Spectrophotometric Determination of Sulfacetamide and Sulfamethaxazole in Aqueous Solution Using Tetracyanoethylene Reagent

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الملخص

تم وصف طريقة طيفية يسيرة وحساسة لتقـدير الـسلفاسيتامايد و السلفاميثاكـسازول بأشكالها النقية وفي مستحضراتهما الصيدلانية. تعتمد الطريقة على التفاعل في المحلول المائي مع كاشف رباعي سيانوأثيلين بوجود بيكاربونات الصوديوم لتكوين نواتج صفراء اللون تمتلك أقصى امتصاص عند الأطوال الموجية ٣٥٥ و٣٥٦ نـانوميتر لكـل مـن الـسلفاسيتامايد و السلفاميثاكسازول على التوالي. أمكن تطبيق قانون بير ضمن مدى التركيـز ١-٣٠ و١-٢٥ مايكروغرام مل$^{-١}$ وبامتصاصية مولارية 10575 و 13146 لتر مول$^{-١}$سم$^{-١}$ في حين كانـت دلالة ساندل ٠,٠٢٤٠ و٠,٠١٩٢ مايكروغرام سم$^{-٢}$ لكل من المركبين الدوائيين على التوالي. كما وجد أن تلك النواتج تتكون بنسبة ١:١ . وتـم تطبيـق الطريقـة بنجـاح فـي تقـدير السلفاسيتامايد و السلفاميثاكسازول في مستحضراتهما الصيدلانية وكانت النتـائج متفقـة مـع المحتوى الأصيل وطريقة الإضافة القياسية.

Abstract

A simple and sensitive spectrophotometric method has been developed for determination of sulfacetamide and sulfamethaxazole. The method is based on the reaction of these drugs in aqueous solution with tetracyanoethylene reagent in the presence of sodium bicarbonate to produce yellow colored species measured at 355 and 356 nm for sulfacetamide and sulfamethaxazole respectively. Beer's law obeyed over the concentration range1-30 and 1-25 µg ml^{-1} with molar absorpitivity of 10575 and 13146 L.mol^{-1}.cm^{-1} and sandell index of 0.0240 and 0.0192 µg.cm^{-2} for the above drugs respectively. It was found that these products were formed in ratio of 1:1. The method was applied successfully to the

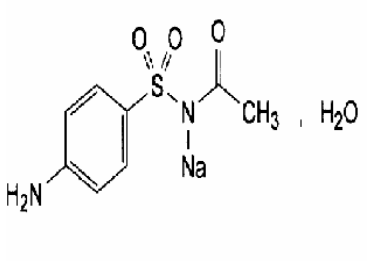
assay of sulfacetamide and sulfamethaxazole in their pharmaceutical formulations and was agreed well with the certified values and with standard addition procedure.

Key words: *Spectrophotometry, Sulfacetamide, Sulfamethaxazole, aqueous solution, Tetracyanoethylene*

Introduction

Sulfacetamide [N(4-aminophenyl)sulphonyl] acetamide(1) and sulfamethaxazole [4-amino-N(5-methylisoxazol-yl) benzene sulphonamides(II) are N-substituted derivatives of sulfanilamide which are widely used in the treatment of urinary tract infection burn therapy, conjunctivitis and chloroquine resistant malaria. They are also the drugs of choice for the treatment of nocardiosis toxoplasmosis, severe travelers diarrhea and meningococcal infections[1]. Sulfamethaxazole and sulfacetamide have been widely used for both prevention and treatment of diseases and as feed additives to promote growth in animal feeding operations and concentrated animal feeding operations[2]. Sulfonamides as additives are used at the level of 100 mg kg^{-1}[3].

Various methods have been reported for the determination of sulphonamides. These include gravimetric[4-5], titrimetric[6], potentiometric[7], amperometric[8] polarographic[9], chromatographic [10-11], flame spectroscopic[12], and fluorimetric[13], methods. Several spectrophotometric methods using various reagents such as 2,3-dichloro-5,6-dicyano-1,4-benzoquinone[14], 7,7,8,8 tetracyano quinodimethane[15], chloranil[16], 8-hydroxyquinoline [17], acetyl acetone-formaldehyde [18], furfuraldehyde [19], and N-(1-naphthyl) ethylene diamine [20], have been reported for determination of sulphonamide derivatives.



