# New spectrophotometric determination sulfamethoxazole drug via various analytical methods in pharmaceutical formulation

[1]Dalia M. Jameel, [1]Raghda S. AlSayed, [2]Nisreen Kais Abood

[1]Department of Chemistry, College of Science, Al-Nahrain University ,Baghdad Iraq
[2] Department of Chemistry, College of Science, Mustansiriyah University Baghdad, Iraq
Daliamahmood34@yahoo.com

## Abstract

For the determination of sulfamethoxazole (SMZ) in pure and pharmaceutical formulations, new simple sensitive spectrophotometric methods have been devised. The first technique involved forming an ion pair complex between the basic nitrogen of the SMZ medication and Eriochrome black (EBT) in an acidic buffer solution, and extracting the created complex with chloroform to get a persistent reddish-orange colored dye with a maximum wavelength of 490 nm. Beer's law was followed, and 0.9998 was the correlation coefficient. $0.471\times104$L. $mol^{-1}.cm^{-1}$was the molar absorptivity, and the detection limit was 0.177 g/mL. Dispersive liquid-liquid extraction (DLLE) is a second method for estimating a trace amount in an aqueous solution product using an ion pair and a UV-visible spectrophotometer with a maximum wavelength of 490 nm. The concentration range was 0.5-6 g/mL, with a correlation coefficient of 0.9995, $0.889\times10^{5}$ $L.mol^{-1}.cm^{-1}$, a molar absorptivity of ,the detection limit of 0.013 g/L, and a pre-concentration factor of 25 and an enrichment factor of 18.20. Sulfamethaxazole in pharmaceutical formulations was successfully determined using the provided methodology.

Keywords: sulfamethaxazole, ion pair complex, Dispersive liquid-liquid extraction, pharmaceutical formulation and spectrophotometer

## Introduction

Sulfamethoxazole (SMZ) belongs to the sulfonamide family of antibacterials, and its chemical name is 4-Amino-N-(5-methyl-3-isoxazolyl)-benzene sulfonamide, with the atomic formula $C_{10}H_{11}N_3O_3S$ and a molecular mass of (253.279 $g.mol^{-1}$). The drug's basic structure is illustrated in Fig 1. . The development of resistance has hampered its use., and it is now primarily used

in combination with trimethoprim [1, 2, 3]. Co-trimoxazole is a combination of sulfamethoxazole and trimethoprim that is used to treat a wide range of bacterial infections, including middle ear infections, genito-urinary tract infections, respiratory tract infections like bronchitis, and enteric infections. Pneumocystis carinii pneumonia, toxoplasmosis, and nocardiosis are the most common uses presently. The most prevalent side effects of this medication combination are gastrointestinal problems (mostly nausea and vomiting) and skin responses [4, 5]. According to a literature review, only a few analytical procedures for Sulfamethoxazole analysis have been published. Some spectrophotometric methods [6,10], HPLC [11,18], As For an example, Flow Injection Analysis (FIA) [19] and Micellar Electro Kinetic Capillary Chromatography (MEKC) [20]. The purpose of this study is to develop an improved spectrophotometric approach based on the ion pair reaction with the new reagent EBT for estimating trace concentrations utilizing dispersive liquid-liquid extraction.

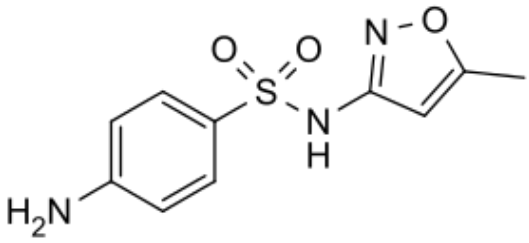



Fig. 1: Structure of ulfamethoxazole

## Experimental: Materials and Methods

**Instruments** : The absorbance was measured using a Spectrophotometric single-beam UV-visible 295 (Lasany-India) with quartz cells of 1 cm and 0.5 cm. The pH of the buffer solution was checked using a Hanna pH-meter device (pH 211) from Romania.

**Chemicals and Reagents:** The chemicals were given by Merck and were of analytical purity. The quality control laboratory received sulfamethaxazole (the general company for the manufacture of medicines and medical supplies - Samarra).

## General procedure for ion pair

1.0 mL aliquots of 1000 mg sulfamethoxazole was transferred into a 20 mL conical flask , then added 1.0 mL1000 mg $mL^{-1}$ of 2.167 $\times 10^{-3}$ M EBT(Erio chrome black) . After that 1 mL of buffer has been added to the solution and completed to 20 mL with distilled water and transfer to funnel separation. The ion-pair have been obtained with 10 mL of chloroform and shaking 5 min, the absorbance of colored ion-pair measured at wavelength 490 nm.

