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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF FLUTICASONE PROPIONATE AND MUPIROCIN IN A COMBINED TOPICAL DOSAGE FORM

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Keywords:

Fluticasone Propionate, Mupirocin, RP-HPLC, Validation

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ABSTRACT: The objective of the present study was to develop and validate a precise and accurate reversed-phase high-performance liquid chromatography for simultaneous estimation of for Fluticasone Propionate (FUP) and Mupirocin (MUP) in combined topical dosage form as per ICH guidelines. Chromatographic separation was achieved using HPLC Shimadzu, Japan, with column syncronis C_{18} (250 × 4.6 mm, 5 µm). The mobile phase was comprised of 0.01% OPA: Acetonitrile (30:70v/v) (pH: 5) pumped at a rate of 1.0 mL/min. About 20 µL of sample solutions were injected and monitored at 232 nm. Column temperature and sample compartment were maintained at 25° and 5°, respectively. Repeatability, intra, and inter-day precision results were well within the tolerable limits. The linearity was found for range 2.5µg/mL - 7.5µg/mL and 100µg/mL - 300 µg/mL for FUP and MUP respectively. The correlation coefficient of linearity was found to be 0.998 and 0.994 for FUP and MUP, respectively. The limit of detection was found to be 0.3527, and 0.5077and limit of quantification was found to be 1.0690 and 1.538 for FUP for MUP, respectively. This method appeared to be rapid, easy, accurate, specific, and robust. Therefore, the method could be applied for regular examination.

INTRODUCTION: [1-5] Atopic Dermatitis (AD), also known as atopic eczema, is a type of inflammation of the skin (dermatitis). It results in itchy, red, swollen, and cracked skin. Clear fluid may come from the affected areas, which often thicken over time. The condition typically starts in childhood with changing severity over the years. In children under one year of age, much of the body may be affected. Treatment involves avoiding things that make the condition worse, daily bathing with the application of a moisturizing cream afterward, applying steroid creams when flares.

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Flutibact Skin Ointment (10gm) s manufactured by Glaxo Smith Kine is used in Atopic dermatitis, containing fluticasone 0.05% w/w and Mupirocin 2% w/w. Fluticasone Propionate **Fig. 1**, a medium potency synthetic corticosteroid, is used topically to relieve inflammatory and pruritic symptoms of dermatoses and psoriasis, intranasally to manage symptoms of allergic and non-allergic rhinitis while Mupirocin **Fig. 2** used to treat bacterial infection of impetigo due to: *Staphylococcus aureus* and *Streptococcus pyogenes*. Mixed anti-inflammatory glucocorticoids and antibacterial agents, MUP, which inhibits synthesis of protein of the bacteria by binding to iso-leucyl tRNA- synthetase.

It is active against gram-positive and some gram-negative bacteria. Fluticasone Propionate indicates that it is in the medium range of potency as compared with other topical corticosteroids.

