----Raf. Jour. Sci., Vol.17, No. 4 Chemistry, Special Issue, pp.25-35, 2006----

Histidine as a New Coupling Agent for The Spectrophotometric Assay of Sulphadiazine – Application to Pharmaceutical Preparations

Nabeel S. Othman **Raeeid M. Kadder**

Department of Chemistry
College of Science
Mosul University

(Received 26/3/2006 , Accepted 8/5/2006)

ABSTRACT

A simple and sensitive spectrophotometric method for the assay of sulphadiazine (SDA) in aqueous medium is described, the method is based on the formation of a yellow coloured azo dye from the diazotisation of SDA followed by a coupling reaction with histidine in alkaline medium of sodium hydroxide. The intensity of absorbance for the resulting yellow azo dye was measured at 423 nm and the azo dye was stable at least for 55 minutes. Beer's law is obeyed in the concentration range of 10 – 240 μg of SDA in a final volume of 25 ml, with a molar absorptivity of 1.75×10^4 $L.mol^{-1}.cm^{-1}$, a relative error of +0.34 to +0.44% and a relative standard deviation of ±0.34 to ±0.87%, depending on the concentration level of SDA.

The method has been successfully applied for the assay of SDA in burn cream and in a veterinary injectable solution.

الهستدين كعامل اقتران جديد للتقدير الطيفي للسلفادايازين – تطبيقـات على مستحضرات صيدلانية

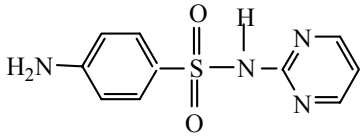
الملخص

يتضمن البحث طريقة طيفية بسيطة وحساسة لتقدير السلفادايازين (SDA) في الوسط المائي. تعتمد الطريقة على أزوتة السلفادايازين ثم اقترانه مع الهستدين لتكوين صبغة آزوية صفراء اللون فـي وسـط قاعدي من هيدروكسيد الصوديوم ثم قياس شدة الامتصاص للصبغة الناتجة عنـد الطـول المـوجي 423 نانوميتر. الصبغة الناتجة مستقرة لمدة 55 دقيقة على الأقل وان حدود تطبيق قانون بير كانت فـي مـدى التركيز من 10 - 240 مايكروغرام/ 25 مل من الـ SDA و قيمة الامتـصاصية المولاريـة كانـت 1.75×10^4 لتر.مول$^{-1}$.سم$^{-1}$، والخطأ النسبي تراوح بين 0.34+ و 0.44+ % والانحـراف القياسـي النسبي بين ± 0.34 و ± 0.87 % اعتمادا على مستوى تركيز الـ SDA. تم تطبيق الطريقة بنجاح في تقدير السلفادايازين في مرهم معالجة حروق الجلد وفي سائل معد للحقن البيطري.

INTRODUCTION

Sulphadiazine (2-Sulphanilamidopyrimidine) is one of the many known sulphonamides drugs (Jaime and William, 1998).

It is absorbed readily from the gastrointestinal tract to yield reproducible blood levels, the solubility of sulphadiazine in urine makes it suitable for the treatment of



Sulphadiazine

E. coli infections in the urinary tract (Romero et al., 1995)

The important role in chemotherapy is played by sulphadiazine and its effects (treatment of pneumocystis pneumonia, chronic bronchitis, meningococcal meningitis, acute otitis media) in the various situations makes the assay of sulphadiazine of utmost importance(Walker and Edwards, 1999).

Many analytical methods for the determination of sulphadiazine (SDA) have been developed, most of them included diazotization of sulphadiazine and then coupling with different coupling agents such as: α-naphthylamine (Jing et al., 2003), iminodibenzyl (Nagaraja et al., 2002), 3-aminophenol (Nagaraja et al., 2003) and primaquine phosphate (El-Sayed, 1999). Other methods are either based on the formation of charge transfer (CT) complex between sulphadiazine and alizarin derivatives (Amin et al., 1995) and with phenosaphranine (Al-Attas, 2003), or an oxidative coupling reaction of sulphadiazine with different reagents and oxidizing agents such as phenothiazine and hypochlorite (Abdine et al., 1979), phenothiazine and ceric ion (Al-Abachi and Al-Talib, 1994) and 4-amino-N, N-dimethylaniline and dichromate (Al-Abachi and Al-Talib, 1995).