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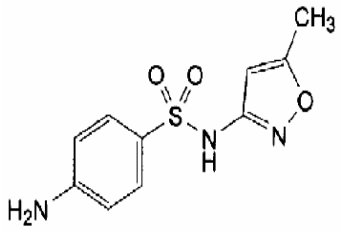


II

The goal of this study is developing simple and sensitive spectrophotometric method for determination of sulfacetamide and sulfamethaxazole in pure form and in their pharmaceutical preparations with TCNE reagent in aqueous solution.

General procedure

Accurately measured volumes of sulfacetamide and sulfamethaxazole solution containing 1-30 sulfacetamide and1-25 sulfamethaxazole μg ml^{-1} in a final dilution were transferred into a series of 25 ml calibrated flasks. A 1.2 ml of 5×10^{-3}M $NaHCO_3$ followed by addition of 1.2 and 1.0 ml of 1×10^{-2} M TCNE. The solutions were mixed and diluted to the mark with distilled water and left for10 min at room temperature. The absorbance was measured at 355and 356 nm for sulfacetamide and sulfamethaxazole respectively against their respective reagent blanks.

Procedure for the determination of sulfamethaxazole and sulfacetamide in pharmaceutical preparations.

Analysis of* sulfamethaxazole *tablets:

Five tablets of sulfamethaxazole contents (each tablet containing 400 mg) were weighed and finely powdered, an accurately weighed portion of the powder equivalent to 400 mg of pure sulfamethaxazole was dissolved in a minimum amount of ethanol and distilled water. The residue was filtered through Whatmann no. 42 filter paper into 500 ml standard flask and the filtrate was diluted to the mark by repeated washing with distilled water. Aliquots of this solution containing 1-25 μg ml^{-1} of sulfamethaxazole were transferred into 25 ml volumetric flasks and treated in the same way as the standard. The concentration of each drug per tablet was determined using its respective calibration graph constructed for pure drug by following the general procedure**.**

***Analysis of sulfamethaxazole suspension*:**

Two bottles of sulfamethaxazole contents (each bottle containing 200 mg) were mixed and accurate amount equivalent to 200 mg was dissolved in a minimum amount of ethanol and diluted to 250 ml in calibrated flask with distilled water. This solution was analyzed as described for tablet and followed the general procedure to assay the amount of sulfamethaxazol in the suspension.

Analysis of sulfacetamide drop:

Drop container of sulfacetamide (20%) was diluted to 250 ml by distilled water in a calibrated flask. This solution was diluted as needed and followed the general procedure to assay the amount of sulfacetamide in the drop.

Experimental

Apparatus

All absorption measurement were made on a Shimadzu UV-610 double beam spectrophotometer with 1cm.quartz cells.

Materials and reagents

All reagents used were analytical grade and obtained from Fluka and BDH companies. Tetracyanoethylene (TCNE) was recrystalized twice in chlorobenzene and 0.129g was dissolved in 100 ml of absolute ethanol to produce [1×10^{-2} M]. This solution was prepared daily. Sulfacetamide and sulfamethaxazole were obtained from the state company for drug industries and medical appliances Nineveh-Iraq. Solutions were prepared in concentration of 250 μg ml^{-1} in distilled water. Sodium bicarbonate of 5×10^{-3} M was prepared in distilled water.

Result and Discussion

Preliminary investigation

It was found that TCNE reagent reacted with sulfacetamide and sulfamethaxazole in the presence of sodium bicarbonate producing yellow colored species with maximum absorption at 355 and 356 nm for above drugs respectively. The blank reagent has two bands with maximum absorption at 400 and 285 nm (Fig1,2). The spectrophotometric properties of the colored product as well as the different experimental parameters effecting the color development and its stability were carefully studied and optimized.