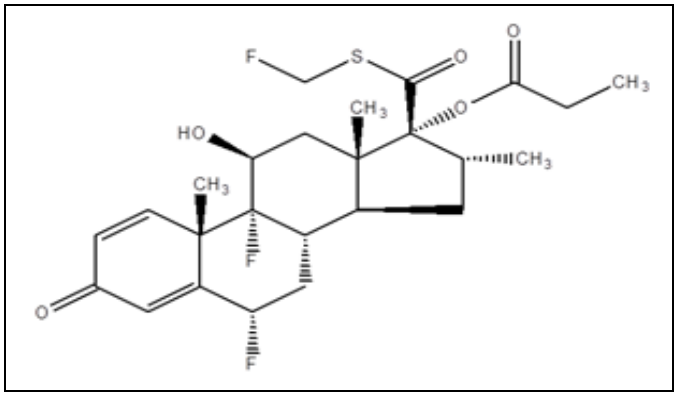

FIG. 1: CHEMICAL STRUCTURE OF FLUTICASONE PROPIONATE

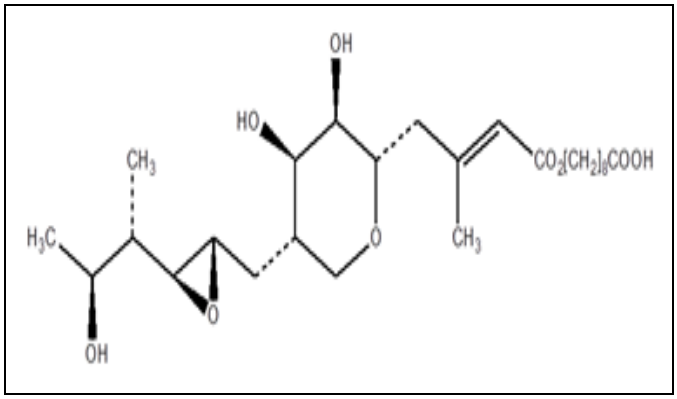

FIG. 2: CHEMICAL STRUCTURE OF MUPIROCIN

MATERIALS AND METHOD:

Materials: [7-9] Fluticasone Propionate (API) has been procured from Vamsi Labs Pvt. Ltd.And Mupirocin (API)has been received as gift samples from Glenmark pharmaceuticals, Mumbai. HPLC grade Acetonitrile, Methanol, and Water has been procured from Fischer scientific, Mumbai. HPLC grade orthophosphoric acid has been procured from RFCL limited. AR grade Potassium dihydrogen phosphate has been procured from Merck, and Sodium hydroxide has been procured from SD fine chem.

Method: [10-15]

Selection of Solvent: Based on solubility studies Acetonitrile and water mixture in the ratio of 50:50 was selected for method development of both the drugs Fluticasone Propionate and Mupirocin.

Selection of Wavelength: Standard solutions of Fluticasone Propionate and Mupirocin 10 µg/mL of each were prepared in acetonitrile: water mixture (50:50) as a solvent. Each solution was scanned between 200-400 nm using Methanol as a blank. The point at which both drugs show common absorbance (isosbestic point) was selected as a wavelength for determination in overlay spectra of both drug as shown in **Fig. 3**.

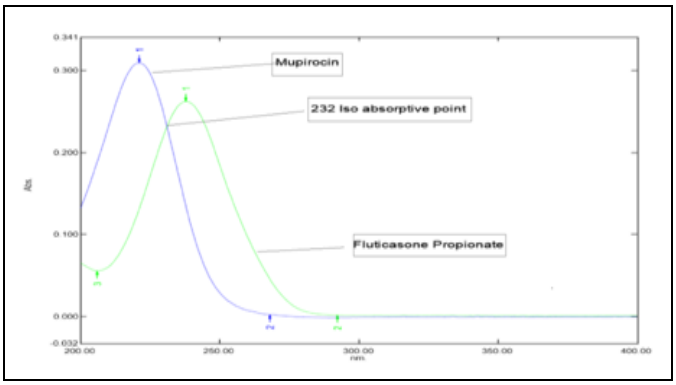

FIG. 3: OVERLAY SPECTRA OF FLUTICASONE PROPIONATE AND MUPIROCIN

Selection of Mobile Phase and Optimization of Chromatographic Condition:

Selection of Mobile Phase: Depending upon the solubility of the drugs, various solvent were tried as mobile phase for separation of Fluticasone Propionate and Mupirocin.

Preparation of Stock Solution for Fluticasone Propionate and Mupirocin: Accurately weighed 5 mg of Fluticasone Propionate and 20 mg of Mupirocin was transferred separately into 100 mL and 10 volumetric flasks respectively, dissolved and diluted up to mark with diluent. It gives a stock solution having a concentration of 50µg/mL Fluticasone Propionate and 2000µg/mL of Mupirocin, respectively.

Preparation of Working Standard Solution for Fluticasone Propionate and Mupirocin: From the stock solution of Fluticasone Propionate (50 µg/mL) 0.5, 0.75, 1.0, 1.25, and 1.5mL of aliquots were transferred in five different 10 mL volumetric flask and from the stock solution of Mupirocin (2000 µg/mL) 2.5, 3.75, 5.0, 6.25, and 7.5mL of aliquots were transferred in five different 10 mL volumetric flask and volume was made up to mark with the methanol to prepare 2.5, 3.75, 5.0, 6.25, and 7.5mL µg/mL of the Fluticasone Propionate and 100, 150, 200, 250 and 300 µg/mL of the Mupirocin.

Preparation of Mobile Phase: 0.1% OPA buffer was prepared (1.0 mL of ortho-phosphoric acid was diluted to 1000mL with HPLC grade water) and pH adjusted to 5 with NaOH and sonicated for 20 min, and acetonitrile was added.

