New development method for sulfamethoxazole Analysis in Pharmaceutical formulation using continuous Turbidity Flow Injection Technique

Abbas.L.Azeez[1], Salam A.H.Al-Ameri[2] and Aktham N. Jasim[3]

University of Mustansiriyah, College of science, Department of chemistry[1,2], Collage of Basic Education, Al- Mustansiriyah University, Science Department[3]

Abas_abas168@yahoo.com

Abstract

This method uses a sensitive and novel methodology to accurately measure sulfamethoxazole in its pure and pharmaceutical form. The method is based on the formation of a white precipitate by the reaction of sulfamethoxazole and phosphomolybdic acid in an acidic medium. As a result of this reaction, the drug's interaction with the reagent is observed and measured using a home-made Ayah 6Sxl-T-2D Cell detector and turbidity flow injection. The ideal chemical parameters were optimized and chosen, including the phosphomolybdic acid concentration (0.5 mmol/L), acidity (acetic acid), and the flow rate (3.2, 2.8 ml/min) for the carrier and reagent streams, respectively, Sample volume as well as the delayed coil reaction were determined and adjusted. Sulfamethoxazole showed a linear range of (0.06-2.6 mmol/L) and a correlation coefficient of r = 0.9855. At the calibration graph's lowest concentration, the limit of detection (LOD) is (0.0141 mmol/L), and the limit of quantification (LOQ) is (0.0466 mmol/L).

keywords. Sulfamethoxazole, Phosphomolybdic acid, Turbidity Flow injection Technique

Introduction

Sulfamethoxazole (Sulfa), Sulfamethoxazole is benzene sulfonamide,4aminoN(5methyl 3 isoxazolyl);N1(5 Methyl 3 Isoxazolyl)sulfanilamide [(1)]. Usually, it is used to treat urinary tract infections. It can also be utilized instead of medicines including amoxicillin to treat sinusitis [(2,3)]. It is the preferred medication for pneumocystis pneumonia, which primarily affects persons with HIV and can also be treated with toxoplasmosis [(4)]. In the literature, a number of analytical methods have been developed for determining these pharmaceutical compounds. Chromatographic techniques are among the best. Very effective liquid chromatography (HPLC). It is utilized by several researchers [(5,6)]. Many researchers explain how they used a wide range of analytical techniques to determine the dosage of pure tablets in both the model and the real sample. Analytical methods, such as gas chromatography (GC) and liquid chromatography (LC), are usually applied in Liquid chromatography is the primary method used to separate and analyze compounds in mixtures[(7,8,9)]. Figure 1.

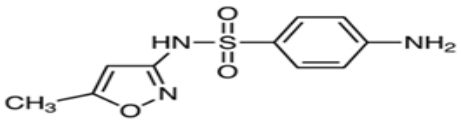

Figure 1: Chemical structure of sulfamethoxazole[(10)]

Materials and methods

Chemicals and reagent

The chemicals used are all analytical gradeand distilled water was used in all dilutions . A 10 mmol/L standard solution of sulfamethoxazole with a molecular weight of 253.28 g/mol was prepared; and 0.7 mmol/L of phosphomolybdic acid (BDH) molar mass 1825.25 g/mol.

Apparatus

Peristaltic pump with variable speed and four channel silicon tube (Ismatec, Switzerland). The utilized solar cell analyzer contains a flow cell that has been irradiated with six snow white light emitting diode LEDs at a 2 mm path length. The detector consists of two solar cells that transmit signals through the sample with a length of 60 mm. The flow injection system's sample is introduced through the injection valve. By use of a voltage recorder, the response signals are plotted[11,12.13)]

Methodology

To determine sulfamethoxazol utilizing phosphomolybdic acid as a precipitating agent, the two-line manifold system design (Figure 2) was attached to a solar cell detector. Preliminary experimental concentration of the precipitating agent was (0.2 mmol/L), which mixed with (2 mmol/L) sulfa to produce a white precipitate. In order to carry sulfamethoxazol (2 mmol/L) from the 150 µL sample volume and mix with the second line (1.3 ml/min) at the point in which the two lines meet, the first line (the carrier), which is water at a flow rate of 1.5 ml/min, is connected to an injection valve.

