Spectrophotometric Determination of Sulphamerazine Via Diazotization and Coupling with m-Hydroxyaniline

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Abstract

A simple and accurate spectrophotometric method has been developed based on diazotization and coupling reactions. The method relied on the diazotization of sulphamerazine (SMEZ) via reaction with HNO\(_2\) obtained from the reaction of hydrochloric acid with sodium nitrite. The un-reacted nitrous acid destroyed via a reaction with sulphamic acid. The formed diazotized sulphamerazine (D-SMEZ) was coupled with m-hydroxyaniline to produce yellow azo dye, which gives the highest absorption at 450 nm. The formed azo dye is freely soluble in water and has good stability. The linearity is observed by drawing absorbance of various amounts of sulphamerazine from 0.5 to 25 \(\mu\)g.mL\(^{-1}\), which exhibits molar absorptivity of 1.4592x10\(^5\) l.mol.\(^{-1}\)cm\(^{-1}\) and Sandell's sensitivity factor 0.001811 \(\mu\)g.cm\(^{-2}\). The low detection limit, low quantitation limit, relative error, and percent relative standard deviation have been estimated, and their values are 0.0022 and 0.0076 \(\mu\)g.mL\(^{-1}\) as well as 0.02 to 0.2\%, respectively. The present method has successfully been applied to determine sulphamerazine in trisulphoprim injection (veterinary drug).

Keywords: Sulphamic acid, Diazotization, m-Hydroxyaniline, Sulphamerazine.

Introduction

Sulpha drugs are antibacterial agents mostly prepared synthetically from the main reagent sulphanilamide or \(p\)-aminobenzene sulphonamide, which are also known as sulphanilamide. Sulphonamide have got wide medical importance and is often identified as a sulpha drug. They have a wide range of effects and are generally used to treat bacterial infections, including Gram-positive and Gram-negative bacteria [1]. Although they have been replaced by a range of other antibiotics such as Penicillin, Terramycin, Chloromycetin, and Aureomycin, however; sulpha drugs still have wide medical applications and have a great space in the pharmaceutical industry [2,3].

The importance of sulpha compounds as medicines is for both humans and animals. In the veterinary field, sulpha drugs have been widely used for dressing wounds and preventing infection after surgical operations as well as in pneumonia treatment and many other diseases. Sulpha drugs are given to animals in an optimal amount (full dose), and consequently, it leads to an increased rate of resistance by microorganisms [4].

Sulphamerazine (SMEZ) is one of the famous sulpha drugs. It is used as an antibiotic for bacterial infections that exhibits a bacteriostatic effect and is used in veterinary medicine for some communicable illnesses.
and treatment of the various animals [5]. SMEZ is a white in colour or slightly yellowish to white-crystalline powder. The storage of SMEZ needs to be protected from light. It is slightly soluble in ethanol, acetone, and soluble in water. The solubility increased in an acidic medium, but there are some difficulties in dissolving SMEZ in ether and chloroform because of its slight solubility. The molecular formula of SMEZ is C_{11}H_{12}N_{4}O_{2}S, and its chemical structure is given in scheme 1.

![Scheme 1. Chemical structure of Sulphamerazine.](image)

Several techniques or methods have been listed in literature for assaying Sulphamerazine as pure form and/or in dosages, these include HPLC [6-8] SPE-HPLC [9], LC-PDA [10], capillary electrophoresis [11], capillary zone electrophoresis [12], dual-cloud point extraction [13], electrochemical sensor [14], voltammetric determination [15], spectrophotometric methods [16-20] and SPE - UV-Visible spectrophotometry [21].

The main purpose of the present work is to suggest an accurate and simple spectrophotometric method for assaying SMEZ in its formulation via diazotization and coupling with the proper reagent.

**Materials and Methods**

**The materials**

All analytical reagents used in the current work were of high purity.

Sodium nitrite, m-hydroxyaniline, sulphamic acid, and hydrochloric acid were submitted from Fluka Company, while SMEZ submitted as a gift from Samara Drug Industries Company.

**Apparatus**

The main apparatus used in this method are UV-VIS Hach DR6000 Spectrophotometer with quartz cells and a sensitive digital balance type of HR – 200 AND to achieve the accurate weight of reagents.

**Solutions**

All solutions were prepared by dissolving appropriate amounts in distilled water as a solvent.

**The solution of Sulphamerazine (100 μg mL⁻¹)**

100 mg of SMEZ powder was taken and dissolved with 100 mL of distilled water in a 100 mL volumetric flask.

