Application of Trifluoperazine Hydrochloride as a Chromogenic Reagent for Spectrophotometric Determination of Sulphacetamide Sodium – Application to Ophthalmic Preparations

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ABSTRACT

A simple, rapid and sensitive spectrophotometric method for the determination of sulphacetamide sodium (SAS) was described. The method based on the oxidative coupling reaction of trifluoperazine hydrochloride (TFP) with SAS using ammonium ceric sulphate (ACS) as oxidizing agent in acidic medium to form a water soluble product, that is stable and has a maximum absorption at 560 nm. Beer's law is obeyed in a concentration range of 1-22 $\mu g.ml^{-1}$ SAS with a molar absorptivity of 7.0×10^3 $L.mol^{-1}.cm^{-1}$, a relative error of -0.56 to $+2.33\%$ and a relative standard deviation of ±2.56 to $\pm4.56\%$ depending on the concentration of SAS. The proposed method has been successfully applied to the determination of SAS in various ophthalmic preparations.

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$^{1-}$.   22   1

$\%+2.33$ -0.56 $^{1-}$ $.^{1-}$. $^310\times7.0$

. $\%\pm4.56$ ±2.56

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INTRODUCTION

Sulfa drugs were among the first pharmaceutical agents to be used clinically against infection. Although they have largely been replaced by safer and more powerful antibiotics but they are still prescribed for infections of urinary tract. (McMurry, 2004).

Sulphacetamide sodium (SAS), N-sulphanilyl acetamide mono sodium salt is very soluble in water it is especially suited as a solution for repeated topical applications in the local management of ophthalmic infections susccptible to sulphonamide therapy, (Jaim and William, 1991).

Various spectrophotometric methods have been used in the determination of SAS. These methods included oxidative coupling reaction using different reagent and oxidizing agents such as, phenothiazine and hypochlorine (Abdine et al., 1979), 3-methylbe-nzothiazolin-2-one hydrazone using iron (III) ion as oxidizing agent (El-Kommos and Emara, 1988), promethazine hydrochloride and hypochlorite (Al-Abachi et al., 1990), phenothiazine and ceric ion (Al-Abachi and Al-Talib, 1994), 4-amino-N, N-dimethy-laniline and dichromate (Al-Abachi and Al-Talib, 1995) and phenothiazine and sodium metaperiodate (Al-Talib and Qasim, 1997).

Another spectrophotometric methods included diazotisation of SAS and coupling the corresponding diazotised with different coupling agents such as N-(1-naphthyl) ethylenediamine (Bratton and Marshall, 1939), chromotropic acid (Hohlein, 1967), ethylacetoacetate (Belal et al., 1978), phloroglucinol (Krishna and Sastry, 1979), thymol (Grabowska and Weclawska, 1979), sodium 4-aminosalicylate (Krishna and Sastry, 1980), 8-hydroxyquinoline (Ismail, 1986), orcinol (Sadana et al., 1987), primaquine phosphate (El-Sayed, 1999), Iminodibenzyl (Nagaraja et al., 2002), 3-aminophenol (Nagaraja et al., 2003) and pyrogallol (Othman, 2005).

The purpose of the investigation reported in this paper is to evaluate a simple spectrophotometric method for the determination of SAS based on it is oxidative coupling reaction with trifluoperazine hydrochloride in the presence of ceric ion as oxidizing agent yielding highly coloured dye in acidic medium .Application part included determination of SAS in different ophthalmic preparations.

EXPERIMENTAL

Apparatus:

All spectral and absorbance measurements were performed on Shimadzu UV-Visible-160 double beam recording spectrophotometer using 1 cm silica cell. pH meter type Philips PW 9420 was used for pH reading.

Reagents:

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

Solutions:

SAS solution, 500 μg.ml^{-1}: This solution was prepared by dissolving 0.05 g of SAS in 10 ml of ethanol to increase solubility and diluted to 100 ml in a volumetric flask with distilled water.

Ammonium ceric sulphate (ACS) solution, 4×10^{-3}M: This solution was prepared by dissolving 0.253 g of ammonium ceric sulphate dihydrate in 100 ml of 0.05 N sulphuric acid solution.

Trifluoperazine hydrochloride(TFP) solution, 1.5×10^{-2} M: This solution was prepared by dissolving 0.721 g of TFP in 100 ml of distilled water.

Ophthalmic solution: A suitable volume (0.5 ml) of the ophthalmic solution containing exactly 0.05 g of SAS was treated with 5 ml of ethanol and diluted to 100 ml with distilled water to give 500 μg.ml^{-1} SAS, different volumes of this solution latter was used to be determinated by proposed method.

