Spectrophotometric determination of Sulphamethoxazole Via Charge Transfer Complex Formation Reaction

Thabit S. Al-Ghabsha
College of Pharmacy
University of Mosul

Theia'a N. Al-Sabha
Department of Chemistry
College of Education
University of Mosul

Mohammed S. Al-Enizzi
Department of Chemistry / Education College for Girls
University of Mosul

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الخلاصة

تم وصف طريقة طيفية بسيطة وحساسة لتقدير السلفاميثوكسازول بهيئته النقية وفي مستحضراته الصيدلانية. تعتمد الطريقة على تفاعل السلفاميثوكسازول كمادة مانحة للالكترونات مع أورثو–كلورانيل كمادة مستقبلة للالكترونات لتكوين معقد الشحنة المنتقلة ذي اللون البنفسجي الذي يمتلك أقصى امتصاص عند الطول الموجي 539 نانوميتر. لقد وجد عند الظروف المثلى أن قانون بير ينطبق بحدود 2–60 مايكروغرام/مللتر بامتصاصية مولارية 3.675×10^3 لتر/مول.سم. كما وجد حد الكشف والحد الكمي 0.371 و 1.236 مايكروغرام/مللتر على التوالي. كان معدل نسبة الاسترجاعية 100.95% والانحراف القياسي النسبي ≤ 1.06% . تم دراسة طبيعة المعقد وميكانيكية التفاعل. طبقت الطريقة بنجاح في تقدير السلفاميثوكسازول في مستحضراته الصيدلانية على شكل أقراص وشراب بدقة وتوافق جيدين.

Abstract

A simple and sensitive spectrophotometric method for the determination of sulphamethoxazole in pure as well as in dosage form is described. The method is based on the reaction of sulphamethoxazole as electron donor with o-chloranil as electron acceptor to form violet colored charge transfer complex having maximum absorption band at 539 nm. Under the optimized reaction conditions, Beer's law was obeyed in the

range of 2-60 μg ml^{-1} with molar absorptivity 3.675×10^3 L $mol^{-1}cm^{-1}$. The limits of detection (LOD) and limit of quantitation (LOQ) were 0.371 and 1.236 μg ml^{-1} respectively. The accuracy and precision of the method were satisfactory; the average recovery % was 100.95 % and values of relative standard deviations ≤1.06 %. The stoichiometry of the reaction was studied, and the reaction mechanism was postulated. The proposed method was successfully applied to the determination of sulphamethoxazole in its pharmaceutical tablets and syrup with good accuracy and precisions.

Keywords: Charge transfer; Spectrophotometry; *o*-chloranil; sulphamethoxazole

Introduction

Sulfamethoxazole(3-p-aminobenzenesulphonamido-5-methylisoxazole)(SMZ) belongs to the sulfonamides group of chemotherapeutics which using in both systemic and urinary infections. Generally, it is combined with trimethoprim in commercial drugs[1,2]. Numerous methods have been developed for the determination of sulphamethoxazole and trimethoprim in combination present in pharmaceutical preparations[3-7]. Few spectrophotometric methods have been reported for determination of SMZ alone. These methods are based on the reaction of sulphamethoxazole with dimethylaminocinnamaldehyde[8], 1-2-naphthaquinone-4-sulfonate[9], 7,7,8,8-tetracyanoquinodimethane[10], 1-naphthol[11], phenosafranine[12], phloroglucinol[13], dopamine in the presence of molybdate ions[14], p-dimethylaminobenzaldehyde and 8-hydroxyquinoline[15].

The methods that are based on charge-transfer complexation are usually sensitive, rapid and simple to perform. Charge transfer reactions have been widely used for the determination of electron donating compounds through interaction with π-acceptors.

This work describes a simple, rapid and sensitive spectrophotometric method for the determination of SMZ, in its pure form and pharmaceutical formulations containing trimethoprim and other excepients, by exploiting its electron donating property. The method is based on the charge transfer complexation reaction of SMZ with *o*-chloranil.

