

Spectrophotometric Estimation of Sulfacetamide Sodium Using N,N-Diethyl-p-phenylenediamine Reagent

Thukaa M. Abdulrazzaq

Saad H. Sultan

Department of Chemistry/ College of Science/ University of Mosul

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corresponding author:

Saad H. Sultan

saad.hasani@uomosul.edu.iq

ABSTRACT

A simple, accurate and sensitive spectrophotometric method was described for the determination of sulfacetamide sodium in an aqueous solution. The method is based on the oxidative coupling reaction of N,N-diethyl-p-phenylenediamine with sulfacetamide sodium in the presence of potassium dichromate as an oxidizing agent in an acidic medium to form a violet-colored product, which has a maximum absorbance at 546 nm. Beer's law is obeyed in the concentration range of 1.25-75 $\mu\text{g.ml}^{-1}$ of sulfacetamide sodium, with a molar absorptivity of $0.76 \times 10^4 \text{ L.mol}^{-1}.\text{cm}^{-1}$ and sandell's sensitivity value of $0.03344 \mu\text{g.cm}^{-2}$. The relative error values are in between -2.01 and -0.14 while the relative standard deviation values are in between 0.34 and 1.55. The proposed method was applied successfully to assay sulfacetamide sodium in its pharmaceutical preparations as eye drops and ointment.

Keywords: Sulfacetamide sodium, oxidative coupling reaction, N,N-diethyle-p-phenylendiamine reagent, spectrophotometry.

INTRODUCTION

Sulfa medicines are among the oldest antibiotics manufactured and used to treat infections. They are also known as sulfonamides. The reason for its importance is due to its effectiveness in treating bacterial infections and because it provided information about how antibiotics work in treating these infections (Csaky, 1979). Despite the discovery of other antibiotics such as penicillin, sulfa drugs are still used today in the medical field (Carey, 1996). The sulfa drugs act as anti-bacterial metabolites during inhibiting the bacterial enzyme that synthesizes the vitamin folic acid from the para-aminobenzoic acid molecule, which is essential for the transfer of methyl groups in the process of biological production of methionine and nitrogenous bases within bacteria, and when taking sulfa drugs, they undermine inside the body, it turns into sulfanilamide a compound that binds to a bacterial enzyme, and thus prevents the formation of folic acid, and thus leads to bacterial death (Jacqueline and Melvin, 1990; Robert *et al.*, 2001; Suchoki, 2004). It has been observed that the human body is not affected as a result of sulfa drugs, because the body does not make folic acid, but rather obtains it from the food it eats, especially leafy vegetables (Rang and Dale, 1987).

Sulfacetamide sodium (SAS) belongs to a broad-spectrum antibiotic of the sulfonamide family (Safronova *et al.*, 2020). SAS is widely used in medicine due to its excellent inhibitory effect on the growth of many types of bacteria (Al-Safar and Othman, 2020). SAS is a white crystalline powder free soluble in water and partially soluble in ethanol and not soluble in ether and chloroform, it melts at 257°C (Al-Uzri and Fadil, 2017). And it is chemically N-[(4-aminophenyl)sulfonyl]acetamide monosodium salt monohydrate ($\text{C}_8\text{H}_9\text{N}_2\text{NaO}_3\text{S} \cdot \text{H}_2\text{O}$) (Fig. 1) and it is N-substituted derivatives of sulfanilamide and compete with p-aminobenzoic acid in enzymatic synthesis of dihydrofolic acid (Ayad *et al.*, 2012).

