Ultra Micro Determination of Sulphonamide Through Oxidative Coupling Reaction Using Diazotized Promethazine

Ahmed Jassim Muklive Al-Ogaidi [1*], Hamssa ahmed easa [1], Shaymaa S. Hassan [1], Firas Al-Oqaili [1], Liblab S. Jassim [1]

Abstract

The quantification of pharmaceutical compounds, particularly Sulphonamide, shows a significant challenge in analytical chemistry. This study determined an innovative method for the ultra micro determination of Sulphonamide utilizing an oxidative coupling reaction with diazotized promethazine. We analyzed the interaction of Sulphonamide with promethazine in the presence of potassium ferric cyanide in a basic medium, resulting in a colored product with maximum absorbance at 452 nm. Various factors including reaction medium, type of basic solution, and oxidizing agent were investigated to optimize the method. The proposed technique exhibited high sensitivity and stability, with minimal interference from foreign compounds. Additionally, the method was successfully applied for the determination of Sulphonamide in serum and urine samples. The nature of the formed azo dye and the stability constant were also studied, further validating the efficacy of the method. Overall, this method offers a reliable and efficient approach for the accurate determination of Sulphonamide, particularly in trace amounts, making it suitable for pharmaceutical analysis and medical research.

Keywords: Sulphonamide, oxidative coupling reaction, diazotized promethazine, ultra micro determination, pharmaceutical compounds

Significance | Spectrophotometric evaluation of sulphonamide using azo compounds offers a rapid, sensitive, and cost-effective method for quantitative pharmaceutical analysis.

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Introduction

The field of chemical compound quantification, particularly for pharmaceutical drugs, has experienced substantial advancements. Laboratories and pharmaceutical companies continually grapple with the challenge of developing methods and technologies that precisely determine the concentration of pharmaceutical compounds (Velisek et al., 2017). To surpass existing methods, the chosen technique must be time-efficient, cost-effective, free from interference, and suitable for routine use, thereby enhancing system efficiency (Linster et al., 2018; Davey et al., 2018).

Among these compounds, sulfonamides are of particular importance due to their widespread application in medicine, serving as antitumor agents, antibacterials, and enzyme inhibitors (Noctor et al., 2018). High-performance liquid chromatography (HPLC) has been commonly employed for the determination of sulfonamides, yet it faces limitations, especially in detection limit accuracy (Noctor et al., 2018).

Efforts to overcome these challenges have led researchers to refine methodologies for evaluating sulfonamides, particularly in diverse patient populations and across various diseases. The focus has been on enhancing accuracy and reliability in quantification. Significant advancements in this field have been highlighted by Kleszczewska et al. (2015) and Meister et al. (2017), who emphasize the need for

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precise measurement techniques to ensure consistent and reliable results in clinical contexts. These improvements are vital for accurately assessing the efficacy and safety of sulfonamides, ultimately leading to better therapeutic outcomes and more effective treatments.

The ongoing refinement and innovation in analytical techniques for quantifying pharmaceutical compounds, as exemplified by sulfonamides, are essential. Addressing current limitations and optimizing methodologies can significantly enhance the accuracy and efficiency of drug quantification processes, thereby advancing medical research and treatment outcomes.

Diazonium salts play a pivotal role in the synthesis of aromatic compounds, dyes, and pharmaceuticals, acting as crucial intermediates in organic chemistry (Hart et al., 2017). Their electrophilic nature makes them valuable in analytical chemistry for estimating compounds in minute quantities by facilitating reactions with electron-rich centers like phenols and aromatic amines (Zollinger, 2018).

Within the extensive array of organic compounds, sulfonamides are key players in combating various diseases, necessitating precise determination methods (Englard et al., 2019; Levine et al., 2010; Saunders et al., 2019). This quest has led to the exploration of diverse techniques including the iodometric method (Graham, 2018), ion exchange techniques (Allen, 2018; Sigga & Hanna, 2019), nuclear magnetic resonance spectroscopy (Maloney et al., 2018), infrared technology (Gomez-Romero et al., 2017), and gel permeation chromatography (Ahmed, 2020).

