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Simultaneous Estimation Of Formoterol Fumarate Dihydrate and Fluticasone Propionatein Dry Powder Inhalation Formulation By RP-HPLC

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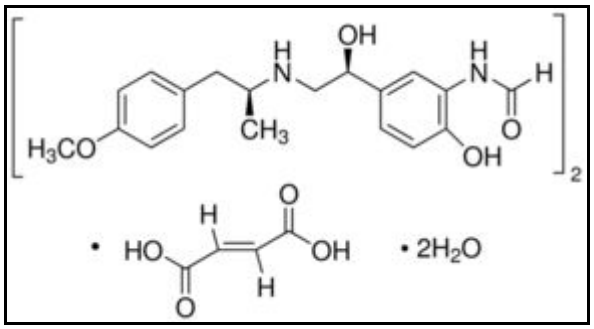
Abstract: A simple isocratic reversed phase high performance liquid chromatographic (HPLC) method has been developed for the simultaneous determination of Formoterol fumarate dehydrate and Fluticasone propionate in dry powder inhalation formulation. The separation was achieved by HiQSil C18HS, 250×4.6mm i.d., 5µmcolumn, acetonitrile: 0.01 M ammonium dihydrogenphosphate bufferpH 3.5 adjusted with *o*-phosphoric acid (80: 20 v/v) as mobile phase, at a flow rate of 1mL/min. The detection was carried out at 215 nm. Retention time of Formoterol fumarate dihydrate and Fluticasone propionate was found to be 4.892 and 9.183min, respectively. The method has been validated for linearity, accuracy and precision. Linearity for Formoterol fumarate dehydrate and Fluticasone propionate were in the range of 2.4-7.8µg/mLand 10-90µg/mL, respectively. The mean recoveries obtained for Formoterol fumarate dehydrate and Fluticasone propionate were found to 99.48% and 99.54 %, respectively. Developed method was found to be accurate, precise, selective and rapid for simultaneous determination of Formoterol fumarate dihydrate and Fluticasone propionate in dry powder inhalation formulation.
Keywords : Formoterol fumarate dihydrate, Fluticasone propionate, HPLC, Validation.

Introduction

Asthma is a common disease that causes inflammation of the bronchial tubes or airways that carry air to lungs. Common symptoms of the disease include wheezing, shortness of breath, coughing and chest tightness. Formoterol fumarate dihydrate(FFD) and Fluticasone propionate(FP) is a combination therapy used for the treatment of asthma. Formoterol fumarate dihydrate, chemicallyN-[2-Hydroxy-5-(1-hydroxy-2-{[2-(4-methoxyphenyl)-1-methylethyl] amino} ethyl) phenyl] formamidefumarate, is along-acting β_2-agonist, often used in the management of asthma and chronic obstructive pulmonary disease (COPD). Formoterol contains bronchodilators, which make the inhale and exhale process easier by relaxing the narrowed airways.

Fluticasone propionate, chemically,S-(fluoromethyl) 6α,9-difluoro-11β,17-dihydroxy-16α-methyl-3oxoandrosta-1,4-diene-17β-carbothioate, 17-propionate, is asynthetic corticosteroid, often used to treat asthma and allergic rhinitis. Fluticasone propionate is corticosteroid with mainly glucocorticoid activity. Fluticasone contains corticosteroids that help reduce swelling and inflammation in the airways. It is used by powder or aerosol inhalation for the prophylaxis of asthma. Both drugs are official in IP, BP, EP and USP[1-4]. The chemical structures of Formoterol fumarate dehydrate and Fluticasone propionateare shown in Fig.1a and Fig. 1b.



