Determination of Some Important Antibacterial Drugs Using Alizarins and Thymol Blue Uv-visible Spectrophotometry

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Abstract: Simple and rapid spectrophotometric procedures have been established for the quantitation of antibacterial drugs such as Metronidazole and Tinidazole. The procedures are based on the reaction between the examined drugs (MD and TD) and alizarin red S(I), alizarin flourine blue(II) and thymol blue(III) in distilled water producing ion-pair complexes which can be measured at the optimum wavelength 420nm for reagent I for both drugs, 391nm for (MD) and 306nm for (TD) for reagent II, respectively and 434nm for reagent III for both drugs. The optimization of the reaction conditions is investigated. Beer’s law validation, accuracy, precision and other aspects of analytical merit are presented in the text. The proposed methods are applied for the determination of the analytes in their pure forms and in pharmaceutical preparations. The results were in good agreement with those obtained by the official and reported methods.

Keywords: Spectrophotometry; Ion-pair complexes; Metronidazole; Tinidazole; Alizarins; Thymol blue and Dosage forms.

INTRODUCTION

Metronidazole is chemically 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethanol. It is a nitroimidazole anti-infective medication used mainly in the treatment of infections caused by susceptible organisms, particularly anaerobic bacteria and protozoa, it is a drug used for the treatment of trichomonal vaginitis, amebiasis, giardiasis, and certain anaerobic bacterial infections in humans. Metronidazole is thought to improve portosystemic encephalopathy through a reduction in the concentration of ammonia-producing bacteria in the intestine. Several methods have been applied in the literature for the determination of metronidazole. Among the several analytical methods are spectrophotometrically, HPLC, RP-HPLC and voltametry. Tinidazole is chemically 1-(2-ethylsulfonyl-ethyl)-2-methyl-5-nitroimidazole. It is active against protozoa and anaerobic bacteria and is used like metronidazole in a range of infections. The drug is reported to hydrolyze quantitatively in alkaline conditions to 2-methyl-5-nitroimidazole and under photolytic conditions, the drug yields intermediate, rearrangement, and degradation products. Several analytical methods for tinidazole have been developed so far such as HPLC, LC-MS, capillary electrophoresis, spectrophotometry, voltammetry, and electrochemical methods.

In order to continue our work for using alizarin derivatives and thymol blue for drug analysis, a simple, accurate, economic, sensitive, more essential and less-time consuming spectrophotometric method for the determination of the antibacterial drugs (Metronidazole and Tinidazole) under investigation in pure and in their dosage form are performed.

MATERIALS AND METHODS

Apparatus: A SHIMADZU UV 160-A is a double beam UV-Visible recording spectrophotometer with a 10 mm quartz cell was used for all spectrophotometric measurements, a HANA microprocessor pH meter 8417 was used for checking the pH of buffer solutions.

Materials: Metronidazole stock solution (10⁻³M), was prepared by dissolving 0.017116 gm of metronidazole in small amount of anhydrous formic acid in 100 ml measuring flask then completed with distilled water to the mark. Tinidazole stock solution (10⁻³M), was prepared by dissolving 0.06182 gm of tinidazole in small amount of anhydrous formic acid in 100 ml measuring flask then completed with distilled water to the mark. Amriya Pharm. Ind., Alexandria, Egypt supplied metronidazole in pure form and their pharmaceutical formulation, [Amrizole oral suspension (it was labeled to contain
Table 1: Quantitative parameters for the complexation of MD and TD with alizarin derivatives (I, II) and thymol blue (III).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MD</th>
<th>TD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>λ&lt;sub&gt;max&lt;/sub&gt; (nm)</td>
<td>420</td>
<td>391</td>
</tr>
<tr>
<td>pH</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>reagent (ml)</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>time (min.)</td>
<td>0-60</td>
<td>0-60</td>
</tr>
<tr>
<td>molar absorptivity</td>
<td>0.005</td>
<td>0.048</td>
</tr>
<tr>
<td>sandell sensitivity</td>
<td>0.509*10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>0.580*10&lt;sup&gt;-3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Standard division</td>
<td>0.336</td>
<td>0.295</td>
</tr>
<tr>
<td>Specific absorptivity</td>
<td>1*10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>1*10&lt;sup&gt;-6&lt;/sup&gt;</td>
</tr>
<tr>
<td>Regression equation</td>
<td>Y = a + b + c</td>
<td></td>
</tr>
<tr>
<td>Slope (a)</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Intercept (c)</td>
<td>0.24</td>
<td>0.078</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.998</td>
<td>0.986</td>
</tr>
<tr>
<td>R.S.D. (%)</td>
<td>1.676</td>
<td>0.567</td>
</tr>
<tr>
<td>Range of error</td>
<td>0.8</td>
<td>0.8</td>
</tr>
</tbody>
</table>

120 ml where 200 mg is equivalent to 5 ml) Batch NO.: 351167]. Medical Union Pharmaceuticals, Abu-Sultan, Ismailia, Egypt supplied tinidazole in pure form and their pharmaceutical formulation, [Protozole tablets where each tablet contains 500 mg tinidazole].