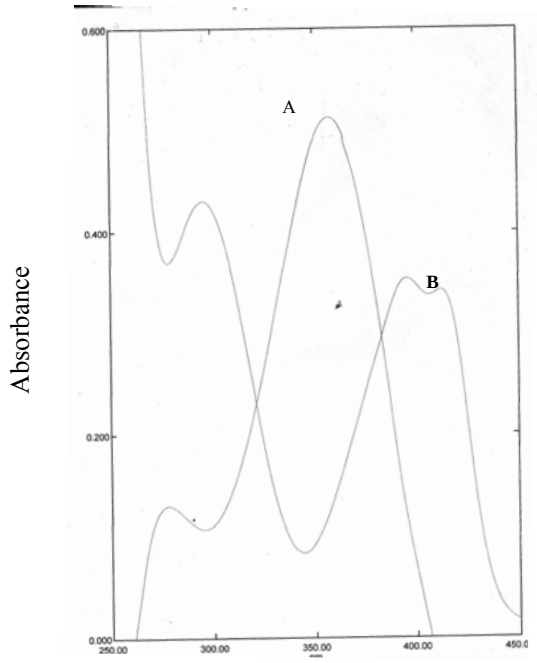
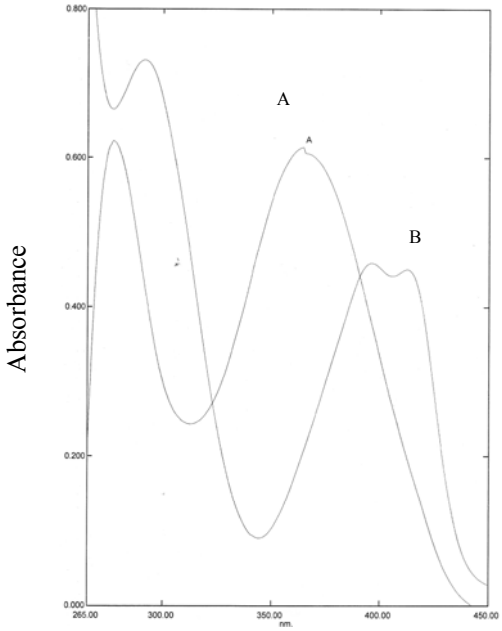


Fig1. Absorption spectra of (a) sulfacetamide (10μg/ml) with TCNE vs. reagent blank (b) reagent blank vs. distilled water



Wavelength nm

Fig 2. Absorption spectra of (a) sulfamethaxazole (10µg/ml) with TCNE vs. reagent blank (b) reagent blank vs. distilled water

Study of the optimum reaction conditions

The effect of various parameters on the absorption intensity of the color formed was studied and the reaction conditions were optimized.

Effect of pH and buffer solution

The effect of pH on the absorption of the product was studied using different pHs of HCl and NaOH ranged from 2 to12. It was found that the product formed in the final pH ranged between 5 and 6 by addition of 1ml of 1×10^{-4}M NaOH to the studied drugs, and found decreases in absorbance through addition of HCl, which may be attributed to the liberation of hydrogen cyanide. Therefore different buffers of pH 5-6 are prepared to examine the sensitivity. It was found that there is negative effect on the color intensity.

Effect of bases

Different bases such as sodium hydroxide, potassium hydroxide, calcium hydroxide, sodium carbonate and sodium bicarbonate were examined in order to obtain high sensitivity, it was found that sodium bicarbonate gave maximum color intensity for both drugs(Table 1), therefore, it was selected as abase for the reaction medium. The optimum amounts of sodium bicarbonate was also studied and found to be 1.2 ml of 5×10^{-5}M for both drugs (Fig 3,4).