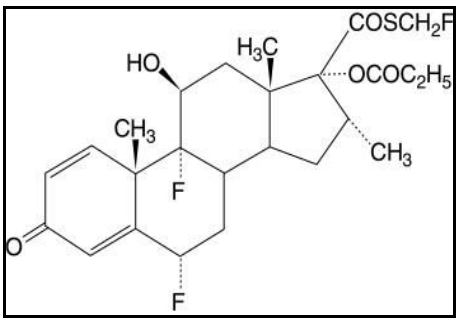


Fig. 1a Chemical structure of FFPFig.1bChemical structure of FP

Literature survey revealed that various analytical methods such as spectrophotometry[5-9], HPLC[10-18], HPTLC[19]and NMR[20]have been reported for determination ofFormoterol fumarate dehydrate (FFD) and Fluticasone propionate (FP)in bulk drug formulationsor combination with other drugs. Hence the objective of the present workis to develop a simple, precise, accurate, validated reverse phase HPLC for the simultaneous determination of Formoterol fumarate dihydrate and Fluticasone propionatein dry powder inhalation formulations

Materials and Methods

Chemicals and reagents

Formoterol fumarate dihydratewas a kind gift of Vasmi Labs Ltd. (Solapur, India) and Fluticasone propionatewas provided by Aarti Industries Ltd. Palghar, (Thane, India). Pharmaceutical formulation of capsule Maxiflo-100 Rotacapscontaining6 mcg of FFD and 100 mcg FPwas purchased from local market.All chemicals and reagent used were of HPLC grade and were purchased from Merck Chemicals, Mumbai, India**.**Double distilledwater was used throughout the study.

Instrumentation

The HPLC system consisted of Intelligent HPLC pump model (Jasco PU 2080 Plus) with sampler programmed at 20 µL capacity per injection was used. The detector consisted of a UV/ VIS (Jasco UV 2075 Plus). Data was integrated using Jasco Borwin version 1.5, LC-Net II/ADC system.

Chromatographic conditions

The mobile phase consisted of acetonitrile: 0.01 M ammonium dihydrogenphosphate (80:20 %v/v), pH of which is maintained at3.5 using ortho-phosphoric acid. The mobile phase was always freshly prepared and filtered through whatman filter paper No.41 and degassed by ultrasonicator. Chromatography wasperformed at ambient temperature by pumping the mobile phase at a flow rate of 1.0 mL/min. Thecolumn effluent was monitored at 215 nm.

Preparation of Standard stock solution

Accurately weighed FFD (6 mg) and FP (100 mg) were transferred to 100 mL volumetric flask and dissolved in, and then diluted to the mark with mobile phase. Appropriate dilutions were made with mobile phase to produce working solutions in the concentrations range 2.4-7.8 µg/mL and 10-90 µg/mL for FFP and FP, respectively. 20µL of samples were injected into the chromatographic system and peak areas were measured.

Preparation of Sample solution

Powder from twenty capsules (Maxiflo-100 Rotacaps containing 6 µg of FFD and 100 µgof FP per capsule, manufactured by Cipla Ltd.) were weighed, their mean weight determined, and crushed to fine powder. An amount of powder was transferred into a 10mL volumetric flask containing 5mL of mobile phase and mixed

well. The solution was ultrasonicated for 30 min, and then diluted to 10mL with mobile phase. The solution was filtered through whatman filter paper No.41 and appropriate dilutions were made with mobile phase.From the dilution, 20 µL was injected into the sample injector under the optimized chromatographic conditions. Area of each peak was measured at selected wavelength. The amount of each drug present in the sample was determined by comparing mean peak areas with that of the standard.

Method Validation

Specificity

The specificity of the method was ascertained by analysis of drug standards and samples. Theidentities of the peak for FFP and FP were confirmed by comparing the t_R with those of standards.

Linearity

Linearity is generally evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. For determining linearity, calibration curves were plotted over a concentration range of 2.4-7.8 µg/mL and 10-90 µg/mL for FFP and FP, respectively. A 20 µL of sample solution was injected into the chromatographic system using fixed volumeloop injector. Chromatograms were recorded. All measurements were repeated three times for each concentration and calibration curve was constructed by plotting the peak areas of analyte versus the corresponding drug concentration.

Limit of detection and limit of quantitation

The LOD and LOQ were calculated according to the 3.3 σ/s and 10 σ/s criteria, respectively; where σ is the standard deviation of the peak area and s is the slope of the corresponding calibration curve.

Precision

The precision of the proposed method was assessed as intraday and interday precision by preparing three different sample solutions at low, medium and high concentrations, which were freshly prepared and analyzed daily. These experiments were repeated 3 different days over a period of a week to evaluate day-to-day variability (interday precision).

