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Original Research Paper

## STABILITY INDICATING HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF MOMETASONE FUROATE AND FORMOTEROL FUMARATE IN COMBINED DOSAGE FORM

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### ABSTRACT

A simple, specific, accurate, and stability indicating reversed-phase high-performance liquid chromatographic method was developed for the simultaneous determination of formoterol fumarate and mometasone furoate using a Enamal C18 column and a mobile phase composed of acetonitrile: 0.05 M orthophosphoric acid: methanol (60:30:10 v/v), pH 3. The retention times of formoterol fumarate and mometasone furoate found to be 1.68 min and 7.17 min, respectively. Linearity was established for of formoterol fumarate and mometasone furoate in the range of 3-9 µg/ml and 100-300 µg/ml, respectively. The percentage recoveries of formoterol fumarate and mometasone furoate were found to be in the range of 98.66-99.31%. Both the drugs were subjected to acid and base hydrolysis, oxidation, photolytic, and thermal degradation conditions. The degradation products of formoterol fumarate and mometasone furoate were well resolved from the pure drug with significant differences in their retention time values. This method can be successfully employed for simultaneous quantitative analysis of formoterol fumarate and mometasone furoate in bulk drugs and formulations.

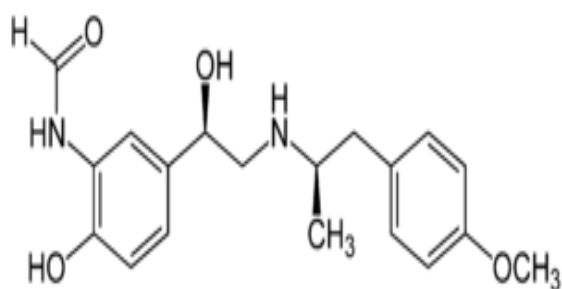
**Keywords:** Formoterol fumarate, Mometasone furoate, Degradation products, Stability indicating HPLC method, Dosage form.

### INTRODUCTION<sup>1-7</sup>

Formoterol fumarate (FF) is N-[2-hydro-5-(1-hydro-2-{{1-(4-methoxyphenyl) propan-2-} amino} ethyl) phenyl] formamide (figure 1). It is a white crystalline, soluble in ethanol and methanol, slightly soluble in water, practically insoluble in acetonitrile. FF is long-acting selective  $\beta_2$ -adrenergic receptor agonist ( $\beta_2$ -agonist). Inhaled FF acts locally in the lung as a bronchodilator. FF is official in Indian Pharmacopoeia, which recommends a high performance liquid chromatographic (HPLC) method for its analysis. Mometasone furoate (MF) is (11 $\beta$ , 16 $\alpha$ )-9, 21-dichloro-11-hydroxy-16-methyl-3, 20-dioxopregna-1, 4-dien-17-yl 2-

furoate (fig. 2). It is a white crystalline powder, soluble in acetone and dichloromethane and slightly soluble in ethanol. Practically insoluble in water. MF is a corticosteroid demonstrating potent anti-inflammatory properties. The precise mechanism of corticosteroid action on allergic rhinitis is not known. 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. The

combination of these two drugs are used to reduce the death caused by FF. The combination of these



**Figure 1:** Chemical structure of FF

Literature survey reveals that many analytical methods are reported for determination of FF and MF individually and combination with other drugs like UV, HPLC, Ratio Derivative Spectrophotometry but are no method available for the simultaneous estimation of FF and MF by stability indicating HPLC method.<sup>8-21</sup>

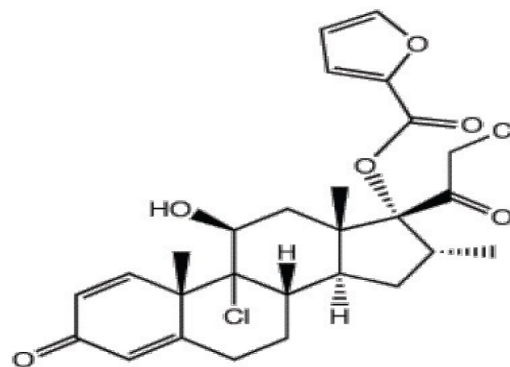
## MATERIALS AND METHODS

FF and MF working standard were procured from Intas Pharmaceutical and West Coast Pharmaceutical, respectively. Combination product of FF and MF (Label claim: 6 µg and 200 µg, respectively) Formost 200 (Manufacture: Cadila) was purchased from the local pharmacy. Methanol, water, Orthophosphoric acid (OPA), sodium hydroxide, hydrochloric acid, and hydrogen peroxide were used of HPLC grade and purchased from Merck Specialties Pvt. Ltd., Mumbai. The chromatographic analysis was performed using Lc solution, C18 column (250×4.6 mm, 5 µm particle sizes) as a stationary phase.

