A New Spectrophotometric Method for the Determination of Ketoconazole Based on the Oxidation Reactions

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A new spectrophotometric method is proposed for the determination of ketoconazole in pharmaceutical preparations. The method is based on the coupled redox-complexation reactions, which proceed in the ketoconazole-iron (III) and 1,10-phenanthroline systems. A linear calibration graph was obtained between 1.6-16.0 ppm of ketoconazole. The proposed method is simple, rapid and sensitive. The procedure was successfully applied for the determination of ketoconazole in tablet, cream and shampoo samples.

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Ketoconazole, cis-1-acetyl-4-[4-[2-(2,4-dichlorophenyl)-2-(1H-imidazole-1-ylmethyl)-1,3-dioxolon-4-yl] methoxy piperazin (KC), is a highly effective broad spectrum antifungal agent. It is used to treat a wide variety of superficial and systemic mycoses and has the advantage over other imidazole derivatives of producing adequate sustained blood levels following oral administration. Moreover, it has been found that ketoconazole may cause changes in cytochrome P-450 dependent monooxygenase activities, as well as in epoxide hydrolase. Thus, the determination of ketoconazole in biological specimens and dosage forms has been the subject of considerable interest.

Due to the vital importance of ketoconazole determination in pharmaceutical preparations and in biological fluids, several chromatographic, spectroscopic and electrochemical methods have been reported. However, some of these methods need expensive equipment and/or are time consuming. In this article, we report a new simple, sensitive and inexpensive method for the determination of KC from pharmaceutical preparations.

We have recently studied the electrooxidation of KC in aqueous and non-aqueous media. Based on the electrochemical results obtained, it was found that KC is initially oxidized reversible with the loss of one electron to KC⁺ cation radical, which can be stabilized by resonance, results in the observed pink-red color product. The stability of the cation radical is completely dependent on the conditions of solution, so that it gradually decays via a chemical reaction in polar solvent and/or in weak acidic aqueous media. Also, we found that, KC⁺ can be further oxidized with the loss of the second electron to give some stable products. On the basis of results mentioned above, we were interested to investigate the chemically oxidation of KC in solutions in order to develop a spectrophotometric method for the determination of KC.

In the present work, a new spectrophotometric method was developed for the determination of KC based on the coupled redox-complexation reaction, which proceed in the KC-Fe(III) and 1,10-phenanthroline system. The resulting colored complex between Fe(II) and 1,10-phenanthroline was determined at 512 nm.

**Experimental**

**Apparatus**

A LKB model 4054 UV-Vis recording spectrophotometer equipped with 10 mm matched silica cells was used for all spectral measurements. The pH values were determined with a WTW moltilab 540 I onalyzer (Germany) pH/mV meter using a combined electrode.

**Reagents**

All of the chemicals used in this study were of the highest purity available and used without further purification. Triply distilled water was used throughout. Reagent grade ketoconazole and its tablets, creams containing 200 mg and 2% of the drug were obtained from Behvazan pharmaceutical company, Rasht, Iran. Ketoconazole shampoo samples (2%) was a pharmaceutical preparation from Shapha Pharmaceutical company, Tehran, Iran. Analytical grade 1,10-orthophenanthroline, FeCl₃ and Cetyltrimethylammonium bromide (CTAB) were purchased from Merck Company.

A working standard solution of 0.001 M of KC was prepared by dissolving 0.0267 g of pure drug in 50 ml of water containing a few drops of hydrochloric acid (about 0.04 M) followed by further diluting of 5 ml of this solution to 50 ml.

**General Procedure**

An accurate ml volume of standard or sample solution containing an appropriate amount of ketoconazole was pipetted...
into a 50 ml volumetric flask and 5 ml of Fe (III) 0.01 M was added. The resulting solution was mixed well, and allowed to stand for 10 min. Then, 25 ml of 1.10-phenanthroline 0.2% and 5 ml of acetate buffer solution 1 M was added and diluted to the mark with distilled water (pH=5.0). The absorbance of the solution was measured against a reagent blank at 512 nm after 10 min.

