Rafidain Journal of Science
https://rsci.mosuljournals.com

Vol. 31, No.1, pp. 23-34, 2022

Development of Spectrophotometric Method to Assay Sulfadiazine in Pure and in Pharmaceutical Dosage form through Diazotization and Coupling Reaction

Dheyaa T. Azeez **Salim A. Mohammed**
Department of Chemistry/ College of Science/ University of Mosul

Article information

p-ISSN: 1608-9391
e -ISSN: 2664-2786

Received:14/6/2021
Accepted:11/9/2021

DOI: 10.33899/rjs.2022.172925

corresponding author:
Dheyaa T. Azeez
Dheyaathamerazeez@gmail.com

Salim A. Mohammed
salimsalih813@yahoo.com

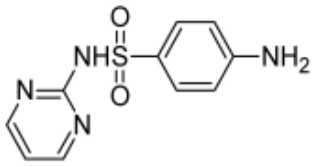
ABSTRACT

This study involves the development of a sensitive accurate and low-cost spectrophotometric procedure to analyze sulfadiazine as pure and in its dosage forms. In this method sulfadiazine was diazotized with an equimolar solution of sodium nitrite in the presence of hydrochloric acid to form corresponding diazonium salt followed by coupling with phloroglucinol reagent in basic solution of NaOH to produce a yellow water-soluble azo dye that has maximum absorption at 417 nm versus reagent blank. Under optimum conditions. The linearity of the method obeyed Beer's law in the concentration range 0.25-15 µg/ml with an excellent determination coefficient (R^2= 0.9992) and molar absorptivity 3.81×10^4 l/mol.cm. The detection limit (LOD) and quantification limit (LOQ) have also been estimated and their values were found to be 0.192 and 0.563 µg/ml, respectively. A relative error% was calculated and found in the range -2.75% to 4.02%, while the precision (RSD%) was estimated as ≤ 1.36%. The stoichiometry of the resulting azo dye was found to be 1:1 sulfadiazine: phloroglucinol with stability constant 0.37×10^7 l/mol. The suggested procedure was applied to the analysis of sulfadiazine in cream and veterinary drugs (powder and injection).

Keywords: Sulfadiazine, Diazotization, Phloroglucinol, Spectrophotometry.

INTRODUCTION

Sulfonamides are a class of drugs that have been commonly used as antibacterial agents in veterinary practice and in medicine especially in the treatment of eye infections and urinary tract infections as a prophylaxis of rheumatic fever (Petri, 2001). Sulfadiazine (SDZ), is one of the common sulfonamide's groups in clinical practice, chemically it is [4-amino-N -pyrimidin-2-yl-benzenesulfonamide], (John and John, 2004), whereas its chemical structure is:



$C_{10}H_{10}N_4O_2S$
M.Wt. =250.3g/mol

SDZ is a synthetic agent that has been used in human and veterinary therapy about 60 years (Pecorelli *et al.*, 2004) . It eliminates bacteria that cause infections by inhibiting the formation of folic acid inside the cell of bacteria (Vandana *et al.*, 2011). SDZ has been employed to those prophylaxis furthermore in the medication of contamination for parasitic protozoa called toxoplasmosis gondii and also in the medicine for contamination brought about because of pneumocystis carinii, an extracellular lung pathogen, which makes pneumonia for immune-compromised hosts. (Vree *et al.*, 1995). The literature survey revealed that several methods have been reported for direct or indirect estimation of SDZ, they include: potentiometry (Ayman *et al.*, 2009), Nanozyme-labeled biomimetic immunoassay (He *et al.*, 2020), capillary zone electrophoresis (Berzas *et al.*, 2001), flow injection-flame atomic absorption (FI-FAAS) device (Haji Shabani *et al.*, 2011), tandem mass spectrometric detection, (Forti and Scortichini, 2009), ICP-atomic emission spectroscopy (Xiao-Ling *et al.*, 2010) and immune-chromatographic assay (Wang *et al.*, 2007). However, most of these methods either require several manipulation steps, sophisticated, cloud point extraction and expensive instruments which may not be available in every laboratory or they had high detection limit.

Several important spectrophotometric methods have also been employed to assay of SDZ in bulk and in its pharmaceutical preparations. Some of these methods based on the diazotization coupling reaction by using various organic coupling reagents such as, histidine (Othman and Kadder, 2006), oxine (Naik *et al.*, 2007), pyrogallol (Naser *et al.*, 2018), 2,6-dihydroxybenzoic acid (Mohammed and Zebary, 2013) and 4-amino-2-hydroxy acetophenone (Al-Rufaie, 2016). Other methods are either based on the oxidative coupling reactions of SDZ with p-N,N-diethylphenylenediamine sulphate using KIO_4 as oxidizing agent (Nagaraja *et al.* , 2010) . In addition, determination of SDZ via charge transfer complex formation with phenosaphranine (Al-Attas, 2003) have been also reported. SDZ was also estimated through the oxidation of SDZ with permanganate solution in alkaline medium producing bluish-green colour of manganate MnO_4^{2-} appeared absorption peak at 610 nm (Siddiqui *et al.*, 2013). In the present investigation, a phloroglucinol (Phl) reagent has been employed to develop a simple , fast and inexpensive visible spectrophotometric method for the assay of SDZ in pure form and in its pharmaceutical drugs. This method was based on the reaction of Phl reagent with diazotized SDZ to yield a water-soluble yellow dye in a basic solution that has been employed to assay of SDZ in burn cream, powder and veterinary injectable solution.

