SPECTROPHOTOMETRIC ASSAY OF SOME SULPHONAMIDE DRUGS VIA OXIDATIVE COUPLING[+]

Saladdin M.AL. Talib[*] **Deia. A.A Youns[**]** **Razan Y. Q. Al – Abachi[***]**

Abstract

A simple, rapid and sensitive spectrophotometric method for the determination of some sulphonamide drugs in aqueous solution is described. The method is based on the oxidative coupling reaction of phenothiazine with sulphonamide drugs in the presence of N-chlorosuccinimide as oxidizing agent forming an intense green, water- soluble dye and has a maximum absorption at 610 nm. The molar absorptivities range from 16.23×10^3 to 25.99×10^3 $l.mol^{-1}.cm^{-1}$ and Sandell sensitivities range from 0.986×10^{-2} to 1.542×10^{-2} µg $.cm^{-2}$. The method is applied successfully for the determination of sulphonamide drugs in bulk and some of their pharmaceutical preparations with comparable to the standard method (Bratton-Marshall).

Keywords:- Sulphonamide drugs determination , phenothiazine , spectrophotometric .

المستخلص:-

يتضمن البحث طريقة طيفية بسيطة وسريعة وحساسة لتقدير بعض أدوية السلفانوميد في محلول مائي. تعتمد الطريقة على تفاعل أقتران تأكسدي لأدوية السلفانوميد مع الفينوثايزين بوجود N- كلوروسكسيناميد كعامل مؤكسد حيث تكونت صبغة خضراء ذائبة في الماء تعطي أعلى امتصاص عند طول موجي مقداره 610 نانوميتر. تراوح معامل الامتصاص المولاري بين 16.23×10^3 الى 25.99×10^3 لتر. مول$^{-1}$. سم$^{-1}$ وتراوحت حساسية سيباندل بين 0.986×10^{-2} الى 1.542×10^{-2}. مايكروغرام. سم$^{-2}$. طبقت الطريقة بنجاح في تقدير أدوية السلفانوميد غير المعبئة وبعض مستحضراتها الصيدلانية بمقارنة النتائج مع الطريقة القياسية (براتون – مارشال) .

Introduction:-

Sulphonamides were discovered in 1908; over 5,000 closely related compounds have been synthesised. of these, only very few have proved to be clinically useful . The sulphonamide drugs are active against both gram positive and gram negative bacteria (bacteria that are stained purple (positive) or stained pink (negative) by Gram's solution respectively. In most instances, they are bacteriostatic) that is, they inhibit the growth and multiplication of the bacteria, but do not kill them[1-3].

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[*] Professor / Medical Technical Institute- Mosul

[**] Lecturer / Medical Technical Institute- Mosul

[***] Asst. Professor / Medical Technical Institute- Mosul

Various methods have been reported for the determination of sulphonamides with phenothiazine and hypochlorite or cupper (II)[4], ceric ion[5], benzoyl peroxide[6] and sodiummetaperiodate[7] .

The objective of the present investigation reported in this paper was to evaluate a spectrophotometric method for the determination of sulphonamides based on the reaction of sulphonamides with phenothiazine (thiodiphenylamine) in the presence of N-chlorosuccinimide forming intensly colored products. A comparative study of this method with the popular Bratton – Marshall method[8] was made .

Material and methods:-

All chemicals used are of analytical grade. All sulphonamide drugs were obtained in highly pure form and in pharmaceutical preparations from the state drugs industry (SDI) Sammara-Iraq.

Standard sulphonamide solution (500μg.ml^{-1}) : 0.1000g of each sulphonamide drug were dissolved in 10 ml ethanol and diluted to 200 ml with distilled water .

Dilute standard sulphonamide solutions (50 μg .ml^{-1}) were prepared by simple dilution of the appropriate volume of the each sulphonamid solution (500 μg .ml^{-1}) with distilled water .