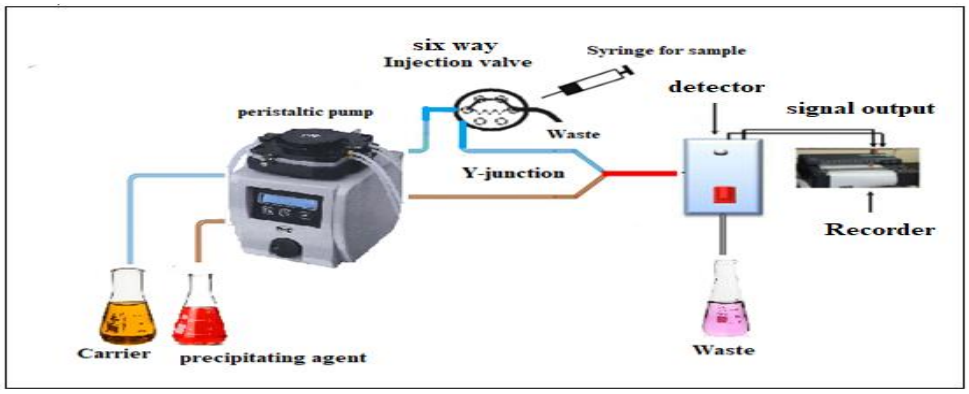

Figure 2.Two-line manifold system design phosphomolybdic acid as reagent; sulfamethoxazole as an estimated drug.

1- Chemical Variables

1.1.Precipitating Reagents

Variable injections of phosphomolybdic acid in the range of 0.04-6 mmol/L are used to optimize the amounts of precipitated reagents. Using distilled water as the carrier, the reagent flow rate was 1.3 mL/min and the carrier stream line was 1.5 mL/min. During reagent experiments, samples were taken with a 150µL sample volume, the valve open, and a 2 mmol/L sulfamethoxazole concentration. According to Figure 3, the response profiles for phosphomolybdic acid increase when the precipitation reagent is increased up to 0.5 mmol/L due to formation of precipitate particulate that might an increase the reflection of incident light and that refers to increase on attenuation of light intensity; but even so, when the concentration is increased more than 0.5 mmol/L for phosphomolybdic acid, the light intensity decreased[14]. Therefore, 0.5 mmol/L of phosphomolybdic acid was chosen as the optimal concentration.

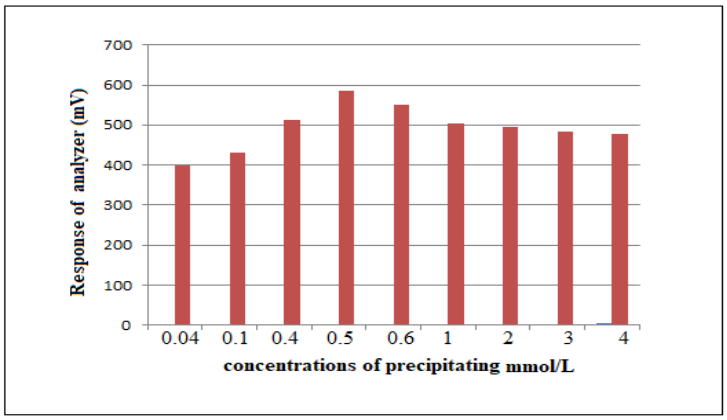

Figure 3. Variation of concentrations of precipitating reagents (phosphomolybdic acid)

1.2 Effect of kinds acidity solutions

To determine the sulfamethoxazole by reaction with phosphomolybdic acid, several kinds of solutions containing 10 mmol/L of HCl, H_2O, H_2SO_4, and CH_3COOH were created. These solutions It provides as a carrier instead of distilled water. Additional study conditions include a flow rate 1.5 mL/min for the carrier 1.3 mL/min for reagents and sample volume of 150 µL. Because it gave the best response, acetic acid was chosen as the best carrier. There for acetic acid 10 mmol/L is the perfect choice for the sulfamethoxazole -phosphomolybdic acid turbidity system. Table 1, figure.4.

Table.1: Effect of medium solution as a carrier stream on phosphomolybdic acid to determination of sulfamethoxazole

Acid Conc. [10 mmol/L]	Response (mV)
H_2O	588
HCl	280
H_2SO_4	591
CH_3COOH	661

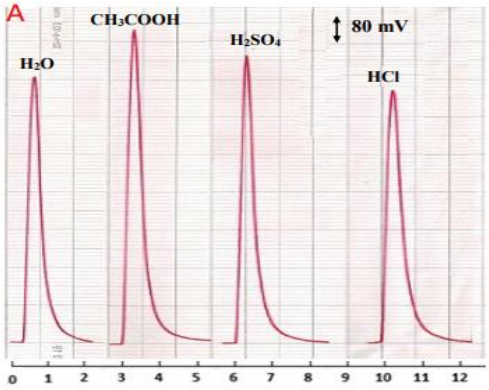

Figure.4: Response profile of media effect on Sulfa- phosphomolybdic acid turbidity system

