Spectrophotometric Determination of Oxymetazoline Hydrochloride Based on the Oxidation Reactions

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Abstract

Simple, sensitive and reproducible spectrophotometric method was developed for determination of oxymetazoline hydrochloride in pure as well as in pharmaceutical drop formulations. The method is based on the reduction of ferric to ferrous ions followed by complexation with 1,10-phenanthroline to produce an orange red chromogen that has maximum absorption at 510 nm and obeyed Beer’s linearity in the concentration range of 0.1-7 µg/ml, with molar absorptivity 5.74×10^4 l.mol^{-1}cm^{-1}, accuracy (average recovery %) 100.53 % and precision better than 1.6. The developed method was successfully applied to the determination of oxymetazoline hydrochloride in bulk and pharmaceutical formulations without any interference from common excipients.

Keywords: Oxymetazoline hydrochloride; 10-Phenanthroline; Spectrophotometry.

Introduction

Oxymetazoline hydrochloride (OMZH), [6-tert-butyl-3(2-imidazolin-2-il)methyl]-2-4-dimethylphenol, (Figure 1) [1], belongs to non-selective adrenergic drugs which has been used as eye and nose drops and acting on adrenergic receptors causing a strong vasospam leading to an increase of a blood pressure [2]. Oxymetazoline is used to treat epistaxis and eye redness due to minor irritation [3,4].

Various analytical techniques have been used for the determination of OMZH which include high-performance liquid chromatography [2,5-8], liquid chromatographic-mass spectrometry [9], chemiluminescence [10] and potentiometry [1,11]. These techniques require sophisticated instruments and expensive reagents, and involve several manipulation steps and derivatization reactions. However, spectrophotometric

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techniques continue to be the most preferred method for routine analytical work due to their simplicity and reasonable sensitivity, along with significant economical advantages. Literature survey revealed few spectrophotometric methods using various reagents for determination of OMZH including 2,6–dichloroquinone–chlorimide in the presence of an oxidant \cite{12}, 2,4,6-tris(2-pyridyl)-5-triazine in the presence of Fe(III) \cite{13}, ceric sulfate in the presence of perchloric acid \cite{14}, sodium cobaltinitrite in acetic acid medium \cite{15} and ammonium reineckate \cite{16}. Some of these methods are time-consuming, extraction procedures or heating and require strictly controlled reaction conditions. Others are less sensitive. The aim of the present work is to provide simple, sensitive, and rapid spectrophotometric method for determining of OMZH in pure form as well as in pharmaceutical drop formulations based on the oxidation of OMZH with ferric salt in acidic medium and subsequent complexation of ferrous ion produced with 1,10-phenanthroline reagent.

**Experimental**

**Apparatus**

All absorption measurements were made on a Shimadzu UV-210A double-beam spectrophotometer.

**Reagents**

All reagents used were analytical grade and were obtained from Fluka and BDH companies.

_**Fe(III) solution (0.025 M)**_ was prepared by mixing 1.2054 g of Ferric ammonium sulphate (NH₄ Fe (SO₄)₂·12 H₂O) with 5 ml distilled water and 2 ml of 1 M HCl and diluted to 100 ml in a volumetric flask with distilled water.

_1,10-Phenanthroline solution; o-phen (0.05 M)_ was prepared by dissolving 0.9 g of 1,10-phenanthroline in ethanol and diluted to the mark in a 100ml-volumetric flask with ethanol.

_Standard solution of oxymetazoline hydrochloride; OMZH (100 μg ml⁻¹)_ was prepared by dissolving 0.01 g of pure OMZH, provided by state company for Drug Industries and Medical appliance-(SDI) Sammara-Iraq, in distilled water and diluted to the mark in 100 ml-volumetric flask and stored in amber colored bottle in refrigerator. The solutions were diluted as needed.

**Recommended procedure**

Aliquots of standard drug solution of OMZH were transferred into a series of 25 ml calibrated flasks. To each of these were added 0.5 ml of NH₄Fe(SO₄)₂·12H₂O solution and 2 ml of o-phen solution. The solutions were heated in a water bath adjusted at 70°C for 40 min. Then cooled, diluted up to the mark with distilled water and mixed well. The absorbance of complex was measured at 510 nm against reagent blank.
Procedure for OMZH assay in nasal drops

Five nasal drop containers were mixed well and an accurately measured volume solution, equivalent to about 5 mg of OMZH was transferred into a 100 ml calibrated flask and diluted to the mark with distilled water. A liquid sample of the drug solution was analysed as described in recommended procedure.