### Preparation of Mobile Phase

Mobile Phase comprising Acetonitrile: Orthophosphoric acid (0.05 M): Methanol in the ratio of 60:30:10. Mobile phase was prepared by mixing 600 ml acetonitrile, 300 mL Orthophosphoric acid (0.05 M) and 100 ml of Methanol. This mixture was sonicated and filtered through 0.45 µm membrane filter and used as a mobile phase. To prepare 0.05 M orthophosphoric acid solution take 4.9 ml of concentrated orthophosphoric acid in to 1000 ml

two drugs is used when asthma is not controlled with long term asthma control medicine.



**Figure 2:** Chemical structure of MF

volumetric flask and dilute up to 1000mL with water.

### Preparation of Diluent

Diluent was prepared by using 600 ml acetonitrile, 300 ml water and 100 ml Methanol. This mixture was sonicated and filtered through 0.45 µm membrane filter and used as a diluent.

### Preparation of Standard Stock Solution of FF

An accurately weighed quantity of standard FF (10 mg) powder and transferred to 10 ml volumetric flask and made up to mark with diluent (1000 µg/ml).

### Preparation of Standard Stock Solution of MF

An accurately weighed quantity of standard MF (100 mg) and transferred to 100 ml volumetric flask and was made up to mark with diluent (1000 µg/ml)

### Preparation of Working Solution of FF

Pipette out 1 ml from stock solution (1000 µg/ml) and transferred in to 10 ml volumetric flask and made up to the mark with diluent (100 µg/ml).

### Chromatographic Condition

- Column: C18 Enable (25 cm \* 4.6 mm, 5µ)
- Mobile phase: Acetonitrile: 0.1% OPA : MeOH (60:30:10)
- Injection volume: 20 µl
- Flow rate: 1.0 ml/min
- Detection wavelength: 248 nm
- Temperature: 25°C

### Forced Degradation Studies

#### Acid Degradation

Solution of FF and MF for acid degradation study was prepared using 0.1 N HCl for 30 min to facilitate acid degradation. It was diluted to prepare (6:200 µg/ml FF and MF respectively) with the diluent. The solution was then sonicated for 10 min followed by filtration through 0.45 µm filter and then injected into HPLC. The chromatogram was obtained and the degraded peak was separated from the analyte.

#### *Alkali Degradation*

Solution of FF and MF for base degradation study was prepared using 0.1 N NaOH for 30 min to facilitate base degradation of FF and MF. It was diluted to prepare (6:200 µg/ml FF and MF respectively) with the diluent. The solution was then sonicated for 10 minutes followed by filtration through 0.45 µm filter and then injected into HPLC. The chromatogram was obtained and the degraded peak was separated from the analyte.

#### *Oxidation Degradation*

Solution of FF and MF for oxidation degradation study was prepared using 3% H<sub>2</sub>O<sub>2</sub> for 24 hr to facilitate oxidative degradation of FF and MF. This solution was kept inside the water bath maintained at a temperature of 70°C. Samples were withdrawn at an interval of 30 minutes. It was diluted to prepare (6:200µg/m FF and MF respectively) with the diluent. The solution was then sonicated for 10 minutes followed by filtration through 0.45µm filter and then injected into HPLC. The chromatogram was obtained and the degraded peak was separated from the analyte

#### *Thermal Degradation*

Thermal degradation was performed by placing FF and MF bulk drug in the incubator at 100°C. 10mg of both the sample was added to two different 10 ml volumetric flask. Add 5 ml of diluent and sonicated for 5 min and make up volume which give the concentration of 1000 µg/ml for FF and MF. Further dilution gives the concentration 6:200 µg/ml of FF and MF respectively. It was sonicated and filtered through 0.45µm filter and injected into HPLC. The chromatogram was obtained and the degraded peak was separated from the analyte

#### *Photo Degradation*

API of FF and MF were exposed to sun light and UV light to determine the effects of light radiation on the stability of FF and MF in the solid state. Approximately 100 mg of FF and MF were spread on a petri dish in 2 mm thick layer. All samples for photo stability testing were placed in direct sun light and UV light for 1 week. The powder was removed and final concentration was made of 6:200 µg/ml of FF and MF respectively. It was sonicated and filtered through 0.45 µm filter and injected into HPLC. The obtained chromatogram was analyzed for any degradation undergone during the time.

#### **Method Validation**

##### *Specificity*

Specificity was performed by taking the chromatogram of the diluent, FF (6 µg/ml), MF (200 µg/ml) and binary mixture of FF and MF(6 µg/ml and 200 µg/ml FF and MF respectively) .

##### *Linearity Solutions*

For preparing linearity solutions, pipetted out 0.3, 0.45, 0.6, 0.75 and 0.9 ml from working solution of FF (100 µg/ml) and 1.0, 1.5, 2, 2.5 and 3.0 ml from working solution MF (1000 µg/ml) and transferred in to 10 ml volumetric flask and made up to the mark with diluent. So the obtained concentrations were 3, 4.5, 6, 7.5, 9 µg/ml and 100, 150, 200, 250, 300 µg/ml of FF and MF respectively.

##### *Precision*

Precision was performed by taking six replicates of (6:200 µg/ml for FF and MF respectively) for repeatability. For interday and intraday precision (4.5, 6, 7.5 : 150, 200, 250 µg/ml for FF and MF respectively) were injected for three times for consecutive three days and three times in a day respectively.

