DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR SIMULTANEOUS ESTIMATION OF MUPIROCIN AND MOMETASONE FUROATE IN TOPICAL FORMULATION BY RP-HPLC

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ABSTRACT

Purpose: To develop simple, precise, rapid RP-HPLC (Reverse-phase High Performance Liquid Chromatography**)** method for estimation of marketed combination formulation of Mupirocin and Mometasone Furoate. **Methods:** The Chromatographic separation was achieved on a reversed-phase Phenomenax-luna C18 (250 x 4.6mm, 5 µm) column using a mobile phase consisting of acetonitrile : Sodium dihydrogen phosphate buffer (pH 6.8) (70:30 v/v) at a flow rate of 1 mL/min and UV detection at 240 nm. Developed methods were validated according to ICH Q2 (R1) guidelines. The methods were found to be linear between the range of 10 - 60 µg/ml for Mupirocin and 1 – 6 µg/ml for Mometasone Furoate. **Results:** An intra-day precision for Mupirocin was found to be 0.6788% - 0.9820% and for Mometasone Furoate 0.6758%- 0.7532%, inter-day precision for Mupirocin was found to be 0.9775% - 1.1277% for Mometasone Furoate 0.9114% - 1.0765%. Accuracy was determined by recovery studies and showed % recovery between 98 to 102%. **Conclusions:** A simple, accurate and precise HPLC method was developed and validated for the routine analysis of Mometasone Furoate and Mupirocin in ointment topical formulation. The results make known that the proposed method could be successfully useful for the routine analysis and quality control of pharmaceutical dosage forms containing Mometasone Furoate and Mupirocin.

NOVELTY OF THE WORK - The proposed HPLC method was validated as per ICH guidelines. The drug was extracted from an ointment and there is no any interference of excipients was founded. The results of the recovery studies performed show the high degree of accuracy of the proposed method, hence proposed method will useful to routine analysis of Mometasone Furoate and Mupirocin.

KEYWORDS: Mometasone Furoate, Mupirocin, Method development, Validation, RP –HPLC method.

INTRODUCTION:

Mometasone Furoate [MF]**,** 9, 21 – dichloro-11b, 17 – dihydroxy-16a-methyl-pregnane-1, 4 – ,lodiene – 3, 20 – dione 17 – (2 – furoate ester), (Fig. 1) is a synthetic glucocorticoid with anti-inflammatory, anti-allergy effect. It is effective for various skin diseases. Mometasone Furoate is a topical corticosteroid; it has anti-inflammatory, anti-pruritic, and vasoconstrictive properties. Corticosteroids act by the induction of phospholipase A2 inhibitory proteins. Mometasone Furoate is official in IP[5], BP[7], USP[6].

Mupirocin (MUP) is an antibacterial agent produced by fermentation. Chemically it is (*E*)-(2*S*, 3R, 4R, 5S)-5-[(2*S*, 3S, 4S, 5S)-2, 3-epoxy-5-hydroxy-4-methylhexyl] tetrahydro-3, 4-dihydroxy-β-methyl-2*H*-pyran-2-crotonic acid, ester with 9-hydroxynonanoic acid Mupirocin is official in IP[5], BP[7], USP[6].

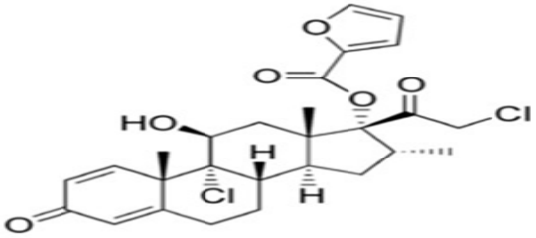

Fig No. 1 : Mometasone Furoate

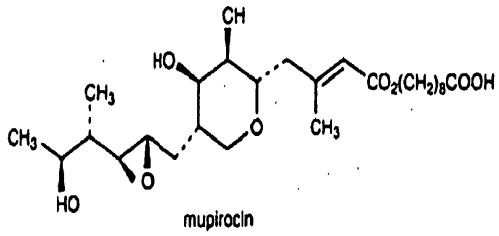

Fig No. 2 : Mupirocin

Literature survey reveals that there are several methods were reported for the estimation of Mupirocin and Mometasone Furoate individually as well as in combination with other drugs. As no method is reported for Mupirocin and Mometasone Furoate in combination, the aim of the present study was to develop accurate, precise and sensitive method for the simultaneous estimation of Mupirocin and Mometasone Furoate in combined dosage form by RP-HPLC method.

