is stable for at least 60 min., which is a sufficient period to perform several measurements.
The flowing table shows a summary of the studied optimal conditions for the determination of sulfasalazine by the proposed method.

Table (12) Summary of optimal conditions for the method

Parameters	Optimization condition
Reagent used	8 amino-2-naphthalen sulfonic acid,
Amount of reagent used (ml)	0.5
Oxidizing agent	Potassium periodate
Amount of oxidizing (ml)	0.5
Base used	NaOH
Amount of NaOH 1.0 M used	1.2
Solvent Medium	Aqueous
Time of oxidation, min.	5
λ_{max}(nm)	457
Stability period, min.	$\geq 60\ min$

The final absorption spectrum:
By following the optimal conditions per fuse mention (12), the drawing of the final absorption spectrum of the colored results which formed product, which formed from the reaction of 50 µg / ml of sulfasalazine with 0.5 ml of potassium periodate (0.01M) and 0.5 ml of the reagent 8-amino-2-naphthalene sulfonic acid (0.01) M, after 5 minutes 1.2 ml of sodium hydroxide (1M) was added.The volume was complete to the mark with distilled water in a volumetric flask of 10 ml, and the absorbance of the product was measured at the wavelength of 457 nm against its mock solution, which gave little absorption at the same wavelength. Figure (2) illustrated this.

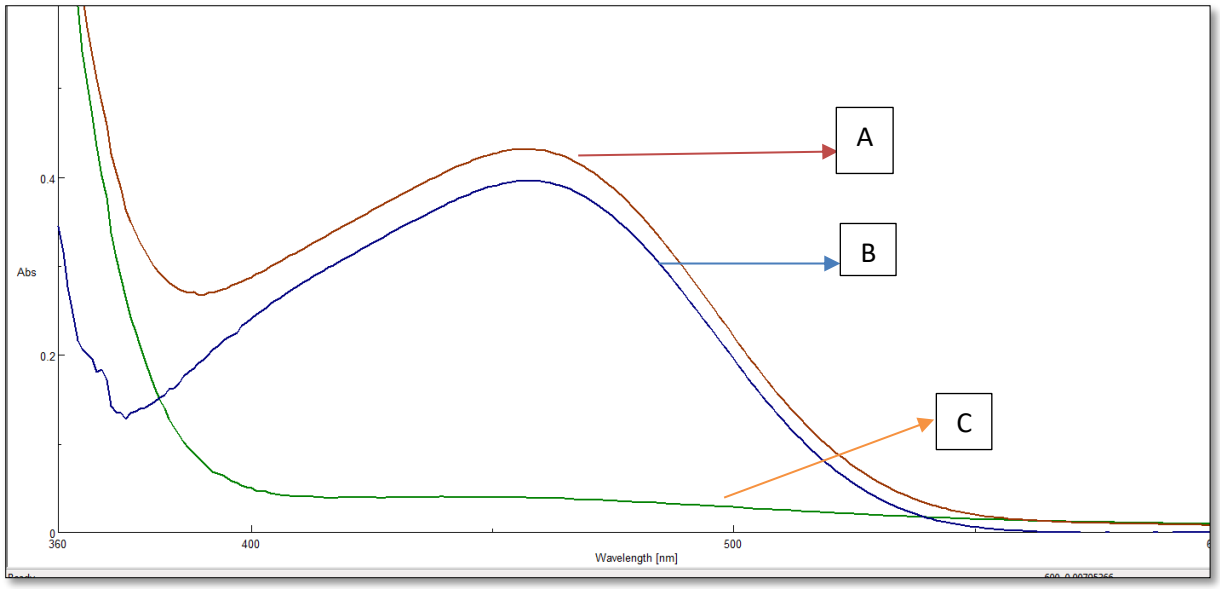


Figure (2) The final absorption spectrum of 50 µg / 10 ml sulfasalazine measured (A) versus distilled water, (B) versus blank solution, and (C) blank versus distilled water.