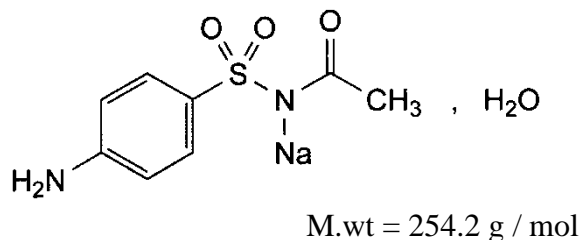


Fig. 1: Chemical structure of sulfacetamide sodium monohydrate

Sulfacetamide sodium was determined using different analytical methods, Spectrophotometrically (Talib *et al.*, 2009; Jassam *et al.*, 2021). Electrochemically (Parshina *et al.*, 2022), or chromatographically tandem-mass (Tamošiūnas *et al.*, 2009), HPLC (Deng *et al.*, 2016). Due to the medicinal importance of the sulfacetamide drug and simplicity of the spectrophotometric methods in assay of different pharmaceuticals (Yassin and Othman, 2022; Hasan and Sultan, 2022; Sultan and Majed, 2020; Aziz and Sultan, 2019) so, the present work has been adopted for the determination of SAS in its pharmaceutical formulations using oxidative coupling reaction.

EXPERIMENTAL

Apparatus and instrumentation

All absorbance measurements were done by using a double-beam UV-Visible spectrophotometer (JASCO V- 630) with 1.0 cm glass cells. Professional benchtop pH meter TRANS BP3001 was used for the pH measurements.

Chemical reagents

All chemicals and analytical reagents used in this study were selected with a high degree of purity.

Preparation of material solutions

SAS solution ($500 \mu\text{g}.\text{ml}^{-1}$): This solution was prepared by dissolving 0.05 g of pure SAS in enough amount of distilled water, then complete the volume to 100 ml by distilled water using volumetric flask.

Sulphuric acid solution (0.1 M): This solution was prepared by diluting 0.86 ml of concentrated acid with distilled water in 100 ml volumetric flask.

N,N-Diethyl-p-phenylenediamine dihydrochloride monohydrate reagent solution (5×10^{-3} M): This solution was prepared daily by dissolving 0.1093 g of N,N-diethyl-p-phenylenediamine in enough amount of ethanol and the volume was completed to 100ml in a volumetric flask with the same solvent and kept in a dark container.

Potassium dichromate solution (4×10^{-3} M): A 0.1177 g of potassium dichromate was dissolved in enough amount of distilled water with stirring and completed the volume to mark level in 100ml volumetric flask with distilled water.

Additives solutions ($10 \mu\text{g} / \text{ml}$): Preparing these solutions by dissolving 0.01 g of each additive's solution compound in 100 ml of distilled water using 100 ml volumetric flask, followed by dilution 10 ml of the later solution to 100 ml by distilled water.

Pharmaceutical solution for eye drops ($500 \mu\text{g} / \text{ml}$): This solution was prepared by withdrawing 1 ml of the eye drops (Apisulfa-10/Amman Pharmaceutical Industries Co. Jordan) (10%, each 1 ml contains 0.1 g of SAS) and diluted to 100 ml by distilled water, then taking 50 ml of the later solution and diluted to 100 ml with distilled water.

Ointment solution ($500 \mu\text{g}/\text{ml}$): This solution was prepared by weighting 0.5 g of the eye ointment (Predmacin 10% /Linda-a-vetha ,Portugal) (each 1 g contains 0.1 g of SAS) and then dissolving it in 10 ml of ether and quantitatively transferred to separating funnel followed by addition of 40 ml of ether, and start extraction process by three batches of 25 ml distilled water then collect the aqueous layers containing SAS and transfer it to a volumetric flask of 100 ml capacity, then completing the volume with distilled water.

