

*Research Article*

**Simultaneous Equation Spectrophotometric Methods for Simultaneous Estimation of Formoterol Fumarate Dihydrate and Fluticasone Propionate in Pure Drug and Pharmaceutical formulations.**

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*Received 27 August 2017; received in revised form 20 September 2017; accepted 20 September 2017*

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**ABSTRACT**

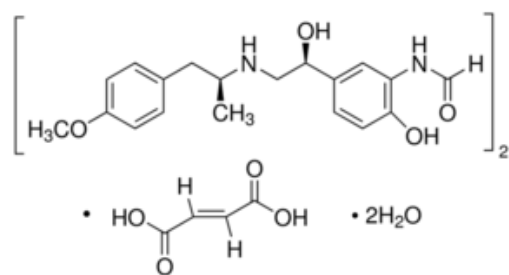
A simple, accurate, sensitive and economical spectrophotometric method have been developed and subsequently validated for determination of Formoterol Fumarate Dihydrate (FFD) and Fluticasone Propionate (FP) in bulk and pharmaceutical formulation. For the simultaneous equation method, the estimation of FFD and FP was carried out at 215nm ( $\lambda_{\max}$  of FFD) and 236 nm ( $\lambda_{\max}$  of FP), respectively. Calibration curves of FFD and FP were found to be linear in the concentration ranges of 6-36  $\mu\text{g/mL}$  and 20-120  $\mu\text{g/mL}$  for FFD and FP, respectively.

**KEYWORDS**

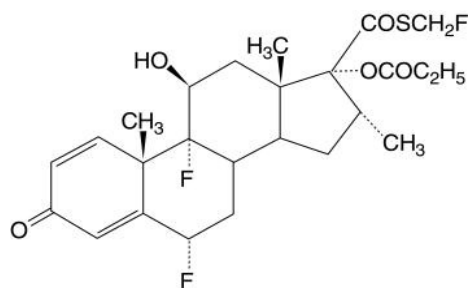
Formoterol Fumarate Dihydrate, Fluticasone Propionate, simultaneous equation method, Validation.

## 1. 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 methoxy phenyl) - 1-methylethyl] amino] ethyl) phenyl] formamide fumarate, is a long-acting  $\beta_2$ -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) 6 $\alpha$ ,9-difluoro-11 $\beta$ ,17-dihydroxy-16 $\alpha$ -methyl-3oxoandrosta-1,4-diene-17 $\beta$ -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 FFP.



**Fig.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 simultaneous equation spectrophotometric method for simultaneous estimation of formoterol fumarate dihydrate and fluticasone propionate in pure drug and pharmaceutical formulations.

## 2. MATERIALS AND METHODS

### 2.1. 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 containing 6 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.

### *2.2. Instrumentation*

UV-visible double beam spectrophotometer, Make: JASCO spectrophotometer, model V-550 with a pair of 10 mm matched quartz cells was used for experiments. The absorption spectra of reference and test solution were carried out in a 1 cm quartz cell over the range of 200-400 nm.

### *2.3. Preparation of working standard stock*

Accurately weighed quantity of Formoterol Fumarate Dihydrate (10 mg) and Fluticasone Propionate (10 mg) was transfer into two separate 100 ml volumetric flask, dissolved in 25 ml of methanol and diluted up to the mark to get standard stock solution of concentration 100 µg/ml for each drug.

### *2.4. Preparation of sample solutions*

From the above stock solution of concentration of 100 µg/ml, serial dilutions were done so as to get sample solution of concentration range from 1 µg/ml to 16 µg/ml for both drugs individually.

### *2.5. Determination of absorption maxima*

From the standard stock solutions of FFD and FP (100 µg/ml) pipette out 1 ml of each in two separate 10 ml volumetric flask and make up the volume to get a concentration of 10 µg/ml each. Both the solutions were scanned in the spectrum mode over the range of 200-400 nm. FFD showed an absorbance peaks at 215 nm, whereas FP indicated at 236 nm. For simultaneous equation method, the absorbance maxima 215 nm and 236 nm were selected for analysis of FFD and FP respectively. (Overlain spectra : Fig. 2)

### *2.6. Preparation of standard calibration curve*

The absorbance of serial dilutions was recorded for simultaneous equation method, at 215 nm and 236 nm and calibration curve was plotted.

### *2.7. Simultaneous equations Method*

For the simultaneous equations were formed.  $A_1 = 432 \times (C_x + 1260) \times y$  and

$$A_2 = 331 \times (C_x + 1349) \times C_y,$$

where  $C_x$  and  $C_y$  are concentrations of FFD and FP respectively in g/100ml in the sample solution.  $A_1$  and  $A_2$  are the absorbance of the mixture at 215nm and 236nm, respectively. The concentration of  $C_x$  and  $C_y$  can be obtained.

### *2.8. Assay Procedure for Marketed formulation*

20 capsules of marketed formulation of FFD and FP corresponding to 6 µg and 100 µg (Maxiflo-100 Rotacaps) respectively were weighed; their average weights determined. The correct amount of drug powder equivalent to label claim was weighed and transferred to 10 ml volumetric flask, dissolved in methanol. The volume was then made up to the mark using same solvent. The filtrate was having concentration 6 µg/ml for FFD and 100 µg/ml for FP. Absorbance of this sample solution was recorded for simultaneous equations at 215 nm and

236 nm. Result of analysis of capsule formulation shown in Table.1.