The present method involves the diazotisation of sulphadiazine followed by coupling reaction with histidine to form a yellow dye that has been proved successfully for the assay of sulphadiazine in burn cream and in a veterinary injectable solution.

EXPERIMENTAL

Instruments:

All spectrophotometric measurements are performed on Shimadzu UV-Visible Recording Spectrophotometer UV-160 by using 1 cm silica cell, pH meter type Philips PW 9420 is used for pH readings.

Reagents:

All chemicals used in this investigation are of analytical – reagent grade, and SDA standard material is provided from the general establishment for medical appliance and drugs / SDI – Samaraa / Iraq.

Solutions:

Sulphadiazine (SDA) solution, 100 μg.ml^{-1}: This solution is prepared by dissolving 0.01 g of sulphadiazine in 10 ml of ethanol and diluting with distilled water to a 100 ml in a calibrated volumetric flask.

Histidine solution, 0.1% (w/v): This solution is prepared by dissolving 0.1 g of histidine (Fluka) in 100 ml distilled water.

Sodium nitrite solution, 1% (w/v): This solution is prepared by dissolving 1 g of sodium nitrite (BDH) in 100 ml distilled water.

Sulphamic acid solution, 3% (w/v): This solution is prepared by dissolving 3 g of sulphamic acid (Fluka) in 100 ml distilled water.

Hydrochloric acid solution, 1N: This solution is prepared by diluting 8.3 ml of concentrated acid to a 100 ml with distilled water.

Sulphaprim (Veterinary injectable solution, 20% SDA), 100μg.ml^{-1}: A 0.5 ml of the veterinary injectable solution is treated with 10 ml of ethanol and diluted to a liter with distilled water in a calibrated volumetric flask.

Flammazine cream(1% Ag.SDA), 100μg.ml^{-1}: The follow extraction method is applied:- to 1 g of cream added 50 ml ether shake well and transfer the mixture to a separating funnel, then extract the Ag.SDA to the aqueous layer with 25 ml of distilled water (three times). Collecte the aqueous layer and filter, dilute the filtrate to 100 ml with distilled water in a volumetric flask, the final solution obtained is 100 μg.ml^{-1} of Ag. SDA (The United States Pharmacopeia, 1995).

Procedure and calibration graph:

To a series of 25 - ml calibrated flasks transfer 0.1 – 3.0 ml of 100 μg.ml^{-1} SDA solution, then 1 ml of 1N hydrochloric acid and 0.3 ml of 1% (w/v) sodium nitrite solution are added and the mixture is allowed to stand for 1 minute and then 0.1 ml of 3% (w/v) sulphamic acid solution is added with occasional shaking for 2 minute. After that a 2 ml of 0.1% (w/v) histidine solution and 2 ml of 1N sodium hydroxide are added.

After the volumes are completed to the mark with distilled water, the absorbance is measured at 423 nm against the reagent blank after 5 minutes. A linear calibration graph is obtained over the concentration range of 10 – 240 μg / 25 ml SDA and a concentration above 240 μg / 25 ml gives a negative deviation (Fig. 1). The molar absorptivity has been found to be 1.75×10^4 l.mol^{-1}.cm^{-1}.