## General procedure of the DLLME method

In a beaker, a 1 mL aliquot of the produced dye from the ion-pair complex was prepared, along with a 2 mL organic solvent mixture containing ethanol as a dispersive solvent and chloroform as an extraction solvent, and swiftly injected into the aqueous phase of the dye from ion-pair via micro syringe. The injection procedure promotes the production of hazy samples, which aids in the separation of solution. After that, spin for 5 minutes at 4000rpm. The blank solution was created in the same way.

## Solution for the Analysis of Sulfamethoxazole in Pharmaceutical Preparations

### In Tablets

The contents of 10 tablets were pulverized and mixed together thoroughly. A specified amount of fine powder was carefully weighted to provide an equivalent of 500 mg for tablets, and the mean value of the weight of one tablet was obtained. About 10 mL of 0.4 M HCL was added,and transferred to a 100mL volumetric flask, and the solution was agitated vigorously for 5 minutes before being diluted to the mark with distilled water to get 100 g.ml-1. The solution was filtered with Whatman filter paper No. 41 before use to eliminate any suspended or undissolved materials, with the first portion of the filtrate being discarded. Working solutions were created after dilutions with distilled water.

### In Syrup

There are 5 mL of syrup in each (200 mg of sulfamethoxazole with 40 mg of trimethoprim). To generate 100 g.mL-1 SMZ solutions, a precise volume (0.25 mL) was placed in a 100mL volumetric flask, swirled with 10 mL of 0.4 M HCl, allowed to stand for 5 minutes, then diluted to the mark with distilled

water. The solution was filtered with Whatman filter paper No.41 before use to eliminate any suspended or undissolved materials, with the first portion of the filtrate being discarded. Working solutions were prepared fresh and analyzed following the stated procedure after dilutions with distilled water.

## Result and discussion

The ion-pair complex of sulfamethoxazole with EBT as a reagent, which is reddish-orange in color and has a wavelength of 490 nm, is the subject of the basic study. Figure 2 shows the product's absorption spectra in comparison to a blank.

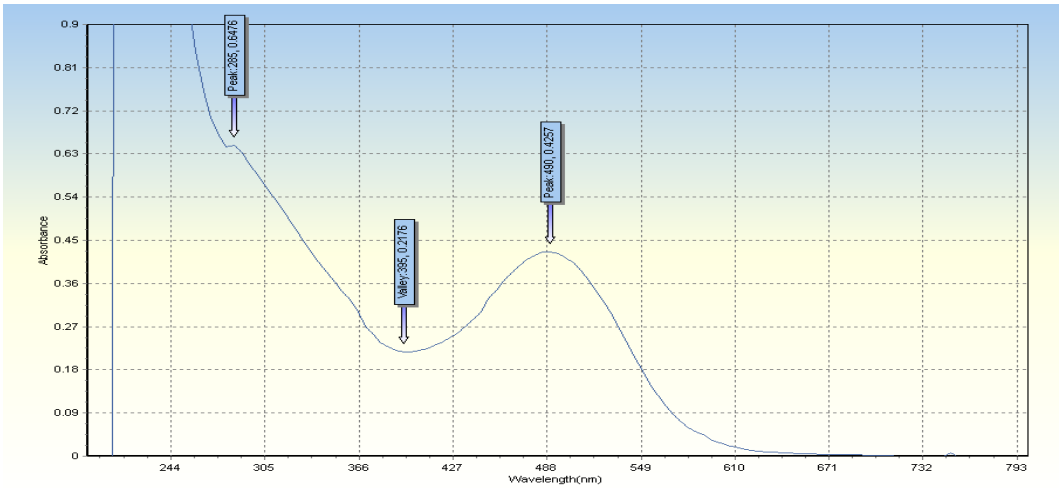
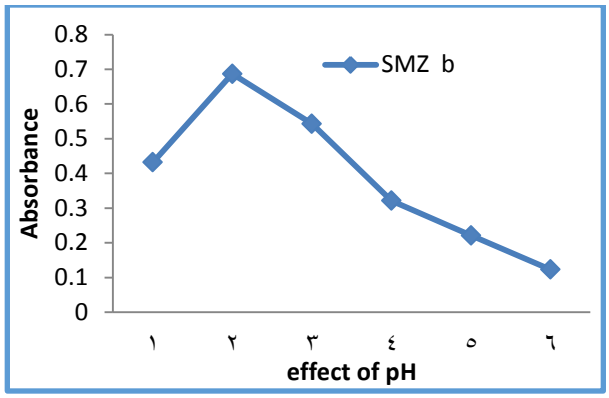
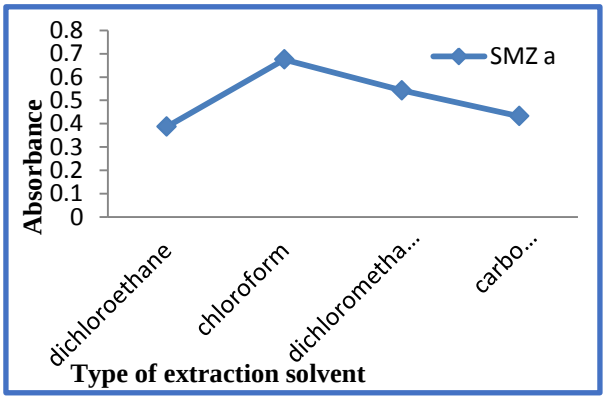


