SIMULTANEOUS ESTIMATION OF MOMETASONE FUROATE AND FORMOTEROL FUMARATE BY HPLC METHOD IN ROTACAPS

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ABSTRACT

Objective: To develop and validate a simple and sensitive RP-HPLC method for the simultaneous determination of mometasone furoate (MOM) and formoterol fumarate (FOR) in pharmaceutical dosage forms.

Methods: In RP-HPLC method, chromatographic separation was achieved using a mixture of a solvent system consisting of methanol-water (pH 3.5) in the ratio of 85:15 % v/v at a flow rate of 1 ml/min and detection was carried out at 225 nm.

Results: The run time for the simultaneous estimation of drugs for the proposed method was 10 min as drugs eluted at 5.217 min (MOM) and 8.650 min (FOR). The linearity was found in the range of 33.33-299.97 µg/ml and 1-9 µg/ml for MOM and FOR, respectively. The values of limit of detection and limit of quantification were 3.634, 0.266 µg/ml and 11.014, 0.807 µg/ml which indicates the sensitivity of the method for the estimation of MOM and FOR, respectively. The results of recovery studies for both the drugs were within the range i.e. 98.87-101.48 % which indicates the precision of the method. The proposed method was validated according to ICH guidelines.

Conclusion: The proposed RP-HPLC method was found to be sensitive and precise because of the low LOD, LOQ and % RSD values (<2). The proposed work does not require acetonitrile and ion pairing reagent as compared to the reported methods. Therefore, method can be used preferably for routine analysis due to its simplicity and economic advantages.

Keywords: RP-HPLC, Mometasone furoate, Formoterol fumarate, Analytical method

INTRODUCTION

Management of asthma is improved by combining inhaled corticosteroids with long-acting β₂-agonists. Inhalation permits effective delivery of the drugs in high concentration to target sites within the lung, minimizing systemic exposure [1]. Combinational therapy of mometasone furoate (MOM) and formoterol fumarate (FOR) has been used in the treatment of COPD and asthma as it produces the additive effects for improving the symptoms, lung functions and reduces exacerbation in patient [2]. MOM (fig. 1) is a white crystalline powder, soluble in acetone, dichloromethane and slightly soluble in ethanol and a highly potent synthetic chlorinated glucocorticosteroid [3]. FOR (fig. 2) is a white crystalline, soluble in ethanol and methanol, slightly soluble in water, practically insoluble in acetonitrile. It appears to be more effective than shorter acting β₂-agonist in the treatment of nocturnal and exercise induced asthma. Moreover, it acts locally in the lung as a bronchodilator [4].

The literature survey reveals that several analytical methods have been published for the estimation of MOM alone and in combination with other drugs like fusidic acid [5], oxymetazoline [6] terbinafine HCl, nadifloxacin [7], eberconazole nitrate [8], miconazole, hydrocortisone [9], ketoconazole [10], salicylic acid [12] etc. Some of these methods include HPLC, GC, supercritical fluid chromatography and UV spectrophotometry. Various methods have also been reported for the estimation of FOR alone [13] and in combination with other drugs like budesonide [14, 16], tiotropium bromide [15] including HPLC, GC, and UV spectrophotometry [13-17]. A spectrophotometric and HPLC method for the simultaneous determination of MOM with FOR has also been reported in meter dose dosage form [17-19]. In one of the reported method, the mobile phase contained ion pairing reagents which will decrease the column life and was less sensitive. In the second method use of 60% ACN may increase the cost of analysis. The present study proposes a new RP-HPLC method using a mixture of methanol and water as a mobile phase for simultaneous estimation for MOM and FOR in rotacaps with the run time of 10 min.

MATERIALS AND METHODS

Instrumentation

Younglin (S. K ) gradient HPLC system combined with UV detector and Software-Autocho-3000 was used. Toshcon Ultrasonic Cleaner (Sonicator) of model SW 4 was used for sonication purpose. Digital Balance of Model Adventurer Pro AVG 264C (0.0001 gm to 260 gm) was used for weighing purpose. Digital pH meter of Model: S901 was used for pH measurement.

