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VALIDATED METHOD DEVELOPMENT FOR ESTIMATION OF FORMOTEROL FUMARATE AND MOMETASONE FUROATE IN METERED DOSE INHALATION FORM BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A simple, precise, accurate, and stability-indicating method is developed and validated for analysis of formoterol fumarate and mometasone furoate in metered dose inhalation formulations. Separation was achieved on a reversed-phase C₁₈ column (150 mm X 4.6 mm i.d., 5µm) using a mobile phase consisting of Sodium dihydrogen orthophosphate buffer/acetonitrile (50:50,v/v) at a flow rate of 1.0 mL/min and UV detection at 220 nm. This method is validated according to United States Pharmacopeia requirements for new methods, which include accuracy, precision, selectivity, robustness, and linearity and range. This method shows enough selectivity, accuracy, precision, and linearity and range to satisfy Federal Drugs Administration/International Conference on Harmonization regulatory requirements. The current method demonstrates good linearity over the range 0.01-0.20 mg/mL of formoterol fumarate with r² 0.999 and 0.40-6.00 mg/mL of mometasone furoate with r² 0.999. The average recovery of the method is 99.9% of formoterol fumarate with a relative standard deviation of 1.94% and 101.5% of mometasone furoate with a relative standard deviation of 0.81%. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters and by changing analytical operators has proven that the method is robust and rugged.

Keywords: Formoterol Fumarate, Mometasone Furoate, HPLC, Antiasthmatic.

INTRODUCTION

Formoterol fumarate^{1,2} and Mometasone furoate³ is a long-acting beta2-agonist used in the management of asthma and/or chronic obstructive pulmonary disease (COPD). Inhaled formoterol/mometasone works like other beta2-agonists, causing bronchodilatation by relaxing the smooth muscle in the airway so as to treat the exacerbation of asthma. Formoterol fumarate chemical name is N-[2-hydroxy-5-[1-hydroxy-2-

[1-(4-methoxyphenyl) propan-2-ylamino] ethyl] phenyl] formamide phenylethylamine derivative with one phenolic hydroxyl and one secondary amino group. Mometasone furoate is a corticosteroid used as an anti-inflammatory³ and chemical name is (11β, 16α)-9, 21-dichloro-11-hydroxy-16-methyl-3, 20-dioxopregna-1, 4-dien-17-yl 2-furoate. The aim of this study Formoterol fumarate and Mometasone furoate is latest combination of anti asthmatic drugs. It is

available in dry powder inhalation and metered dose inhalation form. Both the drugs were individually official in Indian pharmacopoeia⁴, United States pharmacopoeia⁵ and British pharmacopoeia.⁶