Phenothiazine solution (5×10^{-4}M):0.0199 g of phenothiazine was dissolved in 200 ml ethanol.

N-Chlorosuccinimide solution (3×10^{-3}M) : 0.0801g of N-chlorosuccinimide was dissolved in 200ml distilled water.

Procedure:-

Into a series of 25-ml volumetric flasks, transfer 6 ml of 5×10^{-4} M phenothiazine . Add increasing volumes of sulphonamide working solution (50μg.ml^{-1}), followed by 2ml of 0.1N hydrochloric acid and 2ml of 5×10^{-2} M N-chlorosuccinimide. Dilute the solution to the mark with distilled water and allow the reaction mixture to stand for 30 minute. Measure the absorbance at 610nm against reagent blank using 1cm cells.

Procedures for Pharmaceutical Preparations tablets :- Weigh and finally powder 10 tablets. Extract an accurately weighed portion of the powder equivalent to about 0.1g of sulphonamide with 20ml of ethanol; shake and warm the solution if necessary. Filter the solution into 200-ml calibrated flask, wash the residue with 10ml of ethanol and diluted to the volume with distilled water to obtain 500μg. ml^{-1} of sulphonamide. Dilute the solution with distilled water to obtain 50μg. ml^{-1} of sulphonamide. Use 5ml of this solution for reaction described in the procedure .

Ophthalmic Solution:- Dilute a suitable volume of the ophthalmic solution containing about 0.1g of sulphonamide with 30ml of ethanol and distilled water to obtain a solution

containing 500μg.ml^{-1} of sulphonamide. Dilute the solution with distilled water to obtain 50 μg.ml^{-1} of sulphonamide. Use 5ml of this solution for reaction described in the procedure.

Results and Discussion

A green colored oxidative coupling product with an absorption maximum at 610nm is formed when sulphonamide was allowed to react with phenothizaine in the presence of N-chlorosuccinimide. The absorption spectra of the resulting colored products are shown in Fig 1 and their spectral characteristics are summarized in Table 1.