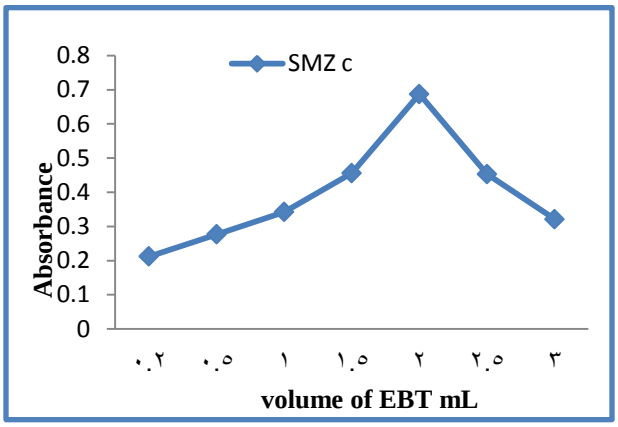

**Fig. 2: Absorption spectrum ofSMZ( a) SMZ solution ( b) blank solution( c) dye of SMZ+EBT**

## Study the optimization of ion pair reaction

Different types of extraction solvents (dichloroethane, chloroform, dichloromethane, and carbon tetrachloride) were studied, and the chloroform presented increased effectiveness for the color intensity. When using different types of pH buffers [NaAc-AcOH (pH 3.6-5.6), NaOAc-HCl (pH 1.0-2.2), the results that were higher than pH 6 were not repeatable. As can be seen in fig3b, pH 2 results in a high absorption. The best result was attained in 2 mL, as shown in fig3c, using varying volumes of EBT (0.2-3) mL. The optimal period to give high absorbance 2 minutes was studied using different shaking times (1-5) minutes, as illustrated in fig3d.



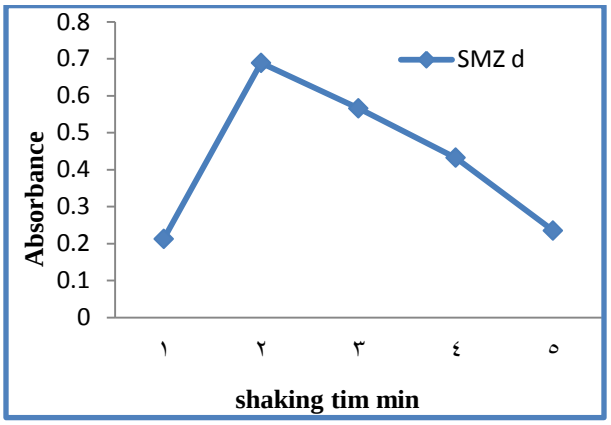




Fig.3:The effect of experimental conditions ( a):effect type extraction solvent (b): effect of pH (c): volume of EBT d: shaking time

## Stoichiometric Ratio Determination

The stoichemistry of the ion pair reaction between SMZ and EBT was investigated using the mole ratio method. (Fig.4) shows that the reddish-orange dye is formed in the ratio1:1 (ETB reagent[R]: SMZ [D]).