Experimental

Apparatus

Shimadzu UV-1650 PC UV-Visible spectrophotometer equipped with a 1.0-cm path length silica cell**,** Philips PW (9421) pH-meter with a combined glass electrode was used for pH measurements**,** All calculations in the computing process were done in Microsoft Excel for Windows. Weighing was carried out on a balance type of Mettler H 54 AR.

Chemicals

Sulphamethoxazol and its pharmaceutical formulations (tablet and syrup) were kindly provided by state company for Drug Industries and Medical Appliance-(SDI) Sammara-Iraq. o-Chloranil was obtained from MOLEKULA and other chemicals were obtained from Fluka and BDH companies. All solvents were analytical reagent grade and water was distilled.

Working standard solution of sulphamethoxazol: 250 μgml^{-1} sulphamethoxazole solution was prepared by dissolving of 25 mg of its pure form in 5 ml ethanol and diluted to 100 ml with distilled water in a volumetric flask. Further dilution, to obtain 100 μgml^{-1}, has been prepared.

Reagent solution: 5×10^{-3} M o-chloranil solution was prepared by dissolving 0.123 g in absolute ethanol and diluted to 100 ml in a calibrated flask with the same solvent.

Basic solutions: 0.01 M sodium carbonate and sodium hydroxide were prepared in distilled water.

Recommended procedure

Aliquots of the working solution of sulphamethoxazol (2-60 μgml^{-1}) were transferred into a series of 5 ml calibrated flasks. Then, 1ml of 5×10^{-3} o-chloranil and 0.75 ml of 0.01 M Na_2CO_3 were added and the solutions were diluted to the mark with ethanol. The absorbance was measured at 539 nm at room temperature against reagent blank.

Procedure for pharmaceutical formulations

Tablet

Ten tablets (each tablet containing 400 mg sulphamethoxazole) were accurately weighed and pulverized. A portion of the fine and homogenized powder equivalent to 400 mg sulphamethoxazol was accurately weighed and dissolved in about 10 ml of water-ethanol (50:50 v/v) mixture with mixing and heating for 5 min, then filtered with Whatmann filter paper no.1. The filtrate was diluted to the 100 with distilled water in a volumetric flask obtaining 4000 μgml^{-1}. a suitable volume was diluted, and the above procedure was followed.

Syrup

The appropriate volume of the syrup containing SMZ equivalent to 10 mg was transferred into a 100 ml measuring flask, diluted, filtered and made up to the mark with distilled water. An aliquot of the solution was analyzed, as described in recommended procedure.

Results and discussion

The proposed method involves the and reaction of SMZ with o-chloranil reagent in the presence of Na_2CO_3 to form a violet colored charge transfer complex having maximum absorption at 539 nm. This wavelength was used for all subsequent measurements. The absorption spectra of the reaction product are shown in Figure 1. The corresponding reagent blank have low absorbance at this wavelength.

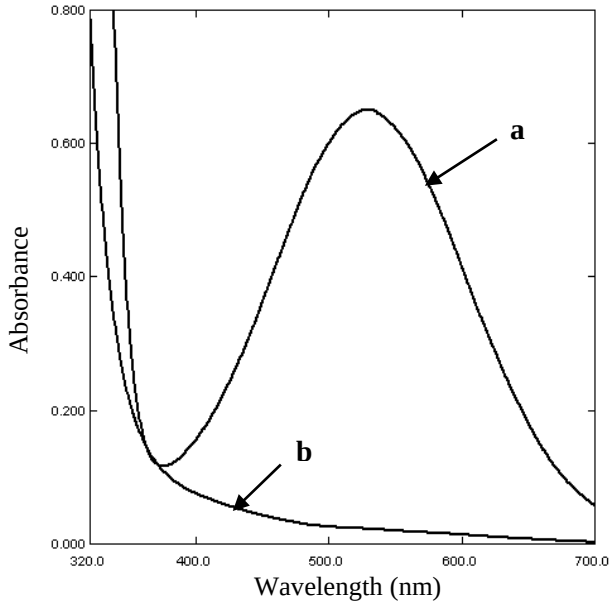


Figure 1: Absorption spectra of (a) SMZ (20 µg ml^{-1}) complex with *o-CA* reagent (5×10^{-3}M) in the presence of 0.01 M Na_2CO_3against reagent blank and (b) reagent blank against distilled water.