MATERIALS AND METHODS:

INSTRUMENTATION

HPLC of Shimadzu (LC-20AD Prominence Liquid Chromatography) with Phenomenax-luna C18 (250 x 4.6mm, 5µm) (Spincotech Pvt. Ltd.) Column was used for chromatographic separation. Its contain Rhenodyne valve with 20µl fixed loop injector and UV Detector (LC-20AD). The ultrasonic bath of Equitron Agilent 1200 Infinity Series was used for sonication. Analytical balance of Wenstara 13-220 having weighing capacity of 0.01 – 200 gm were used for the study. Mometasone Furoate (West-coast Pharma. Ahmedabad), Mupirocin (West-coast Pharma. Ahmedabad) and Methanol were used in the study.

REAGENTS AND CHEMICALS:

Analytical pure samples of Mometasone Furoate and Mupirocin were obtained as a gift samples from West coast, Ahmedabad. These samples were used without further purification. Semisolid formulation "MATOS-M" manufactured by West coast Pharmaceutical -Ahmedabad, was purchased from the local market containing MF (5 mg) and MUP (100 mg) per ointment (5gm).

PREPARATION OF STANDARD SOLUTIONS:

- **For Stock solution of Mupirocin**: Accurately weigh 10 mg of Mometasone Furoate and transferred to a 100 ml volumetric flask and diluted with acetonitrile(100 µg/ml).
- **For Stock solution of Mometasone Furoate**: Accurately weigh 10 mg of Mometasone Furoate and transferred to a 100 ml volumetric flask and diluted with acetonitrile (100 µg/ml).

SELECTION OF WAVELENGTH:

The standard solution of Mometasone Furoate (2 µg/ml) and Mupirocin (40µg/ml) in Acetonitrile was prepared and was scanned separately in UV region of 200 to 400 nm and overlain spectra were recorded. Overlain spectra showed 248 nm as the λmax of Mometasone Furoate and 220 nm as the λmax of Mupirocin. But both the drugs showed good absorption at 240 nm, so it was selected as detection wavelength.

CALIBRATION CURVE FOR MOMETASONE FUROATE AND MUPIROCIN

For Mupirocin:

An aliquots of 1, 2, 3, 4, 5 and 6 ml of stock solution of Mupirocin (100 µg/ml) were pipette out in six different 10 ml volumetric flasks and further diluted to attain concentration of about 10, 20, 30, 40, 50 and 60 µg/ml respectively.

Graph of Area Vs Concentration was plotted.

For Mometasone furoate:

An aliquots of 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 ml of stock solution of Mometasone furoate (100 µg/ml) were pipettes out in five different 10 ml volumetric flasks and further diluted to attain concentration of about 1, 2, 3, 4, 5 and 6 µg/ml respectively.

Graph of area Vs Concentration was plotted.

METHOD VALIDATION

LINEARITY AND RANGE:

The linearity response was determined by analyzing 6 independent levels of calibration curve in the range of 10 - 60 µg/ml and 1- 6 µg/ml for MUP and MF respectively (n = 3).

The calibration curve of area vs. respective concentration was plotted and correlation coefficient and regression line equations for MUP and MF were calculated.

PRECISION

(A)Repeatability:

Aliquots of 3 ml of working standard solution of MUP (100 µg/ml) were transferred to a 10 ml volumetric flask. Aliquots of 0.3 ml of working standard solution of MF (100µg/ml) were respectively transferred to a 10 ml volumetric flask. The volume was adjusted up to mark with Acetonitrile to get 30 µg/ml solution of MUP and 3 µg/ml solution of MF. The Area of solution was measured six times and % RSD was calculated.

