Validation of sensitive spectrophotometric method for determination of Salmeterol xinafoate and Fenoterol hydrobromide via o-Phenanthroline and iron complexation

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ABSTRACT

A Simple, accurate and sensitive spectrophotometric method has been established and validated for the determination of two selective beta-2 agonist drugs namely; Salmeterol xinafoate and Fenoterol hydrobromide in their pure forms and pharmaceutical preparations. The proposed method is based on the ability of studied drugs to reduce iron (III) in (o-phenanthroline)-iron (III) complex to form highly stable colored tris (o-phenanthroline)-iron (II) complex that can be measured spectrophotometrically at 510 nm. Different variables affecting the reaction were studied and optimized to ensure maximum sensitivity of the method. The developed method was found to obey Beer’s law in the concentration range of (0.7-7.0) and (1.0-8.0) µg mL⁻¹ with quantitation limits 0.523 and 0.705 µg mL⁻¹, for Salmeterol and Fenoterol, respectively. The suggested method was completely validated according to the ICH guidelines and successfully applied for determination of the studied drugs in their pharmaceutical preparations with high accuracy.

INTRODUCTION

Salmeterol xinafoate (SAL); (RS)-5-salicyl alcohol 1-hydroxy-2-naphthoate and Fenoterol hydrobromide (FEN);1-(3,5-Dihydroxyphenyl)-2 - (4-hydroxy-a-methylphenethylamino) ethanol hydrobromide, are selective beta-2 agonist drugs that are widely used for the treatment of Asthma and chronic obstructive pulmonary diseases due to their direct sympathomimetic action on the smooth muscles (Martindale, 2005). Many analytical procedures have been reported in the literature for determination of the cited drugs in their pharmaceutical preparations as well as biological fluids. These methods include Spectroscopic (Abounassif and Abdel-Moety, 1989; Chowdary and Devala Rao, 1997; Thea Suferment, 1998; Chowdary and Devala Rao, 1999; AL-Malaq et al., 2000; Kumar et al., 2000; Reddy et al., 2000; El-Shabrawy et al., 2003; Beyene et al., 2004; Eid, 2007; Zamuner et al., 2008; Samir et al., 2012; Soliman and Abdel Moey, 2014), HPLC(Nayak et al., 1996; Wang et al., 2007; Siluk et al., 2008), HPTLC (Kasaye et al., 2010; Ahmed and Youssef, 2011)and electrochemical methods (Radi, 2006). However most of these methods require sophisticated instruments, high cost reagents, or require the use of organic solvents which have harmful effects on the analysts.

So there was a need for the development of new sensitive, simple and cost effective method for the determination of cited drugs in their dosage forms that can be used in routine work analytical laboratories.
Preparation of standard solutions

Stock standard solutions (1.0 mg mL\(^{-1}\)) of each studied drug were prepared by dissolving appropriate amount of authentic powder in methanol and distilled water for Salmeterol xinafoate and Fenoterol hydrobromide, respectively. Further dilutions were made using distilled water for preparing standard working solutions.

General analytical procedure

Into a series of 10-mL stoppered test tubes, appropriate volumes of working drug solutions covering final concentration range (0.7-7.0) and (1.0-8.0) µg mL\(^{-1}\) for SAL and FEN, respectively were added and followed by the addition of 2 mL (Phen) reagent, shaken well and transferred to thermostatically controlled water bath kept at 70 \(^\circ\)C for 15 minutes. The tubes were cooled using ice bath and their contents were transferred quantitatively into 10-mL volumetric flasks and completed to the volume with distilled water. The red color formed was measured at 510 nm against reagent blank treated similarly omitting drug addition.

Preparation of pharmaceutical dosage forms

Metrovent\(^\circ\) inhaler labeled to contain 25 µg SAL per actuation was shaken well and 200 actuations (the whole content of the inhaler) were actuated in 100mL beaker containing 40mL of methanol. The mouth piece of the inhaler was immersed beneath the methanol to ensure complete delivery of the drug without any waste. The contents of the beaker was gently warmed at 50\(^\circ\)C in thermostatically controlled water bath to expel propellants, the contents of the beaker was then transferred into 50mL volumetric flask and completed to the volume with methanol to obtain final concentration of 100µg mL\(^{-1}\), and then general analytical procedure was followed.

Bronotrol\(^\circ\) syrup labeled to contain 2.5mg/5 mL FEN was prepared by transferring 100mL of syrup into a 250-mL separating funnel, 5-mL of concentrated ammonia were added and (FEN) was extracted with 30-mL chloroform (5mL×6 times). The chloroform layer containing the studied drug was collected over anhydrous sodium sulfate organic layer, evaporated to dryness and the residue was dissolved in methanol and transferred into 50-mL volumetric flask and completed to the volume with distilled water to obtain final concentration of 1mg mL\(^{-1}\), and then general analytical procedure was performed.

