**RESEARCH ARTICLE**

# Synthesis of New Azo Compound and its Application for Spectrophotometric Determination of Sulfamethoxazole and Extraction using Cloud Point Extraction

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**ABSTRACT**

In this study, we synthesized new sulfamethoxazole azo derivative by converted the SMZ drug to diazonium salt and coupling it with salbutamol in the alkaline medium to form an orange water-soluble azo dye that has a maximum absorption at $\lambda_{max}$ 450 nm. This reaction was used in the spectrophotometric determination of SMZ drug. It was obeyed to beer-lambert,s low over concentration between (10–100 $mg.L^{-1}$) with LOD (0.507 $mg.L^{-1}$), LOQ (1.5 $mg.L^{-1}$) and molar absorptivity (2332.2 $L.mol^{-1}.cm^{-1}$). The approach indicated great sensitivity for the assessment of chosen medicine. Another method, a new cloud point extraction approach, was successfully used to extract the SMZ medication in its pure form as well as in pharmaceutical formulations. Due to its features and structure, non-ionic surfactant, also known as 2-[4-(2,4,4-trimethylpentan-2-yl) phenoxy] ethanol, was chosen as the green extraction solvent in this study. The effect of several parameters on the CPE of Sulfamethoxazole, including as the type and volume of surfactant, salt, temperature, and incubation duration, was thoroughly researched, and a set of ideal conditions was established. The correlation coefficient (R2) for the calibration curve was found to be 0.9956. The limit of detection (LOD), limit of quantitation (LOQ) and molar absorptivity were 0.122 $mgL^{-1}$, 0.403 $mg.L^{-1}$ and 11319.41 $L.mol^{-1}.cm^{-1}$, respectively.

**Keywords:** Non-ionic surfactant, Sulfamethoxazole, Cloud point extraction, Spectrophotometry, Pharmaceutical formulations.

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**Conflict of interest:** None

## INTRODUCTION

Sulfomethoxazole is chemically known as 4-amino-N-(5-methyl-3-isoaxzoyl) benzene sulfonamoide (F.wt: 253.3 g/mol-1, Figure 1). Sulfamethoxazole is an antibacterial medication used to treat various bacterial infections in humans and other animals.[1] It is usually used for many infection treatment such as middle ear infections, acute urinary tract infections, eye infections, diarrhea, and bronchitis and as prophylaxis of rheumatic fever.[2-5] Several techniques are utilized for determination of sulfamethoxazole (SMZ) such as, High-performance liquid chromatography (HPLC),[3-5] high-performance thin layer chromatography (HPTLC),[6] solid phase extraction (SPE),[7] flow injection,[8-10] micellar electrokinetic capillary chromatography (MEKC),[11] GC,[12] voltammetry[13] and spectrophotometric methods.[14-17] Cloud point extraction has grown in popularity in comparison to other extraction techniques due to the advantages of low organic solvent consumption, fast recovery, low cost, high enrichment factor, and rapid phase separation.It is appealing because it reduces the employing and exposures to the solvent, as well as the time and costs of extraction and removal that have been used for pre-concentration of sulfomethoxazole after the formation of an azo compound that is poorly $H_2O$-soluble.[18-24] The aim of this work, is synthesis of azo derivative of sulfomethoxazole with salbutamol using azo coupling reaction and use this reaction for determination of sulfomethoxazole spectrophotometrically, then extraction with cloud point extraction technique.

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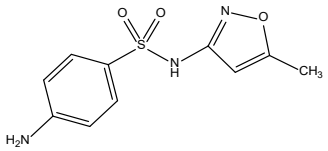

