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Article

Development of a Spectrophotometric Method Based on a Schiff Base for the Determination of Sulfasalazine in Bulk Drug Substance and Pharmaceutical Dosage Forms

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Abstract: A simple, accurate, and sensitive spectrophotometric method was established for the quantitative determination of Sulfasalazine (SSZ) based on Schiff base formation. The method involves the condensation reaction between Sulfasalazine and terephthalaldehyde in an acidic medium, producing a stable, water-soluble yellow-colored product. The resulting Schiff base exhibits a maximum absorption peak at (480 nm) and A UV–visible spectrophotometer was used to carry out the spectral measurements. The results showed that absorbance increased with increasing sulphasalazine concentration within the range (3–120 μg mL^{-1}), with a linear relationship in accordance with Beer–Lambert's law. The molar absorptivity was recorded as 5,982.73 L/mol^{-1} cm^{-1}, whilst the Sandel sensitivity was 0.0665 μg/cm^{-2}. The limit of detection (LOD) was 0.0592 μg mL^{-1}, whilst the limit of quantification (LOQ) was 0.1974 micrograms/ml. When the method was applied to commercial sulphasalazine tablets, it yielded accurate results, indicating its suitability for the quantitative analysis of pharmaceutical preparations.

Keywords Sulfasalazine;Terephthalaldehyde ; Spectrophotometric ; Schiff s bases , Beer's law.

1. Introduction

Sulphasalazine is classified as a disease-modifying antirheumatic drug (DMARD) and is widely used in the treatment of a number of autoimmune diseases, particularly rheumatoid arthritis and inflammatory bowel disease [1]. This drug consists of a complex of sulphapyridine and 5-aminosalicylic acid (5-ASA), and its therapeutic effect is attributed to its anti-inflammatory properties, which have made it a commonly used medication in clinical practice for the treatment of these conditions [2]-[6]. In its pure form, sulphasalazine appears as a powder ranging in colour from light yellow to yellowish-brown, It is characterise by low solubility in water and alcohol, whilst it dissolves well in dilute alkali hydroxide solutions and is slightly soluble in dichloromethane [7]. Sulfasalazine was developed in 1942 to combine 5-ASA with sulfapyridine, an antibiotic [8]. The molecular structure of sulfasalazine is presented in Figure 1 :

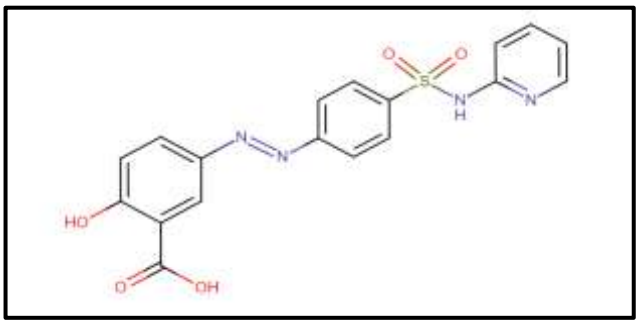

Figure 1. Molecular structure of Sulfasalazine.

The molecular formula is C18H14N405S and molar mass 398.39 g.mol-1 and melting point of around 240 to 245 °C [9] , A review of the scientific literature indicated that several analytical techniques are available to determine sulfasalazine as an example HPLC (high performance liquid chromatography) [10]-[12] , smartphone-based colorimetric [13] , UV spectrophotometry method [14] NMR [15], LC-MS/MS(liquid chromatography /mass spectrometry) [16]-[17] , fluorescence [18] ,electro chemical detection of SSZ used to modify a glass carbon electrode [19], [20] or carbonpast electrode [21], [22],Bismuth nanoparticles carbon [23] and spectrophotometric methods using different reagent(1,2 naphtha quinoline 4-sulphonate sodium) [24] ,P-Nitro aniline [25].

In this study the Terephthalaldehyde reagent was used in ,spectrophotometric determination of Sulfasalazine, the goal of this research was to develop a swift ,sensitive, simple and efficient process for quantitative estimation of sulfasalazine in its pure and pharmaceutical formulations.