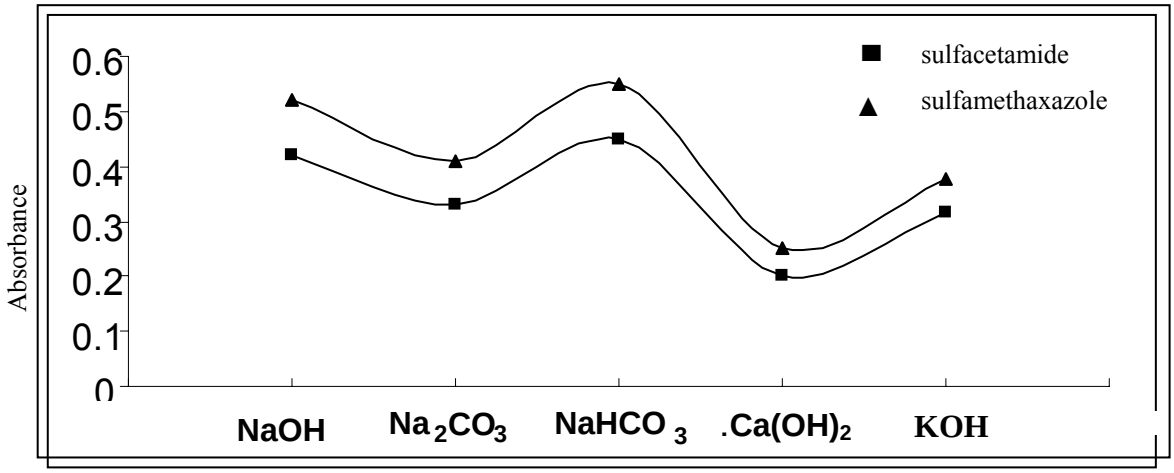


Fig 3. Effect of bases on the absorption of 10µg/ml of drugs with TCNE

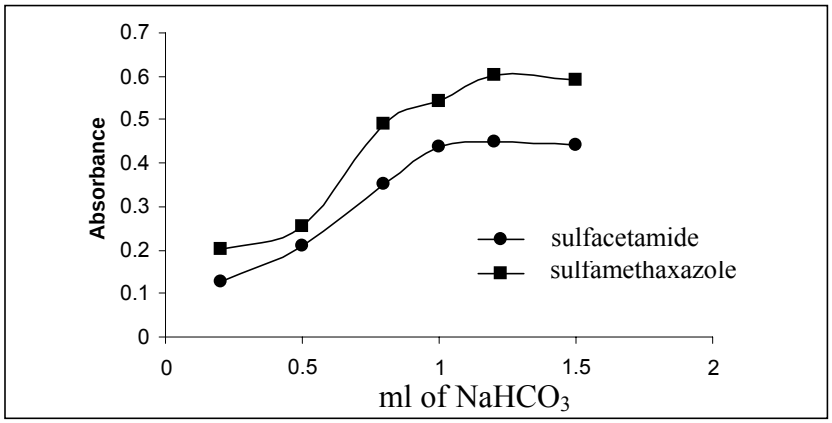


Fig4. Effect of $NaHCO_3$ on the absorption of 10µg/ml of drugs with TCNE

Effect of temperature and time on the color development

The reaction time was determined by following the color development in ice-bath, at room temperature and in thermostatically controlled water bath at different temperatures. The absorbance was measured against blank treated similarly. It was observed that the sensitivity of both drugs reached maximum after10 min at room temperature (22°C) and remain constant for more than 90 min. This temperature and reaction time were chosen for color development (Fig 5)

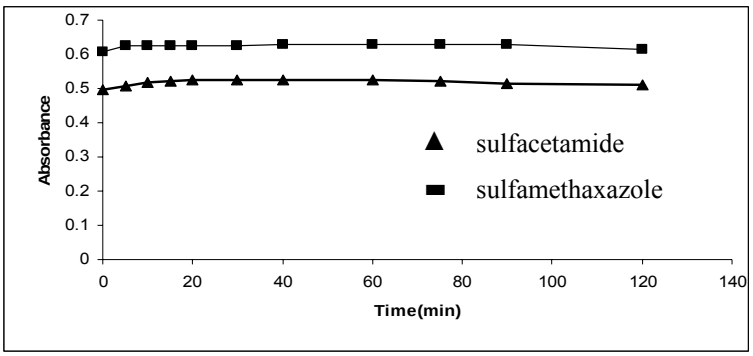


Fig5. Effect of reaction time on the absorption of drugs with TCNE at room temperature

Effect of TCNE concentration

The effect of changing the TCNE concentration on the absorbance of solution containing a fixed amount of sulfacetamide or sulfamethaxazole was studied. It is evident that the absorbance increases with increasing TCNE concentration and reached maximum on using 1.2 and1 ml of 1×10^{-2}M TCNE for sulfacetamide and Sulfamethaxazol respectively. Therefore, this volume of this concentration was used in all subsequent experiments (Fig 6).