**Figure 1:** Structure of sulfomethoxazole(SMZ).

## MATERIALS AND METHODS

### Apparatus and Chemicals

Spectrophotometric measurements were performed utilizing a UV-visible 160 single beam UV is spectrophotometer equip with 1.0 cm quartz cell. The FTIR Spectra were achieved on a Shimadzu model FTIR-8400. A metlar pH meter was used to record the pH values. Infrared spectrum was obtained using shimadzu spectrophotometer. Both chemicals and reagents were used without being purified further. Sulfamethoxazole (SMZ) and salbutamol drugs were purchased from the state company for drugs industry and medical equipment in Samarra, Iraq, which is a general company for the manufacture of medicines and medical supplies. Standard solution of SMZ (500 mg.$L^{-1}$) was prepared by dissolving 50 mg in distilled water and diluting to the mark with D.W in a 100 mL volumetric flask. Stock solution of salbutamol (500 mg.$L^{-1}$) was prepared by dissolving 50 mg in D.W and diluting to the mark in the volumetric flask (100 mL). All surfactants (Triton$X^{-100}$, tween 80, tween20 and SDS) were prepared by dissolving 10 g of surfactant in the 100 mL of D.W.

### Preparation of SMZ Tablet Solution

A SMZ (Metheprim and supreme D.S) (400 mg) supplied from SDI, Samarra, Iraq was powdered and an equivalent amount was transferred to a volumetric flask (100 mL) to prepare a solution (500 mg.$L^{-1}$). The solution was centrifuged for 4 min at 3000 rpm and filtered. The solution was completed to (100 mL) using D.W.

### Synthesis of SMZ Azo Compound[25]

To SMZ (0.003 mol) ice, conc. HCl (0.9 mL), and a solution of $NaNO_2$ (0.0033 mol) in $H_2O$ (8 mL) were subsequently added and the mixturewas stirred at 0–5°C for 6 minutes to form a diazonium salt. To a solution of salbutamol (0.003 mol) in water (20 mL) 10% aq. NaOH (3 mL) and then the solution of the diazonium salt were subsequently added at 0–5°C. The product formed was filtered off, washed with small portions of water, and dried in air to give the orange compound. Formula: $C_{23}H_{28}N_4O_6S$; Mwt: 503.18 g/mol; Yield : 85%; m.p: 230–234°C; FTIR ($cm^{-1}$): OH (3504), $CH_{or}$(3099), $CH_{al}$(2983), NH(3104),C=N(1618), C=C(1600); Mass spectra(m/z): 503, 386, 289, 208, 156.

### A General Procedure of Diazotization

The excellent technique was to develop to prepared azo coupling solution by add (1-mL) of sulfomethoxazole 500 mg $L^{-1}$ in the volumetric flask (10 mL) immersed in an ice bath (0–5°C), add 1.2 mL of hydrochloric acid acid (1:1) and step by step add (0.8 mL) of (1%) $NaNO_2$ solution and wait for 20 minutes. To remove the excess nitrite, add (1.4 mL) of a 4% urea solution, constantly stirring, followed by adding 0.8 mL of 500 mg $L^{-1}$ salbutamol. Finally, add (2 mL) sodium hydroxide (25%) and diluted the mixture with D.W to 10 mL. The azo dye solution has orange colored which have absorbance at 450 nm.

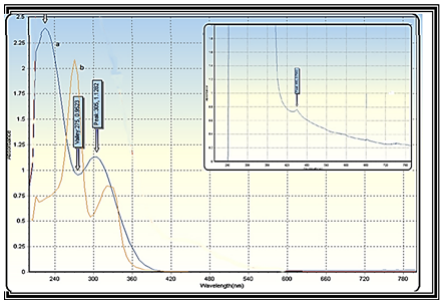

**Figure 2:** Absorption spectra of the sulfomethoxazolew azo compound.

### Cloud Point Extraction Technique of Sulfomethoxazole (SMZ)

The main method is based on azo-coupling reaction of SMZ with nitrous acid coupled with SAL. Concentrations range from 2.0 to 13.0 mg.$L^{-1}$ of azo compound formed (SMZ) were placed in the centrifuge tubes (10 mL), followed by (1.5 mL) of 10% TX-100 was added and completed it utilizing distilled water to the mark. The solutions were immersed in the water bath for 50 minutes at 80°C. The got solutions were centrifuged for 7 minutes at 4000 rpm,then cooled for 30 minutes in an ice bath to increase the viscosity of the surfactant-rich phase. The organic phase (surf- rich phase) was dissolved, diluted to 2 mL with MeOH, and transferred into 1-cm quartz cell. At 450 nm, the absorbance solution was measured. The solution of blank was submitted to the same method without (SMZ) medication.