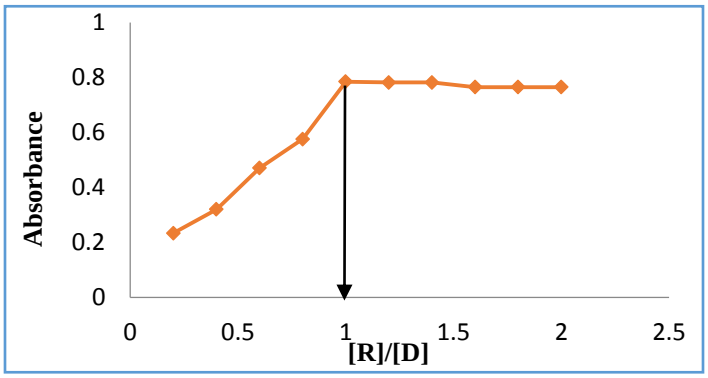



Fig.4: mole ratio for ion pair SMZ coupled with EBT

From the mole ratio method shown, the ratio between the drugs and reagents was 1:1, the proposed formula for the resulting dye produced are therefore as follows [21] shown in fig5.

**The possible reaction path may be written**

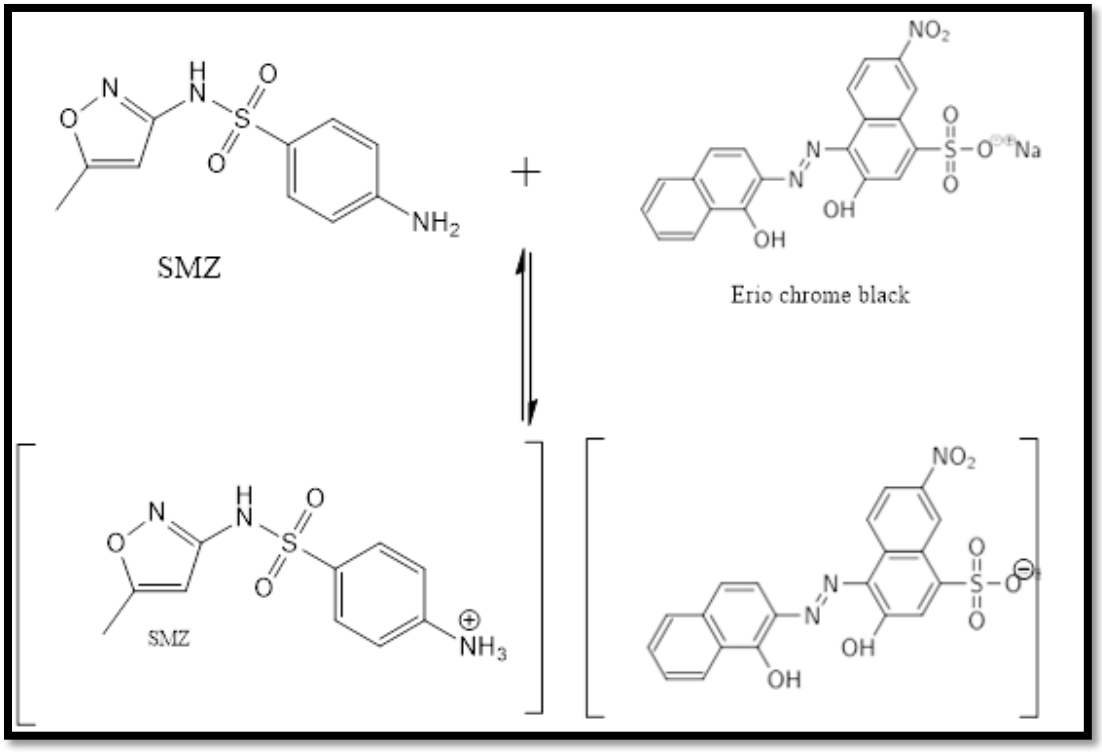



**Fig.5: suggest mechanism of SMZ reaction with EBT**

**Study the optimum experimental conditions of DLLME for estimation Amoxcillin**

To achieve characterization, many types of extraction and disperser solvents were studied, including dichloromethane, dichloromethane, carbon tetrachloride, and chloroform as extraction solvents. As shown in fig.6a, chloroform was the best solvent for high- intensity of absorbance. The absorbance of various disperser solvents, such as ethanol, methanol, acetone, and acetonitrile, was investigated. Ethanol gave the highest absorbance, as shown in Fig 6b. The value of the preconcentration factor and the sensitivity of

the DLLME method are strongly influenced by the volume of the organic phase; the results show that chloroform has the best extraction ability among the studied solvents when 2mL of it is mixed with ethanol in a 1:3 ratio; increasing the volume of chloroform in this ratio reduces the absorbance value of the extracted dye. This is due to the dilution effect, which lowers the analyte concentration. It was determined that as the volume of ethanol was increased;, the absorbance grew, but then quickly decreased, as seen in Fig 6c. Different centrifugation speeds (1000-6000 rpm) and times (1-7 min) were studied to get great separation; the results show that 5 minutes at 4000 rpm gave higher absorbance; therefore, they were fixed in experiential result shown in Fig. d &e 6.