##### *Accuracy*

Accuracy was performed at three level (80%, 100%, and 120%) by taking 3:100 µg/ml of FF and MF respectively as 100%. To determine the accuracy, standard addition was used.

##### *Limit of Detection and Limit of Quantization*

Limits of detection (LOD) and limit of quantification (LOQ) were estimated by using following equations:

$LOD = 3.3 \times \text{Standard deviation of intercept} / \text{Slope of calibration curve}$  and

$LOQ = 10 \times \text{Standard deviation of intercept} / \text{Slope of calibration}$

### Robustness

The robustness of the developed method was established by deliberating varied chromatographic conditions such as Flow rate and ratio of mobile phase.

### Analysis of Marketed Formulation

Twenty capsules were accurately weighed and powdered. The quantity of the powder equivalent

to 60 mg FF and 200 mg MF was accurately weighed and transferred into 100 ml volumetric flask. The volume made up to the mark with mobile phase. The solution was filtered through whatman filter paper. The both solutions of FF and MF were further diluted with the mobile phase to obtain final concentrations of 6:200  $\mu\text{g/ml}$  for FF and MF, respectively.

## RESULTS AND DISCUSSION

### Stability Results

The results obtained in acidic degradation, alkaline degradation, oxidative degradation, thermal degradation and photolytic degradation are depicted as chromatograms and given in figure 3, 4, 5, 6 and 7 respectively .

