Stability indicating LC method for estimation of Formoterol fumarate and Mometasone furoate in respicaps dosage form

H K Jain*, 1, P R Bhangale1 & S S Satam2
1Department of Quality Assurance Techniques, STES’s Sinhgad College of Pharmacy, Vadgaon (Bk.), Pune 411 041, India
2Watson Pharma Pvt. Ltd., Ambernath, Thane 421 506, India
Email: hemantkjain2001@yahoo.co.in

Received 30 July 2013; accepted 9 June 2016

A stability indicating high-performance liquid chromatographic (HPLC) method for simultaneous estimation of Formoterol fumarate and Mometasone furoate in respicaps has been represented. The chromatographic conditions employed for the estimation includes a reversed phase Inertsil C8 column (250 × 4.6 mm, 5µ) at 25°C, a mixture of ammonium acetate buffer solution (0.05M, pH 5.0) and acetonitrile (30:70) as mobile phase at 0.8mL/min flow rate and UV detection at 247 nm. The retention time of Formoterol fumarate and Mometasone furoate are found to be 3.06 min and 8.67 min, respectively. Linearity range for Formoterol fumarate and Mometasone furoate are found at 0.75 -2.25 µg/mL and 25-75 µg/mL, respectively with good correlation coefficients. The sample solution undergoes significant degradation under acidic, basic and oxidation stress conditions. Chromatograms of the stress studies indicate that obtained peaks were spectrally pure during peak purity studies. The method is validated as per ICH guidelines for precision, accuracy and robustness studies. Results suggest that the developed methods can be efficiently used for routine quality control analysis as well as stability indicating assay of Formoterol fumarate and Mometasone furoate in respicaps.

Keywords: Formoterol fumarate, Mometasone furoate, Simultaneous estimation, Stability indicating assay method, HPLC, Validation

Formoterol fumarate dihydrate (FF) is chemically, N-[2-Hydroxy-5-[(1RS)-1-hydroxy-2-[(1RS)-2-(4-methoxy-phenyl)-1-methylethyl]amino] ethyl]phenyl] formamide(E)-butanedioate dihydrate1 (Fig. 1). FF is a selective long acting β2 agonist belongs to bronchodilator class, which is used to treat asthma by relaxing bronchial smooth muscles2,3. FF is official in USP4, EP5, IP6 and BP7. Mometasone furoate (MF) is chemically known as 9, 21-dichloro-11β-hydroxy-16α-methyl-3, 20-dioxopregna-1, 4-diene-17-yl furan-2-carboxylate8 (Fig. 2). MF is a synthetic corticosteroid which suppresses bronchial inflammation, increases peak expiratory flow rate and prevent episodes of acute asthma3,9. MF is official in BP10, USP11 and EP12. Combined dosage form of FF and MF represents two different classes of medication and has different effects on clinical, physiological and inflammatory indices of asthma7.

Literature survey revealed that several analytical methods have been reported including HPLC13-16, GC17 and UV spectrophotometry18 for the estimation of FF either in single component or in multi-component dosage form. Various methods have been published for estimation of mometasone furoate alone or in combination with other drugs including UV spectrophotometry19 and supercritical fluid chromatography20.

Improper storage may be cause of expiration of drug before expiry date and formation of degradation products, which may be inactive, less active and sometimes toxic. Therefore, determination of such degradation products is important. A stability indicating assay plays vital role for determination of the degradation products formed during stress condition21,22. However, no stability indicating RP-HPLC assay method has been reported so far, for determination of formoterol fumarate and mometasone furoate in respicaps. Hence, the aim of the present work was to develop a stability-indicating RP-HPLC method for simultaneous estimation of Formoterol fumarate and Mometasone furoate in respicaps23,24.

