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Simultaneous Estimation of Formoterol Fumarate Dihydrate and Fluticasone Propionate in Dry Powder Inhalation Formulation by HPTLC

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ABSTRACT

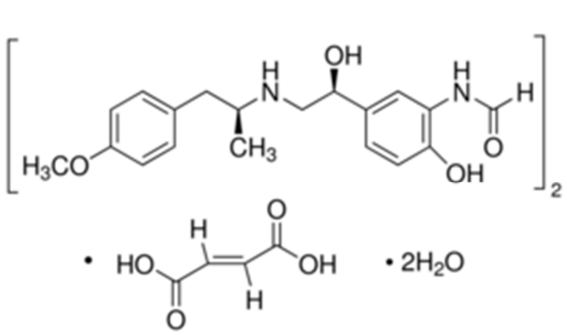
High performance thin layer chromatographic (HPTLC) method has been developed and validated for simultaneous analysis of Formoterol fumarate dihydrate and Fluticasone propionate in dry powder inhalation formulation. Chromatographic separation was achieved on aluminum foil plates precoated with silica gel 60F_{254}, with toluene: ethyl acetate: formic acid (7: 3: 0.1 % v/v/v) as mobile phase. Detection was performed densitometrically at 215 nm. The R_F of Formoterolfumarate dihydrate and Fluticasone propionate were 0.19 ± 0.10and 0.41± 0.10, respective ly. Linearity was found to be in the concentration range of 50-350ng/spot for Formoterol fumarate dehydrate and 50-350ng/spot for Fluticasone propionate, accuracy (99.28 % for Formoterol fumarate dehydrate and 99.46 % for Fluticasone propionate) and specificity, in accordance with ICH guidelines. The methods can be used for routine simultaneous analysis of Formoterol fumarate dihydrate and Fluticasone propionate in dry powder inhalation formulation.

Keywords: Formoterol fumarate dihydrate, Fluticasone propionate, HPTLC, Validation.

INTRODUCTION

Formoterol fumarate dihydrate (FFD) and Fluticasone propionate (FP) is a combination therapy used for the treatment of asthma. Formoterol fumarate dihydrate, chemically N-[2-Hydroxy-5-(1-hydroxy-2-{[2-(4-methoxyphenyl)-1-methylethyl] amino} ethyl) phenyl] formamide fumarate, is a long-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 a synthetic 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 dihydrate and Fluticasone propionate are shown in Fig.1a and Fig. 1b.



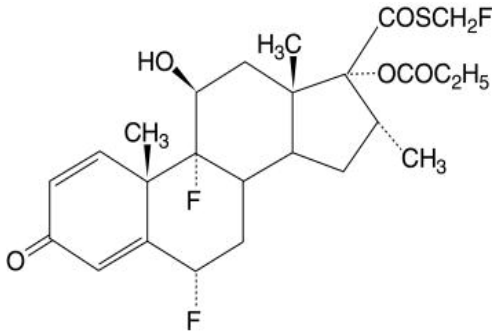


Fig. 1a Chemical structure of FFPFig.1b Chemical structure of FP

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 of Formoterol fumarate dihydrate (FFD) and Fluticasone propionate (FP) in bulk drug formulations or combination with other drugs. Hence the objective of the present work is to develop a simple, precise, accurate, validated HPTLC for the simultaneous determination of Formoterol fumarate dihydrate and Fluticasone propionate in dry powder inhalation formulations

MATERIALS AND METHODS

Chemicals and reagents

Formoterol fumarate dihydrate was a kind gift of Vasmi Labs Ltd. (Solapur, India) and Fluticasone propionate was provided by Aarti Industries Ltd. Palghar, (Thane, India). Pharmaceutical formulation of capsule Maxiflo-100 Rotacaps containing6 µg of FFD and 100 µg FP was purchased from local market. All chemicals and reagent used were of AR grade and were purchased from Merck Chemicals, Mumbai, India**.**

Instrumentation

The HPTLC system (Camag Sonnenmattstr, Mutenz, Switzerland) consisting of a Linomat V semi-automatic spotting device, a glass twin-trough TLC chamber (20×10 cm), a TLC scanner-III, a data station with winCATS (V 1.4.7) software and an HPTLC syringe (100 µL capacity; Hamilton Company, Bonaduz, Switzerland) was used for thin layer chromatographic studies. Linear ascending development was carried out in a twin trough glass chamber (20cm x 10 cm, 10 x 10cm).