1.3. Effect of acetic acid concentration on response profiles

The ideal experimental setup that had previously been achieved was utilized (2 mmol/L of 0.5 mmol/L sulfamethoxazole-phosphomolybdic acid system and 150 µL sample volume). prepare a range of CH3COOH concentrations (4, 8, 12, and 18) mmol/L. 1.5 ml/ minutes for the carrier stream and 1.3 mL/minutes for the reagent stream, respectively. In Figure 5, response profiles are shown. At increasing CH3COOH concentration up to 8 mmol/L, the increase in peak height can be seen clearlly, This increase is due to the accumulation of large sediments particles therefore increase the reflectance of light. At a high concentration of CH_3COOH (more than 8 mmol/L) leads to a reduce peak response, due to large precipitated particles dispersed[15]. Therefore a 8 mmol/L CH_3COOH is chose as the beast carrier stream.

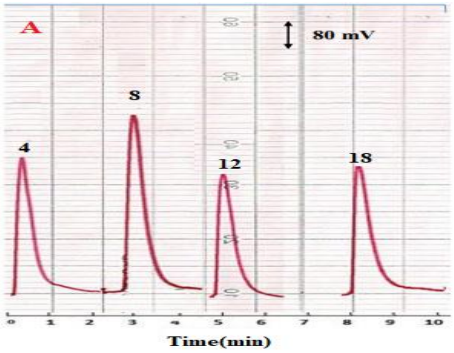

Figure 5. Effect of variable Concentration of CH_3COOH on response profile of sulfamethoxazole -phosphomolybdic acid- CH_3COOH system.

2. Physical parameters

2.1. Effect of Flow Rate

To choose the preferred flow rate within the range (0.4-4.0) mL min^{-1} for the carrier stream and (0.3-3.0) mL min^{-1} for reagent lines with a 150 µL sample loop. Through other optimal conditions, namely: 2 mmol/L sulfamethoxazole - 0.5 mmol/L phosphomolybdic acid - 8 mmol/L CH_3COOH reaction. It was observed that 3.2, 2.8 mL/min for carrier and reagent stream respectively using phosphomolybdic acid were the optimal flow rates. There was an increase in the peak width of the base (Δt_B) at lower flow rate due to increased dispersion. While at higher flow rates (3.2, 2.8) mL min^{-1} the flow rate (for carrier and detector stream respectively) decreased slight in peak height response due to precipitating particles leaving the flow measurement cell in a short time[16]. Therefore, optimal flow rates at 2.8 mL/min were chosen due to less dispersion. Figure 6.

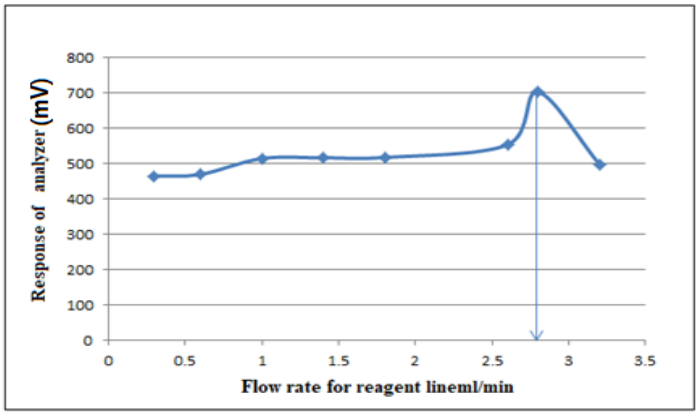

Figure 6. Effect of flow rate of reagent variation on peak response

2.2. Effect variable of sample volume

The effect of a variable sample volume loop with a range of (40-200 µL) and a 200 mm long Teflon tube with a diameter of 1 mm is up to 10000 mm. In according to figures 7,8 it was found that using a system of sulfamethoxazole (2 mmol/L), phosphomolybdic acid (0.5 mmol/L), and CH3COOH (8 mmol/L) at the optimal flow rate resulted in an increase in sample volume that increased the signal response up to 150 µL.

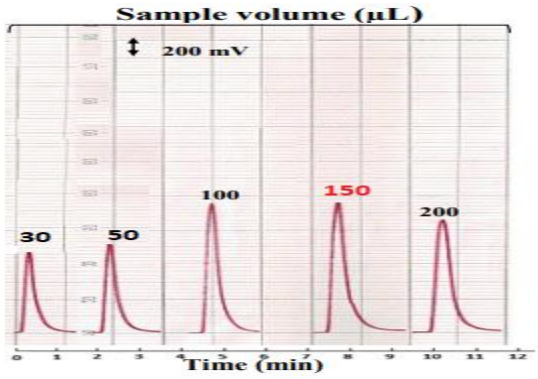

Figure 7: Effect of sample volume on response profile versus time using variable of sample volume.

