ORIGINAL ARTICLE

Validated chromatographic methods for the simultaneous determination of Mometasone furoate and Formoterol fumarate dihydrate in a combined dosage form

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Mometasone furoate; Formoterol fumarate dihydrate; TLC-densitometry; High performance liquid chromatography; Isocratic elution

Abstract
Two chromatographic methods were developed and validated for the simultaneous determination of Mometasone furoate (MO) and Formoterol fumarate dihydrate (FOR). Combination of MO and FOR is used for the treatment of asthma in patients suffering from reversible obstructive airway disease. The first chromatographic method was based on using aluminum TLC plates pre-coated with silica gel GF254 as the stationary phase and chloroform:ethyl acetate:methanol:toluene:formic acid (5:2:2:2:0.1, by volume) as the mobile phase followed by densitometric measurement of the separated bands at 233 nm. The second method is a high performance liquid chromatographic method for the separation and determination of MO and FOR using reversed phase C18 column with isocratic elution. The mobile phase composed of methanol:0.5% ammonium acetate pH adjusted with acetic acid (80:20, v/v) at a flow rate of 1.0 mL/min. Quantitation was achieved with UV detection at 220 nm. The specificity of the developed methods was investigated by analyzing the pharmaceutical dosage form. The validity of the proposed methods was assessed using the standard addition technique. The obtained results were statistically compared with those obtained by the reported methods, showing no significant difference with respect to accuracy and precision at p = 0.05.

1. Introduction

Asthma is a chronic inflammatory disorder of the airways. During asthma attacks, the smooth muscle cells in the bronchi constrict, the airways become inflamed and swollen, and breathing becomes difficult.1 Therefore one of the ways of treating it is a combination of inhaled corticosteroids to reduce the inflammation of the airways and prevent the loss of lung functions2 with long acting β2 agonists (LABA) which acts locally on the lung as a bronchodilator and relaxes muscles

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in the airways to improve breathing. An example of this combination is Mometasone furoate (MO), (9α,21-dichloro-1β,17-dihydroxy-16α-methylpregna-1,4-diene-17-yl furan-2-carboxylate) (Fig. 1a) which acts as a corticosteroid and Formoterol fumarate dihydrate (FOR), (N-[2-hydroxy-5-[(1RS)-1-hydroxy-2-[(1RS)-2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyllformamidine(E)-butenedioate dehydrate) (Fig. 1b) which acts as a long acting β2 agonist.

Literature survey reveals that MO and FOR are official drugs in European Pharmacopoeia, also MO is official in United States Pharmacopeia. Several analytical methods have been reported for the determination of MO alone or in combinations with other drugs including, spectrophotometry, TLC and HPLC. Besides, several methods have been reported for the determination of FOR alone or in combinations including, non aqueous titration, spectrophotometry, voltammetry, capillary electrophoresis, and HPLC. The aim of this work is to develop simple chromatographic methods for the simultaneous determination of MO and FOR in pharmaceutical dosage form.

2. Experimental

2.1. Instruments

The thin-layer chromatographic (TLC) system consisted of a Camag Linomat autosampler (Muttenz, Switzerland), a Camag microsyringe (100 µL) and a Camag 35/N/30319 TLC scanner with winCATS software; an ultraviolet (UV) lamp with a short wavelength at 254 nm (Desaga, Wiesloch, Germany); and TLC plates precoated with silica gel GF254 (10 × 20 cm, 0.25 mm thickness (Merck, Darmstadt, Germany)).

Shimadzu HPLC system consisted of a pumping system (model LC-10 AD vp), an ultra-violet variable wavelength detector (model SPD-10A vp), Degasser (model DGU-12A) and System controller (model SCL-10A vp) Equipped with a prominence autosampler (model SIL-20A) (Shimadzu, Kyoto, Japan). An Inertsil ODS-3 column (5 µm, 250 mm × 4.6 mm i. d.) was used as stationary phase (GL Sciences, Tokyo, Japan).

2.2. Materials and reagents

2.2.1. Pure standard

Mometasone furoate was kindly supplied by SIGMA Pharmaceutical Industries, Cairo, Egypt, its purity was found to be 100.12 ± 0.762 according to the official method.

