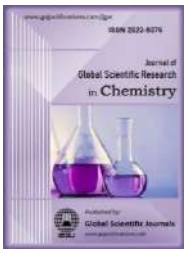
Journal of Global Scientific Research 8 (2) 2022/ 2936-2946

Contents lists available at www.gsjpublications.com

Journal of Global Scientific Research in Chemistry

journal homepage: www.gsjpublications.com/jgsr



Spectrophotometric Determination of Sulphacetamide Sodium in Its Pharmaceutical Preparations Using Azo-Coupling Reaction

Thukaa M. Abdulrazzaq, Saad H. Sultan

Department of Chemistry, College of Education for Pure Sciences, University of Mosul, Mosul, Iraq.

ARTICLE INFO

Received: 21 Dec 2022,
Revised: 23 Dec 2022,
Accepted: 2 Jan 2023,
Online: 28 Feb 2023,

Keywords:
sulphacetamide sodium,
diazotization and coupling reaction,
thymol reagent,
spectrophotometry

ABSTRACT

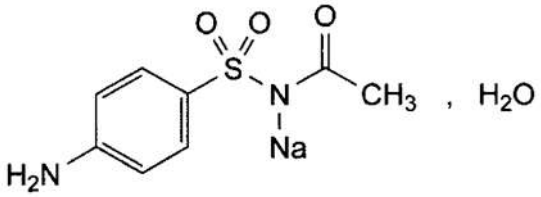
The determination of sulphacetamide in its pharmaceutical preparation was described as an accurate, easy, and sensitive spectrophotometric assay. The procedure depends on diazotization of sulphacetamide via reaction with sodium nitrite to form diazonium salt in an acidic environment that combined with thymol reagent in an alkaline media to give an orange coloured azo-dye, which is stable and soluble in water, and has maximum absorption at 474 nm . Beer's law is obeyed in the concentration range of 0.125-20 µg.ml-1 of sulphacetamide with a molar absorptivity of 3.4×104 l.mol-1.cm-1and sandell's sensitivity value of 0.00732 µg.cm-2.The relative error is in between -2.36 and -0.03 ,relative standard deviation from 0.75 to 1.28 . The method was successfully applied for determine of sulphacetamide sodium in pharmaceutical preparations, eye drops and ointment.

1. Introduction

Sulfa drugs are known as sulfonamide compounds are function class of bacteriostatic antibiotics still which used today for the treatment of bacterial infection and those caused by microoganisms. It is were main sours of treatment against bacterial infection before introduction of penicillin in 1941. These compounds have acquired much consideration because of their deferent biological processes in agricultural areas and pharmaceutics. These compound used in therapy of conjunctivitis, meningitis, malaria, bacillary dysentery, trachoma, streptococcal pharyngitis, toxoplasmosis, nocardiasis [1]. Sulfonamides are benzenoid amino compounds derived from sulfanilic acid,and they were the most frequently recommended antibiotic agent to treatment human and veterinary infections due to their low cost and capability for remedy of various bacterial infections. Sulphacetamide an important member of sulfonamide group and its sodium salt is effective in the treatment acne, pityriasis versicolor [2]. It is widely used in the therapy of infections especially of patients intolerant to antibiotics[3]. Where they are prevent the biosynthesis of folic acid prepation from p-aminobenzoic acid and are so named because of the substituted of the carboxyl group in p-aminobenzoic acid with sulfa derivative and subsequently lead to bacteria death [4]. Sulphacetamide sodium (SAS) is white crystalline powder, fast soluble in water and little soluble in alcohol and acetone, it melts at 257 C^0. It has age

Corresponding author:
Email address: saad.hasani@uomosul.edu.iq
doi: 10.5281/jgsr.2023.7704608
2523-9376/© 2023 Global Scientific Journals - MZM Resources. All rights reserved.

half of about 7 hours and it is derivative of sulfanilamide which used in the treatment of urinary tract infection and burn therapy, it is an antibiotic that is used as cream to treat skin to or acne and seborrheic dermatitis and as eye drops for the therapy of trachoma infection because itsn't causes irritation in high dosage[5,6,7]. Appellation chemical of sulphacetamide sodium is(N−[(4 − aminophenyl)sulfonyl]acetamide), whereas its chemical structure is given in Fig . (1)[9].



