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2,5-dimethoxyaniline as a New Coupling Agent for the Spectrophotometric Determination of Sulfamethoxazole by Diazotization-Coupling Reaction

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ABSTRACT

A simple, rapid, sensitive, selective, and accurate method for the spectrophotometric determination of sulfamethoxazole (SMZ) in bulk and in dosage forms. The method is based on diazotization of primary amine group of SMZ with sodium nitrite and hydrochloric acid followed by coupling with 2,5-dimethoxyaniline (DMA) in aqueous mildly acidic medium to form a stable orange-yellow azo dye, showed a maximum absorption at 475 nm. Beer's law was obeyed over the concentration range of 0.1-8 ppm with a molar absorptivity 5.11×10^4 $L.mol^{-1}.cm^{-1}$. Sandell's sensitivity, limit of detection (LOD), and limit of quantification (LOQ) are 0.005 $\mu g.cm^{-2}$, 0.017 ppm, and 0.06 ppm respectively. The method has been successfully applied to the determination of (SMZ) in bulk and in its pharmaceutical preparations, oral suspension, and tablet with very good recoveries 99.35-100.2%.

Keywords: Spectrophotometry, Sulfamethoxazole, Diazotization-coupling, 2,5-dimethoxyaniline.

INTRODUCTION

Sulfamethoxazole (SMZ) is a white or almost white, crystalline powder which is practically insoluble in water, freely soluble in acetone, sparingly soluble in ethanol. It dissolves in dilute solutions of sodium hydroxide and in dilute acids. Its systematic chemical name 4-Amino-N-(5-methylisoxazol-3-yl)benzenesulphonamide.[1] SMZ is a member of the sulfonamide family of antibiotics. It has a broad spectrum of antibacterial activity against both Gram-positive and Gram-negative aerobic bacteria, also being effective against protozoa, and has been used successfully for the treatment of bacterial infections, including those of the respiratory and urinary tract[2]. SMZ is used in human and veterinary medicine to fight infectious diseases and in animal feeds to promote livestock growth[3]. The British Pharmacopoeia BP [1] recommend a diazotization (sodium nitrite) titration with potentiometric detection of the end-point for the evaluation of the raw material and tablet dosage form. For oral suspension recommend a spectrophotometric method based on azo-coupling reaction with N-(1-naphthyl)ethylenediamine dihydrochloride. Various analytical methods have been developed for the determination of this drug. These methods include chromatographic [4-7], electrochemical [8-10], spectrophotometric [11-14], specofluorimetric [15-18], and flow injection analysis [19-22].

The aim of present work was to develop simple, sensitive and selective spectrophotometric method based on diazotization-coupling reaction with the new reagent 2,5-dimethoxyaniline (DMA) for the determination of SMZ in bulk as well as pharmaceutical formulations.

MATERIALS AND METHODS

Instruments : All spectral and absorbance measurements were carried out on a double-beam UV–Visible spectrophotometer (Shimadzu, Japan) model UV-1650 PC with quartz cell of 1 cm path length, which connected to computer have the software UV-Prob version 2.21.

Reagents: All chemicals used were of analytical reagent grade purity. Standard reference sulfamethoxazole was obtained from (SAFA Pharmaceutical Industries Co, Iraq). Pharmaceutical preparations containing SMZ obtained from the commercial market.

Solutions: All aqueous solution were prepared using deionized water. SMZ stock standard solution 250 ppm was prepared by dissolving SMZ in 5 mL absolute ethanol in 100 mL volumetric flask and then complete to the mark with deionized water. Working standard solutions were freshly prepared by diluting the stock solution with deionized water to obtain the appropriate concentration. Sodium nitrite (BDH) aqueous solution 0.01M. Hydrochloric acid (BDH) aqueous solution 1M. Sulfamic acid (BDH) aqueous solution 0.2 M. 2,5- dimethoxyaniline (Sigma-Aldrich) 0.01 M prepared using absolute ethanol (GCC). Dosage form aqueous solutions 1000 ppm.