Chemicals and reagents

Standard drugs of MOM and FOR were obtained as gift samples from Sun Pharmaceutical Industries Ltd., Vadodara (Gujarat) India. The marketed formulation (Evocort®, Cipla Ltd.) of rotacaps containing MOM (200 µg) and FOR (6 µg) was purchased from local market. All the chemicals and reagents used were of HPLC/analytical grade.
Chromatographic conditions

The separation and quantitation of MOM and FOR were made on a 250 mm × 4.6 mm (id.) thermo (5 μm particle size) reversed phase C18 analytical column. The mobile phase was prepared by mixing methanol-water (pH 3.5 with 0.05 % orthophosphoric acid) in the ratio of 85:15 % v/v. The flow rate was set at 1 ml min⁻¹. All detection was carried out at ambient temperature. The injection volume was 20 μl. The detector was set at 225 nm wavelength. Data acquisition was performed by Autochro-3000 software.

Preparation of standard solution

MOM (333 mg) and FOR (10 mg) were weighed accurately and transferred to a 100 ml volumetric flask containing 25 ml methanol and sonicated. It was further diluted up to the mark with methanol to get 3330 μg/ml of MOM and 100 μg/ml of FOR, respectively and labelled as a standard stock solution. Further dilution was made to get required concentration.

Analysis of sample formulation

The powder content of twenty rotacaps (Each Rotacap contain FOR: 6 μg; MOM: 200 μg) was accurately weighed. A portion of powder (403.83 mg) equivalent to 0.1 mg of FOR and 3.33 mg of MOM was transferred to a 10 ml volumetric flask containing 5 ml methanol. The solution was sonicated for 15 min and made up to mark with methanol and filtered through 0.45 μm membrane filter. Further suitable aliquots of above sample formulation were diluted using mobile phase to obtain the concentration of both the drugs within the linearity range. The solutions were injected and chromatograms were recorded using the proposed RP-HPLC method. Based on the peak area of analytes, percentage assay of the formulation was calculated.

Method validation

The developed method for the simultaneous determination of MOM and FOR was validated for specificity, linearity, precision, accuracy, sensitivity, robustness and system suitability according to the International Conference on Harmonization guidelines [20].

Specificity

The selectivity of the RP HPLC method was checked by comparison of the chromatograms obtained from the samples and the corresponding placebo. The resolution factor between the MOM and FOR was 11.63 indicating that the method remained selective for both the drugs under test conditions.

Linearity and range

Linearity was evaluated by linear regression analysis. The linearity of the method was studied by analyzing different aliquots of binary mixture of standard solutions in the range of 3.33–299.97 μg/ml and 1–9 μg/ml for MOM and FOR, respectively in five replicates. Calibration graphs were plotted using peak areas versus concentration. The results were subjected to regression analysis by the least squares method to calculate the values of slope, intercept and correlation coefficient.

Precision

It was studied by carrying out repeatability, intraday and interday precision. Repeatability study was evaluated by analyzing the solution (99.99 μg/ml and 3 μg/ml of MOM and FOR) six times in HPLC system and % RSD was calculated. Intraday and interday precision was carried out by analyzing three replicate injections of MOM at different concentration 99.99, 166.65, 233.31 μg/ml and FOR 3, 5, 7 μg/ml on the same day and different day which was expressed in term of % RSD.

