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Development and Validation of Analytical Method for Simultaneous Estimation of Mometasone Furoate and Fusidic Acid in Pharmaceutical Dosage Form

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ABSTRACT:

A reversed-phase liquid chromatographic method has been developed and validated for estimation of Mometasone furoate and Fusidic acid in cream form. RP-HPLC method, Column used was C18 (150 x 4.6 mm i.d.,5µm) with mobile phase containing 10 mM ammonium formate in water pH 5.5:Methanol (65:35 %v/v). The flow rate (1.0 ml/min) and wavelength (240 nm). The retention time was found to be 4.325 mins and 8.109 mins of Fusidic acid and Mometasone furoate respectively. Correlation co-efficient for Fusidic acid and Mometasone furoatewas found to be 0.999. Assay result of marketed formulation wasfound to be in 99.3 % and 98.3 % for Fusidic acid and Mometasone furoate respectively . The proposed method was validated with respect to linearity, accuracy, precision androbustness. Recovery was found in the range of 99.5 %– 101.7 %. Statistical Analysis proves that the developed methods weresuccessfully applied for the analysis of pharmaceutical formulations and can be used for routine analysis of drugs in QualityControl laboratories.

KEYWORDS Mometasone furoate, Fusidic acid, HPLC, analytical method development, validation

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INTRODUCTION:

The IUPAC name of the Fusidic acid3 is ent-(17Z)-16α-(Acetyloxy)-3β-11β-dihydroxy-4β,8,14-trimethyl-18-nor-5β,10α-cholesta-17(20),24-dien-21-oic acid hemihydrate.Withmolecular formula and molecular weightC31H48O6, 1/2H2Oand 525.7 g.mol−1respectively.
The molecular structure of the drug is given in Fig.1

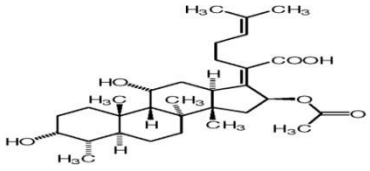

Figure 1: Chemical structure of Fusidic acid

Fusidic acid is used as a antibacterial 4.

The IUPAC name of the Mometasone furoate1,2 is 9,21-Dichloro-11β-hydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-17-yl furan-2-carboxylate.withmolecular formula and molecular weight C22H28Cl2O4 and 521.4 g.mol−1 respectively.

The molecular structure of the drug is given in Fig.2

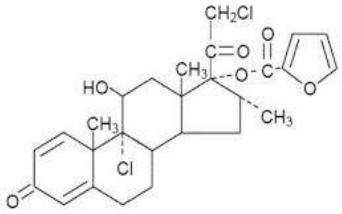

Figure 2: Chemical structure of Mometasone furoate

- Mometasone furoate is a glucocorticosteroid used as topically to reduse inflammation of the skin
- Mometasone furoate has anti-inflammatory, antipruritic, and vasoconstrictive properties.
- Corticosteroids are thought to act by the introduction to phospholipase A2 inhibitory proteins, collectively called lipocortins.
- It is postulated that these proteins control the biosynthesis of potent mediators of inflammation.[5]

However no HPLCmethod has been reported till date for theestimation of Mometasone furoate and Fusidic acid using the RP-HPLC method. The present paper describesthe analytical method development and validation of estimation ofMometasone furoate and Fusidic acid inPharmaceutical dosage form using RP-HPLC . The proposed methodare optimized and validated as per ICH guidelines.[6]

Materials and methods

Materials:

a) Instruments

- Analytical Weighing Balance
- Sonicator
- FT-IR spectrophotometer
- HPLC system

b) Glasswares

- Beaker
- Conical flask
- Measuring cylinder
- Petri dish
- Pipette
- Volumetric flask

c) Chemicals

Standard Mometasone furoate Gifted by Intracin pharmaceutical Pvt. Ltd, nadiad. Standard Fusidic acid Gifted by Intracin pharmaceutical Pvt. Ltd, nadiad. The commercial fixed dose combination Momoz-F was procured from local market. All solvents (HPLC grade) were obtained from S.D.fine chemical.

d) Method

Chromatographic method

Methods

Working Standard preparation

Solution<u>Preparation of Mometasone furoate : (10.0 µg/ml)</u>

About 10 mg of Mometasone furoate API was weight and dissolve in 100 ml of. methanol. Further diluted 5 ml of this solution to 50 ml with methanol.

