**Baghdad Science Journal** **Vol.7(1)2010**

# Safety method, Spectrophotometric Determination of Sulfamethaxazole drug in bulk and Pharmaceutical Preparations

*Saadiyah A. Dhahir** *Amira .H. Hamed** *Maha K. Salman**
*Rafah K.Ahmed **

Date of acceptance 1/3 / 2010

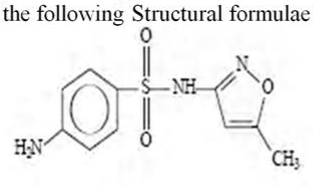
## Abstract

A simple, cheap, fast, accurate, Safety and sensitive spectrophotometric method for the determination of sulfamethaxazole (SFMx), in pure form and pharmaceutical dosage forms. has been described The Method is based on the diazotization of the drug by sodium nitrite in acidic medium at 5C° followed by coupling with salbutamol sulphate (SBS) drug to form orange color the product was stabilized and measured at 452 nm Beer's law is obeyed in the concentration range of 2.5-87.5 µg $ml^{-1}$ with molar absorptivity of $2.5x10^4$ L $mole^{-1}$ $cm^{-1}$. All variables including the reagent concentration, reaction time, color stability period, and sulfamethaxazole /salbutamol ratio were studied in order to optimize the reaction conditions. No interferences were observed Results of analysis were validated statistically and by recovery studies. These methods are successfully employed for the determination of sulfamethaxazole in some pharmaceutical preparations.. The developed method is easy to use and accurate for routine studies relative to HPLC and other techniques.

**Key words: Spectrophotometric; Sulfamethaxazole, pharmaceutical formulations.**

## Introduction

Sulfamethoxazole is 4-Amino-N- (5-methyl-3-isoxazolyl) benzene sulfonamide with the following Structural formulae [1,2]



It is a sulfonamide bacteriostatic antibiotic. Sulfonamides are structural analogs and competitive antagonists of *para*-aminobenzoic acid (PABA). [3,4] are used in the treatment of urinary track infections and eye infections The drug has been determined by a variety of analytical techniques such as titrimetric method[5,6], high performance liquid chromatography [7-9], gas chromatography [10] and spectrophotometry[11-13]

Aromatic amines containing drugs such as the sulfonamides were determined by a diazotization reaction [14]. It is based on the conversion of the free aryl amine into a diazonium salt at 0-5C° by a reaction with nitrous acid; the salt rapidly forms an azo dye with a chromogenic reagent, such as phenolic aromatic compounds

In the present study the authors had developed simple and sensitive spectrophtometric methods for the determination of Sulfamethoxazole in pharmaceutical formulations, based on he coupling of his diazotized with SBS, which results in the formation of orange colored products in alkaline medium.

*Baghdad University / College Science for women / Chemistry department.

## Material and Methods:

### Apparatus

All spectrophotometric measurements were carried out using Computerize UV-Visible, shimadzu T60U Spectrophotometer; silica glass cell of 1 cm thickness was used throughout this study.

### Materials

All chemicals used were of analytical reagent grade Sulfamethaxazole and salbutamol sulphate standard material were provided from state company for Drug Industries and Medical appliance (SDI) Sammara-Iraq.of (99% purity) and stander solution of 100 µg $ml^{-1}$ both of them were freshly prepared by dissolving 0.025gm of SBS and sulfamethaxazol in 20ml absolute ethanol and then diluted with distilled water to the mark with 100 ml volumetric flacks respectively. Sodium nitrite (99.8 purity) from (BDH) and stander solution of 1% was prepared. Sodium Hydroxide of (98% purity) from (RDL), solution of 1M was prepared by dissolving 4 gm in 100 ml distilled water, 100 ppm of varies interferences and 1M both of HCl, sulfuric acid and phosphoric acid were used These solutions are stable for a period of 3 d when refrigerated (4 °C).

### Recommended analytical procedure

Different aliquots of sulfamethaxazole standard stock solution equivalent to 4-80 µg ml were transferred into a series of 10 ml volumetric flasks, with 0.5ml of 1% sodium nitrite and 0.75 ml of 1M HCl The flasks were cooled then added 1.25ml of salbutamol with 1.ml of 1M sodium hydroxide solutions the volume was made up to the mark with distilled water. The absorbance was measured at 452 nm against a blank solution prepared in the same method but without sulfamethaxazole.

### Analysis of dosage forms

***Tablets*:** ten tablets were weighed and finely powdered. A weighed amount of the powder containing 400 mg of SFMx (equivalent to one tablet) was dissolved in 50 ml of volumetric flasks and diluted up to the mark.