(B) Intraday precision:

Aliquots of 2, 3 and 4 ml of working standard solution of MUP (100 µg/ml) were transferred to a series of 10 ml volumetric flask. Aliquots of 0.2, 0.3, and 0.4 ml of working standard solution of MF (100µg/ml) were respectively transferred to the same series of 10 ml volumetric flask. The volume was adjusted up to mark with acetonitrile to get 20, 30 and 40 µg/ml solution of MUP and MF. Solution was analyzed 3 times on the same day area and % RSD was calculated.

(C) Interday Precision:

Aliquots of 2, 3 and 4 ml of working standard solution of MUP (100 µg/ml) were transferred to a series of 10 ml volumetric flask. Aliquots of 0.2, 0.3, and 0.4 ml of working standard solution of MF (100µg/ml) were respectively transferred to the same series of 10 ml volumetric flask. The volume was adjusted up to mark with acetonitrile to get 20, 30 and 40 µg/ml solution of MUP and 2, 3 and 4 µg/ml solution of Mometasone Furoate.

Solution was analyzed 3 times on the 3 different days Area and % RSD was calculated.

SPECIFICITY:

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present. Typically, these might include impurities, degrades etc. A solution of placebo in mobile phase was injected and the chromatogram showed no inferring peaks at retention time of the two drugs. The chromatogram of placebo was compared with those acquired from standards.

LIMIT OF DETECTION (LOD):

The LOD is estimated from the set of 5 calibration curves used to determine method linearity.

The LOD may be calculated as,

LOD = 3.3 ?SD/Slope

Where,

SD = the standard deviation of Y- intercept of 5 calibration curves.

Slope = the mean slope of the 5 calibration curves.

LIMIT OF QUANTIFICATION (LOQ):

The LOQ is estimated from the set of 5 calibration curves used to determine method linearity.

The LOD may be calculated as,

LOQ = 10 ?SD/Slope

Where, SD = the standard deviation of Y- intercept of 5 calibration curves.

Slope = the mean slope of the 5 calibration curves.

ACCURACY:

To study the accuracy of the proposed methods, recovery studies were carried out by standard edition method at three different levels (80%, 100%, 120% of the test concentrations as per ICH guidelines). A known amount of drug was added and percentage recoveries were calculated. The results of recovery studies were satisfactory.

ROBUSTNESS:

Robustness of the method was determined by small, deliberate changes in mobile phase ratio and detection wavelength. Typical changes include the mobile phase ratio changed to 70±2 v/v for acetonitrile and detection wavelength changed to 240±2 nm.

ASSAY OF MARKETED FORMULATION:

For the analysis of marketed semisolid formulation, 5 g ointment was weighed accurately and an amount equivalent to 5 mg of Mometasone furoate and 100 mg of Mupirocin was weighed and dissolve in 50 mL methanol with the aid of ultrasonicator for 15 min and solution was filtered through Pre-filter + PVDF (0.45µm) into a 100mL volumetric flask and volume was made up to mark with methanol as a diluents. The solution was suitably make up with methanol, Pipette out 0.2 ml from this solution and transfer into 10 ml volumetric flask and diluted up to the mark with acetonitrile to give a sample solution having strength of 1 µg/ml of Mupirocin and 20µg/ml of Mometasone furoate.

