



## Research Article

### **DEVELOPMENT AND VALIDATION OF ULTRA PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE ANALYSIS OF PULMONARY DRUG PRODUCT CONTAINING FORMOTEROL FUMARATE AND FLUTICASONE PROPIONATE**

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#### **ABSTRACT**

An ultra-performance liquid chromatographic assay method was developed for the simultaneous determination of Formoterol fumarate and Fluticasone propionate in dry powder inhalation formulation. The separation was achieved on Acquity UPLC HSS C18 (50 mm x 4.6 mm x 1.8  $\mu$ m) column. The mobile phase consists of water ( $p^H$  adjusted to 2.5 with orthophosphoric acid): acetonitrile (40:60 v/v) pumped at a flow rate 0.4 ml/min. The column temperature was maintained at 35 °C and the detector was monitored at a wavelength of 223 nm. The injection volume was 10  $\mu$ l with a total run time of 3.5 min. The retention times of Formoterol fumarate and Fluticasone propionate was found to be 0.58 $\pm$ 0.01 min and 1.84 $\pm$ 0.01 min respectively. The calibration curves were linear in the concentration range of 3-9  $\mu$ g/ml and 50-150  $\mu$ g/ml of Formoterol fumarate and Fluticasone propionate respectively ( $r^2 = 0.999$ ). The percentage recoveries were found to be 99.43-99.84 % for Formoterol fumarate and 99.44-100.51 % for Fluticasone propionate. The limit of detection was found to be 0.028  $\mu$ g/ml & 0.15  $\mu$ g/ml and limit of quantitation was found to be 0.085  $\mu$ g/ml & 0.47  $\mu$ g/ml for Formoterol fumarate and Fluticasone propionate respectively. The most sensitive UPLC method was developed for the estimation of dry powder inhalation containing varied strengths of Formoterol fumarate and Fluticasone propionate. The developed method was validated for system suitability, specificity, accuracy, precision, linearity, limit of detection, limit of quantitation and robustness according to International Conference on Harmonization (ICH) guidelines.

**Keywords:** Formoterol fumarate, Fluticasone propionate, pulmonary drug product, UPLC, Method development, Method validation.

#### **INTRODUCTION**

Formoterol fumarate, chemically (E)-but-2-enedioic acid; N-[2-hydroxy-5-[(1S)-1-hydroxy-2-[[[(2S)-1-(4-methoxyphenyl)propan-2-yl]amino]ethyl]phenyl]formamide, is a long-acting  $\beta_2$ -agonist. It is used in the treatment of asthma and chronic obstructive pulmonary disease (COPD).

Fluticasone propionate, chemically S-(fluoromethyl)-6 $\alpha$ ,9-difluoro-11 $\beta$ ,17-dihydroxy-16 $\alpha$ -methyl-3-oxoandrost-1,4-diene-17 $\beta$ -carbothioate-17-propionate, is a synthetic steroid with glucocorticoid receptor activity. It is used to treat asthma and allergic rhinitis.

Extensive literature survey revealed that there were ultraviolet spectroscopic methods for the estimation of Formoterol fumarate alone<sup>1,2</sup> and with other combinations.<sup>3,4</sup> An ultraviolet spectroscopic method has been developed for Fluticasone propionate<sup>5</sup> and in combination with Salmeterol xinafoate.<sup>6</sup> Few RP-HPLC methods were reported for the estimation of Formoterol fumarate<sup>7</sup> and with other combinations.<sup>8-12</sup> An HPTLC method has been reported for quantification of Fluticasone propionate with Salmeterol xinafoate.<sup>13</sup> A bioanalytical method was also reported for the estimation of Formoterol fumarate in human urine.<sup>14</sup> There were few RP-HPLC methods for the simultaneous estimation of Formoterol fumarate and Fluticasone propionate.<sup>15-17</sup> In these methods the retention time of analytes was more and even the run time was more than 10 min there by increasing the solvent consumption and leading

to longer analysis time. In addition to this, no UPLC method has been reported for the assay of specified drugs in pharmaceutical formulation. Therefore, it was thought appropriate to develop an ultra-performance liquid chromatographic procedure that serves as a rapid, accurate and simple method for the simultaneous estimation of Formoterol fumarate and Fluticasone propionate in bulk and pharmaceutical formulation.