Experimental Section
Agilent 1200 series HPLC System equipped with Empower & Chemstation softwares, inbuilt solvent degasser system, Quaternary pump, UV visible/photodiode array detector, variable injector...
and auto sampler, was employed for this study. Schimadzu UV-1800 spectrophotometer with matched quartz cells, was used for selection of wavelength for detection. Weighing of the drug was performed using an Electronic Balance (Sartorius ME5). Equiptronics EQ-614 pH meter and Spectralab UCB-40 Ultrasonicator were also used for this work.

Reagents and materials

The active pharmaceutical ingredients of Formoterol fumarate dihydrate and Mometasone furoate were supplied as gift sample by Zydus Cadila Pvt. Ltd., Ahmedabad (India). Respicaps (Formost 200, each capsule contains 6 µg of Formoterol fumarate dihydrate and 200 µg of mometasone furoate) were procured from the market, HPLC grade acetonitrile, ammonium acetate buffer, glacial acetic acid were obtained from Merck specialties Pvt. Ltd., Mumbai (India), Acrodisc PSF GHP 0.45 µm nylon filters were purchased from Pall Life Science limited, Mumbai (India). High purity water was generated by using Milli-Q Plus water purification system (Merck Millipore).

Method

Chromatographic conditions

Chromatographic separation was achieved on a reversed phase Inertsil C₈ column at 25°C using mobile phase consisting of a mixture of ammonium acetate buffer (7.7 g ammonium acetate in 2 L purified water and pH was adjusted to 5.0 with glacial acetic acid) and acetonitrile in the ratio of 30:70 v/v, at 0.8 mL/min flow rate and detection was carried out at 247 nm. The injection volume was 30 µL and sample temperature was maintained at 5±0.1°C.

Preparation of standard stock solutions

Formoterol fumarate dihydrate (6.0 mg of formoterol fumarate) was accurately weighed and transferred into a 200 mL of volumetric flask. About 120 mL of mobile phase was added followed by sonication for 10 min, to dissolve the content. The volume was made up to mark with mobile phase. Similarly, mometasone furoate (20 mg) was accurately weighed and transferred into a 20 mL of volumetric flask. About 10 mL of mobile phase was added followed by sonication for 10 min, to dissolve the content. The volume was made up to mark with mobile phase.

Preparation of working standard solution

Working standard solution containing 1.5 µg/mL of Formoterol fumarate dihydrate and 50 µg/mL of Mometasone furoate, was prepared by transferring 5 mL each of standard stock solution (Formoterol fumarate dihydrate and Mometasone furoate) into a 100 mL volumetric flask and volume adjustment with mobile phase.

Sample preparation

Twenty capsules were accurately weighed and average weight was calculated. Capsule shells were removed. A quantity of powder (equivalent to 30 µg of Formoterol fumarate dihydrate and 1000 µg of Mometasone furoate) was weighed and transferred into 20 mL of volumetric flask. About 15 mL of mobile phase was added to dissolve the content followed by sonication for 30 min with occasional swirling. The volume was made up to mark with same solvent. The solution was filtered through 0.45 µ Acrodisc GHP filter and injected into the HPLC system.

Induced degradation of formoterol fumarate and mometasone furoate

(i) Acid and base induced degradation

A quantity of powder (equivalent to 30 µg of formoterol fumarate and 1000 µg of mometasone
furoate) was weighed and transferred into 20 mL of volumetric flask. About 15 mL of mobile phase was added to dissolve the contents followed by sonication for 30 min with occasional swirling. Then 2 mL of 1 N HCl and 2 mL of 1 N NaOH were added, separately. The contents of volumetric flasks were refluxed on water bath at 80°C for 1 h for acid induced degradation and for 30 min for base induced degradation. The samples were allowed to cool at room temperature, neutralized and volume was made up to mark with mobile phase. These solutions were filtered through 0.45 µ Acrodisc GHP filter and analyzed using HPLC system.

(ii) Degradation by oxidation
The method described above (i) was followed except that 2 mL of 3% H₂O₂ was added in place of HCl.

