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Research Article

Simultaneous Estimation of Mometasone Furoate and Formoterol Fumarate from Pharmaceutical Capsule Formulations by RP-HPLC Using Pda Detector

S. Ashutosh Kumar*[1], Manidipa Debnath[2], Gokavarapu Divya Sravani[3], Mahesh Kumar Singh[4]

[1]Department of Pharmacy, Tripura University (A Central University), Tripura

[2]Inspecting officer (Drugs), The Deputy Drugs Controller, Tripura

[3]Department of Pharmaceutical Analysis and Quality Assurance, A.K.R.G College of Pharmacy, West Godavari

[4]Department of Pharmacy, Tripura University (A Central University), Agartala

***Corresponding author:** S. Ashutosh Kumar, Department of Pharmacy, Tripura University (A Central University), Tripura, Tel: +919640060617; E-mail: ashu.mpharm2007@gmail.com

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Abstract

The present study aimed to develop and validate the simultaneous estimation of mometasone furoate and formoterol fumarate from capsule dosage forms by using RP-HPLC. An isocratic reversed phase High-Performance Liquid Chromatographic (HPLC) method with ultraviolet detection at 254 nm has been developed for the determination of mometasone furoate and formoterol fumarate from capsule dosage forms. Good chromatographic separation was achieved by using a stainless steel analytical column, the Inertsil -ODS C18 (250 X 4.6 mm; 5 µ). The system was operated at 25 ± 2°C using a mobile phase consisted of methanol and phosphate buffer (pH adjusted to 5.8 using ortho phosphoric acid), mixed in the ratio of 55 : 45 at a flow rate of 1.0 mL/minute. The slope, intercept, and correlation coefficient were found to be y = 30294x - 77911, and 0.999 for mometasone and y = 30294x - 77911, and 0.999 for formoterol, respectively. The proposed method was validated for its specificity, linearity, accuracy, and precision. The method was found to be suitable for the quality control of mometasone furoate and formoterol fumarate simultaneously in a bulk samples as well as in a formulations.

Keywords: Formoterol fumarate, Isocratic separation, Method validation, Mometasone furoate, RP-HPLC.

Introduction

Mometasone furoate is (11β, 16α)-9, 21-dichloro-11-hydroxy-16-methyl-3, 20-dioxopregna-1, 4-dien-17-yl 2- furoate (Figure 1). It is a white crystalline powder, soluble in acetone and dichloromethane and slightly soluble in ethanol. It is practically insoluble in water. Mometasone furoate is a corticosteroid demonstrating potent anti-inflammatory properties. The precise mechanism of corticosteroid action on allergic rhinitis is not known. The corticosteroids have been shown to have a wide range of effects on multiple cell types (e.g., mast cells, eosinophils, neutrophils, macrophages, and lymphocytes) and mediators (e.g., histamine, eicosanoids, leukotrienes, and cytokines) involved in inflammation. It is official in Indian Pharmacopoeia and British Pharmacopoeia.

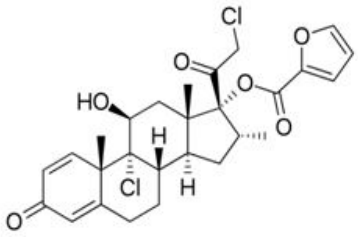

Figure 1: Chemical structure of Mometasone furoate

Formoterol fumarate is N-[2-hydro-5-(1-hydro-2-{[1-(4-methoxyphenyl) propan-2-] amino} ethyl) phenyl] formamide (Figure 2). It is a white crystalline powder, soluble in ethanol and methanol, slightly soluble in water, practically insoluble in acetonitrile. Formoterol fumarate is long-acting selective ß2-adrenergic receptor agonist (ß2-agonist). Inhaled formoterol fumarate acts locally in the lung as a bronchodilator. Formoterol fumarate is official in Indian Pharmacopoeia, which recommends a High Performance Liquid Chromatographic (HPLC) method for its analysis. The combinations of these two drugs are used to reduce the death caused by formoterol fumarate. The combinations of these two drugs are used when asthma is not controlled with long term asthma control medicine [1-7].