#### **MATERIALS AND METHODS**

##### **Apparatus**

Acquity UPLC H-Class system (Waters, Milford, USA) consists of a binary solvent manager, auto sampler and a UV detector. The output signal was monitored and processed using empower software. Sonicator (LMUC-2, Labman scientific),  $p^H$  meter (AD 1020, ADWA) and analytical balance (ER-200A, AFCOSET) were used.

##### **Reagents and chemicals**

Formoterol fumarate and Fluticasone propionate capsules for inhalation (6 mcg of Formoterol fumarate and 100 mcg of Fluticasone propionate) manufactured by Macleods were purchased from local pharmacy. Orthophosphoric acid of analytical reagent grade was purchased from Rankem, New Delhi, India. Acetonitrile, Milli-Q water and methanol of HPLC grade was purchased from Rankem, Maharashtra, India.

### Chromatographic conditions

The chromatographic column used was Acquity UPLC HSS C18 (50 mm x 4.6 mm x 1.8  $\mu\text{m}$ ). The separation was achieved on isocratic mode. The mobile phase consists of water whose  $\text{p}^{\text{H}}$  was adjusted to 2.5 with orthophosphoric acid and acetonitrile in the ratio 40:60 v/v was pumped at a flow rate 0.4 ml/min. The column temperature was maintained at 35°C and the detector was monitored at 223 nm. The injection volume was 10  $\mu\text{l}$  with a total run time of 3.5 min.

### Preparation of diluent

The diluent was prepared by taking water and acetonitrile in the ratio 50:50 % v/v.

### Preparation of standard solution

A standard solution of Formoterol fumarate and Fluticasone propionate was prepared by dissolving 0.6 mg of Formoterol fumarate and 10 mg of Fluticasone propionate in 5 ml of diluent, sonicated for 15 min, filtered and final volume was made up to 10 ml with diluent. From this 1 ml was taken and made up to 10 ml with diluent to get a final concentration of 6  $\mu\text{g}/\text{ml}$  and 100  $\mu\text{g}/\text{ml}$  of Formoterol fumarate and Fluticasone propionate respectively.

### Preparation of sample solution

From a blend of 10 capsules, weight equivalent to 60  $\mu\text{g}$  of Formoterol fumarate and 1000  $\mu\text{g}$  of Fluticasone propionate was taken into volumetric flask containing 5 ml of diluent, sonicated for 15 min, filtered and volume was made up to 10 ml with diluent to get a final concentration of 6  $\mu\text{g}/\text{ml}$  and 100  $\mu\text{g}/\text{ml}$  of Formoterol fumarate and Fluticasone propionate respectively.

## RESULTS AND DISCUSSION

### Method development

Several chromatographic conditions were tried for better separation and resolution. A number of trials were performed with different solvents in different ratios over a wide  $\text{p}^{\text{H}}$  range, with different flow rates and column temperatures to get good, sharp peaks with better retention times for efficient resolution between two peaks. Satisfactory results were achieved in terms of retention time, resolution, symmetry and sensitivity on isocratic trial using Acquity UPLC HSS C18 (50 mm x 4.6 mm x 1.8  $\mu\text{m}$ ) column with a mobile phase of water ( $\text{p}^{\text{H}}$  adjusted to 2.5 with orthophosphoric acid): acetonitrile (40:60 % v/v) pumped at a flow rate 0.4 ml/min. The injection volume was 10  $\mu\text{l}$  and the detection were carried out at 223 nm using UV detector with a run time of 3.5 min, at a column temperature 35°C. The typical UPLC chromatograms for simultaneous determination of Formoterol fumarate and Fluticasone propionate from standard preparation and sample preparation were shown (Figure 1 & 2). The retention time of analytes were reduced drastically due to the small particle size of column packing material (1.8  $\mu\text{m}$ ) which enhances number of theoretical plates. The proposed UPLC method was found to be rapid, specific and selective for the assay of Formoterol fumarate and Fluticasone propionate.