Procedure and calibration graph:

To a series of 25 ml calibrated flasks, transfer 1 ml of TFP reagent (1.5×10^{-2} M), an increasing volumes (0.05-1.4 ml) of SAS (500 μg.ml^{-1}) are added, shaking the solutions, followed by 3 ml of ACS solution (4×10^{-3} M) and the volumes are completed to the mark with distilled water, after 15 minutes the absorbances are read at 560 nm against reagent blank. The calibration graph is linear over the range 1-22 μg.ml^{-1} (Fig. 1).The apparent molar absorptivity reffered to SAS, has been found to be 7.0×10^{3} L. mol^{-1}. cm^{-1}.
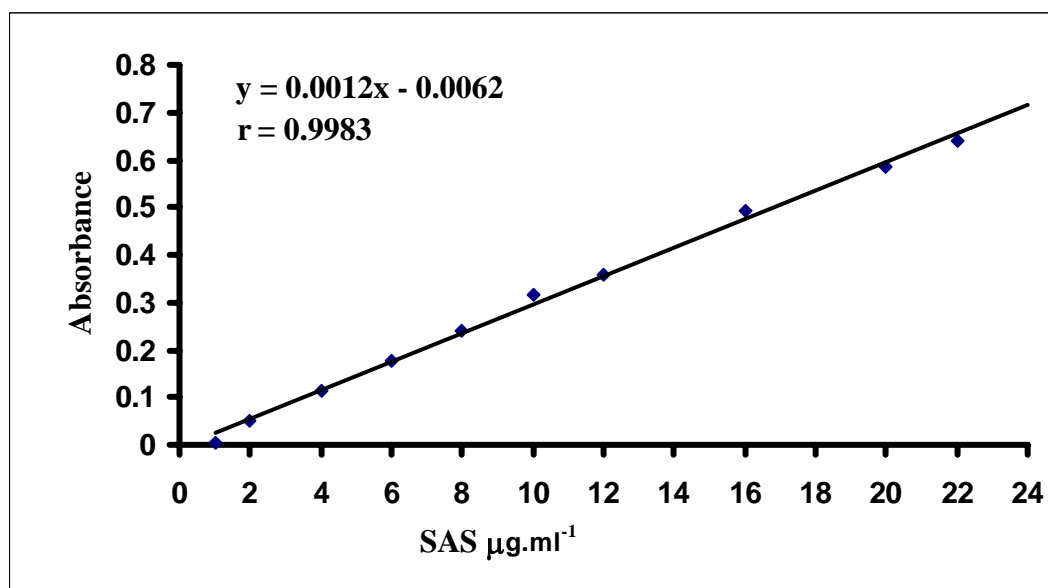

Fig. 1: The calibration graph for SAS.

Results and discussion:

The effect of various variables on the colour development is tested to establish the optimum conditions, the results indicated that the reaction between SAS and TFP in the presence of an oxidizing agent (ACS) produce highly coloured product which can be utilized as a suitable assay procedure for SAS in different ophthalmic preparations by spectrophotometric determination. The violet product has a maximum absorption at 560 nm (Fig. 2).
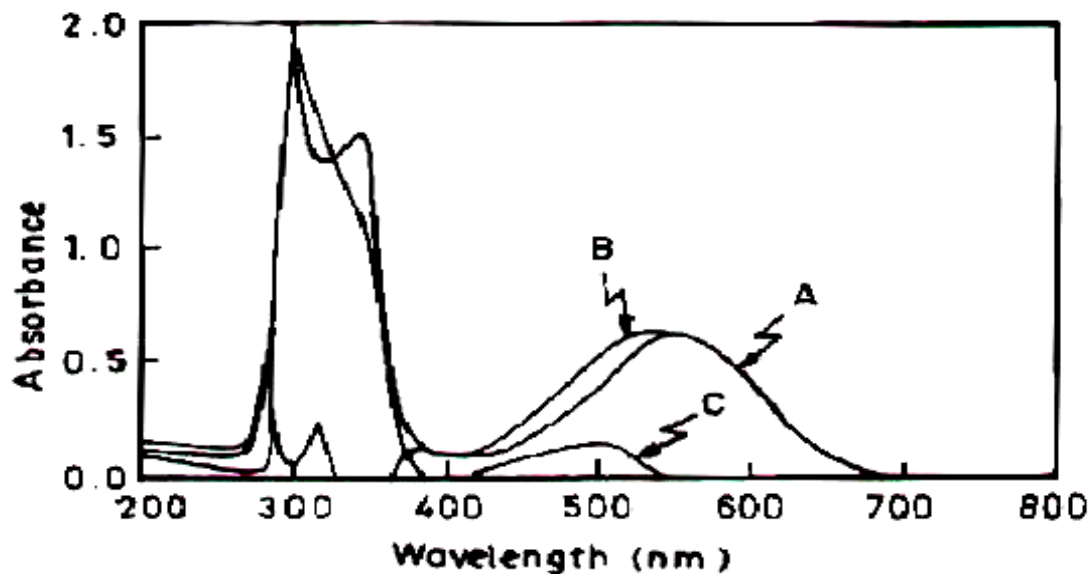
Fig. 2 : Spectrum absorbance for 20 $\mu g.ml^{-1}$ SAS treated according to the recommended procedure and measured against (A) blank, (B) distilled water and (C) blank measured against distilled water.