M.Wt = 254.2 g/mol

Fig 1 : Chemical structure of sulphacetamide sodium monohydrate

Several methods were published for sulphacetamide sodium assay using different analytical techniques, such as: Spectrophotometey [10,11,12], Chromatography [13,14,15] and Electrochemical [16,17]. Actually spectrometry still simple and sensitive technique that which available in most analytical laboratories , so the aim of this work was development of simple and sensitive spectrophotometric method for the determination of sulphacetamide sodium in aqueous solution using diazotization and coupling reaction, and then applying the proposed method to assay the studied drug in its pharmaceutical preparation in the form of eye drops or ointment.

2. Experimental

Apparatus

All absorption spectra and absorbance measurements were done by using adouble beam UV-Visible spectrophotometer (Jasco V-630) with 1.0 cm glass cells. Professional Benchtop pH meter TRANS BP3001 was used for the pH measurement.

Reagents and Chemicals

Analytical reagent grad was utilized for all compounds employed in this study.

SAS solutions stock (500 µg.ml^{-1}):

Prepared by dissolving 0.05 gm of pure SAS in enough amount of distilled water, then complete the volume to 100 ml by distilled water using volumetric flask and the solution was stored in dark flask.

SAS solution working solution (50 µg.ml^{-1}):

This solution was prepared by dilution 10 ml of SAS stock solution with distilled water then complete volume to 100 ml using volumetric flask and stored in dark flask.

Solution of sodium nitrite (1.0 % w/v)

This solution was made by mixing 50 ml of distilled water with 0.5 gram of sodium nitrite (BHD).

Solution of sulphamic acid (3.0 % w/v)

This solution was made by mixing 50 ml of distilled water with 1.5 gm of sulphamic acid (BHD).

Solution of hydrochloric acid (1M)

In a volumetric flask with a capacity of 100 ml, 8.6 ml of concentrated acid were diluted with distilled water to create this solution.

Thymol reagent solution (0.1% w/v)

This solution prepared by dissolving 0.1 gm of thymol reagent (BOH) in enough amount of ethanol with stirring then complete the volume to mark level in 100 ml volumetric flask stored in dark flask.

Sodium hydroxide solution (1M)

This solution prepared by with dissolving 4 gm of NaOH (sigma) in 100 ml distilled water using volumetric flask, and then solution was kept in plastic container.

Additives solution (1000 µg/ml)

This solution were prepared by dissolving 0.01 gm of additives compounds in 100 ml of distilled water using 100 ml volumetric flask

Eye drops solution (50 µg/ml)

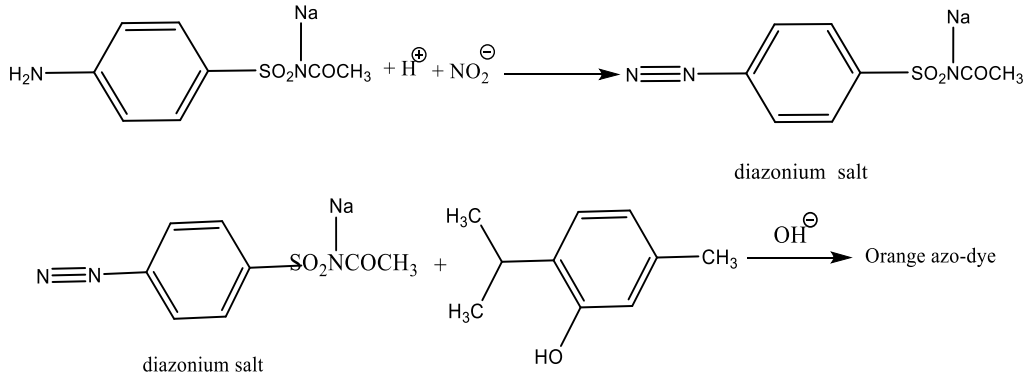
This solution was prepared by pipetting 1 ml of the eye drops10% solution (each 1 ml contains 100 mg of SAS) and diluted to 100 ml by distilled water , then taking 5 ml of the later solution and diluted to 100 ml with distilled water .