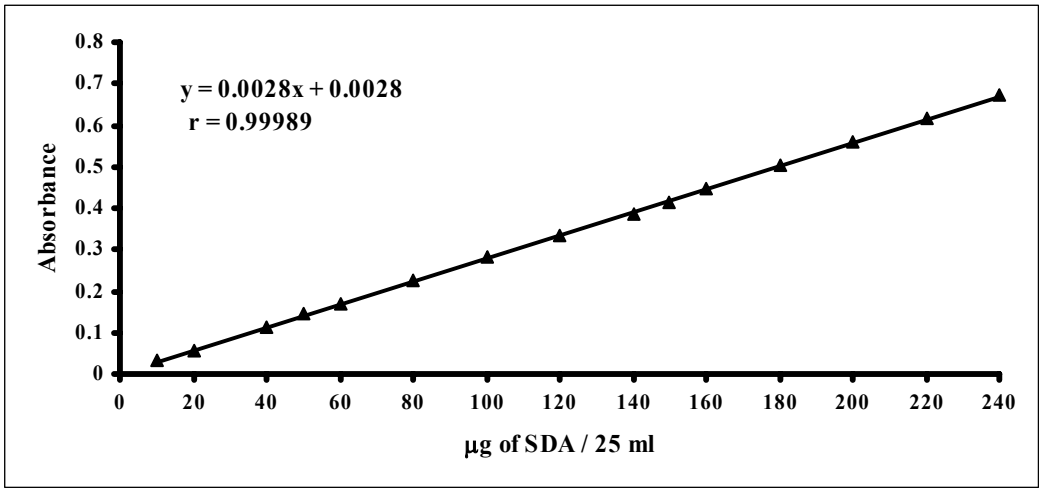


Fig. 1: Calibration graph of SDA determination.

RESULTS AND DISCUSSION

During the investigations, a 100µg of SDA is taken and the final volumes are brought to 25 ml with distilled water.

Effect of diazotisation acid:

Different amounts and types of acids have been used in diazotisation of SDA, the results show that 1 ml of 1N hydrochloric acid solution give the best results (Table 1).

Table 1 : Effect of acids.

1N Acid solution used	Absorbance / ml of acid added				
	0.5	**1**	**1.5**	**2**	**3**
HCl	0.180	0.233	0.192	0.037	0.030
HNO_3	0.174	0.220	0.188	0.034	0.025
H_2SO_4	0.171	0.212	0.181	0.030	0.007
CH_3COOH	0.156	0.175	0.162	0.027	0.003

Effect of sodium nitrite amount and time:

The maximum absorbance reading is obtained by adding 0.3 ml of 1% sodium nitrite with 1 minute reaction time (Table 2).

Table 2 : Effect of sodium nitrite amount and time.

ml of $NaNO_2$ solution(1%)	Absorbance / minute standing time					
	0	**1**	**2**	**3**	**4**	**5**
0.1	0.146	0.163	0.176	0.186	0.152	0.150
0.2	0.170	0.181	0.196	0.188	0.184	0.182
0.3	0.196	0.241	0.235	0.199	0.197	0.194
0.4	0.178	0.177	0.176	0.172	0.170	0.168
0.5	0.165	0.167	0.158	0.158	0.157	0.156

Effect of sulphamic acid amount and time:

The excess of nitrous acid is removed by the addition of sulphamic acid solution (Bladyga and Bourne, 1999). The effect of sulphamic acid amount and time has been studied.(Table 3).

Table 3 : Effect of sulphamic acid amount and time.

ml of Sulphamic acid solution (3%)		Absorbance/minute standing time				
		0	1	2	3	4
0.00	Sample = S	0.181	0.199	0.196	0.195	0.190
	Blank = B	0.018	0.019	0.020	0.019	0.018
0.05	S	0.211	0.220	0.234	0.233	0.232
	B	0.012	0.014	0.020	0.022	0.023
0.10	S	0.261	0.266	0.277	0.275	0.274
	B	0.005	0.006	0.002	0.007	0.004
0.20	S	0.242	0.244	0.244	0.245	0.244
	B	0.005	0.006	0.002	0.007	0.004
0.25	S	0.232	0.237	0.237	0.237	0.235
	B	0.012	0.013	0.016	0.017	0.019

The results in the table 3 indicate that 0.1 ml of sulphamic acid solution (3%, w/v) with 2 minute as standing time for the reaction give the most suitable effect on the intensity of the dye with corresponding low reagent blank absorbance.