A 500 μg of SAS in final volume 25 ml (20 $\mu g.ml^{-1}$) was used for optimization.

Effect of Ph:

The preliminary experiments have shown that SAS can give coloured dye with TFP in presence ACS in acidic medium only. So that ACS solutions ($4 \times 10^{-3}M$) were prepared by dissolving the accurate weight in different concentrations of sulphuric acid solution ($5 \times 10^{-4} - 0.2$ N).

The results indicates that using of solution of ACS which prepared by using 0.05 N sulphuric acid solution was the most suitable acid solution for optimum pH 2.33 to produce the highest intensity (Table 1).

Table 1: Effect of pH on absorbance.

Normality of H_2SO_4	5×10^{-4}	1×10^{-3}	5×10^{-3}	1×10^{-2}	2×10^{-2}	0.05	0.1	0.2
Absorbance	0.306	0.329	0.428	0.459	0.468	0.483	0.318	0.166
Final pH	2.85	2.80	2.77	2.68	2.58	2.33	2.20	2.03

Effect of oxidizing agent

The effect of several oxidizing agents ($4 \times 10^{-3}M$) on intensity of the coloured dye were investigated in acidic medium (pH 2.33) the results is shown in Table (2).

Table 2: Effect of oxidizing agent.

Oxidozing agent*	ACS	N-Bromo succinimide	KIO_3	K_2CrO_4	$K_2Cr_2O_7$
Absorbance	0.480	0.272	0.350	0.077	0.112

* 3ml added

The results illustrated in Table (2). Indicated that ACS give the more sensitive reaction (highest intensity).

The effect of different volumes (0.5 - 4 ml) of ACS solution (4×10^{-3}M) on the colour intensity has been studied it was observed that 3 ml of ACS is the more suitable amount since it gives the highest intensity, therefore the volume 3 ml was recommended for the subsequent experiments.

Effect of TFP concentration:

Various volumes of TFP (1.5×10^{-2} M) were tested the final results indicated that using 1 ml of TFP solution give maximum absorbance of the coloured product (Table 3).

Table 3: Effect of reagent amount.

ml of TFP (1.5×10^{-2} M)	0.5	1	2	3	4
Absorbance of 500 µg SAS in 25 ml	0.400	0.582	0.537	0.462	0.292

Order of addition of reagents:

The order of additions of reagents was examined. The results (Table 4), indicate that the order (III) of addition of reagents was the optimum order it gives the highest intensity of the product.

Table 4: Effect of order of addition.

Order	I	II	III	IV	V	VI
Absorbance	0.499	0.429	0.582	0.422	0.423	0.428

I. SAS (S) + TFP (R) + ACS (O).
II. S + O + R.
III. R + S + O.
IV. R + O + S.
V. O + R + S.
VI. O + S + R.

Effect of temperature and time:

The effect of temperature on the colour intensity of the resulting dye was investigated. The results indicated that absorbance of the dye decrease when the colour developed at 60°C (in water bath) and there is no significant different when the colour developed between 0°C (in ice bath) to room temperature (Table 5).

Table 5: Effect of temperature and time on absorbance.

Temperature °C	Absorbance/minute								
	5	**10**	**15**	**20**	**25**	**30**	**35**	**40**	**45**
0	0.486	0.573	0.596	0.607	0.608	0.608	0.608	0.609	0.609
R.T.*	0.433	0.506	0.580	0.583	0.584	0.584	0.590	0.595	0.595
60	0.622	0.601	0.576	0.506	0.465	0.404	0.343	0.287	0.228

*R.T. = room temperature

The room temperature (25±3) is recommended for the subsequent experiments.