Oinment solution (50 µg/ml)

This solution was prepared by weighting 0.5 gm of the ointment 10 % (each 1 gm contains 100 mg of SAS) then dissolve in 10 ml of ether and quantitatively transferred to separative funnel followed by addition of 40 ml of ether, and start extraction process by three batch of 25 ml distilled water then collect the aqueous layer which contains SAS and transfer it to a volumetric flask of 100 ml capacity, and dilute to the mark by distilled water , then taking 10 ml of the obtained solution and diluted to 100 ml with distilled water.

The general principle of the method

The principle of proposed method depends on the diazotization of the medicinal compound sulphacetamide by sodium nitrite in the presence of hydrochloric acid to form the corresponding diazonium salt, which is combined with thymol reagent in a basic medium to give an orange azo – dye, according to the following equations:



3. Results and Discussion

The effect of different conditions affecting the absorption of the resulting colored product was studied using 1.0 ml (50 µg/ml) of SAS in final volume 20 ml.

Effect of Acidity

One of the requirements of the diazotization process is the presence of acid to form diazonium salt, the effect of different types and quantities of acids were studied. The results in table (1) shows that addition 1 ml of hydrochloric acid at concentration of 1 M gave the best absorbance of the formed azo-dye, so it was chosen in subsequent experiments.

Table 1: choosing the type of acid

Type of acid Used (1M)	Abs./ ml of acid				
	0.25	0.5	1	1.5	2
HCl	0.3332	0.3416	0.3542	0.3521	0.3203
H_2SO_4	0.3256	0.3305	0.3311	0.3229	0.0543
HNO_3	0.3432	0.3459	0.3521	0.3228	0.0503
CH_3COOH	0.0826	0.0841	0.0600	0.0547	0.0410

Effect of sodium nitrite quantity and reaction time

The effect of the sodium nitrite was studied with the time needed for diazotization process, table (2) shows that addition 0.5 ml of sodium nitrite 1% solution with a time of three minutes gave the higher absorption of the formed dye, so it was adopted in subsequent experiments.

Table 2 : Effect of sodium nitrite and reaction time

ml of $NaNO_2$(1%) solution	Absorbance / min.				
	0	1	2	3	5
0.25	0.3385	0.3362	0.3359	0.3486	0.3250
0.5	0.3425	0.3393	0.3440	0.35940	0.3494
0.75	0.3420	0.3351	0.3389	0.3334	0.3444
1	0.3402	0.3329	0.3385	0.3378	0.3359

Sulphamic acid quantity and reaction time effects

Table (3) demonstrates that the best dye absorbance was achieved when 0.5 ml of sulphamic acid was added with a reaction period of three minutes. As a result, this method was used in following tests.

Table 3 : Effect of amount of sulphamic acid and reaction time

ml of (3.0 %) Solution of sulphamic acid	Variable symbol	Abs. / time minutes				
		0	1	2	3	5
0.25	S B	0.3262 0.0145	0.3449 0.0232	0.3437 0.0234	0.3439 0.0209	0.3405 0.0240
0.5	S B	0.3425 0.0187	0.3432 0.0222	0.3462 0.0020	0.3519 0.0034	0.3369 0.0022
0.75	S B	0.3490 0.0035	0.3265 0.0041	0.3462 0.0143	0.3509 0.0068	0.3501 0.0309
1.0	S B	0.3389 0.0042	0.3499 0.0031	0.3509 0.0063	0.3411 0.0085	0.3408 0.0344

Effect of thymol reagent amount

The effect of coupling agent quantity on the absorbance of the formed azo-dye was studied and the results in table (4) show that adding 1.5 ml of 0.1% thymol solution gave the highest absorbance for the produced dye with the best value of determination coefficient (R^2) , so it was selected for subsequent experiments.