## RESULTS AND DISCUSSION

The diazotization reaction of sulfomethoxazole with nitrous acid and coupling with salbutamol as a reagent, the orange colored at $\lambda_{max}$ 450 nm, appears to be the fundamental study.

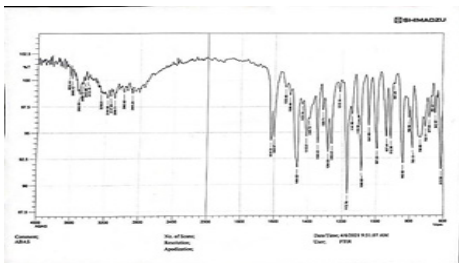

**Figure 3:** FTIR of SMZ azo compound.

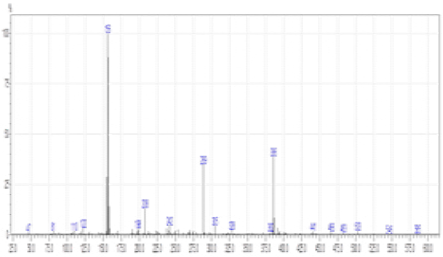

**Figure 4:** Mass spectra of SMZ azo compound.

**Table 1:**Effect of acid type.

| *Type of acid* | *Abs.* |
|---|---|
| HCL | 0.150 |
| $CH_3COOH$ | 0.135 |
| $H_2SO_4$ | 0.117 |
| $HNO_3$ | 0.103 |

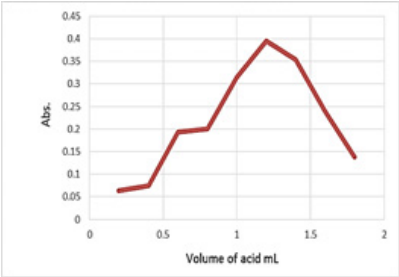


**Figure 5:** Effect volume of acid.

Figure 2 shows absorption spectra of product against the blank solution.

### Synthesis and Characterization of SMZ Azo Compound

We synthesized new sulfamethoxazole azo derivative by converted SMZ drug to diazonium salt utilizing conc. HCl and sodium nitrate, then coupling with salbutamol as reagent. In the solid-state their IR spectra reveal the presence of OH, C-$H_{or}$, C-$H_{al}$, NH, C=N and C=C vibration at 3504, 3099, 2983, 3104, 1618 and 1600 $cm^{-1}$, correspondingly.

### Optimization of the System

The reaction conditions must be optimized to get the most out of the technique. In order to achieve optimal experimental conditions, various parameters were investigated. The parameters were optimized by making them constant and then optimizing one at a time. For the formation of diazonium salt, the effects of various acids such as HCl, $HNO_3$, $H_2SO_4$ and acetic acid were investigated, and the results are shown in Table 1. In the range of (0.2–1.8 mL), the effect of acid volume on the developed at a fixed concentration of azo compound solution was examined. Figure. 5 shows that 1.2 mL of HCl was the best volume.

Since the amount of sodium nitrite will affect the maximum absorbance signal, different amounts of (1.0%) in the range of 0.2–1.8 mL were added. The results revealed that 0.8 mL of NaNO2 (1.0%) solution provided the strongest absorbance signal, which was used to carry out the following measurements, (Figure 6). A different amount of urea solution 4% in the range of 0.2–2.0 mL was added to remove the excess nitrous acid. Figure 7 shows the findings, which showed that 1.4 mL of urea solution gave the highest absorbance and was used to make the subsequent measurements.