**Sodium nitrite solution, 0.3 w/v %**

300 mg of sodium nitrite was dissolved in 100 mL distilled water in a volumetric flask.

**m-Hydroxyaniline, 0.1 w/v %**

100 mg of m-hydroxyaniline was dissolved in 100 mL distilled water in a volumetric flask.

**Sulphamic acid, 0.5 w/v %**

500 mg of sulphamic acid was dissolved in 100 mL distilled water in a volumetric flask.

**The hydrochloric acid solution, 1M HCl**

This solution was prepared by diluting 8.6 mL of concentrated HCl (Aldrich) to 100 mL with distilled water in a volumetric flask.
The solution of the veterinary formulation (100 μg. mL⁻¹)

1 mL of the veterinary medicinal product trisulphoprim (injection, 100 mg SMEZ 100 mL⁻¹) was taken and diluted with 100 mL of distilled water. Then 10 mL of the diluted solution was taken and further diluted to 100 mL with distilled water.

The method

A standard process method and calibration curve were prepared after establishing practically optimal conditions for the determination of SMEZ as follows: Increasing volumes of SMEZ solution containing 0.5-25 μg.mL⁻¹ were added to a series of volumetric flasks (10 mL), then 0.5 mL of HCl solution (1M) and 0.3 mL of 0.3% sodium nitrite solution added, and the solutions were left for 4 min with shaking, after that 1.5 mL of sulphamic acid solution (0.5%) was added, and the solutions left for 5 min to destroy the excess of sodium nitrite. After that, 2 mL of m-hydroxyaniline reagent solution (0.1%) was added, and completed the volumes with distilled water to the mark. The absorbance of the solutions against the blank solution was measured at 450 nm.

Results and Discussion

The effect of different parameters on the absorbance of the resulting yellow dye was studied using 1 mL of 100 μg SMEZ solution in a final volume of 10 mL.

The general principle of the method

SMEZ was converted to the Sulphamerazine - diazonium salt (D-SMEZ) by reacting with acidic sodium nitrite (prepared HNO₂ instantaneously). The un-reacted amount of sodium nitrite was removed using sulphamic acid [22]. The D-SMEZ was coupled with m-hydroxyaniline to form a yellow-coloured azo dye.

Selection of coupling agent

A number of organic compounds have been examined in coupling reaction with D-SMEZ, and the results are shown in Table 1.

Table 1. The selection of appropriate coupling reagent.

<table>
<thead>
<tr>
<th>Coupling agent soln. (0.1%)</th>
<th>Structure</th>
<th>Absorbance</th>
<th>λ_max (nm)</th>
<th>Colour of azo dye</th>
<th>ε (l/mol.cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroxine</td>
<td><img src="image" alt="Thyroxine structure" /></td>
<td>0.321</td>
<td>450</td>
<td>Yellow</td>
<td>4.29x10⁴</td>
</tr>
<tr>
<td>Catechol</td>
<td><img src="image" alt="Catechol structure" /></td>
<td>0.532</td>
<td>470</td>
<td>Orange</td>
<td>5.86x10⁴</td>
</tr>
<tr>
<td>m-Hydroxy-aniline</td>
<td><img src="image" alt="m-Hydroxy-aniline structure" /></td>
<td>0.823</td>
<td>450</td>
<td>Yellow</td>
<td>9.07x10⁴</td>
</tr>
</tbody>
</table>
It is evident from the results in Table 1 that the use of m-hydroxyaniline in coupling gave the highest absorbance and thus was chosen in the subsequent experiments.

**Acid types used in the diazotization process**

One of the requirements for the diazotization process is the presence of acid in creating the corresponding diazonium salt. 1 mL of each type of acid, i.e., nitric acid, hydrochloric acid, acetic acid, and sulphuric acid, was added separately at a concentration of 1 M to 1 mL of 100 μg SMEZ /10 mL solution.

It has been found from the results that the best medium of reaction is achieved by using HCl with a high absorbance of the resulting yellow azo dye. Thus, HCl has been selected for use in the subsequent experiments.

**Optimization of HCl amount in the diazotization process**

The effect of adding different quantities of HCl (1 M) to different concentrations of SMEZ solution (3-20 μg / mL) has been studied. The results indicated that the diazotisation reaction needs 0.5 mL as an optimum volume of HCl by giving the highest absorbance of a yellow azo dye. Therefore, 0.5 mL HCl (1 M) has been recommended for the next experiments.