Table 4 :Effect of reagent amount

ml of Th (0.1%)sol.	Absorbance/ SAS (µg)										
	25	50	75	100	125	150	175	200	225	250	R^2
0.25	0.1908	0.3340	0.5133	0.6564	0.8324	0.8904	1.1450	1.2207	1.3368	1.4800	0.9934
0.5	0.1943	0.3161	0.5747	0.7077	0.8779	1.0337	1.2065	1.3546	1.5224	1.6655	0.9975
1.0	0.2828	0.3541	0.5224	0.7242	0.8840	1.0452	1.2288	1.3954	1.5501	1.7333	0.9972
1.5	0.2310	0.3593	0.5849	0.7703	0.9533	1.1010	1.3139	1.5020	1.6790	1.8563	0.9990
2.0	0.2507	0.3442	0.5351	0.7417	0.9032	1.0708	1.2562	1.5492	1.5728	1.7757	0.9928

Effect of basicity

The results of the preliminary investigation show that the color of the formed azo-dye only appears in the basic medium; therefore, the impact of the quantity of strong and weak bases solution on the absorption of the resulting dye was studied. Table (5) demonstrates that using 2 ml of sodium hydroxide resulted in a higher absorbance of the colored product, so it was used in the following experiments.

Table 5 : Effect of sort & amount of different bases

Base used (1M)		Absorbance / ml of base added				
		1	1.5	2	2.5	3
NaOH	A	0.2185	0.3660	0.3677	0.3510	0.3429
	λ_{max}(nm)	379	474	476	476	474
	pH	2.50	12.90	13.25	13.65	13.77
KOH	A	0.2005	0.3401	0.3452	0.3415	0.3438
	λ_{max}(nm)	388	476	476	476	476
	pH	1.94	2.98	13.61	13.82	13.96
Na_2CO_3	A	0.2072	0.3237	0..3292	0.3168	0.3418
	λ_{max}(nm)	376	476	475	476	476
	pH	8.31	10.36	10.69	10.84	11.16
$NaHCO_3$	A	0.0545	0.2528	0.2466	0.2368	0.2307
	λ_{max}(nm)	386	375	374	375	374
	pH	2.75	6.42	6.62	6.77	7.19

Dye Stability

The stability of the resulting dye was studied by measuring the absorbance against the blank solution at different time intervals and for two different concentrations, results in the table (6) shows that the dye was formed after addition of reaction component and dilution to the mark and remains stable for at least for one day.

Table 6: Dye stability

Time (min)	Absorbancs/µg of SAS	
	50	100
After dilution	0.3498	0.6680
5	0.3529	0.6822
10	0.3582	0.6864
15	0.3584	0.6877
20	0.3597	0.6887
25	0.3544	0.6888
30	0.3565	0.6868
35	0.3582	0.6872
40	0.3581	0.6892
45	0.3583	0.6888
50	0.3588	0.6880
55	0.3586	0.6888
60	0.3588	0.6894
2 hour	0.3579	0.6873
24 hour	0.3570	0.6881

The effect of surfactant

Different type of surfactants were studied, it was found that surfactants decreased the absorbance as it is clear in the table (7) so they omitted in this study.

Table 7 : The effect of surfactant

Surfactant solution 3 ml of (1%)	Absorbance/order of addition	
	λ_{max}	A
SDS	475	0.3224
Triton x-100	475	0.3316
CPC	482	0.3030
Without	474	0.3529

Effect off 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 has been studied. The results in table (8) show that there is no noticeable overlap of these compounds in the estimation of SAS, that is mean the possibility of estimating sodium sulphacetamide in presence of these compounds using the proposed method.

Table 8 : Effect of additives

Foreign compound	Recovery (%) of 50 µg SAS / µg of foreign compound added		
	0.25	0.5	1.0
Prednisolone acetate	97.16	98.42	96.44
Benzalconium chloride	96.90	97.02	97.07

Final absorption spectra

The diazonium salt is formed by reaction the medicinal compound sulphacetamide sodium with nitrite sodium in the acidic medium, which is combined with the thymol reagent in the alkaline medium to form a stable orange dye which soluble in water and gives the highest absorption at wavelength 474 nm compared to the blank solution, which gives very weak absorption at the same wavelength and figure (2) shows the final absorption spectrum of the formed dye against its blank solution.