Solution preparation ofFusidic acid : (200 µg/mL)

10 mg of Fusidic acid API was dissolved in 10 ml of diluent, further diluted 2 ml to 10 ml with diluent to get 200µg/ml concentration of Fusidic acid in solution.

Sample Preparation for marketed formulation[7]:

5gm of Cream dosage form containing Mometasone (0.1% w/w) and Fusidic Acid (2% w/w) was accurately weighed which contains equivalent to 10mg of Mometasone and 200mg of Fusidic Acid was transferred to 50mL of volumetric flask and then about 30mL of diluent was added into it. It was shaked for 30 minutes by mechanical means and then sonicated for 15 minutes. The solution was cooled up to room temperature and volume was made up to 50mL with diluent. The solution was mixed properly and it was filtered through 0.45µ Nylon syringe filter. 5mL of filtrate was diluted up to 50mL with diluent to get final concentration as 10µg/mL of Mometasone and 200µg/mL of Fusidic Acid. This sample was injected.

Fusidic acid: 200 ppm

Mometasone furoate: 10 ppm

METHOD VALIDATION

Chromatographic conditions and System SuitabilityParameters:

Pumps: Mode of chromatography: Reversed PhaseChromatography

Mode of Elution: Isocratic

Flow Rate: 1.0 ml/min

Oven: Oven Temperature: 35° ± 2°C

Detector: Type: uv detector

Wavelength: 240 nm

Column: Waters symmetry C-18, 150X4.6 mm, 5µ

Sample Volume: 10 µl

Run time: 10 min

Mobile Phase: 10 mM ammonium formate in water pH 5.5:Methanol(65:35 %v/v)

System Suitability Parameters:

Table 1: System Suitability Parameters

Sr. no.	System suitability parameter	Fusidic acid	Mometasone furoate
1	Retention times (R_T)	4.398	8.107
2	Theoretical plates (N)	7469	6325
3	Resolution (R_S)	-	4.7
4	Tailing factor (A_S)	1.0	1.0
5	% RSD	0.6	0.9

Linearity and Range (n=3):

The linearity response was determined by analyzing 5independent levels of calibration curve in the range of100-300 µg/ml for Fusidic acid and 5-15 µg/ml for Mometasone furoate.

The plot of peak area against concentration was plotted. Correlation coefficient and regression line equationswere calculated. Linearity range was established throughconsideration of required practical range and accordingto each drug concentration present in thepharmaceutical product, to give accurate, precise andlinear results**.**

Precision

Repeatability

Repeatability was determined by analyzing standardsolution ofFusidic acid having the concentration 200 µg/ml and Mometasone furoate having the concentration 10 µg/ml. Scanned these solutions six times in a day. Theresults were reported in terms of % RSD (relativestandard deviation).

Intraday Precision

The intra-day precision of the proposed method wasdetermined by measuring the corresponding responses 3times on the same day for 3 different concentration of Fusidic acid for 100,200 and 300 µg/ml and Mometasone furoate for 5,10 and 15 µg/ml. The results werereported in terms of % RSD.

Interday Precision

The inter-day precision of the proposed method wasdetermined by measuring the corresponding responseson 3 different days over a period of 1 week for 3 different.

concentration of Fusidic acid for 100,200 and 300 µg/ml and Mometasone furoate for 5,10 and 15 µg/ml. Theresults were reported in terms of % RSD.