2. Materials and Methods

2.1. Instrumentation:- Spectrophotometric analyses were conducted using a T90 UV–Visible spectrophotometer (PG Instruments Ltd., UK) equipped with 1 cm quartz cells. An analytical balance (Sartorius 210S, Kern) was used for accurate weighing of chemicals, while a water bath was utilized to maintain the required reaction temperature.

2.2. Reagents and Materials:- In all experiments, chemicals and solvents of analytical grade produced by Aldrich and Fluka were used without further processing. A standard sulphasalazine solution with a concentration of 1,000 μg $mL^{-1}$was prepared by dissolving 0.100 g of the substance in 5 ml of dimethylformamide (DMF), the volume was then made up to 100 ml with distilled water, and all standard solutions were prepared from this solution by appropriate dilution. A terephthalaldehyde solution with a concentration of 1.0×10^{-2} M. was also prepared by dissolving 0.1341 g of the reagent in ethanol and then making up the volume to 100 ml with distilled water. The working solution of sulphosalazine at a concentration of 300 μg mL^{-1} was prepared by diluting the standard solution, whilst 0.1 M. solutions of hydrochloric, nitric, sulphuric and acetic acids were prepared fresh prior to use in all experiments.

2.3. Sample preparation:- For the analysis of the pharmaceutical formulation, ten Salazopyrin EN tablets, each labeled to contain (500 mg of Sulfasalazine), were accurately weighed, giving a total weight of (12.6239 g). The tablets were ground into a homogeneous powder; a quantity of (0.07574 g) of the powder was then weighed out and placed in a volumetric flask with a capacity of (100 ml). The powder was first dissolved in (5 mL of dimethylformamide (DMF)), then diluted with distilled water and filtered to remove any insoluble excipients. Finally, The sample was made up to the final volume with distilled water in a volumetric flask to produce a sulphasalazine solution with a concentration of 300 micrograms per millilitre; the required working solutions were then prepared by diluting this with distilled water.

3. Results and Discussion

The analytical basis of the method was established through the reaction of sulphasalazine with terephthalaldehyde in an acidic medium, generating a yellow Schiff base with a spectral response that was utilised for the quantitative determination of the drug.The resulting product exhibits a maximum absorption peak at (480 nm), which was used for quantitative analysis. Graduated volumes (0.1–4.0 ml) of a sulphasalazine solution at a concentration of 300 micrograms/ml were taken and dispensed into separate 10-ml volumetric flasks. Then, 2.0 ml of a terephthalaldehyde solution at a concentration of 1.0×10^{-2} molar was added to each vial, followed by 1.0 ml of a hydrochloric acid solution at a concentration of 0.1 molar. After making up the volume with distilled water, the contents were mixed thoroughly to ensure the reaction was complete and a yellow colour had formed. Finally, the absorbance values were recorded at a wavelength of 480 nm, using the blank reagent solution as a reference. To obtain the optimum analytical performance, the experimental variables affecting Schiff base formation—including reagent volume, acid concentration, reaction medium, and other relevant parameters—were systematically investigated and optimized.

Optimization of Reaction conditions:-

1. Effect of the volume of the terephthalaldehyde solution

The effect of the volume of the terephthalaldehyde solution within the range (0.1–4.0 ml) was investigated by monitoring the change in spectral response. The results showed that using 2.0 mL of the reagent yielded the highest absorbance value at 480 nm, indicating that the Schiff base formation reaction was complete under the experimental conditions adopted. Therefore, this volume was used in all subsequent experiments, as illustrated in Figure (2).

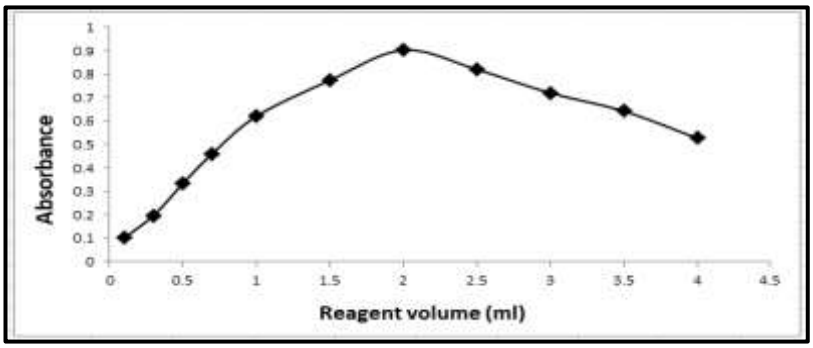

Figure 2. The effect of reagent amount on absorbance.