Accuracy

Recovery studies were carried out by the addition of standard drug to pre-analyzed sample solution (FOR: 3 μg/ml; MOM: 99.9 μg/ml) at three different levels: 50, 100 and 150 % to validate the accuracy parameter. The result of the accuracy study was assessed based on the percentage of standard FOR and MOM recovered from the formulation using following formula.

\[ \% \text{Recovery} = \left( \frac{\text{Amount of drug found after addition of standard drug}}{\text{Amount of drug found before addition of standard drug}} \right) \times 100 \]

Sensitivity

Sensitivity was evaluated by calculating the limit of detection and limit of quantification of FOR and MOM using the following equation as per ICH guidelines.

\[ \text{LOD} = 3.3 \times \frac{\sigma}{S} \]
\[ \text{LOQ} = 10 \times \frac{\sigma}{S} \]

Where, \(\sigma\) = Standard deviation of the response,
\(S\) = Slope of the calibration curve.

Robustness

Robustness was checked based on slight alteration in some critical parameters to allow routine laboratory use. It was performed by making slight alteration in proportion of methanol in mobile phase (85±2% v/v), flow rate (1±0.1 ml/min) and buffer pH (3.5±0.2 units). The solutions were analysed, values of peak area and retention time were recorded.

System suitability

System suitability tests were performed to confirm that the instrument was in appropriate condition for the analysis to be performed. Six replicates of the standard solution was injected and chromatograms were recorded to confirm the suitability of the chromatograph. Peak area reproducibility, number of theoretical plates, resolution and tailing factor were recorded.

Fig. 3: HPLC chromatogram of MOM and FOR under optimised condition
RESULTS

For developing well suited RP-HPLC method for analysis, parameters like detection wavelength, mobile phase composition, optimum pH and concentrations of the standard solution were comprehensively studied. The working standard solution was scanned in the range of 190-400 nm. At 220 nm, both the drugs gave satisfactory absorbance with due consideration in difference of their concentration and absorbance intensity. Several trials were carried out with different ratios of methanol and water for optimization of the mobile phase. Water: methanol in the ratio of 15:85 % v/v gave good resolved peaks, numerous trials of mobile phase ratios of methanol and water for optimization of the mobile phase.

Water: methanol in the ratio of (85:15 % v/v) gave optimum peaks with a flow rate of 1.0 ml/min. MOM [retention time (tR) 8.650 min] as shown in fig. 2. Numerous trials of mobile phase in the pH range of 3-5 were also tried. A solvent system of methanol-water (pH adjusted to 3.5 with 0.05 % ortho phosphoric acid) in the ratio of (85:15 % v/v) gave optimum peaks with a flow rate of 1.0 ml/min. MOM [retention time (tR) 8.650 min] was eluted first followed by FOR [retention time (tR) 9.150 min] as shown in fig. 2.

Validation of the developed method

The developed method was validated as per ICH guidelines. The specificity analysis revealed the HPLC method did not suffer any interferences from the formulation excipients, because no other peaks were observed at the retention times of MOM and FOR.

The linearity range was optimized by analyzing the solution of MOM and FOR at different concentration range. The calibration curve was constructed by plotting concentration of standard solution against mean peak area and the regression equation was computed. The goodness of fit (R²) was found to be 0.9997 for both the drugs, indicating a linear relationship between the concentration of analyte and peak area, as shown in table 1. The LOD and LOQ values were, 3.634, 11.014 µg/ml and 0.266, 0.807 µg/ml respectively for MOM and FOR which indicates the sensitivity of the method.

The intra-day and inter-day reproducibility values, expressed as percent relative standard deviation were less than 2 and percent relative standard deviation of repeatability study was less than 1, which ensures the reliability of results as shown in table 1.

Accuracy was determined by comparing the amount found (concentration) with the amount added (concentration). The mean percentage recoveries were calculated for MOM and FOR. The results are shown in table 2 which indicates that there was no interference from the excipients. All parameters described under robustness studies were analysed, but no significant changes were found in retention time, peak area and symmetry of the peaks as mentioned in table 3. It was found that the values of system suitability parameters were within the acceptable limits as recommended in ICH guidelines (table 4).

The amount found in percentage which were close to 100 and relatively low % RSD values shows that the developed method was successfully applied to analyze MOM and FOR in rota caps (table 5).