Reagents: All the reagents and solvents used were of analytical grades. All solutions were freshly prepared.

- Alizarin red S, 9,10-dihydro-3,4-dihydroxy-9,10-dioxo-2-anthracenesulfonic acid sodium salt (I), a stock solution (10<sup>-3</sup> M) was prepared by dissolving 0.0179 gm in 50 ml distilled water in a measuring flask.
- Alizarin fluorine blue, 3,4-dihydroxynroquin-2-yl-methylimino-diacetic acid (II), a stock solution (10<sup>-3</sup> M) was prepared by dissolving 0.019267 gm in 50 ml distilled water in a measuring flask.
- Thymol blue (III), thymolsulfonphthalein, a stock solution (10<sup>-3</sup> M) was prepared by dissolving 0.019267 gm in 50 ml distilled water in a measuring flask.
- 2M NaOH solution.
- An acetic buffer solution was prepared by add NaOH solution for adjust the pH to 100 ml acetic acid (0.1N) then completed to 250 ml with distilled water in a measuring flask.

BDH supplied alizarin derivatives which made in England and finchemie K.-H.Kallies KG supplied thymol blue which made in Germany.

General procedure: 1.0 ml of metronidazole and tinidazole solution (10<sup>-3</sup>M) was added to 0.5 ml of reagents (I, III) for both MD and TD while for reagent II (0.5 ml for MD, 1ml for TD) in 10 ml measuring flask containing 5 ml buffer solution of pH 7 for all cases except for (TD) drug with reagent II add 5 ml buffer solution of pH 5 and completed to the mark with distilled water. The absorbance was measured at the optimum wavelength as shown in (Figs. 1 - 6) and as recorded in (Table 1) against a water blank.

Application to various dosage forms: For amrizole syrup (metronidazole) filterate it through a whatmann N0.1 paper (500 µg/ml) and take 0.8 ml (0.8 ml is equivalent to 20 mg) of the clear solution then completed to 100 ml with distilled water in a measuring flask. For protozole tablet (tinidazole), at least 10 tablets of the drug were weight into a small dish, powdered and mixed well. A portion equivalent to 30 mg was weight and dissolved in 100 ml distilled water in a measuring flask, shaken well and filtered through a whatmann No.1 filter paper (500 µg/ml). The general procedures are applying to the drugs content of these clear solution.
RESULTS AND DISCUSSION

Optimization: Careful investigations were carried out to establish the most favorable conditions to achieve maximum colour intensity in the quantitative determination of the examined antibacterial drugs (MD and TD). The absorption spectra of the drugs and their complexes with alizarin derivatives (I,II) and thymol blue (III) under the optimum conditions are shown in (Figures 1 - 6) and recorded in (Table 1). The absorption band of MD and TD complexes are located at 420 nm for reagent (I) for both drugs, 391 nm for MD and 306 nm for TD, respectively for reagent (II ) and 434 nm for reagent (III) for both drugs.

However, in all instances the absorbance was measured at this $\lambda_{max}$ against a water as a blank under identical conditions. The influence of each of the following variables on the reaction was tested. The reaction mechanism of metronidazole and tinidazole with alizarin derivatives (I,II) and thymol blue (III) was proposed in scheme 1 and 2.

Effect of pH: Different buffer media (universal, phosphate, borate and acetate buffer solutions) were examined to achieve maximum colour intensity. Acetate buffer proved to be the most favorable one due to its highly absorbance values in addition to instantaneously formation of the ion-pair complex without affecting the
absorbance in the pH ranges (3-12). The optimum pH was 7 for all cases except for reagent II with (TD) the optimum pH was 5, as shown in (Table 1).

Effect of the reagent concentration: The effect of reagent was investigated by taking various amount of reagent added to an aliquot of solution containing 1 ml of drug under investigation (MD, TD) and follow of the procedure of each reagent, the volume of reagent was increased from 0.5 to 3 ml. The better absorption was observed with the addition of 0.5 ml for all cases except for (TD) with reagent II 1 ml was the best as shown in (Table 1).