(iii) Thermal degradation
The method described above (i) was followed without the addition of H₂O₂ or HCl/NaOH. The sample was refluxed on a water bath previously maintained at 80°C for 3 h.

(iv) Photolytic degradation
The method described above (i) was followed without the addition of H₂O₂ or HCl/NaOH. The sample was exposed to UV light for 12 h.

Detection of impurities
The same method as described under sample preparation was followed and the resulting solution was kept at room temperature for 24 h referred as no treatment or control sample.

Results and Discussion

HPLC method development and optimization
Optimization of the chromatographic conditions was done by performing several trials in order to develop a stability indicating assay method for simultaneous estimation of Formoterol fumarate and Mometasone furoate. Development of this method was challenging due to extreme ratio (3:100) of the drugs present in the combined dosage form. Detection wavelength was selected at 247 nm on basis of significant absorption of both drugs. Three different chromatographic columns were tried namely Zorbax-SB-C8 (150 × 4.6 mm, 5 µm), thermosil BDS (250 × 4.6 mm, 5 µm), Inertsil ODS (250 × 4.6 mm, 5 µm). The Zorbax-SB-C8 and thermosil BDS columns have lower retention time for formoterol fumarate. Several mobile phases were attempted containing different composition of buffers (sodium dihydrogen phosphate, ammonium acetate at different pH between 3 to 6), acetonitrile, methanol and water by isocratic and gradient elution. Here, chromatogram obtained by mobile phase containing acetonitrile, water containing acetic acid (50:50 v:v); sodium dihydrogen phosphate buffer and acetonitrile (84:16 v:v) were not showing satisfactory results. Finally, optimized conditions for better chromatographic separation were achieved on the basis of peak shape, resolution, theoretical plates and tailing factor using Inertsil C8 (250 × 4.6 mm, 5µm) column and 0.05M ammonium acetate buffer, pH 5.0 and acetonitrile in the ratio of 30:70 v/v as mobile phase with isocratic elution. System suitability test were carried out on freshly prepared standard solution of the drugs as per guidelines. Retention times of Formoterol fumarate and Mometasone furoate were found to be 3.065 and 8.639 min, respectively. The theoretical plates of Formoterol fumarate and Mometasone furoate were found to be 4094 and 12705, respectively and the value of USP resolution was found to be 21.98. The values of USP tailing were also found within acceptance criteria.

Calibration curves
The linearity of response was evaluated for a set of five different standard solutions containing 0.75-2.25 µg/mL and 25-75 µg/mL for Formoterol fumarate and Mometasone furoate, respectively. The calibration curves for both components were constructed by simple linear regression between mean peak area and corresponding concentration. The regression data indicated that response was linear for both components over above mentioned concentration range. The values of correlation coefficient slope and intercept for calibration curve of Formoterol fumarate was found to be 0.999, 49851 and -401.36, respectively. Similarly, values of correlation coefficient, slope and intercept for calibration curve of Mometasone furoate were found to be 0.999, 101266 and 15174, respectively.

Validation of the method
The proposed method was validated for following parameters.

Precision
Repeatability
Intra-day precision was measured in terms of repeatability of method. Repeatability of sample measurement was carried out by injecting six different sample preparations from same homogenous blend of marketed sample and
measuring the peak areas. Percent relative standard deviation (%RSD) of assay of Formoterol fumarate and Mometasone furoate in repeatability was found to be 0.754% and 0.819%, respectively and %RSD of retention time of formoterol fumarate and mometasone furoate was found to be 0.0245% and 0.0314%, respectively (Table 1). This study shows that intra-day precision of the method is satisfactory as % relative standard deviation is within prescribed limit.

