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Spectrophotometric Determination of Sulphadiazine Using 2,4 –dinitrophenylhydrazine as Coupling Reagent

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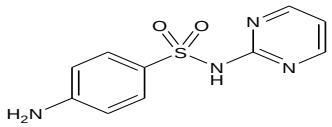
Abstract

A rapid, sensitive and selective spectrophotometric method was developed for determination of sulphadiazine in aqueous solution. The method is based on the oxidative coupling reaction with 2,4-dinitrophenylhydrazine (2,4-DNPH) in a basic medium in the presence of potassium periodate to produce an intense orange colour product, soluble in water, stable and absorbs at 486 nm. The linearity of Beer's law is in the range 3-15µg/ml of sulphadiazine. The molar absorptivity, Sandell's sensitivity index, and detection limit were $2.23x10^4$liter.mol^{-1}. cm^{-1}, 0.0222 µg.cm^{-2} and 0.0307µg/ml respectively. The RSD value was 0.506 – 0.116 % depending on the concentration value. This method is applied successfully to the determination of sulphadiazine veterinary injection liquid solution (VAPCOTRIM) with average recovery of not less than 99.5%.

Introduction

Sulphadiazine, 4 - amino – N - pyrimidin – 2 - yl - benzenesulfonamide, $C_{10}H_{10}N_4O_2S$ the molecular weight is 250.3 g/mol ,and the chemical structure is[1,2] :



It is from the group of antibiotic drugs ,which is one of the oldest and still widely used sulfonamides, a group of synthetically produced antibiotics, which is introduced in 1939[3], and has been used in veterinary and human therapy over 60 years[4,5]. various analytical methods are used for the determination of sulphdiazine such as spectrophotometric methods[6-16], electrochemical methods [17-20], flow-injection method [21-23]. high performance liquid chromatographic methods (HPLC)[24-26], In this research a simple, accurate and sensitive spectrophotometric method is introduced for determining sulphdiazine in pure form as well as in veterinary injection liquid solution (bio prime) based on the oxidative coupling using 2,4-dinitrophenylhydrazine (2,4-DNPH) in the presence of potassium periodate as oxidizing agent in basic medium.

Experimental part

Apparatus

Double-beam spectrometer type: Spectro UV-VIS double beam model UVD-3000 / UVD-3200 and Balance of type: DHAUS are used

Reagents and chemicals used

All chemicals and analytical reagents used in this research were of high purity .

Chemicals	Chemical Structure	Company
Sulphadiazine	$C_{10}H_{10}N_4O_2S$	SDI
2,4 Di nitro phenyl hydrazine	C6H6N4O4	Fluka
Potassium periodate	KIO_4	BDH
Sodium Hydroxide	NaOH	Fluka

Preparation of solutions

1- Standard sulphadiazine solution,1000 µg/ml

This solution was prepared by dissolving 0.1000 g of sulphadizine in amount of distilled water and the volume was diluted to 100ml with distilled water in a volumetric flask. Tweenty five ml aliquot of this solution was diluted to 100 ml with distilled water, to obtain a solution with a concentration of 250 µg/ml (3.99× 10-3 M).This solution was prepared to be used for one month.

2- Solution of 2,4-dinitro phenylhydrazine reagent (0.01M)

The solution was prepared by dissolving 0.1981g of 2,4-dinitro phenyl hydrazine in 5 ml of concentrated sulphuric acid and the volume was completed to 100 ml in a volumetric flask with distilled water.

3 - Potassium periodate solution (0.01M)

A 0.2300 g of potassium periodate was dissolved in amount of distilled water using ultrasonic bath and the volume was completed to 100 ml in a volumetric flask with distilled water.

4- Sodium hydroxide solution, (approximate concentration 1.0 M)

The solution was prepared by dissolving 4.0 g of sodium hydroxide in 100 ml of distilled water in a volumetric flask . Solutions of lower concentration were prepared by appropriate dilution

5- Solution of Sulphadiazine injection formulation (1000 µg/ml) Veterinary injection liquid solution

Each 1.0 ml contains 40 mg of sulphdiazine. The solution was prepared by pipetting an equivalent of 0.100g from sulphdiazine and the volume was completed to 0.5 ml with distilled water to obtain a solution with a concentration of 1 mg/ml. A solution of 250 µg/ml was prepared by dilution of 25 ml of the above solution by distilled water in a volumetric flask of 100 ml.