Optimization of experimental conditions

The optimum conditions for the color development of the *o-CA*-SMZ complex were established by varying the parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of colored species. The following experiments were conducted for this purpose and conditions so obtained were incorporated in general procedure.

Effect of solvents

Different solvents such as methanol, ethanol, acetonitrile, acetone and water as medium for the reaction, between SMZ (20 µgml^{-1}) and *o-CA* (1ml of 5×10^{-3} M), in the presence of NaOH (1ml of 0.01M) in final volume of 5 ml, have been tried in order to achieve maximum sensitivity and complex stability. The absorbance of solutions were measured against corresponding blank after 5 min at room temperature. As shown in table1. It was found that on using water as solvent for sulphamethoxazole and ethanol as solvent for *o-CA* and dilution with

ethanol were gave maximum color intensity and recommended in this method. However; dilution with water gave turbid solutions.

Table 1: Effect of solvents on the absorbance of *o-CA*-SMZ complex

* Sample against blank

SMZ (20 µgml^{-1}) dissolved in	*o-CA* Dissolved in	Dilution by	λ_{max} nm	Abs. S*	Abs.B **
Water	Methanol	Water	-	turbid	turbid
Water	Methanol	Methanol	-	turbid	turbid
Methanol	Methanol	Methanol	446	0.137	0.278
Methanol	Methanol	Water	_	turbid	turbid
Water	Ethanol	Water	525	0.121	0.052
Water	Ethanol	Ethanol	539.5	0.140	0.039
Ethanol	Ethanol	Ethanol	523.5	0.092	0.084
Ethanol	Ethanol	Water	367	0.110	0.411
Water	Acetone	Water	-	turbid	turbid
Water	Acetone	Acetone	541	0.118	0.058
Acetone	Acetone	Acetone	553	0.014	0.055
Acetone	Acetone	Water	580	0.019	0.034
Water	Acetonitrile	Water	445	0.082	0.106
Water	Acetonitrile	Acetonitrile	409	0.078	0.120
Acetonitrile	Acetonitrile	Acetonitrile	495.5	-0.014	0.093

** Blank against solvent at λ_{max} of complex

Effect of pH

The effect of pH on the absorption of the complex was studied using different pH values. It was observed that the complex was formed with low sensitivity at pH 3.34, but this sensitivity was increased by addition of NaOH and reached its maximum absorption at pH 5.1 (Fig.2). Therefore different buffers of pH 5.1 were prepared to examine the sensitivity. A negative effect was observed on the color intensity.

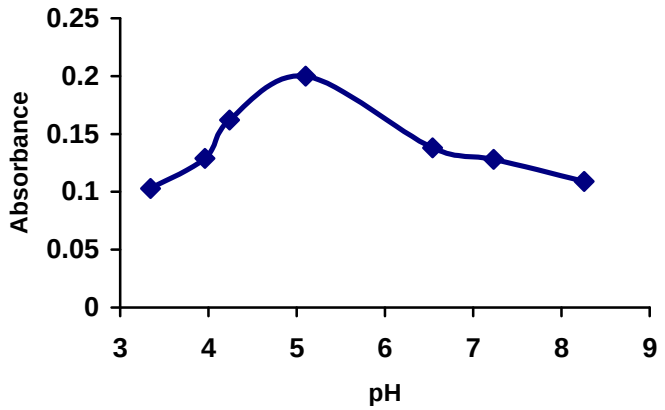


Figure 2: Effect of pH on the absorption of 20 µgml^{-1} SMZ complex with *o-CA*

Effect of bases

To obtain high sensitivity for the complex, different bases such as sodium hydroxide, potassium hydroxide, sodium carbonate and sodium bicarbonate with fixed volume and a concentration of 0.01M were examined by addition to a fixed amount of SMZ. It was found that sodium carbonate gave maximum color intensity (Figure 3), and the optimum amounts of this base were found to be 0.75 ml which was used in the subsequent experiments.

