NEW SPECTROPHOTOMETRIC METHOD FOR THE ASSAY OF SULFADIAZINE DRUG BASED ON DIAZOTIZATION COUPLING REACTION

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Abstract: A sensitive, simple and rapid spectrophotometric procedure for the assay of trace quantities of sulfadiazine (SDZ) drug as bulk and in diluted solution is characterized. The procedure depends on the diazotization reaction which is produced by coupling SDZ with (4-amino-2-hydroxy acetophenon) (AHA) to produce an intense colored complex spectrophotometrically determined at 410 nm. Beer’s law was applied in the range of concentration 0.5 – 15 ppm; the molar absorptivity and Sandell’s sensitivity were $2.8484 \times 10^4$ L mol$^{-1}$ cm$^{-1}$ and 0.008 μg cm$^{-2}$ respectively. The method limit of detection (LOD) was 0.443 μg mL$^{-1}$ and LOQ (the method limit of quantitation) was 0.249 μg mL$^{-1}$. The procedure is not based on solvent extraction and the additives and excipients do not significantly influence the developed procedure.

Keywords: new spectrophotometric procedure; diazotization reaction; sulfadiazine drug.

Introduction

Sulfa derivative drugs are widely used in medicine because of their inhibitory effect on the growth in many bacteria.$^1$ That was considered to be the major cause of death before the discovery of sulfa drugs and other antibiotics.$^2$ It acts by inhibiting the production of folic acid inside the
bacterial cell. Sulfadiazine (SDZ) is used for the treatment of urinary tract infections.

All sulfa derivatives with inhibitory effects on bacterial growth contain one benzene ring with amino group and sulfate group. Among the most important of these derivatives is sulfadiazine. The drug has the molecular formula C\textsubscript{10}H\textsubscript{10}N\textsubscript{4}O\textsubscript{2}S and the chemical structure presented in Figure 1. The molar mass of drug is 250.3 g mol\textsuperscript{-1}.4

![Figure 1. The chemical structure of sulfadiazine (SDZ).](image)

The IUPAC recommended nomenclature for SDZ is 4-amino-N-pyrimidin-2-yl benzene sulfonamide. In its pure form is a white, pinkish-white or yellowish-white crystal or crystalline powder, water insoluble, very slightly soluble in ethyl alcohol, slightly soluble in acetone. It is soluble in dilute mineral acids and in alkali hydroxides. The melting point is 255 °C and it was reported to be unstable when exposed to air or light. SDZ is included as active component in several pharmaceutical products including floumizin cream and tablets.5-7 Currently, there are multiple analytical methods developed and applied for identification and quantification of SDZ in pharmaceutical preparations or in biological fluids. The main procedures for SDZ assay are based on direct or indirect spectrophotometric analysis based on Schiff base, redox, charge-transfer and diazo-coupling reactions followed by subsequent measurement of absorption of the colored complex compounds obtained after applying the above mentioned reactions.8-21 Other analytical procedures for SDZ assay are based on amperometric titration,22 potentiometric direct analysis using ion-selective electrodes,23
potentiometric titration, differential pulse polarography, differential scanning calorimetry, thin layer chromatography, reversed-phase high performance liquid chromatography, capillary zone electrophoresis, sequential injection chemiluminescence, Fourier transform Raman spectroscopy, spectrofluorimetric and nuclear magnetic resonance.

The aim of this study was to develop a new sensitive, simple and rapid spectrophotometric method for the SDZ assay based on the azo-coupling reaction between SDZ and 4-amino-2-hydroxy acetophenon (AHA) to form an intensely colored complex reaction product. The reaction conditions for obtaining the colored product together with analytical performance parameters (method precision and accuracy) were further tested for the SDZ assay in its pure form and in several pharmaceutical preparations with different doses of the SDZ active component.

Experimental

Materials

The chemicals used in the procedure were of high degree of purity and did not need purification. Their solutions were prepared as follows.

-Sulfadiazine: SDZ pure substance was purchased from the state company for drug industries and medical appliances (SDI) Samara – Iraq. 100 ppm stoc standard solution of SDZ was prepared by dissolution of 0.01 g SDZ in 5 mL of ethanol and the volume was completed to 100 mL with deionized water in a volumetric flask. The solution was transferred to a dark flask and it was stable for more than one month. The working solutions were prepared by subsequent dilution of this stoc solution.
New spectrophotometric procedure for the assay of sulfadiazine in pharmaceutical preparations

-Sodium nitrite NaNO₂ laboratory reagent was supplied by BDH Chemicals Ltd and a 0.05 M solution was prepared by dissolution of 0.345 g of pure substance in 100 mL deionized water.