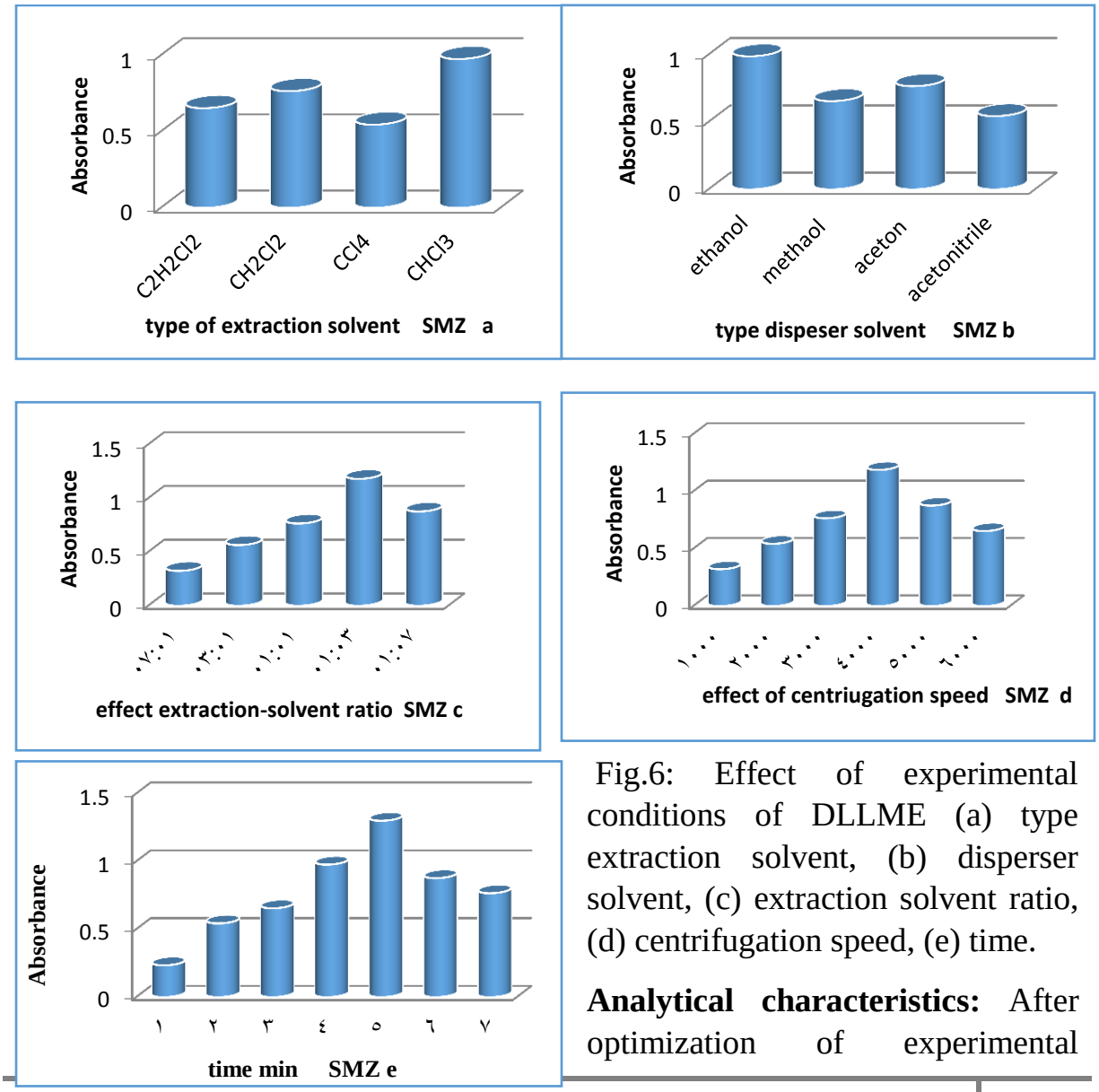



Fig.6: Effect of experimental conditions of DLLME (a) type extraction solvent, (b) disperser solvent, (c) extraction solvent ratio, (d) centrifugation speed, (e) time.