Formoterol fumarate dihydrate was kindly supplied by NOVARTIS pharmaceuticals, Cairo, Egypt, its purity was found to be 100.02 ± 0.592 according to the reported method.

2.2.2. Pharmaceutical dosage form

Dulera® Inhalation aerosol (Batch No. GLG122) labeled to contain 100 µg of MO and 5 µg of FOR per actuation, was manufactured by (MERCK & CO. INC, White House Station, USA) and obtained from the American market.

2.2.3. Chemicals and reagents

All chemicals used throughout the work were of analytical grade and solvents for HPLC were of HPLC grade. These included methanol (Sigma-Aldrich, Belgium), chloroform (Sigma-Aldrich, Belgium) and double distilled deionized water (Otsuka, Cairo, Egypt). Ethyl acetate, toluene, formic acid and ammonium acetate were purchased from Al-Nasr Pharmaceutical Chemicals Co., Cairo, Egypt.

2.2.4. Standard solutions

– Standard stock solution of MO: 1.0 mg/mL in methanol.
– Standard stock solution of FOR: 1.0 mg/mL in methanol.

2.2.5. Working Solutions

For TLC-spectrodensitometric method: Working solution of FOR (200 µg/mL) was prepared from its stock solution using methanol as a solvent.

For HPLC method: Working solutions of MO (400 µg/mL) and FOR (100 µg/mL) were prepared from their respective stock solutions using mobile phase as a solvent.

2.3. Procedures

2.3.1. Construction of the calibration curves

2.3.1.1. For TLC-spectrodensitometric method. Accurately measured aliquots of MO stock standard solution (1 mg/mL) and FOR working solution (200 µg/mL) were spotted onto TLC plates using Camag Linomat autosampler with microsyringe (100 µL). The plates were then developed by the ascending technique using chloroform:methanol:ethyl acetate:toluene:formic acid (5:2:2:2:0.1, by volume) as a mobile phase. The plates were then removed and air-dried. The chromatogram was scanned at 233 nm. Calibration curves representing the relationship
between integrated peak area and the corresponding concentrations of each of MO (2–14 μg/band) and FOR (0.1–5 μg/band) were plotted.

2.3.1.2. For HPLC method. Aliquots equivalent to 100–3000 μg of MO and 10–500 μg of FOR were accurately measured and transferred from their working solutions into a set of 10-mL volumetric flasks and the volumes were completed to the mark with the mobile phase (methanol: 0.5% ammonium acetate pH 5.7 (80:20; v/v)). A 20-μL aliquot of each solution was injected into an Inertsil ODS-3 column (5 μm, 250 mm × 4.6 mm i.d.), using the mobile phase, at flow rate 1.0 mL/min and UV detection at 220 nm. Two calibration curves were constructed by plotting the relative peak area, using 100 μg/mL of MO and 25 μg/mL of FOR as the external standards, against the corresponding concentrations of each drug.

2.3.2. Application to pharmaceutical formulations

The actuator after shaking was inverted and placed in a beaker containing 4 mL methanol, and then the beaker was covered tightly. Ten actuations were delivered in the beaker, then the actuator was washed with methanol. The solution was transferred accurately into 10-mL volumetric flask and the volume was completed with methanol to prepare dosage form solution containing 100 μg/mL MO and 5 μg/mL FOR. For TLC-spectrodensitometric determination of both drugs, 40 μL was applied onto TLC plates.

For HPLC analysis, dosage form solution was prepared as mentioned above but completing the volume with the mobile phase instead of completing with methanol and then injected to the column. The procedure was completed as mentioned under construction of calibration curves for each method. The concentration of MO and FOR was calculated from the corresponding regression equations.

3. Results

Several trials were conducted to develop the optimum chromatographic conditions for the sufficient separation of both drugs including chloroform:methanol (2:8, v/v), methanol:toluene (8:2, v/v) and chloroform:methyl acetate:toluene (5:4:3, by volume) but bad resolution was obtained. The results of the TLC system were satisfactory when using chloroform:methyl acetate:methanol:toluene:formic acid (5:2:2:2:0.1, by volume) as the mobile phase. \( R_f \) values were found to be 0.81 ± 0.02 and 0.17 ± 0.02 for MO and FOR, respectively as shown in (Fig. 2). This separation allows the determination of MO and FOR at 233 nm without any interference from each other.