RESULTS AND DISCUSSION: Different trials have been taken during optimization of the method where the list has been shown in **Table 1**.

Different Trials:

TABLE 1: SELECTION OF MOBILE PHASE OF MIXTURE

S. no.	Mobile Phase	Ratio %v/v	Retention Time (min)		Remark	Figure no.
			MUP	FLP		
1	Water : tetrahydrofuran : 1.05% ammonium acetate (pH5.7)	35:15:50	5.786	-	Mupirocin peak was observed but tailing was observed and Fluticasone Propionate peak was not observed	4.27
2	Methanol: Phosphate buffer	20:80	4.585	-	Mupirocin peak was observed but system suitability criteria not followed and Fluticasone Propionate peak was not observed.	4.28
3	Acetonitrile: 0.1M phosphate buffer (pH 6.3)	28:80	4.528	-	Mupirocin peak was observed and Fluticasone Propionate peak was not observed.	4.29
4	Buffer 0.1% OPA : ACN: Methanol	35:50:15	1.861	4.794	Mupirocin and Fluticasone Propionate peak was observed. All system suitability parameters were not as per criteria.	4.30
5	0.1%OPA Buffer: CAN	65:35	2.454	12.592	Peak separation was good with high resolution. RT prolonged Theoretical plates and tailing factors for both drugs were as per system suitability criteria	4.31
6	0.1%OPA Buffer: CAN	30:70	2.969	6.793	Peak separation was good with high resolution. Theoretical plates and tailing factors for both drugs were as per system suitability criteria.	4.32

Selection of Optimized Chromatographic Condition: Selection of optimized chromatographic conditions based on different trials.

Optimized Chromatographic Condition by Trial and Error Method:

Column: Syncronis C18, 250 mm × 4.6 mm, 5 µm.

Flow Rate: 1.0 mL/min.

Wavelength: 232 nm

Injection Volume: 10 µL

Run Time: 8 min

Mobile Phase

A. 0.1% OPA Buffer **B.** Acetonitrile

Mobile Phase Ratio: 30: 70 % v/v

The results of the optimized chromatographic conditions have been shown in **Table 2**. Where both the drugs eluted, as shown in **Fig. 4**.

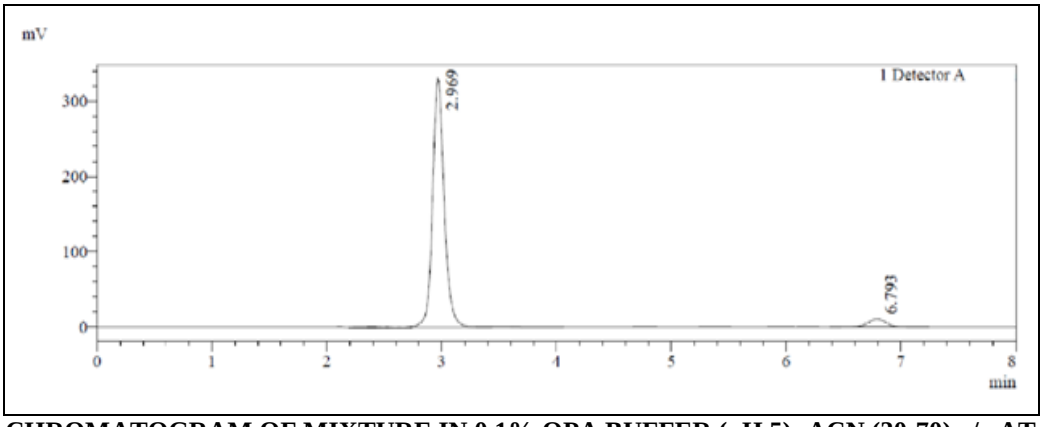

FIG. 4: CHROMATOGRAM OF MIXTURE IN 0.1% OPA BUFFER (pH 5): ACN (30:70) v/v AT 232 nm

TABLE 2: RESULT OF OPTIMIZED CHROMATOGRAPHIC CONDITION

Drug	Retention time (min)	Area (mv)	Theoretical plates	Tailing Factor	Resolution
Mupirocin	2.969	2309495	6067	1.148	15.833
Fluticasone Propionate	6.793	115638	8499	0.952	

Validation of RP-HPLC Method:

System Suitability Test: In this solution of Fluticasone Propionate and (5µg/mL) and Mupirocin (200µg/mL) was prepared. Parameters such as tailing factor, theoretical plate, resolution, reproducibility (% RSD, retention time, area) were determined, as shown in **Fig. 5**. The results of the system suitability have been shown in **Table 3**.