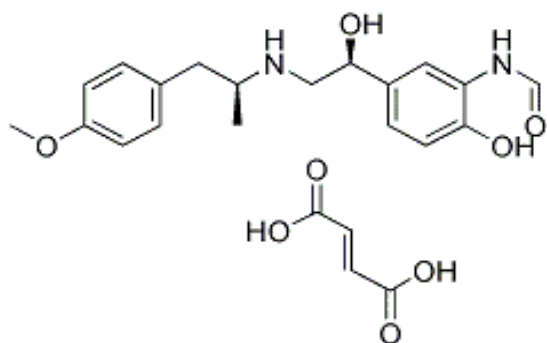


Figure 1: Structure of Formoterol Fumarate dehydrate

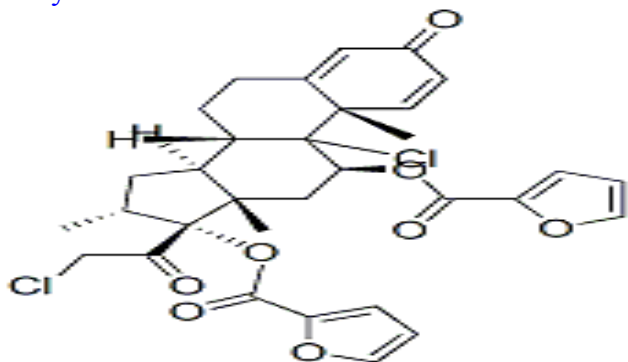


Figure 2: Structure of Mometasone Furoate

The current work presents a reversed phase and stability-indicating method for analysis of formoterol fumarate and mometasone furoate drugs in metered dose inhalation. The method is simple where reversed-phase-LC with isocratic elution and UV detection was used. Validation of the method was performed according to the requirements of United States Pharmacopoeia for assay determination, which includes accuracy, precision, (repeatability and intermediate precision (ruggedness)), selectivity, robustness, and linearity and range. Additionally, in order to meet the regulatory guidance of the Federal Drug Administration/International Conference on Harmonization (ICH, Q2 (R1))⁷, formoterol fumarate and mometasone furoate was degraded forcibly in acidic, basic, and thermal at 60°C.

EXPERIMENTAL

Chemicals

Acetonitrile HPLC grade was from Merck specialties private limited (Mumbai, India).

Methanol HPLC grade was from Merck specialties private limited (Mumbai, India). Sodium dihydrogen orthophosphate monohydrate (AR Grade), Decane Sulfonic acid sodium salt (AR Grade), Orthophosphoric acid (AR Grade) purchased from Merck specialties private limited (Mumbai, India) and double distilled water were used in analysis. Working standards of pharmaceutical grade formoterol fumarate and mometasone furoate were obtained as generous gifts from Hetero Labs Ltd (Hyderabad, India) and was used as such without further purification.

Apparatus

HPLC system (Shimadzu system, Japan) with a detector (SPD-20A/20AV Series-Prominence), equipped with a pump (LC-20 AD, 228-45000-32), auto sampler (SIL-10AF), column compartment (CTO-10A VP), and solvent rack (SIL-20) was employed during this study. The LC-solutions software was employed. The chromatographic analysis was performed on C₁₈ column (150 mm X 4.6 mm i.d., 5µm) (Thermo Scientific Corporation, Karlsruhe, Germany).

HPLC Chromatographic Conditions

Phosphate buffer was prepared by dissolving 1.38 g of Sodium dihydrogen orthophosphate monohydrate and 1.22 g of decane sulphonic acid of sodium salt monohydrate in 1000 mL of water, and adjusting the pH to 3.0 with dilute phosphoric acid solution. Filtered and degassed mixtures of acetonitrile and buffer (different volume fractions) were tested as mobile phases for Formoterol fumarate and mometasone furoate analysis. Different flow rates (0.5, 1.0, and 1.5 mL/min) were also tested. UV detection was performed at 220 nm and injection volume was 100 µL.

Standard Solutions

Stock standard solution of formoterol fumarate and mometasone furoate were prepared by dissolving a quantity of formoterol fumarate and mometasone furoate equivalent to 12.0 mg of formoterol fumarate and 40.0 mg of mometasone furoate in 100 mL of mobile phase to obtain a solution having known concentration of 120.0

mg/mL formoterol fumarate and 400 mg/mL mometasone furoate.

Nominal (working) standard solutions were prepared by diluting 1 mL of formoterol fumarate stock standard solution and 10 mL of mometasone furoate stock standard solution to 50 mL mobile phase to obtain a solution having a known concentration of 2.4 mg/mL formoterol fumarate and 80 mg/mL mometasone furoate. Transferred 5.0 mL of above solution into a 100 mL volumetric flask dilute to volume with mobile phase. Those solutions having known concentration of 0.12 mg/mL formoterol fumarate and 4.00 mg/mL mometasone furoate.