Recommended Procedure and Calibration Graph: Transfer increasing volumes of working SMZ solution, covering the range 2.5-200 µg (0.1–8 ppm), into a series of 25-mL volumetric flasks. Add 0.5 mL of 1M HCl and the mixtures are shaken. Then 1 mL of 0.01 M sodium nitrite solution is added and the mixtures are allowed to stand for 2 minutes. Then 1.5 mL of 0.2 M sulphamic acid solution was added and the mixtures allowed to stand for 2 minutes. After that 3 mL of 0.01 M (DMA) was added and the volumes completed to the mark with deionized water. After 5 minutes measure the absorbance against a reagent blank, prepared in the same manner but containing no SMZ, at 475 nm using 1-cm cells.

Procedure for dosage forms

Tablet: Five tablets (400mg and 800mg SMZ/tablet) were weighed and finely powdered. A portion of the powder equivalent to 100 mg of the drug was weighed and dissolved in 20 mL absolute ethanol then transferred into 100 mL volumetric flask shaken well and completed to the mark with the deionized water. The solution shaken well, filtered and an aliquot of the filtered drug solution was then treated as done in the recommended procedure.

Oral suspension: The content of two containers of SMZ oral suspension (200 mg SMZ/5mL) was mixed well and 5 mL of the suspension mixture was quantitatively transferred into 200 mL volumetric flask and dissolved in 40 mL absolute ethanol shaken well and completed to the mark with deionized water. An aliquot of the filtered solution was then treated as done in recommended procedure.

RESULTS AND DISCUSSION

Chemistry: Azo dye derivatisations are the most widely applied reaction for the chemical derivatisation of drugs. In this method, the drug contain a free primary amino group will diazonated and coupled with a coupling component contain a powerful electron-releasing group, generally -OH,-NR_2, or -NH_2. Typically, coupling with phenols is carried out in mildly alkaline solution, and with amines in mildly acidic solution [23]. These groups not affect on the rate of reaction only, but act as an auxochrome which

enhance the spectral properties. Among the auxochrome groups it is found that amino group and its derivatives exert a greater effect than hydroxyl group [24].

Choice of coupling reagent: A critical study of several coupling reagents, most of which have not previously been used, was made. The reagents tested are: m-hydroxy benzoic acid, 2,4-dimethylphenol, 2,5-dimethoxyaniline, 1,2-phenylenediamine, 1,3-phenylenediamine, and o-tolidine. Useful analytical results obtained by 2,5-dimethoxyaniline. This reagent gave a stable water-soluble azo dye with SMZ. High intensity of the azo dye formed with a good color contrast and relatively rapid coupling rates. Therefore, this reagent was selected and the optimum conditions of its reaction with SMZ was further studied.

Spectral characteristics: Absorption spectrum of the orange-yellow azo product with maximum absorption at 475 nm is shown in fig.1. The reagent blank has practically negligible absorption at this wavelength. Hence, all measurements were made at this wavelength against reagent blank.

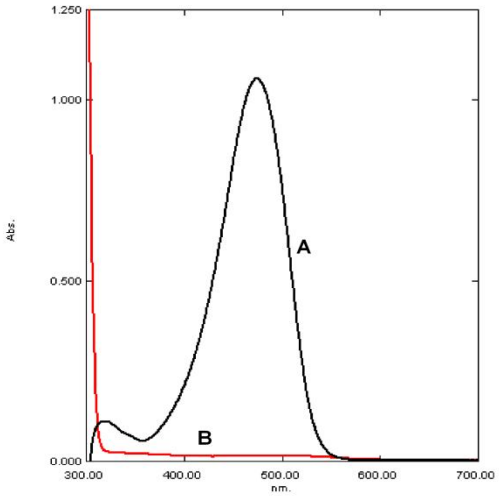


Fig. 1: Absorption spectra of A: (5 ppm) of SMZ treated as described under procedure and measured against DMA reagent blank and B: the DMA reagent blank measured against distilled water.