RESULTS AND DISCUSSION

SAL and FEN contain phenolic and alcoholic hydroxyl groups which are known for their effective reducing power(Pesez, 1974; Firuzi et al., 2005). The reduction of ferric to ferrous had been widely used for spectrophotometric determination of phenolic drugs in many occasions (Reddy et al., 2000; Gousuddin et al., 2011). In this study, we utilized the presence of phenolic and hydroxyl groups in the studied drugs to quantitatively reduce the Fe (III) in the Fe (III)-o-Phenanthroline complex to form stable red colored chromogen corresponding to Fe (II) o-Phenanthroline complex (Fig 2) that can be measured spectrophotometrically at 510 nm, Fig 3.

Optimization of variables

Different experimental parameters affecting reaction development and stability of the reaction products were carefully studied and optimized. These factors were changed individually, while the others were kept unchanged. These factors include volume of Phen reagent, heating time and temperature, pH of the reaction media and effect of diluting solvents.
Effect of heating time and temperature

Different temperatures were studied to select the best conditions for the developed procedure. The reaction was allowed to proceed at room temperature and it took more than two hours to develop the desired color which is not convenient for routine analysis, so in order to save time, the reaction was performed in a thermostatically controlled water bath at different temperatures and different time intervals. It was found that best conditions for the suggested method are heating the reaction medium at 70 °C for 15 minutes. Longer periods of time did not affect color development and higher temperatures caused slight decrease in the absorbance, Fig (5 and 6).

Effect of reagent volume

Different volumes of (Phen) reagent were added to obtain maximum color development and hence achieve high sensitivity of the method. It was found that 2 mL of the reagent gave maximum absorbance and any further increase did not affect the color development, Fig 4.

Effect of pH

It was reported that the reaction of o-phenanthroline with iron proceeded better in acidic pH (Homoda et al., 2016). However, the effect of pH on the reaction products was also studied and the results showed no effect on the color development. So for simplicity of the method no buffer was added and the reaction was kept to proceed in acidic medium provided by HCl content in Phen reagent.

Effect of diluting solvent

Different solvents such as water, methanol, ethanol, acetone, acetonitrile and DMF were studied in order to select the
most suitable solvent for dilution. Methanol and ethanol did not affect the absorbance significantly while acetone and DMF caused turbidity in the flask affecting the color of the complex. So water was used as the best solvent that gave better results making the method simpler and more economic.

Validation of the proposed method

The proposed spectrophotometric method was validated according to the ICH guide lines regarding accuracy, precision, Limits of quantitation and detection and robustness in order to prove that the performance characteristics of the method meet the requirements for the intended analytical applications (ICH Guidelines, 2005).

Linearity and concentration range

In this work, the general analytical procedure was performed on a series of standard drug solutions having concentrations ranging from (0.7-7.0) and (1.0-8.0) µg mL⁻¹ for Salmeterol and Fenoterol, respectively. The whole set of experiments were carried out within this range to ensure the validation of the proposed procedure. Linear calibration was obtained by plotting the Absorbance at 510 nm versus the drugs concentration within the specified range. Statistical treatment of the data was carried out using linear regression analysis and the analytical parameters were calculated, the correlation coefficient was 0.9996 for FEN and SAL indicating good linearity, Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SAL</th>
<th>FEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ max (nm)</td>
<td>510</td>
<td>510</td>
</tr>
<tr>
<td>Concentration range (µg mL⁻¹)</td>
<td>0.7-7.0</td>
<td>1.0-8.0</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9996</td>
<td>0.9996</td>
</tr>
<tr>
<td>Determination coefficient (r²)</td>
<td>0.9993</td>
<td>0.9992</td>
</tr>
<tr>
<td>Slope</td>
<td>0.1144</td>
<td>0.1024</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.1136</td>
<td>0.09205</td>
</tr>
<tr>
<td>SD the intercept (Sa)</td>
<td>0.00598</td>
<td>0.00722</td>
</tr>
<tr>
<td>SD the slope (Sb)</td>
<td>0.00156</td>
<td>0.00144</td>
</tr>
<tr>
<td>LOD (µg mL⁻¹)</td>
<td>0.173</td>
<td>0.232</td>
</tr>
<tr>
<td>LOQ (µg mL⁻¹)</td>
<td>0.523</td>
<td>0.705</td>
</tr>
</tbody>
</table>

LOD: Limit of detection, LOQ: Limit of quantitation.