EXPERIMENTAL

Apparatus

A double beam UV-visible spectrophotometer (JASCOV-630) with 1.0-cm quartz cells was used for all absorbance measurements and absorption spectra. While a professional Benchtop pH meter BP3001 was used for the pH measurements.

Chemical Reagents

All chemical substances used were of the highest purity available and were obtained from Fluka, BDH and Merk companies. The standard material of SDZ was provided from the state company for drug industries and medical appliances (SDI), Samarra-Iraq.

-SDZ Stock Standard Solution 500 µg/ ml: A 0.0500 g of pure SDZ was dissolved in about 5 ml of ethanol and the volume was then made up to 100 ml with distilled water in a 100 ml calibrated flask. This solution was kept in a dark bottle. Similarly, a working standard solution (50 µg /ml = 1.9976×10^{-4} M) of SDZ was freshly prepared by diluting 10 ml of the stock solution with distilled water in a 100 ml calibrated flask (Mohammed and Zebary, 2013).

Phloroglucinol (Phl) Solution 0.1% w/v: A 0.100 g of Phl reagent was dissolved in small portions of distilled water and the volume was made to 100 ml in a calibrated flask with the same solvent. The solution was then transferred to a dark bottle.

Sodium nitrite stock solution 1.9565×10^{-3} M: This solution was prepared by dissolving 0.0135 g of $NaNO_2$ in 100 ml distilled water using a volumetric flask. Similarly, a working solution 1.9976×10^{-4} M of $NaNO_2$was then prepared by diluting 10.2 ml of the stock solution with distilled water in a 100 ml calibrated flask.

Recommended Procedure and Calibration Curve

Into a series of 20 ml calibrated flasks, 0.1 to 7 ml of 50 µg/ml pure SDZ solution, an equimolar of 1.9976×10^{-4} M $NaNO_2$ and 1ml of 1M hydrochloric acid solutions were added. The contents were mixed thoroughly and kept constant at room temperature for 5 min then followed by adding 1.5 ml of 0.1% Phl reagent and 2 ml of 1M sodium hydroxide solutions. The contents were Shaked well and made up to the mark with distilled water. The absorbance of the azo dye formed was recorded at 417 nm against blank solution that was prepared similarly but without drug. A linear relationship between absorption and SDZ concentration in the range 5−300 µg of SDZ/20 ml was obtained Fig. (1). The apparent molar absorptivity and Sandell's sensitivity were found to be 3.81×10^4 l/mol.cm. and 0.00657 µg/cm^2, respectively.

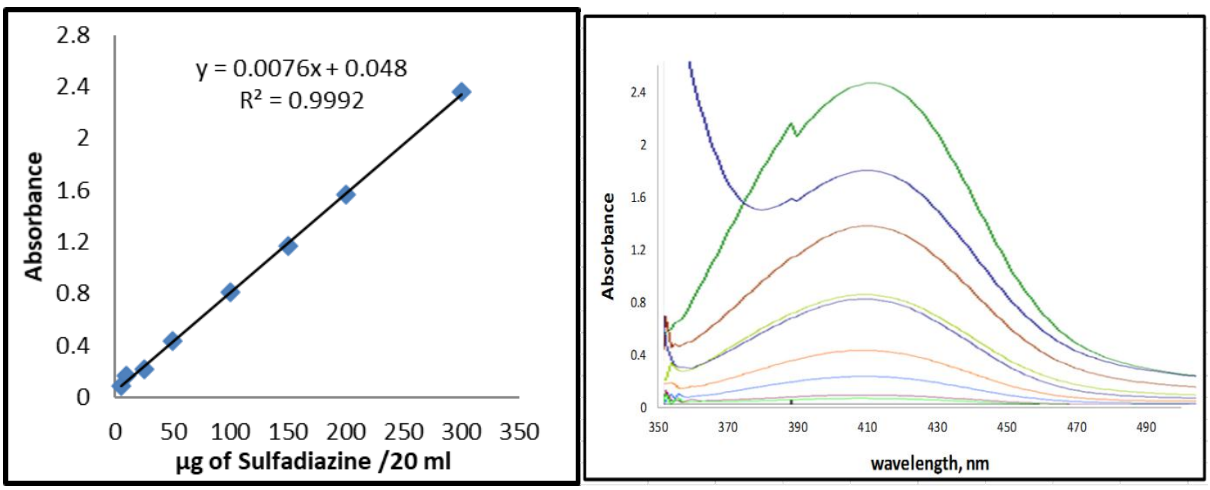


Fig. 1: The calibration curve for SDZ determination

Recommended Procedure to Assay SDZ in Drug

Injection solution:

The injection container (each ml of injection contains 200 mg SDZ and 40 mg trimethoprim, TMP) was mixed well to get a homogeneous solution. A 1ml of the injection solution was diluted with distilled water in 100 ml calibrated flask to prepare 2000 µg/ml SDZ solution and similarly a 10 ml of it was pipetted into a 100 ml calibrated flask and with distilled water made up to the mark (this is stock solution). A working solution of injection containing 50 µg/ml of SDZ was prepared by diluting 25 ml of the stock solution with distilled water using 100 ml calibrated flask. An aliquot of the diluted drug solution was then treated as done in recommended procedure.