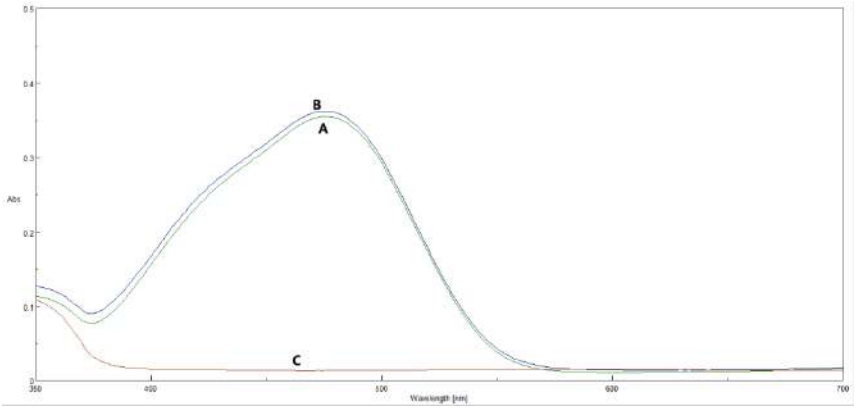


Figure 2 : Absorption spectrum for 50 µg / 20 ml from sulfacetamide (A) Versus blank (B) Versus distilled water (C) Blank versus distilled water.

Approved working method and standard curve

After fixing the practical optimal conditions to preparation calibration curve for the determination of sulphacetamide sodium, as follows: increasing volumes of sodium sulphacetamide solution are added to a series of 20 ml volumetric flask, then addition 1.0 ml of 1 M hydrochloric acid solution and 0.5 ml of 1% sodium nitrite solution are added and left for three minutes with shaking after that, 0.5 ml of 3.0 % sulphamic acid solution was added, also left solutions for three minutes with occasionally shaking, followed by addition of 1.5 ml thymol reagent solution, finally 2 ml of sodium hydroxide solution was added and the volumes were supplemented with distilled water to the mark, and the absorbance was measured at 474 nm against the blank solution . Figure (3) illustrated the linearity of Beer′s law over concentration range of 0.125-20 µg of SAS/ml, higher concentration give negative deviation from Beer′s law . The molar absorptivity and Sandell′s values were 3.4×10^4 $l.mol^{-1}.cm^{-1}$and 0.00732 $\mu g.cm^{-2}$,while the values of LOD&LOQ were 0.00605 and 0.02018 $\mu g.ml^{-1}$ respectively.

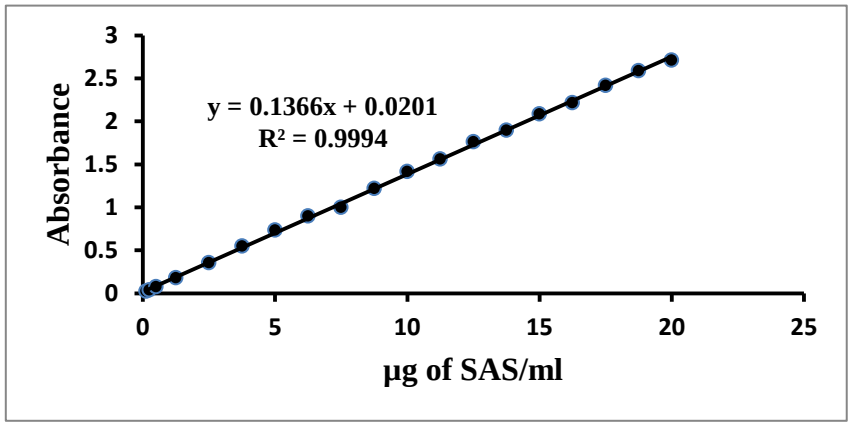


Figure (3) : Calibration curve of SAS estimation

The nature of the dye formed

To find out the correlation ratio of the diazonium salt corresponding to sodium sulphacetamide with thymol reagent, the continuous change method (Job's method) was applied, and the figure (4) revealed that the coupling ratio between SAS and thymol was 1:1 .