**Intermediate precision**

Intermediate precision of the proposed method was demonstrated by performing the experiment by two different analysts, on two different days, using two different instruments. Percent relative standard deviation (%RSD) of assay of Formoterol fumarate and Mometasone furoate was found to be 0.437% and 0.814%, respectively and %RSD of retention time of Formoterol fumarate and Mometasone furoate was found to be 0.0399% and 0.0715%, respectively (Table 1). This study shows that intermediate precision of the method is satisfactory.

**Robustness**

Robustness of the proposed method was studied by applying the method for determination of Formoterol fumarate and Mometasone furoate in sample preparation, with deliberate small changes in method parameters such as mobile phase composition, flow rate, change in pH of buffer, column oven temperature. Such variations did not have significant impact on the results obtained (Table 2) and %RSD of area response as well as retention time was within acceptance criteria.

**Accuracy**

The accuracy of the analytical method was established by performing recovery experiments in triplicate across its range. The results of recovery studies are given in Table 3. The results indicated that the individual recovery of Formoterol fumarate and Mometasone furoate ranges from 98.2 to 101.8% and 98.0 to 100.3%, respectively, which are within accepted limit. The values of % relative standard deviation Formoterol fumarate and Mometasone furoate were found to be 1.88% and 1.23%, respectively. These results suggested accuracy of these developed method.

**Analysis of marketed formulation**

The developed method was successfully applied for simultaneous analysis of both drugs in respicaps. The assay results are presented in Table 4. Contents of formoterol fumarate and mometasone furoate were found to be 99.88% and 101.05%, respectively with satisfactory %RSD value. It is noted that degradation after 24 hour (at room temperature) of Formoterol fumarate and Mometasone furoate had occurred in the marketed formulation with total degradation as 0.46% and content for Formoterol fumarate and Mometasone furoate was found to be 97.94% and

---

**Table 1 — Results of intra-day and intermediate precision data obtained for the method**

<table>
<thead>
<tr>
<th>Condition</th>
<th>% Assay</th>
<th>Retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FF</td>
<td>FF</td>
</tr>
<tr>
<td>Intra-day precision</td>
<td>Average</td>
<td>98.99*</td>
</tr>
<tr>
<td></td>
<td>STDEV</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>% RSD</td>
<td>0.75</td>
</tr>
<tr>
<td>Intermediate precision</td>
<td>Average</td>
<td>99.16</td>
</tr>
<tr>
<td></td>
<td>STDEV</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>% RSD</td>
<td>0.43</td>
</tr>
</tbody>
</table>

* n = 6

**Table 2 — Results of robustness and ruggedness of method**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Variations</th>
<th>Resolution factor</th>
<th>Tailing factor</th>
<th>* % Assay</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Variations</td>
<td>Resolution factor</td>
<td>Tailing factor</td>
<td>* % Assay</td>
<td>% RSD</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.9mL</td>
<td>24.23</td>
<td>1.29</td>
<td>1.035</td>
<td>98.50</td>
</tr>
<tr>
<td></td>
<td>0.65mL</td>
<td>27.05</td>
<td>1.25</td>
<td>1.04</td>
<td>98.63</td>
</tr>
<tr>
<td>Column oven temperature</td>
<td>20ºC</td>
<td>24.52</td>
<td>1.30</td>
<td>1.12</td>
<td>98.41</td>
</tr>
<tr>
<td></td>
<td>30ºC</td>
<td>24.61</td>
<td>1.26</td>
<td>1.06</td>
<td>98.62</td>
</tr>
<tr>
<td>pH (± 0.2 units of the set pH)</td>
<td>pH 4.8</td>
<td>24.65</td>
<td>1.27</td>
<td>1.04</td>
<td>98.63</td>
</tr>
<tr>
<td></td>
<td>pH 5.2</td>
<td>23.67</td>
<td>1.22</td>
<td>1.04</td>
<td>98.58</td>
</tr>
<tr>
<td>Mobile phase composition</td>
<td>Buffer:ACN 40:60</td>
<td>24.55</td>
<td>1.24</td>
<td>1.04</td>
<td>98.65</td>
</tr>
<tr>
<td></td>
<td>Buffer:ACN 25:75</td>
<td>24.42</td>
<td>1.26</td>
<td>1.04</td>
<td>98.63</td>
</tr>
</tbody>
</table>

*The data indicates average of triplicate (n=3) determinations.
JAIN et al.: STABILITY INDICATING LC FOR FORMOTEROL FUMARATE & MOMETASONE FUROATE

This study indicated suitability of the method for routine quality control analysis of both components in pharmaceutical dosage form.