**Analytical characteristics:** After optimization of experimental

conditions, prepared of the ion pair dye and DLLME curve by plotting absorbance different concentrations SMZ 2-50 and 0.5-6 µg / mL respectively show in Fig.7and Table 1 shows the parameter characteristic for regression equation of ion pair and DLLME techniques

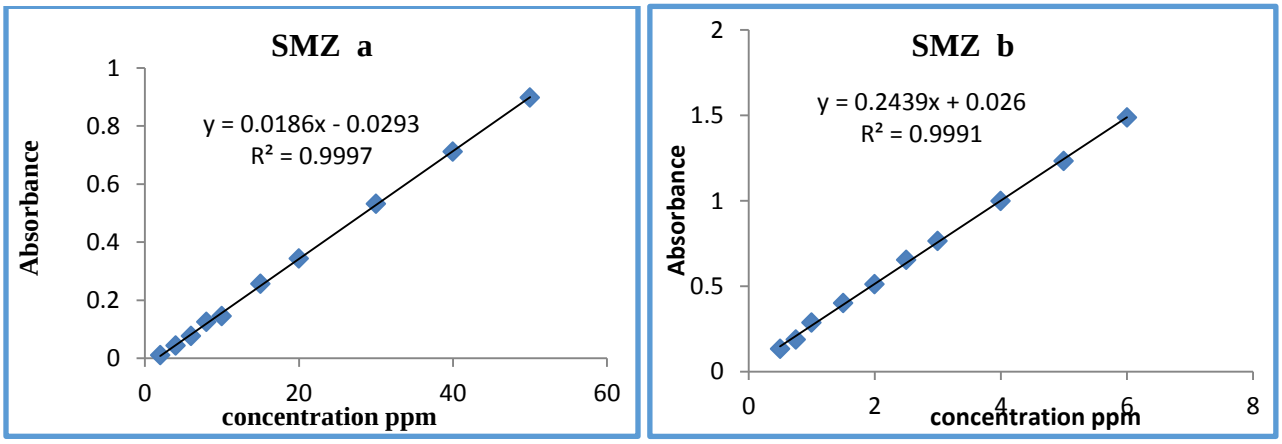



**Fig. 7: Calibration curve of SMZ: (a) ion pair ( b) DLLME**

**Table 1: shows a characteristic parameter for the suggested ion pair and DLLME of the SMZ drug's regression equation.**

| Parameters | Ion pair | DLLME |
|---|---|---|
| $\lambda_{max}$ nm | 490 | 490 |
| Color | Reddish-Orange | Reddish-Orange |
| Regression equation | Y=0.0186X-0.0293 | Y=0.2439X+0.026 |
| Linearity range( µg/mL) | 2-50 | 0.5-6 |
| Correlation Coefficient (r) | 0.9998 | 0.9995 |
| Ɛ(L.mol$^{-1}$.cm$^{-1}$) | $0.471\times10^{4}$ | $0.889\times10^{5}$ |
| Sandal' sensitivity (µg . cm$^{-2}$) | 0.054 | 0.004 |
| Slope (b) | 0.0186 | 0.2439 |
| Intercept(a) | -0.0293 | 0.026 |
| Limit of detection(µg/mL) | 0.177 | 0.013 |
| Limit | 0.537 | 0.041 |

| | | |
|---|---|---|
| quantification(µg/mL) | | |
| Enrichment Factor (EF) | ------ | 18.20 |
| Pre-concentration factor (PF) | ------- | 25 |

LOD = $3.3\times SD_b/S$, $SD_b$= the standard deviation of intercepts of regression lines [22]

## Precision and accuracy

The proposed methodologies ion pair reaction and DLLME were studied for accuracy and precision under ideal conditions using varied concentrations, measuring absorbance at a minimum of five readings per concentration. (RE percent) -, (R percent), and (RSD percent ) are used to determine precision and accuracy, as illustrated in Tables 2 and 3.

**Table 2 shows the precision and accuracy of the proposed approaches for estimating pure samples.**

| **Ion pair method** | | | | | | |
|---|---|---|---|---|---|---|
| **Drug** | Amount of drugs µg /ml | | Relative* Error % | Recovery* % | Average Recovery% | RSD%* (n=5) |
| | Taken | Found | | | | |
| **SMZ** | 10 | 9.970 | -0.30 | 99.70 | 99.94 | 0.54 |
| | 20 | 19.99 | -0.05 | 99.95 | | |
| | 30 | 30.05 | 0.16 | 100.16 | | |
| **DLLME method** | | | | | | |
| **SMZ** | 2 | 2.01 | 0.50 | 100.50 | 99.63 | 0.37 |
| | 4 | 4.01 | 0.25 | 100.25 | | |
| | 6 | 5.89 | -1.83 | 98.166 | | |

* Average of five times, E% = relative error Found-taken /taken ×100, Rec% =recovery, and RSD%=relative standard deviation.