Tablet and Cream Sample Solutions
An accurately weighed amount of KC cream or finely powdered KC tablet was dissolved in water containing a few drops of HCl 1 M. The excipients were separated by filtration and the filter paper was washed three times with water. The filtrate and washing solutions of the tablet or cream samples were transferred quantitatively into 100-ml calibrated flask and diluted to the mark with water, and the spectrophotometric procedure was followed.

Shampoo Sample Solution
An accurately weighed portion of the ketoconazole shampoo equivalent to 10 mg of the drug was dissolved in water containing 1 ml of HCl 1 M and 0.5 ml of CTAB 0.01 M, and the volume was made to 100 ml in a volumetric flask. An accurate ml volume of the resulting solution was pipetted into 100 ml volumetric flask and the recommended procedure for the determination of KC was followed. The method of standard addition was used for the accurate determination of the KC content.

Results and Discussion

As a continuation of our works dealing with study of electrooxidation and chemical oxidation of KC, we attempted to improve a new spectrophotometric method for the determination of KC based on its oxidation reaction with Fe (III) ions. In preliminary experiments, it was observed that by addition of Fe (III) ions to an acidic solution of ketoconazole, at first a pink-red solution was obtained which became colorless during a few minutes. These observations were of completely according to the potentiostatic oxidation of ketoconazole to unstable pink-red ketoconazole cation radical, KC·+.22,24 On the other hand, it was found that the color of the chemically produced cation radical was immediately disappeared in the presence of excess Fe(III), probably due to further oxidation of KC·+ to colorless dication (KC2+). Therefore, because of instability of the colored ketoconazole cation radical, we have employed the amount of Fe(II) ions obtained from the reaction mixture to the determine the amount of ketoconazole. Moreover, due to the ability of Fe(II) ions to create stable, colored complexes with aromatic ligands, 1,10-orthophenanthroline (Phen)26 was selected to indirect spectrophotometric determination of KC. The run of occurring oxidation-complexation processes can be represented according to following reactions:

\[
\text{KC} + \text{Fe(III)} \rightleftharpoons \text{(KC} \text{ or KC}_2^+) + \text{Fe(II)}
\]

The result of the reactions is a colored [Fe(Phen)]\textsuperscript{2+} complex which absorbs at 512 nm.

In order to optimize the proposed spectrophotometric method, the effect of some experimental variables was studied. The optimization was made using 1.25×10\textsuperscript{-3} M sample solution of KC at 512 nm. Studies were performed by altering each variable in turn while keeping the others constant.

**Optimization of Chemical Variables**

The composition of iron(III)-1,10-phenanthroline-pH of solution and temperature have strong influence on the peak height. The effect of pH for the quantitative determination of KC with the proposed oxidation-complexation reaction was studied over the range of 2-6. At pH<2, the intensity of the absorption of the Fe(II)-phen complex decreases most probably due to difficult oxidation of protonated forms of KC by Fe(III). On the other hand, the dissolution of KC is very poor at higher pH values (pH>6). Therefore, an acetate buffer solution (pH 5) was used for further studies. The concentration of buffer solution did not considerably alter the absorption of solution. Thus, a 0.1 M of acetate buffer was selected.

[Fig. 1 The effect of the Fe(III) concentration on the absorbance of 15 ppm KC.]

[Fig. 2 The effect of the volume of 0.2% 1,10-phenanthroline on the absorbance peak height of 15 ppm KC.]

The effect of the Fe(III) concentration was studied in the range 1.0×10\textsuperscript{-4}-1.5×10\textsuperscript{-3} M. As can be seen from Fig.1, the analytical signal increased with the increase in reagent concentration up to 1.5×10\textsuperscript{-3} M, above which it remained virtually constant. Therefore, the concentration selected was 1.5×10\textsuperscript{-3} M.

