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# Development Method for Spectrophotometric Analysis of Sulfamethoxazole Using Vanilline Reagent

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors designed the study. Author PAJ performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors TNAS and NSM managed the analyses of the study. Author NSM managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

This study involves a development method for spectrophotometric analysis of sulfamethoxazole (SMZ) with vanillin reagent in acidic medium. The reaction mechanism was proposed by Schiff's base reaction of the amine group in SMZ with the carbonyl group in vanillin. The spectrum of SMZ complex showed high absorbance at 399.09 nm following the Beer–Lambert law in the concentration levels ranging from 5 to 80 μg.mL$^{-1}$ with good sensitivity. The stability constant of the product was determined. The values of relative standard deviation (≤ 6.63) and recovery (> 98.73%) indicated a reasonably precise and accurate method with a good limit of detection and quantification. The present method was compared with other spectrophotometric methods in the literature. The procedure of the method showed to be simple since no need for temperature control and solvent extraction. The suggested method was used to determine of SMZ in commercial pharmaceutical drops and compared with the certified method.

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*Keywords: Spectrophotometry; sulfamethoxazole; vanillin; Schiff's base reaction.*

## 1. INTRODUCTION

Sulphamethoxazole (SMZ) is chemically described as 4-amino-N-(5-methyle-3 isoxazoyl) benzene sulphonamide ($C_{10}H_{11}N_3O_3S$, M.W 253.3 $g.mol^{-1}$) (Fig. 1). It is a white crystalline powder, belongs to sulphonamides bacteriostatic antibiotic drugs and usually used for many infection treatments such as eye infections, middle ear infections, bronchitis, acute urinary tract infections, diarrhea, and as prophylaxis of rheumatic fever. Frequently, the formulation products contain a mixture of sulfonamide with other drugs to increases the potential of the product such as sulphamethoxazole and trimethoprim product which is known as co-trimoxazole [1]. Searching the literature showed various instrumental methods for the analysis of sulpha drugs such as, GC [2], HPLC [3-5], flow injection [6-8], HPTLC [9], micellar electrokinetic capillary chromatography (MEKC) [10], voltammetry [11], solid phase extraction (SPE) [12] and spectrophotometric methods [13-16]. Some of the reported spectrophotometric methods require long heating times and a lot of data manipulation, which makes some difficult for their application as standard routine methods.

The work reported here describes a spectrophotometric analysis of sulphamethoxazole in bulk form and pharmaceutical formulation by condensation reaction with vanillin reagent in acidic medium to form Schiff's base product at room temperature. The present method was compared with other spectrophotometric methods in the literature for the determination of SMZ.

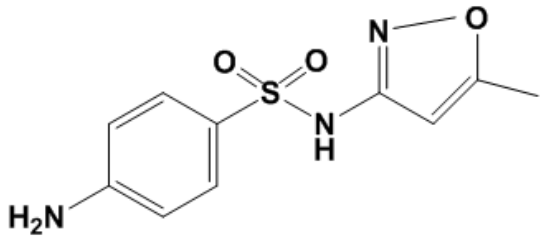



**Fig. 1. Sulfamethaxazole structure**

## 2. MATERIALS AND METHODS

### 2.1 Instruments

Perkin Elmer double beam spectrophotometer (Lambda 25 UV- VIS) was used with silica cuvettes (1 cm).

### 2.2 Chemicals

High purity analytical materials were used for the preparation of the following:

Vanillin solution (11%): prepared daily and used immediately by dissolving of 5.5 g of 4-hydroxy-3-methoxy benzaldehyde in 50 mL absolute methanol.

SMZ standard (250 $\mu g.mL^{-1}$) : a 0.025 g of pure sulphamethoxazole was dissolved in 20 mL mixture of water and acetone in the ratio of 1: 30 then made up to 100 mL by D.W. This stock solution was used for the preparation of working solutions.

Sulfuric acid solution (0.1 M): a suitable volume of concentrated $H_2SO_4$ was diluted with distilled water.

### 2.3 Recommended Procedure

Increased volumes of stock solution of sulphamethoxazole were taken (covering the range of 5.0-80 $\mu g.mL^{-1}$), then 1.6 mL of 11 % vanillin and 1.0 mL of concentrated $H_2SO_4$ (0.1 M) were added with final dilution with D.W. The absorbance measurement of series solutions was carried out at 399.09 nm at room temperature beside reagent blank.