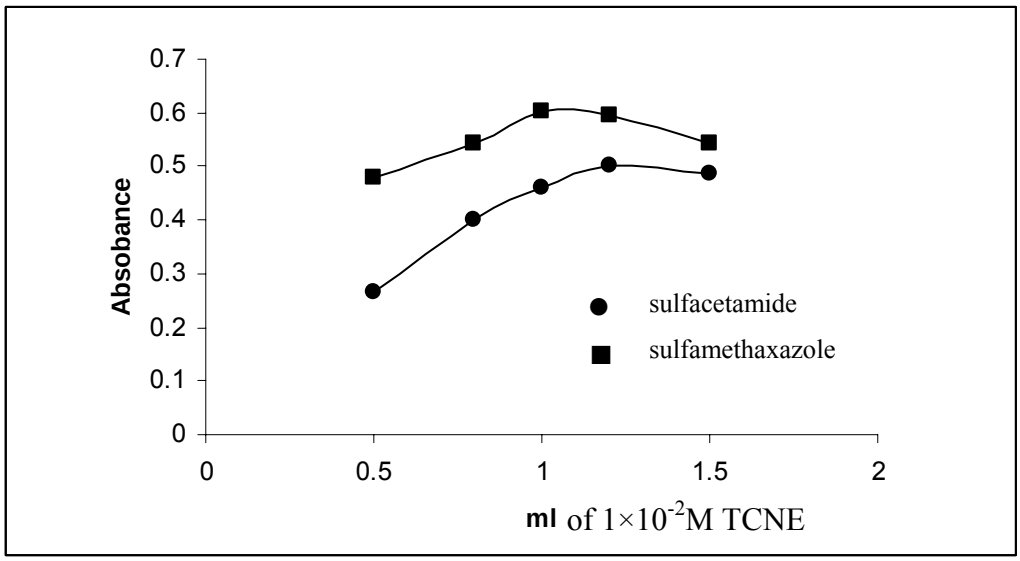


Fig 6. Effect of 1×10^{-2}M TCNE on the absorption of drugs with TCNE

Effect of surfactant

Effect of various surfactants including cetylperidinum chloride (CPC), Tween 20, Triton x-100, sodium dodecyl sulphate (SDS) and cetyltrimethyl ammonium bromide (CTAB)] have been examined. It was found that these surfactants either decreased the absorbance or produced turbid solution.

Effect of order of addition

To obtain optimum results, the order of addition of reagents should be followed as given under the recommended procedure, otherwise a loss in color intensity was observed.

However; the optimum reaction conditions for developing the color intensity of TCNE- drug products are summarized in (Table 1).

Table 1: Optimum reaction conditions of TCNE reagent with sulfacetamide and sulfamethaxazole

Drug	λ_{max} (nm)	Temp (°C)	Development time (min)	Stability period (min)	$NaHCO_3$ 5×10^{-3}M (ml)	TCNE 1×10^{-2}M (ml)	Final Ph
Sulfacetamide	355	22	10	90	1.2	1.2	5.9
Sulfamethaxazole	356	22	10	90	1.2	1	5.9

Quantification

The absorbance of the formed products conforms to Beer's law in the concentration ranges of 1-25 and 1-30 μgml^{-1} for sulfacetamide and sulfamethaxazole respectively (Fig7).