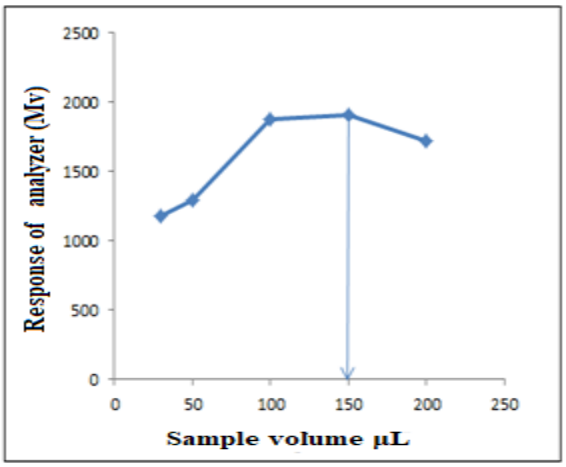

Figure 8 . Height of detector response with versus variable of sample volume.

3.Calibration curve for sulfamethoxazole

The calibration curve of sulfamethoxazole. was achieved by using the optimum chemical and physical parameters for system. A set of sulfamethoxazole solution ranging from (0.07 – 2.5 mmol/L) for sulfamethoxazol- phosphomolybdic acid- CH_3COOH system. Figure 9 shows the response profile of the scatter plot using a solar cell detector, while. Table 2 summarizes all the results, including measurements to estimate the response value obtained from linear regression analysis, linearity percentage, correlation coefficient (r), coefficient of determination (r^2). Figure 10.

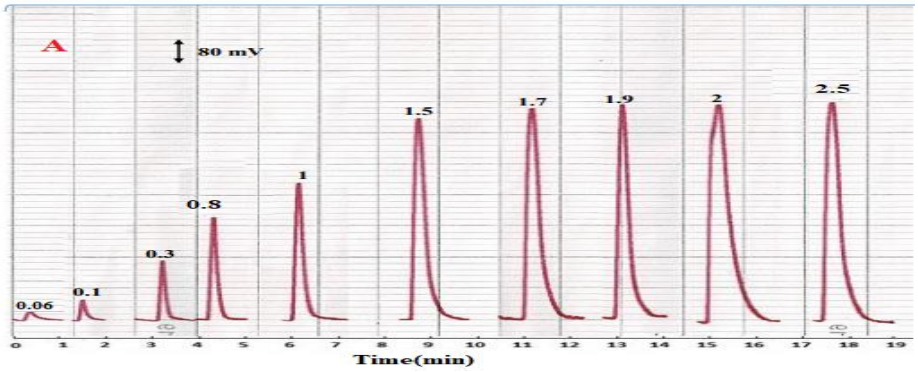

Figure.9. shows the response profile of the scatter plot using a solar cell detector

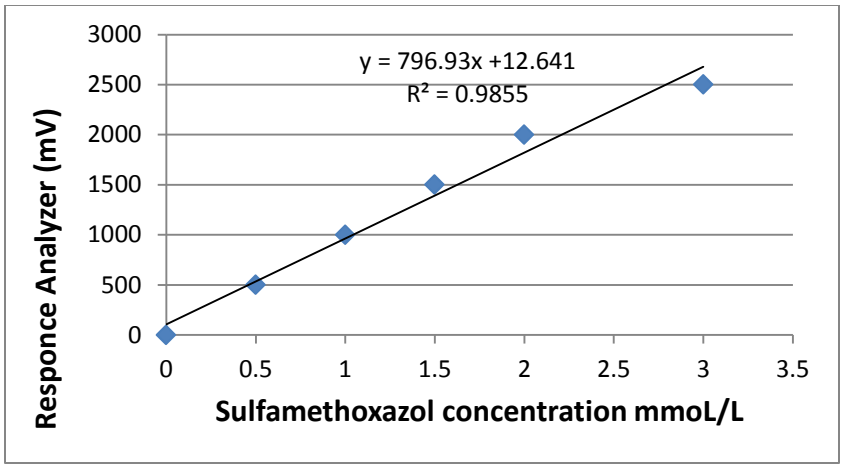

Figure 10: calibration curve for determination of sulfamethoxazole at range [0.06-2.6 mmol/L] against concentration using sulfamethoxazol -phosphomolybdic acid (0.5 mmol/L)-CH_3COOH (8 mmol/L) system.