Flamalife Cream (each 100 g cream contains 1g silver SDZ):

A 1.0 g of flamalife cream was dissolved in 5 ml of ethanol and transferred to 50 ml ether, shake well and moved to 50-ml isolating channel. SDZ was then removed multiple times with 25 ml refined water. The watery layer was gathered, separated and finished to 100 ml with refined water in utilizing volumetric carafe (United States Pharmacopeia, 1995). Each mL of this solution containing 100 µg SDZ. An aliquot of diluted solution of the drug was then analyzed using the recommended procedures.

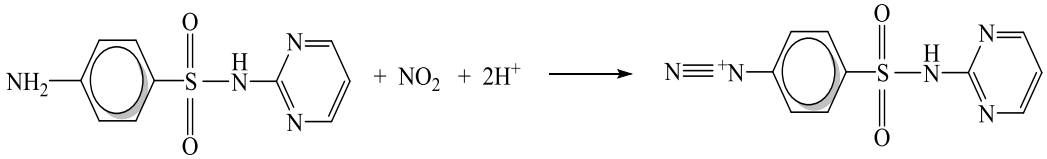
Powder:

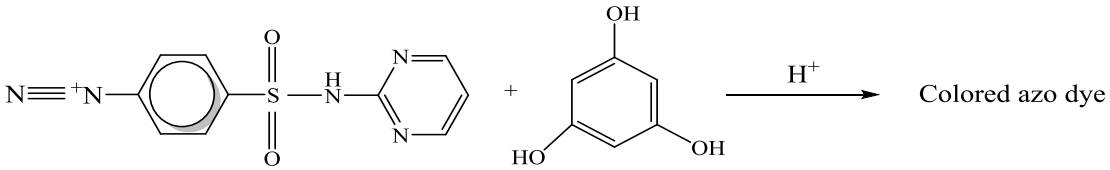
An accurately weighed 0.025g of the drug powder (each 1g powder contains 400 mg SDZ and 80 mg TMP) was dissolved in about 10 ml of distilled water. The resulting solution was then mixed well, filtered and diluted to 100 ml with distilled water in a calibrated flask. Each ml of this solution contains 100 µg of SDZ. The recommended procedure was then followed for the determination of SDZ by taking an aliquot of diluted solution of the drug.

RESULTS AND DISCUSSION

Preliminary conditions

Under the response condition, SDZ was reacted with an equimolar of $NaNO_2$ in acidic solution to give the relating diazonium salt, which goes through a diazotization procedure with Phl as a coupling agent to produce the azo dye (Scheme 1):





Scheme 1

The azo dye gave maximum absorption at 417 nm against blank solution. The dye intensity has been found to be proportional to the SDZ amount that originally present in the solution.

Optimum Conditions for the Reaction

Effect of Different Amounts of Various Acids on the Absorbance

The reaction of diazotisation requires acid solution. Therefore, the effect of different amounts of various acidic solutions (1M) such as, HCl, H_2SO_4, HCOOH, HNO_3 and CH_3COOH has been examined on the dye colored. The experimental results are shown in Fig. (2).

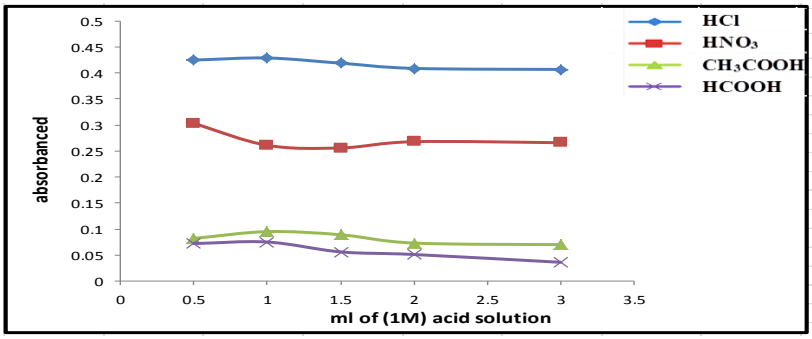


Fig. 2: Effect of different amounts of various acids on absorbance of azo dye

The data in Fig. (2) illustrate that HCl was convenient as acidic medium for a maximum stability and sensitivity and 1ml of 1M HCl with occasionally shaking for 5 and 6 min were found to be suitable, therefore 5 min was selected for the subsequent investigation. The results are explained in Fig. (3).

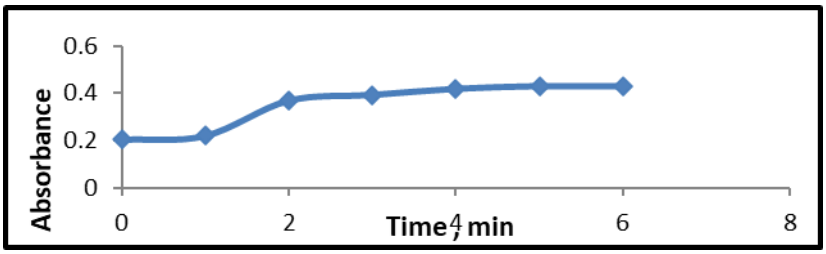


Fig. 3: Influence of diazotization time on the dye absorbance

Effect of Phl Amount

The influence of various amounts (0.5-2.0) ml of Phl reagent (0.1%) on the azo dye absorbance has been investigated. The results in Fig. (4) indicated that 1.5 ml of Phl (0.1%) are the most suitable volume to give high absorbance and good determination coefficient value (R^2=0.9995) therefore, it was selected for coupling reaction.