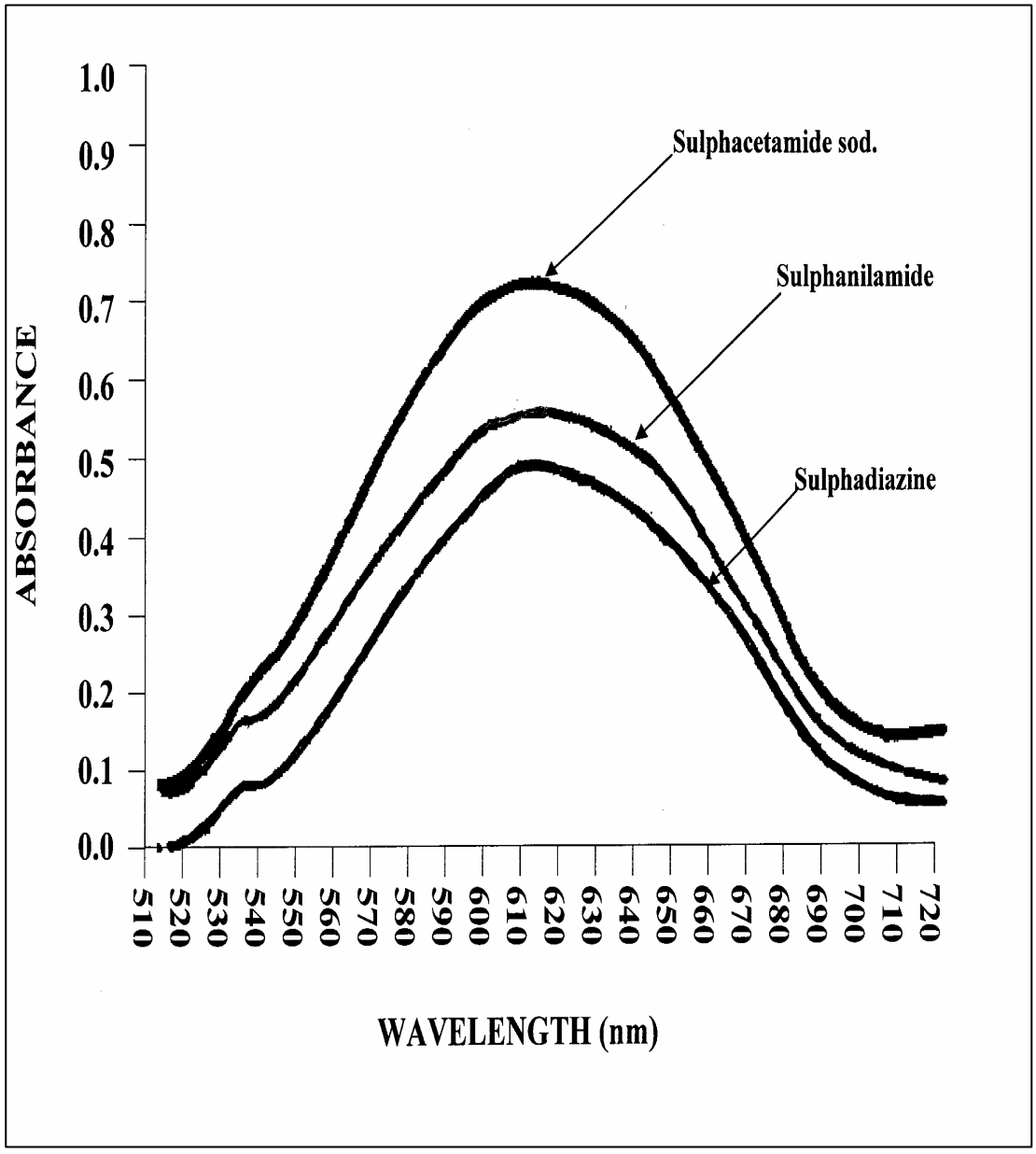

Fig. 1

Table 1: Spectral characteristics of the products from the reaction of phenothiazine with sulphonamide drugs.

Compound	λ max(nm)	(l. mol^{-1} . cm^{-1})	Sandell sensitivity (µg. Cm^{-2})
Sulphanilamide	610	1.746×10^4	0.986×10^{-2}
Sulphadiazine	610	1.623×10^4	1.542×10^{-2}
Sulphacetamide sodium	610	2.599×10^4	1.01×10^{-2}

The influence of various reactions variables were tested to establish the optimum conditions for the proposed procedure and the most suitable values of the variables tested based on absorbance obtained in each case.

When various concentrations of phenothiazine in ethanol were added to a series of 25ml volumetric flasks and diluted to the mark with distilled water turbidity was observed in the case of the phenothiazine concentration more than 1.2×10^{-3} M that may be due to the phenothiazine is insoluble in water. The highest and most reproducible absorbances were obtained by using 6ml of 5×10^{-4}M phenothiazine solution in ethanol .

The effect of pH on the sensitivity of the colored reaction product was investigated in the range of 2 – 8 (tartaric acid and sodium hydroxide buffer solution). Only in acidic medium the reaction was carried out . Finally the reaction was carried out in various acids (hydrochloric acid , sulphuric acid , phosphoric acid , acetic acid , oxalic acid and citric acid) to establish which was the most suitable acid for the reaction. The experimental data showed that hydrochloric acid (2ml of 0.1N) was the most suitable acid.

The concentration of N-chlorosuccinimide was found to affect markedly the colour intensity. The optimum concentration of N-chlorosuccinimide was 2ml of 5×10^{-2} M.

The calibrated flasks are placed in different temperatures ranged from 0C° to 70Cº for 30 minutes. No remarkable change in the absorbance through this range. Therefore , it is recomended to proceed the reaction at room temperature.