Table 2: summary of calibration curve and Limit of detection, Limit of quantitation for sulfamethoxazole drugs using developed method

pharmaceutical compounds	Linearity range (mmol/L)	r^2	slope	Intercept	LOD $X=3S_B/slope$	LOQ $X=10S_B/slope$
sulfamethoxazol	0.06-2.5	0.9855	796.93	12.641	0.0141	0.0466

4-Repeatability

Using various sulfa concentrations as 0.2, 0.8 mmol/L, a follow-up investigation was conducted for the described technique. The response time profile at ideal parameters is repeatedly measured, Figure 11A&B, table 3.

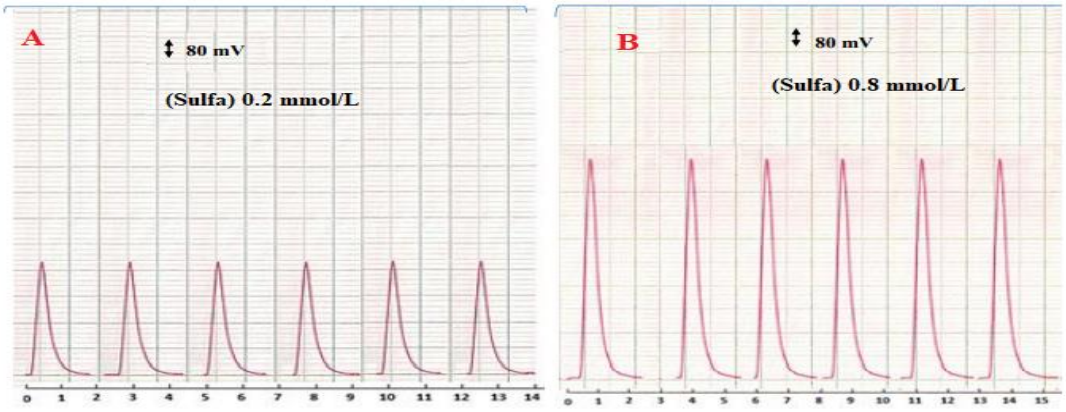

Figure 11: Response profile for six successive repeatable measurements of Sulfa in different concentration: **A**-0.2 mmol/L and **B-** 0.8 mmol/L using sulfa-phosphomolybdic acid system.

Table 3: Repeatability of Sulfa at optimum parameters.

No.of injection	Sulfa mmol/L	Response of detector ȳi (mV)	S.D	RSD %	Confidence interval of the average at (95%) ȳi ± t $\sigma/\sqrt{n}$
phosphomolybdic acid					
6	0.2	74	0.101	0.158	74±0.106
6	0.8	338	0.351	0.107	338±0.371

4. Application of the metho

Sulfamethoxazol in commercially available tablets has been effectively determined using the suggested method. Under optimal conditions, the FIA was injected three times with the two concentrations of the drug's stock solution that were created by serial dilution: 1, 2 mmolL-1. The results show that the recommended method for sulfamethoxazol determination in its formulation has been successfully used, and there have been no excipient (drug additive) interaction. Table 4.

Table 4. Application of the method for measurement of the pharmaceutical compounds at two concentration in different pharmaceutical companies.

Commercial Name, Company	Dosage of sulfamethoxazol	Conc. taken (mmol/L)	Conc. found (mmol/L)	Confidence interval of the average at (95%) $\bar{y}i \pm t\sigma/\sqrt{n}$	RSD %	Recovery
Septrin tablet	400 mg	0.7	0.68	0.68±0.0084	1.18	97.14
		2	2.04	2.04±0.0252	2.00	102.00
Kindi tablet	400 mg	0.7	0.69	0.69±0.0192	2.17	98.57
		2	2.01	2.01±0.0011	2.01	100.50

5-Conclusion

The suggested techniques are simple, sensitive, and rapid. The use of the suggested methods to analyze sulfamethoxazol in pure and pharmaceutical formulations is based on the creation of a white precipitate for an ion- pair compound when the reaction of sulfamethoxazol with phosphomolbdic acid in an acidic media. It showed that the newly developed method is just as effective as the traditional method and an alternative method to many other methods for the analysis of sulfamethoxazole.

6-Acknowledgment

The authors thank Prof. Essam Mohamed Ali Shaker and Prof. Dr. Negm Turkey Awadi, for a locally made Ayah 6Sxl-T-2D Cell detector.

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