Results and Discussion

Ferric salts play a prominent role in the spectrophotometric determination of many pharmaceutical drugs. It acts as an oxidant. The amount of Fe(II) can be determined using o-phen reagent. These properties have been utilized to develop spectrophotometric methods for the determination of OMZH.

Absorption spectra

OMZH undergoes oxidation by Fe(III). The Fe(II) formed readily combines with o-phen to form a red colored complex of tris o-phen — iron (II) chelate (ferroin) \([Fe(o-phen)_3]^{2+}\), which has absorption maximum at 510 nm, as shown below:

\[
OMZH + Fe^{3+} \rightarrow Fe^{2+} + o-phen \rightarrow \text{Red color determined spectrophotometrically}
\]

Under the experimental conditions the reagent blank showed a negligible absorbance at the corresponding \(\lambda_{\text{max}}\). The absorption spectra of colored complex for \([Fe(o-phen)_3]^{2+}\) is shown in figure 1.

![Absorption spectra](image)

**Figure 1:** Absorption spectra of: (A) Fe(III)- o-phen with OMZH (2.0 \(\mu\)g ml\(^{-1}\)) against (B) reagent blank.
Optimum reaction conditions

In order to optimize the proposed spectrophotometric method, the effects of some experimental variables were studied. The effects of reagents were studied by measuring the absorbances of solutions containing 2 μg ml\(^{-1}\) of OMZH and varied amounts of the reagent separately.

Effect of temperature and reaction time

The reaction time was determined by following the color development at room temperature and in a thermodynamically controlled water-bath at different temperatures. The absorbance was measured at 5 min intervals against reagent blank treated similarly. The formation of colored complex was slow at room temperature and required longer time for completion. Hence, efforts were made to accelerate the reaction by carrying out the reaction at higher temperatures. It was observed that the maximum absorbance was obtained when heating the reaction mixture to 70 °C for 40 min (Figure 2). The stability of complexes was found constant after cooling to room temperature for more than 1 hr.

Effect of Fe \(\text{III}\) concentration

The effect Fe (III) concentration on a fixed amount of OMZH and o-phen was investigated. It was found that 0.025 M concentration of ferric ion gave a maximum absorbance. Also; the amount of this concentration was studied and figure 3 showed that the absorbance increased and reached maximum by using 0.2-0.7 ml of 0.025 M ferric solution. 0.2 ml was considered as optimal amount.

![Figure 2: Effect of time and temperature on the absorbance of 2 μg ml\(^{-1}\) OMZH](image)

Effect of Fe III concentration

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![Figure 2: Effect of time and temperature on the absorbance of 2 μg ml\(^{-1}\) OMZH](image)
Effect of o-phen reagent concentration

The effect of o-phen concentration on the absorbance of the complex was investigated. It was found that 0.5-0.8 ml of 0.025 M o-phen give maximum absorbance; 0.5 ml was used in subsequent experiments. Above this concentration the absorbance was decreased as shown in figure 4.

Effect of order of addition

To obtain optimum results the order of addition of reagents should be followed as given under the general procedure, otherwise a loss in color intensity was observed.

Quantification

In order to investigate the range in which the colored complex adhere to Beer's law, the absorbance of the complex was measured at 510 nm after developing the colour by following the suggested procedure for a series of solutions containing increasing amounts of OMZH drug. The Beer's law limits, molar absorptivity and Sandell's sensitivity values were evaluated and are given in table 1. This indicates that the method is sensitive. The linearity was represented by the regression equation and

Figure 3: Effect of 0.025 M Fe (III) amount on the oxidation of 2 μg ml⁻¹ OMZH in the presence of o-phen.

Figure 4: Effect of o-phen concentration on the absorbance of 2 μg ml⁻¹ OMZH in the presence of Fe(III)
the corresponding correlation coefficient for OMZH determined by the proposed method represents excellent linearity \((R^2=0.9992)\). The relative standard deviation (RSD) and accuracy (average recovery %) for the analysis of five replicates of each of the three different concentrations of OMZH indicates that the method is precise and accurate.

**Table 1**: Summary of optical characteristics and statistical data for the proposed method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Values of method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer's law limits (µg ml(^{-1}))</td>
<td>0.1-7.0</td>
</tr>
<tr>
<td>Molar absorptivity (l.mol(^{-1}). cm(^{-1}))</td>
<td>5.74×10(^{4})</td>
</tr>
<tr>
<td>Sandell's sensitivity (µg ml(^{-2}))</td>
<td>0.005</td>
</tr>
<tr>
<td>Average recovery (%)**</td>
<td>100.53</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9992</td>
</tr>
<tr>
<td>Regression equation ((Y) *)</td>
<td></td>
</tr>
<tr>
<td>Slope, (a)</td>
<td>0.1935</td>
</tr>
<tr>
<td>Intercept, (b)</td>
<td>0.0028</td>
</tr>
<tr>
<td>RSD**</td>
<td>± 1.6</td>
</tr>
</tbody>
</table>

* \(Y = aX + b\), where \(X\) is the concentration of OMZH in µg ml\(^{-1}\).
** Average of five determinations.