The reaction time was determined by following the development of colour at different times. Maximum absorption was obtained after 30 minute . The colour obtained was stable for at least 6 hr.

The reaction has been shown to be dependent on the order of the addition of reagents to the sample solution . It was found that the use of the following order; phenothiazine solution , sample solution , hydrochloric acid and N-chlorosuccinimide solution obtain optimum results.

Analytical Application:-

Typical calibration data for the three sulphonamides investigated obtained form linear regression analysis of absorbance reading vs. concentration of each drug (Fig.2) gave the slopes , intercepts and correlation coefficients presented in Table 2.

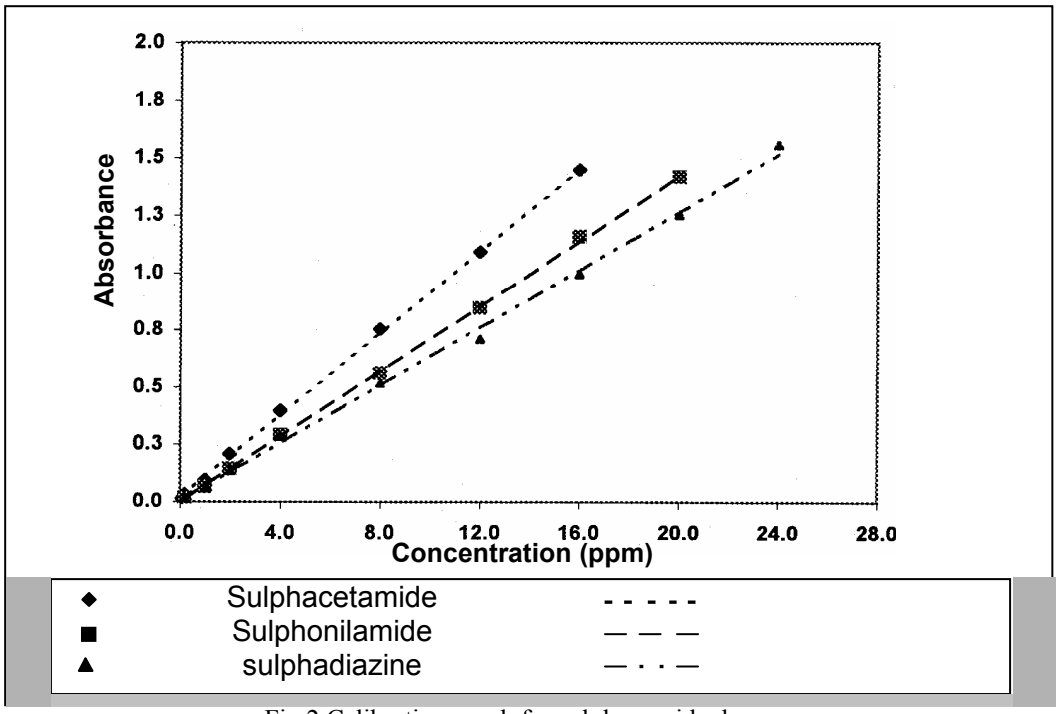

Fig 2 Calibration graph for sulphonamide drugs

Table 2: Analytical data for phenothiazine colour reaction with sulphonamide drugs.

Compound	Linear range µg.ml^{-1}	Intercept	Slop µg^{-1}	Correlation coefficient
Sulphanilamide	0.2-20	-0.0005	0.0712	0.9996
Sulphadiazine	0.2-24	+0.0017	0.0633	0.9978
Sulphacetamide sodium	0.1-16	+0.0182	0.0898	0.9994

The linear calibration graphs obtained for the sulphonamides in vestigated can also be used for calculation of concentrations. To estimate the accuracy and precision of the method, six replicate determinations were made at three different concentrations (1,8,16µg ml^{-1}). The results indicated that the present method is accurate and precise (Table 3).