HPLC method was also tried to separate MO and FOR, therefore several trials have been undertaken to reach the optimum stationary/mobile phases matching. Good chromatographic separation of the two drugs in their binary mixtures could be achieved using an Inertsil ODS-3 column (5 μm, 250 mm × 4.6 mm i.d.), with a mobile phase consisting of (methanol: 0.5% ammonium acetate (80:20, v/v) pH 5.7 adjusted by glacial acetic acid) at flow rate 1.0 mL/min, followed by UV detection at 220 nm, (Fig. 3). Calibration curves were plotted for both TLC and HPLC for the determination of the cited drugs.

An overall system suitability testing was calculated (Table 1) to determine whether the operating system performed properly.

![Figure 2](image-url)  
**Figure 2** TLC chromatogram of a resolved mixture containing 0.5 μg/band of FOR and 10 μg/band of MO.
The proposed methods were validated according to International Conference on Harmonization (ICH) guidelines (Table 2). The table also shows the assay parameters of the regression equations and the ranges of concentration.

The proposed methods were successfully applied for the determination of MO and FOR in Dulera® inhaler. The results shown in Table 3 were satisfactory. The validity of the proposed methods was assessed by applying the standard addition technique, no interference due to excipients was observed as shown from the results in Table 3.

The results obtained by applying the proposed methods for the analysis of pure MO and FOR compared to those obtained by applying the official5 and reported methods,19 respectively, they showed no significant difference regarding accuracy and precision Table 4.

Table 1  Parameters required for system suitability test of TLC-densitometric and HPLC methods.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TLC</th>
<th>HPLC</th>
<th>Reference values (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time (tR) [min]</td>
<td>MO 6.925</td>
<td>FOR 2.717</td>
<td></td>
</tr>
<tr>
<td>Retardation factor (Rf)</td>
<td>0.81</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>Tailing factor (T)</td>
<td>0.89</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>Capacity factor (K')</td>
<td>4.76</td>
<td>7.343</td>
<td>1 &lt; K' &lt; 10</td>
</tr>
<tr>
<td>Selectivity factor (α)</td>
<td>3.23</td>
<td>2.273</td>
<td>α &gt; 1</td>
</tr>
<tr>
<td>Resolution factor (Rs)</td>
<td>12.8</td>
<td>12.27</td>
<td>Rs &gt; 2</td>
</tr>
<tr>
<td>Column efficiency (N)</td>
<td>3503</td>
<td>2485</td>
<td>N &gt; 2000</td>
</tr>
<tr>
<td>HETPa [mm]</td>
<td>0.071</td>
<td>0.100</td>
<td></td>
</tr>
</tbody>
</table>

HETPa = height equivalent to theoretical plates (length of column in mm/N).
4. Discussion

Planar chromatography with precise application of the samples and computer controlled evaluation and quantification of the developed chromatograms has been considered to be a reliable technique for purity control and for quantitative drug testing. Therefore the aim of this work is to develop simple, accurate, rapid, specific and valid spectro-densitometric method. This separation allows the determination of MO and FOR without any interference from each other. A polynomial relationship was found to exist between the integrated peak area of the separated spots at the selected wavelength (233 nm) and the corresponding concentrations of MO and FOR, in the range of 2–14 µg/band and 0.1–5 µg/band for MO and FOR, respectively.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TLC</th>
<th>FOR</th>
<th>HPLC</th>
<th>FOR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Range</strong></td>
<td>2–14 µg/band</td>
<td>0.1–5 µg/band</td>
<td>10–300 µg/mL</td>
<td>1–50 µg/mL</td>
</tr>
<tr>
<td><strong>Linearity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>Slope 1 = −0.1286</td>
<td>Slope 1 = −0.3501</td>
<td>0.0083</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Slope 2 = 3.6519</td>
<td>Slope 2 = 4.5039</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>3.0026</td>
<td>0.2202</td>
<td>0.1688</td>
<td>−0.008</td>
</tr>
<tr>
<td>SE of intercept</td>
<td>1.7826</td>
<td>0.3482</td>
<td>0.0048</td>
<td>0.0005</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9999</td>
<td>1</td>
<td>0.9999</td>
<td>1</td>
</tr>
<tr>
<td>Accuracy (mean ± SD)</td>
<td>100.10 ± 0.1039</td>
<td>99.96 ± 0.970</td>
<td>100.24 ± 0.494</td>
<td>100.24 ± 0.819</td>
</tr>
</tbody>
</table>