***Oral Solution*** 6.25 ml was taken from container containing 200 mg of SFMx was transferred into 50 ml volumetric flasks and diluted up to the mark with distilled water. Working standard was prepared by suitable dilution and the recommended procedure was used for SFMx for its determination

## Results & Discussion

### Absorption Spectra

An orange -colored oxidizing coupling product with absorption maximum at 452 nm is formed when Sulfamethaxazole was allowed to react with Salbutamol in basic medium sodium hydroxide. Figure 1 shows the spectra of orange product formed so; the maximum absorption at 452 nm is used in all subsequent experiments.

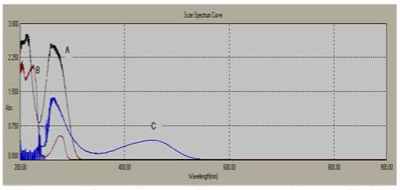



**Figure 1.** Absorption spectra of (a) Sulfamethaxazole versus distilled water, and (b) reagent blank (SBS) versus distilled water (C) Sulfamethaxazole/Salbutamol Dye against reagent blank

### *Study of the Optimum Reaction Conditions*

The effect of various parameters on the absorption intensity of the dye formed

was studied and the reaction conditions are optimized.

**Effect of Base:** It was found that the presence of a base led to increase the intensity of the produced product, therefore some bases such as NaOH, was examined and was found that 1 ml of this base give high sensitivity which selected in subsequent experiments.

*Effect of acidity*

The effect of acidity on the diazotization reaction was studied in the range 0.25-1.25ml of 1 M HCl, The minimum time required for diazotization was 2 min. Diazotization was carried out at room temperature and the optimum acidity for the formation of diazonium ion was fixed to 0.75ml of 1M. HCl..

**Effect of sodium nitrite:** The optimum concentration of sodium nitrite solution was found to be 0.5 ml of 1% solution of sodium nitrite.

*Effect of coupling agent*

The effect of varying the concentration of coupling reagent was studied using the proposed procedure and adding 0.25-2.0 ml of 500 $\mu gml^{-1}$ of SBS to a series of drug solutions. It was found that maximum and stable color was formed with 1.25 ml of SBS solution in final volume of 10 ml.

**Effect of Reaction Time:** The colour intensity reached its maximum after the diazonium salt of Sulfamethaxazole drug had been reacted immediately with salbutamol in the presence of sodium hydroxide and became stable after 3 minutes, therefore 3 minutes development time was selected as optimum in the general procedure. The colour obtained was stable for at least 30 hours.

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

**Effect of Temperature:** The resulting product of the proposed method was studied at different temperatures. The results indicate that the absorbance values remain constant in the temperature range 0-70ºC, whereas, at higher temperatures the absorbance value decrease, indicating the dissociation of the product on prolonged heating. The colored product was stable for more than 6 hours at room temperature Therefore room temperature is selected in this method.

Calibration Graph

Employing the conditions described in the procedure, a linear calibration graph for Sulfamethaxazole is obtained (Figure 2), which shows that Beer's law is obeyed over the concentration range of 2.5-87.5 $\mu gml^{-1}$ with correlation coefficient of 0.9997 and an intercept of 0.003. The conditional molar absorptivity of the orange product formed was found to be $2.5x10^{4}$ $L.mol^{-1}.cm^{-1}$.

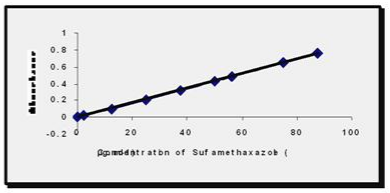



**Figure 2.** Calibration graph of sulfamethaxazole.

*Effect of Organic solvents*

The effete of organic solvents such us methanol, ethanol, acetone, and distill water were studied by using in the dilution and measuring the absorbance the absorbance were found 1.24,1.3,0.602 and 0.855 respectively Distill water found to be the best. , Cheap, and available solvent.

### *Interference*

The effect of some foreign organic compounds, which often found in pharmaceutical products, were studied by adding different amounts organic compounds 1ml of 500µg/ml of sulfamethaxazole. The color was developed following the recommended procedure described earlier.. It was observed that the talc, glucose, starch, were not interfering with the determination at levels found in dosage form.

## Structure of the Dye

The stoicheiometry of the reaction between sulfamethaxazole and SBS was investigated mole ratio method; the results obtained (Figure 3) show that dye 1:1 SFMx to SBS was formed at 452nm.