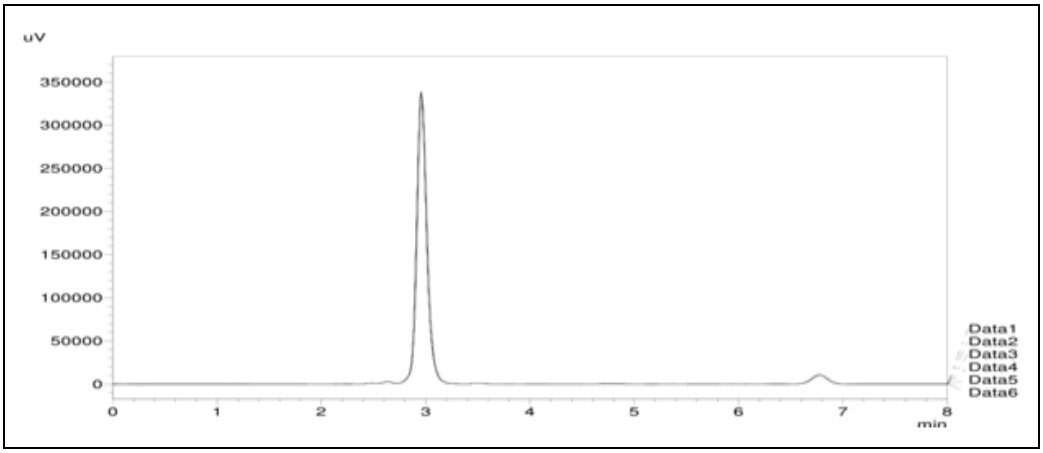

FIG. 5: CHROMATOGRAM OF SYSTEM SUITABILITY

TABLE 3: SYSTEM SUITABILITY DATA OF FLUTICASONE PROPIONATE (5µg/mL) AND MUPIROCIN (200µg/mL)

No. of runs	Retention time (min)		Theoretical plates		Tailing factor		Resolution
	MUP	FUP	MUP	FUP	MUP	FUP	
1	2.969	6.793	3977	8499	1.148	0.952	15.833
2	2.957	6.775	4088	8606	1.168	0.970	16.000
3	2.957	6.773	4121	8722	1.182	0.982	15.867
4	2.956	6.772	4144	8765	1.190	0.990	16.133
5	2.956	6.771	4150	8808	1.194	0.995	16.153
6	2.955	6.770	4159	8831	1.199	0.999	16.183
Avg.	2.958	6.775	4106.5	8705.166	1.1801	0.9813	16.0281
SD	0.0052	0.0086	68.3776	128.5358	0.0191	0.0176	0.1519
% RSD	0.1784	0.1278	1.6651	1.4765	0.0002	1.801	0.9478
Limit	< 2		>2000		< 2		> 2

Specificity: There was no interference of the placebo in the formulation of the chromatogram, as shown in **Fig. 6, 7,** and **8**.