The absorbance was measured using a variety of bases, including NaOH, KOH, and $NH_4OH$, with the results indicating that sodium hydroxide was the best choice.Since the effect of base volume on maximum absorbance can be important, various volumes of 25% sodium hydroxide in the range of 0.2–2.5 mL were added. The results revealed that 2.0 mL of

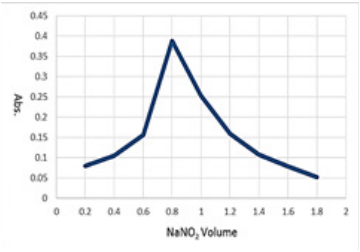


**Figure 6:** Effect of 1% sodium nitrite.

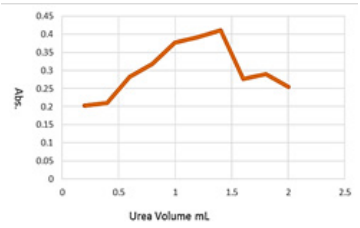


**Figure 7:** Effect volume of urea (4%).

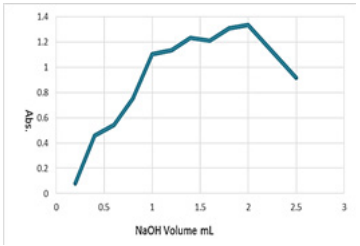


**Figure 8:** Effect of NaOH volume.

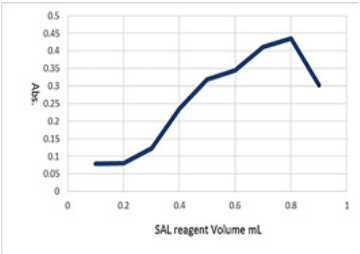


**Figure 9:** Effect of reagent volume on absorbance of azo dye.

NaOH solution produced the best absorbance signal, which was used in the following steps, (Figure 8). Since reagent volume (SAL) will affect maximum absorbance, different volumes of salbutamol solution in the range of 0.1–0.9 mL were added. Figure 9 shows that 0.8 mL of SAL solution provided the best absorbance signal, which was used to complete the following steps.

The addition sequence can cause a significant effect on the form of the azo dye compound. Various studies were carried out with various addition sequences. The results showed that

**Table 2:** Effect of addition sequence on absorbance of azo dye

| *Order of additions* | *Abs.$\lambda_{max}$ 450 nm.* |
|---|---|
| T+R+B | 0.447 |
| T+B+R | 0.202 |
| T+M | 0.005 |

T:(HCl+SMZ+Urea+NaNO2), R:SAL, B:Base, M:(R+B)

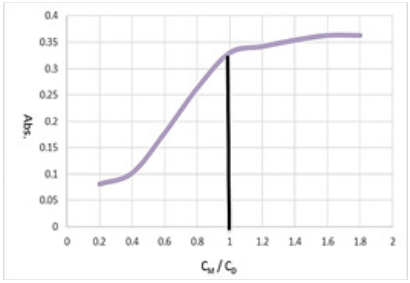


**Figure 10:**Mole-ratio method of SMZ -Reagent.

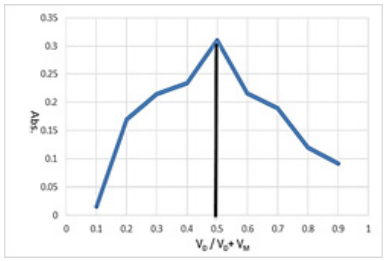


**Figure 11:** Continuous variation method of SMZ -Reagent.

adding (T), then (R), and finally (B) yielded the best results. Table 2 shows the recording of the perfect absorbance signal. Using the continuous variation and molar ratio methods, the nature of the azo compound was demonstrated under the ideal conditions mentioned above. Figure 10 shows an inflection in the plot of absorbance versus molar ratio of SMZ to SAL obtained by varying the SAL concentration. Furthermore, the job method revealed a SAL to SMZ ratio of 1.0 (Figure 11). As a result, the findings revealed the stoichiometric ratio (1:1).

### Calibration Curve

A linear calibration curve was constructed by plotting absorbance vs. SMZ concentration (10.0–100.0 $mg.L^{-1}$), (Figure 12) under ideal conditions established utilizing a spectrophotometric for sulfomethoxazole evaluation.