Effect of reaction time:

The effect of time on the development and stability period of the coloured dye was investigated under the optimum conditions of the reaction.

The maximum colour intensity was reached after about 15 minutes and the absorbance of the coloured dye remained constant for at least another 45 minutes. (Table 6).

The development time 15 minutes was selected as the optimum in the general procedure.

Table 6: Effect of time on absorbance.

SAS μg.ml^{-1}	Absorbance/minutes							
	0	**5**	**10**	**15**	**25**	**35**	**45**	**60**
2	0.009	0.029	0.031	0.051	0.051	0.051	0.051	0.050
4	0.043	0.070	0.077	0.112	0.112	0.112	0.112	0.108
8	0.115	0.175	0.190	0.246	0.246	0.246	0.246	0.246
16	0.268	0.370	0.396	0.458	0.457	0.457	0.457	0.450

Accuracy and precision:

To determine the accuracy and precision of the method, SAS was determined at three different concentrations. The results illustrated in Table (7), indicate that satisfactory precision and accuracy could be obtained.

Table 7: Accuracy and precision of the proposed method.

SAS µg.ml^{-1}	Relative error*, %	Relative standard deviation*,%
4	+2.30	±2.56
8	+2.33	±4.56
12	-0.56	±4.30

* Average of five determination

The nature of the reaction product:

Job's (Fig. 3) and mole-ratio (Fig. 4) methods indicate that the coloured product has a composition of 1:1 SAS to TFP reagent at 560 nm.
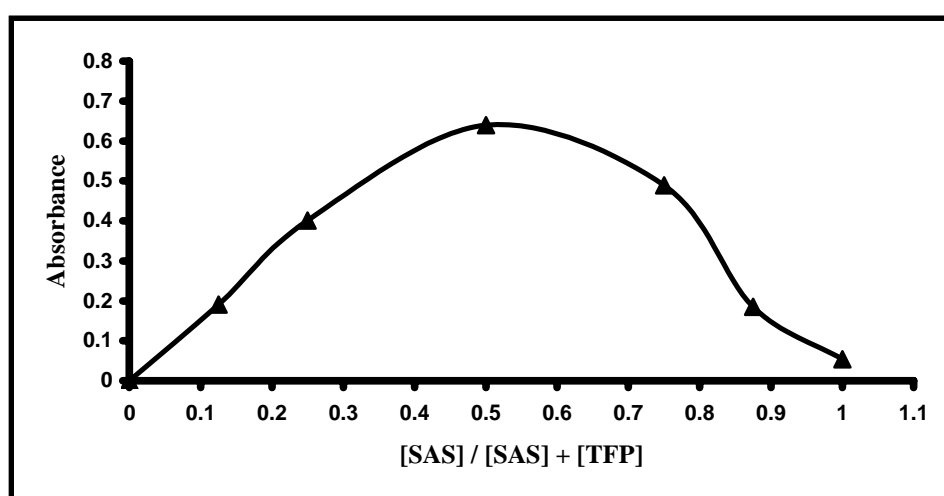

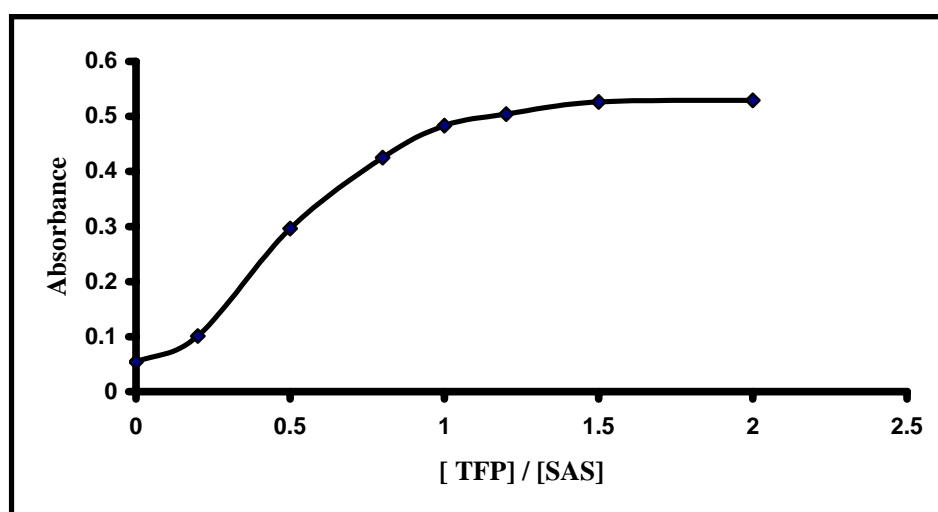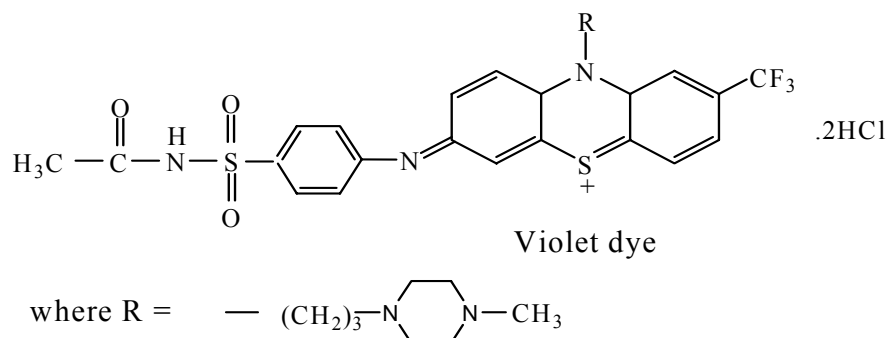Fig. 3 : Job's plot for SAS – TFP coloured product.