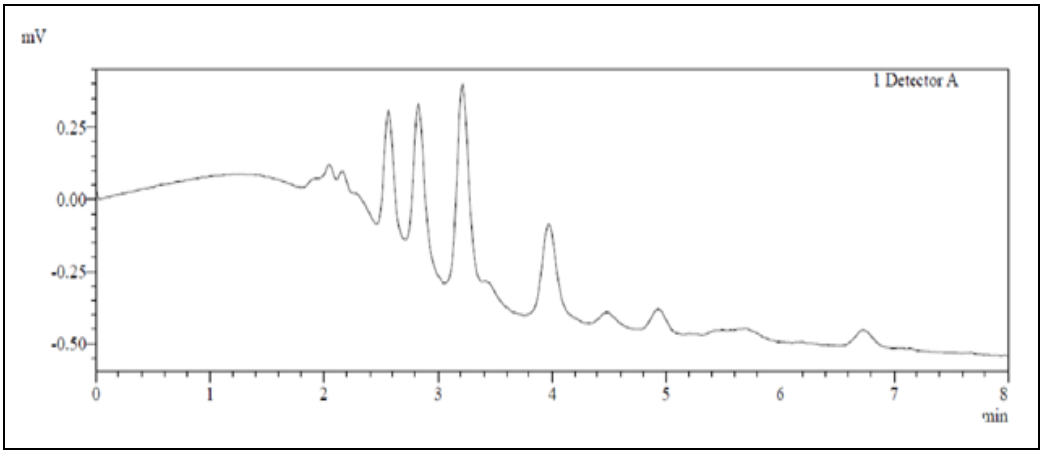

FIG. 6: CHROMATOGRAM OF BLANK

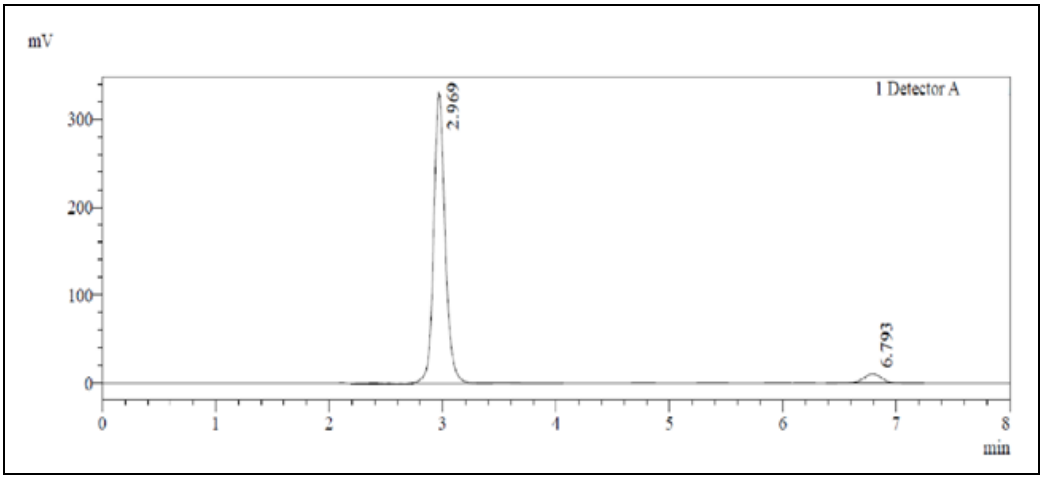

FIG. 7: CHROMATOGRAM OF STANDARD FLUTICASONE PROPIONATE AND MUPIROCIN

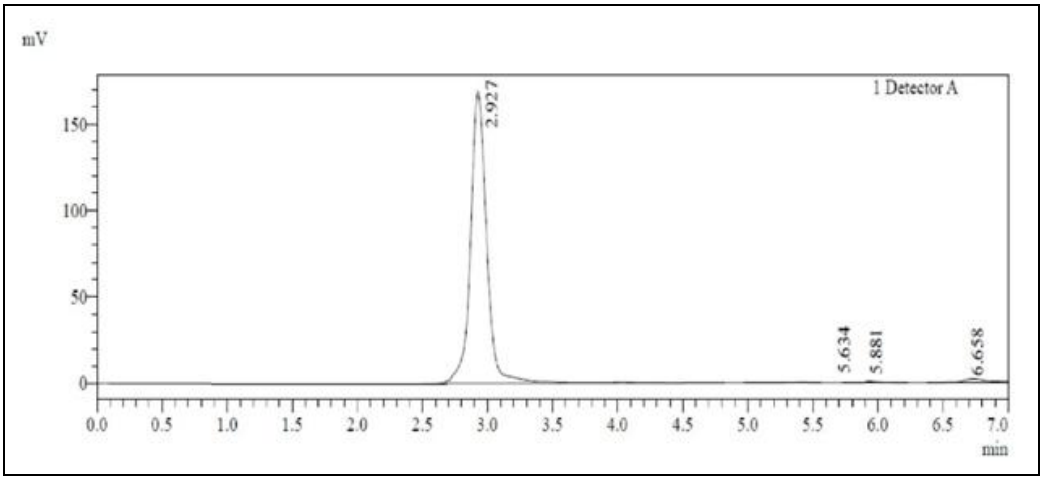

FIG. 8: CHROMATOGRAM OF FORMULATION FLUTICASONE PROPIONATE (5µg/mL) AND MUPIROCIN (200µg/mL)

Linearity and Range: The linearity for Fluticasone Propionate and Mupirocin was found to be in the range of 2.5 to 7.5µg/mL and 100 to 300µg/mL, respectively **Fig. 9, 10,** and **11**.