The commended procedure and calibration curve

The standard curve was prepared depending on the proven optimal conditions, where different volumes (0.05-5) ml of sulfasalazine solution were added to a set of 10 ml volumetric flask with a concentration of (50 µg / ml), and these volumes rang were in the range of 0.25-25 µg / ml , then added 0.5 ml of potassium periodate with of (0.01) M with waiting 5 minutes, then 0.5 of the reagent solution 8-amino-2-naphthalene sulfonic acid were added, after that 1.2 ml of sodium hydroxide solution with a concentration of (1 M) were added, the volume was completed with distilled water, The absorbance of the colored solutions was measured at a wavelength of 457 nm against the blank solution. Figure (3) shows that the calibration curve follows Beer's law in the range of 0.25-20 µg/ml and that there is a deviation in the concentrations that are higher than 20 µg/ml. The value of the molar absorption coefficient is 2.9203×10^{4} l. mol^{-1}. cm^{-1} and the Sandell's sensitivity index is 0.0136 µg/cm^2**.**

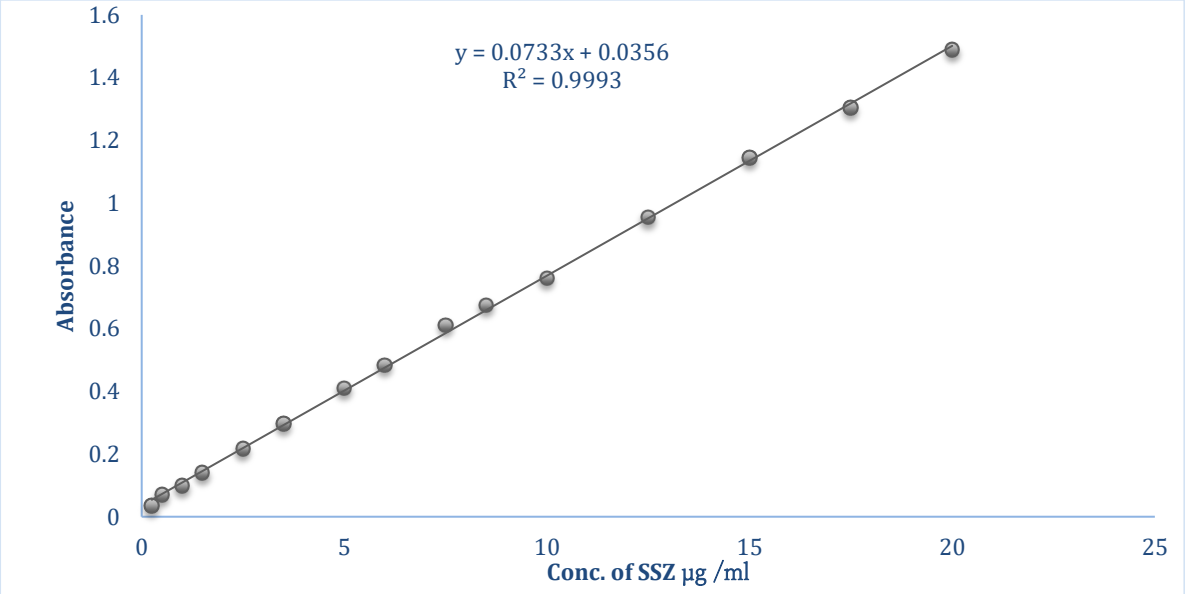


Figure (3) the calibration curve for of sulfasalazine determination

Accuracy and Precision of the method:

The accuracy and precision of the method were calculated for two different concentrations of sulfasalazine solution of (5,10) µg /ml, where the recovery ratio that expresses the accuracy and the relative standard deviation that expresses the precision were calculated. The results in Table (13) indicate that the method is stasis accuracy and precision .

Table (13) Accuracy and compatibility

Amount of SSZ µg, ml Present	Recovery* %	Amount of SSZ µg,ml Found	RE*%	RSD*%
5	99.61	4.980	-0.39	0.548
10	101.06	10.106	1.06	1.15

*Average for five determinations.

Studying the nature of the reaction between sulfasalazine and the reagent

Jobs method (continuous changes) [12] was applied to find the molar ratio of the reaction between the sulfasalazine and the reagent (8-ANS) at an equal concentration ($(1.255\times10^{-2}$ mol/l) and that was achieved by preparing solutions contained volumes of range (0.1-0.9) ml of sulfasalazine solution as well as different volumes (0.1-0.9) ml of the reagent solution (8-ANS) and depending on the proven method, the absorbance was measured

at the wavelength of 457 nm. Figure (5) shows that the reaction ratio between sulfasalazine and reagent (8-ANS) is 1:1.