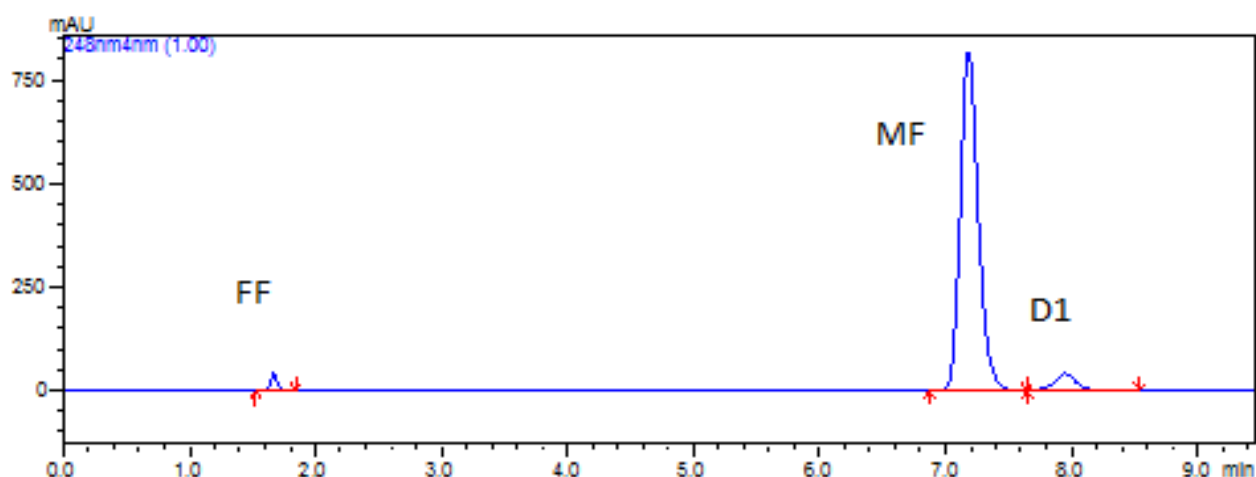


Figure 3: Chromatogram of acidic degradation

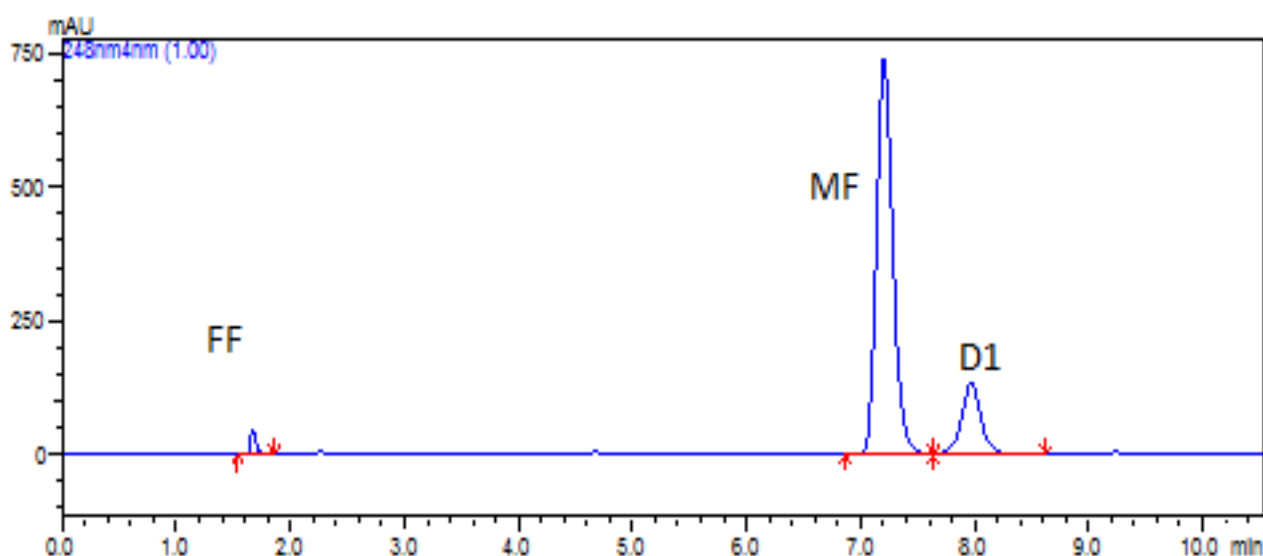
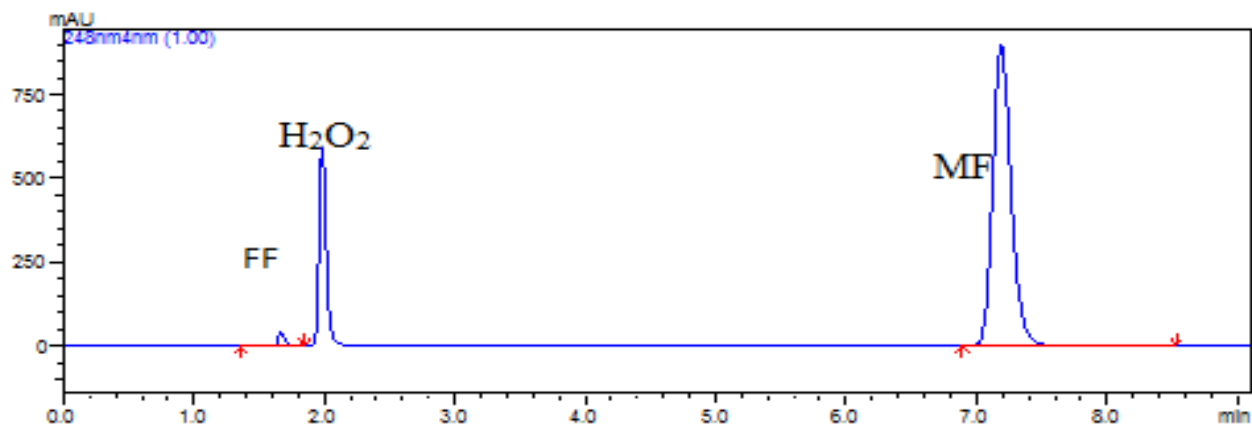
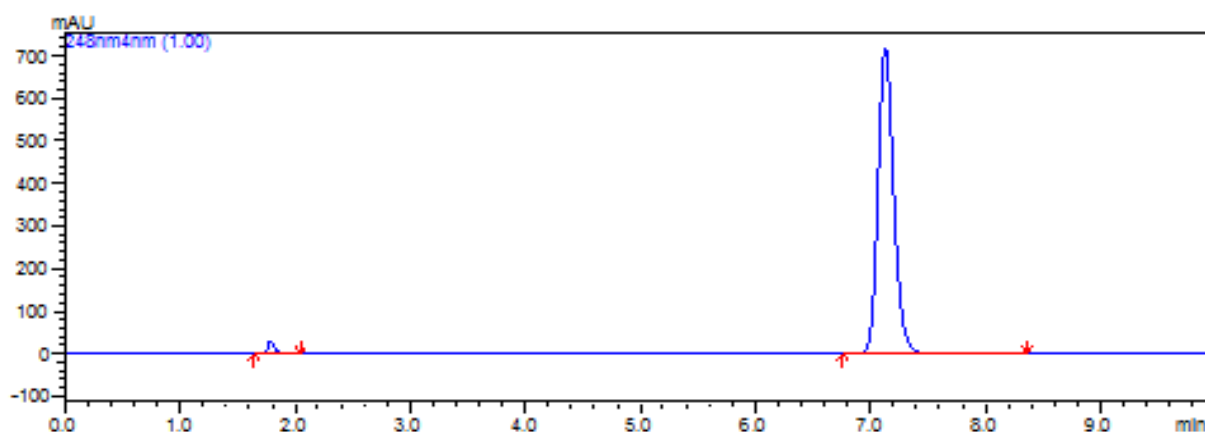


