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Spectrophotometric Determination of Sulfamethoxazole and Sulfadiazine in Pure and Pharmaceuticals Preparation

SAADIYAH A. DHAHIR* and AMAL H. MHEMEED

Chemistry Department, College Science for Women, Baghdad University, Baghdad, Iraq

*Corresponding author: E-mail: sadiataher@yahoo.com

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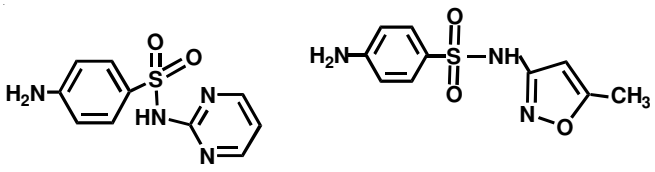
A rapid and sensitive spectrophotometric method is proposed for the determination of sulfamethoxazole and sulfadiazine. This method is based on the diazotization of sulfonamide with sodium nitrite and a coupling reaction of the diazo-compound with thymol. The optimum reaction conditions and other analytical parameters were evaluated. The linear ranges for the determination of sulfamethoxazole and sulfadiazine are 1-10 μg mL^{-1} and 1-7 μg mL^{-1} and their detection limits are 0.008 μg mL^{-1} and 0.007 μg mL^{-1} respectively. There was no interference from commonly utilized tablet excipients. The method has been used to determine sulfamethoxazole and sulfadiazine in pharmaceuticals preparation without separation.

Key Words: Spectrophotometry, Sulfamethoxazole, Sulfadiazine, Diazotization, Thymol.

INTRODUCTION

Sulfonamides are a class of drugs commonly used for their bacteriostatic activity especially in the treatment of urinary-tract infections[1]. Sulfadiazine and sulfamethoxazole (**Scheme-I**) are now available as widely used pharmaceutical products and veterinary practices. They are kinds of sulfonamides used in the treatment of urinary-tract infectious, pneumocystis pneumonia, chronic bronchitis, meningococcal meningitis, acute otitis media and toxoplasmosis[2].



Sulfadiazine **Sulfamethoxazole**

Scheme-I: Structural formulas of sulfadiazine and sulfamethoxazole

The aromatic amines were determined spectophotometry by a diazotization reaction. It is based on the conversion of free primary aryl amine into a diazonium salt by a reaction with nitrous acid, on coupling the salt then rapidly forms an azo-dye with a chromogenic reagent, such as *N*-(1-napthyl)-ethylenediamine (NED)[3], α-naphthylamine[4] and 8-hydroxy-quinoline[5]. The procedure requires the removal of excess nitrous acid by sulfamic acid, the stabilization of intermediary diazonium salt at low temperature. Various methods have been developed and used for the separation and quantitative estimation of sulfonamides sulfadiazine and sulfamethoxazole in food matrices, biological samples and veterinary products and different final dosage forms. These methods include titrimetric method[6,7], high-performance liquid chromatographic (HPLC) technique[7-10]. Thin layer chromatography[11,12], capillary zone electrophoresis[13], liquid chromatography with post column fluorescence derivatization[14] and micellar electrokinetic capillary chromatographic method[15]. In the present study, we succeeded in developing a novel coupling agent for sensitive and selective spectrophotometric determination of the sulfonamide class of drugs based on the coupling of their diazotized form with thymol, which results in the formation of coloured products in alkaline medium.

EXPERIMENTAL

A UV/VIS spectrophotometer (T60U with 1 cm matched quartz cells) was used. Sulfamethoxazole and sulfadiazine chemical reference substances were used from state company for drug Industries and Medical Appliance-(SDI) Samara-Iraq (BDH), standard solutions of pure reference sulfamethoxazole and sulfadiazine100 μg mL^{-1} were freshly prepared by dissolving 0.01 g both of them in 10 mL absolute ethanol and then diluted with distilled water to 100 mL.

Standard solution of 100 μg mL^{-1} of thymol was freshly prepared by dissolving 0.01 g of thymol with distilled water to 100 mL. All other reagents and solvents used were of analytical grade without further purification. Sodium nitrite (99.8 %

purity) and standard solution of 1 % was prepared. 1 M sodium carbonate of (98 % purity) was prepared, 100 μg mL^{-1} of varies interferences and 1 M both of HCl, sulfuric acid, acetic acid nitric acid phosphoric acid, sodium hydroxide, potassium hydroxide, potassium carbonate and ammonium hydroxide were used.