Effect of histidine amount:

The effect of different amounts of 0.1% histidine solution has been studied on the intensity of absorbance at different amounts 5 - 200 μg / 25 ml of SDA. (Table 4).

Table 4 : Effect of coupling agent amount on absorbance.

ml of 0.1% Histidine solution	Absorbance / μg SDA present in 25 ml											r
	5	10	20	40	80	100	120	140	160	180	200	
0.5	0.013	0.021	0.042	0.069	0.151	0.170	0.210	0.233	0.277	0.318	0.360	0.9986
1	0.021	0.030	0.054	0.101	0.183	0.225	0.257	0.324	0.352	0.382	0.458	0.9982
2	0.026	0.034	0.070	0.120	0.227	0.277	0.312	0.377	0.456	0.500	0.540	0.9987
3	0.028	0.035	0.071	0.131	0.260	0.318	0.330	0.397	0.504	0.584	0.660	0.9945
4	0.031	0.036	0.072	0.154	0.277	0.371	0.387	0.479	0.554	0.623	0.704	0.9984

Although the results in the above table indicate that the sensitivity increase with increasing of histidine amount but the stability of the formed dye is decreased, therefore a volume of 2 ml of 0.1% histidine solution in a total volume of 25 ml is selected for subsequent experiments because of the higher value of correlation coefficient (r) and it gives stable azo dye.

Effect of base:

This investigation is showed that the azo dye is formed in alkaline medium, therefore a different types and amounts of strong and weak bases have been studied

(Table 5). The results indicate that the strong bases give high intensity and high colour contrast. A volume of 2 ml of 1N sodium hydroxide (final reaction mixture pH = 12.45) has been selected for the subsequence experiments.

Table 5 : Effect of base on absorbance and colour contrast.

Base used (1N)	Variable	ml of base used *				
		1	2	3	4	5
NaOH	A**	0.117	0.280	0.274	0.236	0.215
	Δλ***	211	213	212	212	211
	pH	4.50	12.45	12.60	12.70	12.80
KOH	A	0.078	0.243	0.271	0.225	0.204
	Δλ	218	216	216	217	218
	pH	4.40	12.75	13.00	13.08	13.20
NH_4OH	A	0.111	0.138	0.138	0.140	0.157
	Δλ	104	104	103	104	102
	pH	2.12	8.30	9.33	9.56	9.90
Na_2CO_3	A	0.035	0.096	0.189	0.219	0.222
	Δλ	150	151	150	150	150
	pH	2.32	8.16	9.10	10.06	10.57
$NaHCO_3$	A	0.006	0.013	0.011	0.011	0.016
	Δλ	19	19	18	18	19
	pH	1.96	6.80	7.30	8.35	8.36
CH_3COONa	A	0.004	0.011	0.017	0.012	0.009
	Δλ	27	27	26	26	26
	pH	1.90	3.85	4.53	4.74	4.95

* When zero ml base added A = 0.012

** A = Absorbance

***$\Delta\lambda = \lambda_{max}S - \lambda_{max}B$ where S = The dye , B = Blank

Effect of time:

The coloured azo dye is developed rapidly after addition of base and attains maximum intensity at room temperature after 5 minutes. The colour is stable for at least 55 minutes and the results are given in table 6.

Table 6 : The effect of time and SDA amount on absorbance.

µg of SDA present	Absorbance / minute standing									
	2	**5**	**10**	**15**	**25**	**30**	**35**	**45**	**55**	**60**
50	0.139	0.146	0.149	0.149	0.150	0.150	0.150	0.150	0.148	0.148
100	0.260	0.270	0.274	0.279	0.279	0.278	0.278	0.278	0.277	0.277
200	0.388	0.401	0.411	0.415	0.415	0.415	0.414	0.414	0.413	0.412

From the above table the stability period is 55 minutes, this stability period is sufficient to allow several measurements to performed sequentially.

Final absorption spectrum:

The absorption spectrum of the yellow azo dye formed from coupling of diazotised SDA with histidine in alkaline medium shows a maximum absorption at 423 nm. The reagent blank has no absorption at this wavelength (Fig. 2).