Approved working method and calibration curve

After establishing the experimentally optimal conditions for the determination of sulfacetamide sodium, the standard curve was prepared as follows: Increased volumes of sulfacetamide sodium were added to a series of 20-ml volumetric flasks, then 1 ml of H_2SO_4 0.1 M and 4 ml of solution 5×10^{-3} M N,N-diethyl-p-phenylenediamine reagent was added followed by 4.5 ml of 4×10^{-3} M potassium dichromate solution with mixing the contents and left for 30 minutes to complete the reaction, then the volume completed with distilled water. The absorbance of the solutions was measured against blank solution at 546 nm. The calibration curve shown in Fig. (2) is in accordance with Beer's law in the range of concentrations ($1.25\text{-}75 \mu\text{g} .\text{ml}^{-1}$) and there is a negative deviation at concentrations higher than $75 \mu\text{g}$ of SAS/ml. The value of the molar absorption of the resulting dye was $0.76 \times 10^4 \text{ L}.\text{mol}^{-1}.\text{cm}^{-1}$. while the value of Sandell's sensitivity is $0.03344 \mu\text{g}.\text{cm}^{-2}$. Limit of detection and limit of quantitation values were 0.24322 and $0.81073 \mu\text{g} .\text{ml}^{-1}$ respectively.

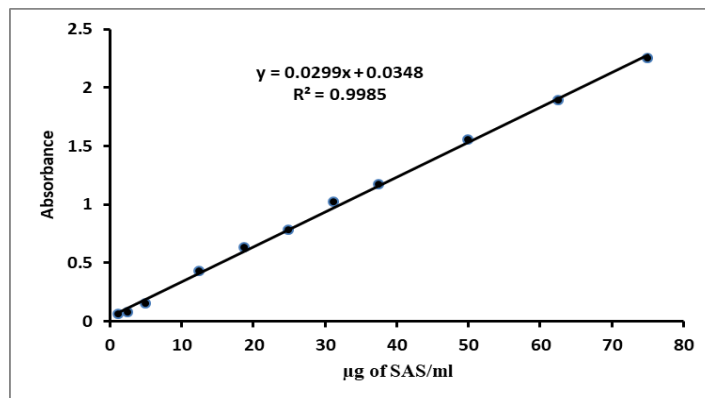
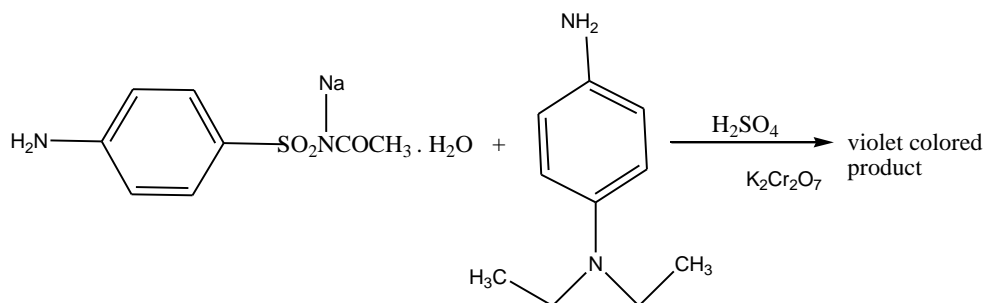


Fig. 2: Calibration curve for SAS determination

RESULTS AND DISCUSSION

The general principle of the proposed method

The principle of the proposed method depends on conjugation of sulfacetamide with the reagent N,N-diethyl-p-phenylenediamine in the presence of the oxidizing agent potassium dichromate in acidic medium, to form violet color product which gives the highest absorption at 546 nm.



Optimum Reaction Conditions

To select optimal conditions for SAS determination, different effects were investigated using 1 ml of pure SAS solution (500 µg) in a final volume 20 ml and the absorbance was measured at 546 nm against the blank solution.

Effect of acid type and quantity of different acids

The study of the acidic medium is necessary for the oxidation reaction to obtain the best results by using different acid solutions with a concentration of 0.1 M and different quantities for each acid with a waiting time of 30 minutes to complete the oxidation process and complete the volume to the mark with distilled water. The results in (Table 1) showed that 1 ml of sulfuric acid gave the highest absorbance value.

