Spectrophotometric method for Determination of sulfamerazine Using 2,4-dinitrophenylhydrazineReagent

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Abstract. This research describes the spectrophotometric method for determination of sulfamerazine (SMZ) in aqueous solution. This study involves the use 2,4-dinitrophenylhydrazine (2,4-DNPH) as a coupling reagent to determine sulfamerazine using potassium periodate (KIO₄) as an oxidative agent in alkaline medium. The coupling produces an orange product with maximum absorption at 489 nm, the concentration range of sulfamerazine solution (2.5-55) μg.ml⁻¹ within limits of beer law with molar absorptivity of 9251 L.mole⁻¹.cm⁻¹ and Sandel index 0.0286 μg.cm². The accuracy and precision of this research was examined by calculating the percentage of recovery and the percentage of the relative standard deviation and it was shown that they ranged between 98.55 - 99.77. sulfamerazine has been successfully determined in pharmaceuticals using the proposed method by a standard addition method and two levels of concentration.

Keywords: Spectrophotometric method, 2,4 – DNPH , sulfamerazine.

Introduction

sulfamerazine (SMZ) is one of the sulfa drugs, which is also called Sulfamethyldiazine and has several names according to the system of IUPAC including (1,2) :

4-Amino-N-(4-methyl–2–pyrimidinyl)benzenesulfonamide

N1-(4-Methylpyrimidin-2-yl) sulfanilamide, and its structural formula

![scheme1: structural formula for sulfamazin](image)
Sulfamerazine is a white or faintly yellowish–white crystalline powder and decompose on exposure to light, molar mass is 264.3 g/mol. Soluble in water, ethanol, acetone and dilute mineral acids and in solutions of alkali hydroxides and carbonates. It is very slightly soluble in ether and chloroform.

Sulfamerazine is an antibiotic drug used for bacterial infection and other cases where it is used in veterinary medicine to treat many infectious diseases of the animal.

Several methods were proposed for estimating sulfamerazine in pharmaceuticals, including: spectrophotometric methods, HPLC, electrochemical impedance spectroscopy, voltammetric and first-derivative photochemically. This research describes the spectrophotometric method for determination of sulfamerazine (SMZ) in aqueous solution using 2,4-dinitrophenylhydrazine (2,4-DNPH) as a coupling reagent and potassium periodate (KIO₄) as an oxidative agent in alkaline medium and successfully applied in pharmaceutical preparations.

**Experimental**

**Devices used**

1 - UV-VIS spectrophotometer double-beam model UVD-3000 / UVD-3200 with quartz cells along a 1 cm path were used for spectral measurements

2 - Balance of type DHAUS

**Chemicals and reagents used**

Chemicals and analytical reagents were selected with a high degree of purity for use in this study and are described in the Table 1.

| No | Substance                  | Chemical formula | Company |
|----|----------------------------|------------------|---------|
| 1  | sulfamerazine              | C₁₁H₁₂N₄O₂S      | Fluka   |
| 2  | Potassium Periodate        | KIO₄             | BDH     |
| 3  | 2,4-Dinitrophenyl hydrazin | C₆H₄N₂O₄        | Fluka   |
| 4  | Sodium hydroxide           | NaOH             | Fluka   |

**Preparation of materials solutions**

1-Standard solution of sulfamerazine, 250 μg/ml (9.459 × 10⁻⁴ M)

An accurately 0.1g of sulfamerazine powder was dissolved in 1 ml of concentrated hydrochloric acid (HCl) then complete to 100 ml with distilled water in a volumetric flask. 25 ml of sulfamerazine solution are diluted to 100 ml with distilled water, to obtain a solution with a concentration of 250 μg/ml. This solution is kept in a dark bottle and it stays stable for at least 10 days.

2-potassium periodate solution (1× 10⁻³ M)

The solution was attended by dissolving 0.1980 g of potassium periodate powder in a quantity of distilled water using ultrasonic and complete the volume to 100 ml in a volumetric flask with distilled water.

3-2,4-dinitrophenylhydrazin solution (3× 10⁻³ M)
The reagent solution was attended by dissolving 0.1981 g of 2,4-dinitro phenyl hydrazine powder in 5 ml of sulfuric acid (H$_2$SO$_4$), the volume was supplemented to 100 ml in a volumetric flask with distilled water, then 30 ml of this solution was diluted to 100 ml with distilled water to give a solution with a concentration of (3 x 10$^{-3}$ M).

4- Sodium hydroxide solution (1.0 M)

The solution of sodium hydroxide was attended through dissolving 4.000 g of sodium hydroxide in 100 ml of distilled water.

5- Solution of SMZ injection formulation (1000 μg/ml)

The solution of veterinary injection liquid (bio prime) (Bioagripharm GmbH-germany), every 1.0 ml contains 100 mg of sulfamerazine, the solution was attended as follows:

The solution was attended by taking an equivalent of 0.100 g from sulfamerazine and the volume was supplemented to 100 ml with distilled water to give a solution with a concentration of 1000 μg/ml. A working solution of 250 μg/ml is attended by dilution of 25 ml of the above solution by distilled water in a volumetric flask of 100 ml.

Results and Discussion

After reaching to optimal conditions, typical absorption spectra were measured. The absorption of the SMZ-2,4-DNPH product was measured versus the blank reagent which was found to achieve the highest absorption at 489 nm while the blank reagent showed zero absorption in this region. Fig. (1) shows the final absorption of the orange product versus the Blank reagent (A), Distilled water (B) and blank reagent versus distilled water (C).

![Absorption spectrum of the colored product](image)

Fig. (1) Absorption spectrum of the colored product.