Results and discussion

Optimization of the experimental conditions

Preliminary investigations

A one ml of 2,4- DNPH reagent was added to 2 ml of standard sulphdiazine solution (250 ug/ml) in the presence of 1 ml of potassium periodate solution in basic medium using 2 ml of 1.0 M sodium hydroxide then diluted with distilled water in a 25 ml volumetric flask to produce an orange color product. Absorption spectrum of the colored dye against its corresponding blank reagents showed maximum absorption at 486 nm .

Selection of the oxidizing agent

The study was conducted by adding 1.0 ml of different types of oxidizing agents (0.01M) to 0.5 ml of 2,4-dinitro phenyl hydrazine solution (0.01M) and 2 ml of sodium hydroxide solution(1.0 M) such as: potassium periodate, potassium iodate and, potassium dichromate and Iron chloride. The results showed that potassium periodate solution gave the highest intensity for colored product at 486 nm so it was selected in subsequent experiments.

Effect of the amount of oxidizing agent

The best amount of oxidizing agent KIO4 (0.01M) was investigated by adding different volumes (0.5- 3 ml) of oxidizing agent to volumetric flasks containing 1ml of SDz (250µg/ml) and 0.5 ml of 2,4-DNPH reagent (0.01M), then addition of 2 ml of 1.0 M sodium hydroxide and the volume was completed to 25ml with distilled water. Results shown in Table (1) indicated that the volume of 1ml of oxidizing agent KIO4 (0.01M) was the optimum amount because of highest absorbance, so it was selected in subsequent experiments.

Table (1) Effect of the amount of oxidizing agent.

ml of KIO_4	0.5	1	1.5	2	2.5	3
Absorbance	0.287	0.720	0.611	0.519	0.447	0.400

Effect of the amount of coupling reagent

The effect of the amount of coupling reagent was studied by adding different volumes (0.3 - 1.5ml) of reagent solution (0.01 M) to the volumetric flasks containing 1 ml of sulphdiazine (250µg/ml) and one ml of oxidizing agent KIO_4 (0.01M), then addition of 2 ml of 1.0 M sodium hydroxide and the volume was completed to 25ml with distilled water. The results were shown in Table (2), it was clear that the volume of 0.5 ml of 2,4-DNPH reagent (0.01M) was the optimum amount.

Table (2) Effect of the amount of coupling reagent.

ml of 2,4-DNPH	0.3	0.5	0.8	1	1.2	1.5
Absorbance	0.456	0.718	0.455	0.402	0.334	0.325

Effect of the amount of sodium hydroxide

The study was conducted to select the best amount of NaOH (1.0 M) by adding different volumes (1- 3.5 ml) of NaOH to volumetric flasks containing 1 ml of SDz (250 µg/ml) and 1.0 ml KIO_4 of 2,4-DNPH reagent 0.5 ml, the volume was completed to 25ml with distilled water. The results shown in Table (3) indicated that the volume of 2 ml of (1M) was the optimum amount.

Table (3) Effect of the amount of sodium hydroxide

ml of NaOH	1	1.5	2	2.5	3	3.5
Absorbance	0.315	0.718	0.783	0.689	0.550	0.524

Effect of oxidation time

The color intensity reached maximum, after drug was reacted with 2,4-DNPH and KIO4 for 5 min in basic medium, therefore, a 5min was sufficient for the oxidation to be completed, so it was adopted in the subsequent experiments.

Table (4) Effect of oxidation time.

Time (min)	Directly	5	10	15	20	25
Absorbance	0.525	0.782	0.660	0.625	0.605	0.599

Effect of time for adding NaOH

Results in table (5) showed that the best time for adding NaOH was five minutes .