Accuracy (% Recovery)

The accuracy of the method was determined bycalculating recovery of Fusidic acid and Mometasone furoate. by the Standardaddition method. Each solutionwas injected in triplicate and the percentage recoverywas calculated by measuring the peak areas and fittingthese values into the regression equation of therespective calibration curves.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit ofquantification (LOQ) were calculated using the standarddeviation of y-intercept of calibration curve (σ) andaverage of slope (S) of the calibration curve.

$LOD = 3.3 \times \sigma / s$

LOQ = 10 × σ /s

Robustness

The robustness was studied by analyzing the sample of Fusidic acid and Mometasone furoate by deliberate variation in the methodparameters. The change in the response was noted.

Robustness of the method was studied by changingdifferent experimental conditions like temperature ofcolumn by ± 2°C, Flow rate by ±0.2 ml/min, Mobile phaseby ± 2 %.

Result

VALIDATION PARAMETER

Linearity and Range

Linear correlation was obtained between peak area andconcentration of Fusidic acid in the range of 100-300µg/ml and Mometasone furoate in the range of 5-15 µg/ml. The linearity of the calibration curves wasvalidated by the value of correlation coefficients of theregression (r).

Table 2: Linearity data for Fusidic acid

% Linearity Level	Concentration (µg/ml)	Mean area	Correlation coefficient
50	100.0	860590	
75	150.0	1291500	
100	200.0	1723235	0.9999
125	250.0	2153405	
150	300.0	2596113	

Table 3: Linearity data for Mometasone furoate

% Linearity Level	Concentration (µg/ml)	Mean area	Correlation coefficient
50	5.0	675512	
75	7.5	1013612	
100	10.0	1354182	0.9999
125	12.5	1692222	
150	15.0	2036700	

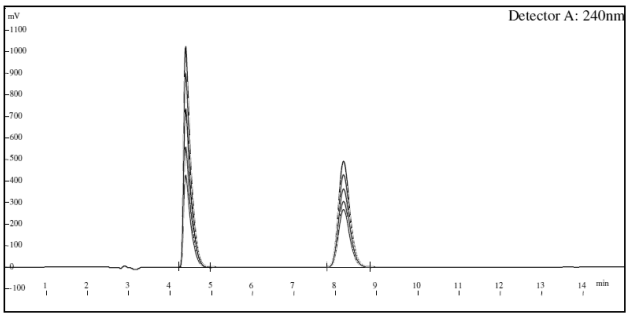

Figure 3: Overlay chromatogram of different concentration of Fusidic acid and Mometasone

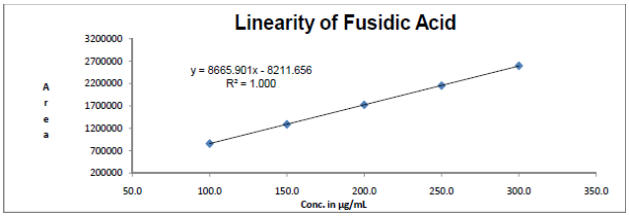

Figure 4: Calibration curve of Fusidic acid

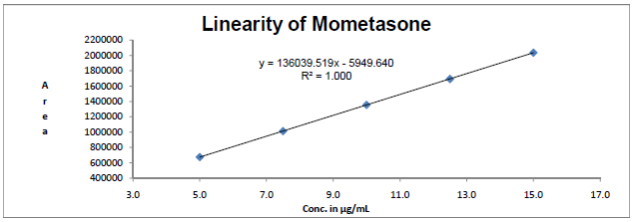

Figure 5: Calibration curve of Mometasone furoate

Precision

Repeatability

The data for repeatability for Fusidic acid is shown inTable-4. The % RSD for Repeatability data was found to be 0.6%.

Table 4: Repeatability of Fusidic acid

Sr. no	Area	Mean	% RSD
1	1699985		
2	1704523		
3	1714589	1707543	0.6
4	1723654		
5	1702555		
6	1699952		

The data for repeatability for Mometasone is shown in Table-5. The % RSD for Repeatability data was found to be 0.9%.