2. Effect of Acid Type

The effect of acid type on the spectral response of the Scheffler base reaction was investigated using several acids. The results showed that hydrochloric acid (HCl) produced the highest absorbance and the greatest stability of the coloured product; it was therefore used in all subsequent experiments. The results are shown in Table (1).

Table 1. Effect of acid type on the absorbance of the reaction product.

Selection of the Acid Type	Abs.	λmax.
Sulfuric acid	0.629	480
Nitric acid	0.712	480
Hydrochloric acid	0.901	480
Acetic acid	0.573	480

3. **Effect of hydrochloric acid volume**

An evaluation was carried out to assess the effect of the volume of hydrochloric acid (0.1 molar) on reaction efficiency, using volumes ranging from 0.1 to 4.0 ml. The results showed that the addition of 1.0 mL yielded the highest spectral response for the product formed; therefore, this volume was adopted for all subsequent measurements, as shown in Figure 3.

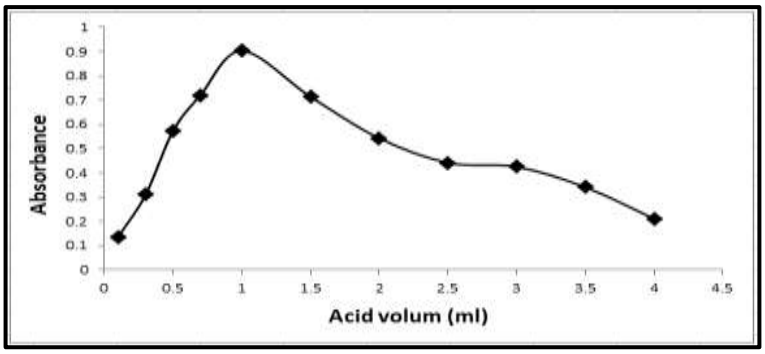

Figure 3. Effect of Hydrochloric Acid Volume.

4. Selection of the optimum temperature

The effect of temperature on the spectral response of the product formed was investigated. The data showed that the highest response was recorded at room temperature (25–30 °C); therefore, this temperature was adopted for all subsequent experiments, as shown in Figure 4.

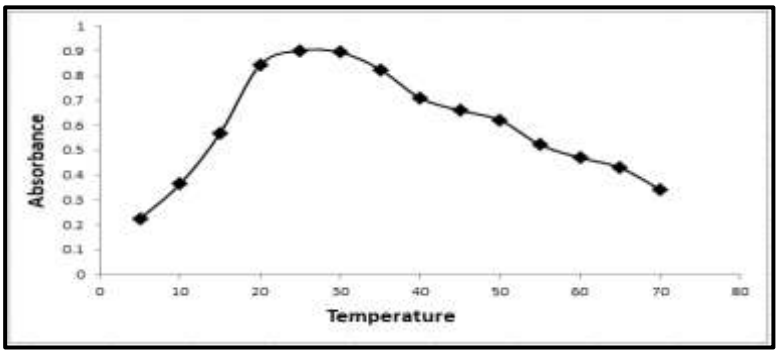

Figure 4. The effect of Temperature.

5. Stability of the Reaction Product

The stability of the resulting product was investigated over various time intervals. Specifically, (2 ml) of sulfasalazine, (2 ml) of terephthalaldehyde reagent , After adding (1.0 ml) of hydrochloric acid, the volume of the reaction mixture was adjusted to (10 ml) using distilled water in a volumetric flask; details are given in Table (2).The product was found to remain stable for approximately 60 minutes (one hour), a duration considered sufficient for performing the necessary calculations.

Table 2. Stability of the Reaction Product.

time / minute	Direct	5 min.	10 min.	15 min.	20 min.	25 min.	30 min.	35 min.	40 min.	45 min.	50 min.	60 min.
Abs.	0.876	0.889	0.901	0.902	0.903	0.9053	0.904	0.903	0.901	0.901	0.90	0.901

6. Effect of the Order of materials Addition

The effect of varying the order in which the reaction components were added was systematically investigated. As shown in Table 3, the highest absorbance was achieved when the materials were added according to sequence (1). Accordingly, this order was selected for all subsequent analytical measurements.