### Investigation of optimization of cloud point extraction for SMZ

The effect of different surfactants, such as TX-100, TW80, TW20, and CTAB, on the separation and extraction of azo compound, was investigated, and the results are shown in Table 3. The effect of TX-100 on azo compound extraction was investigated in the volume range of 0.5–3.5 mL. Increased TX-100 volume to 1.8 mL increased absorbance, but decreased

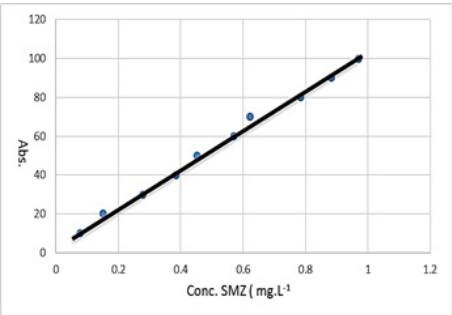


**Figure 12:** Standard calibration curve of SMZ

**Table 3:** Effect type of surfactant on absorbance

| *Surfactant* | *Abs.$\lambda_{max}$ 450 nm.* |
|---|---|
| Triton-X100 | 0.205 |
| Tween 80 | - |
| Tween 20 | - |
| CTAB | - |

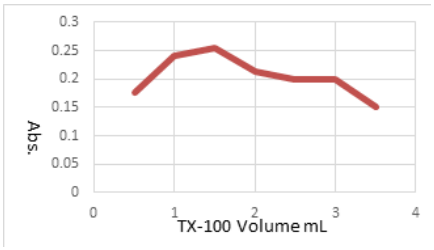


**Figure 13:** Effect volume of (10% v/v) Triton $X^{-1}00$.

at higher volumes.[25] According to the findings, 1.8 mL had the best absorbance, as shown in Figure 13.

The effect of temperature on the extraction efficiency of SMZ is depicted in Figure 12. Due to an improvement in the separation and extraction capacity of TX-100 toword SMZ due to dehydration in the external layer of micelles, the CMC of non-ionic surfactant decreased with temperature, while the percentage of hydrophobic micelles in the surfactant process increased with temperature.[26] Figure 14 showed that the absorbance of SMZ increased from 50 to 80°C, but that the absorbance decreased after 80°C due to increased viscosity. The extraction point cloud is a type of equilibrium extraction. Once the equilibrium was reached, the extraction efficiency was perfected. In the CPE, the shortest incubation time is preferred. As a result, the effect of incubation time on extraction efficiency was investigated in the range of 10 to 70 minutes. Figure 15 shows that when the period time was longer than 50 minutes, the absorbance of the SMZ medication decreased. Within 50 minutes, the extraction equilibrium

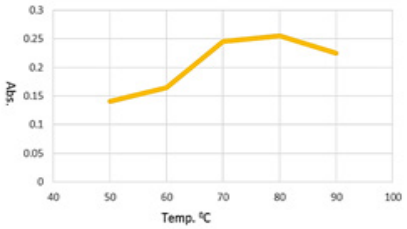


**Figure 14:** Effect of temperature on CPE of AZO-SMZ.

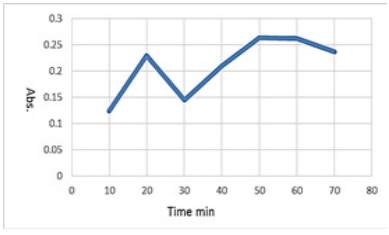


**Figure 15 :** Effect of time on CPE of AZO-SMZ.

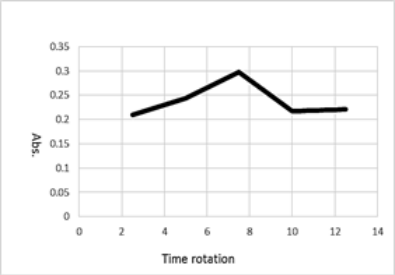


**Figure 16:** Effect of rotation time on CPE of AZO-SMZ.

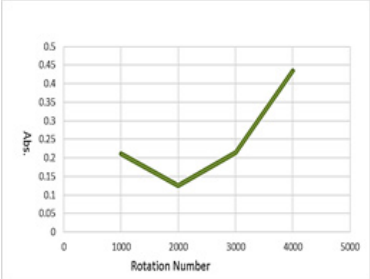


**Figure 17:** Effect of rotation number on CPE of AZO-SMZ.

**Table 4:** Interference study

| *Compound* | *Recovery%* |
|---|---|
| Maltose | 96.3 |
| Sucrose | 93.4 |
| Glucose | 91.7 |
| Galactose | 95.0 |
| Fructose | 94.6 |
| Gum arabic | 96.7 |