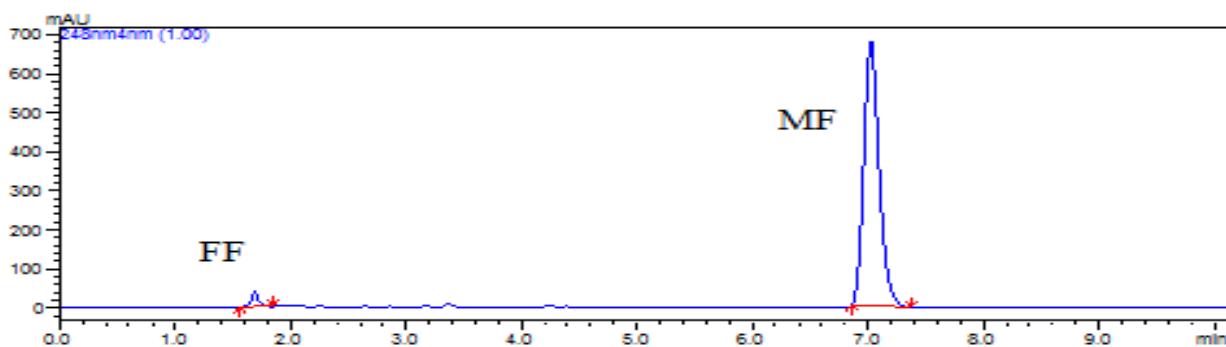
Figure 4: Chromatogram of alkaline degradation



**Figure 5:** Chromatogram of oxidative degradation



**Figure 6:** Chromatogram of thermolytic degradation



**Figure 7:** Chromatogram of photo degradation

**Table 1:** Stability study results of FF

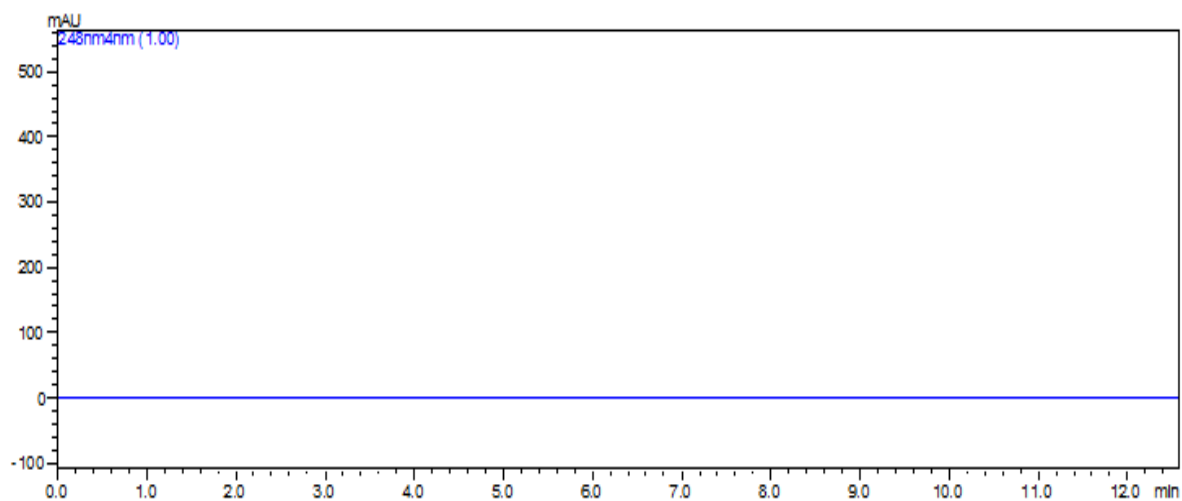
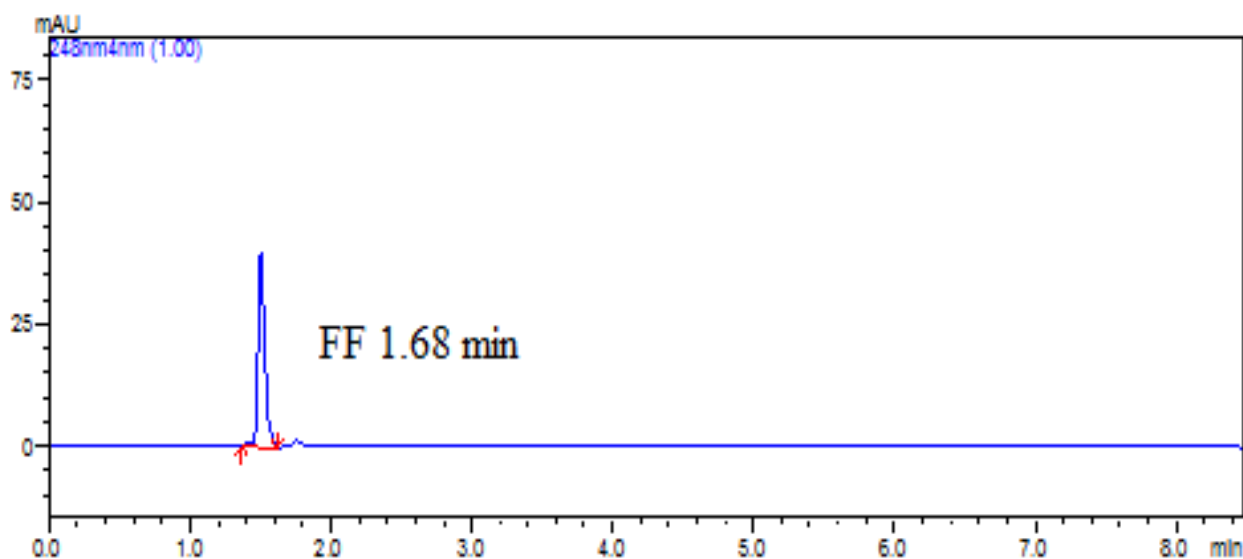
Conditins	Conc. (µg/ml)	Time	Peak Area		% Degraded
			Before Degradation	After Degradation	
Acidic Degradation	6	30 min	139812	137849	1.41
Alkaline Degradation	6	30 min	139812	138643	0.84
Oxidative Degradation	6	24 hr	139812	120321	13.95
Photolytic Degradation	6	1 week	139812	126084	9.82
Thermolytic Degradation	6	1 week	139812	126100	9.81
Neutral Degradation	6	3 Hours	139812	138401	1.01