### Method validation

The developed method was validated for parameters such as system suitability, specificity, linearity, accuracy, precision, LOD, LOQ and robustness according to ICH guidelines for analytical procedures Q2 [R1].<sup>18</sup>

### System suitability

System suitability was checked to verify the system performance. Six replicate samples containing 6  $\mu\text{g}/\text{ml}$  of Formoterol fumarate and 100  $\mu\text{g}/\text{ml}$  of Fluticasone propionate were analyzed using the developed method. The factors such as theoretical plate count, tailing factor and resolution between the peaks were taken into consideration for testing system suitability. The results were presented in Table 1. From the results, it was found that the resolution between two peaks was 25.08 and the tailing factor for both drugs was  $< 2$ . The theoretical plate count was 4474 and 14435 for Formoterol fumarate and Fluticasone propionate respectively. All the parameters were found to be within the limits.

### Specificity

Specificity of the method was confirmed by observing the interferences of blank and placebo at analyte peaks. The blank and placebo were prepared as per test method and injected into the chromatographic column and checked for the interfering peaks at the retention times of analyte peaks and thereby found no interfering peaks at the retention times of analyte peaks. Hence the results prove that the developed method was specific for the estimation of Formoterol fumarate and Fluticasone propionate.

### Precision

Precision of method was verified by repeatability and intermediate precision. Repeatability was checked by injecting six individual homogenous preparations of standard solution under the same operating conditions over a short interval of time (method precision). Intermediate precision of the method was also evaluated on different days (inter day) and with different analysts. Relative standard deviation (RSD) was calculated. The results of precision studies were tabulated in Table 2 & 3. The data obtained from precision studies, it was found that the RSD values were  $< 2$  and hence assure the precision of the developed method.

### Accuracy

Accuracy of the method was determined in triplicate at three concentration levels 50 %, 100 % and 150 % of target assay concentration. Known quantities of drug substances corresponding to the specified level of the label claim were added to the pre-analyzed sample. Each set of addition were repeated three times. The results were expressed as the percentage of analytes recovered by the assay. The recoveries of the drugs from a series of spiked concentrations were presented in Table 4. According to statistical data, the recoveries of drugs were found to be within the specified range of 98-102 %. Hence it can be concluded that the method was highly accurate for the determination of Formoterol fumarate and Fluticasone propionate.

### Linearity

A linear relationship was evaluated across the range of the analytical procedure. It was demonstrated directly on the drug substance by diluting standard stock solution of Formoterol fumarate and Fluticasone propionate. The calibration curves were plotted for Formoterol fumarate and Fluticasone propionate (Figure 3 & 4). They were found linear over the concentration range of 3-9  $\mu\text{g}/\text{ml}$  for Formoterol fumarate, 50-150  $\mu\text{g}/\text{ml}$  for Fluticasone propionate. The data was subjected to statistical analysis using a linear-regression model, the regression equation

and correlation coefficient. The results were tabulated in Table 5. The regression plots revealed the compliance with Beer Lambert's law in the concentration range of 3-9 µg/ml for Formoterol fumarate and 50-150 µg/ml for Fluticasone propionate. The correlation coefficient ( $r^2$ ) was found to be 0.999 which show a good linearity between absorbance and concentration.

#### LOD and LOQ

The detection limit was determined based on the standard deviation of y-intercepts and the slope from set of three calibration plots by using the following formulae.

$$\text{LOD} = 3.3 * \sigma / s$$

$$\text{LOQ} = 10 * \sigma / s$$

Where,  $\sigma$  = the standard deviation of y-intercept of regression lines,  $s$  = the slope of the calibration curves

The detection limits and quantitation limits of the drugs were presented in Table 6 & 7. The LOD and LOQ of Formoterol fumarate and Fluticasone propionate were found to be 0.028 µg/ml & 0.085 µg/ml and 0.15 µg/ml & 0.47 µg/ml respectively. From the results of LOD and LOQ it was concluded that the developed method has good sensitivity.

#### Robustness

The robustness of the developed method was established to make sure that the developed analytical method was unaffected by

small, but deliberate changes in the method parameters. For this, the experimental conditions like flow rate, column temperature and mobile phase ratio were deliberately altered, and the system suitability parameters were evaluated. The solutions were prepared as per the test method and injected at different variable conditions like flow rate ( $\pm 0.1$  ml/min), column temperature ( $\pm 2$  °C) and mobile phase ratio ( $\pm 2$  % organic phase). The results were presented in Table 8. In all the varied chromatographic conditions no, significant differences have been observed in system suitability parameters and were found to be within the limits. The result indicates that the method was unaffected and found to be robust.