Table 1: Results of linearity and precision studies of proposed method

| Parameters                        | MOM              | FOR              |
|-----------------------------------|------------------|------------------|
| Linearity range (µg/ml)           | 33.33-299.97     | 1.9              |
| Correlation coefficient           | 0.9997           | 0.9991           |
| Regression Equation               |                  |                  |
| Slope                             | 18.922           | 51.351           |
| Intercept                         | -24.204          | 6.533            |
| LOD (µg/ml)                       | 3.634            | 0.266            |
| LOQ (µg/ml)                       | 11.014           | 0.807            |
| Precision (%RSD) *                | 0.751            | 0.827            |
| Intra-day (n=3)                   | 1.207            | 1.211            |
| Inter-day (n=3)                   | 1.605            | 1.700            |

* n = number of determinations, % RSD (Percentage relative standard deviation).

Table 2: Results of recovery studies of proposed method

| Level (%) | MOM         | FOR         |
|-----------|-------------|-------------|
|           | Recovery (%) | RSD (%)     | Recovery (%) | RSD (%) |
| 50        | 100.925±0.56 | 0.749       | 99.05±1.813  | 1.830   |
| 100       | 101.482±0.69 | 0.685       | 98.87±1.704  | 1.723   |
| 150       | 101.009±0.985| 0.976       | 99.42±1.683  | 1.692   |

*mean±SD (n=3), SD (Standard deviation), % RSD (Percentage relative standard deviation)

Table 3: Results of robustness studies of the proposed method

| S. No. | Modification     | MOM | Peak Area | FOR | Peak Area |
|--------|------------------|-----|-----------|-----|-----------|
| 1      | Organic phase (85±2% v/v) | 5.134 | 3175.67   | 8.734 | 268.57    |
| 2      | Effect of pH (3.5±0.2 unit) | 5.148 | 3276.76   | 8.698 | 276.54    |
| 3      | % RSD* (<2)      | 5.248 | 3198.66   | 8.675 | 274.59    |
| 1      | Effect of pH     | 1.201 | 1.647     | 0.342 | 1.521     |
| 2      | % RSD* (<2)     | 1.210 | 1.713     | 0.527 | 1.695     |
| 3      | % RSD* (<2)     | 5.234 | 3215.13   | 8.745 | 267.75    |
| 1      | Effect of flow rate | 5.167 | 3156.36   | 8.665 | 270.59    |
| 2      | % RSD* (<2)     | 5.106 | 3193.23   | 8.608 | 264.41    |
| 3      | % RSD* (<2)     | 1.239 | 0.931     | 0.794 | 1.156     |

* (n = 3), % RSD (Percentage relative standard deviation)
The proposed RP-HPLC method was found to be simple, sensitive, precise and cost-effective for simultaneous determination of MOM and FOR in rotacaps on account of low LOD, LOQ and % RSD values (<2). The proposed work contributed advantage in case of precise and cost-effective for simultaneous determination of MOM and 99.596±1.005 for FOR. Moreover, in comparison with the recovery of the formulation was 100.250 ±0.789 for FOR. The proposed RP-HPLC method was found to be simple, sensitive, precise and cost-effective for the analysis of drug in their water and methanol. Hence, the developed RP-HPLC assay method, showed good linearity over the range of 33-300 µg/ml for MOM and 1-9 µg/ml for FOR. The correlation coefficient was found to be greater than 0.998 which was within the limits specified (NLT 0.99). This gives confidence that the method can be used for routine analysis in quality control department for analysing MOM and FOR in rotacaps.

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AUTHORS CONTRIBUTIONS
The complete research work was guided by Dr. A K Seth. Method development and validation works were carried out by Aarti S. Zanwar. Further, the manuscript was drafted by Aarti S. Zanwar and it was edited by Dr. Dhanya B. Sen and Dr. Ashim Kumar Sen. Authors read and approved the final manuscript.

CONFLICT OF INTERESTS
Declared none

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