-Hydrochloric acid (1 M) solution was prepared from 98% HCl solution purchased from GCC.

-Sodium hydroxide supplied by Merck was used to prepare 1 M solution by dissolving 4 g of substance in 100 mL deionized water.

-4-amino-2-hydroxy acetophenone 0.01 M was prepared from the pure laboratory reagent supplied from BDH Chemicals Ltd, by dissolution of 0.075 g in 50 mL absolute ethanol (99.9%, BDH Chemicals Ltd).

To prepare the working solutions a sensitive balance, ice-water bath and a Jenway 3020 pH-meter were used. All spectrophotometric measurements were performed on a double – beam UV-Visible 160 digital recording spectrometer (Japan).

Preliminary investigations

In a series of 25 mL volumetric flasks aliquots of standard solutions of SDZ were added, to obtain final concentrations in the range 0.5 – 15 ppm, followed by addition of 1 mL 1 M hydrochloric acid and 1 mL of 0.01 M sodium nitrite. After ten minutes, to complete the azo-coupling reaction, 1 mL of sodium hydroxide 0.5 M and 3 mL of AHA were added and the volumetric flasks filled up to the mark with deionized water. The obtained solutions were kept for ten minutes at the room temperature and then the absorbance at 410 nm was measured, against reagent blank solution.

Procedure for assay of sulfadiazine in pharmaceutical preparations

A number of pharmaceutical preparations that contain SDZ as active ingredient were analyzed as follows.
**Tablets.** Two tablets (1.0 g sulfadiazine per tablet) of drug are weighed and crushed to powder. Portions of 0.021 g of this powder, which is equivalent to 0.01 g of SDZ, were dissolved in distilled water containing 2 drops of 1 M NaOH solution. The resulting solution was then well mixed, filtered to get clear solution and diluted to 100 mL with deionized water in a volumetric flask. Each mL of the solution contains 100 µg SDZ.

Floumizin cream (Ag.SDZ). To 1.0 g cream (containing 0.01 g of Ag.SDZ) 50 mL of ether were added, shaked well and the mixture was transferred to a separating funnel. The Ag.SDZ was then extracted three times with 25 mL of deionized water. The aqueous layer was collected, filtered and diluted to 100 mL with deionized water in a volumetric flask.

**Results and discussion**

*Effect of reagent volume*

0.01 M AHA volumes in the range 0.5 – 6 mL were tested in the presence of 1 mL 1 M HCl, 1 mL of 0.01 M NaNO₂ and 0.5 mL NaOH 0.5M. The volume of 3 mL was found to give the highest absorption and was applied in subsequent experiments.

*Effect of sodium nitrite volume*

The influence of sodium nitrite volume on the absorption intensity was also studied. Volumes of 0.05 M NaNO₃ between 0.5 and 5 mL at concentration were tested with 3 mL of AHA and 1 mL of HCl solution and 0.5 mL NaOH 0.5 M. It was observed that 1 mL is the optimum for maximum absorption.

*Effect of acid*

Various acids such as H₂SO₄, HCl, HNO₃ and CH₃COOH, all of concentration 1 M were tested. HCl was found to give the highest
absorption of the measured product and a volume of 1 mL 1 M solution was added in the following experiments.

*Effect of reaction time*

It was found that the azo-coupling reaction was complete in 10 minutes and the colored product was stable more than 24 hour.

*Effect of temperature*

The resulted product was examined at different temperatures in the range 0-70 °C. The absorbance values decrease at higher temperatures, presumably due to dissociation of the product. The colored product was stable and has the highest absorbance in the range of temperature 5–30 °C. The subsequent experiments were therefore conducted at the room temperature.

*Absorption spectra*

Figure 2 gives the spectra of the colored product, with the maximum absorption at 410 nm, compared to the blank.

![Absorption Spectra](image)

**Figure 2.** Spectra of the yellow product (4 ppm) (A) and blank (B).

*Calibration curve*

The calibration curve in the optimized conditions for determination of SDZ is showed in Figure 3. The curve is linear in the range of
concentration of 0.5 – 15 ppm, with a correlation coefficient of 0.9993, the slope 0.1138 L mg\(^{-1}\), and an intercept of 0.0545. The molar absorptivity of the yellow product was found 2.8484 \(10^4\) L mol\(^{-1}\) cm\(^{-1}\) and the Sandell’s sensitivity was 0.008 \(\mu\)g cm\(^{-2}\). LOD and LOQ were 0.443 \(\mu\)g mL\(^{-1}\) and 0.249 \(\mu\)g mL\(^{-1}\), respectively.

**Figure 3.** Calibration curve.

**Precision and accuracy**

The method precision and accuracy were calculated for three different SDZ concentrations. The obtained results shown in Table 1, indicate good precision and accuracy.