### 2.4 Procedure for SMZ Assay in Sulfatrime

Ten tablets of Sulfatrime (Middle East Laboratories Co. LTD) were weighed and crushed, then an accurate amount equal to 0.330 g sulphamethoxazol was dissolved in 20 mL mixture of water and acetone in the ratio 1: 30 and well mixed followed by filtration. The volume of the filtrate was made up to 100 mL with D.W to obtain 250 $\mu g.mL^{-1}$. From the latter solution, three replicate at different concentrations were prepared. The drug solution was analyzed as described in the recommended procedure.

## 3. RESULTS AND DISCUSSION

### 3.1 Absorption Spectra

The spectrum of SMZ complex (A) showed maximum absorption at 399.09 nm which used later for all measurements, the blank spectrum (B) has low measurement at this wavelength as shown in Fig. 2.

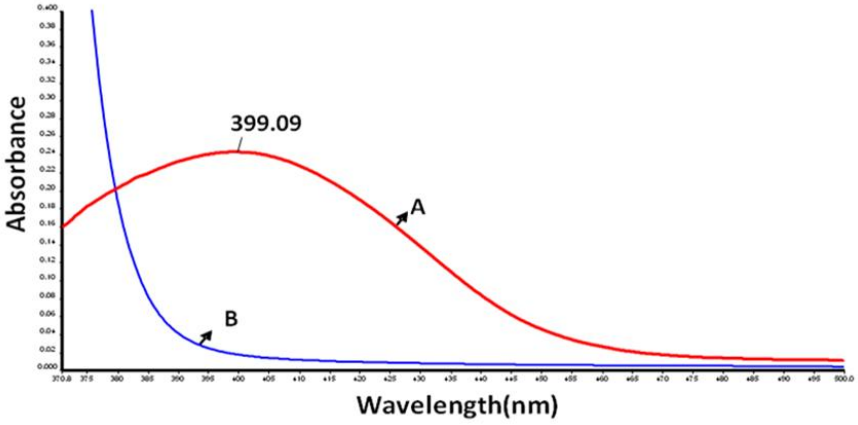



**Fig. 2. Absorption spectra of (a) SMZ complex (b) blank**

## 3.2 Optimization of Proposed Method Conditions

The proposed method was optimized through studying the effect of different variables including solvent, temperature, reaction time, vanillin concentration, pH, acids, different combinations of buffer solutions and their pH, and surfactants.

### 3.2.1 Type of solvent

The effect of the solvent was investigated. Different solvents were tested namely water, acetone, methanol, acetonitrile, ethanol, and 1,4-Dioxane. The procedure was based on the mixing of (2 mL) 250 $\mu g.mL^{-1}$ sulphamethoxazole with 1 mL of 10% vanillin in the presence of 1 mL $HNO_3$ in a final volume of 10 mL. Measuring the absorbance after 5 min at room temperature showed that using water as a solvent for sulphamethoxazole and methanol as a solvent for vanillin and the dilution with water gave maximum absorbance (0.162) at 399.09 nm. Therefore, this system of solvents was recommended in this method.

### 3.2.2 Temperature and time

Based on the development of color intensity, the time of the reaction was determined by measuring the absorbance at 25°C (R.T) and 40°C (using thermostatic water bath) after each 5 min intervals. The results showed high color intensity after 5 min with maximum absorbance (0.166) at room temperature with high stability for more than 100 min. While the absorbance was low at 40°C and continuously decreased which can be interpreted for the decomposition of the SMZ complex product. Therefore, the room temperature was selected.

### 3.2.3 Vanillin concentration

Different concentrations (%) from vanillin reagent were tested using the same amount of SMZ and $HNO_3$. The absorbance increases when vanillin concentration increasing (Fig. 3), and reaches its maximum value when using 1.6 mL 11% which was used in the subsequent work.

### 3.2.4 Type of acid and buffer solution

Due to the reaction of vanillin with sulphamethoxazole was carried out in acidic medium, therefore; the effect of different acids was studied using 1 ml (0.1 m) of each tested acid. Fig. 4 shows maximum absorbance in the presence of sulphuric acid with a final ph value of 1.54. Different buffer solutions at this ph were also tested showing no effect (Fig. 5).

### 3.2.5 Addition order

The best results were obtained when vanillin and sulphamethoxazole were added before adding $H_2SO_4$. Otherwise, a loss in color intensity was observed when changing the order of the addition of reagents. That can be attributed to decomposition SMZ complex or create a new product with different molecular structure and absorption intensity leading to a color change.

