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Analytical Method Development for the Spectrophotometric Determination of Sulfamethoxazole in Bulk Drug and Pharmaceutical Preparation

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Abstract

A spectrophotometric method for the determination of Sulfamethaxazole (SMX) in bulk drug and in solid dosage was developed. The method was based on the condensation reaction between primary aromatic amine group present in drug with aromatic aldehyde, vanillin, to produce a yellow color. The resulting Schiff's base shown maximum absorption at 372nm. The reaction product is stable. The reaction was carried out in acetic acid and in 0.1M perchloric acid medium. The Beer's law was obeyed in the ranges 1.5–40 mg/L with the acceptable correlation coefficient r = 0.9993. The detection limit and limit of detection were 0.19 and 0.24 mg/L of SMX, respectively. The method was successfully applied to the determination of this compound in pharmaceutical formulation.

Keyword: spectrophotometric determination, Sulfamethoxazole, pharmaceutical preparation, Bulk drug

1. Introduction

Sulfamethoxazole is an antibacterial drug which has been used to the treatment of various bacterial infections in humans and other species. it is the sulfonamide drug most commonly used by combination with trimethoprim for the treatment of urinary tract infections or with pyrimethamine for the treatment of Chloroquine-resistant Plasmodium falciparum malaria. Sulfamethoxazole abbreviated SMX.

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Synonyms, 4-Amino-N-(5-methyl-3-isoxazolyl) benzenesulfonamide; N1-(5-Methylisoxazol-3-yl) sulfanilamide, Molecular formula C10H11N3O3S and structural formula shown in figure 1. SMX is available as 500-mg, 1-g tablets and as a 500-mg/5 mL oral suspension and is a White to slightly off-white crystalline powder. It is slightly soluble in water (0.5 g/L) and benzene, slightly soluble in chloroform, diethyl ether, Isopropanol and soluble in ethanol and methanol, Melting point is 167 C^{o} (Gennaro, 1995, Rudy et al., 1973 and Budavari, 2000).

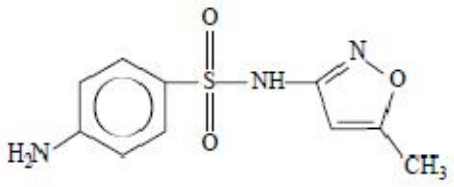

Figure 1: Structural formula of the pure SMX

Methods for the analysis of SMX have been reported. The methods include enzyme immunoassay (Endoh et al., 1994), gas chromatography with atomic emission(Chiavarino et al., 1998),Gas chromatography-Mass spectroscopy (Mooser et al., 1993), TLC (Schlatterer et al., 1982), HPTLC (Van Poucke et al., 1989), LC (Edder et al., 1997 and Mengelers et al., 1993), HPLC (Van der Steuijt et al., 1987, Shao et al., 1993 and Lin et al., 1995), HPLC-FD (Stoev et al., 2000), HPLC- photodiode array detection (Nishimura et al., 1996) and Flow injection sensor (Fernandez de Cordova et al., 2003). The chromatographic techniques were the most widely used .Although procedure is specific, most of it is time consuming and require multistage extraction procedure. In addition to the spectrophotometric methods reported have other disadvantages such as poor sensitivity and use organic solvent. Table 1shows comparison of SMX determination in the present work and other published methods carried out by the same instrument. The aim of this work was to development simple, sensitive and cost – effectiveness UV-Visible spectrophotometric method that could be used to determine sulfamethaxazole in bulk drug and pharmaceutical preparation.

Table 1: Comparison of the Experimental Characteristics of the Present Work with Published Methods

No.	Reagent used	Methodology	λ_{max} nm	Linear rang , ppm	D.L ppm	Ref.
1	2-Naphthol		482	2-13	--	Fazel et al., 2006
	5N HCl	Coupling reaction of drug by diazotizing with reagent in presence 0.1 M NaNO2. Measured of absorbance in acidic medium	271	8-20	--	
2	Phloroglucinol	Coupling of diazotized sulfa drug with reagent in an acidic medium.	420	0.2-2	--	Kanchan et al., 2012
3	Salbutamol Sulphate	The method is based on the diazotization of the drug with salbutamol sulphate to form orang color.	452	2.5-87.5	--	Saadiyah et al., 2010
4	Ethanol	UV-Vis spectrometry of mixture SMX and TMP binary mixtures measured	200-400	2-15	--	Mohmoud et al., 2010
5	Aqueous med.	Using the net analyte signal standard addition method with simultaneous addition of both SMX and TMP.	220-320	--	0.26	Givianrad et al., 2012
6	Pyrocatechol	based on the oxidative coupling organic reaction of SCS and SMX with reagent in the presence of sodium periodate to form red water soluble products.	500	2-26	0.74	Abdul Staar, 2006
7	Vanillin	The method was based on the condensation reaction between primary aromatic amine group present in drug with aromatic aldehyde.	372	1.5-40	0.19	Present work

2. Experimental

(2-1) Apparatus

The absorption spectra and absorbance were recorded and obtained by using computerized UV-Visible spectrophotometer Shimadzu -1800 with 1 cm quartz and glass cells for product analysis and using quartz cell for starting material analysis.

