Spectrophotometric Determination of Sulfathiazole in Different Pharmaceutical Formulations

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ABSTRACT
A simple, rapid and sensitive spectrophotometric method for the determination of sulfathiazole in aqueous solution is described. The method is based on the diazotized coupling reaction of aniline with sulfathiazole drugs. A mixture of an acidic solution (hydrochloric acid) of the sulfathiazole and the chromogenic was treated with sodium nitrite and aniline to form an intense orange water-soluble dye that is stable and has a maximum absorption at 480 nm. It was observed that the method is applicable for the determination of sulfathiazole in Nicotrim, Contri, Biotran and Bactrim forte formulations. The LOD (limit of detection) and LOQ (limit of quantification) for the method were calculated and found to be 0.23 and 0.48 ppm respectively.

Introduction:
Sulfathiazole belongs to the sulfonamide groups of chemotherapeutics. Despite the availability of numerous antibiotics, sulfonamide is still an important drug for therapeutic use, particularly in the treatment of acute urinary
infection (UTI). The use of sulfonamides has increased greatly with the introduction of trimethoprim sulfathiazole mixtures, which represent a synergistic combination of antibacterial agent[1].

Various methods for the analysis of different types of sulfonamides have been reported. These methods are based mainly on titration[2,3], FTIR[4], HPLC[5], spectrophotometry[6,7] and enthalpimetry[8]. However, they are expensive, complicated, and relatively time consuming.

The aim of the present work was to develop a simple, less expensive, and more reliable method for the determination of sulfathiazole in pure and pharmaceutical preparations. The method investigated is based on the spectrophotometric procedure which is not only faster and reliable, but also does not need sophisticated equipment compared to chromatographic techniques and therefore can be used in routine laboratories.

A higher value than the method in the literature was obtained; thus the extraction procedure[9] was adopted to extract trimethoprim from the alkaline solution with chloroform. The remaining procedure for the determination of sulfathiazole was the same as mentioned previously.

Experimental

Instruments

A UNICO UV-2100 spectrophotometer (united products and instruments Inc., Dayton, NJ), water bath, and CD 660 digital pH meter (Walden precision Apparatus, Cambridge, U.K.) were used during this investigation.

Reagents:

Analytical reagent-grade sodium nitrite, aniline concentrated hydrochloric acid, methanol, chloroform, sodium hydroxide and sulfathiazole were used during this work. Drug samples were purchased commercially and used directly.

Solutions:

a) Nitrite solution: 1000-ppm nitrite solution was prepared by dissolving 0.15g sodium nitrite in distilled water and diluted to 100 ml with distilled water. 100-ppm nitrite solution was prepared from 1000 ppm stock solution by dilution.

b) Aniline solution: 10% aniline solution was prepared by mixing 10ml aniline with 50ml methanol and diluted to 100ml with distilled water.
c) Sample solution: 0.1g of finely powdered tablet was dissolved by heating a small amount of distilled water to which 5ml of concentrated hydrochloric acid was added. The resulting solution was diluted to 100ml with distilled water.

d) Standard sulfathiazole solution: 1000-ppm sulfathiazole stock solution was prepared by dissolving 0.1g of authentic standard sulfathiazole in 5ml of concentrated hydrochloric acid and diluted to 100ml with distilled water. 100-ppm sulfathiazole solution was prepared from 1000-ppm stock solution by dilution. Similarly, working standards in the range of 2-10ppm were prepared from 100-ppm solution by dilution.

**Procedure:**

Standard solution in the range of 2-10ppm was taken in separation flasks to each of these added 3ml of 100-ppm nitrite solution and an optimum volume of concentrated hydrochloric acid. The resulting diazotized product was coupled with aniline by addition of 1.5ml of 10% of this reagent. The resulting orange-color azodye was measure spectrophotometrically at 480nm.

For the determination of sulfathiazole in various formulations, the acidic solution of the drug was diazotized and the color developed as mentioned above, absorbance of the colored solution was measured at 480nm. Fig (1) shows the absorption spectra of sulfathiazole. Six replicate readings were taken for each sample. The amount of sulfathiazole present in the drug was determined from the calibration curve using standard sulfathiazole solution.