Table (5) Effect of time for the addition of NaOH

Time (min)	Directly	5	10	15	20	25
Absorbance	0.525	0.782	0.660	0.625	0.605	0.599

Order of additions

The effect of different orders of addition on the absorption of the colored product was studied. Results shown in Table (6) indicating that the addition in the order (SDz + KIO4 + 2,4DNPH + OH) results in a higher absorption of colored product. So it was adopted in subsequent experiments.

Table (6) Order of additions.

Order number	Order of addition	Absorbance
I	SDz+ KIO4 + 2,4−DNPH +OH-	1.125
II	SDz +2,4 DNPH +KIO4 + OH-	0.008
III	2,4 DNPH + KIO4 + SDz +OH-	0.009
IV	SDz + OH + KIO4 +2,4 DNPH	0.660
V	2,4 DNPH+ OH + KIO4 + SDz	0.026

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Effect of temperature

The results in (table7) showed that the optimum temperature range that gave the best absorption was (15 – 25°C) so it was used in subsequent experiments.

Table (7) Effect of temperature

Temperature °C	15	20	25	30	35	40
Absorbance	1.123	1.125	1.122	1.115	1.105	1.095

Effect of the solvents

The effect of the solvents on the formed colored product was studied by dilution with different organic solvents instead of water. The results in (table 8) indicated that the water was a good medium for reaction and gives good absorption value at the wavelength of 486 nm and due to its availability, it was used as the best solvent in the subsequent experiments.

Table (8) Effect of Solvent

Solvent	**Absorbance**	**λmax, nm**
Water	1.126	486
Acetone	1.291	500
Iso probanal	0.734	482

Final absorption spectra

The spectrum (figure 1) of the colored product formed by coupling of 1 ml of sulphdiazine solution (250 µg/ml) with 0.5 ml of 2,4-DNPH (0.01M) in the presence of 1 ml of KIO_4 (0.01M) in basic medium (2 ml,1M NaOH) and temperature at 25°C against its corresponding reagent blank showed a maximum absorption at 486 nm.

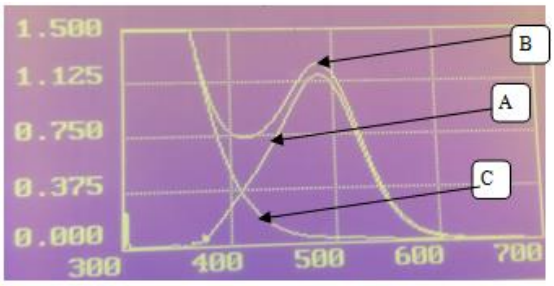

Figure (1): Final absorption spectrum of the colored complex

A : Absorption spectrum of colored complex versus blank. **B**: Absorption spectrum of colored complex versus distilled water. **C**: Absorption of blank versus distilled water.

Procedure for construction of calibration curve

To a series of volumetric flasks (25ml),(0.3-2) ml of (250 µg/ml) of sulfdaizine were transferred, and then 1ml of KIO_4 (0.01 M) and 0.5ml of 2,4-DNPH reagent (0.01M), 2 ml of 1.0M sodium hydroxide solution were added at 25°C. The solutions were left for 5 min to complete the reaction, then the volumes were completed to the mark with distilled water. The absorbance was measured at 486 nm against the blank reagent. Figure (4) illustrated that the calibration curve was linear over the concentration range of 3 - 15 µg/ml while higher concentrations showed a negative deviation from Beer's law. The molar absorptivity value was 2.23×10^4 liter. $mol^{-1}.cm^{-1}$and the Sandell's sensitivity index was 0.011µg/cm^2 .

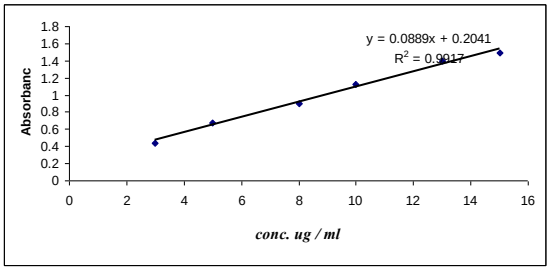

Figure (2): Calibration curve for determination SDz by oxidative coupling with 2,4-DNPH reagent.