The peak areas for linearity of Fluticasone Propionate and Mupirocin have been shown in **Table 4** and **Table 5,** respectively.

TABLE 4: LINEARITY DATA FOR FLUTICASONE PROPIONATE

Concentration of Fluticasone Propionate (µg/mL)	Peak area
2.5	56491
3.75	78622
5	103297
6.25	129146
7.5	153261

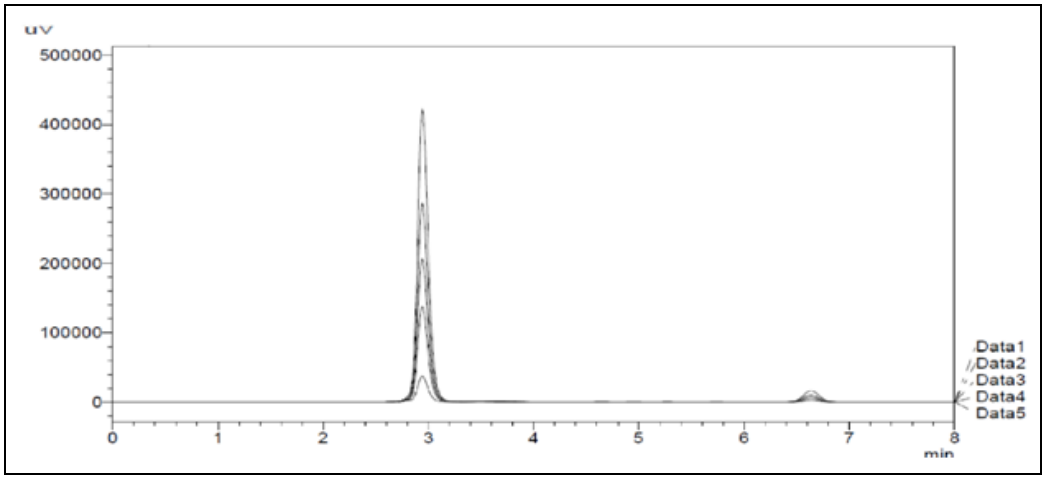

FIG. 9: OVERLAY CHROMATOGRAM OF FLUTICASONE PROPIONATE AND MUPIROCIN

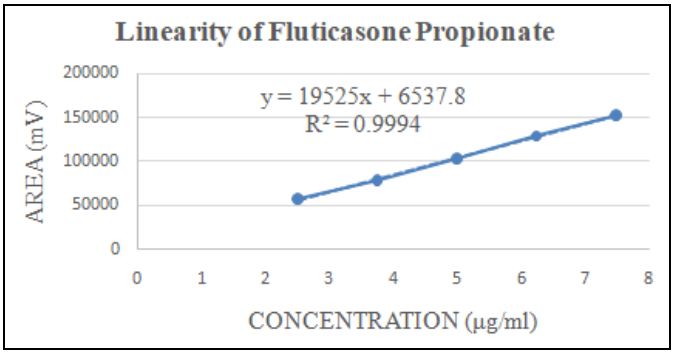

FIG. 10: CALIBRATION CURVE OF FLUTICASONE PROPIONATE

TABLE 5: LINEARITY DATA FOR MUPIROCIN

Concentration of Mupirocin (µg/mL)	Peak area
100	901735
150	1331619
200	1745171
250	2179093
300	2623795

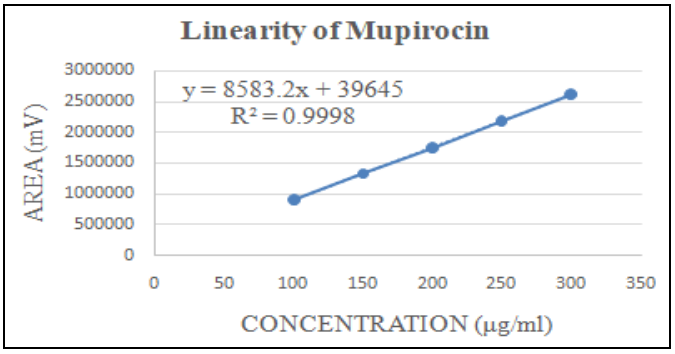

FIG. 11: CALIBRATION CURVE OF MUPIROCIN

Precision:

Repeatability: The data for repeatability of area measurement for Fluticasone Propionate (5µg/mL) and Mupirocin (200µg/mL) based on six measurements of the same solution of Fluticasone Propionate and Mupirocin % RSD was calculated. The peak areas have been shown in **Table 6**.