RESULTS:

SELECTION OF WAVELENGTH:

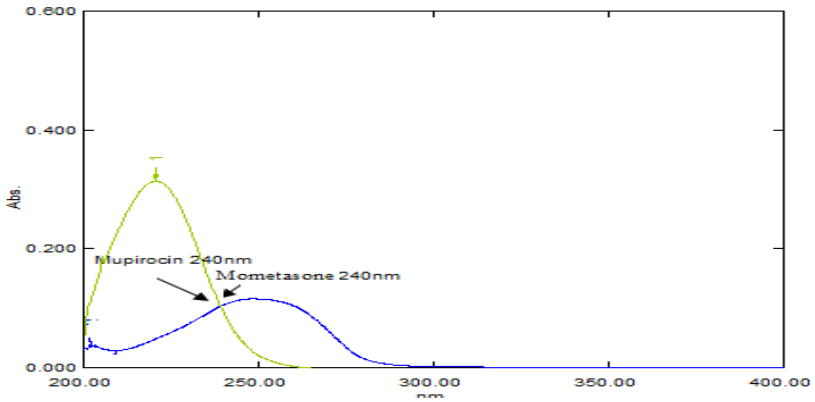

Fig No. 3: Selection of analytical Overlain Spectra of Mupirocin (20 µg/ml) and Mometasone Furoate (1 µg/ml)

CALIBRATION CURVE OF MUPIROCIN AND MOMETASONE FUROATE:

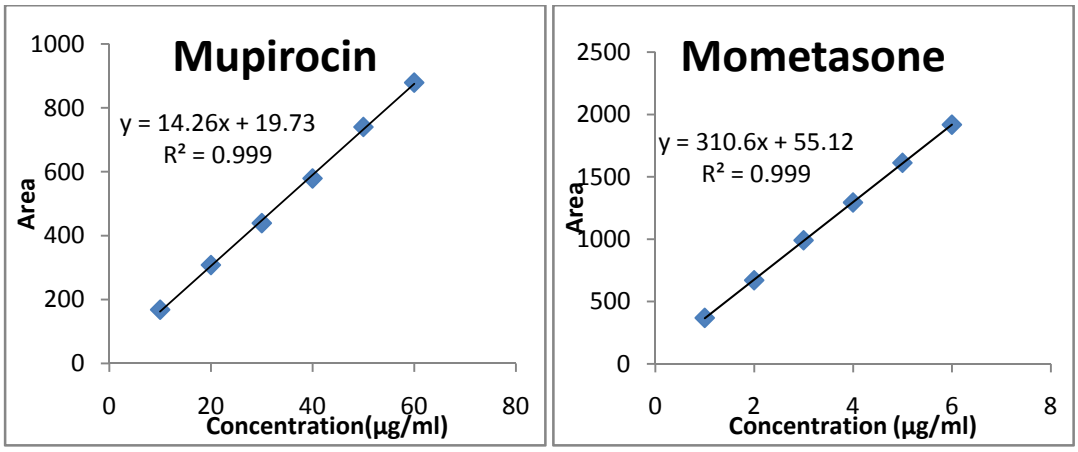

Fig No. 4 : Calibration curve of Mupirocin and Mometasone Furoate

Table 1: Calibration data for (n=3) Mupirocin:

Mupirocin		
Conc.(µg/ml)	**Mean Area (mV*s) ± SD (n=3)**	**% RSD**
10	168.802 ± 1.0571	0.770841
20	311.128 ± 2.7344	0.983875
30	436.336 ± 3.7881	0.409005
40	575.453 ± 5.5994	0.678766
50	743.873 ± 5.6060	0.505208
60	882.406 ± 6.5020	0.511086

Table 2: Calibration data for (n=3) Mometasone Furoate:

Mometasone Furoate		
Conc. (µg/ml)	**Mean Area (mV*s) ± SD (n=3)**	**% RSD**
1	368.833 ± 2.8431	0.626279
2	670.713 ± 6.5989	0.867583
3	978.024 ± 4.0001	0.86817
4	1266.187 ± 8.5944	0.973049
5	1649.391 ± 8.3328	0.753634
6	1918.103 ± 9.8031	0.736853

PRECISION:

Table No. 3 : Repeatability Data (n=6)

Drug Name	**Mupirocin**	**Mometasone Furoate**
Concentration	30 µg/ml	3 µg/ml
Mean area (mV*s) ± SD (n=6)	434.98 ± 2.8284	975.43 ± 5.5655
%RSD	0.650213	0.570572