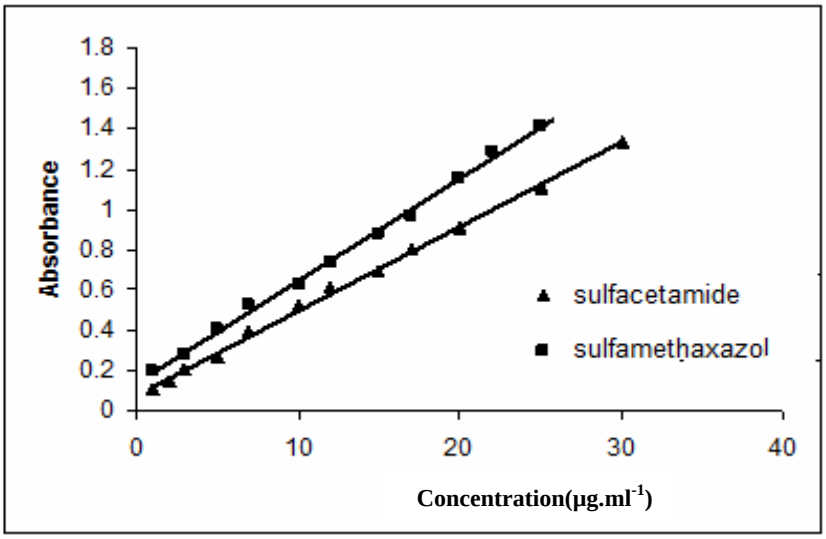


Figure 7. Calibration graphs for (a) sulfacetamide and (b) sulfamethaxazole

The molar absorptivity values are 10575 and13146.mol^{-1}.cm^{-1} and sandell index are 0.0240 and 0.0192 M.cm^{-2} for sulfacetamide and sulfamethaxazole respectively. The linearity was represented by the regression equation and the corresponding correlation coefficients for the studied were calculated representing excellent linearity(Table 2).

Table 2 : Slope, intercept and correlation coefficient for the studied drugs

Drug	Slope	Intercept	Correlation coefficient
Sulfacetamide	0.0416	0.0828	0.9977
Sulfamethaxazole	0.0519	0.1338	0.9975

Precision and accuracy

Six replicate analyses are performed at three different concentrations of each drug. The relative standard deviation and recovery results indicated the high precision and accuracy of the proposed method (Table3).

Table 3: Precision and accuracy of the proposed method

Drug	Amount added (μg/ml)	Recovery* (%)	Average recovery	RSD*
Sulfacetamide	5	100.4	99.7	0.78
	10	100.4		0.68
	15	98.7		0.936
Sulfamethaxazole	5	100.84	100.2	0.454
	10	100.1		0.972
	15	99.86		0.252

* Average for six determinations

Interferences

The effect of interferences by some excipients which often accompanied pharmaceutical preparations were studied by measuring the absorbance of solutions containing 10 μgml^{-1} of using general procedure sulfacetamide or sulfamethaxazole and various amounts of diverse species in a final volume of 25 ml. It was found that the studied excipients do not interfere in the presence of 6 and 8 folds for the determination of sulfacetamide and sulfamethaxazole in their dosage forms respectively. An error of ± 5 % in the absorbance reading was considered tolerable Table(4,5).

Table 4: Effect of excipients for assay of sulfacetamide

Excipient	Recovery % of 10 μgml^{-1}of sulfacetamide per μgml^{-1} excipient added						
	4	10	20	30	40	50	60
Glucose	100.0	100.0	100.3	100.9	101.9	102.6	
Lactose	100.0	100.9	101.7	103.0	104.3	104.9	109.1
Starch	98.8	97.7	97.1	98.8	100.7	103.0	105.7
Arabic gum	99.4	99.4	98.6	97.6	97.1	97.1	96.5
Sodium chloride	100.9	101.5	102.2	103.6	104.7	107.4	

Table 5: Effect of excipients for assay of sulfamethaxazole

Excipient	Recovery % of 10 μgml^{-1}of sulfamethaxazole per μgml^{-1} excipient added								
	٥	١٠	٢٠	٣٠	٤٠	٥٠	٦٠	٧٠	٨٠
Lactose	٩٩,١	٩٩,٤	١٠٠,٣	١٠٠,٣	١٠٠,٩	١٠١,٢٥	١٠٣,٦	١٠٣,٩	١٠٦,٢
Glucose	٩٩,٥	٩٩,٩	١٠١,٣	١٠١,٩	١٠٢,٣	١٠٢,٩	١٠٣,٣	١٠٤,٦	١٠٥,٣
Starch	١٠١,١	١٠٠,٣	١٠٠,١	٩٩,٩	٩٩,٢	٩٨,٩	٩٨,١	٩٧,٣	٩٤,٥
Sodium chloride	١٠٠,١	١٠٠,٩	١٠١,٨	١٠٣,٥	١٠٣,٧	١٠٤,٣٤	١٠٥,٤		
Arabic gum	١٠٠,٠	١٠٠,٣	١٠٢,٩	١٠٣,١	١٠٣,٩	١٠٠,٤٨	١٠٤,٤	١٠٥,3	
Trimethoprim	٩٨,٧	٩٩,٤	٩٩,٦٧	٩٩,٩١	١٠٢,٢	١٠٣,٥	١٠٥,٢		

Application

Applications of the proposed method to the assay of various pharmaceutical samples of sulfacetamide and sulfamethaxazole (drops, syrup and tablet) gave reproducible and accurate results. Further, the proposed method is very economical when compared to electrometrical British pharmacopoeia methods [21]. However; the validity of the method was confirmed by applying the standard addition procedure, (Fig.8,9,10), and the results suggested that there is no interference from any excipients, which are present in commercial dosage forms (Table 6).

