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Original Article

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 accuracy of the method. Relative standard deviation obtained from repeatability and reproducibility studies were less than 2% 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

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INTRODUCTION

Management of asthma is improved by combining inhaled corticosteroids with long-acting β_2-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 glucocorticosteriod [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 β_2 agoinst in the treatment of nocturnal and exercise induced asthma. Moreover, it acts locally in the lung as a bronchodilator [4].

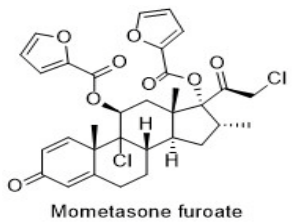

Fig.1: Chemical structure o mometasone furoate

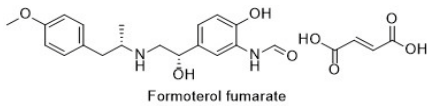

Fig. 2: Chemical structure of FOR formoterol fumarate

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–Autochro-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: S901was 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 (i.d.) thermo (5 µm particle size) reversed phase C_{18} 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 by 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 33.33-299.97 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.

% Recovery = (Amount of drug found after addition of standard drug–Amount of drug found before addition of standard drug)/(Amount of standard drug added) x 100

$$\%\text{Recovery} = \frac{(\text{Amount of drug found after addition of standard drug} - \text{Amount of drug found before addition of standard drug})}{(\text{Amount of standard drug added})} \times 100$$

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.

$$\text{LOD} = 3.3 \times \sigma/S$$

$$\text{LOQ} = 10 \times \sigma/S$$

Where, σ = 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.

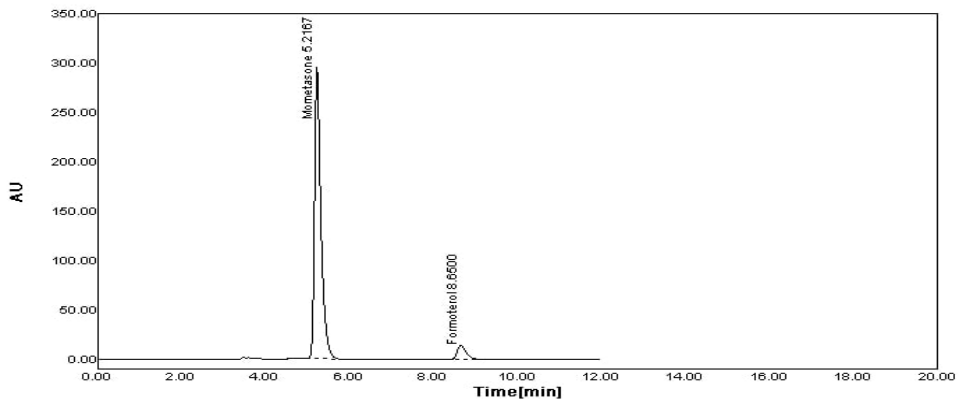

Fig. 3: HPLC chromatogram of MOM and FOR under optimised condition

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, but tailing was observed in FOR peak. 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 (t_R) 5.217 min] was eluted first followed by FOR [retention time (t_R) 8.650 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 (R^2) 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) *		
Repeatability of measurement (n=6)	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±0.756	0.749	99.053±1.813	1.830
100	101.482±0.696	0.685	98.867±1.704	1.723
150	100.889±0.985	0.976	99.426±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		FOR	
		R_t	Peak Area	R_t	Peak Area
1	Organic phase	5.134	3175.67	8.734	268.57
2	(85±2% v/v)	5.148	3276.76	8.698	276.54
3		5.248	3198.66	8.675	274.59
% RSD* (<2)		1.201	1.647	0.342	1.521
1	Effect of pH	5.154	3178.08	8.615	269.05
2	(3.5±0.2 unit)	5.175	3167.65	8.654	272.34
3		5.168	3267.49	8.706	263.52
% RSD* (<2)		0.207	1.713	0.527	1.695
1	Effect of flow rate	5.234	3215.13	8.745	267.75
2	(1±0.1 ml/min)	5.167	3156.36	8.665	270.59
3		5.106	3193.23	8.608	264.41
% RSD* (<2)		1.239	0.931	0.794	1.156

*(n = 3), % RSD (Percentage relative standard deviation)

Table 4: Result of system suitability studies of the proposed method

Parameters	Values		Acceptance criteria
	MOM	FOR	
Peak area (% RSD)*	0.592	0.827	%RSD ≤ 2
Retention Time (%RSD)*	0.277	0.441	%RSD ≤ 2
No. of theoretical Plates (N)	9806.980	7447.98	N>2000
Tailing Factor (T)*	1.182±0.112	1.378±0.144	T ≤ 2
Resolution (R_S)*	-	11.865±0.396	R_s>2

* (n = 6) number of determinations

Table 5: Results of formulation analysis using the proposed RP-HPLC method

Drugs	Labelled amount (µg/rotacap)	Amount found (µg/rotacap)	Amount found (%)*	RSD (%)**
MOM	200	200.501±1.578	100.250±0.789	0.787
FOR	6	5.978±0.737	99.596±1.005	1.009

*mean±SD (n=6), SD (Standard deviation), ** % RSD (Percentage relative standard deviation)

DISCUSSION

In the growing era of international competition for maintaining the standard of products in high commercial and market value, development and validation of analytical method became obligatory. Analytical method development is the process of demonstrating whether an analytical method is acceptable for use in workplace to quantify the concentration of subsequent sample. The method development and validation should be performed as per the protocols and acceptance criteria set out in the ICH guideline Q2 (R1) and used within GMP and GLP environments. In proposed HPLC method, chromatographic separation was achieved on reversed phase mode consisting of a mixture of methanol–water (pH adjusted to 3.5 with 0.05% ortho phosphoric acid) in the ratio of 85:15% v/v on thermo C_{18} column at a flow rate of 1 ml/min. 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 tailing factor for both the peaks was found to be<1.5. The ability of the method to separate and accurately measure the peak of interests which indicate the specificity of the method. This 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 response and concentration are directly proportional. Moreover, the developed method (linearity range of 33-300 µg/ml for MOM and 1-9 µg/ml for FOR) was more sensitive than the reported method (linearity range FOR 13-193 µg/ml and MOM 0.403-6.127 mg/ml). Precision had shown good results which prove that the method can be used for regular analysis. The standard addition and recovery studies were conducted to demonstrate the accuracy of the method. The recovery was found to be in the range of 98-102 %. So, the method can be used for the estimation of MOM and FOR from its dosage form, without any interference. In all deliberately varied conditions, the SD of retention time and peak area of both drugs were found to be within the acceptable limit. By using the above method, assay of the marketed formulation was carried out. The mean percentage recovery of the formulation was 99.60%. Present assay, the amount of both the drugs recovered was found to be 100.250±0.789 for MOM and 99.596±1.005 for FOR. Moreover, in comparison with the method described in the literature the proposed method was found to be simple, sensitive and precise. The developed HPLC method was cost effective for routine quality analysis as the mobile phase utilizes water and methanol. Hence, the developed RP-HPLC assay method, was found to be appropriate for the analysis of drug in their pharmaceutical dosage form.

CONCULSION

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 the low LOD, LOQ and % RSD values (<2). The proposed work contributed advantage in case of HPLC method which was found to be economical because it does not require acetonitrile and ion pairing reagent. So, the developed 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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