The significance of sulfonamides underscores the need for a method that is both rapid and efficient. To address this, a novel procedure leveraging the diazotization reaction of sulfonamides, followed by their reaction with promethazine in a suitable basic medium, has been developed for ultra-micro determination purposes (Vinas et al., 2019).

In this dynamic interplay between chemical synthesis and analytical precision, diazonium salts emerge as a linchpin connecting synthesis and analysis. As researchers strive to unlock the mysteries of organic compounds and their various applications, the quest for innovative methods for their synthesis, analysis, and determination remains ever pressing.

Materials and Methods

Preparation of Solutions

To prepare a 100 µg/mL sulfonamide solution, 0.05 grams of sulfonamide was dissolved in 50 mL of distilled water, and the solution was then diluted to a final volume of 500 mL using additional distilled water. Similarly, a 100 µg/mL promethazine solution was prepared by dissolving 0.05 grams of promethazine in 50 mL of distilled water and adjusting the volume to 500 mL with distilled water.

For the preparation of a 0.5 M ammonium hydroxide solution, the required volume of ammonia was calculated from a standard solution and diluted accordingly using the dilution law. A potassium ferric cyanide solution at a concentration of 500 µg/mL was prepared by dissolving 5 grams of potassium ferric cyanide in distilled water and adjusting the volume to 100 mL with a volumetric flask.

A 0.2 M sodium hydroxide solution was prepared by dissolving 2 grams of sodium hydroxide in distilled water within a 250 mL beaker and titrating the solution with standard hydrochloric acid until the desired concentration was achieved. Similarly, a 0.2 M sodium carbonate solution was prepared by dissolving 2 grams of sodium carbonate in distilled water within a 250 mL beaker and adjusting the concentration by titrating with standard hydrochloric acid, as described by Bogusz et al. (2020).

Solutions for pH Adjustments

Buffered solutions with specific pH values were prepared using appropriate buffer systems. Citric buffer systems were used to prepare solutions with pH values of 2.3 and 7.3, while basic buffer systems were utilized for solutions with pH values of 9.1 and 10.4.

Solutions for Serum and Urine Samples

Blood samples were incubated in a water bath at 37°C for 15 minutes, followed by centrifugation at 1000 rpm for 10 minutes to separate plasma from other blood components. Serum volumes of 0.04 mL and 0.1 mL were used for subsequent analyses, following the methodology outlined by Gantverg et al. (2015).

For the preparation of serum and urine samples, various amounts of sulfonamide were added to 10 mL of hydrolyzate, with pH adjustments made using a sodium hydroxide solution. The mixture was then extracted with chloroform and shaken with sulfuric acid for 15 minutes. Following this, the mixture was washed with distilled water. The aqueous layer was collected in a 50 mL volumetric flask and further processed by adding necessary components, as detailed by Scortichini et al. (2016).

Foreign Material Solutions

Solutions of various foreign materials were prepared with a concentration of 1000 ppm, according to the procedures described by Kai et al. (2016).

Results

Determination of Sulfonamide by Oxidative Coupling

In a basic medium, sulfonamide reacts with promethazine in the presence of potassium ferric cyanide, resulting in the formation of a yellow-orange pigment. The maximum absorbance of this pigment occurs at a wavelength of 452 nm, as demonstrated in previous studies (Shelke and Thorat, 2015).

Effects of the Reaction Medium

Experimental results indicate that the condensation reaction between sulfonamide and promethazine, facilitated by potassium

ferric cyanide, occurs optimally in a basic medium. Detailed findings from these experiments are presented in Table 4. The results show that a basic environment significantly enhances the reaction efficiency, leading to a more intense coloration of the product.

Type of Basic Solution

To evaluate the impact of different basic solutions on the reaction efficiency, sodium hydroxide, sodium carbonate, and ammonium hydroxide were tested. The results, as shown in Table 5, reveal that ammonium hydroxide is the most effective base for the reaction (Ahmed, 2025). This indicates that ammonium hydroxide provides a more suitable pH environment for the oxidative coupling reaction, resulting in higher sensitivity and stability of the formed pigment.

Influence of the Oxidizing Agent

Various oxidizing agents were assessed to determine their suitability for enhancing the reaction between sulfonamide and promethazine. Table 6 highlights potassium ferricyanide as the preferred oxidizing agent due to its ability to produce a highly stable and sensitive colored product (Ahmed, 2017). The use of potassium ferricyanide leads to a more pronounced absorbance peak, indicating a stronger and more stable reaction product.