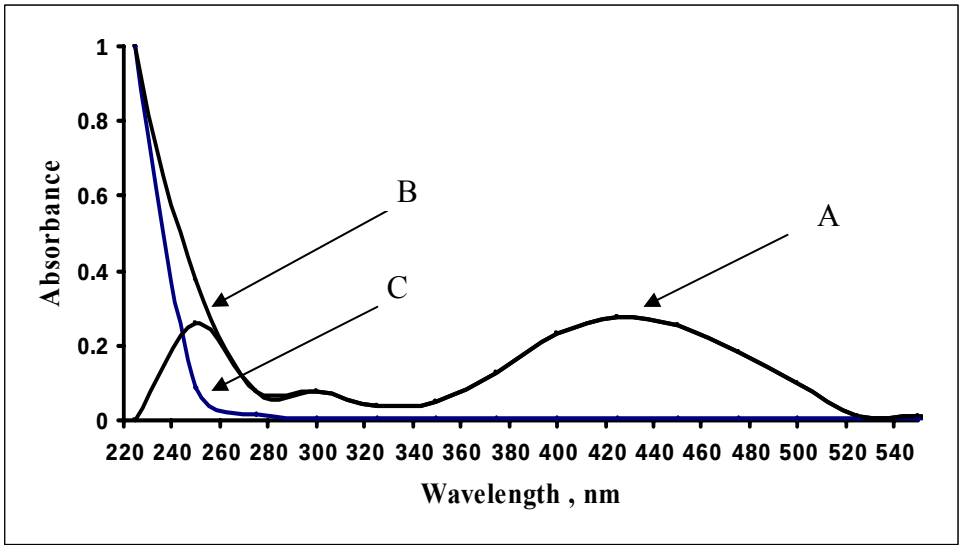


Fig. 2. :Absorption spectra of 100 µg / 25 ml SDA treated according to the recommended procedure and measured against (A) reagent blank, (B) distilled water and (C) reagent blank measured against distilled water

Nature of the dye:

The stoichiometry of the formed azo dye between diazotised SDA and histidine is investigated by applying the continuous variations method (Delevie, 1997) .

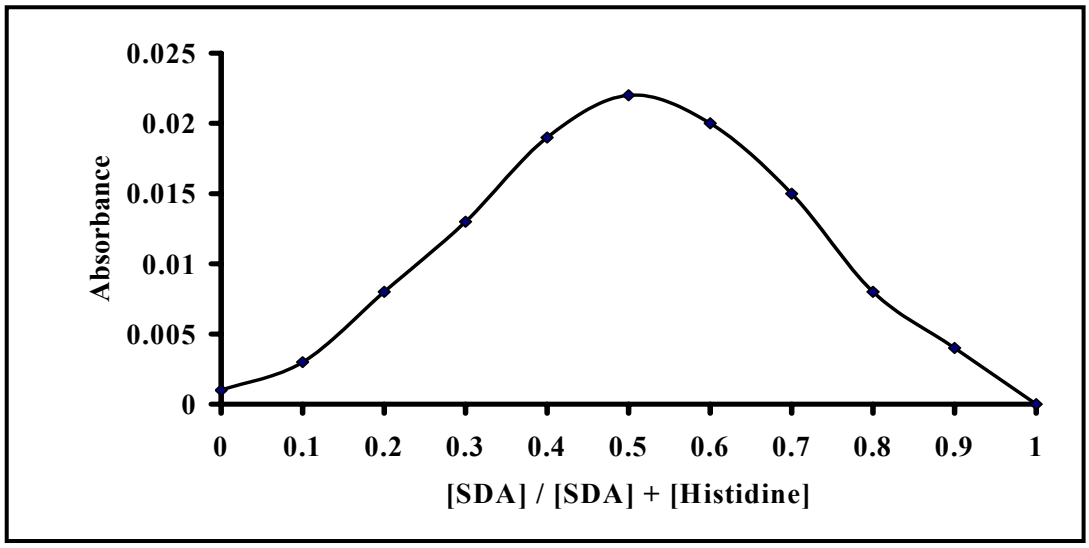
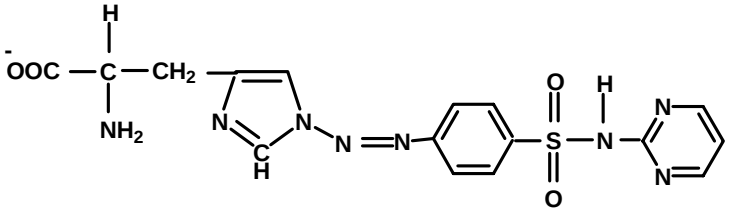