Sample Solutions

Sample A

Took a container, fit in an actuator & prime the valve by wasting first two actuations. Shacked the container for five seconds in between each actuation. Remove the container from its actuator & wash with mobile phase. Clean the valve and valve stem internally and externally with an airline fitted with a narrow jet. Place a disc in clean and dry 100 mL beaker; added about 40 mL of mobile phase. Hold the container in inverted position, shake for 5 seconds and deliver one actuation in beaker through the hole provided in the center of the disc. Similarly deliver further nine actuations in the same beaker with constant shaking for at least five seconds in between each delivery. Transferred the above solution from beaker into a 100 mL volumetric flask. Wash the beaker and disc with mobile phase and transferred into the same volumetric flask. Make up the volume up to the mark with mobile phase. Transferred 5 mL of the above solution in to 25 mL volumetric flask, dilute to volume with mobile phase and mix.

Sample B

Washed the actuator with mobile phase and dry gently. Fit the container in actuator & deliver 10 actuations with constant shaking for at least 5 seconds in between each delivery. Remove the container from actuator, wash the actuator 3-4 times with 10 mL mobile phase, collect in 25 mL

volumetric flask & then make up the volume up to the mark with mobile phase.

Content of Active Ingredient Delivered per actuation = μg of Sample A - μg of Sample B

$$\begin{aligned} & \% \text{ Labeled Amount of Active Ingredient} \\ & \text{Delivered per actuation} \\ & = \frac{[\mu\text{g of Sample A} - \mu\text{g of Sample B}] \times 100}{\text{Label Claim}} \end{aligned}$$

RESULTS AND DISCUSSION

Method Development

Preliminary studies involved trying C₈ and C₁₈ reversed-phase columns and testing several mobile phase compositions were conducted for the separation of formoterol fumarate and mometasone furoate with good chromatographic parameters (e.g. minimized peak tailing and good theoretical plates). A C₁₈ column (150 mm X 4.6 mm i.d., 5 μm) as a stationary phase with a mobile phase of acetonitrile/phosphate buffer pH 3.0 (50:50, v/v) at a flow rate of 1.0 mL/min and detection wavelength of 220 nm afforded the best separation of formoterol fumarate and mometasone furoate.

Method Validation

After method development, validation of the current test method for formoterol fumarate and mometasone furoate were performed in accordance with United States Pharmacopeia requirements for assay determination (category-I: analytical methods for quantitation of active ingredients in finished pharmaceutical products) which include accuracy, precision, selectivity, robustness, and linearity and range .

Linearity and range

To evaluate linearity of the method, six calibration standards of formoterol fumarate containing 0.013, 0.025, 0.064, 0.127, 0.157, and 0.193 mg/mL and mometasone furoate containing 0.403, 0.806, 2.016, 4.031, 4.998, and 6.127 mg/mL were analyzed. A plot of peak areas versus formoterol fumarate and mometasone furoate concentrations was linear in the range from 0.013 to 0.193 mg/mL of formoterol fumarate and 0.403 to 6.127 mg/mL with a

correlation coefficient of 0.9996 and 0.9999. This result demonstrates linearity of this method over the specified range.

Accuracy and percentage recovery

Accuracy of the method was studied by preparing the placebo of the drug formulation according to the formulation procedure. To the required quantity of placebo, a known quantity of formoterol fumarate and mometasone furoate with the same proportion as in the drug formulation was added to get three concentrations (0.064, 0.127, and 0.193 mg/mL of formoterol fumarate and 2.016, 4.031, and 6.127 mg/mL of mometasone furoate). Results have shown that the recovery of formoterol fumarate and mometasone furoate is within 98.2-100.4%, and 101.4-102.3% the RSD is lower than 1.0% (Table 1).

Precision

Repeatability

Repeatability of the method was evaluated by calculating the RSD of the peak areas of six replicate injections for the standard concentration (100%) of formoterol fumarate and mometasone furoate, which were found to be 0.37% and 0.09%. Furthermore, the RSD of the peaks areas of the recovery data analyzed in accuracy study (see Section 3.2.2) for each level was calculated, and it was found to be less than 1.0% for both drugs and each level (0.30%, 0.01%, and 0.17% for 50%, 100%, and 150%, for formoterol fumarate and 0.08%, 0.07%, and 0.01% for 50%, 100%, and 150%, for mometasone furoate respectively), as shown in Table 1. These results show that the current method for formoterol fumarate and mometasone furoate analysis is repeatable.