Table 5: Repeatability of Mometasone furoate

Sr. no	Area	Mean ± SD	% RSD
1	1335247		
2	1325896		
3	1324578	1335190±11675.3	0.9
4	1335874		
5	1356988		
6	1332555		

Intraday precision

The data for intraday precision for Fusidic acid is shownin Table-6. The % RSD for intraday precision was found to be 0.31%.

Table 6: Intraday precision for Fusidic acid

Sr. No.	Concentration (µg/ml)	Mean Area ± SD	% RSD
1	100.0	862060±999.2	0.12
2	200.0	1724580±1639.1	0.10
3	300.0	2598190±10636.3	0.41
Mean			0.31

The data for intraday precision for Mometasone furoate is shownin Table-7. The % RSD for intraday precision was found to be 0.57%.

Table 7: Intraday precision for Mometasone furoate

Sr. No.	Concentration (µg/ml)	Mean Area ± SD	% RSD
1	5.0	672367±2382.3	0.35
2	10.0	1347888±6609.2	0.49
3	15.0	2027289±17532.0	0.86
Mean			0.57

Interday precision

The data for interday precision for Fusidic acid is shownin Table-8. The % RSD for intraday precision was found to be 0.83%.

Table 8: Interday precision for Fusidic acid

Sr. No.	Concentration (µg/ml)	Mean Area ± SD	% RSD
1	100	860566±6780.7	0.79
2	200	1721590±13457.6	0.78
3	300	2593692±23960.6	0.92
		Mean	0.83

The data for intraday precision for Mometasone furoate is shownin Table-9. The % RSD for intraday precision was found to be 0.70%.

Table 9: Interday precision for Mometasone furoate

Sr. No.	Concentration (µg/ml)	Mean Area ± SD	% RSD
1	5.0	669396±4845.3	0.72
2	10.0	1341915±7432.8	0.55
3	15.0	2018311±18982.7	0.94
Mean			0.74

Accuracy

Accuracy of the method was confirmed by recovery studyfrom marketed formulation at three level of standardaddition. Percentage recovery for Fusidic acid and Mometasone furoate was foundto be 99.0 – 100.0%.

Table 10: Recovery Data of Fusidic acid

Accuracy Level %	Set no.	Area	Amount added (mg)	Amount recovery(mg)	%Recovery	Mean	%RSD
	1	1522225	80.02	79.16	98.9		
80 %	2	1531060	80.52	80.19	99.6	99.7	0.8
	3	1529407	79.62	80.0	100.5		
	1	1705059	100.52	100.57	100.0		
100%	2	1691081	100.02	78.94	98.9	99.6	0.6
	3	1696138	99.52	99.53	100.0		
	1	1873446	120.52	120.30	99.8		
120%	2	1871761	120.72	120.10	99.5	100.0	0.6
	3	1875564	119.82	120.55	100.6		

Table 11: Recovery Data of Mometasone furoate

Accuracy Level %	Set no.	Area	Amount added(mg)	Amount recovery (mg)	%Recovery	Mean	%RSD
80 %	1	1188333	4.01	3.94	98.3	99.1	0.7
	2	1191608	3.98	3.96	99.4		
	3	1201213	4.04	4.03	99.7		
100%	1	1319840	5.01	4.92	98.2	99.0	0.9
	2	1334760	5.04	5.03	99.9		
	3	1322173	5.00	4.94	98.8		
120%	1	1467357	6.01	6.02	101.1	99.2	0.8
	2	1458091	6.05	5.96	98.5		
	3	1459143	6.02	5.96	99.1		

Limit of detection and limit of quantification

The Limit of detection (LOD) and Limit of quantitation (LOQ) Fusidic acid and Mometasone furoate as mention below table.