Table 3. The effect Order of Materials addition.

No.	Order of addition	Absorbance
1	**S + T + H**	**0.902**
2	T + H + S	**0.834**
3	H + T + S	**0.868**
4	S + H + T	**0.797**
5	**T + S + H**	**0.755**

(S) Sulfasalazine , (T) Terephthalaldehyde , (H) Hydrochloric acid

7. Establishment of the Calibration Curve

A calibration curve was established by preparing a series of (10 mL) volumetric flasks containing Sulfasalazine at concentrations ranging from (3 to 120 μg mL^{-1}). To each flask, (2.0 mL) of (1.0×10^{-2} M) terephthalaldehyde solution and (1.0 mL) of (0.1 M) hydrochloric acid were added. The reaction mixtures were allowed to stand for (20 min) to ensure complete color development. Subsequently, the solutions were diluted to the calibration mark with distilled water, mixed thoroughly, and their absorbance was measured at (480 nm) against a reagent blank. The calibration curve was constructed by plotting absorbance versus Sulfasalazine concentration. Under the optimized experimental conditions, the method exhibited excellent linearity over the concentration range of (3–120 μg mL^{-1}), with a correlation coefficient (R^2) of (0.9979). The calculated molar absorptivity and Sandell's sensitivity were (5982.73 L mol^{-1} cm^{-1}) and (0.0665 μg cm^{-2}) , respectively. The resulting calibration curve is presented in Figure 5 :

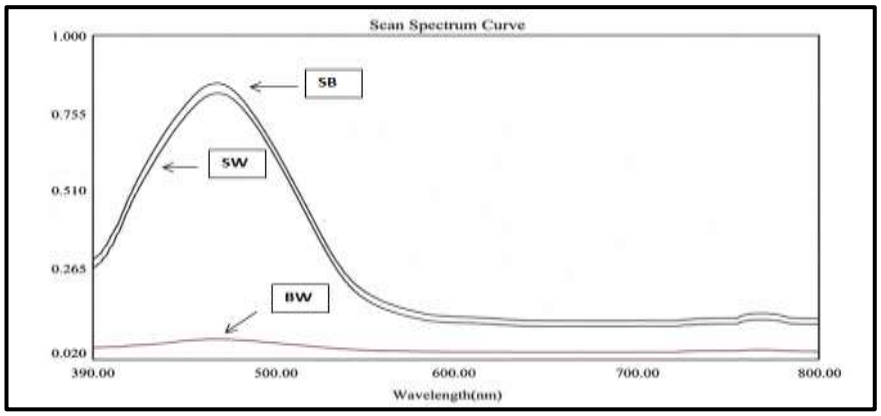

Figure 5. Standard calibration curve of Sulfasalazine.

8. Absorption Spectrum of the Final Reaction Product

The figure (6) displays the absorption spectrum of the colored Schiff base formed from the reaction between sulfasalazine and terephthalaldehyde

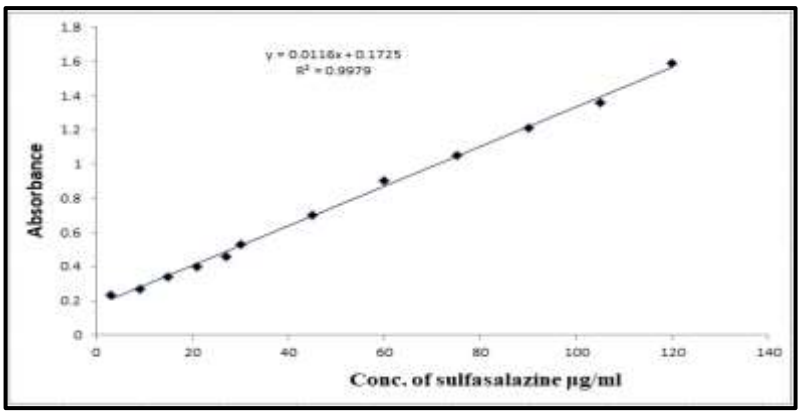

Figure 6. Absorption spectrum of the final reaction product.