**Table 3 shows the precision and accuracy of the suggested method for determining commercial medications.**

| Type of Drugs | Amount of drugs mg Taken | Amount of drugs mg Found | Relative Error % | Recovery % | Average Recovery % | RSD % (n=5) |
|---|---|---|---|---|---|---|
| **Ion pair method** | | | | | | |
| **Bactrim tablet (Roche-France)** | 10 | 10.04 | 0.40 | 100.40 | 100.26 | 0.78 |
| | 20 | 19.97 | -0.15 | 99.85 | | |
| | 30 | 29.95 | -0.16 | 99.83 | | |
| **Cotrim syrup (Asia-Syria)** | 10 | 9.98 | -0.20 | 99.80 | 99.97 | 0.98 |
| | 20 | 19.98 | -0.10 | 99.90 | | |
| | 30 | 30.07 | 0.23 | 100.23 | | |
| **DLLME method** | | | | | | |
| **Bactrim tablet (Roche-France)** | 2 | 1.994 | -0.50 | 99.50 | 99.80 | 0.68 |
| | 4 | 3.976 | -0.75 | 99.25 | | |
| | 6 | 6.04 | 0.66 | 100.66 | | |
| **Cotrim syrup (Asia-Syria)** | 2 | 2.024 | 1.00 | 101.00 | 100.05 | 0.54 |
| | 4 | 3.986 | -0.50 | 99.50 | | |
| | 6 | 5.98 | -0.33 | 99.66 | | |

Table 4 shows that the derived t-values and F-values for SMZ estimation in various pharmaceuticals are fewer than t-tabulated and F-tabulated at 95 percent confidence interval and (n-1) degrees of freedom.

**Table 4: shows a 95 % confidence level comparison of the suggested method to the standard method using t and F statistical tests.**

| Pharmaceutical preparation | Rec% Ion pair complex | Value | | Rec % DLLE method | Value | | Standard method[2][3] |
|---|---|---|---|---|---|---|---|
| | | t | F | | t | F | Rec % |
| sulfamethoxazole pure | 99.94 | 1.10 (2.13) | 7.55 (19.00) | 99.63 | 0.862 (2.13) | 10.79 (19.00) | 100.01 |
| Bactrim tablet (Roche-France) | 100.26 | | | 99.80 | | | 99.89 |
| Cotrim syrup (Asia-Syria) | 99.97 | | | 100.05 | | | 99.91 |

## Conclusion

In this paper, SMZ reacts as an ion pair reaction with EBT and has spectrophotometric characterization for application, for determination the SMZ by using via spectrophotometric methods. These methods have the advantage simple, fast and very sensitive and have good precision and accuracy. The proposed methods were successfully applied for estimation of pure SMZ and pharmaceutical formulation.

## Acknowledgment

The authors are grateful for the spectral analysis services and analytical experiences provided by Mustansiriyah University's Faculty of Science, Chemistry's supplier laboratory.

## References

1. Wormser, G. P.; (1982) "Co-Trimoxazole (rimethoprim-Sulfamethoxazole) An Updated Review of Its Antibacterial Activity and Clinical Efficacy " Drugs, 24(6): 459-518.
2.’SULFAMETHOXAZOLE " (2001); monographs./iarc./fr/ENG/Monographs ...mono; 79: 361.
3. "Sulfamethoxazole" European Pharmacopoeia 5th, Council of Europe, (2005), 2511-2512.
4. Sohrabi, M.; Fathabadi M. and Nouri A.; (2010) "Simultaneous spectrophotometric determination of sulfamethoxazole and trimethoprim in pharmaceutical preparations by using multivariate calibration methods" Journal of Applied Chemical Researches, 3(12): 47-52.
5. Dinça, E.; Kadıoğlub Y.; Demirkayab F. and Baleanuc D.; (2011) "Continuous Wavelet Transforms for Simultaneous Spectra Determination of Trimethoprim and Sulfamethoxazole in Tablets" J. Iran. Chem. Soc., 8(1): 90-99.
6. Givianrad, M. H. and Mohagheghian, M.; (2012) "Net Analyte Signal Standard Additions Method for Simultaneous Determination of Sulfamethoxazole and Trimethoprim in Pharmaceutical Formulations and Biological Fluids" E-Journal of Chemistry, 9(2): 680-692.
7. Upadhyay, K.; Asthana, A. and Tiwari, N.; (2012) "Solid Phase Extractive Spectrophotometric Determination of Some Sulfa Drugs" Asian J. Pharm. Clin. Res. 5: 222-226.
8. Abdulsatar, R. S.; (2009) "Spectrophotometric determination of sulfacetamide sodium and sulfamethoxazole in pharmaceutical preparations by oxidative coupling with pyro catechol" J. Kirkuk Univ. Scient. Stud., 4: 33.
9. Raja, G. V.; Sekaran, C. B.; Teja, D. W.; Madhuri ,B. and Jayasree, B.; (2009) "Simple Spectrophotometric Methods for the Determination of Sulfamethoxazole in Pharmaceuticals Using Folinciocalteau and Orcinol as Reagents" E-Journal of Chemistry, 6(2): 357-360.
10. Nidhal Sher Mohammed1, Theia'a N. Al-Sabha and Payman Abdul-Jabar (2020)Development Method for Spectrophotometric Analysis of Sulfamethoxazole Using Vanilline Reagent. Asian Journal of Applied Chemistry Research 6(2): 41-49.

