Spectrophotometric Determination of Some Sulphonamides in Aqueous Solution Via Azo – Dye Formation Reaction

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Received	Accepted
27/10/2003	15/12/2004

الخلاصة

يتضمن البحث طريقة طيفية لتقدير كميات متناهية في الصغر من السلفونااميدات(ستة مركبات) في محاليل مائية، تعتمد الطريقة على تحويل السلفوناميد إلى مشتق الدايازونيوم المقابل ثم اقترانه مع البايروكالول لينتج صبغة- آزو ذائبة في الماء ومستقرة وذات لون أصفر وتعطي أعلى امتصاص عند طول موجي مقداره 420 نانوميتر. وكانت حدود قانون بير0.4- 14.4 جزء / مليون جزء والخطأ النسبي بين – 2.47 و + 3.41 % والانحراف القياسي النسبي بين 0.51 ± و 2.90 ±% اعتماداً على درجة التركيز.وتم تطبيق الطريقة بنجاح في تقدير السلفاسيتامايد صوديوم في مستحضر دوائي (قطرة للعين).

ABSTRACT

A spectrophotometric method for the determination of trace amounts of some sulphonamides (six compounds) in aqueous solutions is described. The method is based on diazotisation and coupling with pyrogallol to form a yellow coloured, stable and water – soluble azo- dye which shows maximum absorption at 420 nm. Beer's law is obeyed over the concentrations range of 0.4 – 14 ppm. The relative error is –2.47 to +3.41 % and the relative standard deviation is ±0.51 to± 2.9 % depending on the concentration level. The proposed method has been successfully applied to the determination of sulphacetamide sodium in eye drop drug.

INTRODUCTION

The oldest spectrophotometric method for the determination of sulphonamides was based on Bratton – Marshall procedure.(1) It involves diazotisation of sulphonamide with excess of nitrite, destroying the unreacted nitrite, and the product was then coupled with N-(1-naphthyl) ethylenediamine

forming an intense, water – soluble azo- dye which shows a maximum absorption at 545 nm. Several other coupling agents were also recommended, including ethyl-N-2-naphthalene diamine,(2) chromatropic acid,(3) 8-hydroxyquinoline,(4) diaminoacridine,(5) ethyl acetoacetate,(6) phloroglucinol,(7) thymol,(8) sodium 4-aminosalicylate,(9) ethyl hydrogen - hydroxy - (isobutyl amino) benzylphosphate,(10) 3-aminophenol,(11)indol,(12)orcinol(13), N-(1-naphthyl) ethylenediamine in an aqueous solution(14) and primaquine phosphate (15).

Chromogenic reagents such as red and blue alizarin have also been used for the determination of sulphonamides in some pharmaceutical preparations (16).

The coloured reagent p- dimethyl amino cinnamic aldehyde has been used in the determination of some sulphonamides in urine. (17).

The present paper describes a spectrophotometric method for the determination of some sulphonamides based on coupling of the diazotised sulphonamide in basic medium with pyrogallol in aqueous solution forming an intensely azo-dye. Application part included determination of sulphacetamide sodium in eye drop drug.

EXPERIMENTAL

Apparatus:

Absorbance measurements were carried out on a CECIL-CE 1021 digital single beam spectrophotometric and Shimadzu - UV – Visible Recording Spectrophotometric UV – 160, using 1 – cm matched silica cells.

Reagents

All chemicals used were of analytical – reagent grade.

All sulphonamides were obtained in highly pure form and in pharmaceutical preparations from the state drug industry (SDI) Sammera – Iraq.

Standard sulphacetamide sodium solution (500 mg/l)

A 0.1g of sulphacetamide was dissolved in 10 ml absolute ethanol and diluted to 200 ml in a volumetric flask with distilled water.

Working sulphacetamide sodium solution (100 mg/l)

This solution was prepared by simple dilution of standard sulphacetamide sodium solution with distilled water.

Sulphonamides solutions (500 and 100 mg/l)

These solutions were prepared in the same manner as in the preparation of sulphonacetamide solution.

Pyrogallol solution (5×10^{-3} M)

A 0.1576g of pyrogallol was dissolved in 250 ml with absolute ethanol.

Sodium nitrite solution, 0.2%

A 0.2 g of sodium nitrite was dissolved in 100 ml distilled water in a volumetric flask.

Hydrochloric acid solution, 0.3 N

This solution was prepared by appropriate dilution of the concentrated hydrochloric acid with distilled water.

Urea solution, 5%

A 5 g of urea was dissolved in 100 ml distilled water in a volumetric flask.

Sodium carbonate solution, 0.2 M

A 2.12g of sodium carbonate was dissolved in 100 ml distilled water in a volumetric flask.

Foreign compounds solution, 500 mg/l

All foreign compounds solutions were prepared by dissolving 0.05g of each compound in 100ml distilled water.

Samacetamide solution, 100 mg/l.

A 2 ml of eye drops sterile (samacetamide 10%) was diluted to 100 ml in a volumetric flask with distilled water .A 5 ml of the above solution was diluted to 100 ml in a volumetric flask with distilled water.