### *2.9. Method Validation*

The objective of validation of an analytical procedure is to demonstrate whether the procedure is suitable for its intended purpose. The proposed method was validated for various parameters such as Linearity, Accuracy, Precision, Limit of detection (LOD) and Limit of Quantitation (LOQ) according to ICH Q2 (R1) guideline [21].

#### *2.9.1. Accuracy*

The accuracy for the analytical procedure was determined at 80 %, 100 % and 120 % levels of standard solution and results were expressed in terms of % recoveries. Three determinations at each level were performed and % RSD was calculated. The results were tabulated in Table 2.

#### *2.9.2. Precision*

For Intraday and Interday precisions of the method, dilutions of sample were prepared at three concentration levels ( $\mu\text{g/ml}$ ) each in triplicate. These solutions were analyzed respectively three times within one day and three consecutive days. The result of Statistical evaluation for Intraday and inter day precision studies for Q Simultaneous Equation Spectrophotometric Methods.

#### *2.9.3. Limit of detection (LOD) and Limit of quantitation (LOQ):*

The LOD and LOQ were separately determined based on the standard deviation of response of the calibration curve.

$$\text{LOD} = 3.3\sigma/S, \quad \text{LOQ} = 10\sigma/S$$

Where,

$\sigma$  - is the standard deviation of the response of blank,

S - is the slope of calibration curve.

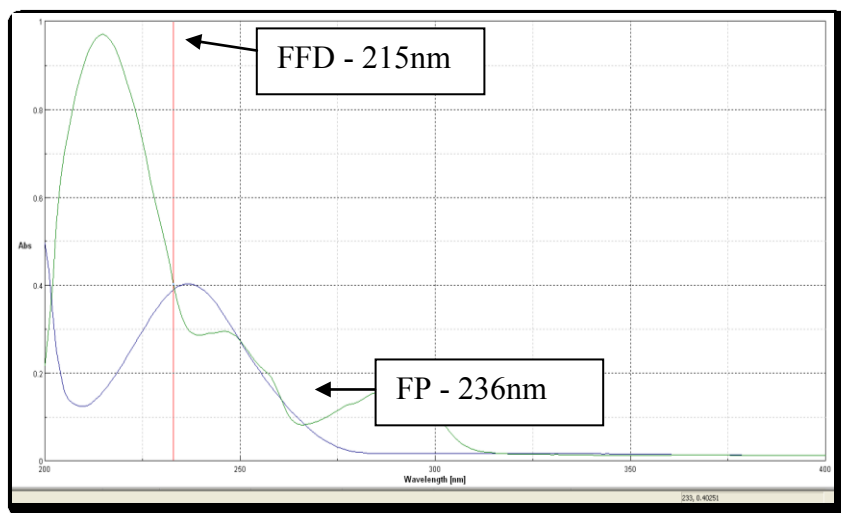
### **3. CONCLUSION**

From the results of Table 3. (Regression analysis and Validation Parameters), the proposed spectrophotometric methods are accurate, precise, economic and reliable for the simultaneous measurement of FFD and FP in combined dosage form. The % RSD for all parameters were found to be less than one, which revealed the validation of new method and assay results obtained by this method are fairly satisfactory. Hence, it can be concluded that the developed UV Spectrophotometric method can be employed successfully as an alternative for HPLC and HPTLC methods for the quantitative estimation of FFD and FP in combined dosage form.

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**Fig. 2.** Overlain UV spectra of FFD & FP.

**Table 1.** Result of analysis of capsule Formulation.

Maxiflo-100 Rotacaps	Label claim (µg/ capsule)	Amount found (µg)	% Content found*	S.D.	%R.S.D.
FFD	6	5.979	99.65	1.5857	1.5919
FP	100	99.50	99.50	1.2494	1.2348

**Table 2.** Result of Accuracy.

Label claim (µg/capsule)	Amount Added (%)	Total amount (µg)	Amount recovered (µg)	(%) Recovery	Mean Recovery (± SD)
FFD 6	80	10.8	10.717	99.23	99.27
	100	12.0	11.923	99.36	± 0.0814
	120	13.2	13.096	99.21	
FP 100	80	180	178.90	99.39	99.35
	100	200	198.88	99.44	± 0.1153
	120	220	218.28	99.22	

**Table 3.** Regression analysis and Validation Parameters.

Parameters	FFD	FP
Linearity Range (µg/mL)	6-36	20-120
Correlation Coefficient	0.996	0.991
Assays %	99.65	99.50

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<b>Precision (%RSD)</b>		
<b>Intraday</b>	0.94	1.11
<b>Interday</b>	1.10	1.12
<b>(% Recovery)</b>	99.27	99.35
<b>LOQ (<math>\mu\text{g/ml}</math>)</b>	5.4	7.5
<b>LOD (<math>\mu\text{g/ml}</math>)</b>	1.8	2.5

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