TABLE 6: REPEATABILITY DATA OF FLUTICASONE PROPIONATE (5µg/mL) AND MUPIROCIN (200µg/mL)

S. no.	Peak Area	
	MUP	FUP
1	1954088	134452
2	1952325	132606
3	1955171	134150
4	1958765	135722
5	1949065	131297
6	1954159	133462
Mean peak area	1953928.83	133593
SD	3201.217	1538.886
% RSD	0.163	1.151

Intraday Precision: The data for intraday precision of area measurement for the standard solution of Fluticasone Propionate (3.75, 5 and 6.25 µg/mL) and Mupirocin (150, 200 and 250 µg/mL), total nine determination were analyzed at three consecutive times on same day and % RSD was calculated. The peak areas have been shown in **Table 7**.

TABLE 7: INTRADAY PRECISION DATA OF FLUTICASONE PROPIONATE AND MUPIROCIN

Drug	Conc. (µg/mL)	Peak area			Mean peak area	S.D	% RSD
		I	II	III			
MUP	150	1451619	1445619	1456226	1451154.67	5318.723	0.366
	200	1855146	1865171	1896243	1872186.67	21427.914	1.144
	250	2282255	2260243	2292823	2278440.33	16621.608	0.729
FUP	3.75	98922	99543	99982	99482.333	532.597	0.535
	5	123022	125885	125255	124720.667	1504.435	1.206
	6.25	147619	149546	149196	148787	1026.544	0.689

Intermediate Precision:

Inter-day Precision: The data for inter-day precision of standard solution of Fluticasone Propionate (3.75, 5 and 6.25 µg/mL) and Mupirocin (150, 200 and 250 µg/mL), total nine determination were analyzed at three consecutive days and % RSD was calculated. The peak areas have been shown in **Table 8**.

TABLE 8: INTER DAY PRECISION DATA OF FLUTICASONE PROPIONATE AND MUPIROCIN

Drug	Conc. (µg/mL)	Peak area			Mean peak area	S.D	% RSD
		Day 1	Day 2	Day 3			
MUP	150	1451619	1431475	1436226	1439773.33	10530.093	0.731
	200	1855171	1845156	1876243	1858856.67	15867.844	0.853
	250	2282823	2255391	2262823	2267012.33	14187.725	0.625
FUP	3.75	99322	99022	98922	99088.666	208.166	0.210
	5	124885	124992	123385	124420.667	898.507	0.722
	6.25	147546	148546	150146	148746	1311.487	0.881

Robustness:

Different Wavelength: Robustness carried out by changing the wavelength, flow rate, and mobile phase ratio % RSD was calculated for Fluticasone Propionate and Mupirocin. The peak areas have been shown in **Tables 9, 10,** and **11**.

TABLE 9: DIFFERENT WAVELENGTH DATA FOR FLUTICASONE PROPIONATE AND MUPIROCIN

Drug	Conc. (µg/mL)	Peak area			Mean peak area	S.D	% RSD
		231 nm	232 nm	233 nm			
MUP	150	1481922	1484922	1483922	1483588.67	1527.525	0.102
	200	2050012	2056012	2043012	2049678.67	6506.407	0.317
	250	2579820	2589820	2575820	2581820	7211.102	0.279
FUP	3.75	84140	86250	84044	84811.333	1246.846	1.470
	5	141887	143987	142780	142884.667	1053.905	0.737
	6.25	177112	177312	176173	176865.667	608.144	0.343

TABLE 10: DIFFERENT FLOW RATE DATA FOR FLUTICASONE PROPIONATE AND MUPIROCIN

Drug	Conc. (µg/mL)	Peak area			Mean peak area	S.D	% RSD
		0.9 mL/min	1.0 mL/min	1.1 mL/min			
MUP	150	1581922	1581922	1601922	1591922	14142.135	0.888
	200	2233987	2263987	2293987	2278987	21213.203	0.930
	250	2697312	2707312	2757312	2732312	35355.339	1.293
FUP	3.75	86620	87820	88820	88320	707.106	0.800
	5	152887	152887	153887	153387	707.106	0.460
	6.25	180780	185780	189780	187780	2828.427	1.506