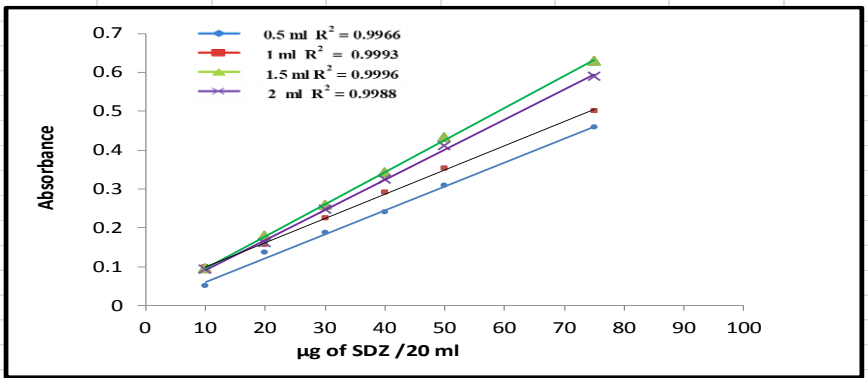


Fig. 4: Effect of reagent amounts on absorbance

The effect of time on the completion of the coupling reaction of diazotized SDZ with Phl reagent has been studied by allowing the solution of the reactants after the addition of Phl solution to stand at room temperature for different times Fig. (5) then the other reagents were added and the absorbance was recorded at 417 nm against the reagent blank.

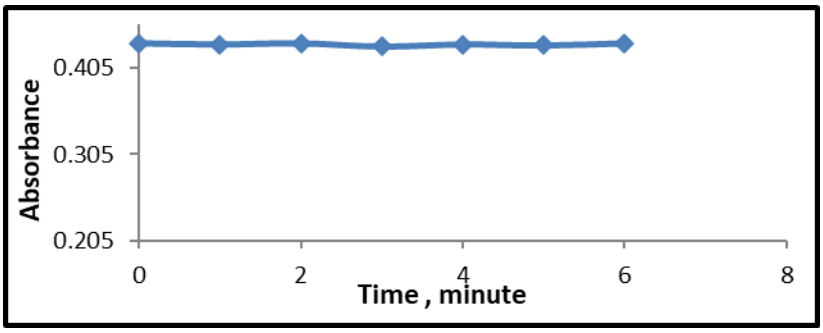


Fig. 5: Effect of coupling reaction time on the absorbance of azo dye

Fig. (5) reveals that the coupling reaction was complete immediately after mixing the diazotized SDZ with Phl reagent and the absorbance remained maximum and stable for at least 7 min. at room temperature.

Effect of alkaline solution

The effect of different amounts 0.5-3 ml of various alkaline solutions (1M) such as, sodium hydroxide, sodium carbonate, sodium bicarbonate and potassium hydroxide has been examined for the purpose of producing azo dye with intense colour and lower blank value. The results in Fig. (6) revealed that maximum sensitivity and stability were obtained only when the reaction was carried out in the presence of sodium hydroxide solution. While, sodium carbonate and sodium bicarbonate exhibited low sensitivity which may be due to pH variation. Therefore, 2.0 ml of 1M sodium hydroxide at (pH=12.1) was found to be optimum.

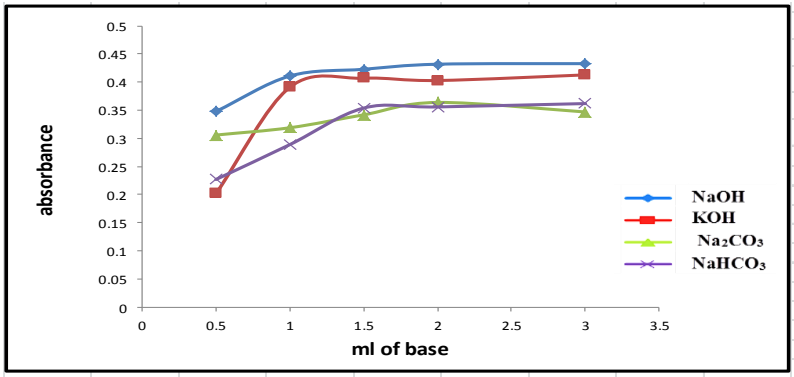


Fig. 6: Effect of different amounts of various bases (1M) on absorbance

Development Time and Stability Period

Under the optimal experimental conditions, the influence of time on the stability of the colored azo dye at 417 nm has been carried out by preparing two different amounts 50 and 150 µg of SDZ. The absorbance was measured at dissimilar pauses of time up to 60 minutes. The results in Fig.(7) illustrate that the absorbance of azo dye reached maximum value after the reaction mixture solution was mixed and the absorbance remained stable for at least 60 minutes at room temperature.

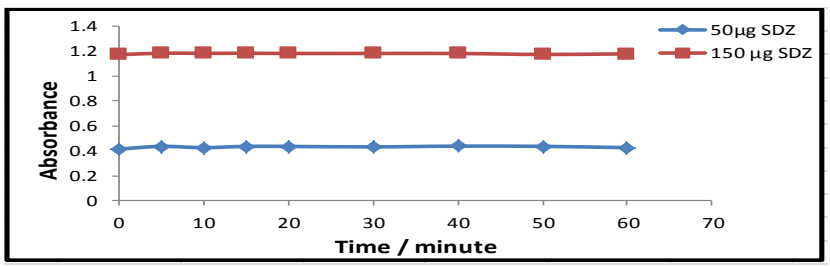


Fig. 7: Effect of time on absorbance.