Table 1: Analytical parameters of the proposed spectrophotometric method for determination of the investigated drugs.

<table>
<thead>
<tr>
<th>Sample No</th>
<th>Taken (µg/ml)</th>
<th>Found (µg/ml)</th>
<th>% Recovery</th>
<th>Taken (µg/mL)</th>
<th>Found (µg/mL)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.70</td>
<td>100.14</td>
<td>1.00</td>
<td>1.00</td>
<td>100.32</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.00</td>
<td>100.02</td>
<td>3.00</td>
<td>3.00</td>
<td>100.25</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3.00</td>
<td>100.04</td>
<td>5.00</td>
<td>5.00</td>
<td>100.16</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5.00</td>
<td>100.11</td>
<td>7.00</td>
<td>6.99</td>
<td>99.87</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>7.00</td>
<td>102.19</td>
<td>8.00</td>
<td>8.01</td>
<td>100.17</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>100.5</td>
<td>100.15</td>
<td></td>
<td>0.94</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>0.70</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mean of three determinations

Accuracy and precision

Accuracy was checked at five concentration levels within the specified range. Three replicate measurements were recorded at each concentration level. The results were presented as percentage recovery ± standard deviation. The obtained results show good agreement between the measured and true value indicating high accuracy of the proposed method, Table 2.

Precision was examined at three concentration levels; three replicate measurements were recorded at each concentration level; both inter-day and intra-day precision were evaluated and their results were summarized in Table 3. The calculated relative standard deviations were below 2 % indicating excellent precision of the proposed procedure at both level of repeatability and intermediate precision.

Table 3: Evaluation of interday and intraday precision of the proposed method for determination of cited drugs.

<table>
<thead>
<tr>
<th>Precision level</th>
<th>Conc (µg mL⁻¹)</th>
<th>% Recovery ± SD</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAL</td>
<td>FEN</td>
<td>SAL</td>
<td>FEN</td>
</tr>
<tr>
<td>Intraday</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>98.78±0.88</td>
<td>99.49±0.66</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>99.37±0.75</td>
<td>99.89±0.41</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>99.81±0.59</td>
<td>99.79±0.39</td>
</tr>
<tr>
<td>Interday</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>99.20±1.1</td>
<td>99.58±0.63</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>99.58±0.73</td>
<td>100.71±1.05</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>99.75±0.30</td>
<td>99.75±0.65</td>
</tr>
</tbody>
</table>

*Mean of three determinations.

Limit of detection (LOD) and limit of quantitation (LOQ)

The limits of detection (LOD) and quantification (LOQ) were calculated based on standard deviation of response and the slope of calibration curve using the equations; LOD=3.3σ/S and LOQ=10σ/S, where S is the slope of the calibration curve and σ is the standard deviation of intercept. The results were presented in Table 1. The limit of quantitation was 0.705 µg mL⁻¹ and 0.523 µg mL⁻¹ for Fenoterol and Salmeterol, respectively indicating high sensitivity of the proposed method compared with the reported reversed phase HPLC method for determination of salmeterol metered dose inhalers (with detection level 2µg mL⁻¹) (Nayak et al., 1996), and the reported spectrophotometric method for Fenoterol (Abounassif and Abdel-Moey, 1989). The high sensitivity of the proposed method gave it the advantage over other methods that require sophisticated and expensive instruments.

Robustness

Robustness of the suggested procedure was assessed by evaluating the influence of small variation in experimental variables (volume of reagent, temperature, and heating time). The results presented in Table 4, indicated that small variations in any of these variables did not significantly affect the performance of the suggested procedure. This gave an indication for the reliability of the proposed method during routine work.
The proposed method was successfully applied for determination of the investigated drugs in their pharmaceutical preparations. The results of the proposed method were statistically compared with those of the reported methods (Abounassif and Abdel-Moey, 1989; Samir et al., 2012) using student’s t-test and F-test with respect to accuracy and precision. There was no significant difference between the proposed and reported methods as the calculated values did not exceed the theoretical values at 95% confidence level, Table 5. This indicates good level of precision and accuracy of the proposed method.

CONCLUSION

In this study, accurate and sensitive spectrophotometric method was established for determination of Salmeterol xinafoate and Fenoterol hydrobromide with limits of detection 0.173 and 0.232 µg mL⁻¹, respectively. The proposed method was successfully applied for determination of investigated drugs in their dosage formulations. The suggested method is simple, cost effective and can be used for routine analysis in any analytical laboratory with good accuracy and precision.

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REFERENCES


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