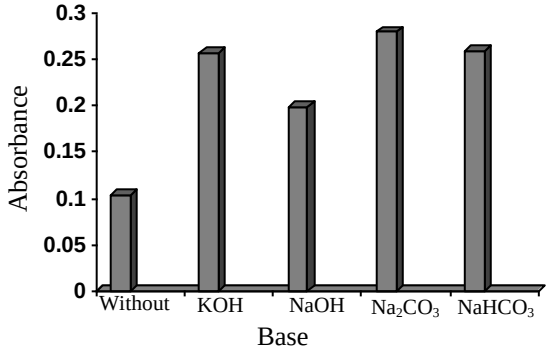


Figure 3: Effect of Different bases on the intensity of 20 µgml^{-1} SMZ complex with *o-CA*

Effect of *o-CA* concentration

The effect of changing the *o-CA* concentration on the absorbance of solution containing a fixed amount of SMZ was studied. It was observed that the absorbance increases with increasing *o-CA* concentration and reached maximum on using 1.0 ml of 5×10^{-3}M *o-CA* (Figure 4). Therefore, this volume of this concentration was used in the subsequent work.

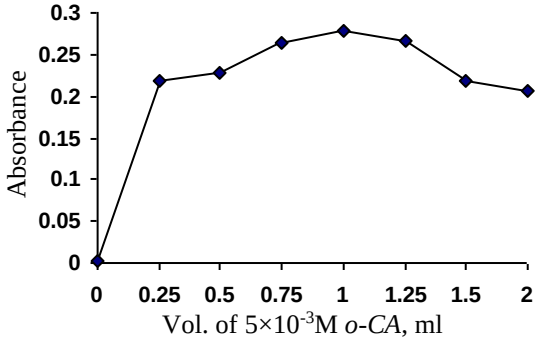


Figure 4: Effect of *o-CA* reagent concentration on absorbance of 20 µgml^{-1} SMZ

Effect of surfactant

Effect of various surfactants including sodium dodecyl sulphate (SDS), cetylperydinum chloride (CPC), cetyltrimethylammonium bromide (CTAB), Tween-80 and Triton x-100 were tested. It was found that these surfactants decreased the absorbance of solutions.

Effect of temperature and reaction time

The reaction time was determined by following the color development at room temperature and in thermostatically controlled water-bath at different temperatures up to 60°C. The absorbance was measured at 5 and 10 minutes intervals against reagent blank treated similarly. It was observed that the complex was formed after addition of *o-CA* immediately at room temperature and no effect of high temperatures on the maximum absorbance and stability of the complex (>5hr), therefore room temperature (25°C) was selected.

Order of addition

The order of addition of reactants on the color development was examined. Maximum sensitivity was achieved when SMZ and *o-CA* were added before adding the sodium carbonate as shown in Fig. 5. Hence, the method was performed in the order: SMZ + *o-CA* + Na_2CO_3.

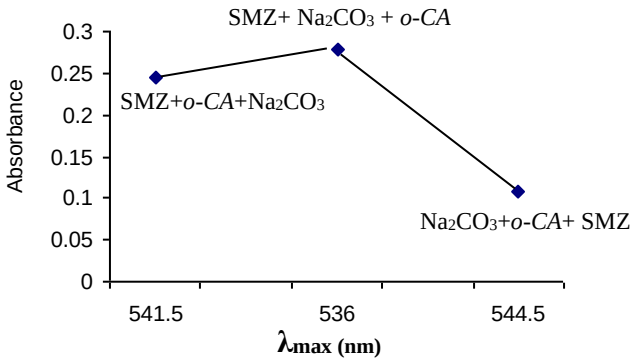


Figure 5: Effect of order of reactants on the absorption of 20 µg/ml SMZ

Quantification

In order to investigate the range in which the colored complex adhere to Beer's law, the absorbance of the complex was measured at 539 nm after developing the color by following the general procedure calibration graph for a series of solutions containing increasing amounts of SMZ. The Beer's law limits and molar absorptivity values were evaluated and given in Table 2, which are indicated that the method is sensitive. The linearity was represented by the regression equation and the corresponding correlation coefficient for the studied determined drugs by the proposed method represents excellent linearity. The relative standard deviation (RSD) and accuracy (average recovery %) for the analysis of five replicates of each three different concentrations for SMZ indicated that the method is precise and accurate. Limit of detection (LOD) are in the accepted range below the lower limit of Beer's law range.