Intermediate precision (ruggedness)

Intermediate precision (also called ruggedness) of the method was also evaluated by analyzing six samples of formoterol fumarate and mometasone furoate by two analysts in the same laboratory using different HPLC systems. Results of this study showed that the RSD of the percentage of formoterol fumarate and mometasone furoate in formoterol fumarate and mometasone furoate metered dose inhalation for the 12 samples (6 samples from each analyst) were 1.25% and

0.98% indicating a good intermediate precision of the method.

Selectivity (stability indicating evaluation)

Selectivity of the method was demonstrated by enhancing degradation of formoterol fumarate and mometasone furoate under liquid and solid stress conditions (acid, base hydrolysis and oxidation, thermal, UV and humidity), to show that formoterol fumarate and mometasone furoate were separated from possible degradation products of formoterol fumarate and mometasone furoate resulted from liquid/solid stress condition. Results have shown that formoterol fumarate and mometasone furoate were stable in hydrogen peroxide solution (it gives no degradation products). Furthermore, it was found that formoterol fumarate and mometasone furoate were stable when standard solution of it were stored at room temperature or in oven at 60°C for one week. On the other hand, formoterol fumarate and mometasone furoate were not stable in acidic and basic solutions; about 40% and 45% of formoterol fumarate and 50% and 40% of mometasone furoate were degraded in hydrochloric acid solution and sodium hydroxide solution, respectively. However, no degradation products were detected.

Robustness

Robustness of the current method was investigated by analyzing three samples of formoterol fumarate and mometasone furoate in metered dose inhalation formulations using the same chromatographic conditions set forth in method development but (a) using flow rate 0.9 and 1.1 mL/min instead of 1.0 mL/min; (b) using column oven temperature 35°C and 45°C instead of 40°C; (c) using buffer pH 2.8 and 3.2 instead of pH 3.0; (d) using acetonitrile/buffer 52:48 and 48:52 instead of 50:50, v/v. RSD of the percentage of formoterol fumarate and mometasone furoate under these conditions is calculated to be less than 2.0% (Table 2). After successful development and validation of this method, it was employed for analysis of formoterol fumarate and mometasone furoate in metered dose inhalation formulations as shown in Figure 3.

Table 1: Accuracy (% recovery) of formoterol fumarate and mometasone furoate in metered dose inhalation formulation at three concentration levels

Formoterol fumarate concentration (mg/mL)	Accuracy (% recovery)				RSD (%)
	Sample 1	Sample 2	Sample 3	Mean	
0.064	98.9	98.7	98.3	98.6	0.30
0.127	98.1	98.2	98.2	98.2	0.01
0.193	100.5	100.2	100.5	100.4	0.17
Mometasone furoate concentration (mg/mL)	Accuracy (% recovery)				RSD (%)
	Sample 1	Sample 2	Sample 3	Mean	
2.016	101.8	102.0	101.8	101.9	0.08
4.031	101.4	101.3	101.3	101.4	0.07
6.127	102.3	102.3	102.3	102.3	0.01