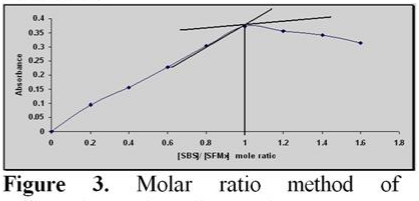



**Figure 3.** Molar ratio method of sulfamethaxazole.:salbutamol Dye

Therefore the formation of the product probably occurs as follows Figure4

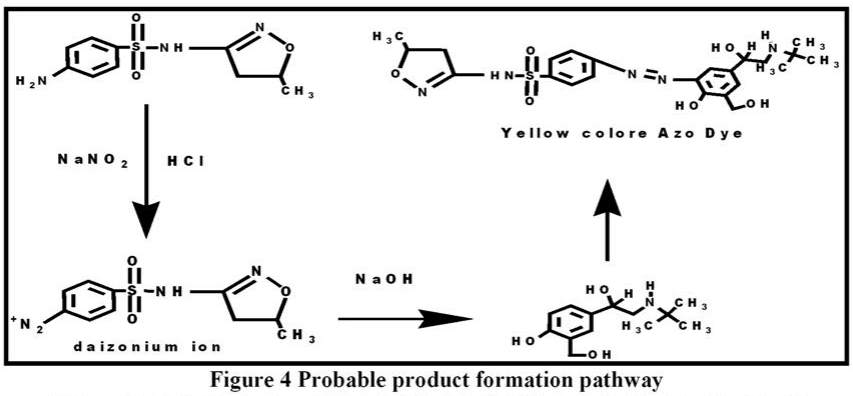



**Figure 4 Probable product formation pathway**

**The product formed was water-soluble, the stability constant was calculated by comparing the absorbance of a solution containing stoicheiometric amount of sulfamethaxazole and SBS The average conditional stability constant of the dye in water under the described experimental conditions was $3.5x10^{-4}$.**

Precision and Accuracy

Sulfamethaxazole was determined at three different concentrations. The results shown in Table 1. A satisfactory precision and accuracy could be obtained with the proposed method.

**Table 1.** Accuracy and precision of the proposed method.

| SFMx Taken | SFMx found | * Recovery% Rec% | Average recovery% Rec% | %E Relative Standard error | Average Relative Standard error %E | Relative Standard Deviation* RSD% |
|---|---|---|---|---|---|---|
| 50 | 49.99 | 99.98 | 100.06 | 0.02 | 0.085 | 0.132 |
| 75 | 75.03 | 100.4 | | 0.04 | | 0.1302 |
| 87.5 | 87.33 | 99.8 | | 0.194 | | 0.1 |

* Average of five determinations

### *Analytical application*

Two types of drug containing sulfamethaxazole (tablet and Oral Solution) have been analyzed and they gave good accuracy and precision, the results obtained were compared successfully with the official method (Table 2). Since F-test and t-test showed that there was no significant difference between the proposed method and the standard method.

Table 2:Application of the proposed method and pharmaceutical preparations for determination of sulfamethaxazole drug

| SBS Sample | SBS ppm | | Recovery% Rec% | * Average recovery% Rec% | Relative Standard Deviation * % RSD% |
|---|---|---|---|---|---|
| | Taken | Found | | | |
| Tablets [a] Metharain | 50 | 50.05 | 100.1 | 99.7 | 0.0833 |
| | 75 | 74.48 | 99.3 | | 0.081 |
| Oral Solution [a]Metharain | 50 | 49.96 | 99.92 | 100.045 | 0.087 |
| | 75 | 75.13 | 100.17 | | 0.0853 |

* Mean of three determinations.
a Marketed by S.D.I, Iraq.

The excellent sensitivity than other spectroscopic methods in literature for the oxidative coupling reaction of sulfamethaxazole .as showed in table (3)

**Table 3**: Comparison of sulfamethaxazole determination in the proposed method and other literature methods.

| Reagent | $\lambda_{max}$ nm | ε ,L mole$^{-1}$ cm$^{-1}$ | Linear range µg.ml$^{-1}$ | Ref. |
|---|---|---|---|---|
| 3-Aminophenol | Not reported | Not reported | 0.05-8.0 | 12 |
| 8-hydroxyquinoline | 500 | 3.38 x $10^4$ | 0.03–0.05 | 15 |
| 2-naphthol | 482 | 1.34 × $10^4$ | Not reported | 16 |
| derivative spectrophotometry | 259 | Not reported | 4–25 | 17 |
| SBS | 452 | 2.5x $10^4$ | 2.5 – 87.5 | **Proposed methods** |

## Conclusion

A simple, rapid, precise and sensitive spectrophotometric method has been developed for the determination of trace amounts of sulfamethaxazole in aqueous solution based on its oxidative coupling reaction with SBS in the presence of sodium hydroxide. The proposed method does not require temperature control or the solvent extraction step; the method was applied successfully on pharmaceutical samples.