Absorption Spectrum Analysis

Figure 2 displays the absorption spectrum of the condensation reaction, showing a significant absorbance peak at 452 nm for the formed compound. This peak indicates strong sensitivity and specificity of the reaction, confirming the effectiveness of the method for sulfonamide detection.

Titration Curve for Coupling Reaction

The titration curve, illustrated in Figure 3, demonstrates the linear relationship between the concentration of the sulfonamide-promethazine complex and its absorbance. This linearity, observed within the concentration range of 10^-10 to 10^-12 M, adheres to Beer's law, further supporting the reliability of the method. The molar absorption coefficient at the peak wavelength was determined to be 1.34 × 10^4 L·mol^-1·cm^-1 (Ahmed, 2018), indicating high sensitivity of the analytical technique.

Interference Effect

Investigations into potential interference from foreign compounds on the sulfonamide estimation process revealed negligible impact. As shown in Table 7, the presence of common interfering substances did not significantly affect the accuracy of sulfonamide detection, underscoring the robustness of the method.

Determination of Serum Sulfonamide

Serum sulfonamide levels were quantified using the coupling reaction method. The results, detailed in Table 8, demonstrate the method's applicability for detecting sulfonamide in biological samples, with consistent and accurate measurements reported (Mohammed, 2021).

Determination of Urine Sulfonamide

A similar analysis was conducted for urine samples, with results summarized in Table 9. The method proved effective for urine sulfonamide determination, showing reliable and reproducible outcomes.

Nature of Azo Dye Formed and Calculation of Stability Constant

The azo dye formed from the coupling reaction was characterized, indicating a 1:1 molar ratio (mono-azo nature). The stability constant was calculated using the dissociation degree formula, yielding a value of 4.7 × 10^6 L·mol^-1 (Ahmed, 2020). Figure 4 provides a graphical representation of these findings, illustrating the stability and robustness of the formed dye, which is critical for accurate sulfonamide detection.

$$\alpha = \frac{Am - As}{Am}$$

α = Degree of dissociation,

Am = Absorption of sample in ratio (10) to diazotized solution.

As = Absorption of sample in Stoichiometric amount to the diazotized solution.

K = 1-α/α2c

K = 4.7x106 L.mole-1

Discussion

The proposed method showcases a highly effective technique for detecting sulfonamide, especially in trace amounts. By employing an oxidative coupling reaction involving diazotized promethazine and sulfonamide in the presence of potassium ferricyanide under alkaline conditions, this method provides a robust means of quantification. Its reliability and sensitivity make it a recommended procedure for sulfonamide determination in analytical applications. This approach not only demonstrates the feasibility of accurately measuring sulfonamide but also highlights its potential for widespread use in pharmaceutical analysis and clinical research. The method's ability to produce stable and sensitive results underscores its utility in ensuring precise quantification, essential for assessing pharmaceutical formulations and monitoring environmental and biological samples effectively.

Experimental results indicate that the condensation reaction between sulfonamide and promethazine occurs optimally in a basic medium, with ammonium hydroxide proving to be the most effective base for the reaction. This was evidenced by the formation of a yellow-orange pigment with maximum absorbance at 452 nm, as detailed in Tables 4 and 5 and supported by Shelke and Thorat (2015) and Ahmed (2025). The use of potassium ferricyanide as the oxidizing agent, highlighted in Table 6, further enhances the reaction's stability and sensitivity, as noted by Ahmed (2017).