Study of the optimization of reaction Conditions

Subsequent studies were performed using 2 ml of sulfamerazine (250 μg / mL), 1.0 ml of 2,4-dinitrophenyl hydrazine (2,4-DNPH) (0.003 M) and 0.5 ml of potassium periodate (0.01 M) in a
basic medium. The absorption spectrum of the colored product versus the blank shows maximum absorption at 489 nm.

**Effect of pH**

A preliminary study was conducted to demonstrate the effect of the basic medium by following the absorption of the colored product at 489 nm using sodium hydroxide solution and found that 2.5 ml sodium hydroxide solution to give better absorption. It was therefore adopted in subsequent studies, Table (2).

| ml of 1.0M NaOH | Absorbance | pH   |
|-----------------|------------|------|
| 0.3             | 0.125      | 9.3  |
| 0.5             | 0.246      | 10.5 |
| 0.7             | 0.384      | 10.9 |
| 1.0             | 0.567      | 11.2 |
| 1.5             | 0.673      | 11.5 |
| 2.0             | 0.745      | 11.7 |
| 2.5             | 0.886      | 11.9 |
| 3               | 0.835      | 12.1 |
| 3.5             | 0.696      | 12.3 |

**Effect of the amount of potassium periodate**

In practice, it was found that the best oxidative agent gave the highest absorption is the potassium periodate which has been used in previous experiments. Subsequently, the effect of the amount of oxidative agent (1× 10⁻² M) was studied. The results shown in Fig. (2) demonstrate that the best volume of the oxidative agent that gives ideal conditions for the reaction is 1.2 ml which has been used in subsequent studies.

![Fig.(2) Effect of amount of KIO₃ on the product absorption.](image)

**Effect of the amount of 2,4-ditrophenyl hydrazine (2,4-DNPH) reagent**

Several experiments were performed to select the best volume of the reagent solution with a concentration (1 × 10⁻² M) using 2ml of sulfamerazine and 1.2 ml (0.01M) of potassium periodate at
room temperature (25 °C) in the basic medium where it was found to 1.8 ml was the best volume of the reagent solution because it gave highest absorption, Fig. (3).

![Graph](image)

Fig.(3) Effect of reagent amount on the color product absorption.

**Effect of oxidation time**

The results shown in Table (3) show that the optimal time for oxidation reaction is 5 min and that the color remains stable for 60 min and therefore, it was used in subsequent studies.

| Time(min) | 5  | 10 | 15 | 20 | 25 | 30 |
|-----------|----|----|----|----|----|----|
| Absorbance| 0.789 | 0.925 | 0.926 | 0.926 | 0.926 | 0.925 |

**Effect of temperature**

The effect of temperature on the absorption of the formed product using optimal conditions obtained from previous experiments was tested. The results of this study showed that the maximum absorption was at 25°C while the sensitivity of the reaction decreased when the temperature increased, thus it was used in subsequent studies, Table(4).

| Temp (°C) | 5  | 10 | 15 | 20 | 25 | 30 |
|-----------|----|----|----|----|----|----|
| Absorbance| 0.178 | 0.352 | 0.436 | 0.612 | 0.925 | 0.841 |

**Calibration curve**

The calibration curve for sulfamerazine form through complexation with 2,4-dinitrophenylhydrazine (2,4-DNPH) showed good linearity at concentration ranges of (2.5-5.5 μg/ml) within limits of beer law with molar absorptivity of 9251 L.mole⁻¹.cm⁻¹ and Sandel index 0.0286 μg.cm⁻², Fig. (4).
Accuracy and precision

To assay the accuracy and precision of this research, the recovery and relative standard deviation (RSD) for the determination of sulfamethazine was examined at two concentrations. Table (5).

Table (5) Accuracy and precision.

| Conc. of SMZ (ppm) | RSD%  | Recovery%  | Average recovery% |
|-------------------|-------|------------|-------------------|
| 5                 | 1.092 | 99.77      | 99.16             |
| 10                | 0.907 | 98.55      |                   |

* Average of five determinations

Stoichiometry of the reaction

Stoichiometry of the reaction between sulfamethazine (SMZ) and 2,4-dinitrophenylhydrazine (2,4-DNPH) was studied through application of mole ratio and continuous variation methods. The concentration of each of the sulfamethazine solution and 2,4-dinitrophenylhydrazine solution in both methods is $9.459 \times 10^{-4} \text{ M}$. Fig. 5 showed that the ratio of SMZ: 2,4-DNPH is 1:1.
The suggested equation of the oxidative coupling reaction for SMZ with 2,4-DNPH reagent in the presence $\text{KIO}_4$ in the basic medium, which gave maximum absorption at 489 nm could be written as the following:

$$\text{SMZ} + \text{DNPH} + \text{KIO}_4 + \text{NaOH} \rightarrow \text{product}$$

Application of the method for the determination of SMZ in Pharmaceuticals

The standard addition method was applied to pharmaceuticals containing sulfamerazine using two levels of concentration to ensure that the proposed method is free from interference, Fig.(6). For the purpose of evaluating the method, the accuracy and precision of the obtained results were calculated, showing that the standard addition method is satisfactory and free of interference, Table (6).
Fig. (6) Standard additions method of the estimation of SMZ in injection

Table (6) Results of standard additions method of the estimation of SMZ in injection.

| Pharmaceutical                  | SMZ Present µg/ml | SMZ Measured µg/ml | RSD% | Recovery, % |
|---------------------------------|-------------------|--------------------|------|-------------|
| bio prime liquid injections      | 2.500             | 2.528              | 1.358| 101.12      |
|                                 | 5.000             | 5.135              | 1.139| 102.70      |

Conclusions

Results of the analysis demonstrated that the suggested method is sensitive to estimate SMZ. This method does not require temperature control, or using various organic solvents, or extraction, and can be applied successfully to estimate SMZ in veterinary pharmaceuticals.

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