Accuracy and precision

Accuracy and precision were studied by measuring absorption at 486nm for three different concentrations of the drug within the limits of Beer's law, the average recovery was (100.26) and the relative standard deviation (0.506–0.116 %) indicating that the method was of high accuracy and precision. The results were shown in Table (9)

Table (9) Results of accuracy and precision.

Conc. of SDz (ppm)	**Recovery*, %**	**Average Recovery*,%**	**RSD* ,%**
5	100.3	100.26	0.506
8	100.3		0.418
10	100.2		0.116

*Average of Six determinations

Detection limit

Detection limit was calculated by measuring the absorption for the lower concentration (3 µg/ml) at optimal conditions at 486 nm. The results were shown in Table (10).

Table (10) Detection limit.

Concentration µg/ ml	$\overline{X}$	S	**D.L µg/ml**
3	0.439	0.00343	0.0703

The nature of the formed product

To know the nature of the formed orange color complex (stoichiometry of drug with the reagent), Job's method and molar ratio method were applied. In both methods, the concentration of each of the standard SDz solution and 2,4-DNPH reagent solution was equal to 1×10^3 M. **In Job's method**, in a series of volumetric flasks (25 ml), different volumes of the drug solution ranging from 1-9 ml and different volumes (9-1 ml) of reagent solution were mixed. One ml of potassium periodate (0.01 M) and 2 ml 1 M of sodium hydroxide solution were added and volumes were completed to the mark with distilled water. The absorbance was measured at 486 nm against the blank reagent. The results in (Figure 3) showed that the ratio was 1:1.

In molar ratio method, volume of 1 ml of the standard drug solution (1×10^3 M) in a series of volumetric flasks (25 ml) was transferred and different volume 0.3- 3ml of 2,4-DNPH reagent solution, 1 ml of potassium periodate (0.01) and 2 ml

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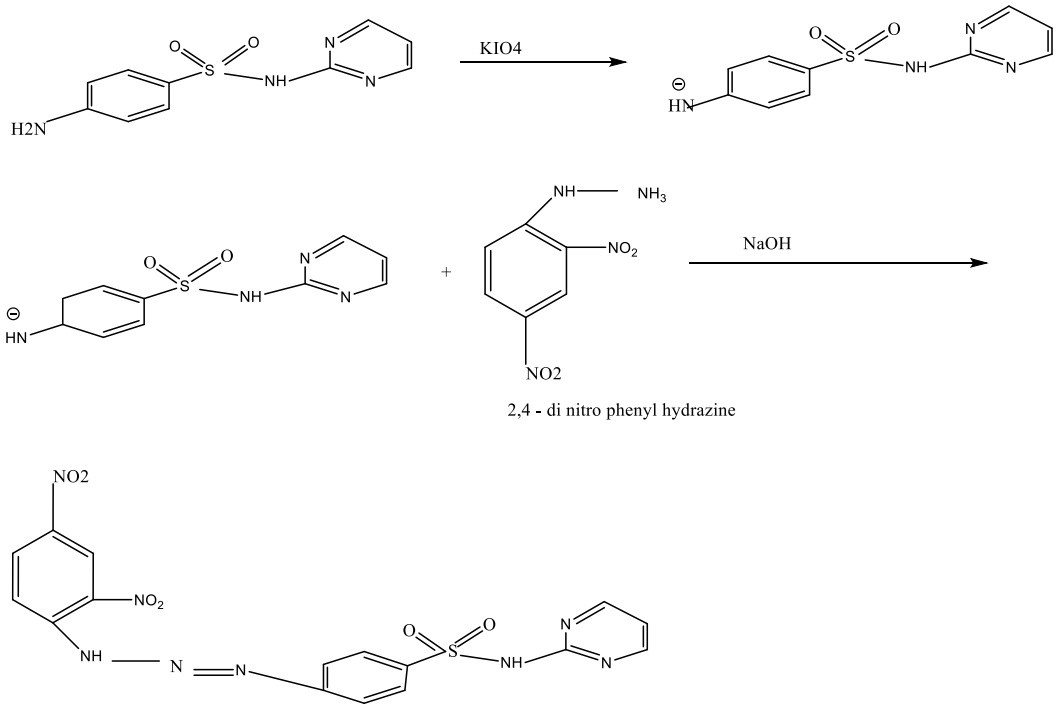
1.0 M of sodium hydroxide solution were added. The volumes were completed to the mark with distilled water and the absorbance was measured at 486 nm against the blank reagent. The results in Figure (4) showed that the molar ratio was 1:1 which was in agreement with the Job's method results. Scheme 1 showed the expeted formed complex structure.