Aliquots of standard sulfonamide solutions (sulfamethoxazole and sulfadiazine) were transferred into 10 mL calibrated flasks followed by 0.5 mL hydrochloric acid to each. After cooling in an ice bath, 0.5 mL of sodium nitrite (1.0 % m/V) was added under swirling. The solutions were allowed to stand for 5 min, then 0.5 mL of sulphamic acid was added for excess of nitronuim ion the solutions were allowed to stand for 5 min, then 0.5 mL of thymol was added, with 0.5. mL of sodium hydroxide (1 M) for sulfamethoxazole or 0.5. mL of potassium carbonate. (1 M) for sulfadiazine The solution was made up to the mark with distilled water mixed thoroughly and after 5 min the absorbance was measured at 417 nm for sulfamethoxazole and 469 nm sulfadiazine against a reagent blank and the calibration graph was constructed.

Analysis of dosage forms

Metheprim tablets: These formulations were purchased from local commercial sources, the state company for drugs industrial and medical application Ninawa (N.D.I) and used for the analysis: Each tablet contain 80 mg trimethoprim and 400 mg sulfamethoxazole. Twenty tablets were powdered and mixed thoroughly. An amount equivalent to 100 mg of powdered was then dissolved in 5 mL ethanol and complete to 100 mL with distill water. and appropriate aliquots of the solution were treated as mentioned above in the general procedure.

Oral solution: Co-trimoxazole suspension from Pharaonia Pharmaceuticals Egypt, Alexandria, this drug contain 40 mg trimethoprim and 200 mg sulfamethoxazole each 5 mL of drugs contain 200 mg of sulfamethoxazole, 0.25 mL was transferred into 100 mL volumetric flasks dissolved in 5 mL ethanol and filtered and diluted up to the mark with distilled water. Working standard was prepared by suitable dilution and the recommended procedure was used for sulfamethoxazole for its determination.

Gel cream: Canting silver sulfadiazine from India Pharmaceuticals, 1 g from drug was transferred in to separation funnel and shaking with 5 mL ethanol for 15 min and then added 3 mL ethanol and shaking for 15 min and added 2 mL and shaking for 15 min, then the organic layer was separated from the water layer and dissolved in 100 mL volumetric flasks 50 mL ethanol and diluted up to the mark with distilled water.

RESULTS AND DISCUSSION

A new spectrometric method was developed for the determination of sulfadiazine and sulfamethoxazole. The method depends upon diazotization of the sulfa drugs followed by coupling with thymol in basic solution.

Absorption spectra: Sulfa drugs sulfamethoxazole and sulfadiazine could be readily diazotized in acidic medium and the diazonium cation would then coupling with a molecule of thymol in basic medium as a coupling agent to produce a yellow coloured azo product.

The coloured reaction products were developed as mentioned in the general procedure and the absorption maxima were found to be 417 nm for sulfamethoxazole and 469 nm for sulfadiazine drug. Figs. 1 and 2 are shows the absorption spectrum of azo dye of sulfamethoxazole and sulfadiazine. The results coloured product was found to be stable for about more one day. The value of absorbance decreased above 70 ºC. Hence, room temperature was preferred for the experiments.

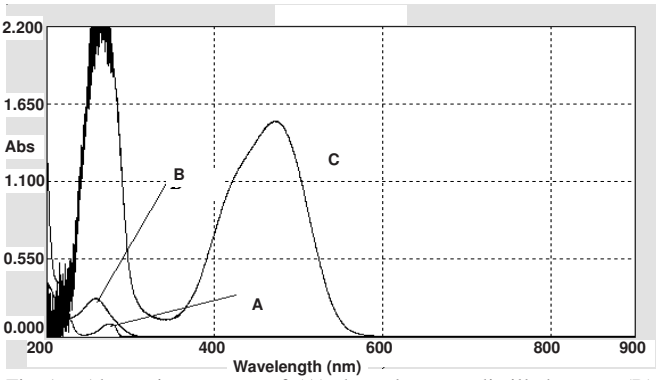


Fig. 1. Absorption spectra of (A) thymol *versus* distilled water, (B) sulfamethaxazole *versus* distilled water and (C) Azo dye against reagent blank