Table 2: Robustness testing of the formoterol fumarate and mometasone furoate

Parameter	Content of formoterol fumarate assay (%)				RSD (%)
	Sample 1	Sample 2	Sample 3	Mean	
Flow rate (mL/min)					
0.9	96.5	98.2	97.2	97.3	0.88
1.0	101.2	99.9	100.2	100.4	0.68
1.1	98.7	99.7	99.8	99.4	0.61
Column temperature (°C)					
35°C	94.2	95.8	95.2	95.1	0.85
40°C	101.2	101.2	102.4	101.6	0.68
45°C	97.0	98.5	98.0	97.8	0.78
Buffer pH 3.0					
2.8	96.4	99.1	95.8	97.1	1.81
3.0	99.8	102.8	99.4	100.7	1.85
3.2	94.0	97.5	95.8	95.8	1.83
Mobile Phase composition (v/v)					
48:52	99.4	102.5	101.3	101.1	1.55
50:50	99.0	99.1	99.6	99.2	0.32
52:48	99.2	102.5	102.1	101.3	1.78
Parameter	Content of mometasone furoate assay (%)				RSD (%)
	Sample 1	Sample 2	Sample 3	Mean	
Flow rate (mL/min)					
0.9	102.6	102.4	101.8	102.3	0.41
1.0	103.7	103.1	103.4	103.4	0.29
1.1	100.7	103.0	104.6	102.8	1.91
Column temperature (°C)					
35°C	102.8	102.9	102.4	102.7	0.26
40°C	103.0	103.0	102.6	102.9	0.22
45°C	101.0	101.2	100.9	101.0	0.15
Buffer pH 3.0					
2.8	102.0	102.5	101.7	102.1	0.40
3.0	101.5	101.7	101.2	101.5	0.25
3.2	101.4	100.1	102.0	101.2	0.96
Mobile Phase composition (v/v)					
48:52	100.8	101.8	105.7	101.8	0.93
50:50	101.1	101.1	101.4	101.2	0.17
52:48	101.4	103.0	105.3	103.2	1.90

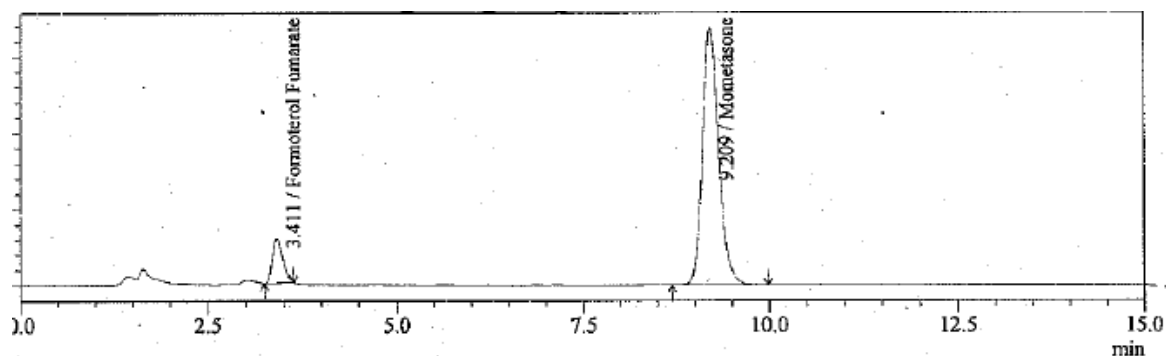


Figure 3: Chromatogram of formoterol fumarate and mometasone furoate in metered dose inhalation formulation. Mobile phase: acetonitrile/sodium dihydrogen phosphate buffer-pH 3.0 (50:50, v/v), flow rate – 1.0 mL/min, injection volume - 100 μ L, Column temperature 40°C. Column: C₁₈, 5 μ m and 15.0 cm-length, 4.6 mm-inner diameter, UV detection: 220 nm. Peaks asymmetry and theoretical plates of formoterol fumarate peak are 1.30 and 2758, and mometasone furoate peak are 1.18 and 8657, respectively.

CONCLUSION

A simple, accurate, precise, and stability-indicating HPLC method was developed and validated for the routine analysis of formoterol fumarate and mometasone furoate in metered dose inhalation formulations. The results of stress testing undertaken according to the International Conference on Harmonization guidelines reveal that the method is selective and stability-indicating.

CONFLICT OF INTEREST STATEMENT

We (the authors of this manuscript) have no financial and personal relationships with other people or organizations that could influence our work.

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