% Assay of Formoterol Fumarate
\[
\frac{AT}{AS} \times \frac{WS}{WT} \times \frac{DT}{100} \times \frac{P \times \text{Avg. Wt. (mg)}}{\text{Label Claim}} \times \frac{804.92}{840.92}
\]

% Assay of Mometasone Furoate
\[
\frac{AT}{AS} \times \frac{WS}{WT} \times \frac{DT}{100} \times \frac{P \times \text{Avg. Wt. (mg)}}{\text{Label Claim}}
\]

where:
- AT = Peak Area of sample preparation
- AS = Peak Area of standard preparation.
- WS = Weight of working standard taken in mg.
- WT = Weight of sample taken in mg.
- DS = Dilution of Standard solution.

DT = Dilution of sample solution.

P = Percentage purity of working standard

804.92 = Mol. Wt of Formoterol fumarate (factor)

840.92 = Mol. Wt of Formoterol fumarate dihydrate (factor)

 Degradation behavior and stability indicating property

No treatment sample and rest of the stress condition samples were evaluated against standard solution and %degradation was calculated. The values of %assay, %degradation and peak purity are given in Table 5 and representative chromatogram is shown in Fig. 3. The chromatogram of no treatment sample indicates that single degradation product was obtained at retention time (RT) of 8.914 min. The chromatogram of acid degraded sample showed sufficient degradation (Fig. 4a); here mometasone furoate had higher degradation as compared to formoterol fumarate. The major degradation peaks
Fig. 3 — Typical chromatogram of formoterol fumarate and mometasone furoate standard solution

Fig. 4 — (a) Chromatograms of acid hydrolysis studies of formoterol fumarate and mometasone furoate; (b) Chromatograms of base hydrolysis studies of formoterol fumarate and mometasone furoate; (c) Chromatograms of oxidation studies of formoterol fumarate and mometasone furoate
were appeared at RT of 7.0, 8.87 and 10.27 min. The chromatogram of alkali degraded sample showed that both drugs were found to be highly labile to alkaline hydrolysis (Fig. 4b). The major degradation peaks were appeared at RT of 4.6, 8.87 and 10.32 min. Sufficient degradation was observed during oxidation and major degradation peaks were appeared at RT of 7.0 and 8.84 min (Fig. 4c). Thermal degradation study showed that both drugs had not significant degradation and degradation peak was appeared at RT of 3.62 min. The photolytic degradation study indicated that both drugs are relatively stable after long term exposure and single degradation peak was appeared at RT of 8.9 min. In each condition, the peak purity of both drugs as determined by diode array detector was greater than 0.98. This study indicated the specificity of the method.

Conclusion
The developed RP-HPLC method can be employed for stability indicating assay as the method successfully separated the drug substances from their degradation products. The proposed method has linear response for both drugs in stated range. Validation studies proved that developed method is specific, precise, accurate and robust for simultaneous analysis of both components. Results suggested that the developed methods can be efficiently used for routine quality control analysis of formoterol fumarate and mometasone furoate in respicaps.

Acknowledgement

Authors thank Watson Pharma Pvt. Ltd. Ambernath (India) for providing the facilities and valuable guidance to carry out this work. Authors also thank Zydus Cadilla Pvt. Ltd., Ahmadabad (India) for providing gift sample of formoterol fumarate dihydrate and mometasone furoate API for this research study.

References