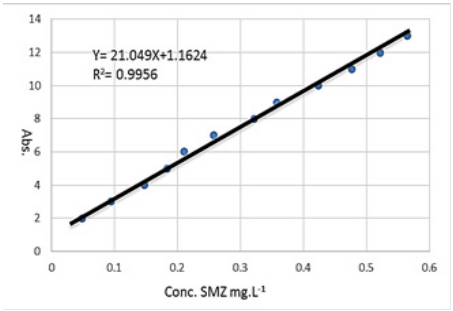


**Figure 18:** Calibration curve of CPE of SMZ drug.

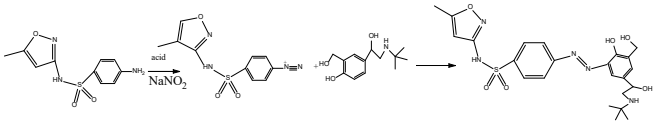


**Figure 19:** The proposed mechanism of diazotization reaction.

can be achieved. Figures 16 and 17 show that a 7.0 minute centrifugation at 4000 rpm was necessary for efficient CPE. To reduce the viscosity of the surfactant-rich phase, a variety of solvents were tested, including methanol, ethanol, water, dioxane, and $CH_2Cl_2$. 2 mL methanol was chosen to provide an adequate volume of sample for transferring and measuring the sample's absorbance at 450 nm. The procedure's selectivity was investigated by separating 1-mL of the sample solution, which contained SMZ drug, and 1-mL (1000 $mg.L^{-1}$) of maltose, sucrose, glucose, galactose, fructose, and Gum arabic, and extracting it under ideal experimental conditions.The obtained results in Table 4 and the recovery values show that the various compounds present at moderate concentrations do not cause any significant interference. The findings indicate that the

**Table 5:** Analytical parameter of cloud point extraction method

| *Parameters* | *Before CPE* | *After CPE* |
|---|---|---|
| $\lambda_{max}$ nm | 450 | |
| Color | Orange | |
| Regression equation | Y=0.010X-0.032 | Y= 21.049X+1.1624 |
| Linearty range(mg/mL) | $10^{-1}00$ | $2.0^{-1}3$ |
| Correlation Cofficient ($R^2$) | 0.9980 | 0.9956 |
| Ɛ($L.mol^{-1}.cm^{-1}$) | 2332.2 | 11319.41 |
| Sandell's sensivity (µg. $cm^{-2}$) | 0.1 | 0.0211 |
| Slope (b) | 0.010 | 21.049 |
| Intercept(a) | 0.032 | 1.1624 |
| Limit of detection(µg/$mL^{-1}$) | 0.507 | 0.122 |
| Limit of quantification(µg/$mL^{-1}$) | 1.5 | 0.403 |
| C.L.for the slope($b \pm ts_b$) at 95% | $0.01 \pm 5.463*10^{-4}$ | $21.049 \pm 0.00225$ |
| C.L.for the intercept($a \pm ts_a$) at 95% | $0.032 \pm 0.0338$ | $1.1624 \pm 0.0185$ |
| Standard error for regression line ($S_{y/x}$) | 0.0213 | 0.0119 |
| *C.L for Conc.$X_1$ µg $mL^{-1}$at 95% | $19.03 \pm 0.0087$ | $3.13 \pm 0.011$ |
| *C.L for Conc.$X_{2\mu g}$ $mL^{-1}$at 95% | $39.73 \pm 0.098$ | $5.06 \pm 0.0024$ |
| *C.L for Conc.$X_3$ µg $mL^{-1}$at 95% | $58.93 \pm 0.135$ | $6.76 \pm 0.049$ |

*Before CPE ($X_1$=20, $X_2$=40, $X_3$=60) and after CPE ($X_1$= 3.0, $X_2$= 5.0, $X_3$=7.0)