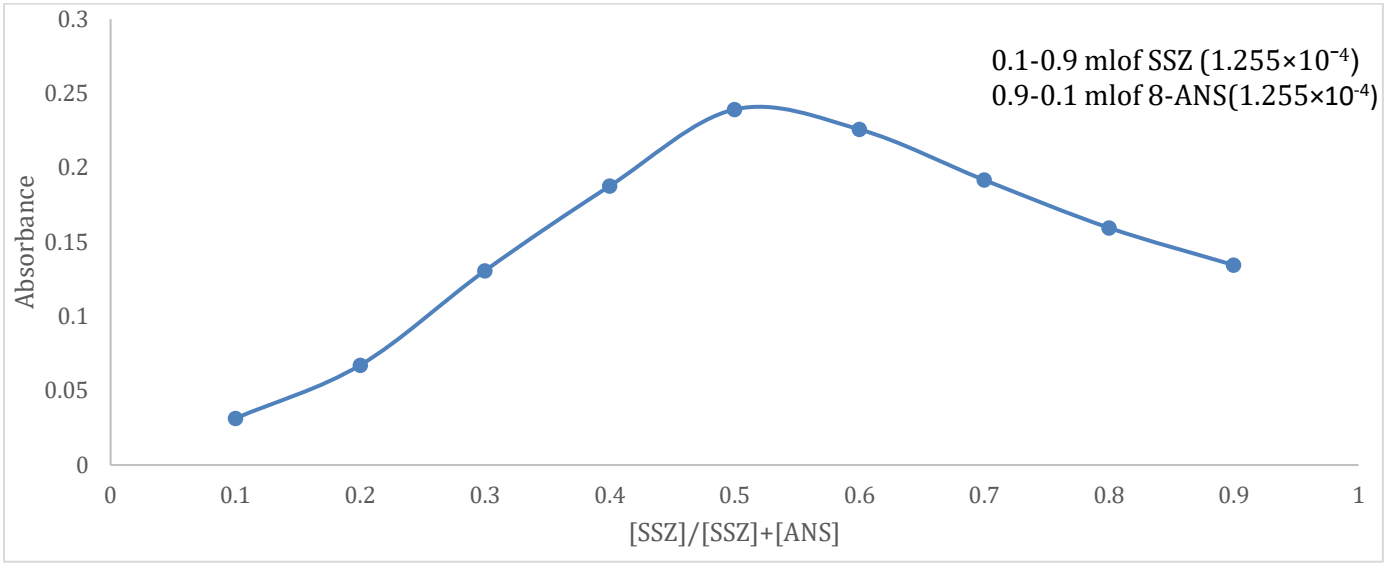


Figure (4) the jobs method curve

To verify this ratio, the method of molar ratios was applied by adding variable volumes (0.25-2.5) of the reagent solution (ANS) at a concentration of 1.255×10^{-2} molar, to be series of volumetric flask counting 1 ml of SSZ by following and debating on the proven method obtained results confirmed that the actual ratio of the reaction of sulfasalazine with the reagent 8-amino-2-naphthalene sulfonic acid is 1:1, as shown in Figure (6).

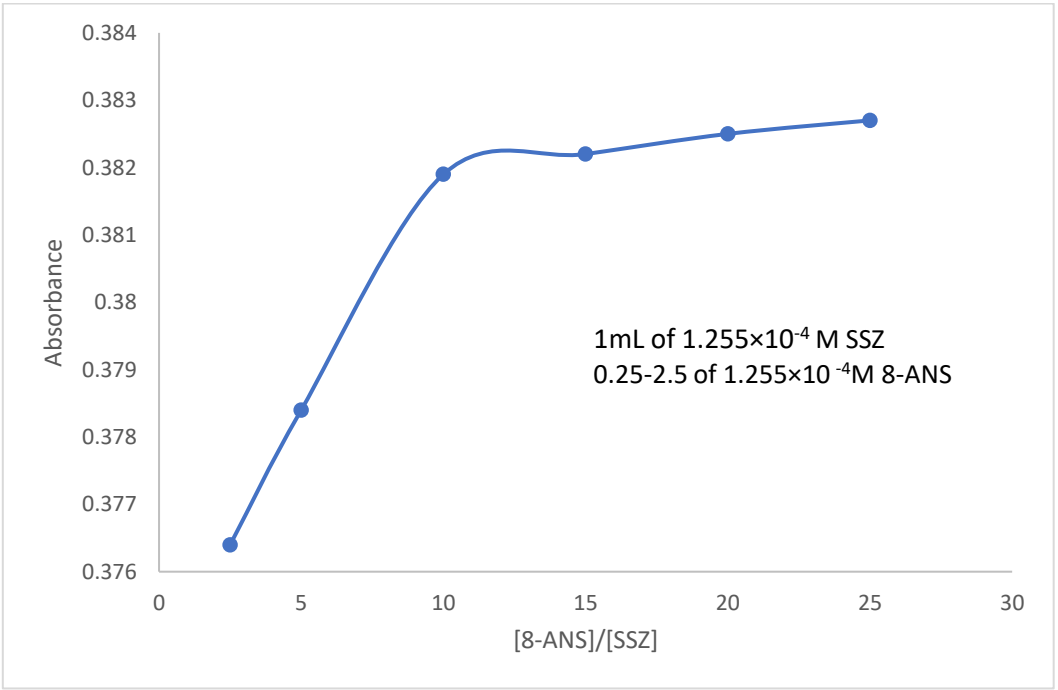


Figure (5) the molar ratio method

The according to the results fig 4 and fig 5 the suggested formula for the colored product can be retain as following