TABLE 11: DIFFERENT MOBILE PHASE RATIO DATA FOR FLUTICASONE PROPIONATE AND MUPIROCIN

Drug	Conc. (µg/mL)	Peak area			Mean peak area	S.D	% RSD
		29.71	30:70	31:69			
MUP	150	275013	278508	272050	275190.333	3232.650	1.174
	200	342088	347055	351020	346721	4475.357	1.290
	250	429076	422273	427088	426145.666	3498.027	0.820
FUP	3.75	2199920	2216188	2250268	2222125.333	25693.758	1.156
	5	2930123	2978033	2980327	2962827.667	28346.287	0.956
	6.25	3520624	3580848	3590602	3564024.667	37901.167	1.063

TABLE 12: DATA OF LOD AND LOQ

Parameter	Fluticasone Propionate	Mupirocin
SD of the Y-Intercepts of 5 Calibration curve	2611.221	6542.4512
Mean slope of 5 calibration curve	24426	42519
LOD (µg/mL)	0.3527	0.5077
LOQ (µg/mL)	1.0690	1.5387

LOD and LOQ: LOD and LOQ were calculated, and peak areas are shown in **Table 12** as follows:

Accuracy: Accuracy of the method was confirmed by the recovery study. % recovery of both Fluticasone Propionate and Mupirocin was found between 98% to 102%. The results have been reported in **Table 13**.

TABLE 13: ACCURACY DATA FOR FLUTICASONE PROPIONATE AND MUPIROCIN

Drug	% Level	Amt. of Sample taken (µg/mL)	Amt. of Standard spiking (µg/mL)	Total Amt. (µg/mL)	Conc. Found (µg/mL)	% Recovery	% recovery (mean ±SD)	% RSD
MUP	I	200	100	300	298.87	99.62	98.74±0.78	0.078
	(50%)	200	100	300	294.36	98.12		
		200	100	300	295.55	98.51		
	II	200	200	400	401.27	100.31		
	(100%)	200	200	400	398.99	99.74	99.95±0.31	0.312
		200	200	400	399.24	99.81		
	III	200	300	500	498.87	99.77	99.49±0.36	0.366
	(150%)	200	300	500	495.42	99.08		
		200	300	500	498.15	99.63		
FUP	I	5	2.5	7.5	07.49	99.86	99.41±0.65	0.66
	(50%)	5	2.5	7.5	07.48	99.73		
		5	2.5	7.5	07.40	98.66		
	II	5	5	10	09.89	98.90		

(100%)	5	5	10	09.98	99.80	99.06±0.66	0.67
	5	5	10	09.85	98.5		
III	5	7.5	12.5	12.45	99.60	99.90±.014	0.14
(150%)	5	7.5	12.5	12.55	100.4		
	5	7.5	12.5	12.48	99.84		

DISCUSSION: RP-HPLC method was developed using 0.01% OPA: Acetonitrile (30:70) pH 5 as a mobile phase flow rate 1.0mL/min and detection wavelength was 232 nm and retention time was found to be for 6.774 min and 2.995 min for Fluticasone Propionate and Mupirocin respectively. The linearity of the developed method was found to be nearer to 1, in the range 2.5-7.5µg/mL and 100-300µg/mL for Fluticasone Propionate and Mupirocin respectively. % RSD was found to be < 2 for repeatability, precision, and robustness. %assay was found to be 100.04-101.08% and 99.04-100.1% for Fluticasone Propionate and Mupirocin, respectively. The % recovery was found to be 99.06-99.9% for fluticasone propionate and 98.74-99.95% for Mupirocin, respectively. Validation of the developed method was done as per ICH guidelines, and these results show the validation parameters within the range according to ICH guidelines.

CONCLUSION: The developed method was simple, precise, accurate, and reliable for the simultaneous estimation of Fluticasone Propionate and Mupirocin in combined dosage form as per ICH guidelines. The % RSD of all results is less than 2% that shows a high degree. Hence, the proposed method was simple, easy, cost-effective, and can be used for routine analysis of Fluticasone Propionate and Mupirocin in the combined dosage form.

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CONFLICTS OF INTEREST: There are no conflicts of interest of any author regarding any of the work done.

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