![Absorption spectra](image)

**Fig (1): Absorption spectra.**
Results and Discussion

The chemical reaction involved in this procedure is shown in Figure(2)\textsuperscript{[10]}.

\[
\text{H}_2\text{N} \begin{array}{c}
\text{S} \\
\text{H} \\
\text{N} \\
\text{S}
\end{array} \text{H} + \text{NO}_2^- + 2\text{H}^+ \rightarrow \text{N} \begin{array}{c}
\text{N} \\
\text{S} \\
\text{H}
\end{array} \text{S} \begin{array}{c}
\text{N} \\
\text{N}
\end{array}
\]

Sulfathiazole

Diazotized Sulfathiazole

\[
\text{N} \begin{array}{c}
\text{N} \\
\text{S} \\
\text{H}
\end{array} \text{S} \begin{array}{c}
\text{N} \\
\text{N}
\end{array} + \text{NH}_2 \rightarrow \text{N} \begin{array}{c}
\text{N} \\
\text{N}
\end{array} \begin{array}{c}
\text{O} \\
\text{S} \\
\text{O}
\end{array} \text{NH} \begin{array}{c}
\text{S} \\
\text{N}
\end{array}
\]

Aniline

Azo dye

Figure(2): Chemical reaction of product

Optimization of parameters:

For the maximum formation of azodye, a volume of 100 ppm of nitrite solution was optimized. The results at Table (1) shows 3ml of 100 ppm nitrite solution was optimum volume for maximum formation of azodye, the azodye synthesized in acidic medium therefore the volume of concentrated hydrochloric acid was also optimized the results at Table (2) shows 1.5 ml of concentrated hydrochloric acid was found to be the optimum volume, as well as the results at Table (3) demonstrate 1.5 ml of 10% aniline was necessary for
coupling to produce azodye. The coupling reaction between diazotized sulfothiazole and aniline was slow at room temperature, therefore heating the mixture for suitable time to increase the rate of coupling by using water bath at boiling temperature. The results at Table (4) illustrates heating for 4 minutes it is suitable for favorable rate of coupling.

At optimum condition studied the effect of sulfathiazole concentration on the formation of azodye, very low concentration used to calculate limit of detection (LOD) and the limit of quantification (LOQ). The results of Table (5) shows this effect through the absorption value\(^{[11]}\).

The method investigated was applied for the determination of sulfathiazole in different brands of cotrimoxazole at optimum conditions (Table (6)). From the results of Table (6) shows the method is applicable directly to sulfathiazole determination in the formulation of Septran(Glozo SmithKline, London, U.K.) only. Fig (3) shows the calibration graph for determination of sulfathiazole.

![Calibration graph for determination of sulfathiazole.](image)

Higher values than those found in the literature were obtained for sulfathiazole in other formulations such as Nicotrim (Reckitt Benckiser, Berkshire, U.K), Biotran (Geofman, Karachi, Pakistan), Contri (unexolabs, Lahore, Pakistan), and Bactrim forte (Roche, Basel, Switzerland).

This could be due to the presence of trimethoprim and other excipients present in these formulations. To make the method applicable to other formulations, it was coupled with a solvent extraction procedure to separate
sulfathiazole from trimethoprim and other interfering excipients used in these formulations. It was observed that the method is applicable for the determination of sulfathiazole in Nicotrim, contri, Biotran and Bactrim forte formulation.

The LOD and LOQ for the method were calculated and found to be 0.23 and 0.48 ppm respectively.

**Conclusion:**

A spectrophotometric method has been investigated for the determination of sulfathiazole in various formulations. The method is directly applicable to certain formulations, while for others, it should be used after solvent extraction. The method is comparable in simplicity and reproducibility and is more sensitive than other published methods.