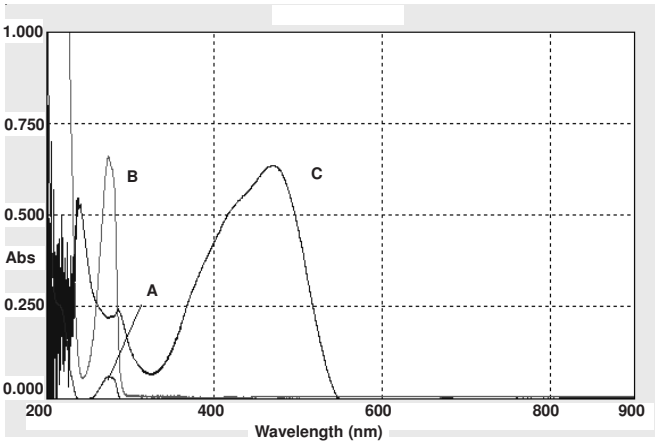


Fig. 2. Absorption spectra of (A) thymol *versus* distilled water, (B) sulfadiazine *versus* distilled water and (C) Azo dye against reagent blank

Optimization of the reaction conditions: The effect of various parameters on the absorption intensity of the dye formed was studied and the reaction conditions are optimized. The factors affecting colour development, reproducibility, sensitivity and conformity with Beer's law were investigated with sulfamethoxazole and sulfadiazine.

Effect of acid concentration: The effect of acidity on the diazotization reaction was studied in for sulfamethoxazole, and sulfadiazine in the range of 0.1-1 mL from 1 M HCl, HNO_3, H_2SO_4, CH_3COOH and H_3PO_4. The maximum diazotization was obtained in 0.5 mL of HCl and the formation of the azo-dye was reached its maximum absorbance after about 5 min; so 0.5 mL of 1 M HCl, was used in this study for sulfamethoxazole, and sulfadiazine. As had been noticed previously[16].

Effect of base in this study was investigated the effect 0.1-1 mL from 1 M of NaOH, KOH, Na_2CO_3, K_2CO_3, NH_4OH on the intensity of the produced product. For sulfamethoxazole and sulfadiazine. It was found that the presence of 0.8 mL of

1 M of NaOH led to increase the intensity of the produced product for sulfamethoxazole and0.6 mL of 1 M K_2CO_3 for sulfadiazine. Therefore these bases which give high sensitivity were selected in subsequent experiments.

Effect of sodium nitrite: The optimum concentration of sodium nitrite solution was found to be 0.3 mL of 1 % solution of sodium nitrite for sulfamethoxazole and sulfadiazine.

Effect of temperature: The effect of different temperature on diazotization and coupling was studied for two selected of sulfa drugs. It was found that diazotization at 0 ºC and at room temperature produced the same colour intensity. Therefore, working at room temperature (25 ºC) was preferred. It was found at higher temperatures the absorbance value decrease, indicating the dissociation of the product on prolonged heating.

Effect of diazotization and coupling time: Since diazotization for 3 min or more gave the same results, 3 min was selected. The azo dye was formed almost instantaneously after the addition of thymol and reached its maximum absorbance after 5 min; then 5 min developing time was used in this study. The colour obtained was stable for at least 24 h.

Effect of coupling agent: The effect of varying the concentration of coupling reagent was studied using the proposed procedure and adding 0.1-1 mL of 100 µg mL^{-1} of thymol to a series of drug solutions of both sulfamethoxazole and sulfadiazine. It was found that maximum and stable colours were formed with 0.5 mL of thymol solution for both sulfamethoxazole and sulfadiazine in final volume of 10 mL.

Effect of organic solvents: The effect of organic solvents such as methanol, ethanol, acetone and distilled water were studied by using in the dilution and measuring the absorbance the absorbance were found 0.186, 0.155, 0.143 and 0.671 respectively distilled water found to be the best.