SB = sample solution vs blank solution

SW = sample solution vs distilled water

BW= blank solution vs distilled water

9. Accuracy and precision

The reliability of the proposed analytical method was evaluated in terms of accuracy and precision by performing six replicate measurements at (480 nm) for three Sulfasalazine concentrations selected from within the Beer's law range. The method exhibited satisfactory analytical performance, yielding a mean recovery of (99.54%) , which reflects its excellent accuracy and precision. The detailed results are listed in Tables (4) and (5). Table 4 includes the theoretical and measured concentrations, together with the percentage recovery and relative error (Er%), whereas Table(5) presents the calculated values of the limit of detection (LOD) and limit of quantification (LOQ).

Table 4. Evaluation of Accuracy and Precision.

Concentration of Sulfasalazine µg /ml	Concentration of Sulfasalazine measured *	Recovery.*%	Er. %
15	**14.3**	**95.33**	**−4.66**
30	**30.9**	**103.3**	**3.3**
90	**89.3**	**99.99**	**-0.77**

*N=6

Table 5. Results of (L.O.D) and (L.O.Q).

Concentration. µg /ml	L.O.D µg/ml	L.O.Q µg/ml
3	**0.0592**	**0.1974**

10. Stoichiometry of the Reaction Product

To determine the molar ratio of the product formed, the Job method and the molar ratio method were used, employing solutions of sulphasalazine and terephthalaldehyde of equal concentration (1.0×10^{3} molar) In the J method, equal volumes of the two solutions were mixed in 10 ml volumetric flasks, followed by the addition of 1.0 ml of hydrochloric acid, and the volume was made up to the mark with distilled water before spectrophotometric measurements were carried out.. the absorbance of the resulting solutions was measured at (480 nm) , The experimental results, presented in Figure (7),

revealed that Sulfasalazine reacts with terephthalaldehyde in a (1:1), confirming the stoichiometric composition of the formed Schiff base.

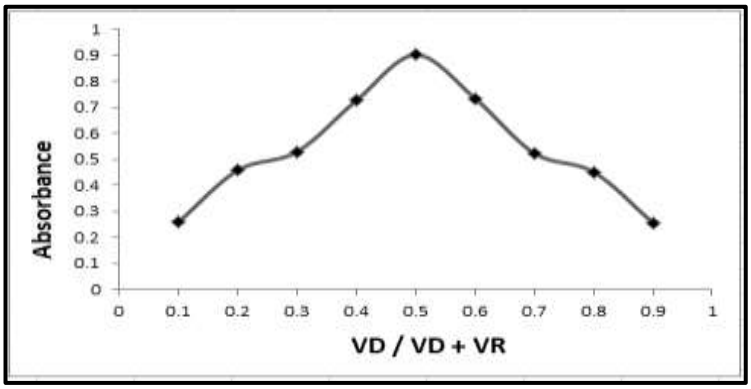

Figure 7. Continuous variations in output method (Job's method).

In the molar ratio method, a fixed volume (2.0 ml) of sulphasalazine solution (1.0×10^{-3} molar) was used, with graduated volumes (0.5–4.5 ml) of terephthalaldehyde solution added to 10-ml volumetric flasks. Then, 1.0 mL of hydrochloric acid was added, and the volume was made up to the mark with distilled water before measuring the absorbance at 480 nm using the blank reagent solution as a reference. The obtained data agreed well with those obtained by the continuous variation (Job's) method, demonstrating that sulfasalazine and terephthalaldehyde react in a (1:1) stoichiometric ratio. The results are illustrated in Figure 8 :

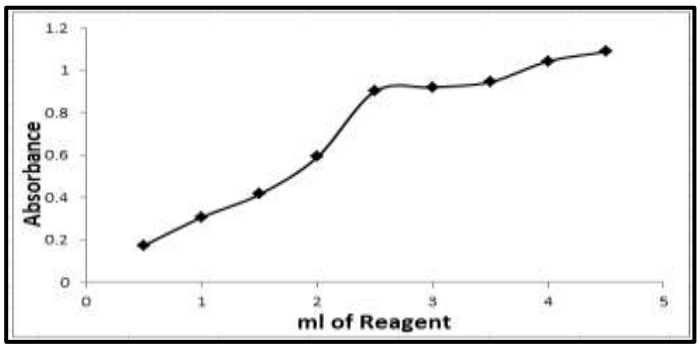

Figure 8. Molar ratio method for the product.