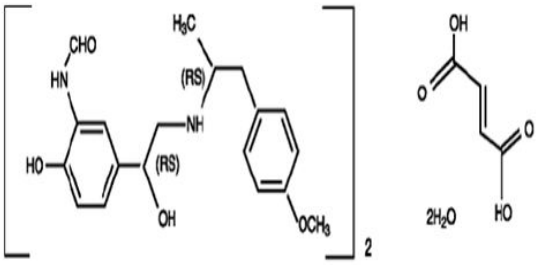

Figure 2: Chemical structure of Formoterol fumarate

The literature survey reveals that many analytical methods are reported for determination of mometasone furoate and formoterol fumarate individually and combinations with other drugs like UV, HPLC, Ratio Derivative Spectrophotometry [8-21]. But there are no methods available for the simultaneous estimation of mometasone furoate and formoterol fumarate by HPLC method. Hence, the aim of our presented study is to develop and validate the simultaneous estimation of mometasone furoate and formoterol fumarate from capsule dosage forms.

Experimental Design

Instrumentation: A Waters Alliance 2695 separation module equipped with a 2487 UV detector was employed throughout this study. Column that was employed in the method was Inertsil -ODS C_{18} (250 X 4.6 mm; 5 µ). The samples were injected with an automatic injector. The 20 µL volume of sample was injected. The input and output operations of the chromatographic system were monitored by Waters Empower 2software. The flow rate selected was 1.0 mL per min. The detection was done at 254 nm. The temperature and run time was monitored at 25 ± 2°C and 10.0 min respectively. The ultra violet spectra of the drugs used for the investigation were taken on a Lab India UV 3000 spectrophotometer for finding out their λ_{max} values. Solubility of the compounds was enhanced by sonication on an ultra sonicator (Power Sonic 510, Hwashin Technology).All the weighings in the experiments were done with an Afcoset electronic balance. The Hermle microlitre centrifuge Z100 (model no 292 P01) was used for the centrifugation process and Remi equipments (model no- CM101DX) Cyclomixer was used.

Reagents and materials: The reference sample of mometasone furoate and formoterol fumarate was supplied by Micro Labs, Bangalore and Yarrow Chem Products, Mumbai, respectively. HPLC grade water (prepared by using 0.45 Millipore Milli-Q) was procured from Standard Reagents, Hyderabad. HPLC grade acetonitrile and methanol were purchased from Merck, Mumbai. The chemicals used for preparation of buffer include potassium dihydrogen phosphate (Finar Chemicals, Ahmedabad), ortho phosphoric acid (Standard Reagents, Hyderabad). 0.45 µ membrane filters (Advanced Micro Devices Pvt. Ltd., Chandigarh, India) were used for filtration of various solvents and solutions intended for injection into the column.

Glassware: All the volumetric glassware used in the study was of Grade a quality Borosil. Optimization of the chromatographic conditions: Several modifications in the mobile phase were made by changing proportions of acetonitrile, methanol and water. Various modifiers were used "such as chloroform, Tetrahydrofuran (THF), ethanol, Isopropyl Alcohol (IPA), n-Hexane, and dichloromethane", with a 5 µ particle size column, used for separation initially. However, the best resolution of 2.76 was observed by using methanol with phosphate buffer (pH 5.8) in the ratio of 55: 45, much above the desirable limit of USP resolution 2.0. The retention time obtained for mometasone furoate and formoterol fumarates are 2.89 and 3.942 min., respectively. Preparation of phosphate buffer: The buffer solution was prepared by dissolving 7.0 grams of "potassium dihydrogen phosphate" in 900 mL HPLC grade water in a 1000 mL clean and dry flask. The mixture was stirred well untill complete dissolution of the salt. Further 100 mL of water was added and the pH was adjusted to 5.8 using ortho phosphoric acid.

Preparation of mobile phase: The mobile phase was prepared by mixing 550 mL of HPLC grade methanol and 450 mL phosphate buffer (pH 5.8) in a clean and dry flask. The mixture was degassed in ultra sonicator for 5 minutes. The resultant mobile phase was filtered through 0.45 µ membrane filter (Advanced Micro Devices Pvt. Ltd., Chandigarh, India) under vacuum.