Table 1: Effect of acids type and quantity of different acids

Acid solution Used (0.1M)	Variables	Absorbance / ml of acid used				
		0.25	0.5	1	1.5	2
HCl	λ	533	547	547	546	546
	A	0.6142	0.6191	0.6173	0.6631	0.6352
H ₂ SO ₄	λ	547	546	546	546	546
	A	0.6247	0.6620	0.6734	0.6604	0.6368
CH ₃ COOH	λ	556	554	553	552	552
	A	0.2102	0.2316	0.4232	0.4440	0.522
H ₃ PO ₄	λ	550	547	546	547	546
	A	0.5651	0.6195	0.6198	0.6202	0.6084

Effect of coupling reagent amount

The effect of the coupling reagent was studied by reacting different volumes of reagent solution with different amounts of SAS in the presence of oxidizing agent (potassium dichromate) and 1 ml of 0.1 M sulfuric acid in a final volume of 20 ml and the absorbance of the solutions was measured at 546 nm against blank solution (Table 2).

Table 2: Effect of reagent amount

Amount of Reagent (5×10^{-3} M), ml	Absorbance / μ g of SAS/ 20 ml						
	125	250	375	500	750	1000	R ²
1	0.1138	0.1853	0.2776	0.3440	0.4808	0.5999	0.9957
1.5	0.1567	0.2633	0.3780	0.4730	0.6814	0.8284	0.9950
2	0.1898	0.3393	0.4702	0.6250	0.8443	1.0439	0.9930
2.5	0.2289	0.4025	0.5383	0.7663	1.0269	1.2725	0.9919
3	0.2327	0.3977	0.5610	0.7274	1.0158	1.2334	0.9935
3.5	0.2352	0.3933	0.5897	0.7639	1.0840	1.3344	0.9944
4	0.2559	0.4023	0.6114	0.7632	1.0921	1.3875	0.9981
4.5	0.2245	0.3858	0.5606	0.7170	1.0422	1.2785	0.9959

The results listed in (Table 2) indicate that 4 ml of reagent gives the highest values of absorbance and determination coefficient for the colored product, so it was approved in subsequent experiments.

Effect of oxidizing agent amount

The effect of the oxidizing agent amount was studied by adding a different volumes of the oxidizing agent $K_2Cr_2O_7$ ranging from (1-5 ml) to 20 ml volumetric flask containing different amount of SAS solution and 4 ml of the reagent and 1 ml of 0.1 M sulfuric acid, leave solutions for 30 minutes and then complete the volumes to the mark with distilled water and measure the absorbance of solutions against its blank solution at 546 nm. (Table 3).

Table 3: Effect of oxidizing agent amount

Amount of oxidizing agent. (4×10^{-3} M), ml	Absorbance / μ g of SAS						
	125	250	375	500	750	1000	R ²
1	0.1270	0.2444	0.3076	0.4400	0.5696	0.6729	0.9921
1.5	0.1787	0.3312	0.4634	0.5758	0.7727	1.0247	0.9956
2	0.1828	0.3624	0.5122	0.6880	0.9603	1.2241	0.9966
2.5	0.2108	0.4099	0.5527	0.7698	1.1130	1.3988	0.9969
3	0.2499	0.4170	0.6280	0.7711	1.1509	1.3998	0.9948
3.5	0.2189	0.4602	0.6015	0.7815	1.2064	1.572	0.9979
4	0.2032	0.3983	0.5814	0.7580	1.1214	1.5463	0.999
4.5	0.2237	0.4055	0.6005	0.7653	1.1638	1.5214	0.9997
5	0.2372	0.4386	0.5996	0.7939	1.1893	1.5422	0.9995

The results are shown in (Table 3) revealed that 4.5 ml of reagent N,N-diethyl-p-phenylenediamine gave a good absorbance of the formed colored product as well as perfect value of determination coefficient and low blank value.