Absorption Spectrum

Under the optimum reaction conditions, SDZ was reacted with equimolar of $NaNO_2$ solution in the presence of HCl solution to give the corresponding diazonium salt. The diazonium salt was then coupled with Phl reagent in a basic solution of NaOH. A yellow water-soluble azo dye was obtained which showed maximum absorption at 417 nm versus the colorless reagent blank Fig. (8). The intensity of the azo dye formed was found to be proportional to the amount of SDZ originally present in the solution.

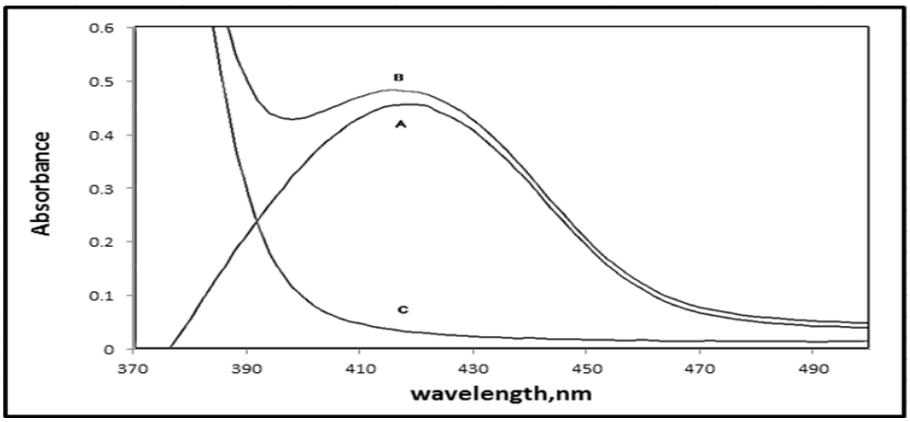


Fig. 8: Absorption spectra of 50 ppm SDZ treated according to the recommended procedure versus (A) reagent blank , (B) distilled water and (C) blank solution measured versus distilled water

Quantification

The limits of Beer′s law, molar absorptivity (ε_{max}), accuracy (recovery%), precision (RSD%), conditional stability constant (Hargis, 1988), LOD and LOQ values were calculated and the data are summarized in (Table 1), which indicates that the proposed method is sensitive, precise and accurate. Linearity was represented by the regression equation and the corresponding determination coefficient (R^2) for SDZ determined by the proposed method represents the excellent linearity.

Table 1: **Summary of optical characteristics and statistical data for the proposed method**

Parameters	Data
Limits of Beer's law , µg/ml	0.25-15
λmax , nm	417
εmax , l/mol.cm	3.81×10^4
Range of recovery* , %	97.48% to 104.02%
Relative error range* , %	- 2.75% to 4.02%
RSD* , %	≤ 1.36%
Sandall's sensitivity , µg/cm^2	0.00657
Determination coefficient (R^2)	0.9992
Average of stability constant (K) , l/mol	0.37×10^7
LOD , µg/ml	0.192
LOQ , µg/ml	0.563
Slope (a)$^{\#}$	0.0076
Intercept (b)$^{\#}$	0.0480

Note: Regression equation X = a Y + b , where Y is [SDZ] in µg/ml.

*Average of five determinations

Composition of the Azo Dye

The stoichiometry of the product was investigated under the established conditions by applying the continuous variation (Job's) and the mole ratio methods (Delevie, 1997). In continuous variation method, volumes 0.5–4.5ml of 1.9976×10^{-4} M portions of SDZ (VS) were diazotized using equimolar of 1.9976×10^{-4} M sodium nitrite and 1 ml of 1M HCl and coupled according to the recommended procedure with the corresponding complementary volume of 1.9976×10^{-4} M Phl solution (VR) to give a total volume of 5 ml for (VS+VR) in 2 ml of 1M NaOH and diluted to 20 ml with distilled water. In mole ratio method, increased volumes 1–4 ml of 1.9976×10^{-4} M Phl solution (VR) were added to a 1 ml of 1.9976×10^{-4} M of SDZ (VS) which was diazotized by using 1 ml of 1.9976×10^{-4} M sodium nitrite in presence of 1ml of 0.8 M HCl, 2 ml of 1M NaOH were added and the absorbance was recorded at 417 nm after dilution to the mark with distilled water. The results obtained in Fig. (9) are revealed that the azo dye was formed by a 1:1 combining ratio of diazotized SDZ to Phl.