(2-2) Reagent

The Chemical and reagents which are used for preparation of solutions in present study are analytical grade.

(2-2-1)CH_3COOH 0.1M solution

Is obtained by made a suitable diluting the concentrated glacial acetic acid with distilled water.

(2-2-2)4-Hydroxy-3-methoxybenzaldehyde 10%w/v

Solution was prepared by dissolving 10g of reagent in 100 ml glacial acetic acid solution.

(2-2-3)Sulfamethaxazole (100 mg /L) solution

It was prepared by dissolving 10 mg of SMX in 100 ml glacial acetic acid.

(2-2-4) Perchloric acid 0.1 M solution

Prepared by dilution with water of appropriate volume of concentrated acid to get 0.1 M solution.

(2-2-5) Pharmaceutical Tablet

Is obtained from commercial sources.

(2-3) Procedures

(2-3-1)Procedure for calibration curve

Varying aliquots (0.15, 0.3, 0.6, 1, 1.5, 2, 2.5, 3, 3.5 and 4)ml of 100mg/L SMX standard solution corresponding to(1.5-40)mg/L of drug were accurately transferred into a series of 10ml volumetric flasks and total volume was set up to 5 ml by adding glacial acetic acid. To each flask, 1 ml of 0.1M $HClO_4$wasadded and 1.5 ml of %10 vanillin was also added. The content was mixed well and then diluted to 10 mL with glacial acetic acid. After allowed to reaction for 45 minute to completed, the absorbance of each solution was measured at 372 nm.

(2-3-2)Procedure for sample preparation priorto analysis

Ten tablets were powdered and their containing 4 g of SMX were transferred in to a beaker and dissolved in 0.1 M glacial acetic acid, then transferred into a 1000 mL volumetric flask and diluted to the calibration mark with the acid. Afterward, appropriate volume of the sample solution was taken and diluted into 100 mL volumetric flask with acid to obtain the drug concentration of about 10, 20 and 30mg/L.

3. Results and Discussion

Vanillin is soluble in methanol and glacial acetic acid while was insoluble in H_2SO_4 and water. In methanol there was colorless, while in acetic acid both vanillin and SMX were found to dissolve and development yellow colored product by reaction. Therefore Acetic acid has been used to prepare vanillin and SMX solution. The condensation reaction between SMX solution in glacial acetic acid and the Vanillin displayed a yellow color whereas the SMX and Vanillin exhibiteda colorless solution separately. The drug was also found to reaction with 4-Hydroxy-3-methoxybenzaldehyde in presence perchloric acid for the protonation of the carbonyl group (oxygen) and thereby leaving the carbonyl group (carbon) positive charge formatting an of imine by condensation reaction between NH_2 group in SMX, which donates pair of electrons to the carbon present in the carbonyl group of 4-Hydroxy-3-methoxybenzaldehyde and giving water and proton as by products. Scheme (2) shows suggested reaction as below.

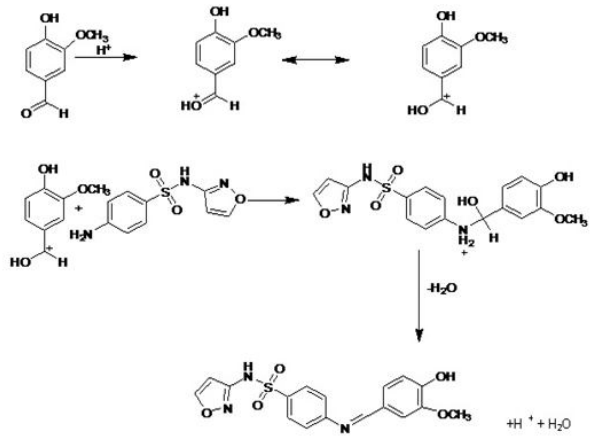

Figure 2: Shows that the Reaction Scheme between the Antibiotic and the Vanillin in Presence of 0.1M Perchloric Acid

(3-1) Spectral Characteristics

The starting materials maximum wavelength has been studies. The observed peak of drug at 273 nm, while the Vanillin have three peaks observed at 233, 285 and 312 nm respectively. The product formed between the drug and vanillin detected by a yellow color and is highest observed peak at 372 nm as shown in figure3.In this method, blank solution absorbance maximally at 266 nm, therefore this wavelength was used as analytical wavelength for all measurements in this study.