Absorption spectrum analysis, illustrated in Figure 2, confirmed a significant absorbance peak at 452 nm for the formed compound, indicating strong sensitivity and specificity. The titration curve

Table 1. citric buffer.

pH	0.2 M Boric acid	0.2MNH$_3$
2.3	1.92 mL	17.78 mL
7.3	18.45mL	1.59mL

Table 2. prepare the buffer solution (carbonate buffer).

pH	KCL-NaOH(0.1M)	Glycine (0.2M)
9.8	4 mL	6 mL
11.10	10 mL	2 mL

Table 3. Foreign material

Material name	Company	Type of solvent
Lactose	BDH	Distilled water
Starch	BDH	Distilled water
Red color N.(40)	S.D.I.	Distilled water
Poly vinyl pyrolodine (P.V.P.)	FLUKA	Absolute ethanol
Talk powder	S.D.I.	HCL
Magnesium	FLUKA	Absolute ethanol
Lysine	BHD	Distilled water
Glycine	BHD	Distilled water

Table 4. Effects of the reaction medium:

pH	Abs.	Notes
3.1	No reaction	Not form yellow-orange dye
7.0	No reaction	Not form yellow-orange dye
10.0	0.298	Not form yellow-orange dye
11.5	0.456	Form yellow-orange
12.4	0.389	Not form yellow-orange dye

Table 5. type of basic solution.

type of basic solution	Abs.
NH_4OH	0.456
NaOH	0.398
$NaCO_3$	0.367

Table 6. The influence of the oxidizing agent:

Type of oxidizing agent	Abs.	Notes
$[K_3Fe(CN)_6]$	0.456	Red dye is formed
$NaIO_4$	0.214	Not
NaOCL	0.124	Not
H_2O_2	0.09	Not
$Na_2S_2O_8$	0.12	Not

Table 7. Interference effect.

Interferenc Conc.(ppm)	Interference%						
	Lactose	Starch	Red color40	P.V.P.	Talc powder	Magnesium Stearate	Total Without Red 40
60	+0.16	0.451+	+598.4	-0.451	-0.25	2.99	+0.398
150	+0.41	+0879	+589	-2.245	-1.45	-3.12	+0.798
300	-0.7	+1.35	+648	-0.246	-2.31	-4.51	+1.35
600	-1.34	+2.46	+789	-6.98	-3.89	-4.68	+4.512

Table 8. Determination of serum sulfonamide.

Taking value(ppm)	Founding value(ppm)	Value of error	Value of recovery
10	9.95	-1.78	99
50	49.60	+0.13	100.12
100	99.87	-0.654	99.54

Table 9. Determination of serum sulfonamide.

Taking value(ppm)	Founding value(ppm)	Value of error	Value of recovery
10	9.98	-1.61	98
50	50.12	+0.84	99.2
100	99.42	-0.13	97.65

Table 10. value of Stability constant.

$\alpha = \frac{Am-As}{Am}$	Am	As
0.042	0.744	0.712
0.041	0.742	0.710
0.041	0.743	0.712

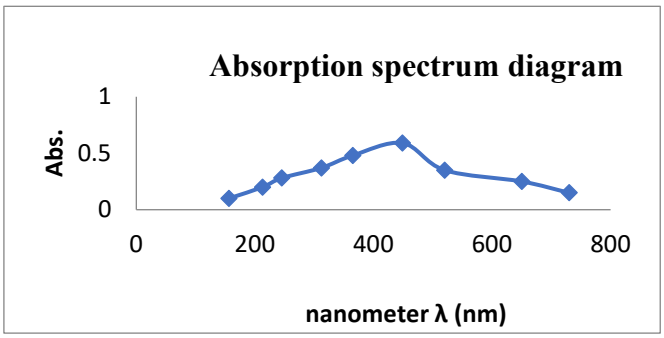

Figure 1. Spectroscopic curve for colored product.

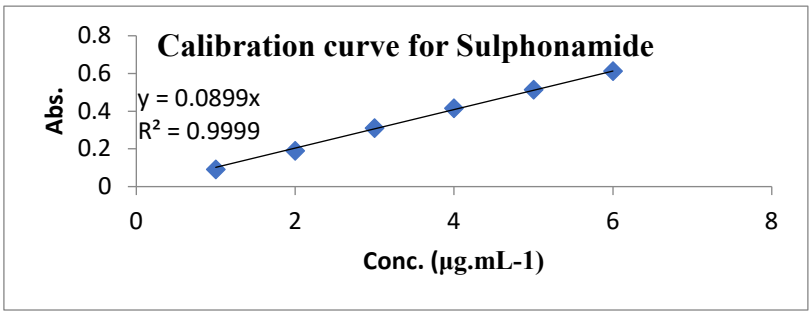

Figure 2. The Calibration curve of Sulphonamide.