Table No. 4 : Intraday Precision Data for Mupirocin and Mometasone Furoate**:**

Mometasone Furoate			**Mupirocin**		
Conc. (µg/ml)	**Mean Area (mV*s) ± SD (n=3)**	**%RSD**	**Conc. (µg/ml)**	**Mean Area (mV*s) ± SD (n=3)**	**% RSD**
2	669.0467 ± 4.52178	0.67585	20	313.5 ± 2.1213	0.6788
3	977.3533 ± 7.02670	0.71895	30	437 ± 4.2426	0.9708
4	1263.187 ± 9.51548	0.75329	40	576.5 ± 5.6568	0.9820

Table No. 5 : Interday Precision Data for Mupirocin and Mometasone Furoate :

Mometasone Furoate			**Mupirocin**		
Conc. (µg/ml)	**Mean Area (µV*s) ± SD (n=3)**	**%RSD**	**Conc. (µg/ml)**	**Mean Area (µV*s) ± SD (n=3)**	**% RSD**
2	672.0467 ± 6.9301	1.0311	20	313.527 ± 3.5355	1.1277
3	982.0247 ± 10.5717	1.0765	30	434.012 ± 4.2426	0.9775
4	1263.1873 ± 11.5128	0.9114	40	575.542 ± 6.3639	1.1058

SPECIFICITY

It is proven by comparing the chromatogram of blank (mobile phase), standard solution and test preparation solution to show that there was no any interference of excipients with the peak of MUP and MF.

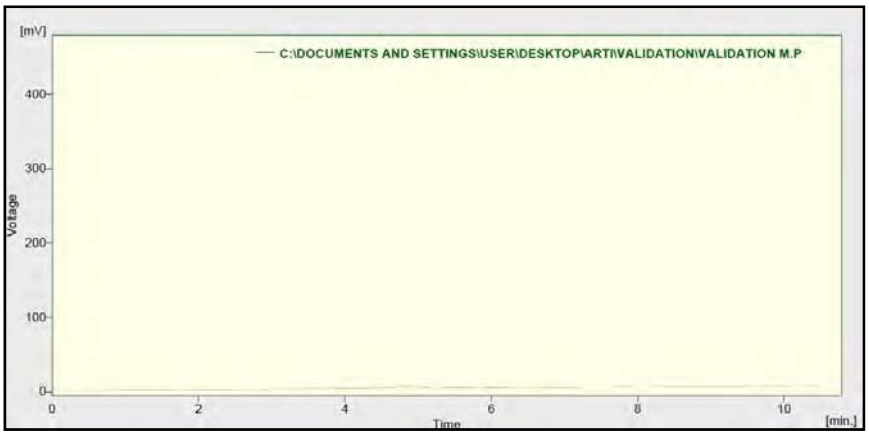

Figure 5: Specificity Chromatogram of Blank (Mobile Phase)