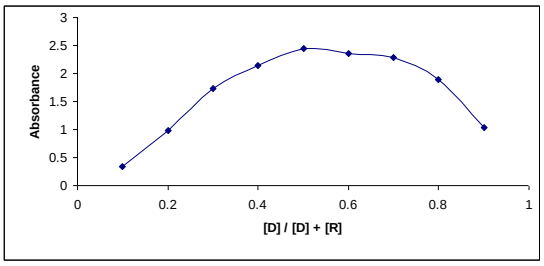


Figure (3) Job's method

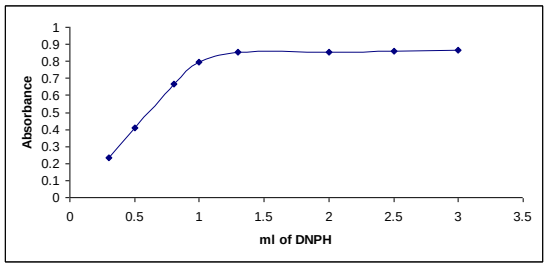


Figure (4): Molar ratio method

Applications

Direct method

In this method, different volumes (0.5, 0.8ml) of a pharmaceutical formulation solutions (250 µg/ml) were transferred to 25 ml volumetric flasks and treated as in construction of calibration curve The absorbance was measured at 486 nm for six times were calculated. Table (11) showed the efficiency and success of the developed method for the determination of SDz in its pharmaceutical formulation, the average recovery was 99.75 %.

Table (11) Direct method for determination of SDz

Conc. Of SDz (ppm)	RE*,%	Recovery*, %	Average Recovery*, %	RSD*, %
5	- 1.47	99.8	99.75	0.212
8	- 0.222	99.7		0.510

*Average of Six determinations

Standard additions method

To prove that the developed method was free from interferences, method of standard additions was applied for determining of SDz in its pharmaceuticals. Different volumes (0.5, 1ml) of a pharmaceutical formulation solutions (250 µg/ml) were transferred to six volumetric flasks (25 ml) for each volume, then increasing volumes of 250 µg/ml of SDz standard solution were added leaving the sixth flask without addition. The solution was treated as in construction of calibration curve. The absorbances were measured at 486 nm (Figure 5) showed the results of this method.

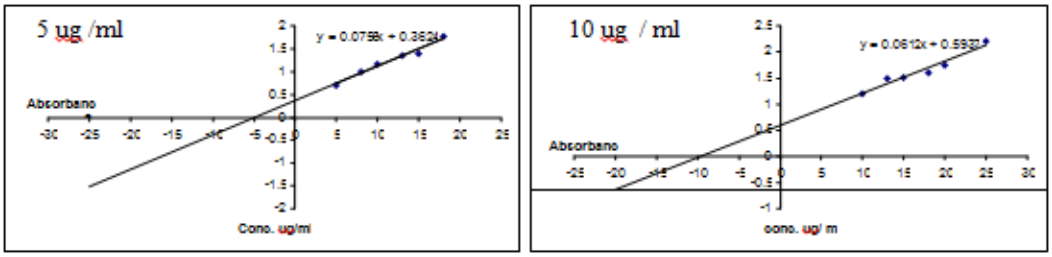


Figure (5): Standard additions curve for the determination of SDZ in injection.

Table (12) Results of standard additions method.