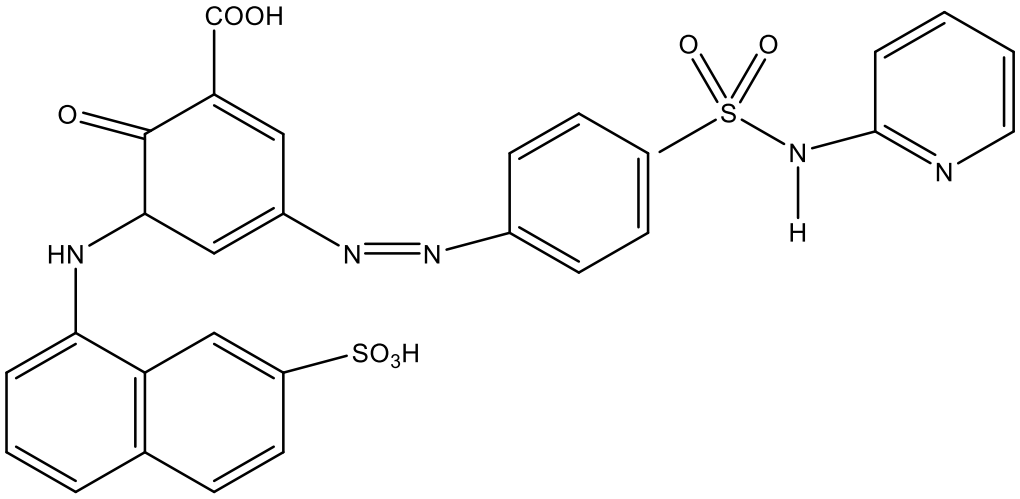


Figure (6) the chemical figure for colure dye.

Applied part:

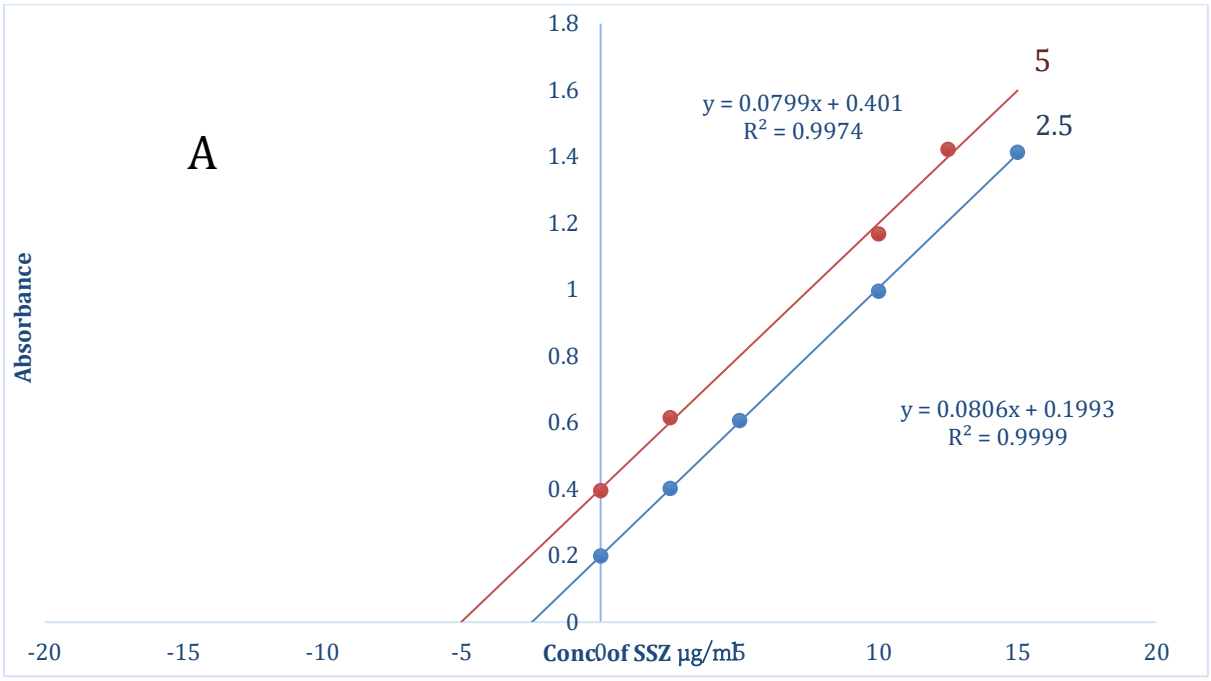
The proposed method was applied to the pharmaceutical preparation (tablets) from two different origins, by taking two different concentrations (2.5, and 5) µg /ml of the pharmaceutical preparation solution prepared previously and the result are fixed in Table (14), The obtained results confirmed the success of the proposed method in the determination of sulfasalazine in pharmaceutical preparations.