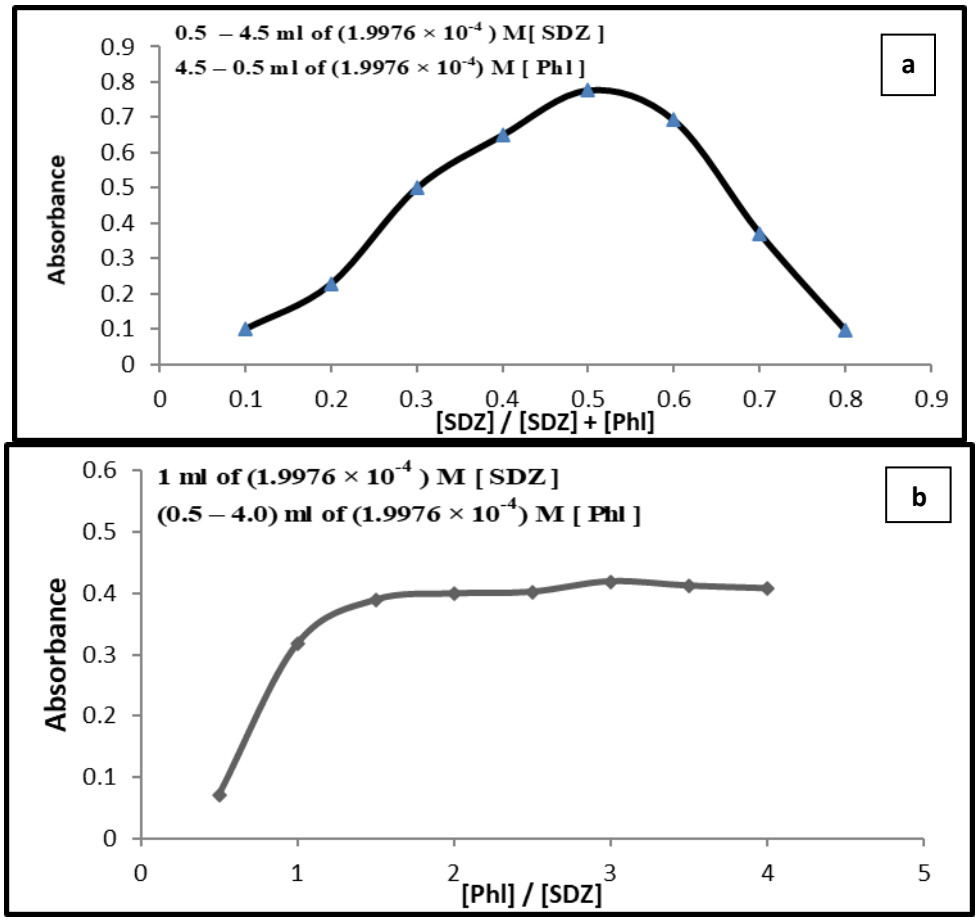


Fig. 9: Determination of the stoichiometry of the reaction by (a) A continuous variation and (b) mole ratio plots for coupling of SDZ with Phl under the optimum conditions.

The structure of the azo dye according to the results obtained in Fig. (9), can be represented as follows Fig. (10):

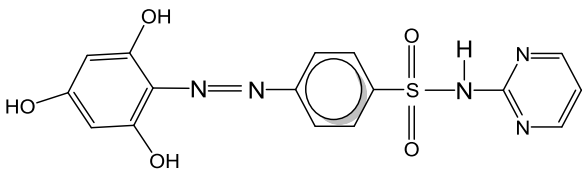


Fig. 10: **The composition of yellow azo dye**

Application of the Method

The present method was applied successfully to the analysis of SDZ in various pharmaceutical preparations containing SDZ (cream, veterinary powder and injection). The results are summarized in (Table 2) reveal that the proposed procedure is in good agreement and with the declared content.

Table 2: Determination of SDZ in pharmaceutical preparations

Drug	SDZ Present (µg)	SDZ Found (µg)	Relative error (%)*	Recovery (%)*	RSD%*
Vapcotrim injection 200 mg SDZ / 1 ml (Jordan)	20	19.80	-1.00	99.00	1.17
	50	51.84	3.68	103.68	1.33
	100	101.48	1.48	101.48	0.96
	200	201.96	0.98	100.98	1.21
Intertrim-480 veterinary powder 400 mg SDZ / 1 g (Holland)	20	19.65	- 1.75	98.25	1.36
	50	52.01	4.02	104.02	0.84
	100	98.98	-1.02	98.98	0.29
	200	199.5	- 0.25	99.75	1.05
Flamalife cream 1g Ag- SDZ/100g (Holland)	20	19.45	- 2.75	97.25	1.18
	100	99.77	- 0.23	99.77	0.69
	200	197.89	- 1.06	98.94	1.11

*Average of four determinations

Evaluation of the suggested procedure

Standard additions method was followed to check the validity of the suggested procedure which proves that the recommended method can be successfully applied for determining SDZ without interferences. The data are shown and listed in Fig. (11) and (Table 3).