**Interference**

The extent of interferences by some excipients, which often accompanied pharmaceutical preparations, were studied by measuring the absorbance of solutions containing 2 µg ml\(^{-1}\) of OMZH and various amounts of diverse species in a final volume of 25 ml. It was found that the studied excipients do not interfere in the determination of OMZH in its dosage forms. An error of 5.0 % in the absorbance readings was considered tolerable. Typical results are given in table 2.

**Table 2**: Effect of excipients for assay of OMZH

<table>
<thead>
<tr>
<th>Excerpt</th>
<th>Recovery (%) of 2 µg ml(^{-1}) OMZH per µg excerpt added</th>
<th>4</th>
<th>8</th>
<th>20</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride</td>
<td></td>
<td>100</td>
<td>100.6</td>
<td>102</td>
<td>104.1</td>
</tr>
<tr>
<td>Acacia</td>
<td></td>
<td>99.7</td>
<td>98.1</td>
<td>96.6</td>
<td>97.6</td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td>102</td>
<td>100.19</td>
<td>99</td>
<td>98.5</td>
</tr>
<tr>
<td>Lactose</td>
<td></td>
<td>103.7</td>
<td>100.2</td>
<td>100.5</td>
<td>105</td>
</tr>
<tr>
<td>Starch</td>
<td></td>
<td>102.6</td>
<td>102</td>
<td>101.6</td>
<td>102.1</td>
</tr>
</tbody>
</table>

**Analytical applications**

The proposed method was successfully applied for determination of OMZH in its pharmaceutical preparation as nasal drops. The obtained results were compared statistically by a Student's \(t\)-test for accuracy with the official method \([1]\) (depending on potentiometric titration for pure drug using 0.1 M perchloric acid) at the 95 % confidence level with five degrees of freedom. The results obtained indicated that the experimental \(t\)-test (2.212) was less than the theoretical value (2.776), indicating that the method is accurate. Moreover, the validity of the method was confirmed by
applying the standard addition procedure. As shown in table 3 and figure 5, the results showed that the proposed method was free from interferences.

Table 3: Assay of OMZH in pharmaceutical nasal drops using the proposed method, standard addition procedure and comparison with the official method.

<table>
<thead>
<tr>
<th>Procedure applied</th>
<th>Pharmaceutical preparation</th>
<th>Drug amount present (μg ml⁻¹)</th>
<th>Recovery (%)</th>
<th>Average Recovery (%)</th>
<th>Drug content found</th>
<th>Certified value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proposed method</td>
<td>Nazodren* drop</td>
<td>1</td>
<td>98.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>99.30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>97.43</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oxymet** drop</td>
<td>1</td>
<td>100.49</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>100.66</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>103.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard addition procedure</td>
<td>Nazodren drop</td>
<td>1</td>
<td>101.40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>103.50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oxymet drop</td>
<td>1</td>
<td>97.30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>102.40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>British pharmacopoeia method</td>
<td>Bulk drug</td>
<td>200 mg</td>
<td>100.90</td>
<td></td>
<td>102.00</td>
<td>200 mg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>103.1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Marketed by LABORATE (INDIAN)
** Marketed by PHARAONIA (EGYPT)

Figure 5: Standard addition plots of (a) Oxymet drop and (b) Nazoden drop

Stoichiometry and reaction mechanism

In the present work the stoichiometric ratio for the oxidation of OMZH by Fe(III) was investigated applying the continuous variation (Job's) and mole ratio methods [17], using equimolar solutions of each (1×10⁻³M). As seen in figure 6, it was found that OMZH forms a product with Fe(III) in the ratio 1:2 respectively. However; the probable reaction mechanism based on the reported method is given in scheme 1.
Conclusion

The proposed method is simple and more sensitive than most of the previously reported spectrophotometric methods. The statistical parameters and the recovery test data indicate the high reproducibility and accuracy of the proposed method. Analysis of authentic samples containing OMZH showed no interference from common additives and auxiliary substances. Hence, this method can be considered for the determination of OMZH in both pure form and in pharmaceutical preparations.
References