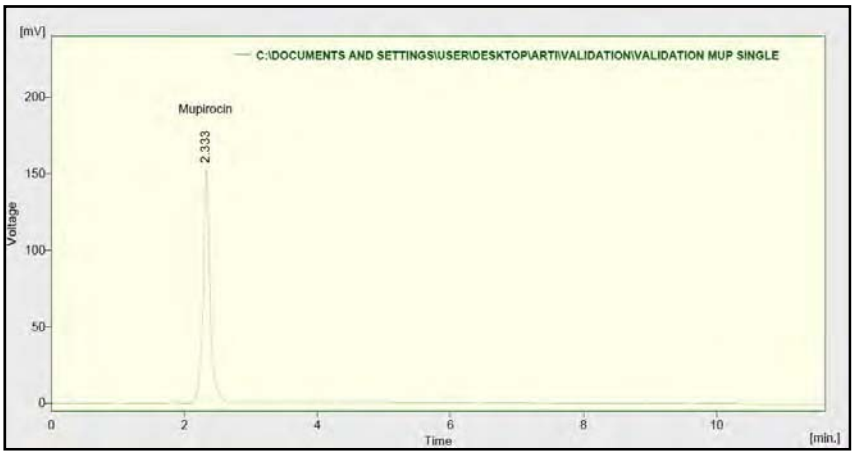

Figure 6: Chromatogram of Mupirocin

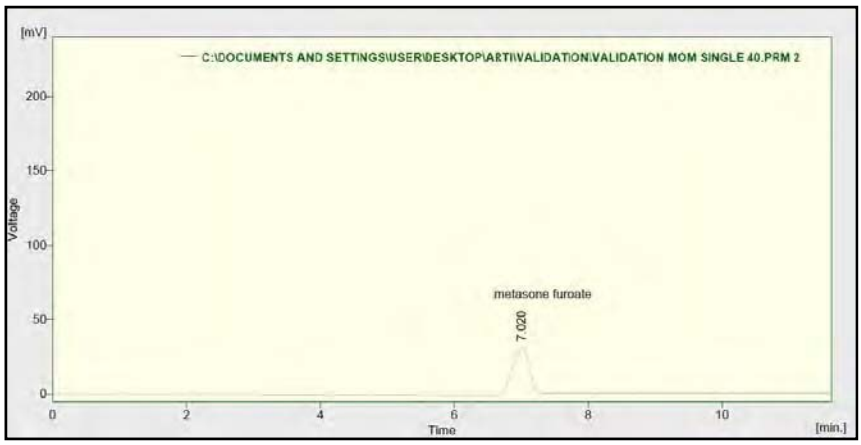

Figure 7: Chromatogram of Mometasone Furoate

Table No.6: System Suitability Test

System Suitability Parameters	Proposed Method		Standard Values
	MUP	MOM	
Retention times (min)	2.31 ± 0.01	7.06 ± 0.01	-
Theoretical plates (N)	1408.4 ± 0.8	3280.2 ± 1.6	Greater than 2000
Resolution (R_S)	11.98 ± 0.08		Greater than 2
Tailing factor	1.65 ± 0.07	1.32 ± 0.04	Not greater than 2.0

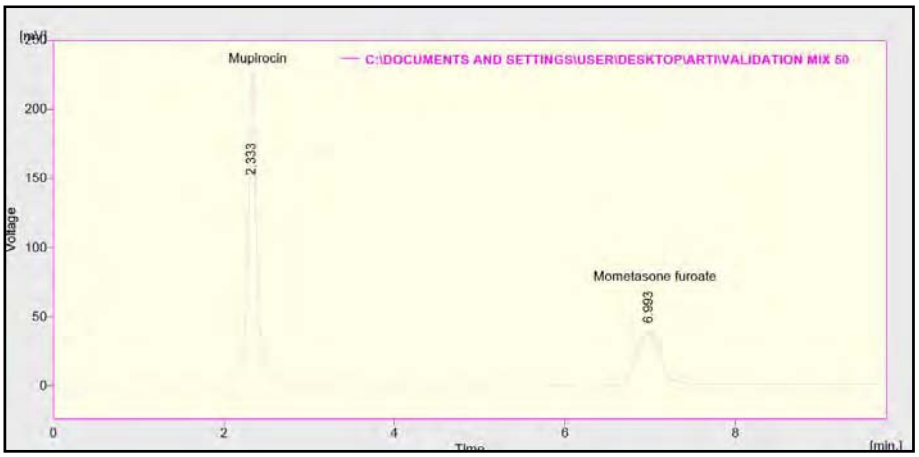

Figure 8: Optimized Chromatogram of Mupirocin (50 µg/ml) and Mometasone Furoate (5 µg/ml).