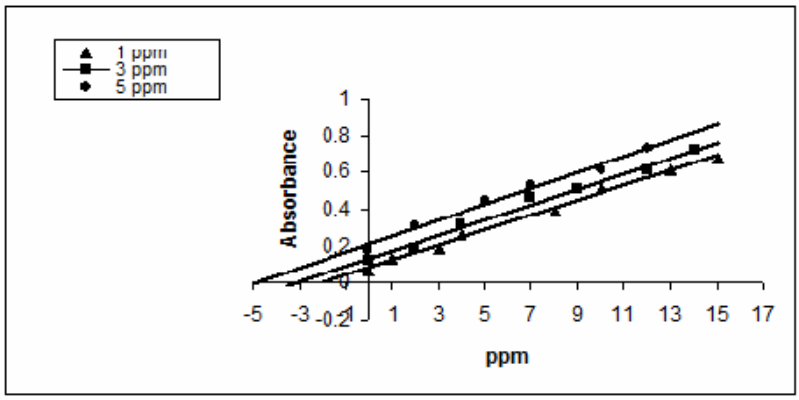


Fig.8 Standard addition plots for the recovery of 1,3,5 µg/ml of sulfacetamide in drop

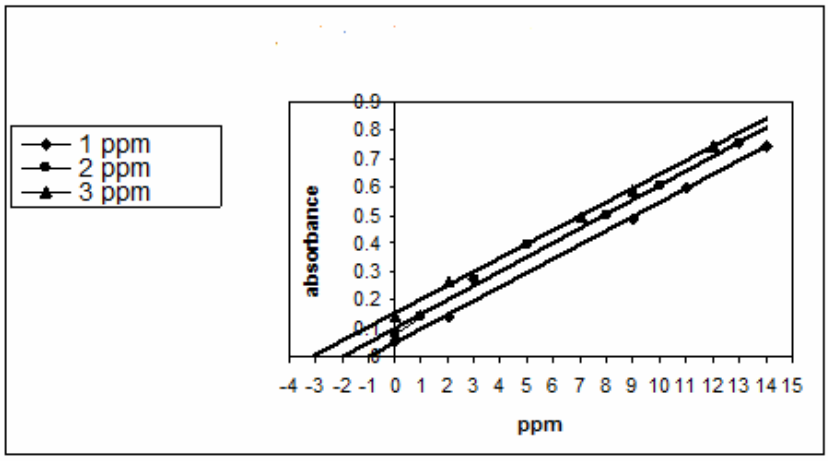


Fig.9 Standard addition plots for the recovery of 1,2,3 µg/ml of sulfamethaxazole in tablet

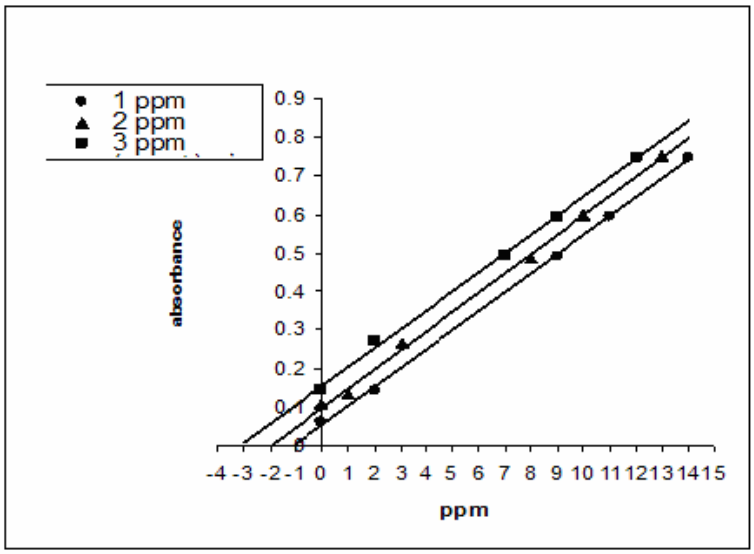


Fig.10 Standard addition plots for the recovery of 1,2,3 µg/ml of sulfamethaxazole in syrup

Table 6: Assay of sulfamethaxazole and sulfacetamide drugs in some pharmaceutical formulations by the proposed method and standard addition procedure.