## 3.3 Quantification

Table 1 shows a summary of method parameters indicating the sensitivity, excellent linearity, precision, and accuracy of the offered method with good LOD and LOQ.

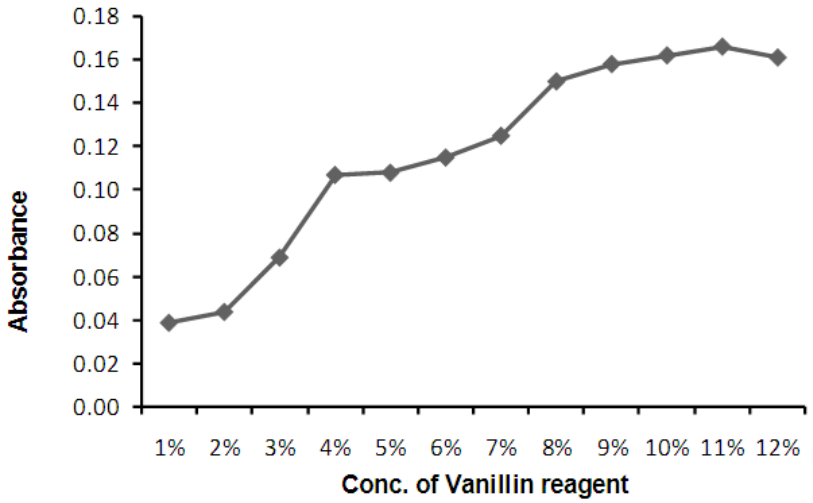



**Fig. 3. Effect the concentration of vanillin reagent**

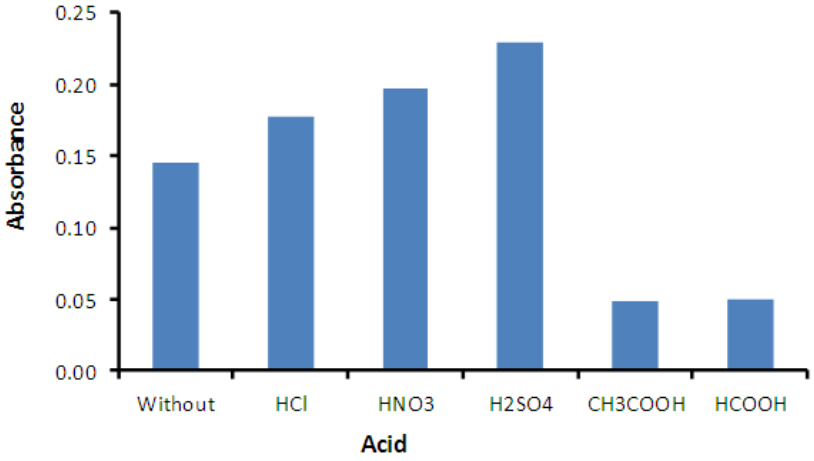



**Fig. 4. Effect type of acid**

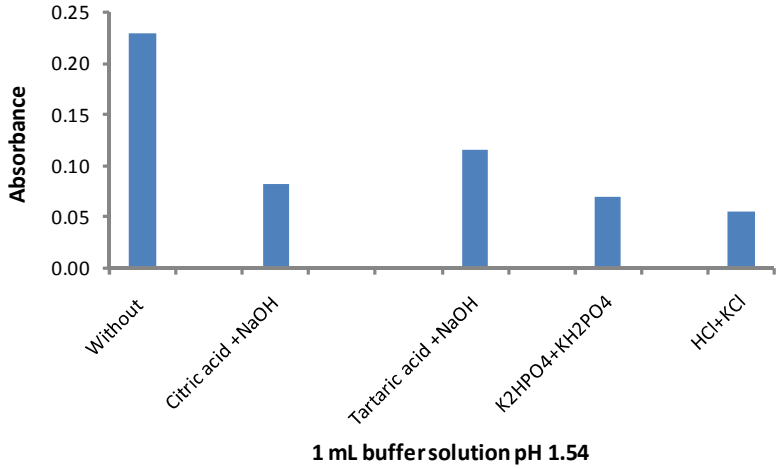



**Fig. 5. Buffer solution effect**

**Table 1. Summary of studied method parameters**

| Parameter | Value |
|---|---|
| Beer's law range | 5 – 80 µg. $mL^{-1}$ |
| Sensitivity (α) | 1.1 × $10^3$ L $mol^{-1}$ $cm^{-1}$ |
| Correlation coefficient | 0.9955 |
| Slope | 0.0042 |
| Intercept | 0.0250 |
| RSD% (n=5) | ≤ 6.36 |
| Average recovery % (n=5) | > 98.73 |
| LOD | 2.829 µg.$mL^{-1}$ |
| LOQ | 8.575 µg.$mL^{-1}$ |