Table No. 7: LOD and LOQ data of Mupirocin and Mometasone Furoate

Drug Name	LOD (µg/ml)	LOQ (µg/ml)
Mometasone Furoate	0.0174	0.0517
Mupirocin	0.2716	0.8450

ACCURACY:

Table No. 8 : Accuracy Data

Drug name	Level of addition	Amount spiked (µg/ml)	Total amount (µg/ml)	Total amount obtained (n=3) ±SD	% Recovery±SD
Mupirocin (20 µg/ml)	80 %	16	36	35.97 ± 0.25	99.91 ± 0.720
	100 %	20	40	40.01 ± 0.26	100.02 ± 1.055
	120 %	24	44	43.85 ± 0.22	99.65 ± 0.440
Mometasone Furoate (1 µg/ml)	80 %	0.8	1.8	1.79 ± 0.38	99.44 ± 0.035
	100 %	1	2	1.98 ± 0.42	99.01 ± 0.901
	120 %	1.2	2.2	2.11 ± 0.39	99.09 ± 0.191

ROBUSTNESS:

The value of % RSD less than 2 revealed the robustness of the method. The robustness of the method was evaluated by:

- Changing detection wavelength (±2nm)
- Changing mobile phase ratio (±2ml)

Table No. 9: Robustness Data of Variation in Detection Wavelength

Wavelength (nm)	Mupirocin (Amount Taken 100µg/ml)		Mometasone Furoate (Amount Taken 5µg/ml)	
	Amount Found (100µg/ml)	%Assay (n=3) ± SD	Amount Found (5µg/ml)	%Assay (n=3) ± SD
238	100.12	100.12 ±0.445	5.08	101.62 ±0.912
240	99.97	99.97 ±0.987	4.98	99.68 ±1.012
242	99.94	99.94 ±0.191	4.96	99.20 ±0.679

Table No. 10: Robustness Data for Variation in Mobile Phase (v/v)

Change in Mobile Phase Ratio (ACN: Buffer)	Mupirocin (Amount Taken 40µg/ml)		(Amount Taken 40µg/ml)	
	Amount Found (100µg/ml)	%Assay (n=3) ± SD	Amount Found (5µg/ml)	%Assay (n=3) ± SD
68 : 32	99.98	99.98±0.487	4.98	99.68±1.034
70 : 30	100.14	100.14±0.897	5.01	100.2±0.456
72 : 28	100.08	100.08±0.934	4.99	99.80±1.108

Table No. 9: Optical Regression Characteristics And Summary of Validation Parameters.

Parameter	Mupirocin	Mometasone
Beer's Law Limit (µg/ml)	10 – 60	1 – 6
Regression equation (y = mx +c)	y = 14.26x + 19.73	y = 310.6x + 55.12
Correlation Coefficient (r^2)	0.999	0.999
Repeatability (% RSD, n=6)	0.650213	0.570572
Interday (n=3) (% RSD)	0.9114 - 1.0765	0.9775 – 1.1277
Intraday(n=3) (% RSD)	0.6788 - 0.9820	0.6758 - 0.7532
LOD(µg/ml)	0.0174	0.2716
LOQ(µg/ml)	0.0517	0.8450
Accuracy	99.65 -100.02%	99.01 – 99.44%

APPLICATION TO PHARMACEUTICAL DOSAGE FORM:

Applicability of proposed method was tested by analyzing the Pharmaceutical dosage form.

Table No. 10 : Applicability to Pharmaceutical dosage form Data:

Drug	Label claim	Amount found(mg) (n=3) ± SD.	%Label Claim ± SD.
Mupirocin	100 mg	99.26 ± 0.99	99.26%±0.99
Mometasone Furoate	5mg	4.98 ± 0.10	99.89%±0.21

DISCUSSION: Mupirocin and Mometasone Furoate are both commonly prescribed drugs for dermatoses. Mupirocin is bacteriostatic at low concentrations and bactericidal at high concentrations and Mometasone Furoate is a synthetic glucocorticosteroid, anti-inflammatory effect. So, Addition of these two drugs is used in skin disorders like psoriasis, eczema, impetigo etc. Rapid, accurate and precise RP-HPLC method was developed and validated for simultaneous estimation of both these drugs. This method developed in acetonitrile. The plot of area versus respective concentration was found to be linear in the concentration range of 10 - 60 µg/ml for Mupirocin and 1 – 6 µg/ml for Mometasone furoate. This method can be successfully applied for the simultaneous estimation of Mupirocin and Mometasone Furoate in topical formulation.

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