Optimization of reaction conditions

The effects of the various parameters on the absorption intensity of the azo dye were studied and the reaction conditions are optimized.

Effect of acid: Different amounts (0.05-5 mL of 1M) of different acids have been examined. A 0.5mL of 1M HCl gives the best results.

Effect of Sodium Nitrite Concentration and Time: The effect of sodium nitrite concentration was tested by using different volumes (0.1-3 mL) of 0.01 M $NaNO_2$ solution. It was found that the addition of 1 mL of $NaNO_2$ solution was required with 2 minute reaction time to obtain a maximum absorbance.

Effect of Sulfamic acid Concentration and Time: The excess of nitrous acid is removed by the addition of sulfamic acid solution. The effect of its concentration was tested by using different volumes (0.1- 4 mL) of 0.2 M sulfamic acid solution. It was found that the addition of 1.5 mL of sulfamic acid solution was required with 2 minute reaction time to obtain a maximum absorbance.

Effect of Reagent Concentration: The effect of reagent concentration was tested by using different volumes (1–5 mL) of 0.01 M 2,5-dimethoxyaniline solution. The results showed that 3 mL of reagent is sufficient for production of maximum and reproducible color intensity.

Effect of Time : The effect of time on the formation of the azo product was investigated by allowing the reaction to proceed for varying times. The results showed that the azo-dye reached maximum absorbance after 5 minutes and remains stable at least for 2 days.

Effect of Temperature: The effect of temperature on the absorption was investigated at different temperatures (1–80°C). The results revealed that the absorbance relatively stable in the temperature range (1–30°C). At higher temperatures, the absorbance value decreased, which was probably due to the dissociation of azo-dye.

Calibration Curve and Sensitivity

Under optimum conditions studied above, standard calibration curves for SMZ-DMA azo product were constructed fig.2, and different parameters of the analytical performance of the proposed method are summarized in table 1

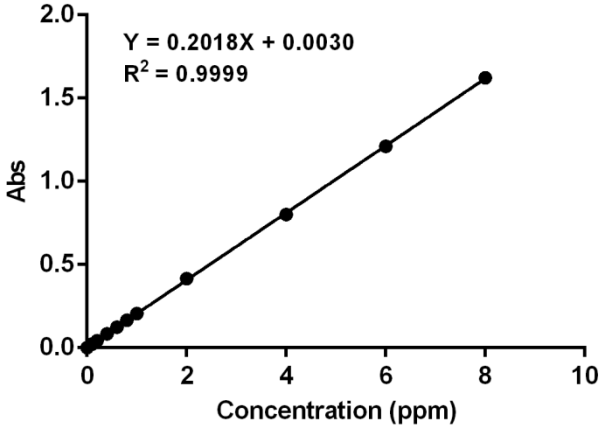
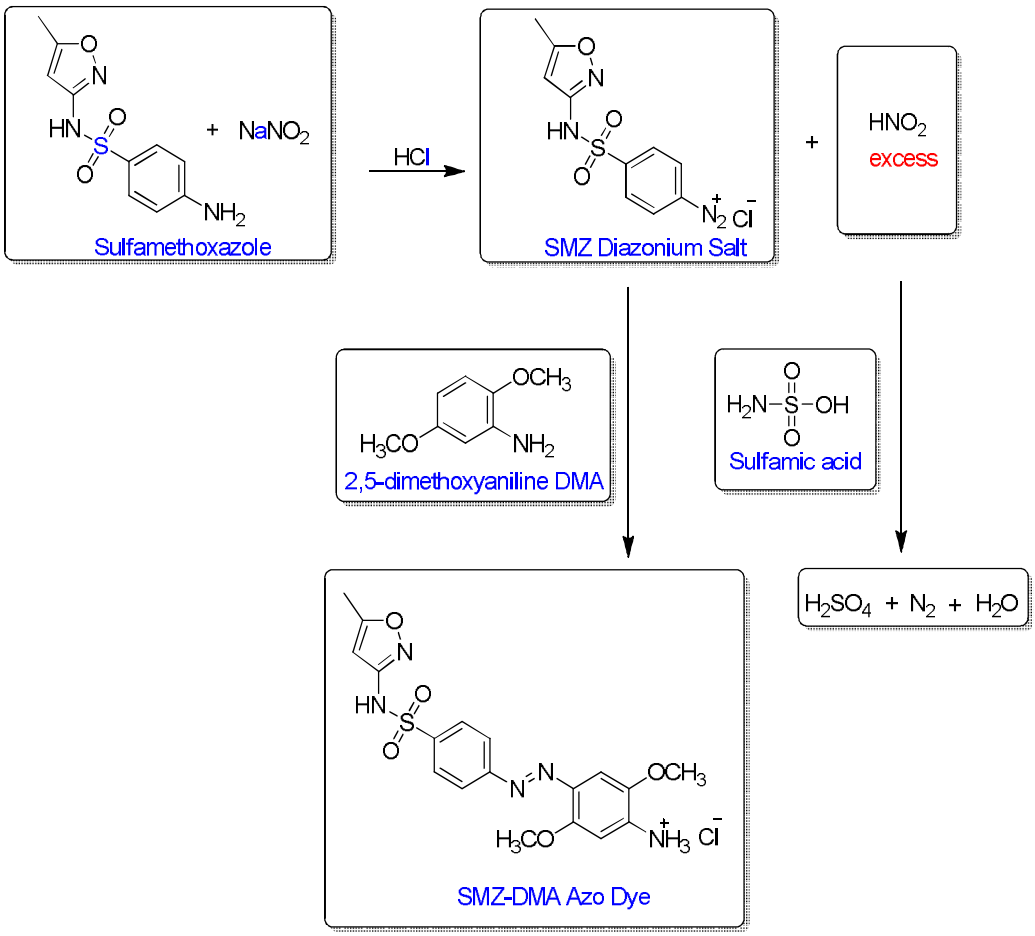