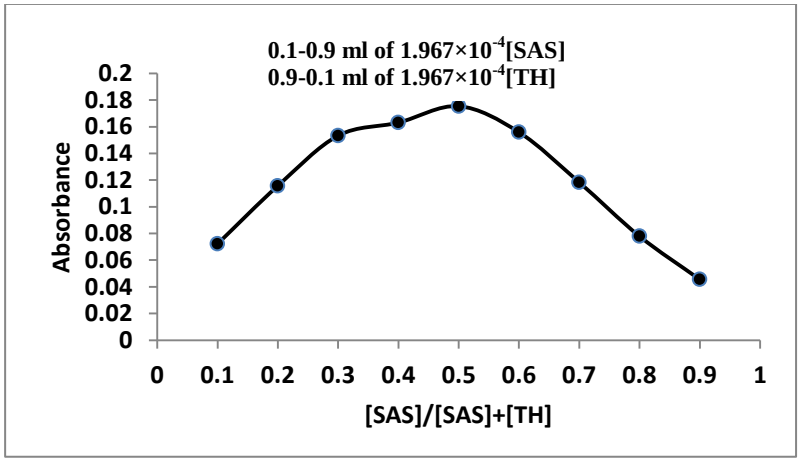
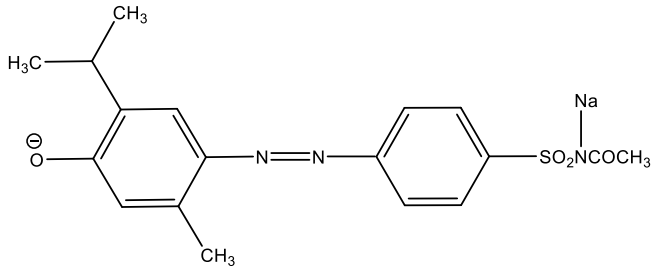


Figure (4) :Job's method curve for azo-dye produced by coupling diazotized SAS with thymol reagent

So, the proposed final structural of the formed azo-dye is as following :



Orange azo-dye(λ_{max} = 474 nm)

Application of the Method

The suggested method was successfully applied to determination drug in commercial preparation (eye drop and ointment), the optained results in table (9) illustrated that the procedure is accurate and reproducible.

Table 9 : The result of applying the method to pharmaceutical preparations

Pharmaceutical Preparations	*SAS Present* (µg)	*SAS Found* (µg)	*Relative error* (%)*	*Recovery* (%)*	RSD **(%)***
Apisulfa-10 sterile eye-drops Amman Pharma.Indust.Co.Ltd(Jordan)	50	49.985	-0.03	99.97	0.75
	100	97.640	-2.36	97.64	1.28
Predmacin 10% ointment Linda-a-vetha (Portugal)	50	49.215	-1.57	98.43	0.96
	100	99.11	-0.89	99.11	0.81

*Average of five determinations

Evaluate the proposed method using standard addition method

For the purpose of proving the efficiency of the proposed method and its success in estimating sulphacetamide sodium and its freedom from interaction of additives in pharmaceutical preparation the standard addition method was applied. The results in the figure (5) and table(10) revealed that the recoveries of SAS in standard addition method is in good agreement with the proposed method, which indicates a good selectivity of the proposed method .

