Spectrophotometric estimation of formoterol fumerate in pharmaceutical formulations

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ABSTRACT

Three simple, accurate, rapid and sensitive methods (A, B and C) have been developed for the estimation of Formoterol Fumerate in its pharmaceutical dosage form. The Method A is based on the formation of orange red colored chromogen, due to reaction of Formoterol Fumerate with p-Dimethyl amino cinnamaldehyde (PDAC) reagent in methanol, which exhibits \( \lambda_{max} \) at 538 nm. Method B is based on the reaction of Formoterol Fumerate with 3-methyl-2-benzothiazolinone hydrazone (MBTH) in the presence of Cerric ammonium sulphate to form a green colored chromogen, which shows maximum absorbance at 635 nm. The Method C is based on the formation of red colored chromogen with Ferric Chloride and 2,2'-bipyridyl which shows absorption maximum at 520 nm. The absorbance-concentration plot is linear over the range of 5-60 mcg/ml for Method A, 5-25 mcg/ml for Method B and 1-12 mcg/ml for Method C. Results of analysis for all the methods were validated statistically and by recovery studies. The proposed methods are economical and sensitive for the estimation of Formoterol Fumerate in bulk drug and in its formulations (Rotacaps).

Key words: Uv-visible spectrophotometry, formoterol fumerate, ferric chloride, p-dimethylaminocinnamaldehyde (pdac), 2,2'-bipyridyl, 3-methyl,2-benzothiazolinone hydrazone,(mbth).

INTRODUCTION

Formoterol Fumerate is chemically (±)-2'-Hydroxy-5'-([(RS)-1-hydroxy-2-((RS)-p-methoxy-alpha-methylphenyl] amino) ethyl] formanilide fumerate. It is official in Japanese and European pharmacopoeias. It is a long acting and potent \( \beta_2 \) agonist, with duration of action up to 12 hrs. Survey of literature reveals that the drug is determined by using HPLC⁵, Gas chromatography⁶, Capillary Electrophoresis⁴, and very few Spectrophotometric methods⁵.⁶. The present study describes simple, sensitive, accurate, rapid and economical Spectrophotometric Methods A, B, C and D for the estimation of Formoterol Fumerate in its formulations (Rotacaps).

EXPERIMENTAL

Instrument

Elico Ultraviolet-Visible double beam spectrophotometer SL-164 with 1 cm matched quartz cells was used for all spectral measurements.
Reagents

All the chemicals used were of analytical reagent grade.

1. 2,2'-bipyridyl- (0.2 M) AR Grade: 780 mg 2,2'- bipyridyl in 25 ml of distilled water.

2. o-Phosphoric acid-(0.2 M) AR Grade:

3. Ferric chloride hexahydrate (0.03 M) AR Grade: 405 mg of Ferric chloride hexahydrate is dissolved in 50 ml of distilled water.

4. 3-methyl 2-benzothiazolinone hydrazone (MBTH) AR Grade - (0.2% w/v): 200 mg of MBTH is dissolved in 100 ml of distilled water.

5. Ceric ammonium sulphate-(0.2 % w/v) AR Grade : 200 mg of CAS in 100 ml of sulphuric acid (0.72 M).

6. p- Dimethylamino cinnamaldehyde- 5%w/v (PDAC) AR Grade : 5 gm of PDAC is dissolved in methanol.

7. Methanol AR grade.

8. Sulfuric acid(0.1N) AR Grade.

Procedure

Standard stock solution was prepared by dissolving 10 mg of Formoterol Fumerate in 1.5 ml of 10% Hydrochloric acid .The volume was made up to 10 ml with distilled water to get a concentration of 1000 mcg/ml. This was further diluted to get the working standard solution of 100 mcg/ml.

Assay procedure

Method A

Aliquots of standard drug solution of Formoterol Fumerate 0.5 – 6.0 ml (100 mcg/ml) were taken and transferred into series of 10 ml graduated test tubes. To each test tube 2ml of methanolic p- Dimethylamino cinnamaldehyde (5%w/v) (PDAC) and 0.5ml of H₂SO₄(0.1N) were added. After thoroughly shaking, the test tubes were set aside for 10 mins, for the reaction to complete. The volumes in each test tube were adjusted to 10 ml with methanol. The absorbances of the solutions were measured at 538 nm against reagent blank, and the calibration curve was plotted. Similarly the absorbance of the sample solution was measured, and the amount of Formoterol Fumerate was determined by referring to the calibration curve.

Method B

Aliquots of standard solutions containing 0.5-8.0 ml (1ml= 50µg/ml) were transferred into series of 10 ml graduated test tubes, 1 ml of MBTH (0.1 % w/v) and 1.0 ml of Ceric ammonium sulphate (0.1% w/v) were added to each test tube. The volume was made upto 10 ml with distilled water. The absorbance of the green colored species was measured at 635 nm against reagent blank. The colored species is stable for 45 min. The amount of Formoterol Fumerate present in the sample solution was computed from its calibration curve.

Method C

Aliquots of standard drug solution of Formoterol Fumerate 0.5-6.0 ml (50 mcg/ml) were taken and transferred into series of graduated test tubes. To each test tube 2 ml of Ferric chloride (0.03M) and 2 ml of 2’2-bipyridyl (0.2M) and 0.2 ml of o-Phosphoric acid were added. The test tubes were allowed to stand in water bath at 70° c for 20 mins. The test tubes were then cooled to room temperature and the solutions were made up to 10 ml with distilled water. The absorbance of the red colored chromogen was measured at 520 nm against reagent blank and a calibration curve was constructed. The absorbance of the sample solution was measured, and the amount of Formoterol Fumerate was determined by referring to the calibration curve.