Effect of order of addition: To obtain optimum results the order of addition of reagents should be followed as given under the procedure for two sulfa drugs, otherwise a loss in colour intensity was observed.

Effect of interference: The effects of some foreign ions which often accompany this drug in pharmaceutical products were studied by adding different amounts of foreign ions to 10 µg/mL of for sulfamethoxazole and sulfadiazine. The colour was developed following the recommended procedure described earlier. It was observed that the Arabic gum, glucose, starch, fructose, acetate, urea, NaCl, benzoic acid, salicylic acid, naphthylamine, *m*-cresol, 2,4-dichloro aniline and *o*-cresol were not interfering with the determination at levels found in dosage form.

Calibration graph: Employing the conditions described in the procedure, a linear calibration graph for sulfamethoxazole and sulfadiazine is obtained, Figs. 3 and 4 shows that Beer's law is obeyed over the concentration range of 1-10 µg/mL for sulfamethoxazole and 1-7 µg/mL sulfadiazine with correlation coefficient of 0.9996 and 0.9997 and an intercept of 0.0471 and 0.0208 respectively. The conditional molar absorptivity of the yalow product formed was found to be (2.6×10^4, 2.1×10^4) mol^{-1} cm^{-1}. 1 for sulfadiazine and sulfamethoxazole, respectively. The per cent relative standard deviations based on five replicates were 0.492, 0.657 for sulfamethoxazole and sulfadiazine other optimal characteristics and statistical data for the sulfadiazine and sulfamethoxazole were listed in Table-1.

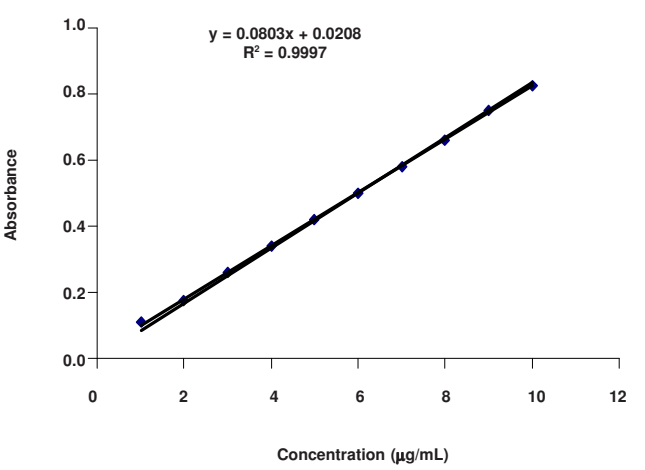


Fig. 3. Calibration graph of sulfamethoxazole

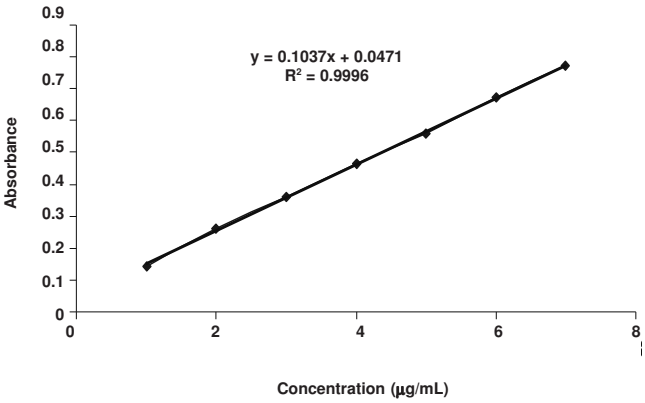


Fig. 4. Calibration graph of sulfadiazine

TABLE-1
OPTIMAL CHARACTERISTICS AND STATISTICAL DATA FOR THE SULFADIAZINE AND SULFAMETHOXAZOLE

Parameter	Sulfadiazine developed method	Sulfamethoxazole developed method
Colour	Yellow	Yellow
λ_{max} (nm)	469	473
Stability constant	3.4×10^{11}	5.6×10^{10}
Beer's law limit a (µg mL^{-1})	1-7	1-10
Molar absorptivity ($mol^{-1}cm^{-1}$ L)	2.6×10^4	2.1×10^4
Sandell's sensitivity (µg cm^{-2})	0.0096	0.012
Slope	0.1037	0.0803
Intercept	0.0471	0.0208
Correlation coefficient	0.9996	0.9997
Limit of quantization (mg mL^{-1})	0.025	0.029
LOD (µg mL^{-1})	0.077	00.087
[a]RSD (%)	0.657	0.492
Average recovery (%)	100.57	100.12