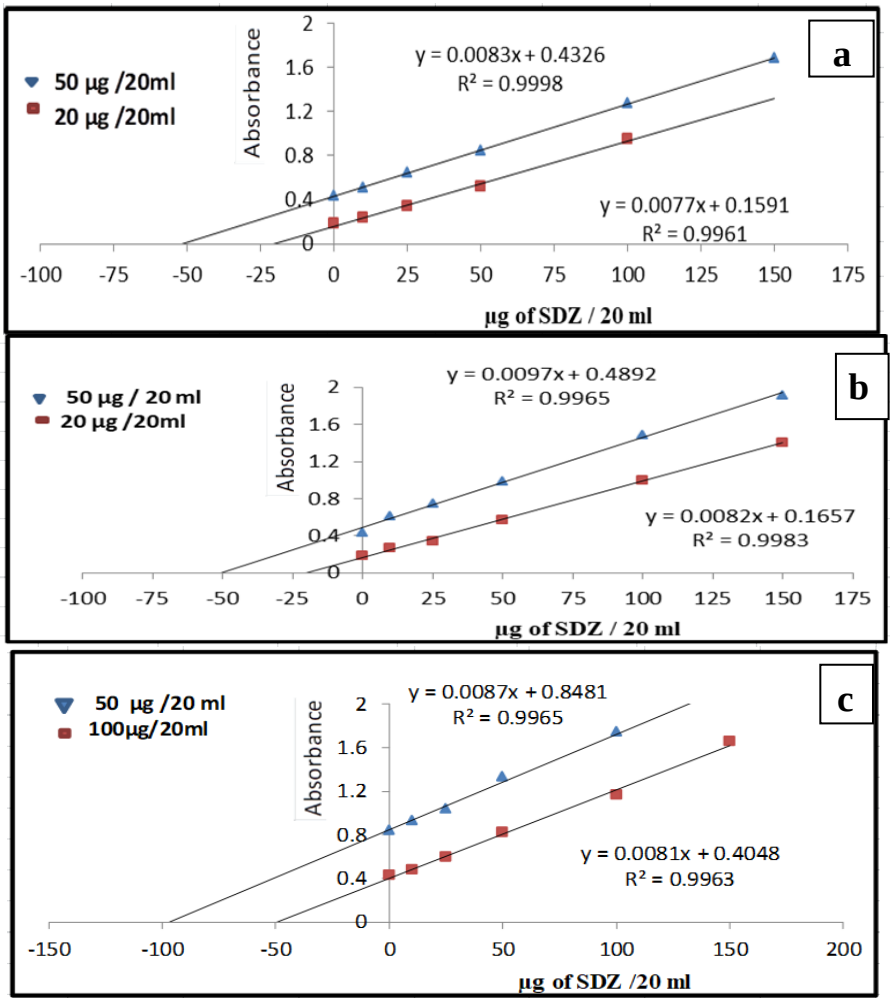


Fig. 11: Calibration graphs of standard addition methods for analysis of SDZ in (a) Intertrim-480 veterinary, (b) Vapcotrim injection and (c) Flamalife cream

Table 3: The results of standard addition methods for analysis of SDZ

Drug	SDZ Present (µg)	SDZ Measured (µg)	Recovery (%)
Vapcotrim injection 200 mg SDZ / 1ml (Jordan)	20	20.66	103.31
	50	52.12	104.24
Intertrim-480 veterinary powder 400 mg SDZ / 1g (Holland)	20	20.21	101.05
	50	50.43	100.86
Flamalife cream 1g Ag- SDZ/100g (Holland)	50	49.97	99.94
	100	97.48	97.48

CONCLUSION

The proposed method for the determination of sulfadiazine in pharmaceutical samples have the advantage to be sensitive, inexpensive and simple since it does not need neither temperature control nor solvent extraction step. The method is also accurate and precise enough to be successfully adopted as an alternative to the existing spectrophotometric method and evaluation of drugs in pharmaceutical preparations.

REFERENCES

Al-Attas, A.S. (2003). Charge transfer complex formation in spectrophotometric and conductometric determination of some sulphonamides. *Saudi. Pharm. J.*, **11**(3), 141-145.

Al-Rufaie, M.M. (2016). New spectrophotometric method for the assay of sulfadiazine drug based on diazotization coupling reaction. *Acta. Chemica. Iasi.*, **24**(2), 88-101.

Ayman, H.K.; Sofia, A.A.; Goreti, M. F. S.; Felismina, T.C.M. (2009). Sulfadiazine potenti-ometric sensors for flow and batch determination of sulfadiazine in drug and biological fluids. *Anal. Sci.*, **25**, 365- 371.

Berzas, N. J. J.; Castaneda, P. G.; Guzman, B. F. J. (2001). Determination of sulfamethoxazole, sulfadiazine and associated compounds in pharmaceutical preparations by capillary zone electrophoresis. *J. Chromatogr. A*, **918**, 205-210.

Delevie, R. (1997). "Principles of Quantitative Chemical Analysis". International Ed., The McGraw-Hill company, Singapore, 498 p.

Forti , A.F.; Scortichini, G. (2009). Determination of ten sulfonamides in egg by liquid chromatography-tandem mass spectrometry. *Anal. Chim. Acta.*, **637**(1-2), 214-229.

Haji Shabani, A.M.; Dadfarnia, S.; Rad, H.F. (2011). Indirect determination of sulfadiazine by cloud point extraction /flow injection- flame atomic absorption (CPE/FI-FAAS) spectrometry. *J. Chin. Chem. Soci*., **58**, 503-508.

Hargis, L.G. (1988). "Analytical Chemistry, Principles and Techniques". Prentice-Hall International, London, pp. 424-427.

He, J.; Li, Z.; Xu, L.; Kong, F.; Xu, Z. (2020). Development of nanozyme-labeled biomimetic immunoassay for determination of sulfadiazine residue in foods. *J. Advances in Polymer Technol.*, Article ID 7647580,1-8.

John, M.B.; John, H.B. (2004). "Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry."12th ed., Lippincott Williams and Wilkins, U K , 237 p.

Mohammed, S.A.; Zebary, H.Y. (2013). Spectrophotometric determination of sulfadiazine via diazotization and coupling reaction - application to pharmaceutical preparations. *Raf. J. Sci.*, **6**(24), 61-73.