Table 2: Assay validation sheet of the proposed methods for the simultaneous determination of Mometasone furoate (MO) and Formoterol fumarate dihydrate (FOR).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TLC</th>
<th>FOR</th>
<th>HPLC</th>
<th>FOR</th>
</tr>
</thead>
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<tr>
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<td>0.1–5 µg/band</td>
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<td>1–50 µg/mL</td>
</tr>
<tr>
<td><strong>Linearity</strong></td>
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<td></td>
<td></td>
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<td>0.04</td>
</tr>
<tr>
<td></td>
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<td>Slope 2 = 4.5039</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>1</td>
<td>0.9999</td>
<td>1</td>
</tr>
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</tr>
</tbody>
</table>

Table 3: Determination of Mometasone furoate (MO) and Formoterol fumarate dihydrate (FOR) in their Dosage form and application of standard addition technique using the proposed methods.

4. Discussion

Planar chromatography with precise application of the samples and computer controlled evaluation and quantification of the developed chromatograms has been considered to be a reliable technique for purity control and for quantitative drug testing. Therefore the aim of this work is to develop simple, accurate, rapid, specific and valid spectro-densitometric method. This separation allows the determination of MO and FOR without any interference from each other. A polynomial relationship was found to exist between the integrated peak area of the separated spots at the selected wavelength (233 nm) and the corresponding concentrations of MO and FOR, in the range of 2–14 µg/band and 0.1–5 µg/band for MO and FOR, respectively.

Table 3: Determination of Mometasone furoate (MO) and Formoterol fumarate dihydrate (FOR) in their Dosage form and application of standard addition technique using the proposed methods.

<table>
<thead>
<tr>
<th>Product</th>
<th>Method</th>
<th>Drug</th>
<th>Recovery% ± RSD</th>
<th>Standard addition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dulera® inhalation aerosol (Batch No. GLG122)</td>
<td>TLC</td>
<td>MO</td>
<td>99.60 ± 0.351</td>
<td>4 µg/band</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FOR</td>
<td>101.00 ± 0.910</td>
<td>0.2 µg/band</td>
</tr>
<tr>
<td></td>
<td>HPLC</td>
<td>MO</td>
<td>100.83 ± 0.630</td>
<td>100 µg/mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FOR</td>
<td>101.16 ± 0.436</td>
<td>5 µg/mL</td>
</tr>
</tbody>
</table>

Table 4: Determination of Mometasone furoate (MO) and Formoterol fumarate dihydrate (FOR) in their Dosage form and application of standard addition technique using the proposed methods.
The regression equations were computed for MO and found to be:

\[ A = -0.1286X^2 + 3.6519X + 3.0026 \quad r = 0.9999 \quad \text{for MO} \]

\[ A = -0.3051X^2 + 4.5039X + 0.2202 \quad r = 1 \quad \text{for FOR} \]

where \( A \) is the integrated peak area multiplied by \((10^{2})\), \( X \) is the corresponding concentration in \( \mu g/\text{band} \) and \( r \) is the correlation coefficient.

The suggested chromatographic system for the HPLC method allows complete base line separation at reasonable time. The linearity of the detector’s response of the studied drugs was determined by plotting a relative peak area (calculated following the external standard technique using 100 \( \mu g/\text{mL} \) of MO and 25 \( \mu g/\text{mL} \) of FOR as the external standards for MO and FOR, respectively) versus concentrations and linear correlation was obtained. The regression equations were computed for MO and FOR and found to be:

\[ A = 0.0083C + 0.1688 \quad r = 0.9999 \quad \text{for MO} \]

\[ A = 0.04C + 0.0011 \quad r = 1 \quad \text{for FOR} \]

where \( A \) is the relative peak area, \( C \) is the corresponding concentration in \( \mu g/\text{mL} \) and \( r \) is the correlation coefficient.