Chromatographic conditions

The experiment was performed on a aluminum packed silica gel 60 F_{254} TLC plates, (20 cm × 10 cm, layer thickness0.2 mm) prewashed with methanol and mobile phase comprising of toluene: ethyl acetate: formic acid (7:3:0.1 %v/v/v). The developing solvent was run up to 80 mm in Camag chamber previously saturated with 10 mL of solvent mixture for10 min. Samples were applied at a distance of 8 mm from lower edge the distance between two bands was 7 mm. The developing solvent was run upto 80 mm, and the development was performed at 25 ± 2°C. The average development time was 15 minutes. After development, the plate was air dried and scanned densitometrically at 215nm with slit dimensions 6.00 x 0.30 mm, using CAMAG TLC scanner 3.

Preparation of Standard Stock Solution

Accurately weighed 10 mg of FFD was transferred to 10 mL volumetric flask, dissolved and diluted up to the mark with methanol to get FFD stock solution containing 1 mg/mL of FFD. Accurately weighed 10 mg of FP was transferred to 10 mL volumetric flask, dissolved and diluted up to the mark with methanol to get FP stock solution containing 1 mg/mL of FP.

Preparation of Calibration Curves:

The combined working standard solution of FFD and FP was prepared by diluting the stock solutions with methanol to prepare mixture of 50ng/µL of FFD and of FP. Aliquots of 1, 2, 3, 4, 5, 6 and 7 µL of working standard solution (corresponding to 50, 100, 150, 200, 250, 300and 350 ng/spot for both FFD and FP, respectively) were spotted on a TLC plate and analyzed. Calibration curve was prepared by plotting peak area of FFD and FP against their respective concentration.

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 methanol and mixed well. The solution was ultrasonicated for 30 min, and then diluted to 10mL with methanol. The solution was filtered through whatman filter paper No.41. The amount of each drug present in the sample was determined by comparing mean peak areas with that of the standard.

Validation of the proposed methods
HPTLC method was validated in compliance with ICH guidelines. The following parameters were validated.

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

Linearity
Standard stock solution of the drug was diluted to prepare linearity standard solutions containing FFD in the concentration range of 50–350ng/spot and 50–350ng/spot for FP, respectively. All measurements were repeated six times for each concentration and calibration curve was constructed by plotting the peak areas of analyte versus the corresponding drug concentration. Standard deviation (SD),slope, intercept and coefficient of determination (r^2) of the calibration curves were calculated to ascertain linearity of the method.

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
Repeatability of measurement of peak area was carried out by repeated scan of the same spot (80ng/ spot each of FFD and FP) seven times without changing the plate position. The % RSD for peak area was calculated. Repeatability of sample application is based on seven-time application of combined standard solution. The % RSD for peak area was computed. Variations of results within same day (intraday precision) and among days (interday precision) are called as reproducibility. The intraday precision (% RSD) was determined by analyzing standard solution of FFD and FP for three times on the same day. The interday precision (% RSD) was determined by analyzing standard solution of FFD and FP for 5 days. The intra- and interday variation for determination of FFD and FPwas carried out at three different concentration levels 100, 200 and300ng/spot for each of FFD and FP.

Accuracy
To evaluate the accuracy of the developed method and to study the interference of formulation additives, 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 proposed HPTLC method was tested for robustness. Three HPTLC conditions were screened: change in amount of toluene in mobile phase composition, change in saturation time and change in solvent run distance.