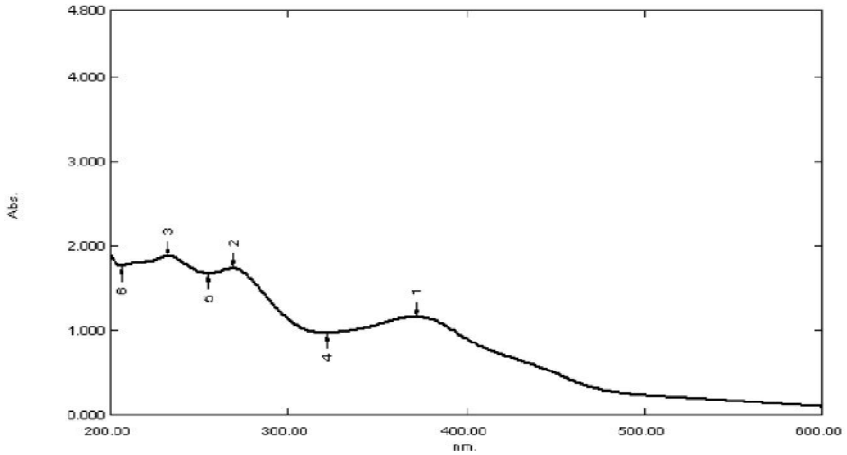

Figure 3: Absorption spectra of Sulfamethaxazole (35 mg/L)/Vanillin (10% W/V) Schiff's basein presence 0.1 M $HClO_4$ (35 mg/L)

(3-2) Optimization Method

A series of preliminary experiments necessary for the rapid and quantitative formation of colored product to achieve the maximum stability and sensitivity was performed in this work. Optimum condition was fixed by varying one parameter at time while keeping other parameters constant in order to follow its effect on the absorbance at 372 nm.

(3-2-1) Effect of Vanillin

The effect of vanillin concentration on the sensitivity of this method was studied by using %10 vanillin and different volume (0.25-3) ml has been used. The maximum absorbance observed between (1-2) ml, which approximately constant. Below and above this rang there is a reductionin absorbance as in table 2.

Table 2: Results of Effect of Volume of Reagent on the Absorbance (16 mg/L SMX)

Volume of %10 vanillin , ml	Absorbance
0.25	0.401
0.5	0.47
1	0.532
1.5	0.538
2	0.536
2.5	0.481
3	0.42

(3-2-2) Effect of acid, Reaction Time and Color Stability

In aqueous medium, SMX failed to give condensation product with vanillin even in the presence of H_2SO_4. Yellow colored product was developed only in glacial acetic acid medium, the intensity of the yellow color wasfound to be increased with addition of concentrated H_2SO_4, however the blank itself showed intense yellow color. There for the use of H_2SO_4 has been excluded.

The sensitivity and the stability of the reaction product were achieved in perchloric acid medium. The effect of perchloric acid was studied by using 0.1 M perchloric acid. The sensitivity of the color increased with increasing in perchloric acid concentration and then decreased. Blank absorption was found to be increased with increasing concentration of perchloric acid (Fig. 5). Hence, 1 ml of 0.1 M acid in a total volume of 10 ml was fixed as the optimum. The reaction was found to be instantaneous and the colored product remained stable for more than one day.

Volume of perchloric acid 0.1 M	Absorbance
0.0	0.04
0.5	0.522
1.0	0.542
1.5	0.504
2.0	0.337

Table 6: Effect of Volume of Perchloric Acid (0.1 M) on the Stability

(3-2-3) Order of Addition

We have studied the order of addition of reactants by following the color development.

Maximum sensitivity was achieved when perchloric acid was added before adding vanillin (Table 7) Hence, the method was performed in the order: SMX+ perchloric acid +vanillin.

Table 7: Effect of Order of Reactants

Number of order of addition	Absorbance
SMX+ Vanillin+ Acid	0.513
SMX+ Acid + Vanillin	0.541
Vanillin + SMX+ Acid	0.508

(3-3) Validation of Proposed Method

A linear relation was found to exist between absorbance and the concentration of SMX in the range (1.5–40) mg/L (Figure4).