By comparing the developed HPLC method with the reported methods, we found that, the developed method is more sensitive and linear over a wider range of concentration 1–50 \( \mu g/\text{mL} \) for FOR and 10–300 \( \mu g/\text{mL} \) for MO than that of the reported method \( 3–9 \mu g/\text{mL} \) for FOR and 100–300 \( \mu g/\text{mL} \) for MO. Also the developed HPLC method shows a shorter retention time, where FOR and MO eluted after 2.7 min. and 6.9 min., while eluted after 3.4 min. and 9.2 min., respectively in the reported method.

4.1. Method validation

The proposed methods were validated according to the ICH Q2 (R1) recommendations. The method was validated for parameters such as system suitability, linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy, precision and selectivity.

4.1.1. System suitability

The system suitability test is an integral part of chromatographic method development and it is used to verify that the system is adequate for the analysis to be performed; the system suitability parameters for MO and FOR were evaluated. The suitability of the chromatographic system was determined according to USP guidelines and with acceptance of the obtained parameter values.

4.1.2. Linearity and ranges

Under the above mentioned experimental conditions, linear relationships were obtained by plotting the drug concentrations either against relative peak areas or integrated peak areas for each drug, for HPLC and TLC methods, respectively.

4.1.3. Accuracy

The accuracy of the proposed methods was validated by analyzing pure samples of each MO, FOR in triplicate. The concentrations of the active drugs were calculated from the corresponding regression equations.

4.1.4. Precision

It was evaluated by calculating intra and inter-day precisions. By repeating the assay of three different concentrations of each of the cited drugs three times in the same day and assaying the same samples in triplicate on three successive days using the developed chromatographic methods and calculating the recovery% and RSD%.

4.1.5. Specificity

The specificity of the developed methods was investigated by analyzing the pharmaceutical dosage form. The spots of the active drugs in the dosage form were confirmed by comparing their \( R_f \) values and densitograms of the spot with that of a standard drugs solutions (in TLC method).

4.1.6. Robustness

It was evaluated by calculating the RSD% of three different concentrations of each of the cited drugs after making a deliberate change in the assay conditions of both TLC and HPLC.

Table 4: Statistical comparison of the results obtained by the proposed methods and the reported methods for the analysis of Mometasone furoate (MO) and Formoterol fumarate dihydrate (FOR).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TLC</th>
<th>HPLC</th>
<th>Reported methods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MO</td>
<td>FOR</td>
<td>MO (5)</td>
</tr>
<tr>
<td>Mean</td>
<td>100.10</td>
<td>99.96</td>
<td>100.24</td>
</tr>
<tr>
<td>SD</td>
<td>1.039</td>
<td>0.969</td>
<td>0.494</td>
</tr>
<tr>
<td>Variance</td>
<td>1.060</td>
<td>0.939</td>
<td>0.244</td>
</tr>
<tr>
<td>( n )</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Student’s ( t )-test</td>
<td>0.038</td>
<td>0.132</td>
<td>0.531</td>
</tr>
<tr>
<td>( F )-test</td>
<td>(2.228)</td>
<td>(2.228)</td>
<td>(2.228)</td>
</tr>
<tr>
<td></td>
<td>(6.16)¹</td>
<td>(6.16)¹</td>
<td>(4.53)²</td>
</tr>
</tbody>
</table>

¹ The figures in parenthesis are the corresponding theoretical values at \( \rho = 0.05 \).
² Official method is HPLC for MO determination using C₈ column (5 μm, 4.6 × 250 mm), methanol:water (65:35, v/v) as a mobile phase at a flow rate of 1.7 mL/min and UV detection at 254 nm.
³ HPLC method for FOR determination using C₁₈ column (5 μm, 4.6 × 150 mm), sodium dihydrogen phosphate buffer:acetonitrile (50:50, v/v) as a mobile phase. pH = 3 adjusted by diluted ortho-phosphoric acid at a flow rate of 1 mL/min and UV detection at 220 nm.
5. Conclusion

The suggested chromatographic methods provide simple, sensitive, accurate and reproducible methods for quantitative analysis of MO and FOR in their binary mixtures and pharmaceutical dosage form. The developed TLC method is highly sensitive. It has the advantages of short run time, large sample capacity and use of minimal volume of solvents. HPLC method gives a good resolution between the proposed components within suitable analysis time; it is highly specific but more expensive. The proposed methods have advantage than other published methods of being more sensitive, simple, lower time consuming and easy in application on inhaler dosage form.

Conflict of interest

The authors declare that there are no conflicts of interest.

References