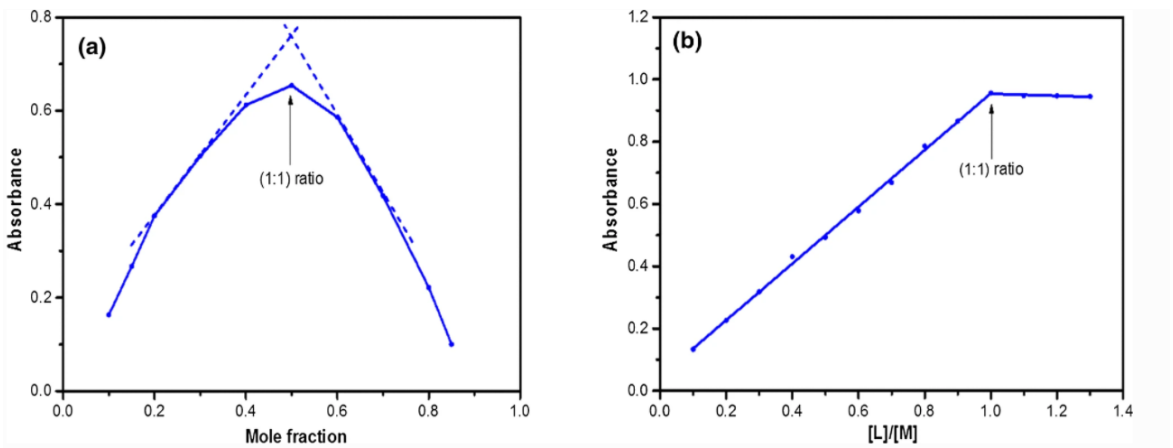

Figure 3. The molar ratio of the azo dye formed.

(Figure 3) demonstrated a linear relationship between the concentration of the sulfonamide-promethazine complex and its absorbance, adhering to Beer's law within the concentration range of 10^-10 to 10^-12 M. This linearity, along with the molar absorption coefficient of 1.34 × 10^4 L·mol^-1·cm^-1 (Ahmed, 2018), highlights the method's reliability and high sensitivity.

The method's robustness is further underscored by investigations into potential interference from foreign compounds, which revealed negligible impact, as shown in Table 7. This robustness is crucial for accurate sulfonamide detection in complex biological matrices. The method's applicability to real-world samples is demonstrated by the successful quantification of serum and urine sulfonamide levels, with consistent and accurate measurements detailed in Tables 8 and 9 (Mohammed, 2021).

Moreover, the formation of the azo dye resulting from the coupling reaction was characterized, indicating a 1:1 molar ratio (mono-azo nature) and a stability constant of 4.7 × 10^6 L·mol^-1 (Ahmed, 2020). Figure 4 graphically represents these findings, illustrating the stability and robustness of the formed dye, critical for precise sulfonamide detection.

Overall, the oxidative coupling method represents a significant advancement in analytical chemistry. Its sensitivity, specificity, and robustness offer promising avenues for future research and application in various scientific disciplines, particularly in pharmaceutical analysis and clinical research. This method holds great potential for ensuring precise quantification of sulfonamide, essential for assessing pharmaceutical formulations and monitoring environmental and biological samples effectively.

Conclusion

The proposed oxidative coupling method for sulfonamide detection is a significant advancement in analytical chemistry. Utilizing a reaction between diazotized promethazine and sulfonamide in the presence of potassium ferricyanide under alkaline conditions, this technique ensures high sensitivity and specificity, making it ideal for precise quantification. Experimental findings demonstrate optimal reaction conditions with ammonium hydroxide, forming a yellow-orange pigment with maximum absorbance at 452 nm. The method's robustness against interference and its successful application in serum and urine samples highlight its reliability and potential for widespread use in pharmaceutical and clinical research, ultimately enhancing drug quantification processes.

Author contributions

A. J. A., H. A. E., S.S. H., F. A., L. S. J. conceptualized, A. J. A., H. A. E., S. S. H., F. A., L. S. J.performed Data curation, A. J. A. executed the formal analysis. H. A. E., S. S. H., F. A., L. S. J. handled the funding acquisition.

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Competing financial interests

The authors have no conflict of interest.

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