RESULTS AND DISCUSSION

Absorption spectra

When a dilute solution of diazotised sulphacetamide and pyrogallol were mixed in carbonate medium a yellow azo-dye was formed. The coloured azo-dye showed maximum absorption at 420 nm in contrast to the reagent blank which showed very low absorption over the region scanned of 390 – 460 nm (Fig. 1).

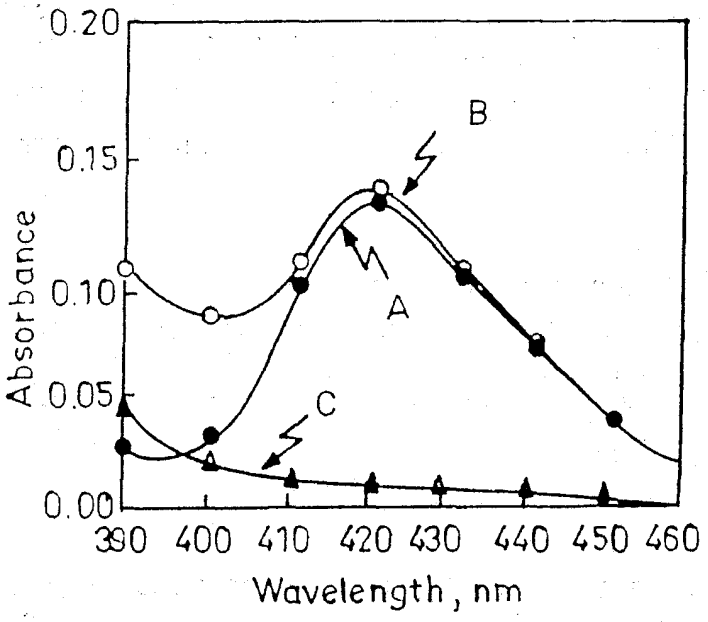


Fig. 1: Absorption spectra of 100 µg

sulphacetamide / 25 ml measured against (A) blank (B) distilled water and (C) blank measured against distilled water.

Study of the optimum reaction conditions

The various parameters affecting the yellow dye product have been studied and the optimum conditions have been selected .

For the subsequent experiments 100 μg of sulphacetamide sodium was taken and the final volumes were brought to 25 ml with distilled water.

Effect of diazotisation acid

When no acid is present, diazotization does not take place even with a large excess of nitrite (18), the effect of the amount of different acids (strong and weak) used for diazotisation of sulphacetamide has been studied. The experimental data revealed that hydrochloric acid was the most suitable of the commonly known acids, 1 ml of 1NHCL solution gives the highest intensity of the azo-dye produced, and therefore this volume is recommended in the subsequent experiments

Effect of nitrite

The effect of nitrite, as a component for the diazotisation of sulphacetamide and hence the intensity of azo- dye formed, has been investigated to verify its optimum amount. The experimental data showed that 1 ml of 0.2 % sodium nitrite solution with 3 minutes as standing time gave a high intensity and it is recommended in the subsequent experiments.

Removal of residual nitrite

The amount of unreacted nitrite should be removed because of undesirable reactions, which result in the disturbance of the production of the azo-dye and a very high blank absorbance, has been observed in basic medium [19].

The experimental data indicated that 1 ml of 5 % urea was found to be satisfactory for destroying the residual nitrite with 5 minutes as standing time.

Effect of pyrogallol amount

The effect of pyrogallol amount on the colour intensity for the azo-dye was studied. The results showed that 1 ml of 5×10^{-3} M of pyrogallol solution was optimum, it gives the highest intensity of the azo-dye produced with corresponding low absorbance of blank, therefore this volume is recommended in the subsequent experiments.

Effect of base

Preliminary experiments have showed that the presence of an alkali in the reaction mixture was essential for developing a more intense yellow colour. In

this respect, ammonium hydroxide, sodium hydroxide, sodium carbonate, sodium bicarbonate and sodium acetate were examined. It was found that the high intensity of the dye was obtained when on using 1.8 ml of 0.2 M sodium carbonate with minimum absorbance of blank at 420 nm.

Effect of time

Table 1 shows the effect of standing time on the intensity of the formed azo-dye.

Table1. Effect of time on absorbance

µg of sulphacetamide sodium/25ml	Absorbance /minute standing time							
	0	5	10	20	30	40	50	60
100	0.113	0.138	0.137	0.135	0.134	0.130	0.128	0.125

The results illustrated in Table 1 show that the colour develops completely within 5 minutes and remains maximum stable for another 25 minutes. The stability period obtained is sufficient to perform many measurements.

Study of interferences

The interfering effects of various compounds usually added as excipients in pharmaceutical preparation were examined by determining 100 µg of sulphacetamide sodium in the presence of 5 fold excess of each of interfering species using the recommended procedure. The results obtained are summarised in Table2.

Table2. Effect of 5 fold excess of foreign compounds on the determination of 100 µg of sulphonacetamide.