The methods were extended for the determination of Formoterol Fumerate from Rotacap formulations, (Foratec 12mcg, Cipla). The total contents of 20 Rotacaps were weighed, powdered and powder equivalent to 100 mcg was dissolved in 10 ml of distilled water containing 1.5 ml of 10% HCl. The above solution was further diluted and analyzed as described, in the above-mentioned methods. The analysis procedure was repeated three times with Rotacap formulations and the results of analysis are shown in Table 2.

Recovery Studies: To ensure the accuracy and reproducibility of the results obtained, adding known amounts of pure drug to the previously analyzed formulated samples and these samples were reanalyzed by the proposed method and also performed recovery experiments. The percentage recoveries thus obtained were given in Table 2.
RESULTS AND DISCUSSIONS

In the present study, the Method A involves quantitative reaction of the drug with PDAC reagent. The reaction is based on the condensation of formoterol fumerate with methanolic p-dimethylaminocinnamaldehyde, in acidic media thereby producing orange red colored chromogen with maximum absorbance of 538 nm. Stability study of the developed chromogen was carried out by measuring the absorbance values at time intervals 15 min for 3 hrs, and it was found to be stable for more than 3 hrs at room temperature. The linearity was found to be in the concentration range of 5-60 mcg/ml.

The Method B is based on the oxidation reaction between MBTH and Cerric ammonium sulphate results on removal of electron gives highly reactive electrophile. This electrophile attacks the aromatic ring containing phenolic-OH group to produce oxidative coupling reaction, which forms a green colored complex with maximum absorbance of 635nm. The linearity was found to be in the concentration range of 2.5-25 mcg/ml. The colored chromogen was stable for 3 hrs.

The Method C is based on the reduction of Ferric chloride to ferrous form by the drug, which forms complex with 2,2'-bipyridyl to yield red colored chromogen, having absorbance maximum at 520 nm. The linearity was found to be in the concentration range of 1-12 mcg/ml.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Method A</th>
<th>Method B</th>
<th>Method C</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_{\text{max}} ) (nm)</td>
<td>538</td>
<td>635</td>
<td>520</td>
</tr>
<tr>
<td>Beer’s law limits</td>
<td>5-60</td>
<td>2.5-40</td>
<td>1-12</td>
</tr>
<tr>
<td>Molar absorptivity (l/mol.cm)</td>
<td>(2.524 \times 10^3)</td>
<td>(2.72 \times 10^3)</td>
<td>(8.36 \times 10^3)</td>
</tr>
<tr>
<td>Sand ell’s sensitivity (micrograms/cm²/0.001 absorbance unit)</td>
<td>0.1242</td>
<td>0.311</td>
<td>0.104</td>
</tr>
<tr>
<td>Regression Equation* (Y)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope (m)</td>
<td>0.008</td>
<td>0.003</td>
<td>0.01</td>
</tr>
<tr>
<td>Intercept (c)</td>
<td>0.0077</td>
<td>0.03</td>
<td>-0.0073</td>
</tr>
<tr>
<td>Correlation Coefficient(r)</td>
<td>0.9999</td>
<td>0.9995</td>
<td>0.9989</td>
</tr>
<tr>
<td>Precision (%Relative Standard Deviation)</td>
<td>0.789</td>
<td>0.269</td>
<td>0.29</td>
</tr>
<tr>
<td>Standard error of mean</td>
<td>0.0174</td>
<td>0.0142</td>
<td>0.0149</td>
</tr>
</tbody>
</table>

*Y=mx+c, where X is the concentration in micrograms/ml and Y is absorbance unit.

Table 2: Assay of formoterol fumerate in rotacap formulations

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Labelled amount (mcg)</th>
<th>Amount taken for 20 rotacaps (mcg)</th>
<th>Amount obtained (mcg)*</th>
<th>%% Recovery by the proposed method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Method A</td>
<td>Method B</td>
<td>Method C</td>
</tr>
<tr>
<td>1</td>
<td>12</td>
<td>240</td>
<td>238.5</td>
<td>240.5</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>240</td>
<td>241.5</td>
<td>241.3</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>240</td>
<td>239.3</td>
<td>239.8</td>
</tr>
</tbody>
</table>

*Average of three determinations.
** After spiking the sample.
concentration of 1-12 mcg/ml. The colored chromogen was stable for 2 hrs.

The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and Sandell's sensitivity are presented in Table 1.

The regression analysis using the method of least squares was made for slope (m), intercept (b) and correlation obtained from different concentrations and the results are summarized in Table 1.

The reproducibility and precision of the methods are very good as shown by the low values of coefficient of variance (CV). The Mean percentage recovery value of 99.5% for Method A, 100.5% for Method B and 100.8% for Method C, indicates non-interferences from the formulation excipients. All the validated parameters are summarized in Table 2.

In conclusion, the proposed methods are simple, sensitive, accurate and economical for the routine analysis of Formoterol Fumarate in bulk and in its formulations (Rotacaps).

ACKNOWLEDGEMENTS

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REFERENCES