Table 2: Summary of optical characteristics and statistical data for the proposed method

Parameter	SMZ
Beer's law limits (μg ml^{-1})	2-60
Molar absorptivity ($l.mol^{-1}. cm^{-1}$)	3.675×10^3
LOD (μg.ml^{-1})	0.371
LOQ (μg.ml^{-1})	1.236
Average recovery (%)*	100.95
Correlation coefficient	0.9994
Regression equation (Y)**	
Slope, *a*	0.0145
Intercept, *b*	0.0101
RSD [a]	≤ 1.06

* Average of five determinations.

** $Y = a\,X + b$, where X is the concentration of drug in μg ml^{-1}.

Interference

The extent of interference by some excipients which often accompany pharmaceutical preparations were studied by measuring the absorbance of solutions containing fixed amount of SMZ and various amounts of diverse species, including trimethoprim which is present the pharmaceutical formulations of SMZ, in a final volume of 5 ml. It was found that the studied excipients up to 22.5 fold excess did not interfere seriously (Table 3). However; an error of 5.0 % in the absorbance readings was considered tolerable.

Table 3: Effect of excipients for assay of SMZ

Exciepient	Recovery % of 20 μg/ml of SMZ per μgml^{-1} Foreign added			
	25	50	100	250
Trimethoprim	97.05	98.75	97.15	99.90
Sodium chloride	99.90	102.90	100.03	95.55
Arabic Gum	100.10	100.55	103.45	96.45
Starch	104.15	101.60	98.85	104.55
Acacia	98.50	104.00	102.40	96.40
Glucose	96.95	96.15	99.00	98.65

Stoichiometry, stability constant and mechanism

The molar ratio of the n-π charge transfer complex formed between the SMZ and *o-CA* reagent was investigated by applying the continuous variation (Job's) and mole ratio methods [16]. The results indicated that

complex was formed in the ratio of 1:1 (Figures 6). This finding supports that the n-π CT complex is formed through amino group. The stability constant (K_{st}) of the complex was determined according to the previous ratio and found 1.897×10^{3} l. mol^{-1}. However; the probable reaction mechanism based on the reported method [17] is given in scheme 1.