Accuracy

To evaluate the accuracy of the developed method and to study the interference of formulationadditives, analytical recovery experiments were carried out by standard addition method, at 80, 100 and 120% level. The experiment was conducted in triplicate. Percentage recovery and relative standard deviation were calculated.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Results and Discussion:

Method development

The HPLC procedure was optimized for simultaneous determination of FFP and FP. Good resolution of both the components was obtained with acetonitrile: 0.01 M ammonium dihydrogen phosphate buffer pH 3.5 adjusted with *o*-phosphoric acid (80: 20 v/v). The flow rate of 1 mL/min was optimum. UV detection was made at 215 nm. At this wavelength FFP and FP can be quantified. Hence, 215 nm determined empirically has been found to be optimum. The average retention times for FFP and FP was found to be 4.892 and 9.183min, respectively.

System suitability

To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. The parameters obtained are shown in Table 1.

Table 1: System Suitability Parameters of RP-HPLC

Parameters	FFP	FP
Retention Time (t_R) in min	4.892	9.183
Resolution (Rs)	--	9.972
Theoretical plates number (N)	7632.83	8643.29
Tailing Factor (T)	1.116	1.130

Specificity

The chromatogram of capsule sample showed peaks at retention time of 4.892± 0.02 and 9.183± 0.02 min. for FFD and FP respectively (Fig. 2), indicating that there is no interference of the excipients present in the capsule formulation.

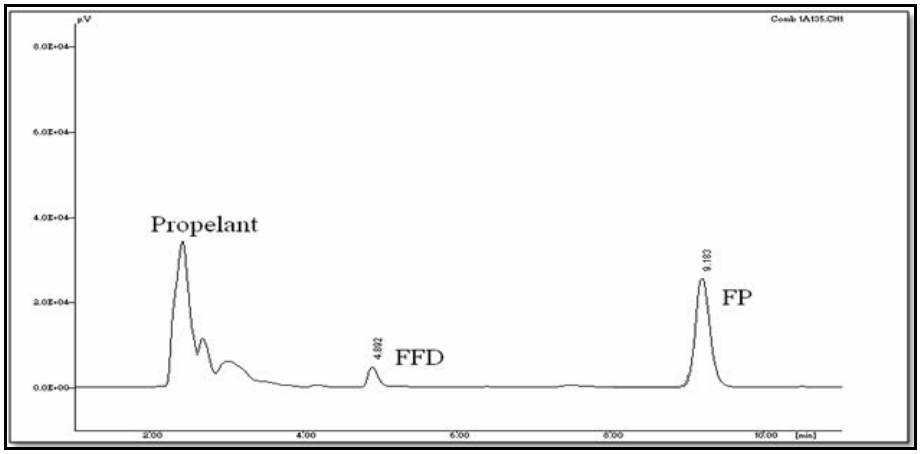
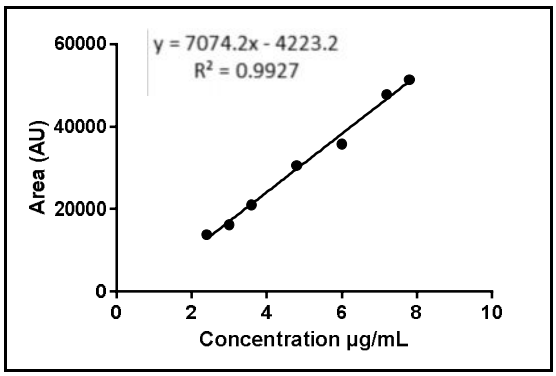


Fig.2: HPLC chromatogram of FFD and FP in capsule formulation

Linearity

Linear regression data for the calibration plots revealed good linear relationships between response and concentration over the ranges 2.4-7.8 µg/mL for FFP and 10-90 µg/mL for FP, respectively. The linear regression equations were Y= 7074.2X –4223.2 (r2= 0.9927) for FFP and Y= 25877X + 5070 (r2= 0.9936). The plots obtained from linear regression analysis are given in Fig.3 for FFP and Fig. 4 for FP, respectively.