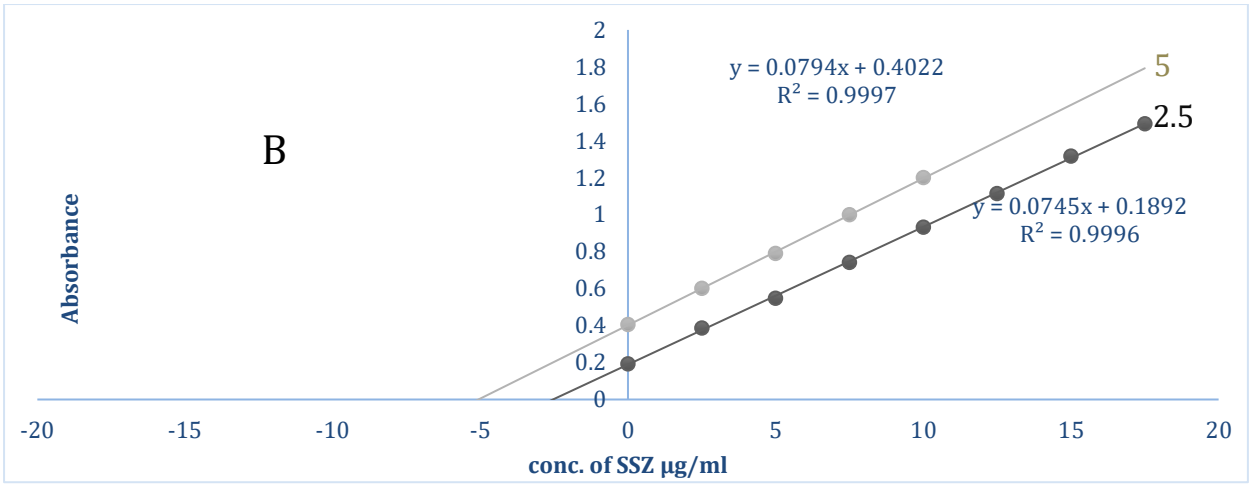
Table (14) apply the proposed method for estimation of sulfasalazine

Pharmaceutical Preparation	Amount of SSZ present µg/mL	Recovery, * %	RSD, *%	RE*%	Amount of SSZ Found µg/mL
Salazopyrin 500 mg/tab SSZ Turkey	2.5	99.96	1.65	-0.04	2.499
	5	99.60	0.49	-0.396	4.980
Salazopyrin, Tablet (500 mg) SSZ, Sweden	2.5	96.40	3.59	-3.60	2.41
	5	99.98	0.71	-0.01	4.999

Evaluate using the stander addition method

To prove that the proposed method is selective and successful in the determination of sulfasalazine in pharmaceutical preparations, and there is no interference with additives exist in pharmaceutical preparations the standard addition method was applied at two concentrations (2.5, and 5) µg / mL,and the result in figures as shown in Figures (7) and table (15) indicated that the stander addition method is in good agreement with proposed method





Figures (7) The standard addition of Swedish medicine (A) the standard addition of Swedish tab. (B) The stander addition of turkey tab.

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Table (15) The results of the standard addition method of sulfasalazine

Pharmaceutical preparations	Amount of SSZ Taken µg/mL	Amount of SSZ measured µg/mL	Recovery %
Salazopyrine, Tablet (500 mg), Sweden	2.5	2.47	98.8
	5	5.01	100.2
Salazopyrine, Tablet (500mg), Turkey	2.5	2.53	101.2
	5	5.06	101.2

Conclusion:

A spectrophotometric method was developed for the determination of sulfasalazine, the method was based on the oxidative coupling reaction between sulfasalazine and the reagent 8-amino-2-naphthalene sulfonic acid in the presence of potassium periodate as an oxidizing agent. The method was successfully applied to the pharmaceutical preparation (tablets).

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