Fig. 2: Calibration graph for SMZ determination using DMA as coupling reagent.

Table 1: Analytical features of the procedure developed for the determination of SMZ

Parameter	***Proposed Method***
Regression equation	Y= 0.2018 X + 0.0030
Slope	0.2018
Correlation coefficient	0.9999
Linear Range (ppm)	0.1 – 8
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	5.11×10^4
Limit of detection (LOD) (ppm)	0.017
Limit of quantification (LOQ) (ppm)	0.06
Sandell's sensitivity, S ($\mu g\ cm^{-2}$)	0.005
Reproducibility (%)	0.49
Recovery (%)	99.70

Nature and stability constant of the dye product: The stoichiometric ratios were determined using Job's method of continuous variation [25]. The results obtained show a 1:1 drug to reagent product was formed. The formation of dye may probably occur as given in scheme.1. The stability constants of the dye product using equation cited in [26]. The result show that the stability constant is $2.43 \times 10^9\ M^{-1}$, which indicate a stable dye products are formed through the reaction of SMZ drug with DMA.



Scheme 1: Proposed mechanism of the reaction between SMZ and DMA.

Interference study: The extent of interference by common excipients frequently found with SMZ drug in pharmaceutical formulations were determined. These excipients include Tween 80, PVP, acacia, mennitol, lactose, sucrose, benzoic acid, aspartate, microcrystalline cellulose, talc, starch, and magnesium stearate. The study done by measuring the absorption of a synthetic sample solutions containing 8 ppm of SMZ and excess amounts (10-fold excess) of each excipient solution. Also the effect of active ingredient Trimethoprim were studied by using different amount of Trimethoprim with constant quantity of SMZ. An error of 5.0% in the absorbance readings was considered tolerable; none of these substances interfered seriously.