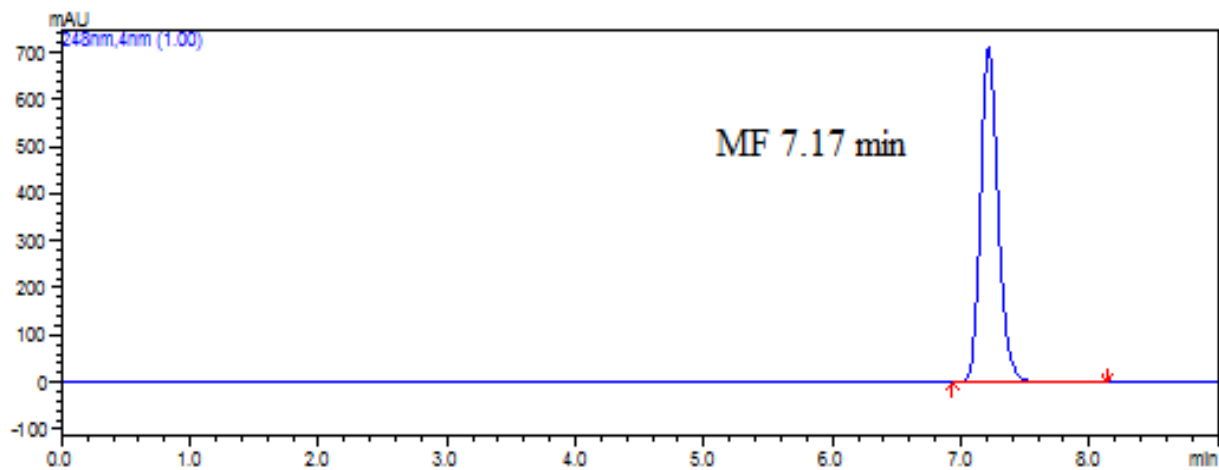
**Table 2:** Stability Study Results of MF

Condition	Conc. ( $\mu\text{g/ml}$ )	Time	Peak Area		% Degraded
			Before Degradation	After Degradation	
Acidic Degradation	200	30 min	8618985	6439165	25.30
Alkaline Degradation	200	30 min	8618985	7377015	14.41
Oxidative Degradation	200	24 hr	8618985	8042403	0.67
Photolytic Degradation	200	1 week	8618985	6375311	26.04
Thermolytic Degradation	200	1 week	8618985	7504162	12.93
Neutral Degradation	200	3hours	8618985	8592372	0.31

### Specificity

Chromatogram of diluent was shown that there is no interfeare from the diluent.

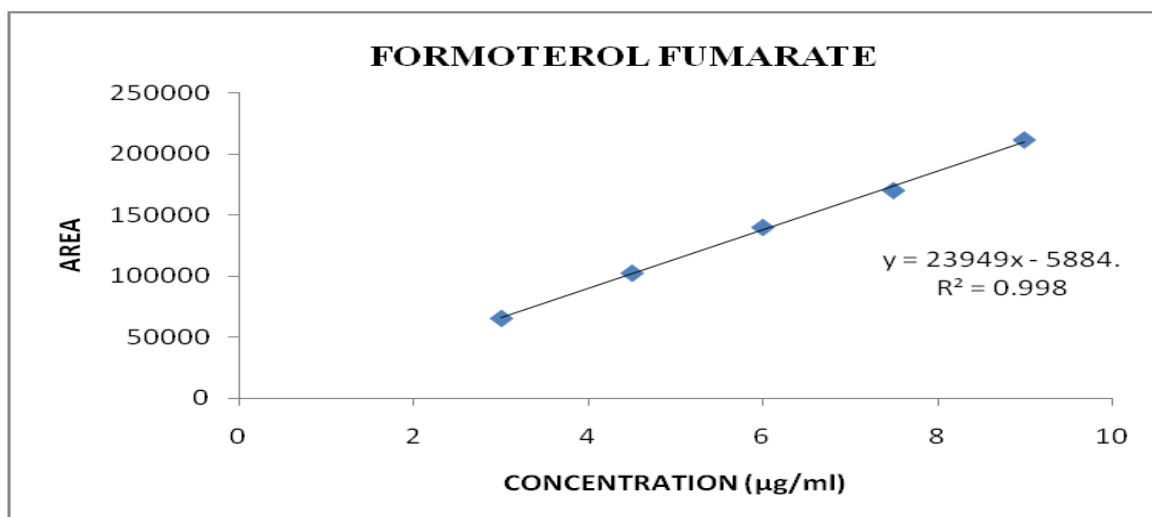
**Figure 8:** Chromatogram of Diluent**Figure 9:** Chromatogram of FF (6  $\mu\text{g/ml}$ )