Effect of time and temperature: The optimum reaction time was determined by following the colour intensity. The experiment shows that the absorbance was stable for a period of time (0- 60 min.) except for thymol blue the maximum absorbance was obtained at 10 min. for (MD ) and at 30 min. for (TD), respectively. It is clear that all conditions studied were optimized at room temperature (25±4°C ).

Sequence of addition: The optimum sequence was defined by following the colour intensity and maximum absorbance on changing the sequences of addition of drug, reagent and buffer. The best condition was {buffer solution first - the reagent - then the drug} for the maximum absorbance and stability.

Interference: For the determination of metroidazole with reagent (I) there are an no interference was observed from the presence of Starch, Urea, Citric acid monohydrate, Sucrose, Ammonium chloride, Nickel (II) chloride and potassium sulphate.
Fig. 7: Linearity of absorbance to concentration of metronidazole with alizarin red S

Fig. 8: Linearity of absorbance to concentration of tinidazole with alizarin red S

Fig. 9: Linearity of absorbance to concentration of metronidazole with alizarin fluorine blue

For the determination of metronidazole with reagent (II) there are an interference was observed from the presence of Ammonium chloride, Nickel (II) chloride, potassium sulphate and no interference observed from the presence of Starch, Urea, Citric acid monohydrate and Sucrose.

For the determination of metronidazole with reagent (III) there are no interference was observed from the presence of Starch, Citric acid monohydrate, Sucrose, Ammonium chloride and no interference observed from the presence of Urea, Nickel (II) chloride and potassium sulphate.

For the determination of tinidazole with reagent (I) there are an interference was observed from the presence of Ammonium chloride and no interference observed from the presence of Starch, Urea, Citric acid monohydrate, Sucrose, Nickel (II) chloride and potassium sulphate.

Fig. 10: Linearity of absorbance to concentration of tinidazole with alizarin fluorine blue

Fig. 11: Linearity of absorbance to concentration of metronidazole with thymol blue

Fig. 12: Linearity of absorbance to concentration of tinidazole with thymol blue

For the determination of tinidazole with reagent (II) there are an interference was observed from the presence of Starch, Urea, Sucrose, Ammonium chloride and Nickel (II) chloride and no interference observed from the presence of Citric acid monohydrate and potassium sulphate.

For the determination of tinidazole with reagent (III) there are an interference was observed from the presence of Starch, Urea, Sucrose and Nickel (II) chloride and no interference observed from the presence of Citric acid monohydrate, Ammonium chloride and potassium sulphate.

The results indicate that up to 100-fold excess of them which may present in its pharmaceutical preparations (in case of non-interference absorbance changes by ± 3.0% which is non-interference).
Table 2: Analysis of metronidazole (Amrizole Syrup) and tinidazole (Protozole Tablets) with alizarin derivatives (I, II) and thymol blue (III)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Reagent</th>
<th>Found (µg/ml)</th>
<th>Taken (µg/ml)</th>
<th>R (%) ±S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>29.935</td>
<td>30</td>
<td>99.783 0.213</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>24.51</td>
<td>24.5</td>
<td>100.041 0.383</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>25.05</td>
<td>25</td>
<td>100.2 0.071</td>
</tr>
<tr>
<td>2</td>
<td>I</td>
<td>131.92</td>
<td>132</td>
<td>99.939 0.295</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>33.89</td>
<td>33.857</td>
<td>100.1 0.086</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>14.1</td>
<td>14</td>
<td>100.714 0.374</td>
</tr>
</tbody>
</table>

Analytical data: Beer's law plots were obeyed in the concentration ranges of (8.558-42.79 µg/ml) for Metronidazole and in the concentration ranges of (12.364-74.184 µg/ml) for Tinidazole (Figures 7-12) with a correlation coefficient, molar absorptivity, sandell sensitivity, regression equation and standard deviation obtained by linear least, square treatment of the results and range of error percent are given in (Table 1), recoveries (R%) and standard deviation (±S.D.) are also calculated and recorded in (Table 2).

Analytical applications: The proposed method was successfully applied to the dosage form oral suspension metronidazole (amrizole) and tinidazole tablet (protozole). The results are recorded in (Table 2) compared statistically with the official method [1,2] reveal that the recoveries are in the range (100.714% - 99.783%) reflecting a high accuracy, in addition to the high precision indicated very low values of relative standard deviations. Therefore, it can be concluded that the results of the present method are in high agreement with those obtained by the official method.

CONCLUSION

Alizarin derivatives and thymol blue are a suitable reagents for the determination of antibacterial drugs such as metronidazole and tinidazole in pure form or in its dosage forms. The suggested method is simple, time saving, sensitive and reproducible. Therefore the proposed method can be used advantageously as a routine method for the determination of metronidazole and tinidazole in quality control and industry.

REFERENCES