[a]Average of five determination

Reaction mechanism of the dye: The stoicheiometry of the reaction between both sulfamethoxazole and sulfadiazine with thymol were investigated using job's method[17] and mole ratio method. The results obtained in Figs. 5 and 6 show that 1:1 drug to reagent was formed at 473 nm for sulfamethoxazole and 469 nm sulfadiazine.

The product formed was water soluble, the stability constant was calculated by comparing the absorbance of a solution containing stoicheiometric amount of 6×10^{-4} M both

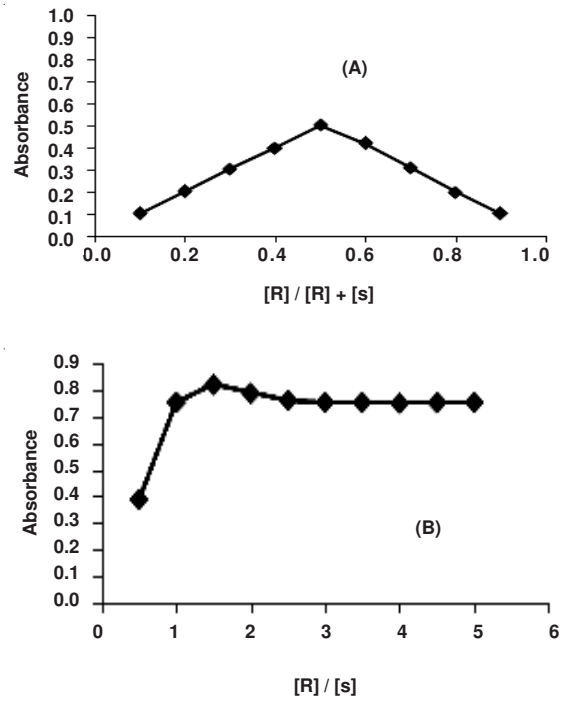


Fig. 5. (a) Job's plot method and (b) molar ratio method of sulfamethoxazole-thymol

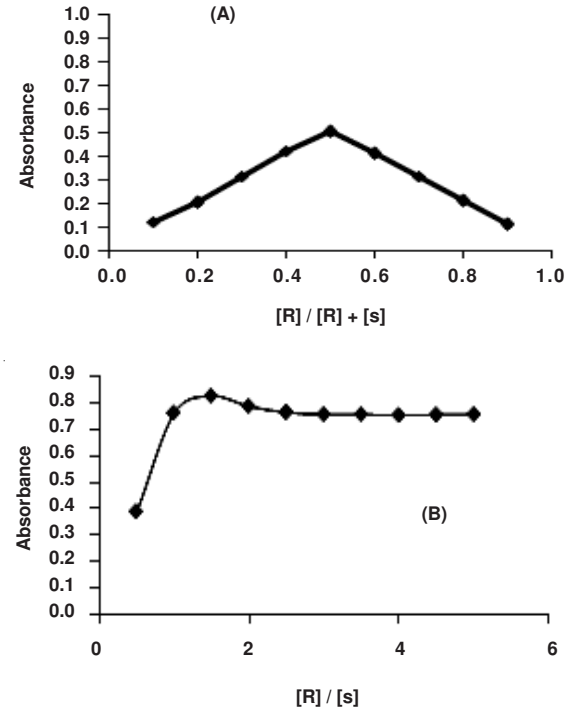
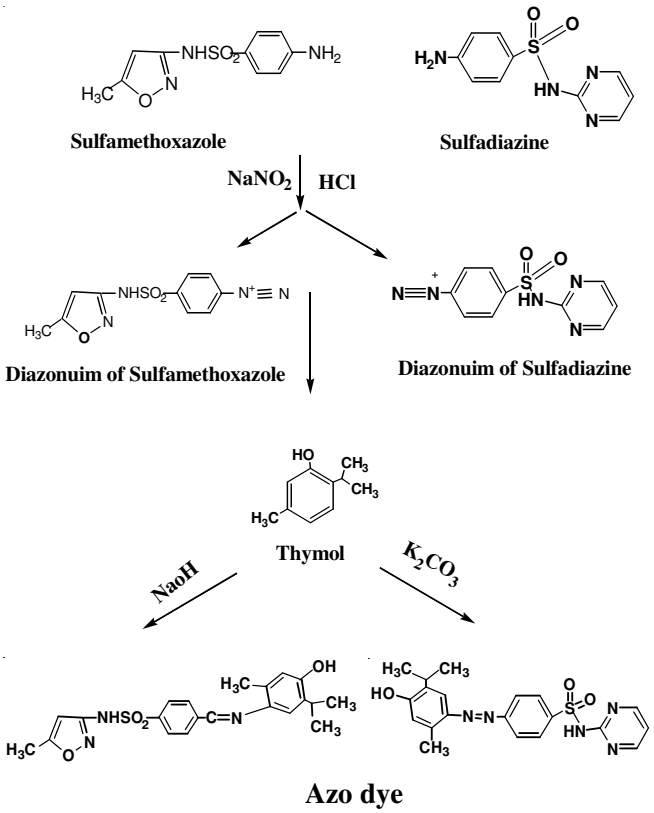