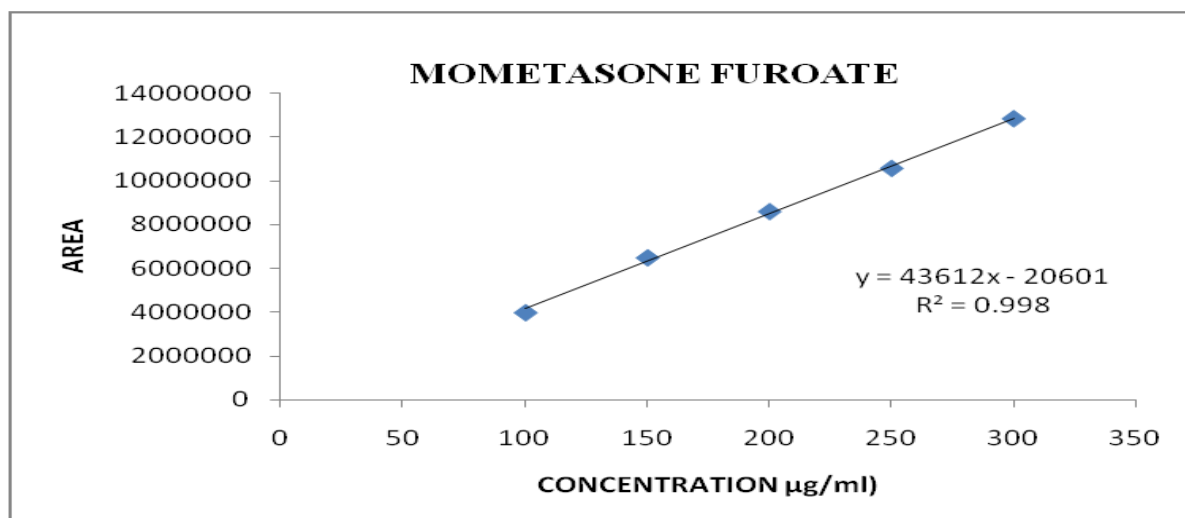
**Figure 10:** Chromatogram of MF (200 µg/ml)

**Linearity**

Linearity was performed and the  $r^2$  value was found to be 0.998 for both the drug FF and MF. The calibration curves (figure 11,12) and data (table 3) are shown below.



**Figure 11:** Calibration curve of FF



**Figure 12:** Calibration curve of MF

**Table 3:** Linearity of FF and MF

Concentration ( $\mu\text{g/ml}$ )		Area $\pm$ SD (n=3)		%RSD	
FF	MF	FF	MF	FF	MF
3	100	65491 $\pm$ 82.1969	4000099 $\pm$ 14142.14	0.125	0.352
4.5	150	102432 $\pm$ 1527.52	6508551 $\pm$ 5567.765	1.486	0.085
6	200	139812 $\pm$ 599.8544	8618985 $\pm$ 6806.850	0.429	0.078
7.5	250	169989 $\pm$ 1732.05	10593233 $\pm$ 152752.5	1.024	1.437
9	300	211332 $\pm$ 1538.46	12860664 $\pm$ 220529.7	0.722	1.734

**Precision***Repeatability*

Precision was performed and the %RSD were found 1.030 and 0.331 for FF and MF respectively (table 4).

*Intraday and Interday*

The %RSD were found to be 0.602-0.717 and 0.335-1.279 for Intraday and 0.928-1.052 and 0.845-1.413 for Interday precision for FF and MF respectively (Table 5 and 6).

**Table 4:** Repeatability data for FF and MF

Concentration		Area	
FF	MF	FF	MF
6	200	138612	8618985
		139245	8658125
		139684	8612254
		139548	8639859
		139658	8654859
		135987	8698989
AVG OF AREA		138789	8647179
SD		1429.877	28668.55
%RSD		1.030252	0.331536

**Table 5:** Intra-day precision data for FF and MF

Drug	Conc. ( $\mu\text{g/ml}$ )	Average (n=3)	SD	%RSD
FF	4.5	102089	732.4391	0.717452
	6	102760	59335.3	0.602456
	7.5	166098	1028.252	0.619064
MF	150	6502986	21821.78	0.335566
	200	8593899	39865.17	0.463878
	250	10692839	136783.2	1.279203



**Table 6:** Inter-day precision data for FF and MF

Drug	Conc. ( $\mu\text{g/ml}$ )	Average (n=3)	SD	%RSD
FF	4.5	109525	1016.782	0.928351
	6	137376	1445.661	1.052334
	7.5	168554	1622.105	0.962365
MF	150	6574864	85451.28	1.299666
	200	8464708	71541.34	0.845172
	250	10828496	153073.3	1.413616