Nagaraja, P.; Shrestha, A.K.; Shivakumar, A.; Gowda, A.K. (2010). Use of p-N,N-diethylphen-ylenediamine sulfate for the spectrophotometric determination of some phenolic and amine drugs. *Acta Pharmaceutica. Zagreb. Croatia.*, **60**(2), 217-227.

Naik, S.D.; Nagaraja, P.; Shrestha, A.K.; Shivakumar, A. (2007). A sensitive spectrophotometric method for the determination of sulfonamides in pharmaceutical preparations. *Acta Pharm.*, **57**, 333-342.

Naser, A.N.; Kasim, M.A.; Zainab, A.K. (2018). New approach for determination of sulfadiazine in pharmaceutical preparations using 4(4-sulphophenyl azo) pyrogallol: Kinetic spectrophotometric method. *Spectrochimica. Acta. Part A Molecular and Biomolecular Spectroscopy*., **201**, 267 – 280.

Othman, N.S.; Kadder, R.M. (2006). Histidine as a new coupling agent for the spectroph-otometric assay of sulphadiazine–application to pharmaceutical preparations. *Raf. J. Sci.*, **4**(17), 25-35.

Pecorelli, I.; Bibi, R.; Fioroni, L.; Galarini, R. (2004). Validation of a confirmatory method for the determination of sulfonamides in muscle according to the European union regulation. *J. Chromatogr. A*, **1032**, 23- 29.

Petri, W. A. Jr. (2001). "Antimicrobial Agents, in Goodman and Gilman's Pharmacological Basis of Therapeutics". (Eds. J.G. Hardman and Lee E. Limbird), 10th ed., McGraw Hill, New York, pp. 1171–1188.

Siddiqui, M.R.; Al-Othman, Z.A.; Rafiquee, M.Z.A.; AlLohedan, H.A.; Ali, M.S.; Ali Khan, M. (2013). Permanganate oxidation of sulfadiazine: Analytical method development and kinetic study. *Analytical Chem. Letters*, **3**(1), 7-17.

United States Pharmacopeia on CD – ROM, (1995). "The National Formulary". 1456 p.

Vandana, K.; Dash, A.K.; Kothapalli, U.; Kishore, T.S.; Harika, L.; Pradhan, K.K. (2011). Method development and validation of sulphadiazine in bulk and pharmaceutical dosage form by UV-Spectrophotometric method. *J. Pharm. and Biol.,* **2**(4),1167-1171.

Vree, T.B.; Schoondermark-van, E.; Verwey-Van Wissen, C.P.W.G.M., Baars, A.M.; Swolfs, A.; Van Galen, P.M.; Amatdjais-Groenen, H. (1995). Isolation, identification and determination of sulfdiazine and its hydroxyl metabolites and conjugates from man and rhesus monkey by high performance liquid chromatography. *J. liq. Chromatogr. B*, **670**(1), 111-123.

Wang, X.; Li, K.; Shi, D.; Jin, X.; Xiong, N.; Peng, F.; Peng, D.; Bi, D.J. (2007). Development and validation of an immunochromatographic assay for rapid detection of sulfadiazine in eggs and chickens. *J. Chromatogr. B*, **847**(2), 289-295.

Xiao-Ling, W.; Qi-Oi, S.; Dong-Mei, L.; Di, G. (2010). Indirect determination of sulfadiazine by ICP-AES of CU precipitation. *Chinese J. Pharm. Anal.,* **30** (12), 2356-2359.

تطوير طريقة طيفية لتقدير السلفاديازين بحالته النقية وفي الاشكال الصيدلانية من خلال تفاعل الازوتة والاقتران

الملخص

تتضمن هذه الدراسة تطوير طريقة طيفية بسيطة و حساسة لتقدير السلفاديازين بحالته النقية وفي اشكاله الصيدلانية. تعتمد هذه الطريقة على ازوتة السلفاديازين مع كمية متكافئة من نتريت الصوديوم بوجود حامض الهيدروكلوريك لتكوين ملح الديازونيوم الذي يقترن بعد ذلك مع كاشف الفلوروكليسينول في وسط قاعدي من هيدروكسيد الصوديوم لتكوين صبغة الآزو ذات اللون الأصفر، المستقرة و الذائبة في الماء والتي أعطت أعلى امتصاص عند الطول ألموجي 417 نانوميتر مقابل المحلول الصوري، وكان مدى الخطية الذي ينطبق على قانون بير تحت الظروف المثلى للتفاعل يقع ضمن مدى التركيز (0.25−15) مايكروغرام/ مللتر، وقيمة معامل التقدير (R^2=0.9992) و كانت قيمة الامتصاصية المولارية للطريقة $3.81x10^4$ لتر/ مول.سم وبلغت قيم حد الكشف (LOD) وحدالتقدير الكمي(LOQ) للطريقة(0.192) و(0.563) مايكروغرام/ مللتر على التوالي، و تراوحت قيم الخطاء النسبي بين (2.75%-) و(4.02%) و بانحراف قياسي نسبي أفضل من(1.36%)، وكانت النسبة التركيبية المولية بين السلفاديازين والكاشف فلوروكلوسينول هي1:1 بقيمة ثابت استقرار (0.37×10^7) لتر/مول، وقد طبقت الطريقة بنجاح لتقدير السلفاديازين في الكريم ومستحضرين بيطريين الحاوية على هذا المركب (المساحيق الدوائية والحقن).

الكلمات الدالة: السلفاديازين، ازوتة واقتران، فلوروكلوسينول، تقدير طيفي.