Diluent preparation: The diluent was prepared by mixing HPLC garde acetonitrile and phosphate buffer (pH 5.8) in the ratio

of 55:45 (v/v). This solution was used for diluting the drug solutions in the study.

Preparation of standard solution: About 20 mg of mometasone furoate was weighed accurately and transferred into a 100 mL clean and dry volumetric flask. Initially, the drug was dissolved with 70 mL of diluent. The solution was sonicated for 15 min for complete dissolution of the drug. The final volume was made up with the same solvent. From the above prepared solution 10.0 mL transferred to a 50 mL clean and dry volumetric flask and it was diluted up to the mark with the same diluent. This stock solution contains 40 µg/mL of mometasone furoate. Similarly, about 20 mg of formoterol fumarate was weighed accurately and transferred into a 100 mL clean and dry volumetric flask. Initially, the drug was dissolved with 70 mL of diluent. The solution was sonicated for 15 min for complete dissolution of the drug. The final volume was made up with the same solvent. From the above prepared solution 10.0 mL transferred to a 50 mL clean and dry volumetric flask and it was diluted up to the mark with the same diluent. This stock solution contains 40 µg/mL of formoterol fumarate.

Preparation of formulation (Capsule) solution: A commercial brand of capsule FORMOST 200 (Manufacture: Cadila) was purchased from the local pharmacy and it was employed for this study. Each capsule contained 6.0 µg of mometasone furoate and 200.0 µg of formoterol fumarate. Twenty capsules were weighed, capsules are emptied and an accurately weighed sample of powdered tablets equivalent to 4.0 mg of mometasone furoate and formoterol fumarate was extracted with small amount of diluent in a 25 mL clean and dry volumetric flask. The solution was shaken well and allowed to stand for 15 min with intermittent sonication to ensure complete solubility of drugs. The contents are made up to the mark with the diluent and filtered through a 0.45µ membrane filter. From this filtrate, further dilution has been made to get a concentration of 40 µg/mL of mometasone furoate and 40 µg/mL of formoterol fumarate. Now the sample of 20 µL was injected and chromatographed. The average of the peak areas was calculated.

Method suitability: The commercial tablet formulation of mometasone furoate and formoterol fumarate namely FORMOST 200 (manufactured by Cadila) was analyzed by the proposed method and the results are shown in Table 1. The values were found to be in good agreement with the labeled amounts, which confirms the suitability of the method for the analysis of the drugs in pharmaceutical dosage forms.

Sr. No.	Formulation	Label claim (µg)	Amount found (µg) (n=3)	% Amount found
1	FORMOST 200 (manufactured by Cadila)	Mometasone furoate (6.0 µg)	6.3 µg	105
		formoterol fumarate (200.0 µg)	202.05 µg	101.25

Table 1: Recovery of mometasone furoate and formoterol fumarate from pahrmaceutical capsules formulations

Result and Discussion

Specificity and Selectivity: An aqueous mixture of mometasone furoate and formoterol fumarate (40.0 and 40.0 µg/mL of mometasone furoate and formoterol fumarate concentration respectively) was prepared and injected into the column and the retention time was checked and any interference at the retention time was checked by comparing the response in the blank. No interference was observed at the retention time for the respective drug. The method was found to be precise and specific. A typical chromatogram of mometasone furoate and formoterol fumarate sample and standard are shown in (Figure 3A and 3 B).

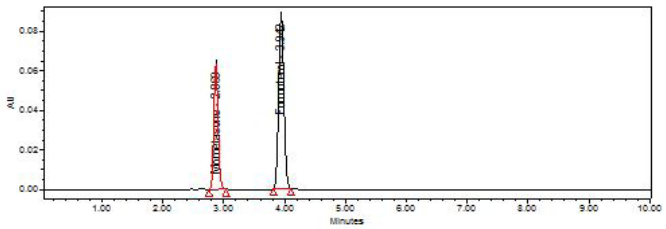

Figure 3 A: A typical chromatogram of mometasone furoate and formoterol fumarate (Sample)