Fig. 4 : Mole ratio's plot for SAS - TFP coloured product.

Therefore, the structure of the formed dye may be written as follows:

Violet dye

where R =   — $(CH_2)_3$—N◯N—CH_3

Application:

The proposed method was applied to determine of SAS in several ophthalmic solutions (eye drops).

Several commercially available eye drops from different origins were examined for their content of SAS (Table 8).

Table 8: Application of the proposed method for the determination of SAS in eye drops.

Eye drops	µg SAS present	µg SAS measured	Recovery*, %
Apisulfa-10 (Jordan)	50	47.5	95
	100	104.0	104
	200	188.0	94
Ocusul-10 (Egypt)	50	52.5	105
	100	105.0	105
	200	186.0	93
Samacetamid (Iraq-SDI)	50	53.5	107
	100	99.0	99
	200	192.0	96

*Average of three determinations

The results illustrated in Table (8), indicated that a good recoveries of SAS were obtained.

Comparison of the methods and t-test:

A comparison between the present method and british pharmacopeia standard method (British Pharmacopeia on CD-ROM, 2000) for the determination of SAS in the three drugs, is based on the t-test to show the ability of using the present method in the determination of investigated drugs (Table 9).

Table 9: Comparison of the methods and experimental t-test values.

| Drug | Pharmaceutical preparation | Recovery*,% | | t.exp |
		Present method	British pharmacopeia method	
Apisulfa-10	Eye drops	97.92	95.67	1.020
Ocusul-10	Eye drops	95.80	95.19	1.040
Samacetamid	Eye drops	97.81	96.49	0.806

*Average of five determinations

The results in Table (9), indicate that the calculated experimental t-values are less than their values in the statistic table at confidence level (95%) and for four degrees of freedom (2.776). These results indicated that there is no significant difference between the present method and the standard method.

Table (10), shows the comparison between some of analytical variables obtained from the present method with that of a recent spectrophotometric method.

Tble 10: Comparison of the methods.

Analytical parameters	Present method	Literature method*
pH	2.33	2.73
Temperature ($^{\circ}$C)	At room temperature	At room temperature
Development time (minutes)	15	30
λ_{max} (nm)	560	550
Medium of reaction	Aqueous	Aqueous
Reagent	Trifluoperazine .2HCl	4-Amino-N,N-dimethylaniline
Beer's law range (ppm)	1 – 22	0.2 – 48
Molar absorptivity (l.mol^{-1}.cm^{-1})	0.7×10^{4}	1.13×10^{4}
RSD (%)	$\leq \pm 4.56$	$\leq \pm 1.17$
Stability of the colour (minutes)	45	60
Colour of the dye	Violet	Violet
Nature of the dye	1 : 1	1 : 2
Application of the method	Has been applied to the assay of SAS in ophthalmic solutions	Has been applied to the assay of SAS in ophthalmic solutions

* Al-Abachi M.Q. and Al-Talib S.M., J. Edu. and Sci.,(1995), 22 , p.172-185.

     The results indicate that the proposed method has a good sensitivity compared with the above literature method.

CONCLUSION

     A simple, rapid and sensitive method has been proposed for the determination of sulphacetamide sodium in pure form and in its pharmaceutical preparation (eye drops).
     The method based on the oxidative coupling reaction of trifluoperazine hydrochloride with sulphacetamide sodium using ACS in acidic medium. The method provides accurate and precise results.

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