RESULTS AND DISCUSSION

Method development
Various solvent systems composed of toluene, ethyl acetate, formic acid or mixture thereof were tried for optimization of mobile phase for HPTLC separation of FFD and FP. But the best resolution and symmetrical peak shapes were achieved using mobile phase system consisting of toluene: ethyl acetate: formic acid (7: 3: 0.1 % v/v/v). The R_F values were found to be 0.19 and 0.41for FFD and FP, respectively.

Specificity
The chromatogram of capsule sample showed peaks at R_F values of 0.19 and 0.41 for FFD and FP respectively (Fig. 2), indicating that there is no interference of the excipients present in the capsule formulation.

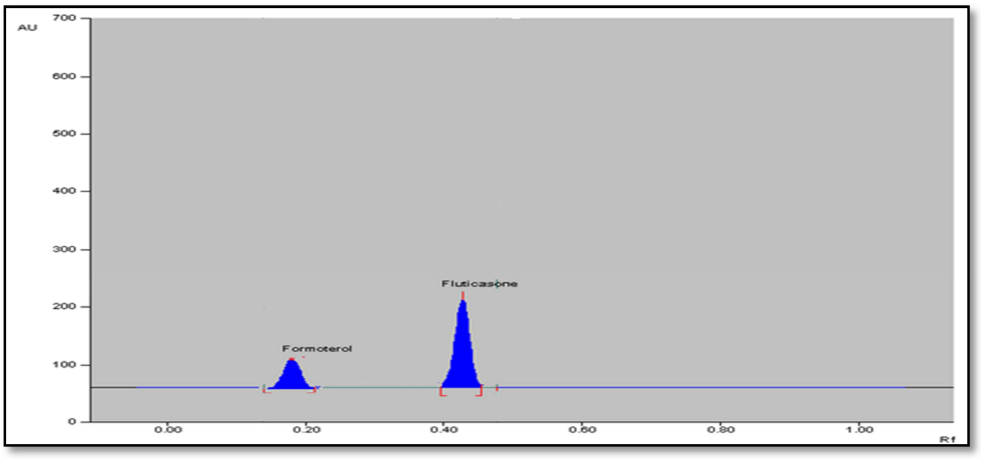


Fig. 2: Typical densitogram of FFD and FP

Linearity

Linear regression data for the calibration plots revealed good linear relationships between response and concentration over the ranges 50-350ng/spot for FFP and 50-350ng/spot for FP, respectively. The linear regression equations were Y= 8.348X +29.069.2 (r^2= 0.9995) for FFP and Y= 14.245X + 167.62(r^2= 0.9991). The plots obtained from linear regression analysis are given in Fig.3 for FFP and Fig. 4 for FP, respectively.