11. Herrera-Herrera A. V.; Hernandez-Borges J.; Afonso M. M.; Palenzuela J. A.; Rodriguez-Delgado M. A.; (2013) “Comparison between magnetic and nonmagnetic multi-walled carbon nanotubes-dispersive solid-phase extraction combined with ultrahigh performance liquid chromatography for the

determination of sulfonamide antibiotics in water samples" TALANTA, 116: 695-703.
12. Waleed M. M.; Khaleel D. H.; Ghada H.; Abdel-Salam R. A.; Haiss A. and Klaus K.; (2013) "Simultaneous Determination of 11 Sulfonamides by HPLC-UV and Application for Fast Screening of Their Aerobic Elimination and Biodegradation in a Simple Test" CLEAN-SOIL AIR WATER, 41(9): 907-916.
13. Pamreddy A.; Hidalgo M.; Havel J. and Salvado V.; (2013) "Determination of antibiotics
(tetracycline's and sulfonamides) in bio solids by pressurized liquid extraction and liquid chromatography-tandem mass spectrometry" journal of chromatographya, 1298: 68-75.
14. Asadi S. and Gharbani P.; (2013) "simultaneous Determination of Sulfamethoxazole and Phthalazine by HPLC and Multivariate Calibration Methods" international of chemistry and chemical engineering international English edition, 32(2): 1-8.
15. Pietron W.; Cybulski W.; Krasucka D.; Mitura A.; Kos K. and Antczak M.; (2013) "Determination of five sulfonamides in medicated feeding stuffs by liquid chromatography with ultraviolet detection" bulletin of the institute, 57(4): 545-552.
16. Hererra-Hererra, A. V.; Hernández-Borges J.; Borges-Miquel T. M. and Rodríguez- Delgado M. Á.; (2013) "Dispersive liquid–liquid micro extraction combined with ultrahigh performance liquid chromatography for the simultaneous determination of 25 sulfonamide and quinolone antibiotics in water samples" J. Pharma. Biomed. Anal., 75: 130-137.
17. Shaaban ,H. and Go´recki, T.; (2011) "Optimization and validation of a fast ultrahighpressure liquid chromatographic method for simultaneous determination of selected sulfonamides in water samples using a fully porous sub-2 μm column at elevated temperature" J. Sep. Sci., 35: 216–224.
18. Liu R.; He P.; Li Z.; and Li R.; (2011) "Simultaneous Determination of Sixteen Sulfonamides in Animal Feeds by UHPLC–MS–MS" Journal of Chromatographic Science, 49: 640-646.
19. Icardo M. C.; Mateo J. V. G.; Lozano M. F. and Calatayud J. M.; (2003) "Enhanced flowinjection– chemiluminometric determination of sulfonamides by on-line photochemical reaction "Analytica Chimica Acta, 499: 57–69.
20. Injac R.; Kočevar N. and Štrukelj B.; (2009) "Optimized Method for Determination of Amoxicillin, Ampicillin, Sulfamethoxazole, and Sulfacetamide in Animal Feed by Micellar Electro kinetic Capillary Chromatography and Comparison with High- Performance Liquid Chromatography" Croat. Chem. Acta, 82 (3): 685–694.

21. MohaumanMohammadMajeedAl-Rufaie(2021) asensitives spectrophotometric method for trace amounts determination of promethazine in drug formulation via ion pair complex formation .MalaysianJournalOfScience40(1):80-92
22.Validation on Analytical Procedures:Text and Methodology –ICH Q2 (R1) –International Conference on Harmonisation, Geneva, Switzerland, 2005.
23. British Pharmacopoeia on CD – ROM.", 3rd Ed., Copyright by System Simulation Ltd. The Stationary office, London, 1999.