Effect of the oxidation time

The required time for completing the oxidation reaction was studied by reacting 500 μ g of SAS according to last optimum conditions in a final volume of 20 ml, and the results are shown in the

(Table 4) indicate that 30 minutes is a sufficient period time to complete the oxidation and coupling process, so it was adopted for subsequent experiments.

Table 4: Effect of time on oxidation

Oxidation time/min.	5	10	15	20	25	30	35
Absorbance	0.4925	0.6135	0.6577	0.6844	0.7229	0.7693	0.7573

Order of additions

The effect of different addition orders of reaction components on the intensity of the resulting-colored product, so a number of experiments were conducted to select the optimal order under conditions were followed in the previous experiments, the obtained results are listed in (Table 5) clear that order **II** gives the highest absorbance, so it was used in the next experiments.

Table 5: Order of additions

Order number	Order of additions	Absorbance
I	SAS+A+OX+R	0.7309
II	SAS+A+R+OX	0.7708
III	SAS+OX+A+R	0.7181
IV	SAS+OX+R+A	0.7651
V	SAS+R+A+OX	0.7583
VI	SAS+R+OX+A	0.7550

Effect of surfactants

Four types of surfactants were studied to determine their effect on the absorbance of the resulting dye by adding 2 ml of each type of surfactant after the completion of other reaction components according to recommended procedure. It was noted from the results shown in (Table 6) that the addition of these substances does not affect positively on the absorbance of the colored product, so it was not recommended in subsequent experiments.

Table 6: Effect of surfactants

Surfactant solution	λ_{max}	Absorbance
SDS (1×10^{-3} M)	546	0.7636
Triton X-100 (1%)	546	0.5800
CPC (1×10^{-3} M)	547	0.7024
CTAB (1×10^{-3} M)	546	0.7637
Without	546	0.7717

Effect of additives

The effect of some compounds that can be added when manufacturing the medicinal preparation of sulphacetamide sodium as preservative substance such as benzalkonium chloride or as medicinal compound such as prednisolone acetate has been studied. The results in (Table 7) show, that there is no noticeable overlap of these compounds in the estimation of the drug compound, that is, the possibility of estimating sodium sulphacetamide in presence of these compounds using the proposed method.

Table 7: Effect of additives on absorbance of formed product

Foreign compounds	Recovery (%) of 500 μ g SAS / μ g of foreign compound added/20 ml		
	2.5	5.0	10.0
Prednisolone acetate	97.99	96.69	96.38
Benzalconium chloride	97.62	99.73	100.05

Colored product stability

The stability of formed dye was investigated by measuring the absorbance after completing volumes to mark with distilled water against the blank solution at period times (Table 8).

Table 8: The stability of colored product

Time (min.)	0	5	10	15	20	25	30
Absorbance	0.7432	0.7573	0.7664	0.7740	0.7784	0.7840	0.7853

The results in (Table 8) show that the formed dye stable for 30 minutes, which can be do enough spectral measurements for SAS assay through it.

Final absorption spectra

The spectrum of the resulting violet colored product formed by coupling of sulfacetamide with N,N-diethyl-p-phenylenediamine reagent in the presence $K_2Cr_2O_7$ in acidic medium, which exhibits maximum absorbance at 546 nm. The final spectrum of 500 μg of SAS/20 mL illustrated in Fig. (3).