#### Applicability of the developed method

The assay of Formoterol fumarate and Fluticasone propionate in dry powder inhalation formulation was performed to check the applicability of the developed method. The standard preparations and sample preparations were made from the pure drugs and formulation respectively. The prepared solutions were injected six times into the chromatographic system. The drugs present in the formulation were estimated by comparing with the reference standards. The average percentage of drugs was calculated, and they were found to be 100.71 % and 100.86 % for Formoterol fumarate and Fluticasone propionate respectively. Hence the developed method was successfully applied for quality control of formulation.

Table 1: Results of system suitability

Analytes	Resolution*	Tailing factor*	Theoretical plates*
Formoterol fumarate	-	1.76	4474
Fluticasone propionate	25.08	1.31	14435

\*Average of six determinations

Table 2: Results of repeatability

N	Formoterol fumarate		Fluticasone propionate	
	Rt	Peak area	Rt	Peak area
Injection 1	0.585	42394	1.830	523362
Injection 2	0.585	42666	1.830	520974
Injection 3	0.586	42261	1.830	523138
Injection 4	0.586	42068	1.833	521626
Injection 5	0.586	41920	1.835	520089
Injection 6	0.587	42146	1.843	524925
Mean		42193		522352
SD		182.9		1777.9
RSD (%)		0.4		0.3

RSD = relative standard deviation; SD = standard deviation; Rt = retention time

Table 3: Results of intermediate precision

Variation		RSD of Formoterol fumarate (%)	RSD of Fluticasone propionate (%)
Inter day	Day 1	0.3	0.7
	Day 2	0.4	1.2
Different analysts	Analyst 1	0.8	0.4
	Analyst 2	1.0	1.3

Table 4: Results of accuracy (Recovery studies)

Analyte	Pre analysed sample concentration (µg/ml)	Amount added (µg/ml)	Amount found (µg/ml)	Recovery (%)	*Mean recovery
Formoterol fumarate	6	3	3.01	100.34	99.43
	6	3	2.97	99.30	
	6	3	2.95	98.65	
	6	6	6.02	100.41	99.84
	6	6	5.96	99.38	
	6	6	5.98	99.73	
	6	9	8.82	98.06	99.43
	6	9	9.04	100.51	
	6	9	8.97	99.74	
Fluticasone propionate	100	50	50.91	101.83	100.51
	100	50	50.27	100.55	
	100	50	49.58	99.16	
	100	100	100.07	100.07	99.56
	100	100	99.61	99.61	
	100	100	99.00	99.00	
	100	150	147.98	98.65	99.44
	100	150	149.31	99.54	
	100	150	150.22	100.15	

\*Average of triplicate determinations

Table 5: Results of linearity

% Level	Formoterol fumarate		Fluticasone propionate	
	Concentration (µg/ml)	Peak areas	Concentration (µg/ml)	Peak areas
50	3.0	20455	50	255081
75	4.5	33243	75	384363
100	6.0	44678	100	522160
125	7.5	55642	125	656294
150	9.0	65797	150	767893
<b>Correlation coefficient</b>		0.999	0.999	
<b>Regression equation</b>		$y = 7417x - 453.6$	$y = 5179x - 665.6$	

Table 6: Results of LOD and LOQ of formoterol fumarate

% Level	Linearity data I	Linearity data II	Linearity data III
50	20455	20486	20371
75	33243	33313	33104
100	44678	44929	44603
125	55642	55749	55857
150	65797	65733	65754
<b>SD of Y intercept (<math>\sigma</math>)</b>	63.39		
<b>Average of slope (s)</b>	7417.6		

Table 7: Results of LOD and LOQ of fluticasone propionate

% Level	Linearity data I	Linearity data II	Linearity data III
50	255081	253298	257024
75	384363	385782	382257
100	522160	524762	520322
125	656294	655798	653506
150	767893	766576	770820
<b>SD of Y intercept (<math>\sigma</math>)</b>	247.38		
<b>Average of slope (s)</b>	5178.6		

Table 8: Results of robustness

Parameter	Formoterol fumarate		Fluticasone propionate	
	Tailing*	Plate count*	Tailing*	Plate count*
Less flow rate (0.3 ml/min)	1.69	3901	1.25	14700
More flow rate (0.5 ml/min)	1.65	3615	1.25	15066
Less column temperature ( $\pm 33^\circ\text{C}$ )	1.62	3453	1.24	14650
More column temperature ( $\pm 37^\circ\text{C}$ )	1.70	3359	1.30	14450
Less organic phase (43:57)	1.75	4141	1.39	12940
More organic phase (42:58)	1.54	3736	1.71	16905