Fig. 3: Continues variations plot for diazotized SDA – Histidine dye .

The results indicate that the azo-dye has been formed in the ratio of 1:1 (diazotised SDA : Histidine), and the azo dye may have the following suggested structure :-



Yellow azo-dye

Accuracy and precision:

To check the accuracy and precision of the present method, a three different concentrations of SDA are determined. The results shown in Table (7), indicate that the method is satisfactory.

Table 7 : Accuracy and precision.

Amount of SDA taken, µg / 25 ml	Relative error, %*	Relative standard deviation, %*
50	+0.340	±0.875
100	+0.440	±0.340
150	+0.36	±0.415

* Average for five determinations

Analytical application:

The proposed method is applied to determine SDA in flammazine (burn cream containing 1% silver sulphadiazine) and in sulphaprim (veterinary injectable solution containing 20% sodium sulphadiazine). On applying proposed procedure, a good recovery is obtained (Table 8).

Table 8 : Analytical applications.

Drug	Pharmaceutical preparation	Certified value (g)	µg SDA present / 25ml	µg SDA found / 25ml	Recovery (%)
Flammazine	Cream	1 % Silver sulphadiazine	50	50.35	100.7
			150	153.15	102.1
			200	202.80	101.4
Sulphaprim	Injection	20 % Sodium sulphadiazine	50	49.80	99.6
			150	153.00	102.0
			200	200.80	100.4

The performance of the proposed method is assessed by calculating the student's t – test compared with the standard method (British Pharmacopeia, 2000). At the 95% confidence limit for four degree of freedom, the calculated t - values do not exceed the theoretical value (2.776). The results in Table (9), indicate that there is no significant difference between the proposed method and the standard method.

Table 9: Analysis of sulphadiazine pharmaceuticals by proposed and standard**.**

Drug	Drug content (µg / 25 ml)	Recovery, (%)*		t-exp.
		Present method	Standard method	
Flammazine	100	100.74	99.80	0.602
Sulphaprim	100	101.14	100.24	0.760

*Average of five determinations

Comparison of the methods

Table 10 shows the comparison between some of analytical variables obtained from the present method with that of the recent spectrophotometric methods.

Table 10 : Comparison of the methods.

Analytical parameters	**Present method**	**Literature method (Mansour, 2002)**	**Literature method (Al-Attas , 2003)**
pH	12.45	3.19	3.0
Temperature (°C)	Room temperature	Room temperature	Room temperature
Development time (minutes)	5	10	10
λ_{max} (nm)	423	440	273
Reagent	Histidine	*m*-Aminophenol	Phenosaphranine
Beer's law range (ppm)	0.4 – 9.6	0.4 – 8.0	1.25 – 15.02
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	1.75×10^4	4.30×10^4	4.53×10^4
RSD (%)	$\leq \pm 0.87$	$\leq \pm 2.34$	$\leq \pm 0.49$
Stability of the colour (minutes)	55	60	60
Colour of the dye	Yellow	Yellow	**- -**
Nature of the dye	1 : 1	1 : 1	1 : 1
Application of the method	Burn cream and veterinary injectable solution	Veterinary suspension and serum	Tablets

The results in Table (10), shows that the suggested method for the determination of sulphadiazine is sensitive.

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