The influence of the volume of 0.2% 1,10-orthophenanthroline in the range of 5-30 ml on the absorbance peaks was examined (Fig. 2). The height of signals grew with the change of reagent concentration up to 25 ml. Above this value, absorbance remained nearly constant. The 25 ml of 0.2% of 1,10-orthophenanthroline was selected as optimal.

After optimization of chemical variables, the influence of temperature over range 25-50 °C was tested. The signal increased directly with the increase in reaction temperature (Fig.3). However, no considerable improvement in linear concentration range and sensitivity was occurred, therefore, 25 °C was selected as optimum temperature. The optimum chemical conditions of investigated reaction are summarized in

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**Table 1**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5</td>
<td>Increased</td>
</tr>
<tr>
<td>Buffer Concentration</td>
<td>0.1 M</td>
<td>Decreased.</td>
</tr>
<tr>
<td>Fe(III) Concentration</td>
<td>1.5×10\textsuperscript{-3} M</td>
<td>Constant.</td>
</tr>
<tr>
<td>Temperature</td>
<td>25 °C</td>
<td>Optimal.</td>
</tr>
</tbody>
</table>
Table 1. These values allowed to obtain good sensitivity and high precision of KC determination.

Table 1. Results of the optimization of the parameters

<table>
<thead>
<tr>
<th>Fe(III)</th>
<th>Phen(0.2%)</th>
<th>pH</th>
<th>Temp.</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>(M)</td>
<td>(ml)</td>
<td>(C)</td>
<td>(min)</td>
<td></td>
</tr>
<tr>
<td>1.5×10⁻³</td>
<td>25</td>
<td>5</td>
<td>25</td>
<td>10</td>
</tr>
</tbody>
</table>

**Beer’s Law Study**

A calibration graph for KC was obtained under the optimum conditions (Fig 4). Beer’s law was obeyed over the concentration range of 1.6–16.0 ppm at 512 nm with molar absorption coefficients (absorptivity) of 4.2×10⁴ l mol⁻¹ cm⁻¹. The detection limit, calculated following the expression a + 3sₓ, was 0.17 ppm. The relative error (95% confidence level) for 10 ppm of drug was 1.2%.

![Fig. 3 The effect of temperature on the absorbance.](image)

**Table 2. Results of determination of ketoconazole in its formulations**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Labeled</th>
<th>Found* (X ± SD) proposed method</th>
<th>Official method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablet</td>
<td>200 mg</td>
<td>210.6 ± 1.98 mg</td>
<td>208 ± 2.0 mg</td>
</tr>
<tr>
<td>Cream</td>
<td>2%</td>
<td>2.05 ± 0.20%</td>
<td>2.10 ± 0.15%</td>
</tr>
<tr>
<td>Shampoo</td>
<td>2%</td>
<td>1.70 ± 0.15%</td>
<td>-</td>
</tr>
</tbody>
</table>

* Average of five replicate measurements

**Determination of Ketoconazole in Shampoo**

To the best of our knowledge, there is no literature report on the determination of KC in shampoo samples. Thus, we have evaluated the accuracy of the proposed method by performing experiments on the samples prepared from dosage form and pure drugs. A mean recovery of 99.5% was obtained from shampoo samples (Table 2). It is interesting to note that the results obtained from the performing of the official USP method (proposed for tablet and cream samples) on shampoo samples were not satisfactory.

**Conclusions**

Figure 4. Calibration graph for the determination of ketoconazole under the optimum conditions.

Figure 5. Potentiometric titration of a ketoconazole tablet sample (equivalent to 108 mg of the drug) dissolved in 30 ml glacial acetic acid with 0.101 M perchloric acid.
The method described provides a simple, fast and reliable means of determining ketoconazole in pharmaceutical preparations. It compares favorably in sensitivity with the most published chromatographic and spectroscopic methods for the determination of ketoconazole, and it can certainly be classed among the most sensitive methods. On the other hand, in terms of simplicity and expense, the method could be considered superior in comparison with the previously reported methods, especially with those based on chromatography and non-aqueous titrations.

Acknowledgments

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References

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