**Table (1): Optimization of the volume of nitrite solution for the formation of azodye**

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Volume of 100-ppm nitrite sol. (ml)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
<td>0.074</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0.106</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>0.153</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>0.156</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>0.152</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>0.146</td>
</tr>
</tbody>
</table>

**Conditions**

\[ \lambda \text{ max} \quad 480 \text{ nm} \]

sulfathiazole solu. 10 ppm
aniline 1.5 ppm
Heating time 4 min

**Table (2): Optimization of the volume of concentrated HCl solution for the formation of azodye**

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Volume of conc. HCl (ml)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
<td>0.149</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0.154</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
<td>0.158</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>0.150</td>
</tr>
<tr>
<td>5</td>
<td>2.5</td>
<td>0.145</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>0.140</td>
</tr>
</tbody>
</table>

**Conditions**

\[ \lambda \text{ max} \quad 480 \text{ nm} \]
sulfathiazole solu. 10 ppm
Na NO₂ (100ppm) 3 ml
aniline 10% 1.5ml
Heating time 4 min

**Table (3): Optimization of the volume of aniline solution for the formation of azodye**

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Volume of aniline (ml)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
<td>0.100</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0.143</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
<td>0.149</td>
</tr>
<tr>
<td>4</td>
<td>2.0</td>
<td>0.143</td>
</tr>
<tr>
<td>5</td>
<td>2.5</td>
<td>0.129</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>0.121</td>
</tr>
</tbody>
</table>

Conditions

λ. max 480 nm
NaNO₂ 3ml
sulfathiazole 10 ppm
Conc. HCl 1.5 ml
Heating time 4 min

**Table (4): Optimization of Heating time for the formation of azodye**

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Heating time (min.)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>0.118</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>0.123</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>0.11</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>0.104</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>0.102</td>
</tr>
</tbody>
</table>

Conditions

λ. max 480 nm
NaNO₂ (100ppm) 3ml
sulfathiazole 10 ppm
Conc. HCl 1.5 ml
Aniline solv. (10%) 1.5 ml
Table (5): Determination of LOD and LOQ for sulfathiazole

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Sulfathiazole conc.(ppm)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.4</td>
<td>0.004</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>0.007</td>
</tr>
<tr>
<td>3</td>
<td>0.6</td>
<td>0.010</td>
</tr>
<tr>
<td>4</td>
<td>0.8</td>
<td>0.012</td>
</tr>
<tr>
<td>5</td>
<td>1.0</td>
<td>0.015</td>
</tr>
<tr>
<td>6</td>
<td>1.5</td>
<td>0.025</td>
</tr>
<tr>
<td>7</td>
<td>2.0</td>
<td>0.039</td>
</tr>
<tr>
<td>8</td>
<td>2.5</td>
<td>0.046</td>
</tr>
<tr>
<td>9</td>
<td>3.0</td>
<td>0.059</td>
</tr>
</tbody>
</table>

Conditions
λ<sub>max</sub> 480 nm
volume of conc. HCl 1.5 ml
volume of NaNO<sub>2</sub>(100ppm) 3ml
volume of aniline solu. (10%) 1.5 ml
conc. of sulfathiazole varied
Heating time 4min
LOQ for concentration 0.475 ppm

Table (6): Quantitative analysis of sulfathiazole in pharmaceutical brands of contrimoxazole by spectrophotometric methods and comparison with titration method

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Brands of contrimoxazole</th>
<th>Spectrophotometric method</th>
<th>Ertraction spectrophotometric method</th>
<th>Titration method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nicotrim 400mg/tab</td>
<td>471.0 ±8.81 gm CV =1.87</td>
<td>418±6.94mg CV= 1.66</td>
<td>416.6±7.88mg</td>
</tr>
<tr>
<td>2</td>
<td>Septran 400 mg/tab</td>
<td>415.91±11.56 mg CV =2.78</td>
<td>374.89±4.57 mg CV = 1.219</td>
<td>416.6±1.99 mg</td>
</tr>
<tr>
<td>3</td>
<td>Contri 400mg/tab</td>
<td>456±12.01 mg CV=2.628</td>
<td>420.09±2.29 gm CV = 1.173</td>
<td>416.0 ±1.99 mg</td>
</tr>
<tr>
<td>4</td>
<td>Biotran 800 mg/tab</td>
<td>451±6.95mg CV=1.538</td>
<td>399.57±4.35 mg CV= 1.09</td>
<td>426.6±2.08mg</td>
</tr>
<tr>
<td>5</td>
<td>Bactrin forte 800 mg/tab</td>
<td>949.46±16.46 mg CV= 1.73</td>
<td>800.39 ±5.18 mg CV=0.647</td>
<td>806±7.96 mg</td>
</tr>
</tbody>
</table>
References


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