**Table 6:** Application of the proposed CPE for the evaluation of Sulfomethoxazole drug

| | *Before cloud point extraction* | | | | | |
|---|---|---|---|---|---|---|
| *Drug* | *Conc. of drug mg.L$^{-1}$* | | *Relative Error%* | *Recov. %* | *Average Recov %* | *RSD% (n=3)* |
| | Taken | Found | | | | |
| Metheprim | 4.0 | 4.07 | 1.75 | 101.75 | | 4.1 |
| | 10.0 | 10.471 | 4.71 | 104.71 | 101.5 | 1.6 |
| | 14.0 | 13.75 | -1.78 | 98.21 | | 0.17 |
| Supreme. D.S. | 4.0 | 4.06 | 1.5 | 101.5 | | 6.0 |
| | 10.0 | 10.22 | 2.2 | 102.2 | 99.3 | 1.71 |
| | 14.0 | 13.19 | -0.57 | 94.2 | | 0.87 |
| After cloud point extraction | | | | | | |
| Metheprim | 3.0 | 3.01 | 0.33 | 100.33 | | 3.17 |
| | 5.0 | 5.00 | 0.06 | 100.06 | 100.49 | 0.57 |
| | 7.0 | 7.07 | 1.09 | 101.09 | | 2.5 |
| Supreme. D.S. | 3.0 | 2.97 | -1.0 | 99.0 | | 4.6 |
| | 5.0 | 4.93 | -1.4 | 98.6 | 99.39 | 3.06 |
| | 7.0 | 7.04 | 0.57 | 100.57 | | 0.93 |

**Table 7:** Comparison the values of LOD and LOQ of the CPE method with various methods reported in the literature

| *Method* | *LOD mg/L* | *LOQ mg/L* | *Ref.* |
|---|---|---|---|
| HPLC | 0.009 | 0.033 | [27] |
| Flow-Injection | 0.05 | - | [10] |
| Spectrophotometric with charge-transfer complexation | 0.589 | 1.964 | [28] |
| Spectrophotometric | 0.5164 | 3.7247 | [29] |
| Spectrophotometric with Diazotization-Coupling Reaction | 0.2345 | 0.7816 | [30] |
| Direct orthogonal shgnal correction-partial least squares | 0.03 | - | [31] |
| Spectrophotometric | 0.021 | - | [32] |
| Spectrophotometric with Schiff's Base Reaction | 0.035 | 0.119 | [15] |
| Means of pH Gradual Change-UV Spectral Data | 0.25 | - | [33] |
| Cloud point extraction with spectrophotometric method | 0.032, 1.1624 | 1.5, 0.403 | Present work |

proposed technique has strong selectivity and that it can be used to accurately test SMZ medication in pharmaceutical formulations (Tables 5 and 6).

### Analytical Performance of the Method

The calibration curve was found to be in the range of 2.0-13.0 mg.L$^{-1}$ under ideal conditions. With a correlation coefficient (R2) of 0.9956, is a good choice. The calibration graph was generated using the average of three replication experiments' absorbance versus SMZ concentration. The quantification limit (LOQ) and detection limit (LOD) were determined using the formulas (10s/b) and (3s/b), respectively, where s represents the standard deviation and b represents the slope of the calibration graph. 0.403 mg.L$^{-1}$ was measured as the LOQ, with a LOD of 0.122 mg.L$^{-1}$.

### Comparison with Literature Studies

The findings of the suggested methodology were compared to those of the published methods. In the assessment of SMZ drug for different samples, Table 7 compares the efficiency of the suggested procedure with that of other approaches. When compared to other approaches, the suggested approach has advantages such as lower LOQ and LOD.

## CONCLUSION

For SMZ drug evaluation, the suggested approach provides a simple, sensitive, and low-cost spectrophotometric technique that may be applied to a variety of samples. The surfactant has been used in pharmaceutical formulations to separate and pre-concentrate SMZ medication. When compared to approaches previously reported using other instrumental approaches, the proposed procedure appears to be more sensitive, safe, easy, rapid, and economical.

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