### 3.4 Interference

The effect of adding some excipients as interference was investigated in the presence of different concentrations (50 - 1500 µg.$mL^{-1}$) including lactose, glucose, acacia, sodium chloride, and starch which commonly are available in pharmaceutical preparations. The recovery results were in the range of 94 to 109% considering this variation is acceptable and no effect of studied excipients.

### 3.5 Stoichiometry Study

To establish the stoichiometry methods of the SMZ complex product, Job's and molar ratio were applied. Fig. 6 indicated a stoichiometric composition of complex to be 1:1. This finding explains that the $NH_2$ group in SMZ is sharing in a Schiff's base product.

### 3.6 Stability Constant

According to the results obtained from stoichiometry methods, stability constant ($K_{st}$) of the complex product was measured through the following equations:

$$K_{st}=1- D/ 4D^3C^2 \quad (1)$$

$$D = a_m - a_s/ a_m \quad (2)$$

Where D is the dissociation degree, C is the complex concentration, $a_s$ is the absorbance of stoichiometric quantities of sulfamethoxazole and vanillin, $a_m$ is the absorbance of the solution containing an excessive quantity of vanillin reagent. Table 2 shows the results of the stability constant ($K_{st}$) indicating good stability.

### 3.7 Reaction Mechanism

The reaction mechanism was proposed by the formation of Schiff's base linkage in the presence of an acid to protonate the carbonyl oxygen of aldehyde group of vanillin to positive charge that gains lone pair of electrons from amine group ($NH_2$) in sulphamethoxazole. That is followed by internal rearrangement to form of Schiff's base called imine giving water and proton as by-products. The tentative reaction scheme was proposed as follow.

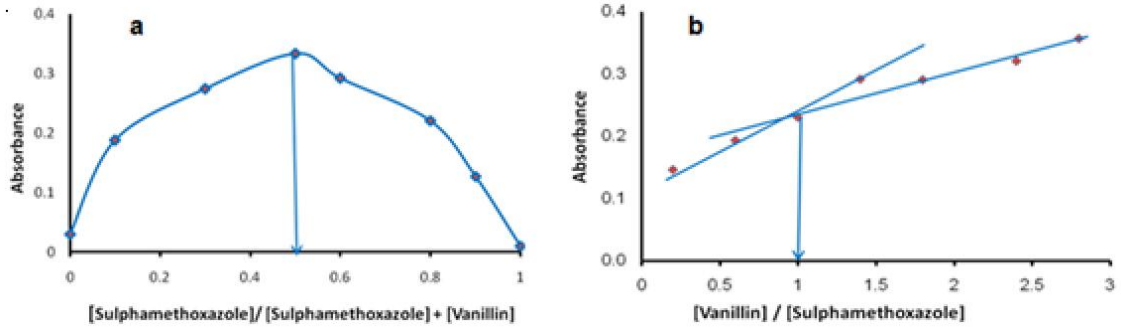



**Fig. 6. (a) Job's method with (b) mole ratio plots of product of sulphamethoxazole with vanillin reagent**

**Table 2. Stability constant of sulphamethoxazole- vanillin product**

| Vol. (mL) | C (M) | Absorbance | | D | $K_{st}$ (L.$mol^{-1}$) | Mean $K_{st}$ |
|---|---|---|---|---|---|---|
| | | $a_s$ | $a_m$ | | | |
| 0.4 | 0.002 | 0.113 | 0.160 | 0.293 | 4.1×$10^3$ | 2.7×$10^4$ |
| 0.8 | 0.004 | 0.312 | 0.335 | 0.068 | 5.04×$10^4$ | |