Fig. 6. (a) Job's plot method and (b) molar ratio method of sulfadiazine-thymol

sulfamethoxazole, sulfadiazine and thymol with that of solution containing the optimum amount of thymol (6×10^{-4} M). The average conditional stability constant of the dye in water under the described experimental conditions was 3×10^6 L^2 mol^{-2}. For sulfamethoxazole-thymol dye and 6.4×10^6 L^2 mol^{-2} for sulfadiazine-thymol dye. The proposed mechanism of reaction between thymol and the sulfonamide drug is illustrated in **Scheme-II**.



Scheme-II: Scheme of the proposed reaction mechanism

Precision and accuracy: sulfamethoxazole and sulfadiazine were determined at three different concentrations the results shown in Table-2. A satisfactory precision and accuracy could be obtained with the proposed method.

TABLE-2
ACCURACY AND PRECISION OF THE PROPOSED METHOD

Pure drugs	Taken (µg/mL)	Found (µg/mL)	Recovery (%)	*Average recovery (%)	*RSD (%)
SFMx	8	8.2	102.50	100.12	0.434
	9	8.9	98.88		0.487
	10	9.9	99.00		0.555
SFD	5	5.1	102.0	100.57	0.771
	6	5.9	98.33		0.645
	7	7.1	101.40		0.555

*Average of five determinations. SFMx = Sulfamethaxazole; SFD = sulfadiazine;

Analysis of pharmaceutical preparations: Two types of drug containing sulfamethaxazole (tablet and oral solution) and sulfadiazine (cream) have been analyzed and they gave good accuracy and precision these applications (Table-3).

The excellent sensitivity than other spectroscopic methods in literature for the oxidative coupling reaction of sulfamethaxazole and sulfadiazine as showed in Table-4.

Evaluate the results of the proposed method: For the evaluating the results of the proposed method comparing with the standard method to determine the efficiency and success in the estimate due to unavailable of the standard method in the British pharmacopoeia, there for standard addition method was used for determination of both sulfamethoxazole and sulfadiazine in pure and pharmaceutical preparation. The results

shown in Figs. 7-9 shows that the results of standard addition method agree well with the proposed method, indicating that the method is selective and free from interference.

TABLE-3
APPLICATION OF THE PHARMACEUTICAL PREPARATIONS OF DETERMINATION OF SULFAMETHAXAZOLE AND SULFADIAZINE DRUGS

Sample preparation	Taken (μg/mL)	Found (μg/mL)	Recovery (%)	Average recovery (%)	*RSD (%)
Tablets [a] metheprim	8	8.6	107.5	100.72	0.956
	9	8.7	96.66		0.858
	10	9.8	98.00		1.260
Oral solution metheprim	8	7.9	98.75	98.95	0.645
	9	9.1	101.11		0.771
	10	9.7	97.00		1.260
Sulfadiazine cream	5	4.9	98.00	99.25	1.260
	6	5.9	98.33		0.956
	7	7.1	101.42		0.771