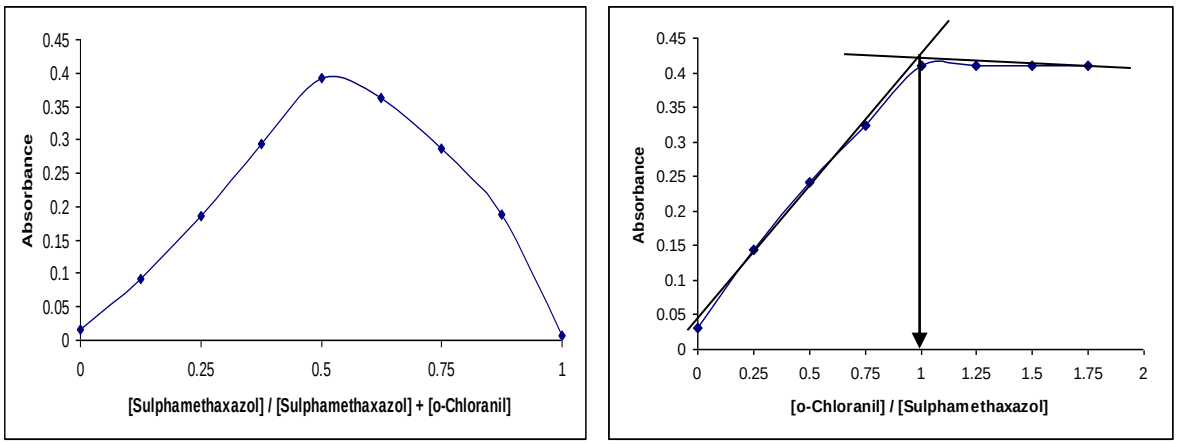
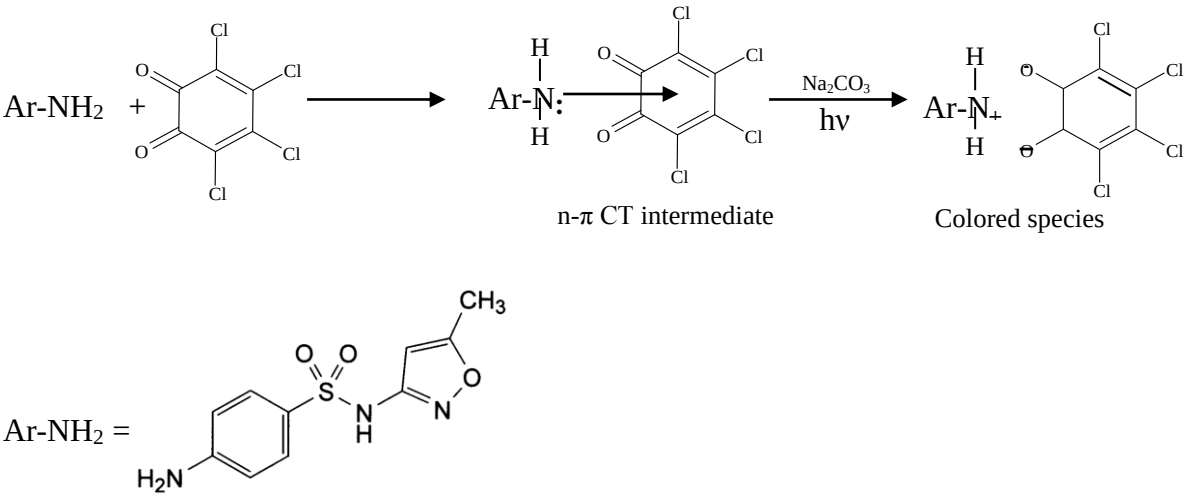


Figure 6:Continuous variations and mole ratio plots for complex of SMZ (1.5×10^{-3}M) and *o-CA* (1.5×10^{-3}M) under the optimum reaction conditions.



Scheme 1: Probable mechanism for the reaction of *o*-CA with SMZ

Analytical applications

The proposed method was successfully applied to determine SMZ in pharmaceutical tablets and syrup preparations. The validity of the method was confirmed by applying the standard addition procedure, (Fig.7) and the results suggested that there is no interference from any excipients, which are present in commercial dosage forms, Table 4.