Type of Drug	SDz present µg/ml	SDz measurd µg/ml	RE %	Recovery, (%)
Injection	5	4.8	96.5	96
	10	9.7		97

Conclusions

Results obtained from this study confirmed that the proposed method was simple, rapid and of good sensitivity for the determination of sulphdiazine. The method was based on oxidative coupling between sulphdaizine and 2,4-dinitrophenylhydrazine reagent in presence of potassium periodate in basic medium to form orange colored dye which was water soluble, stable and showed a maximum absorption at 486 nm. This method does not require temperature control, nor use of organic solvents, or solvent extraction and it can be applied successfully for determination of sulphdiazine in veterinary pharmaceuticals formulation with recovery of not less than 99.5%.

References

[1] British, Ph. (2005). 5th Edn., by system simulation ltd., the stationary office, London, in CD-ROM"

[2] British, Ph. (2009). 6th Edn., by system simulation ltd., the stationary office, London, in CD-ROM"

[3] Harvey, R.A.; Clark, M.A.; Finkel, R.; Rey, J.A. and Whalen, K.; (2012). 5th Edn., Lippincotts Illustrated Reviews: Pharmacology.

[4] https://ar.wikipedia.org/wiki/ Sulphdiazine.

[5] Shackman, M.D.N.H. and Bullowa, M.D.J.G. (1943). Sulphadiazine administered antiumococcus serum in the treatment of pneumococcic pneumonia. Arch Intern Med., **72 (3):**329-345.

[6] AL-doury, S.SH. (2012). spectrophotometric Determination of isoniazid, sulphamethoxazole, sulphacetamide sodiumand sulphdiazine, by Schiff's base Formation:28-32.

[7] Salim, A.M. and Haseeb, Y.S.Z. (2013). Spectrophotometric Determination of Sulfadiazine via Diazotization and Coupling Reaction- Application to Pharmaceutical Preparations. *Raf. J. Sci.*, **24 (6):**61-73.

[8] Mahdi, M.I. and Kadim, K.H. (2015). Spectrophtometric determination of Sulfadiazine in Various Sample by coupling with 2,5-dimethoxy aniline. *Iraqi National Journal of Chemistry*, **15 (1)**.

[9] Amin, A.S. and Zareh, M.M. (1996). Acetylacetone-formaldehyde reagent for the Spectrophotometric determination of some sulpha drug in pure and dosage forms", *Microchem. Acta.*, **124 (3-4):**227-233.

[10] Romero, A.M.; Benito, C.G. and Calatayud, J.M. (1995) Continuous-flow spectrophotometric determination of sulphadiazine by diazotization with in situ preparation of nitrite. *Anal. Chem. Acta*, **308 (1-3):** 451-456.

[11] AL-Anazy, M.S. (2002). Development of spectral methods for the determination of alparasetamaol, histamine and some sulfa drugs.

[12] AL-Korany, R.M. (2006). Spectral estimation of sulfacetamide and sulfadiazine Applications in pharmaceuticals.

[13] Shaheed, H.A. (2015). Atheromdynamic study of Adsorption of Sulfadiazine on tween 20, tween 40. *Journal AL qadisiyah University*, **2 (20):**125-136.

[14] Nagaraja, P.; Naik, S.D.; Shrestha, A.K. and Shivakumar, A. (2007). Asensitive Spectrophotometric method for the determination of sulfonamide in pharmaceutical preparations. *Acta Pharm.*, **57:**333-342.

[15] Nagaraja, A.; Sherstha, K.; Shivakumar A. and Gowda, A.K (2010). Use of N, N-diethyl-p-phenylenediamine sulphate for the spectrophotometric determination of some phenolic and amine drugs. *Acta. Pbarm.*, **60:**217-227.

[16] Nagaraja, P. et al. (2003). 3-Aminophenol as a novel coupling agent for the spectrophotometric determination of sulfonamide derivatives. **58 (12):**1295-300.