**The effect of sodium nitrite amount and time**

The amount of nitrous acid remaining after the diazotization process is not desirable as it acts on nitrification of the coupling agent, which reduces its effectiveness in the main reaction. Therefore, sulphamic acid has been used to remove the remaining nitrous acid by reducing it to inert nitrogen gas [23], as shown in the following equation:

$$\text{HNO}_2 + \text{H}_2\text{NSO}_3\text{H} \rightarrow \text{N}_2 \uparrow + \text{H}_2\text{SO}_4 + \text{H}_2\text{O}$$

The effect of the amount of sulphamic acid and the time required to complete the removal of HNO$_2$ has also been studied. Table 3 shows that adding 1.5 mL of a 0.5% sulphamic acid solution at a standing time (before adding m-hydroxyaniline) of 5 min is the optimum amount and time to destroy the excess of HNO$_2$.

**Effect of sulphamic acid amount and standing time**

Table 2. The amount of sodium nitrite and standing time.

<table>
<thead>
<tr>
<th>Amount of NaNO$_2$ (0.3%) soln.</th>
<th>Absorbance / Standing time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.282 0.322 0.482 0.521 0.587 0.484</td>
</tr>
<tr>
<td>0.2</td>
<td>0.367 0.465 0.552 0.641 0.677 0.513</td>
</tr>
<tr>
<td>0.3</td>
<td>0.432 0.598 0.686 0.721 0.830 0.662</td>
</tr>
<tr>
<td>0.4</td>
<td>0.381 0.456 0.532 0.672 0.734 0.654</td>
</tr>
<tr>
<td>0.5</td>
<td>0.292 0.333 0.481 0.587 0.678 0.568</td>
</tr>
</tbody>
</table>

Table 3. The effect of sulphamic acid and time on removing un-reacted HNO$_2$.

<table>
<thead>
<tr>
<th>Sulphamic acid solution (mL of 0.5%)</th>
<th>Absorbance / Standing time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>0.101 0.201 0.382 0.477 0.582 0.475</td>
</tr>
<tr>
<td>0.5</td>
<td>0.253 0.333 0.461 0.582 0.675 0.521</td>
</tr>
<tr>
<td>1</td>
<td>0.322 0.421 0.521 0.662 0.732 0.656</td>
</tr>
<tr>
<td>1.5</td>
<td>0.486 0.552 0.621 0.701 0.828 0.721</td>
</tr>
<tr>
<td>2</td>
<td>0.417 0.432 0.508 0.654 0.714 0.633</td>
</tr>
</tbody>
</table>
Effect of coupling agent (m-hydroxyaniline) amount.

The effect of the coupling agent (m-hydroxyaniline) on the azo dye's absorbance has been studied. The addition of 2 mL of 0.1% m-hydroxyaniline solution has the best determination coefficient ($R^2$) value. The yellow azo dye has a high absorbance compared with other volumes of 1 and 3 mL.

The effect of pH

The perfect pH of coupling D-SMEZ with m-hydroxyaniline has been noticed that is equal to 2.62, and it has been selected as the medium of the reaction of the components. A turbid solution after 15 min has been observed in adding a base (sodium hydroxide or sodium carbonate), therefore, it is not recommended to add a base.

The effect of adding surfactant

The effect of adding 3 mL of different types of surfactant to the reaction environment in different sequences on the absorbance of the azo dye formed was studied, and from the results of this study, a decrease in absorbance was noticed (the surfactant increased sensitivity or give red-shift); thus surfactant has not been recommended in subsequent experiments.

Stability of the azo dye

The stability of the formed azo dye was studied by measuring the absorbance against the blank solution for different time periods and two different amounts of SMEZ. The results indicate that the yellow azo dye is formed after adding the reactants directly and remains stable for an hour.

Absorption spectrum

D-SMEZ was formed when SMEZ reacts with sodium nitrite in an acidic medium, followed by the reaction with the m-hydroxyaniline reagent to form a yellow azo dye that gives the highest absorption at the wavelength of 450 nm against the blank solution, which shows little absorption at the same wavelength (Fig. 1).

![Absorption spectrum](image)

**Figure 1.** The absorption spectrum (A) formed azo dye against the blank solution, (B) the yellow azo dye against the distilled water, and (C) the blank solution against the distilled water

Standard curve

The relationship between absorbance and concentration of SMEZ is in accordance with the law of linearity (Beer's law) in the concentration range of 0.5 – 25 μg.mL⁻¹, and there was a negative deviation at concentrations higher than 25 μg.mL⁻¹ (Fig. 2). The molar absorption value of the resulting dye was $1.4592 \times 10^5$ L/mol.cm, while Sandell’s index value was 0.001811 μg.cm⁻².