**Table 1.** Method precision and accuracy.

<table>
<thead>
<tr>
<th>Conc. of SDZ mg L(^{-1})</th>
<th>Error (%)</th>
<th>Recovery %</th>
<th>RSD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>-4.30</td>
<td>95.70</td>
<td>0.562</td>
</tr>
<tr>
<td>8.00</td>
<td>+3.20</td>
<td>103.20</td>
<td>0.432</td>
</tr>
<tr>
<td>14.00</td>
<td>+0.24</td>
<td>100.24</td>
<td>0.177</td>
</tr>
</tbody>
</table>

**Stoichiometry of reaction**

The stoichiometry was studied for the reaction of SDZ and AHA by using the mole ratio method and Job’s method. The results showed that 1:1 SDZ to AHA complex was formed at 410 nm\(^{37,38}\). The product is soluble in ethanol. The stability constant of the colored product was calculated by
using the absorbance for solutions containing equal quantity of SDZ and the reagent with optimum quantity (1 mL) of $2.5 \times 10^{-4}$ M and other AHA solution with five times the concentration of the main concentration. The average stability constant of the colored product in ethanol in the optimal experimental conditions was $1.72 \times 10^6$ L mol$^{-1}$.

The colored product was formed between SDZ and AHA probably as shown in Figure 4.$^{39,40}$

![Figure 4. Scheme of the azo - coupling reaction.](image)

**Interferences**

The complex formation in the presence of excipients excipients such as talc, lactose, acacia, starch, sucrose, magnesium stearate, glucose, benzoic acid, aspartate, and polyvinylpirroldone (PVP) was investigated. The excipients were tested at concentrations ten-times higher than SDZ concentration, according to the procedure for the calibration curve (2 mL of 100 mg L$^{-1}$ SDZ and 2mL of each type of excipient and dilution to final volume of 25 mL). The interference was considered acceptable for error lower than ± 2%. The lack of interference from excipients was observed (Table 2).
Table 2. Interference of selected excipients in the determination of SDZ.

<table>
<thead>
<tr>
<th>Interference</th>
<th>Error (%)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Talc</td>
<td>-4.36</td>
<td>95.64</td>
</tr>
<tr>
<td>Lactose</td>
<td>-3.13</td>
<td>96.87</td>
</tr>
<tr>
<td>Starch</td>
<td>+2.85</td>
<td>102.85</td>
</tr>
<tr>
<td>Acacia</td>
<td>-4.44</td>
<td>95.56</td>
</tr>
<tr>
<td>Sucrose</td>
<td>-4.20</td>
<td>95.80</td>
</tr>
<tr>
<td>Glucose</td>
<td>-3.25</td>
<td>96.75</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>+3.50</td>
<td>96.50</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>+2.48</td>
<td>102.48</td>
</tr>
<tr>
<td>Aspartate</td>
<td>-3.35</td>
<td>96.65</td>
</tr>
<tr>
<td>PVP</td>
<td>-4.45</td>
<td>95.55</td>
</tr>
</tbody>
</table>

Method application to pharmaceutical products

The procedure was applied for the assay of pharmaceutical preparations. The results for available formulations of SDZ drugs (average of three determinations) are shown in Table 3.

Table 3. Sulfadiazine determination in pure and dosage forms.

<table>
<thead>
<tr>
<th>Pharmaceutical preparations containing SDZ</th>
<th>Average recovery (%)</th>
<th>Proposed method</th>
<th>Standard method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure (SDZ)</td>
<td>95.63</td>
<td>95.15</td>
<td></td>
</tr>
<tr>
<td>Tablets (100 mg L(^{-1}) SDZ)</td>
<td>96.77</td>
<td>95.35</td>
<td></td>
</tr>
<tr>
<td>Floumizin cream (Ag.SDZ) (100 mg Ag.SDZ L(^{-1}))</td>
<td>97.12</td>
<td>95.41</td>
<td></td>
</tr>
</tbody>
</table>

The standard procedure for drug assay in British Pharmacopoeia (2009) was used.

Conclusions

A sensitive, rapid, precise, and simple spectrophotometric procedure was optimized for the assay of trace quantities of SDZ in aqueous solution.
by using the azo coupling reaction with 4-amino-2-hydroxy acetophenon and hydrochloric acid in the presence of sodium nitrate. The studied procedure needs no solvent extraction or temperature control step; the procedure was successfully applied for the assay of trace quantities commercial SDZ drug.

References


33. B. A. K. Hassoon, determination of sulf drugs by using sequential injection chemiluminescence and application on some types from dosage drug forms “Ph. D. Thesis”, *Baghdad University*, **2005**.