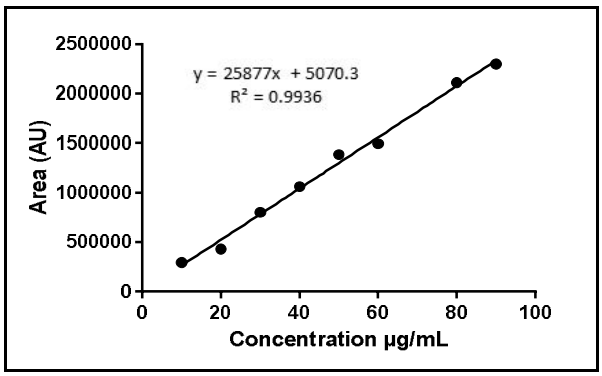


Fig. 3: linear regression for FFP

Fig. 4: linear regression for FP

Limits of Detection and Quantitation

The limits of detection and quantitation were found to be 0.73µg/mL and 2.21 µg/mL respectively, for FFP and 0.89 µg/mL and 2.71 µg/mL for FP. This indicates the method is sufficiently sensitive.

Precision

The precision of the method was expressed as relative standard deviation (RSD, %). The results shown in Table 2 reveal the high precision of the method.

Table 2: Precision studies for FFP and FP (n=3)

Concentration (µg/mL)	Intraday precision			Interday precision		
	Measured conc. (µg/mL)	% RSD	% Content found	Measured conc. (µg/mL)	% RSD	% Content found
Formoterol fumarate dihydrate (FFP)						
2.4	2.39	1.31	99.58	2.38	1.15	99.17
3	2.98	1.20	99.33	2.96	1.31	98.67
3.6	3.57	1.28	99.17	3.56	1.17	98.89
Fluticasone propionate (FP)						
10	9.8	1.14	98.00	9.9	1.10	99.00
20	19.85	1.24	99.25	19.82	1.27	99.10
30	29.87	1.16	99.57	29.85	1.21	99.50

Accuracy

The proposed method when used for extraction and subsequent simultaneous estimation of FFP and FP from dry powder inhalation capsule formulation byspiking with 80, 100, and 120% of additional drug. The added quantities of the individual drugs were estimated by above method. The results of recoverystudies were found to be satisfactory and the resultsare presented in Table 3.

Table 3: Recovery studies for FFP and FP by HPLC method (n=3)

Label claim (µg /capsule)	Amount Added (%)	Total amount (µg)	Amount recovered (µg)	(%) Recovery	Mean (%) Recovery(± SD)
FFP 6 µg	80 100 120	10.8 12.0 13.2	10.75 11.96 13.10	99.54 99.66 99.24	99.48 ± 0.216
FP 100 µg	80 100 120	180 200 220	179.52 199.60 218	99.73 99.80 99.09	99.54 ± 0.391

Robustness

There were no significant changes in the retention times of FFP and FP when the flow rate (± 0.1 mL/min.) and pH (± 0.1) were changed. The low values of the % RSD indicate the robustness of the method, as shown in Table 4.

Table 4: Results of robustness evaluation of FFP and FP (n=3)

Conditions	Level	FFP		FP	
		t_R(min.)	% RSD	t_R(min.)	% RSD
A: Flow rate (±0.1 mL/min.)					
0.9	-0.1	4.974	1.01	9.526	1.14
1	0.0	4.892	1.13	9.183	1.10
1.1	+0.1	4.431	1.16	9.011	1.02
B: pH (± 0.1)					
3.4	-0.1	4.901	1.11	9.195	1.08
3.5	0.0	4.892	1.07	9.183	1.03
3.6	+0.1	4.831	1.14	9.121	1.10

Analysis of marketed formulation

Experimental results of the amount of FFP and FP in dry powder inhalation capsule formulation, expressed as a percentage of label claims were in good agreement with the label claims thereby suggesting that there is no interference from any of the excipients which are normally present in capsules. The mean drug content was found to be 99.80 % for FFP and 99.62 % for FP.

Conclusion

The RP-HPLC method has been developed for the simultaneous estimation of Formoterol fumarate dihydrate and Fluticasone propionate in dry powder inhalation formulation. The developed method is simple, precise, and accurate and does not suffer from any interference due to common excipients. Hence the present RP-HPLC method can be used in the pharmaceutical industry for the routine analysis of simultaneous estimation of Formoterol fumarate dihydrate and Fluticasone propionate in dry powder inhalation formulation.

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