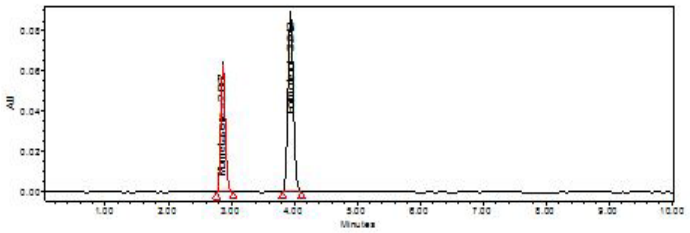

Figure 3 B: A typical chromatogram of mometasone furoate and formoterol fumarate (Standard)

Linearity: In order to find out the linearity range of the proposed HPLC method, curves were constructed by plotting peak areas obtained for the analyte against their concentrations. A good linear relationship ($r^2 = 0.999$) was observed between the concentration of mometasone furoate and formoterol fumarate and their corresponding peak areas. The relevant regression equation was y = 30294x - 77911 (r^2= 0.999) for mometasone furoate and formoterol fumarate y = 30294x - 77911 ($r^2 = 0.999$) (where y is the peak area and x is the concentrations of mometasone furoate and formoterol fumarate (µg/mL)). The data are represented table 2 and 3 and the calibration curves are presented in (Figure 4 and 5).

Sr. No.	Conc. (µg/mL)	Mean Peak Area (n=6)
1	20	412977
2	30	605369

3	40	807564
4	50	1007428
5	60	1210925
6	70	1420989

Table 2: Linearity range for mometasone furoate

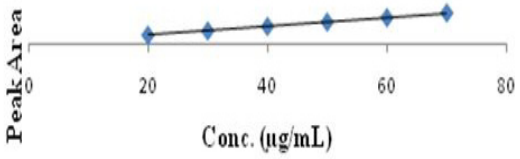

Figure 4: Calibration curve for mometasone furoate

Sr. No.	Conc. (µg/mL)	Mean Peak Area (n=6)
1	20	523467
2	30	829544
3	40	1139272
4	50	1448018
5	60	1728926
6	70	2010675

Table 3: Linearity range for formoterol fumarate

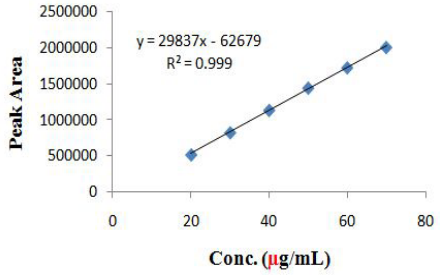

Figure 5: Calibration curve for formoterol fumarate

Precision: Precision is the level of reproducibility of the results as reported between sample analyzed on the same day (intra-day) and samples run on three different days (inter-day). To check the intra and inter-day variations of the method, solutions containing 40.0 µg/mL of mometasone furoate and formoterol fumarate respectively, were subjected to the proposed HPLC method of analysis and results obtained were noted. The precision of the proposed method i.e. the intra and inter-day variations in the peak areas of the drugs solutions were calculated in terms of percent RSD and the results are presented in (Table 4 and 5). A statistical evaluation revealed that the relative standard deviation of the drug at linearity level for 6 injections was less than 2.0.

Injection	Peak Area for mometasone furoate	Peak Area for formoterol fumarate
Injection-1	805783	1152293
Injection-2	801690	1146923
Injection-3	801496	1147283
Injection-4	806432	1152490
Injection-5	797564	1139272
Injection-6	801496	1147283
Average	801593	1147591
Standard Deviation	3262.714	4815.615
%RSD	0.406614	0.419628

Table 4: Intra-day precision of the proposed method for mometasone furoate and formoterol fumarate

% Concentration of spiked	Amount added (mg)	Amount found (mg)	% Recovery	Statistical Analysis of % Recovery	
50% Injection 1	20	19.85	99.25	MEAN	99.88
				% RSD	0.67
50% Injection 2	20	19.96	99.8		
50% Injection 3	20	20.12	100.6		
100% Injection 1	40	39.74	99.35	MEAN	99.81
				% RSD	0.399
100% Injection 2	40	40.08	100.2		
100% Injection 3	40	40.24	100.6		
150% Injection 1	60	59.04	98.4	MEAN	99.19
				% RSD	0.72
150% Injection 2	60	59.62	99.36		
150% Injection 3	60	59.89	99.81		