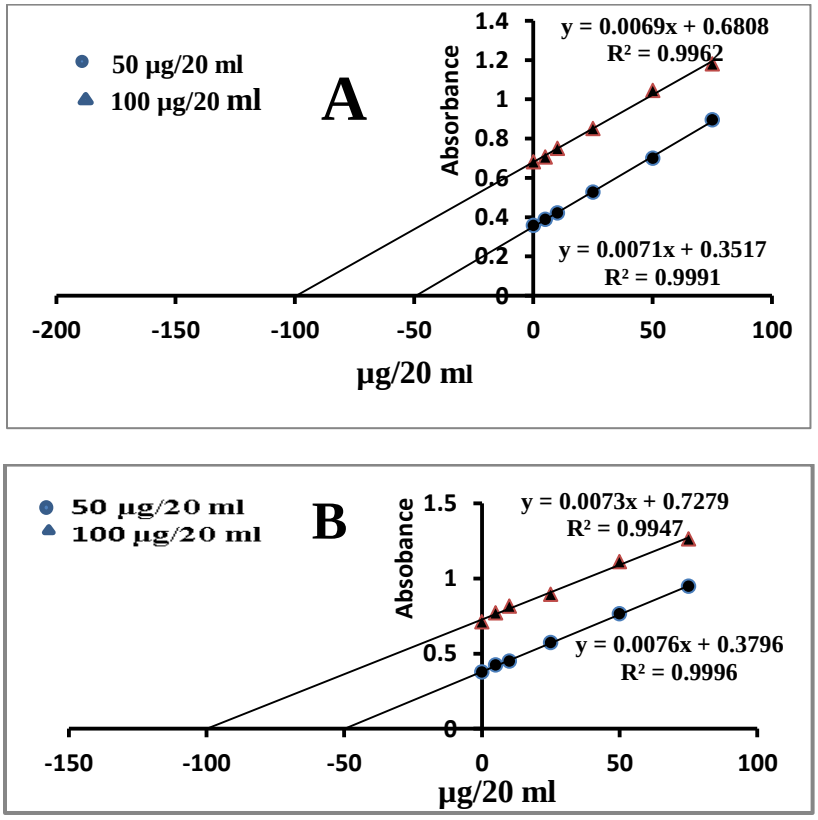


Figure (5) : Standard addition curves for SAS determination in: (A) Eye drops (B) Ointment

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)	50	99.97	99.06
	100	97.64	98.66
Predmacin 10% ointment Linda-a-vetha (Portugal)	50	98.43	99.88
	100	99.11	99.71

Comparison of the Method

Some of the analytical spectroscopic variables of the current proposed method for estimating sulphacetamide sodium were compared with the same variables for other spectroscopic methods and the results are shown in table (11), which indicate that the proposed method has more sensitivity and wide range in concentration , also applied for two preparations .

Table (11): comparison some spectroanalytical of the variables proposed with other spectrophotometric method for the determination of sulphacetamide sodium.

Analytical parameters	Present method	Literature method[5]
Reaction types	Diazotization & coupling	Diazotization & coupling
Reagents	Thymol	m-aminophenol
λ_{max}(nm)	474	436
Medium of reaction	Basic	Basic
Color of the product	Orange	Orange
Beer's law range (ppm)	0.125-20.0	0.4-8.0
Molar absorptivity (L.mol^{-1}.cm)	3.4×10^4	1.6×10^4
Sandell sensitivity (µg/cm^2)	0.00732	1.4×10^{-4}
L.O.D (µg/ml)	0.00605	-------
L.O.Q (µg/ml)	0.02018	-------
Applied pharmaceuticals	Eye drops & Ointment	Eye drops

4. Conclusion

A simple, easy, and accurate spectrophotometric method was proposed for determination of sulphacetamide sodium in aqueous solution depending on diazotization of indeed compound using sodium nitrite in acidic medium followed by coupling with thymol reagent to form intense colored azo-dye which gives high absorbance at 474 nm. The proposed method was validated by applying standard addition method with good results of recovery in its pharmaceutical formulation as eye drops and ointment .

5. Acknowledgment

The authors are grateful to the Department of Chemistry/College of Science/University of Mosul for their supporting to achieve this work .

6. References

[1]. Gulçin, İ., & Taslimi, P. (2018). Sulfonamide inhibitors: a patent review 2013-present. *Expert opinion on therapeutic patents*, *28*(7), 541-549.

[2]. Yadav, S. K., Choubey, P. K., Agrawal, B., & Goyal, R. N. (2014). Carbon nanotube embedded poly 1, 5-diaminonapthalene modified pyrolytic graphite sensor for the determination of sulfacetamide in pharmaceutical formulations. *Talanta*, *118*, 96-103.

[3]. Nagaraja, P., Yathirajan, H. S., Sunitha, K. R., & Vasantha, R. A. (2002). A new, sensitive, and rapid spectrophotometric method for the determination of sulfa drugs. *Journal of AOAC international*, *85*(4), 869-874.

[4]. Castelli, L. A., Nguyen, N. P., & Macreadie, I. G. (2001). Sulfa drug screening in yeast: fifteen sulfa drugs compete with p-aminobenzoate in Saccharomyces cerevisiae. *FEMS microbiology letters*, *199*(2), 181-184.

[5]. Talal, Z. & Bashır, W. A. (2019). Spectrophotometric Determination of Sulfacetamide . The Eurasia Proceedings of Science Technology Engineering and Mathematics , 7 , 385-390 .

[6]. Al-Uzri, W. A., & Fadil, G. (2017). Spectrophotometric Determination of Sulfacetamide Sodium in Pharmaceutical Preparation Using 8-Hydroxy-7-iodoquinoline-5-sulfonic Acid as Chromogenic Reagent. *Asian Journal of Chemistry*, *29*(4), 782.