Table 3: Accuracy and precision of the method

Compound	Mean* recovery (%)	RSD*(%)
Sulphanilamide	+0.9	1.3
Sulphadiazine	-1.7	2.1
Sulphacetamide sodium	+2.6	1.8

* Average of six determinations.

Sulphonamides are usually formulated in tablet form. Therefore , the effect of common tablet excipients and additives on the procedure was investigated. It was found that glucose, lactose, sucrose, starch, magnesium stearate and acacia had no effect on the assay (table 4) .

Table 4: Effect of excipients on the recovery of sulphadiazine by the method.

Excipient	Amount per 10 mg of drug / mg	Recovery * %	R.S.D. %*
Glucose	0.25	100.6	1.83
Lactose	1.00	102.1	1.99
Sucrose	0.25	98.5	1.06
Starch	1.00	101.3	1.75
Acacia	0.25	101.9	1.72

* Average of three determinations.

Table 5 shows the results obtained from the determination of sulpha drugs in pure forms and in some of their dosage forms, both by means of the proposed method and by the Bratton –Marshall procedure. These data indicate a good agreement between the results of the popular Bratton – Marshall method and, consequently, the suitability of the method for quality control analysis due to its rapidity, simplicity, high accuracy and precision (as demonstrated by a good coefficient of variation) . Another favourable characteristic of the present method is that the absorbance of the product formed is stable for at least 6 hr .

Table 5: Assay of sulpha drugs in bulk and dosage forms by the phenothiazine and Bratton-Marshall procedures.

Compound	Average of recovery *	
	Phenothiazine method	Bratton-Marshall method
Sulphanilamide	101.5	98.7
Sulphadiazine	97.8	99.1
Sulphacetamide sod.	100.7	101.3
Sulphadiazine table	99.6	98.6
Sulphacetmide sod. Drops	102.1	100.4

* average of three determinations.

Conclusion:-

A spectrophotometric method for the determination of some sulphonamide drugs in an aqueous solution is described. The method is based on the oxidative coupling reaction of phenothiazine with sulpha drugs in the presence of N-chlorosuccinimide. The proposed method compared favorably with the previous methods, (Table 6) it is considered to be simple (its dose not need heating and extraction) , rapid (30 minute development time) , accurate (relative error $\leq$ 2.6 %) and precise (R.S.D. $\leq$ 2.1%) . The proposed procedure for bulk sulphonamide drugs and some of their pharmaceutical preparations was found to be accurate and precise compared with Bratton – Marshall procedure.

Table 6: Comparison of previous and proposed methods

Analytical parameters	Previous methods					Proposed method
	Phenothiazine (4)		Phenothiazine (5)	Phenothiazine (6)	Phenothiazine (7)	Phenothiazine
Oxidant	Hypochlarite	Cupper (II)	Ceric ion	Benzoyl peroxide	Sodium meta periodate	N-chlorosu-ccinimide
Medium	Ethanol	Chloroform	Aqueous	Aqueous	Aqueous	Aqueous
λ max (nm)	515	515	614	614	612	610
Development time (min)	-	-	15	15	15	30
Stability period (hr)	3	3	6	24	24	6
Linear range ($\mu g.ml^{-1}$)	4-40	4-40	0.2-22	0.1-26	0.05-22	0.1-24
Molar absorptivity $X10^3$ ($l.mol^{-1}.cm^{-1}$)	3.56-8.09	4.64-10.7	15.8-24.0	16.7-24.5	20.1-30.5	16.23-25.99
Relative error %	≤± 1.1	≤± 1.95	≤± 1.77	≤±1.15	≤± 1.5	≤± 2.6
RSD %	<± 2	≤± 2	≤± 1.89	≤± 0.99	≤± 1.8	≤± 2.1
Analytical application	Tablet	Tablet	Tablet, Eye drops, Urine, Blood	Tablet, Eye drops, Urine, Blood	Tablet, Eye drops, Urine, Blood	Tablet, Eye drops

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