Table 6: Accuracy data of the proposed method for mometasone furoate

% Concentration of spiked	Amount added	Amount found	% Recovery	Statistical Analysis of % Recovery	
50% Injection 1	20	19.86	99.3	MEAN	99.46
				%RSD	0.38
50% Injection 2	20	19.98	99.9		
50% Injection 3	20	19.84	99.2		
100% Injection 1	40	39.54	98.85	MEAN	99.76
				%RSD	0.189

100%	40	39.82	99.55		
Injection 2					
100%	40	39.96	99.9		
Injection 3					
150%	60	59.92	99.86	MEAN	100.0067
Injection 1				%RSD	0.136
150%	60	60.08	100.13		
Injection 2					
150%	60	60.02	100.03		
Injection 3					

Table 7: Accuracy data of the proposed method for formoterol fumarate

Limit of detection (LOD) and Limit of quantitation (LOQ): LOD is defined as the smallest level of analyte that gives a measurable response. LOD is based on S/N ratio (signal/noise) typically for HPLC methods. Six replicates of the analyte were measured. The LOQ is the lowest concentration that can be quantified reliably with a specified level of accuracy and precision. It is the lowest concentration at which the precision expressed by Relative Standard Deviation (RSD) is less than 2 % and accuracy expressed by relative difference in the measured and true value is also less than 2 %. In other words, the analyte response is 10 times greater than the noise response. Six replicates of the analyte were analyzed and quantified. The limits of detection for mometasone furoate and formoterol fumarate obtained by the proposed method was 0.34 and 0.25 µg/ml and limits of quantification for mometasone furoate and formoterol fumarate obtained by the proposed method was 1.05 and 0.77 µg/mL.

Robustness: The optimized HPLC conditions were slightly modified to evaluate the robustness of the method. Small variations were made in the mobile phase ratio and flow rate. From the results, it was indicated that the selected factors remained unaffected by small variations in these quantities as well as the method was robust even by change in the mobile phase ± 5 % , flow rate

Sr. No.	Parameters	Furoate	
		Retention Time (min.)	Tailing Factor
1	**Standard**	**2.869**	**1.39**
2	**Flow rate(0.9mL/min.)**	**2.874**	**1.49**
3	**Flow rate (1.1mL/min.)**	**2.878**	**1.38**
4	**Mobile phase(35:65 %v/v)**	**2.789**	**1.27**
5	**Mobile phase(55:45 %v/v)**	**3.012**	**1.44**
6	**Wave length(249nm)**	**2.875**	**1.5**
7	**Wave length(259nm)**	**2.856**	**1.41**

Table 8: Results of the robustness study for mometasone furoate

Sr. No.	Parameters	Formoterol fumarate	
		Retention Time (min.)	Tailing Factor
1	Standard	3.942	1.39
2	Flow rate (0.9 mL/min.)	3.949	1.49
3	Flow rate (1.1 mL/min.)	3.92	1.38
4	Mobile Phase (35:65 % v/v)	3.92	1.27
5	Mobile Phase (55:45 % v/v)	3.812	1.44
6	Wavelength (249 nm)	3.959	1.5
7	Wavelength (259 nm)	3.935	1.41

Table 9: Results of the robustness study for formoterol fumarate

Conclusion

It can be concluded that the proposed RP-HPLC method developed for the quantitative determination of mometasone furoate and formoterol fumarate in bulk samples and in its formulations is simple, selective, sensitive, accurate, precise and rapid. The method was proved to be superior to most of the reported methods. The mobile phases are simple to prepare and economical. The sample recoveries in the formulation were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence this method can easily be adopted as an alternative method to reported ones for the routine determination of mometasone furoate and formoterol fumarate depending upon the availability of chemicals and nature of other ingredients present in the sample. The method also finds use in clinical, biological and pharmacokinetic studies of mometasone furoate and formoterol fumarate.

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