Drug determined	Pharmaceutical preparation	Drug content present (mg)*	Drug content (mg)* found by		Recovery*(%)	
			Direct TCNE method	Standard addition method	Direct TCNE method	Standard addition method
Sulfamethaxazole	Metheprim tablet (SDI)	400	398.87	413.36	99.72	103.34
	Metheprim Syrup (ASIA COMPANY)	200	199.87	209.5	99.94	104.75
Sulfacetamide	Samacetamide Drop (SDI)	20%	19.91	19.86	99.54	99.3

* Average of three determinations.

The Stoichiometry of reaction

The mole ratio of the complexes formed between the drugs and the TCNE was investigated applying the continuous variation (Job's) method [22] using equimolar solutions (2×10^{-3}M) of the drugs and TCNE reagent. The results showed in (Fig11) indicated that the complexes are formed in the ratio of 1:1. This may attributed that the amino group present in drug structures is responsible for the immediate formation of the CT complex with TCNE, and bicarbonate may behaves catalyst for find product formation[23] .According the following reaction mechanism is suggested:

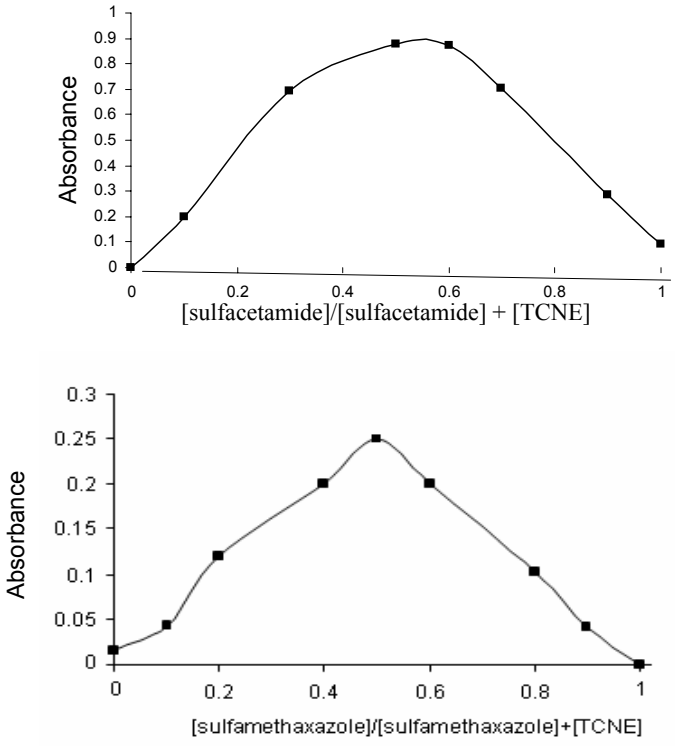
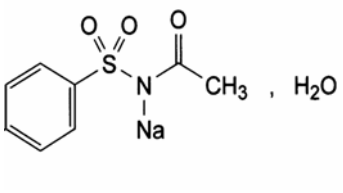


Fig.11 Continuous variation plots for sulfacetamide and sulfamethaxazole with TCNE

NaCN + $NaHCO_3$ / H_2O

R=



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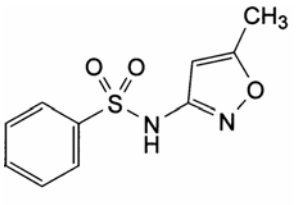

Conclusion:

The proposed method is simple, rapid, sensitive and economical compared to already reported methods and do not require any pretreatment of the drugs or extraction procedure and has a good accuracy and precision. On the other hand, in terms of simplicity and expense, the method could be considered superior in comparison with the electrometrically British Pharmacopoeia method and the previously reported methods, especially with those based on non-aqueous media.

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