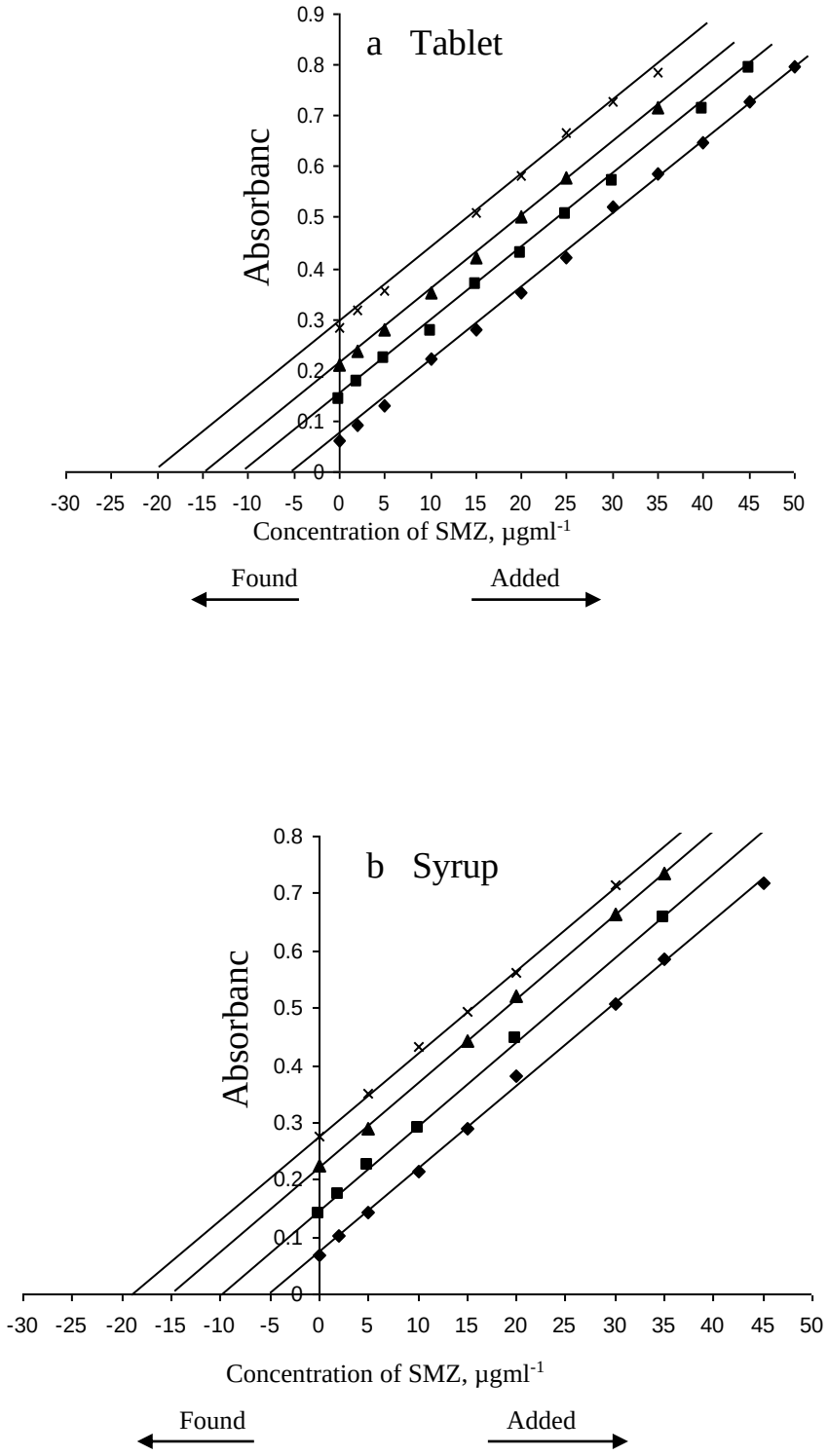


Fig.7: Standard addition plots for the recovery of 5 (♦), 10 (■), 15 (▲) and 20(×) µgml^{-1} of SMZ in tablet (a) and syrup (b).

Table 4: Assay of SMZ in pharmaceutical preparations using the proposed method and comparison with the standard addition method

Pharmaceutical preparation[a]	Direct method				Standard addition method				Certified value (mg)
	Drug amount present ($\mu g\ ml^{-1}$)	Recovery[a] (%)	Drug content found (mg)	Average recovery (mg)	Drug amount present ($\mu g\ ml^{-1}$)	Recovery (%)	Drug content found (mg)	Average recovery (mg)	
Tablet	5	96.68	386.72	393.48	5	96.00	384.00	393.29	400
	15	100.73	402.92		10	101.30	405.20		
	30	98.71	394.84		15	94.94	389.76		
	40	97.36	389.44		20	98.55	394.20		
Syrup	7	106.10	212.20	203.82	5	99.60	199.20	198.28	200
	12	100.40	200.80		10	98.67	197.54		
	25	101.62	203.24		15	97.60	195.20		
	45	99.52	199.04		20	100.60	201.20		

[a] Average of three determinations.

Conclusion

The proposed method is sensitive (trace amounts can be determined), accurate (average recovery range 100.95 %), precise (RSD $\leq$ 1.06) and simple since it does not need neither temperature control nor solvent extraction step. Analysis of authentic samples containing SMZ showed no interference from common additives and auxiliary substances in general. Hence, this method could be considered for the determination of SMZ both in pure form and in pharmaceutical preparations.

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