[17] Braga, O.C.; Campestrini, I.; Vieira, I.C. and Spinelli, A. (2010). Sulphadiazine determination by electrochemical reduction on a glassy carbon electrode. *Journal Braz. Chem. Soc.*, **21 (5):**813-820.

[18] Souza, C.D.; Brage, O.C. and Vieria, I.C. (2008). Elecctrounlytical determination of sulfadiazine and sulfamethoxazol in pharmceuticuts using aboron – deped- diamond electriode. *Sensors actuators Bichemical.*, **135 (1):**66-73.

[19] You, T.; Yang, X. and Wang, E. (1998). Determination of sulphadiazin and sulphamethoxazole by capillary electrophoresis with end column electrochemical detection. *Analyst*, **123**: 2357-2374.

[20] Bashammakh, A.S. (2017). Differential Pulse-dsorptive Cathodic Stripping Voltammetric Determination of Sulfadiazine Drug in Pharmaceutical Formulations and Drug Residue in Wastewater at a Hanging Mercury Dropping Electrode. **42 (1):**183-192|.

[21] Fan, J. et al. (2003). flow-injection spectrophotometric determination of sulphadiazine and sulphamethoxazole in pharmaceutical and urine. *Anal. Sci.*, **3,19 (3):**22- 419.
[22] AL-Abachi, M.Q. and Shaki, I.M.A. (2006). Spectrophotometric Determination of Some Sulphonamide Drugs via oxidative coupling with Phenothiazine and Benzoyl peroxide using flow-injection technique. *National Journal of Chemistry*, **23**: 281-293.
[23] Lattanzio, G. et al. (2008). Chemiluminescence detrmination of sulphadiazine in drugs by flow injection analysis using the peroxyoxalate reaction micellar medium, *Journal Pharm. Biomed. Anal.*, **46 (2)**:5-381.
[24] Croubels, S.; Wassink, P. and Backer, P.D. (2002). Simultaneous determination of sulphadiazine and trimethoprim in animal feed by liquid chromatography with UV and tandem mass spectrometric detaction. *Anal. Chem. Acta*, **473**:183-194.
[25] Wen, Y.; Zhang, M.; Zhao, Q. and Feng, Y.Q. (2005). Monitoring of five sulphonamide antibacterial residues in milk by in-tube solid-phase microextraction coupled to high–performance liquid chromatography. *Journal Agric. Food Chem.*, **53 (22)**:8468-8473.
[26] Vree, T.B. et al. (1995). Isolation, identification and determination of sulphadiazine and its hydroxyl metabolites and conjugates from man and rhesus monkey by high-performance liquid chromatography. *Journal Chromatogr. B*, 111-123.

التقدير الطيفي للسلفاديازين باستخدام كاشف الاقتران 4,2 ثنائي نتروفنيل هيدرازين

نجلاء عبد السلام احمد ، علي ابراهيم خليل
قسم الكيمياء ، كلية العلوم ، جامعة تكريت ، تكريت ، العراق

الملخص

تَضَمَّنَ هذا البحث تطوير طريقة طيفية سريعة وحساسة وانتقائية لتقدير عقار السلفاديازين في الوسط المائي، إذْ اعتمدت الطريقة على تفاعل الاقتران التأكسدي الذي يتم بأكسدة العقار باستخدام بريودات البوتاسيوم إذْ يتكون ناتج برتقالي بأقتران العقارمع الكاشف 4,2 ثنائي نتروفنيل هيدرازين, له أعلى امتصاص عند الطول الموجي 486 نانومتر، وكانت حدود قانون بير في مدى التركيز 3 – 15 مايكروغرام/ مل, وان قيم معامل الامتصاص المولاري والانحراف القياسي النسبي ودلالة ساندل وحد الكشف هي 22.3 ×10^3 لتر.مول$^{-1}$. سم$^{-1}$, 0.505 الى 0.116 %، 0.0222 مايكروغرام.سم−2 و0.0307 مايكروغرام/ مل على التوالي، وتَمَّ تطبيق الطريقة وبنجاح في تقدير بعض المستحضرات الصيدلانية الحاوية على السلفاديازين بآسترجاعية لاتقل عن 99.5%.