**Accuracy**

Accuracy was performed and %recovery were found 97.52- 101.00% and 98.68-101.98% for FF and MF respectively (table 7)

**Table 7:** Result for accuracy of FF

Sample	FF	Average area $\pm$ SD (n=3)	% Recovery $\pm$ SD
1	80%	121533 $\pm$ 1275.17	98.52 $\pm$ 0.392
2	100%	134812 $\pm$ 930.888	97.91 $\pm$ 0.707
3	120%	153771 $\pm$ 477.935	101.00 $\pm$ 0.465

**Table 8:** Result for accuracy of MF

Sample	MF	Average area $\pm$ SD (n=3)	% Recovery $\pm$ SD
1	80%	7677926 $\pm$ 6643.48	98.68 $\pm$ 0.377
2	100%	8618485 $\pm$ 7912.76	99.04 $\pm$ 0.602
3	120%	9764157 $\pm$ 8733.45	101.98 $\pm$ 0.441

**LOD and LOQ**

LOD and LOQ were performed and the limit of detection were found to be 0.15 $\mu\text{g/ml}$  and 1.062  $\mu\text{g/ml}$  for FF and MF respectively. The limit of quantification were found to be 0.47 $\mu\text{g/ml}$  and 3.22  $\mu\text{g/ml}$  for FF and MF respectively (table 9).

**Table 9:** LOD and LOQ

Parameter	FF	MF
SD of y-Intercepts	1130.44	13978.13
Mean Slope	23993.6	43403.33
LOD ( $\mu\text{g/ml}$ )	0.15	1.062
LOQ ( $\mu\text{g/ml}$ )	0.47	3.22

**Robustness**

Robustness was performed and the %RSD were found to be  $< 2$  (table 10).

**Table 10:** Robustness result for variation in Mobile phase Ratio

Ratio of mobile Phase	FF			MF		
	Amount taken ( $\mu\text{g/ml}$ )	Mean Area $\pm$ S.D (n=3)	%RSD	Amount taken ( $\mu\text{g/ml}$ )	Mean Area $\pm$ S.D (n=3)	%RSD
60+29+11	6	164479.3 $\pm$ 1132.301	0.688416	200	9071788 $\pm$ 19851.48	0.2188
60+30+10	6	139580.3 $\pm$ 297.375	0.21305	200	8629788 $\pm$ 24770.26	0.2870
60+31+09	6	166275 $\pm$ 3295.593	1.982014	200	9164898 $\pm$ 30741.21	0.3354

**Table 11:** Robustness result for variation in Flow Rate

Change in Flow Rate	FF			MF		
	Amount taken ( $\mu\text{g/ml}$ )	Mean Area $\pm$ S.D (n=3)	%RSD	Amount taken ( $\mu\text{g/ml}$ )	Mean Area $\pm$ S.D (n=3)	%RSD
1.26	6	137023.7 $\pm$ 437.4521	0.319253	200	8595305 $\pm$ 60073.7	0.6989
1.4	6	139562 $\pm$ 298.161	0.213641	200	8660387 $\pm$ 11518.7	0.1330
1.54	6	138424 $\pm$ 818.1834	0.59107	200	8622885 $\pm$ 28063.3	0.3254

**Table 12:** Estimation of the drugs in FORMOST 200

Drug	Label claim	Concentration obtain ( $\mu\text{g}$ )	% Assay $\pm$ SD
FF	6 $\mu\text{g}$	5.92 $\pm$ 0.44	98.66 $\pm$ 0.52
MF	200 $\mu\text{g}$	198.62 $\pm$ 0.38	99.31 $\pm$ 0.35

**Table 13:** Summary of validation parameters for RP-HPLC method

Sr. No.	Validation Parameter	FF	MF
1	Linearity		
	Regression Equation	y= 23949x - 5884	y= 43612x - 20601
	Regression Coefficient	0.998	0.998
2	Range ( $\mu\text{g/ml}$ )	3-9	100-300
3	Accuracy (%Recovery)	97.91-101.00	98.68-101.98
4	Precision (%RSD)		
	Repeatability	0.419	0.331
	Intraday	0.602-0.717	0.335-1.279
	Inter-day	0.928-1.052	0.845-1.413
5	LOD ( $\mu\text{g/ml}$ )	0.15	1.062
6	LOQ ( $\mu\text{g/ml}$ )	0.47	3.22
7	Robustness (%RSD)		
	Different Ratio	0.213-1.982	0.218-0.335
	Different Flow rate	0.213-0.591	0.133-0.698
8	%Assay	98.66 $\pm$ 0.52	99.31 $\pm$ 0.35

## CONCLUSION

A rapid and efficient stability indicating RP-HPLC method was developed for the estimation of FF and MF in pharmaceutical formulation and their degradation products. The proposed method was demonstrated to be accurate, precise, specific, sensitive, linear and robust based on method validation parameters. Satisfactory results were obtained in separating the peaks of active pharmaceutical ingredients from the degradation products produced by forced degradation. Furthermore, the new method are cost-effective. Thus, it can be used for routine analysis in quality control and any kind of stability and validation studies

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