The values of the limit of detection (LOD) and limit of quantitation were calculated to be 0.0022 μg.mL⁻¹ and 0.0076 μg.mL⁻¹, respectively [24].
Accuracy and precision of the method

Table 4 contains the calculation results, relative error (RE%), and relative standard deviation (RSD%) by taking three different concentrations of 50, 100, and 150 µg SMEZ / 10 mL. The method has good accuracy and precision (RE% from -0.2 to +0.2 and RSD not more than 0.2%). Hence the accomplishment of the proposed method is successful in estimating the SMEZ.

Table 4. The accuracy and precision of the standard curve.

<table>
<thead>
<tr>
<th>SMEZ µg/10mL</th>
<th>Recovery %*</th>
<th>RE %</th>
<th>RSD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>100.2</td>
<td>+ 0.2</td>
<td>0.02</td>
</tr>
<tr>
<td>100</td>
<td>99.8</td>
<td>- 0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>150</td>
<td>99.8</td>
<td>- 0.2</td>
<td>0.07</td>
</tr>
</tbody>
</table>

* Average of five determinations.

The nature of the yellow azo dye

The continuous variation (Job’s method) [25] was used in estimating the composition of the yellow azo dye resulting from the coupling of the D- SMEZ with m-hydroxyaniline (mHA). The procedure includes a number of flasks containing different volumes 2.5-0.5 mL of SMEZ (3.783 x 10^{-4} M) and 0.5-2.5 mL of m-hydroxyaniline reagent (3.783 x 10^{-4} M) solutions, with the addition of the other solutions under optimal conditions. The absorbance was measured at 450 nm, and Fig. 3 indicates that the composition is 1:1.

Scheme 2. The structure of the formed yellow azo dye

Determination of Sulphamerazine contained in the veterinary formulation

The suggested structure of azo dye, as shown in Scheme 2, results from the binding of the D- SMEZ at the para site of the hydroxyl group of m-hydroxyaniline [26].

Table 5. The results of SMEZ in veterinary formulation (injection).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>µg taken</th>
<th>%*</th>
<th>RE %</th>
<th>RSD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veterinary</td>
<td>50</td>
<td>99.8</td>
<td>-0.2</td>
<td>-0.3</td>
</tr>
<tr>
<td>Trisulphoprim (injection)</td>
<td>100</td>
<td>100.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

*Average of five determinations.
Evaluate the results of the proposed method

The standard addition method was applied to estimate SMEZ in its veterinary formulation (trisulphoprim injection), and the results are shown in Fig. 4.

![Graph showing the standard addition curve for estimating sulphamerazine in veterinary injection](image)

Table 6 represents the results of recovery percent of SMEZ by using the standard addition method, which indicates that the method has good accuracy (from -1.95 to +2.40%), accepted recoveries, and is free from interference of additives.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Recovery %</th>
<th>SMEZ µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulphamerazine</td>
<td>5</td>
<td>98.05</td>
</tr>
<tr>
<td>Veterinary formulation</td>
<td>10</td>
<td>102.40</td>
</tr>
</tbody>
</table>

Comparison with literature spectrophotometric method

Table 7 represents the results of the comparison between some of the analytical variables of the suggested method with the same variables of literature diazotization and coupling method.

From the results above in Table 7, the proposed method is the most sensitive and contains the application of veterinary preparation and a wider range of determination compared with the reported in the literature.

Conclusion

In the current research work, a spectrophotometric method is suggested to determine SMEZ, which is usually used to treat and prevent urinary tract infections, treat ear infections caused by certain bacteria, and treat animals by mixing it with other antibiotics. The proposed method is green and environment friendly, as a convenient and well known diazotisation reaction has been carried out to determine SMEZ to give diazonium salt then, followed by its coupling with m-hydroxyaniline. The suggested method is accurate, fast, easy, and precise, and it can assay SMEZ in its formulation (veterinary injection) with satisfactory results. It has been noticed that the formed product has high stability and high sensitivity. In addition, the method could be widely used in many analytical, environmental, and pharmaceutical
laboratories for several reasons, including the licenses of the devices, the ease of maintenance, the cheap price, and ease of use.

Conflict of Interest

The authors declare that they have no conflict of interest for the reported research work.

References