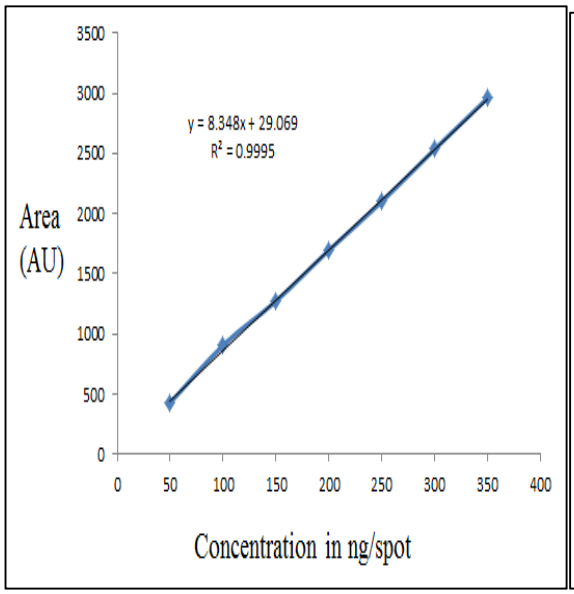


Fig. 3: linear regression for FFP

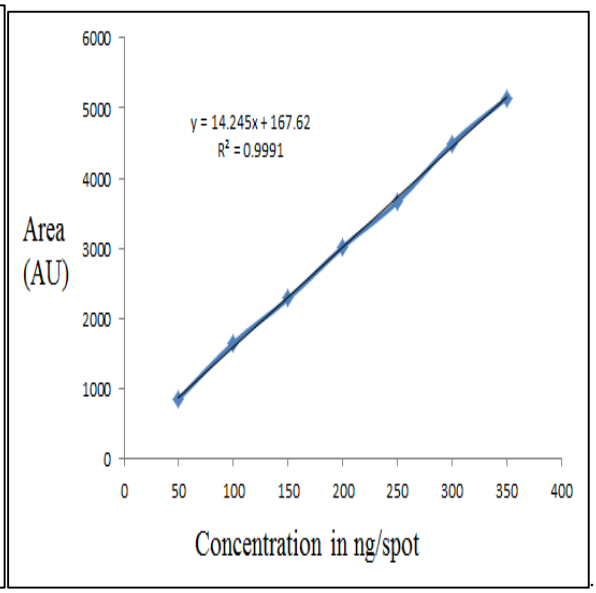


Fig. 4: linear regression for FP

Limits of Detection and Quantitation

The limits of detection and quantitation were found to be 19.60 ng/spot and 41.77 ng/spot respectively, for FFP and 13.71ng/spot and 35.50ng/spot 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 (ng/spot)	Intraday precision			Interday precision		
	Measured conc. (ng/spot)	% RSD	% Content found	Measured conc. (ng/spot)	% RSD	% Content found
Formoterol fumarate dihydrate (FFP)						
100	99.20	1.01	99.20	98.95	1.10	98.95
200	198.9	1.12	99.45	198.2	1.18	99.10
300	298.5	1.13	99.50	298.1	1.15	99.37
Fluticasone propionate (FP)						
100	99.67	1.14	99.67	98.90	1.03	98.90
200	198.8	1.17	99.40	198.1	1.07	99.05
300	297.7	1.10	99.23	297.5	1.11	99.17

Accuracy

The proposed HPTLC method when used for recovery studies for FFD and FP from pharmaceutical formulation after spiking with additional standard drug afforded recovery between 99.05–99.67 % and mean recoveries for FFD and FP from the marketed formulation are listed in Table 3.

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

Label claim (µg /capsule)	Amount Added (%)	Total amount (µg)	Amount recovered (µg)	(%) Recovery	Mean (%) Recovery(± SD)
FFP 6 µg	80	10.8	10.72	99.26	99.28 ± 0.21
	100	12.0	11.94	99.50	
	120	13.2	13.08	99.09	
FP 100 µg	80	180	179.40	99.67	99.46 ± 0.35
	100	200	199.30	99.65	
	120	220	217.9	99.05	

Robustness

The standard deviation of peak the areas was calculated for each parameter and the % RSD was found to be less than 2 %. The low values of the % RSD, as shown in Table 4 indicated robustness of the method.

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

Conditions	Level	FFP		FP	
		R_F	% RSD	R_F	% RSD
A: Change in amount of toluene inmobile phase composition					
Toluene: ethyl acetate: formic acid (6.9: 3: 0.1 % v/v/v)	-0.1	0.18	1.14	0.408	1.12
Toluene: ethyl acetate: formic acid (7.1: 3: 0.1 % v/v/v)	+0.1	0.17	1.10	0.409	1.03
B: Change in saturation time (min.)					
9	-1	0.189	1.10	0.409	1.09
11	+1	0.188	1.13	0.408	1.14
C: Change in solvent run distance (mm)					
79	-1	0.191	1.09	0.411	1.07
81	+1	0.189	1.11	0.409	1.13

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 99.75 % for FFP and 99.64 % for FP.

CONCLUSION

The proposed HPTLC method provides precise, accurate and reproducible quantitative analysis for the simultaneous determination of FFD and FP in Maxiflo-100 Rotacaps. The method was validated as per the ICH guidelines. The robustness of the proposed method was studied and found to be robust at deliberate changes made in experimental conditions. Statistical tests indicate that the proposed HPTLC method reduce the duration of analysis and appear to

be equally suitable for routine determination of FFD and FP simultaneously in in dry powder inhalation formulation in quality control laboratories.

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