\*Average of six determinations

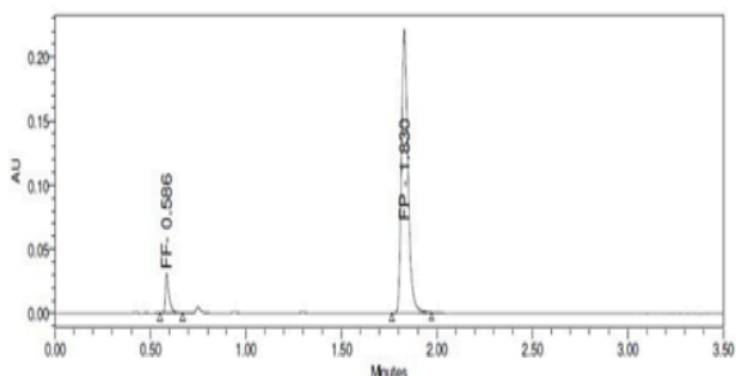


Figure 1: Chromatogram of Formoterol fumarate and Fluticasone propionate standard preparation

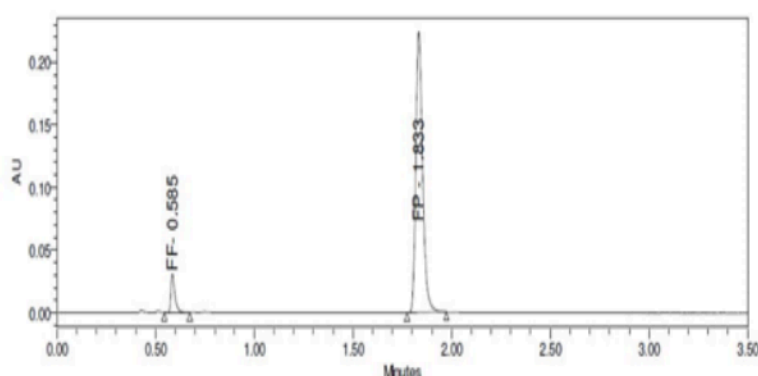


Figure 2: Chromatogram of Formoterol fumarate and Fluticasone propionate sample preparation

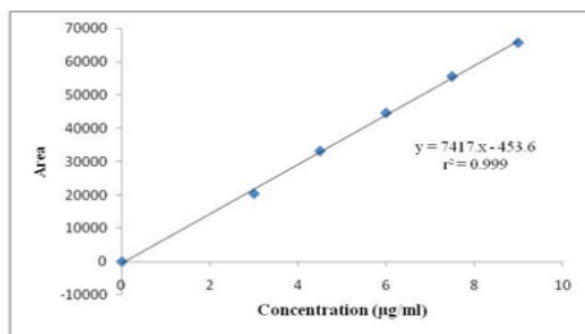


Figure 3: Linearity chart of Formoterol fumarate

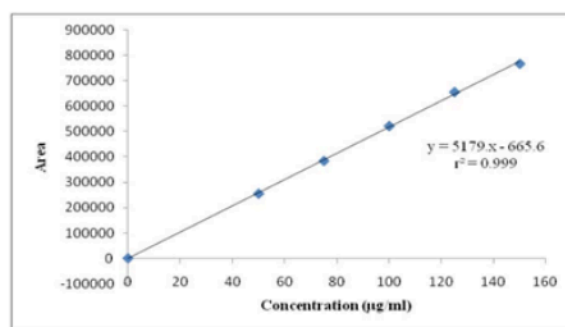


Figure 4: Linearity chart of Fluticasone propionate

## CONCLUSION

The study was undertaken to develop and validate a simple, sensitive and rapid analytical UPLC method for simultaneous estimation of Formoterol fumarate and Fluticasone propionate in dry powder inhalation formulation. The developed method was validated by means of system suitability, specificity, accuracy, precision, linearity, LOD, LOQ and robustness as per ICH guidelines. The results of the study indicate that the proposed UPLC method of analysis can be successfully used in routine analysis of formulation containing Formoterol fumarate and Fluticasone propionate.

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