Table 12: Results of LOD and LOQ

Drug	Fusidic acid	Mometasone furoate
LOD	0.261	0.0130
LOQ	0.790	0.0395

Robustness

The method is found to be robust as the results were notsignificantly affected by slight variation in composition ofmobile phase, Mobile phase pH and flow rate of the mobile phase.

Table 13: Change the ratio of mobile phase

Standard repetitions (n=6)	63 : 37		67 : 33	
	Fusidic acid	Mometasone furoate	Fusidic acid	Mometasone Furoate
Mean Area ± SD	1714657± 11149.94	1337286± 11075.36	1715093± 15866.46	1326308± 15561.18
% RSD	0.7	0.8	0.9	1.2

Table 14: Change the flow rate

Standard repetitions (n=6)	0.8 ml/min		1.2 ml/min	
	Fusidic acid	Mometasone furoate	Fusidic acid	Mometasone furoate
Mean Area ± SD	1874031± 13131.63	1468709± 12842.75	1550991 ±4927.73	1203897± 11976.34
% RSD	0.7	0.9	0.3	1.0

Table 15: Change the Mobile phase pH

Standard repetitions (n=6)	5.4		5.6	
	Fusidic acid	Mometasone furoate	Fusidic acid	Mometasone furoate
Mean Area ± SD	1709679 ±9849.82	1337286± 11075.36	1709279 ±9560.79	1327401± 12219.61
% RSD	0.6	0.8	0.6	0.9

Applicability of the method

The proposed RP-HPLC method was successfully appliedfor determination ofFusidic acid and Mometasone furoate in cream form.The percentage was found to be satisfactory, which iscomparable with the corresponding label claim amount

Table16:RP-HPLC method to Fusidic acid and Mometasone furoate cream formulation

Sr. no.	Sample name	% Assay for Fusidic acid	% Assay for Mometasone furoate
1	Formulation	99.3%	98.3%

CONCLUSION

In Estimation of Fusidic acid and Mometasone furoate in pharmaceutical dosage form,separation was achieved on Waters symmetry C-18, 150X4.6 mm, 5µ at35ºCtemperature by using a mobile phase10 mM ammonium formate in waterpH 5.5:Methanol (65:35 %v/v) at a flow rate of 1.0 ml/min and UV detection was carried out at 240 nm. Data suggests that peak purity index ofthe drug was found to be greater than 0.999, so there isno co-elution of any degradation products with mainpeaks and the results obtained were found within theacceptance criteria. Results of the validation for Fusidic acid and Mometasone furoateof the above method were linear in therange of 100-300 µg/ml and 5-15 µg/ml respectively . The % recovery was found to be 99.0%– 100.0 %.The results of the precision studyindicate that the proposed method shown goodrepeatability with a % RSD of 0.6 % forFusidic acidand % RSD of 0.9 % for Mometasone furoate. Similarly %RSD fromthe intraday precision data was found to be 0.31% for Fusidic acid and 0.57% for Mometasone furoateand %RSD from the Interday precision data werefound to be 0.83% for Fusidic acid and 0.74% for Mometasone furoate. Absolute differencebetween mean assay values of method precision and intermediate precision was found to be less than 2.0 %.Robustness is performed by making changes in flow rate, Mobile phase composition and Mobile phase pH. The assayobtained after proposed changes compared with theassay obtained in normal conditions. According to theacceptance criteria difference in the assay should not bemore than 2%. The results obtained are well within theacceptance criteria. The % assay results of 99.3 % for Fusidic acid and 98.3 % for Mometasone furoate indicates that the proposed method wassuccessfully utilized for the estimation Fusidic acid and Mometasone furoate in pharmaceutical dosage forms. Hence, the method can be termedas robust. Since the results are well within the limit ofacceptance criteria for all validation parameters,therefore the method can be considered as validated andsuitable for intended use. So, the proposed RP-HPLC assay method can be successfullyapplied for the estimation of Fusidic acid and Mometasone furoate in pharmaceuticaldosage form.

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