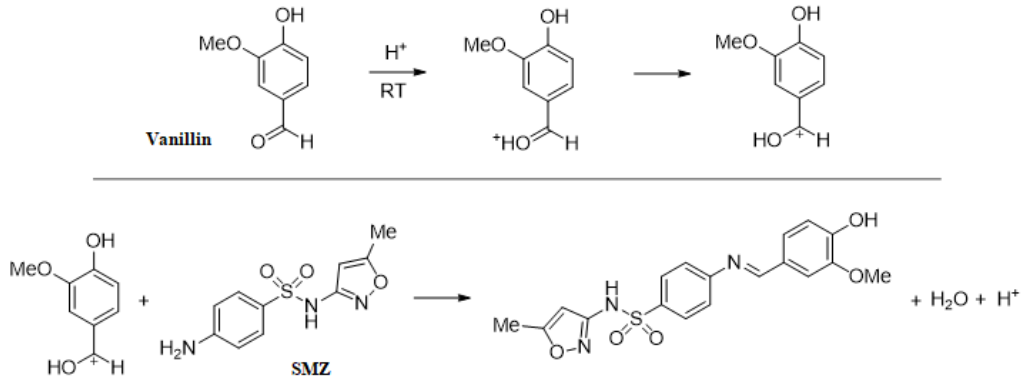
**Table 3. Assay of comparison of sulphamethoxazole in pharmaceutical tablet using different methods**

| Method | Pharmaceuticals | Present (µg. $mL^{-1}$) | Recovery (%) (n=3) | Mean found (mg) | Certified Value (mg) |
|---|---|---|---|---|---|
| Proposed | Sulfatrime | 5 | 90.647 | 363.36 | 400 |
| | tablet | 40 | 90.101 | | |
| | | 75 | 91.772 | | |
| Standard addition | Sulfatrime | 35 | 99.714 | 398.98 | 400 |
| | tablet | 45 | 99.777 | | |
| British pharmacopoeia | Bulk drug | 400 mg | 99.94 | 399.38 mg | 400 mg |

**Table 4. Comparison of the present method in the analysis of sulphamethoxaz with other spectrophotometric methods**

| Analytical parameters | Present method | Literature methods | | | | |
|---|---|---|---|---|---|---|
| | Vanillin | Vanillin [18] | Vanillin [19] | Chromotropic acid [20] | PADMA* [21] | Phenoxazine [22] |
| Type of reaction | Schiff's base | Schiff's base | Schiff's base | Diazotization coupling | Oxidative coupling | Oxidative coupling |
| Wave length (nm) | 399.09 | 372 | 421 | 513 | 550 | 520 |
| Solvent | Water | Perchloric acid | Methanol/HCl | Water | Water | Water |
| Reaction time (min) | 5 | 45 | 10 | Immediately | 10 | 2 |
| Temperature (°C) | R.T | RT | 60-70 | R.T | 60 | R.T |
| Stability time (min) | 100 | ---- | 300 | 20 | ---- | 120 |
| Beer's law limit (µg.mL-$^{1}$) | 5 - 80 | 1.5–40 | 5-50 | 0.5-20 | 5-25 | 0.1-6 |
| Molar absorptivity (L.$mol^{-1}$.$cm^{-1}$) | $1.1\times10^{3}$ | $8.61\times10^{3}$ | $0.4569X10^{4}$ | $3.17\times10^{4}$ | $8.31\times10^{3}$ | $6x10^{4}$ |
| RSD (%) | %6.36 | 0.32-0.55 | 0.1716 | 0.25-1.18 | % 0.6 | 0.5-0.8% |

**N,N-diethyl-p-phenylenediamine sulphate*




**Scheme 1. Mechanism reaction of sulfamethoxazole with vanillin reagent**

### 3.8 Application of Method

Comparing the present method with the authorized method (BP) [17] showed practical values of t-test and F-test (2.122 and 0.0287 respectively) are lower than theoretical values (t=2.920, F=19.00), meaning no significant difference between methods (Table 3).

### 3.9 Comparison with Literature

The present method was compared with other spectrophotometric methods in the literature (Table 4) showing some advantages since it does not require heating, carries out in aqueous medium, has short reaction time with long stability period, and more economic as well.

## 4. CONCLUSION

This method was proposed for the determination of sulphamethoxazole using vanillin reagent through Schiff's base linkage. The method was found to be sensitive since small amounts can be determined with good accuracy and reasonable precision, in addition, the procedure is simple does not need controlling temperature and solvent extraction steps. Analysis of pharmaceutical formulation samples containing sulphamethoxazole showed, in general, no effect of common excipients as interferences. Comparing with other spectrophotometric methods in the literature, the present method does not require heating, it can be carried out in an aqueous medium with a long stability period and more economic. Therefore, the present method can be suggested to determine of sulphamethoxazole in bulk form and formulation preparations.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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