## References

**1.**British Pharmacopoeia Commission (1993) *British Pharmacopoeia 1993*, Vols I & II, London, Her Majesty's Stationery Office, pp. 644, 856–859

**2.**Budavari, S., ed. (2000) *The Merck Index*, 12th Ed., Version 12:3, Whitehouse Station, NJ, Merck & Co. & Boca Raton, FL, Chapman & Hall/CRC [CD-ROM]

**3.** Martindale, The extra pharmacopoeia, 30th Ed., p. 208

**4.** Mitscher L A, Antibiotics and Antimicrobial Agents, Foye's Principles of Medicinal Chemistry, 2002, 5th Ed., 819-862.

**5.** United States Pharmacopoeia, XXIth Revision, National Formulary XVIth ed., 1985, U. S. Pharmacopeial

Convention, Rockville.

**6.** European Pharmacopoeia, Consejo de Europa (III-1975), **1981**, Consejo General de Colegios Oficiales de Farmaceuticos, Spain

**7.** United States Pharmacopeia National Formulary, USP 23, NF 18, **1995**, II, 1464.

**8** El Anwar F M, El Walily A M, Abdel Hay M H and M. El Swify, *Anal Lett*., 1991,**24**, 767–779.

**9** Thomas G K, Millar R G and Antis P W, *JAOAC Int.*, 1997, **80**, 988-995.

**10.** Chivarino B, Crestoni M A, Di-Marzio A and Fornarini S, *J Chromatogr Biomed Appl.***,** 1998, **706**, 269-277.

**11.**Jing F, Yahong C., Suling F., Cunling Y, and Jianji W., Flow-Injection Spectrophotometric Determination of Sulfadiazine and Sulfamethoxazole in Pharmaceuticals and Urine., ANALYTICAL SCIENCES MARCH 2003, VOL. 19 2003 © The Japan Society for Analytical Chemistry

**12**.Nagaraja P, Yathirajan HS, Raju CR, Vasantha RA, Nagendra P, Hemantha Kumar MS..,3-Aminophenol as a novel coupling agent for the spectrophotometric determination of sulfonamide derivatives., Farmaco. 2003 Dec;58 (12):1295-300.

**13.** Nagaraja P, Sunitha K R, Vasantha R A and Yathirajan H S, *Eur J Pharm Biopharm.***,** 2002, **53**, 187-192.132-135.

**14**.Davidson AG. Ultraviolet-visible absorption spectrophotometry. In: Beckett AH and Stanelake JB. (Eds.) *Practical Pharmaceutical Chemistry*. 4th Ed. The Athlone Press, London (1988) 275-337

**15.** Padmarajaiah N, Shailendra D. Naik Ashwinee K.S. Anantharaman S,.A ,Sensitive spectrophotometric method for the determination of sulfonamides in pharmaceutical preparations., *Acta Pharm. 57* (2007) 333–342

**16.** Fazel S. and Leyla A., Determination of Sulfamethoxazole and Trimethoprim in Pharmaceuticals by Visible and UV Spectrophotometry, Iranian Journal of Pharmaceutical Research (2006) 1: 31-36

**17.** S Balyejjusaa, RO Adomeb, and D Musokec, Spectrophotometric determination of sulphamethoxazole and trimethoprim (co-trimoxazole) in binary mixtures and in tablets, Afr Health Sci. 2002 August; 2(2): 56–62.

# تقدير دواء السلفومثوكزول بطريقة طيفيةامنة في المادة النقية وفي المستحضرات الصيدلانية

***سعدية احمد ظاهر* اميرة حسن حمد* مها كاظم سلمان * رفاه خالد احمد****

*جامعة بغداد / كلية العلوم للبنات / قسم الكيمياء ، بغداد ، العراق .

**الخلاصة**

وصفت طريقة لونية امنة وبسيطة ورخيصة وحسّاسة لتعيين تركيزالسلفومثوكزول في المادة النقية وفي بعض المستحضرات الصيدلانية إنّ تعتمد الطريقة على ازوتة السلفومثوكزول باستخدام نتريت الصوديوم في وسط حامضي عند (5 درجة مئوية) ثم ازدواج ملح الدايزونيوم للدواء مع السالبيتمول في وسط قاعدي و الحصول على لون لصبغة الازو البرتقالية عند طول موجي 452 نانومتر, قانون بير وجد مطاعا في مدى تركيز(2.5-87.5) ملغم/لتر مع ظهور قيمةالامتصاصية المولارية بين($2.5x10^{4}$) لتر/مول.سم..وتمت دراسة المتغيرات والمتظمنة تركيز الكاشف ووقت التفاعل واستقرارية الصبغة والنسبة المولية للسلفومثوكزول والسالبيتمول لتحديد الظروف المثلى للتفاعل.لم يلاحظ تاثير للمتداخلات والطريقة طبقت بنجاح لتقدير الدواء في المستحضرات الصيدلانية والطريقة اسهل ومناسبة اكثر من الطرق الروتينية ككروموتغرافا السائلة عالية الاداء والطرق الاخرى