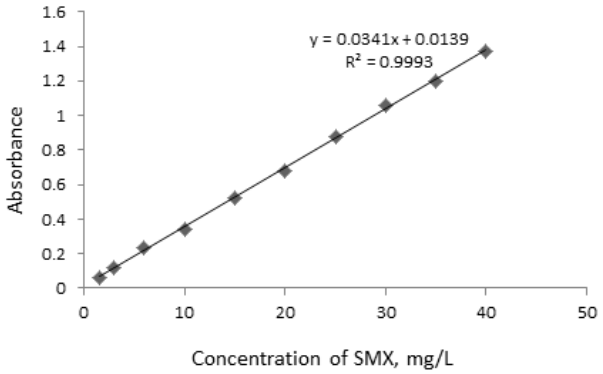

Figure 4: Calibration curve for Sulfamethoxazole using Vanillin in Presence Perchloric Acid

The calibration curve for instrument is linear and is given by an equation of the form:

$S = mc + s_{bl}$

Where S is the signal, Absorbance, at concentration c in mg/L and s_{bl} is the blank (i.e., signal in the absence of analyte). Then m is the slope of the calibration curve and hence the sensitivity (Somenath Mitraand Roman Brukh 2003).

Regression analysis of the Beer's law datausing the method of least squares was made to evaluate the slop, intercept and correlation coefficient (r) and the value is presented in table 8. The optical characteristics such as Beer's law limits, molar absorptivity and Sandell sensitivity values are also given in table 8. Other data described in table 8 calculating by using ICH guidelines for pharmaceutical preparation reported by Oona Mcpolin 2009 about limit of detection (LOD). The Rvalue refer to the acceptable accuracy, and high value of molar absorptivity and low value of Sandell sensitivity, D.L and LOD indicate the high sensitivity of the proposed method.

Table 8: Sensitivity and Regression Parameters. n=5

Parameter	Value
Linear rang	1.5- 40 mg/L
Maximum wavelength ,	372 nm
Molar absorptivity,	8.61×10^3 L.mol^{-1}.cm^{-1}
Sandell sensitivity	$0.029\ \mu g/cm^2$
Detection limit	0.19 mg/L
Standard deviation	0.00216
Limit of detection	0.24 mg/L
Intercept	0.0139
Slop	0.0341
Regression coefficient, R^2	0.9993

(3-4)Accuracy and Precision

To check the accuracy and precision of the method, an assay described under procedure for calibration curve was repeated five times within the day to determine the reproducibility. The assay was performed for three concentrations. The results shown in Table 9 reveal that the accuracy and precision are quite satisfactory. The percentage relative standard deviation %RSD value was < % 0.6 indicating high precision of the method. Accuracy was calculated as percentage relative error between the measured mean concentrations and taken concentrations for SMX (Somenath Mitraand Roman Brukh 2003).

$$\%\text{Accuracy} = \frac{Mean - true\ value}{\text{true value}} \times \%100$$

Percent relative error %RE values of< %2.4 demonstrate the high accuracy of the high accuracy of the present work.

Table 9: Accuracy and Precision

Concentration of SMX taken, mg/L	% Recovery	%RE	%RSD
10	97.9	2.1	0.38
20	98.05	1.95	0.32
30	102.33	2.33	0.55

(3-5) Assay of SMX in Pharmaceutical Formulation

The described procedure was successfully applied to the determination of SMX in pharmaceutical formulation (Metheprim, marketed by S. D. I. – IRAQ). The results obtained are summarized in Table 10. In order to test the result of the existence of a systematic error, the "t"-test is used. The results of the present spectrophotometric method are compared with the standard HPLC method. Five replicated times analysis are performed for the sample by each method. The statistical value of t-test at 95% confidence level for 5degrees of freedom is 2.77. Thus, the conclusion from the result is that the 1.817 calculated value of t_{exp} is < 2.776, thus indicating that no systematic error has occurred. Therefore, the present method has good accurate and precise as the reference method (Janos Inczedy et al. 1997).

Table 10: Results of Determination of SMX in Tablet

Tablet name	Nominal amount, mg/tablet	Concentration of SMX taken, mg/L	% Recovery
Metheprim	400	10	98.92
		20	101.13
		30	99.48

(3-6) Conclusion

The proposed method has been for the determination of Sulfamethaxazole in bulk drug and in pharmaceutical formulation is cheap, simple, sensitive, precise and accurate analytical method. It gives recoveries which indicate its applicability to the analysis of SMX in pharmaceutical, statistical in comparison with the results of the proposed method with the official reported method indicated that there is no significant deference at %95 confidence level.

The proposed spectrophotometric method does not require any expensive equipment and specialized technicians when it compared alongside high performance liquid chromatography. In addition to there is no need to organic solvents in the extraction step.

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