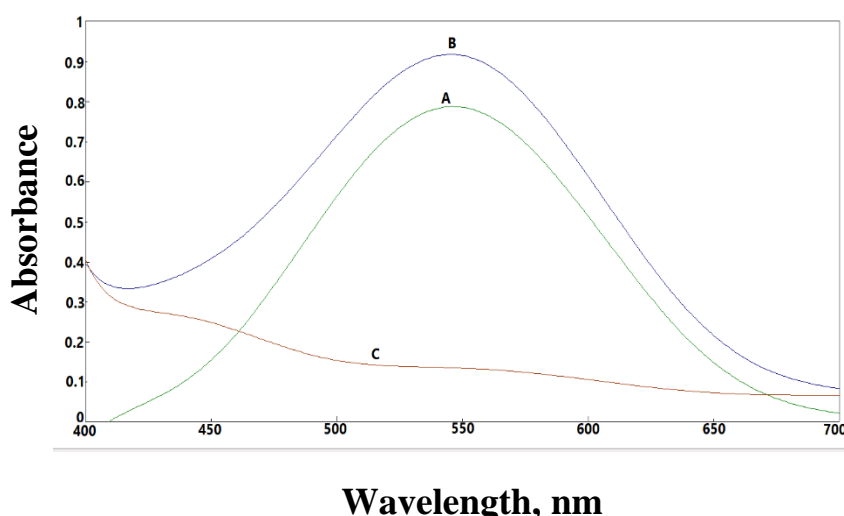


Fig. 3: Absorption spectrum for 500 μg / 20 ml sulfacetamide sodium according to the suggested method conditions: (A) sample versus blank (B) sample versus distilled water (C) blank versus distilled water

The nature of the formed dye

To find out the nature of the formed product and the ratio of the reagent's binding to the sodium sulfacetamide, continuous variation method (Job's method) was applied, the concentration of the SAS solution and the reagent solution N,N-Diethyl-p-phenylenediamine[R] is constant (1.966×10^{-3} M) where different volumes were placed in a series of volumetric flasks with a capacity of 20 ml ranging from (0.1-0.9 ml) and different volumes of the drug solution ranging from (0.9-0.1 ml) of reagent solution were mixed. 1 ml of sulphuric acid (0.1 M) and 4.5 ml of potassium dichromate (4×10^{-3} M) were added and volumes were completed to the mark with distilled water, the absorbance of these solutions was measured after 30 minutes at 546 nm against its blank solution. Fig. (4) shows that the ratio is 1:2 between the drug and the reagent.

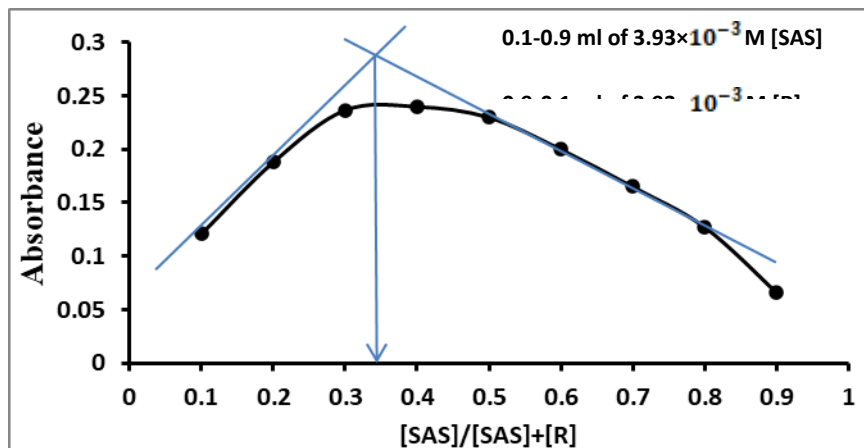


Fig. 4: Job's method curve for SAS-R(reagent) combination ratio

So, the proposed final structural of the formed colored product (Nagaraja *et al.*, 2010) is as following Fig. (5):

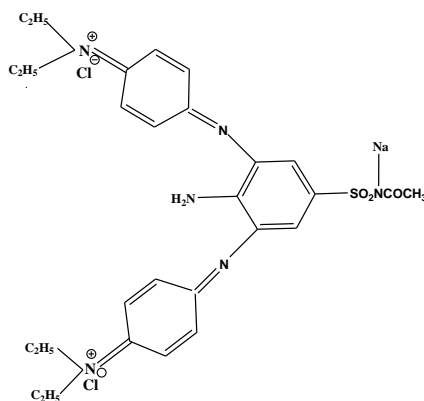


Fig. 5: The proposed structure of the formed colored produced

Application of the method

The suggested method was successfully applied to determination drug in its available commercial preparations as eye drop and ointment. The results in (Table 9) illustrated that the proposed method is an accurate and reproducible and suitable for the determination of sulfacetamide in pharmaceutical preparations.