[7]. El-Ragehy, N. A., Hegazy, M. A., AbdElHamid, G., & Tawfik, S. A. (2017). Validated chromatographic methods for the simultaneous determination of sulfacetamide sodium and prednisolone acetate in their ophthalmic suspension. *Journal of chromatographic science*, *55*(10), 1000-1005.

[8]. Safronova, E., Parshina, A., Kolganova, T., Yelnikova, A., Bobreshova, O., Pourcelly, G., & Yaroslavtsev, A. (2020). Potentiometric multisensory system based on perfluorosulfonic acid membranes and carbon nanotubes for sulfacetamide determination in pharmaceuticals. *Journal of Electroanalytical Chemistry*, *873*, 114435.

[9]. Titova, T. S., Yurova, P. A., Evdokimova, D. D., Kolganova, T. S., Parshina, A. V., Stenina, I. A., & Yaroslavtsev, A. B. (2020). Multisensory Systems Based on Nafion Membranes Modified by PEDOT for the Determination of Sulfacetamide in Aqueous Solutions and Pharmaceuticals. *Membranes and Membrane Technologies*, *2*(6), 417-425.

[10]. Darweesh, S. A., Al-Haidari, I. M. A., Mohammed, A. K., & Dikran, S. B. (2017). Spectrophotometric determinations of sulfacetamide following simple diazotization and coupling with chromotropic acid. *Ibn AL-Haitham Journal For Pure and Applied Science*, *26*(3), 281-295.

[11]. Al-Safar, R. S., & Othman, N. S. (2020, November). Spectrophotometric determination of sulphacetamide sodium via diazotization and coupling reaction. In *IOP Conference Series: Materials Science and Engineering* (Vol. 928, No. 5, p. 052017). IOP Publishing.

[12]. Ayad, M. R., Ali, H. M., & Musa, L. A. (2012). Spectrophotometric determination of sulfacetamide in pure form and pharmaceutical formulations with metol and potassium hexacyanoferrate (III). *Al-Mustansiriyah Journal for Pharmaceutical Sciences*, *12*(2).

[13]. Sayed, R. A., Mohamed, A. R., Hassan, W. S., & Elmasry, M. S. (2022). Earth-friendly-assessed chromatographic platforms for rapid analysis of sulfacetamide sodium and prednisolone acetate in presence of sulfanilamide impurity: Application on ophthalmic formulation and aqueous humor. *Sustainable Chemistry and Pharmacy*, *27*, 100694.

[14]. Wojtowicz, E. J. (1970). A column chromatographic method for the determination of sulfanilamide in pharmaceutical preparations containing sulfacetamide or its sodium salt. *Journal of Pharmaceutical Sciences*, *59*(2), 240-241.

[15]. El-Ragehy, N. A., Hegazy, M. A., Tawfik, S. A., & Sedik, G. A. (2021). Validated chromatographic methods for the simultaneous determination of a ternary mixture of sulfacetamide sodium and two of its official impurities; sulfanilamide and dapsone. *Acta Chromatographica*.

[16]. Ahmed, T. A., Ghandour, M. A., & Khalil, M. M. (2020). Stripping voltammetric determination of Epinephrine applying sulfacetamide modified glassy carbon electrode. *Int. J. Electrochem. Sci*, *15*, 3143-3156.

[17]. El-Ragehy, N. A., Hegazy, M. A., AbdElHamid, G., & Tawfik, S. A. (2018). Validated potentiometric method for the determination of sulfacetamide sodium; application to its pharmaceutical formulations and spiked rabbit aqueous humor. *Bulletin of Faculty of Pharmacy, Cairo University*, *56*(2), 207-212.

[18]. Zeinab, A. S., Aboo. M. A., Nahlaa, S. H. (2022). Removal of Cadmium from Aqueous Solution by Sudanese Natural Zeolite. *Journal of Global Scientific Research*. 7(3): 2201-2205.

[19]. Metwally, M. S. A., El-Bawab, E. M. E., Atty, M. E. A. A., Abdallah, A. M. (2023). Conglutin Gamma, A Lupin Seed Protein Reduces Oxidative Stress in Diabetics. *Journal of Global Scientific Research*. 8(2):2866-2883.