Foreign compounds	The percentage error(%)
Acacia	0.89 -
Glucose	0.66 -
Lactose	1.73 +
Starch	1.03 -
Sucrose	0.95 +

The results in Table2 show that experimentally non of the added foreign compound give an interfering effect presumably because they posses no reactivity towards electrophilic substitution

Recommended Procedure and Calibration Graph

To a series of 25 ml calibrated flasks, transfer increasing volumes of sulphacetamide (100 mg/l) solution to cover the range of 10 – 400 μg, 1 ml of 0.3 N HCl and 1 ml of 0.2% sodium nitrite solution and mixed well, then stand for 3 minutes. Then 1 ml of 5% urea solution was added, mixed and stand for another 5 minutes. Add 1 ml of 5×10^{-3} pyrogallol solution followed by adding 1.8 ml of 0.2 M sodium carbonate. Dilute the solution to the mark with distilled water and allow the reaction mixture to stand for 5 minutes. Measure the absorbance against a reagent blank, prepared in the same way but without sulphacetamide, at 420 nm using 1 – cm cells. The same procedure can be used for the determination of other sulphonamides. The molar absorptivity, the range of linear determinations and Sandell's sensitivity index are shown in (Table3).

Table3. The linear ranges, molar absorptivity and Sandell index for sulphonamides under investigation.

Compound	Linear range (mg/l)	ϵ_{max} $l.mol^{-1}.cm^{-1}\times10^{-3}$	Sandell's sensitivity ($\mu g.cm^{-2}$)
Sulphacetamide sodium	0.4-14	7.24	0.032627
Sulphanilamide	0.4-12	3.62	0.047569
Sulphamerazine	0.4-14	4.53	0.05128
Sulphadimidine	0.4-12	4.49	0.061982
Sulphathiazole	0.4-10	4.71	0.054206
Sulphadiazine	0.4–10	3.85	0.065005

Accuracy and precision

To estimate the accuracy and precision of the method, the sulphonamides were determined at three different concentrations. The results obtained are shown in Table 4 indicating that the method is satisfactory.

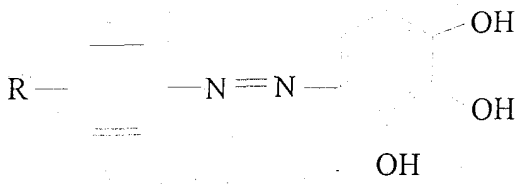
Table4. Accuracy and precision of the proposed method.

Compound	Relative error (%)*, mg/l compound taken			Relative standard deviation (%)*, (mg/l) compound taken		
	1	5	10	1	5	10
Sulphacetamide sodium	+3.38	+0.66	-0.57	±2.74	±1.91	±0.84
Sulphanilamide	+3.41	+0.95	+0.08	±2.81	±1.64	±0.55
Sulphamerazine	+1.87	+2.01	-1.22	±2.28	±1.05	±0.62
Sulphadimidine	-0.79	+2.16	+3.01	±1.95	±1.13	±0.57
Sulphathiazole	+3.03	-0.60	-2.47	±2.90	±2.02	±0.88
Sulphadiazine	+0.55	-1.71	+2.76	±2.14	±1.07	±0.51

*For five determinations.

Nature of the dyes

The stoichiometry of the reaction between diasotized sulphonamides and pyrogallol in carbonate medium was investigated by the mole – ratio and Job's methods, the results showed that the dyes have the composition of 1:1 pyrogallol to diazotised sulphonamides. Hence the dye may have the following structure:



$R = SO_2\text{-}N\text{-}R'$ (R'= changed according to the type of sulponamide)

H

Assay of sulphacetamide in eye drop

The method was applied for the assay of sulphacetamide sodium in samacetamide eye drop by taking 0.2,0.4,0.8,and 1.0ml from samacetamide eye drop solution then the recommended procedure was applied. The results, obtained showed that a good agreement has occurred between the amount of sulphacetamide sodium taken and measured by the recommended procedure (Table5).

Table 5.Assay of sulphacetamide in samacetamide eye drop*.

µg Sulphacetamide sodium present / 25 ml	µg Sulphacetamide sodium measured / 25 ml	%Recovery**
20	20.22	101.12
40	40.80	102.00
80	79.36	99.21
100	101.90	101.90

*The drug from the state company for drug industries and medical appliances
**Average of three determinations.

Comparison of the methods and t-test

A comparison between the present method and the standard method(20)(involving visual titration with sodium nitrite solution using starch-iodide paper as external indicater) for the determination of sulphacetamide in samacetamide eye drop, ,is based on the t-test to show the ability of using the present method in the determination of sulphacetamide in samacetamide eye drop (Table 6).

Table 6 .Comparison of the methods and experimental t-test values

Drug	Recovery% of sulphacetamide sodium*		t-exp.
	Present method	British Pharmacopoeia method	
Samacetamide eye drop	98.20	97.95	2.165

*Average of four determinations.

The results in Table (6) indicate that the calculated experimental t-values are less than their values in the statistic table at confidence level (95%) and for three degrees of freedom (2.353).These results indicated that there is no significant difference between the present method and the standard method .

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