Table 9: The application results for SAS estimation in its pharmaceutical preparations

Pharmaceutical preparation	SAS Present (µg)	SAS Found (µg)	Recovery (%) [*]	Relative error (%)	RSD (%) [*]
Apisulfa-10 sterile eye-drops, Amman Pharma. Indust. Co. Ltd (Jordan)	250	245.5	98.20	-1.8	1.55
	500	497.8	99.56	-0.44	0.34
Predmacin 10% ointment, Linda-a-vetha (Portugal)	250	249.65	99.86	-0.14	0.68
	500	489.95	97.99	-2.01	0.65

^{*}Average of five determinations

Evaluate the result using the standard addition method

For the purpose of proving the efficiency of the proposed method and its success in estimating sulfacetamide sodium in its pharmaceutical preparations, and there is no interference with additives

exist in pharmaceutical preparation, the standard addition method was applied and the results in the Fig. (6) and (Table 10) indicate that the standard addition method is in good agreement with the proposed method.

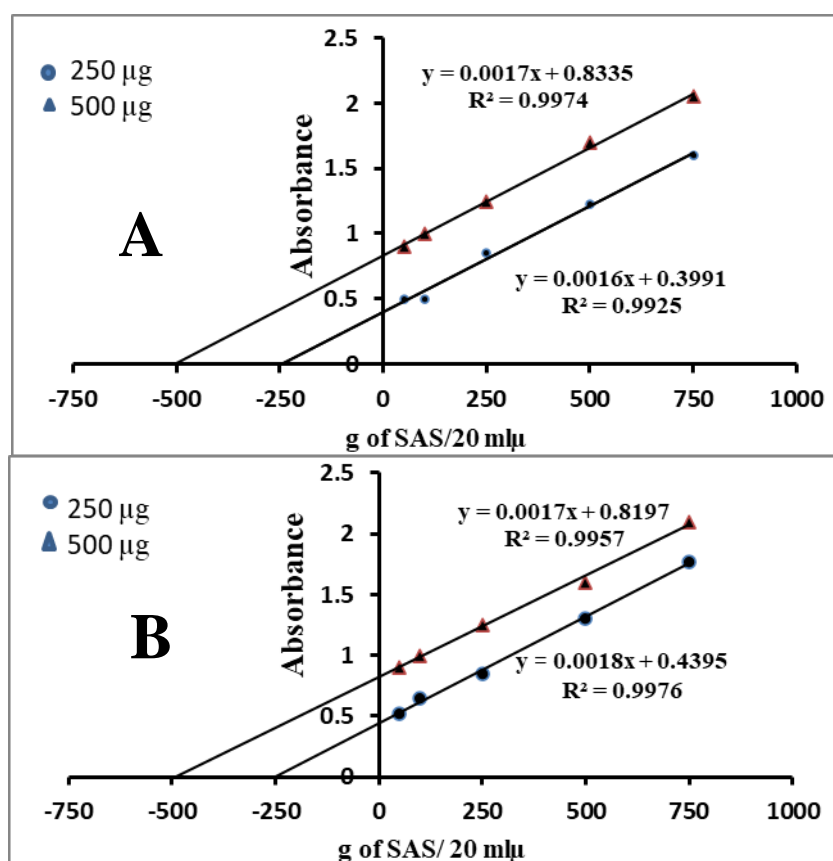


Fig. 6: Standard addition curves for SAS determination in:
(A) Eye drops (B) Ointment

Table 10: Recovery results for SAS in its pharmaceutical preparations by proposed method and standard addition method