The stoichiometric studies confirmed that the reaction between Sulfasalazine and terephthalaldehyde proceeds in a (1:1) ratio The proposed reaction is shown schematically in Figure 9 :

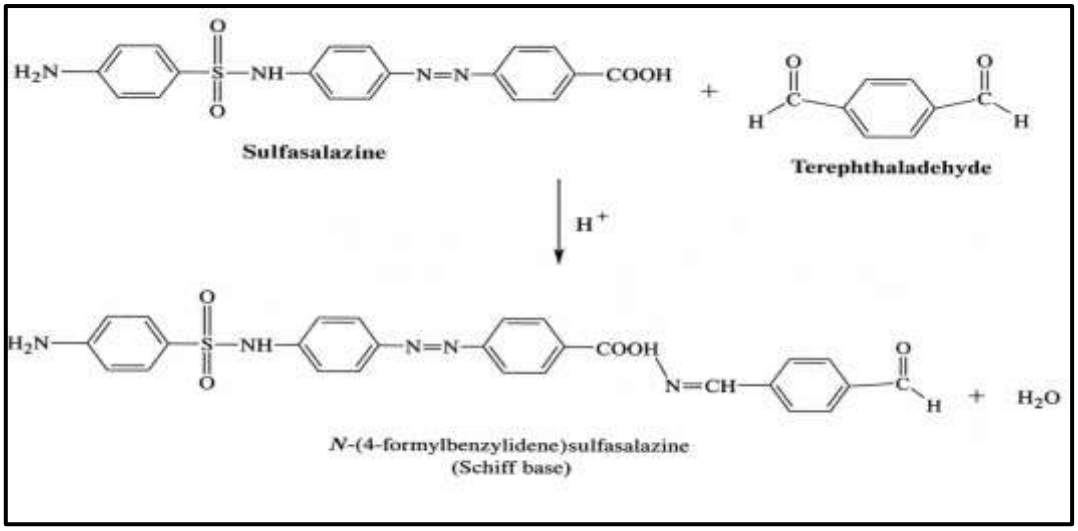

Figure 9. Final reaction equation.

11. Applications

The applicability of the developed method was evaluated by analyzing Sulfasalazine in commercial tablet formulations. Ten tablets (total weight 12.6239 g) were finely powdered, and (0.07574 g) of the powder was dissolved in (5.0 mL) DMF. The solution was diluted to (100 mL) with distilled water, filtered, and the clear filtrate was used to prepare three different concentrations for analysis. The obtained results are presented in Table 6 :

Table 6. Application of the Proposed Method to Sulfasalazine Tablets.

Concentration. of Sulfasalazine μg/mL	Concentration. of Sulfasalazine measured μg/mL*	Er.%
9	8.14	**- 0.095**
30	30.90	**0.03**
75	77.02	**0.026**

* N=3

In the Table (6) shows the application of the Developed Method to the Determination of Sulfasalazine in Pharmaceutical Formulation.

4. Conclusion

A simple, accurate, and sensitive spectrophotometric method was successfully developed for the quantitative determination of Sulfasalazine (SSZ) based on a Schiff base condensation reaction with terephthalaldehyde in an acidic medium. The resulting yellow-colored product is water-soluble and stable, displaying a maximum absorption wavelength (λ_{max}) at **480 nm**.

The proposed method obeys Beer–Lambert's law across a linear concentration range of **3–120 μg mL^{-1}**, with a molar absorptivity of **5,982.73 L mol^{-1} cm^{-1}** and a Sandell sensitivity of **0.0665 μg cm^{-2}**. High sensitivity and precision were confirmed by a Limit of Detection (LOD) of **0.0592 μg mL^{-1}** and a Limit of Quantification (LOQ) of **0.1974 μg mL^{-1}**.

Successful application to commercial Sulfasalazine tablets demonstrated excellent accuracy, indicating that this method is highly suitable for routine quality control and quantitative analysis of Sulfasalazine in pharmaceutical formulations.

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