*Average of five determinations

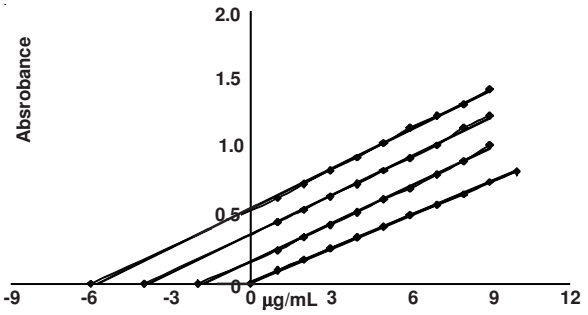


Fig. 7. Standard addition method for determination of sulfamethoxazole tablets

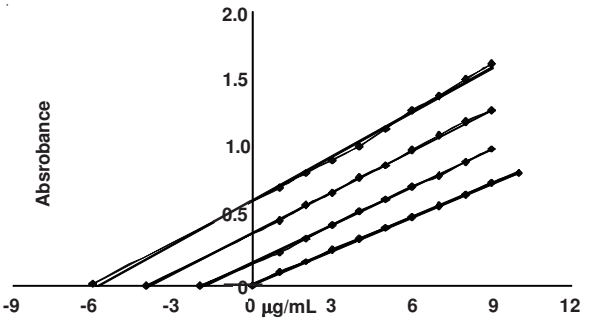


Fig. 8. Standard addition method for determination of sulfamethoxazole oral suspension

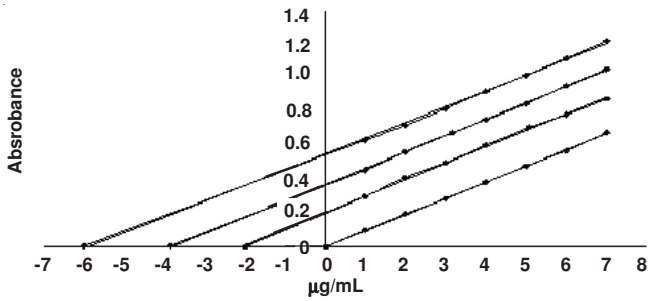


Fig. 9. Standard addition method for determination of sulfadiazine cream

Conclusion

The proposed methods were found to be simple, economical, selective and sensitive. The statistical parameters and recovery study data clearly indicate the reproducibility and accuracy of the methods. Analysis of the samples containing sulfamethoxazole and sulfadiazine showed no Interference from the common excipients. Hence, these methods could be considered for the determination of sulfamethoxazole and sulfadiazine in the quality control laboratories.

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TABLE-4
COMPARISON OF SULFAMETHOXAZOLE AND SULFADIAZINE DETERMINATION IN THE PROPOSED METHOD AND OTHER LITERATURE METHODS

Drugs	Reagent	λ_{max} (nm)	ε ($L\ mol^{-1}\ cm^{-1}$)	Beer's law range (ppm)	Colour of dye	RSD (%)	LOD	Ref.
Sulfamethoxazole	8-Hydroxyquinoline	500	-	o.1-7.0	Red	0.1-0.5	0.03-0.05	5
	Salicylaldehyde	445	-	5-40	Yellow	-	0.06	18
	2-Naphthol	482	1.34×10^4	0.21-0.66	-	-	-	19
	Orcinol	390	-	2-10	-	-	-	20
	o-Phthalaldehyde	340	-	0.01-0.24	-	1.95-2.08	-	21
	Present method	473	2.1×10^4	1-10	Yellow	0.492	0.0087	
Sulfadiazine	α-Naphthalamine	-	-	0.2-20	-	-	0.06	4
	8-Hydroxyquinoline	500	-	0.1-7.0	Red	0.1-0.5	0.03-0.05	5
	Glutaraldehyde	-	-	-	-	3.2-4.6	3.1	22
	Acetylacetone formaldehyde	400	-	4-72	Yellow	1.07	-	23
	Present method	469	2.6×10^4	1-7	Yellow	0.657	0.007	

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