Pharmaceutical preparation	Amount taken, µg	Recovery, %	
		Current method	Standard addition method
Apisulfa-10 sterile eye-drops Amman Pharma. Indust. Co. Ltd (Jordan)	250	98.2	99.7
	500	99.5	98.0
Predmacin 10% ointment Linda-a-vetha (Portugal)	250	99.8	97.6
	500	97.9	96.4

Comparison of the method

Some of the analytical spectroscopic variables and application of the proposed method for estimation of sulfacetamide sodium were compared with the same variables for other spectroscopic methods. Results shown in (Table 11) reveal that the proposed method is analytically significant from point of view, where it has wide range of determination and acceptable sensitivity.

Table 11: Comparison of some analytical variables of the proposed method with other spectroscopic method

Analytical parameter and application	Present method	Literature method*
Type of reaction	Oxidative coupling	Oxidative coupling
Reagent	N,N-Diethyl-p-phenylenediamine	Phenothiazine
λ_{max} (nm)	546	610
Medium of reaction	Acidic	Acidic
Color of the product	Violet	Green
Beer's law range ($\mu\text{g/ml}$)	1.25-75	0.1-16
Molar absorptivity ($\text{L.mol}^{-1}.\text{cm}^{-1}$)	0.76×10^4	2.599×10^4
Sandells sensitivity ($\mu\text{g/cm}^2$)	0.03344	0.0101
LOD ($\mu\text{g/ml}$)	0.24322	/
LOQ ($\mu\text{g/ml}$)	0.81073	/
Method application	Eye drop and Ointment	Eye drop only

*(Talib *et al.*, 2009)

CONCLUSION

A simple spectrophotometric method has been suggested for the determination of sulfacetamide in pharmaceutical preparations based on coupling of the drug with the reagent in an acidic medium in the presence of potassium dichromate, to form a violet-colored dye that gives the highest absorbance at 546 nm. This method is characterized by its ease, speed and sensitivity. In addition, it has been successfully applied to determination of sulfacetamide in its pharmaceutical preparations (eye drops and ointment).

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التقدير الطيفي لصوديوم سلفاسيتاميد باستخدام الكاشف N,N- ثنائي اثيل- بارا- فنيولين ثنائي امين

سعد حساني سلطان

ذكاء محي الدين عبد الرزاق

قسم الكيمياء/ كلية العلوم/ جامعة الموصل

الملخص

في هذا البحث وصفت طريقة طيفية سهلة ودقيقة وحساسة لتقدير صوديوم سلفاسيتاميد في المحلول المائي. تعتمد الطريقة على تفاعل الاقتران التأكسدي للكاشف N,N- ثنائي اثيل- بارا- فنيولين ثنائي امين مع المركب الدوائي صوديوم سلفاسيتاميد بوجود العامل المؤكسد دايكرومات البوتاسيوم في الوسط الحامضي لتكوين ناتج بلون بنفسجي الذي له اقصى امتصاص عند الطول الموجي 546 نانوميتر. اتبعت الطريقة المقترحة لتقدير صوديوم سلفاسيتاميد قانون بير للتركيز ما بين 1.25 الى 75 مايكروغرام. ملتر⁻¹، بمعامل مولارية مقداره 10×0.76 لتر. مول⁻¹ سم⁻¹، بينما بلغت قيمة دلالة ساندل 0.03344 مايكروغرام. ملتر⁻¹. تراوحت قيم الخطأ النسبي ما بين -2.01 و -0.14 وقيم الانحراف القياسي النسبي ما بين 0.34 و 1.55. تم تطبيق الطريقة بنجاح لتقدير المركب الدوائي قيد الدراسة في مستحضراته الدوائية بشكل قطرة العين ومزيج العين.

الكلمات الدالة: صوديوم سلفاسيتاميد، تفاعل الاقتران التأكسدي، الكاشف N,N- ثنائي اثيل- بارا- فنيولين ثنائي امين، طريقة طيفية.