Pharmaceutical applications: The proposed method was applied to analysis four different dosage forms containing SMZ in order to evaluate the analytical usefulness of the spectrophotometric method. The proposed methods were applied successfully to the analysis. Good results with good recoveries and reproducibility's were obtained based on three determinations for three different concentrations of each pharmaceutical preparation table.2. Finally, statistical analysis [27,25] F- and t-test (which were performed using Graph Pad Prism software Version 6.02 for windows 2013), show that there is no significant difference in accuracy between the proposed method and the official BP [28] method table.3.

Table 2: Application of the proposed method to the determination of SMZ in some dosage forms.

Preparation	Concentration taken (ppm)	Concentration found (ppm)	Recovery (%)	RSD[a](%)
METHEPRIM Tablets	2	1.98	99.11	0.72
	6	5.99	99.93	0.13
	8	7.99	99.85	0.16
CO-TRIMOXAZOLE Tablets	2	2.02	101.09	1.02
	6	5.88	98.03	0.12
	8	7.91	98.92	0.11
Cortim Oral Suspension	2	2.02	100.84	1.81
	6	5.90	98.28	0.33
	8	7.96	99.54	0.11
KIMITRIM Oral Suspension	2	1.99	99.85	0.58
	6	5.95	99.19	0.11
	8	8.01	100.16	0.17
a. For three determinations.				

Table 3: Application of the proposed and official methods to the determination of SMZ in pure and some dosage forms.

Preparation	Proposed method		Official method
	Recovery[a] (%)	RSD[a]%	Recovery[a] (%)
Sulfamethoxazole Pure	100.22	0.70	100.20
METHEPRIM Tablets	99.63	0.33	99.80
CO-TRIMOXAZOLE Tablets	99.35	0.41	101.30
Cortim Oral Suspension	99.56	0.75	99.80
KIMITRIM Oral Suspension	99.73	0.28	100.80
Unpaired t test = (2.082) F test = (4.131)			
The value of t (tabulated) at 95% confidence level and for 8 degrees of freedom, two-tailed is 2.31.			
The value of F (tabulated) at 95% confidence level and for 4,4 degrees of freedom, two-tailed is 9.605.			
a For three determinations.			

Comparison of Methods: Table 4 shows the characteristics of spectrophotometric methods based on diazotization-coupling reaction used for the determination of SMZ. We found that reagents (coupler) with amino group are more sensitive than hydroxyl group.

Table 4: Characteristics of methods for the determination of SMZ based on diazotization-coupling reaction.

Reagent	λ_{max} /nm	$\varepsilon \times 10^4$	Linear range/ppm	Remarks	Ref.
NED	537	4.90	0.2-12	Acidic media	[29]
1-naphthol	525	3.37	2-14	Alkaline media	[30]
Salbutamol sulphate	452	2.50	2.5-87.5	Alkaline media	[31]
1-Naphthylamine	525	4.27	-	0.5% SDS media	[32]
NED	545	4.68	-	0.5% SDS media	[32]
Iminodibenzyl	580	4.79	0.05–4.0	Alcohol media	[33]
Orcinol	390	1.48	2 - 10	Acidic media	[34]
3-Aminophenol	460	4.32	0.1-8	Aqueous media	[35]
dopamine hydrochloride	500	2.67	0.1-7	Molybdic acid	[36]
8-hydroxyquinoline	500	3.83	0.2-6	Alkaline media	[37]
2,5-dimethoxyaniline	475	5.11	0.1-8	Aqueous mildly acidic medium	This Work
ε = Molar absorptivity/L.mol^{-1}.cm^{-1}					
NED = N-(1-naphthyl)ethylenediamine dihydrochloride					

APPLICATIONS

This method can be applied for the determination of sulfamethoxazole in bulk and in pharmaceutical preparations (oral suspension and tablet) with very good recoveries 99.35-100.2%.

CONCLUSIONS

The proposed method is found to be simple, rapid, selective and highly sensitive than most of the spectrophotometric methods available in literature. The statistical parameters and the recovery study data clearly indicate the reproducibility and accuracy of the method. Thus the method can be adopted as an excellent spectrophotometric method.

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