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

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ABSTRACT

Mometsone furoate is a corticosteroid (glucocorticoid) having an anti-inflammatory activity. Corticosteroids showed a wide range of effects on various cell types such as mast cells, eosinoplills, neutrophills, macrophages, and lymphocytes.

A validated stability-indicating method has been developed for the simultaneous determination of mometsone furoate (MOM) and salicylic acid (SAA) in a combined dosage form. This method is based on the reversed-phase high performance liquid chromatographic (HPLC) separation of the cited drugs.

The calibration graphs for each drug were rectilinear in the range of 0.25–15 and 12.5–750 mg mL1 for MOM and SAA, respectively using dexamethasone acetate as the internal standard. The proposed HPLC-DAD method was successfully applied in the determination of the investigated drugs in ointment. The method was validated in compliance with ICH guidelines, in terms of linearity, accuracy, precision, robustness, limits of detection and quantitation and specificity.

Keywords: Mometsone Furoate, Salicylic Acid, HPLC-DAD

Introduction

Inhaled mometasone furoate is indicated for prophylaxis of asthma in patients ≥4 years. Applied topically as an ointment, mometasone furoate is indicated for symptomatic treatment of dermatitis and pruritis in patients ≥2 years. Mometasone furoate nasal spray is available both over-the-counter (OTC) and by prescription. The OTC nasal spray formulation of mometasone furoate is indicated for the treatment of upper respiratory allergic symptoms (e.g. rhinorrhea, sneezing) in patients ≥2 years of age. The prescription formulation is indicated for the treatment of chronic rhinosinusitis with nasal polyps in patients ≥18-year-old and for the and prophylaxis of seasonal allergic rhinitis in patients ≥12 years old. It is also approved in combination with olopatadine for the symptomatic treatment of seasonal allergic rhinitis in patients ≥12 years.

Structure

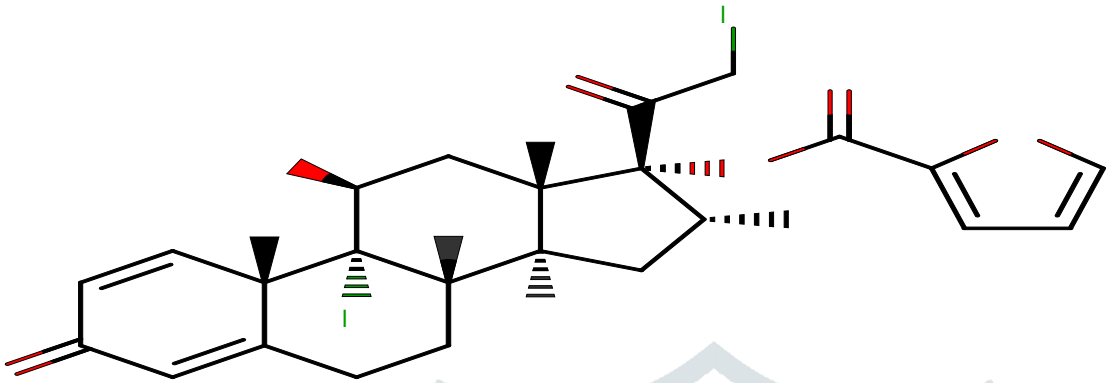


FIG 1: Molecular Structure of Mometasone furoate

Experimental Section

Instrumentation

HPLC of Shimadzu (LC-20AD Prominence Liquid Chromatography) with Phenomenaxluna C18 (250 x4.6mm, 5µm) (Spincotech Pvt. Ltd.) Column was used for chromatographic separation. It contains Rhenodyne valve with 20µl fixed loop injector and UV Detector (LC20AD). 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 (Karnani Pharmaceuticals, Selaque), Mupirocin (Karnani Pharmaceuticals, Selaque) and Methanol were used in the study.

Reagent and Chemicals

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

Analytical Method Validation (AMV)

- Accuracy
- Precision
- Linearity and range
- Selectivity/ Specificity
- Robustness/ Ruggedness
- Limit of detection (LOD)
- Limit of quantitation (LOQ)

Accuracy

Accuracy of the proposed HPLC determination was evaluated from assay result of components. Accuracy was evaluated by performing the assay of samples, and calculated from peak area responses of different samples by component recovery method.

Stock solution was prepared by dissolving accurately weighed portions of 40 mg of CC, MF, and 800 mg of FA in acetonitrile to produce a 200 mL solution.

Appropriate portions of the stock solution were spiked into blank placebo matrix to provide concentrations of 50%, 75%, 100%, 125%, and 150% of target level. Mean recovery of spiked samples was 100.31% for CC, 100.38% for MF, and 100.34% for FA.

Precision

Instrumental precision was determined by six replicate determinations of standard solution, and the relative standard deviations (RSD) were 0.21% for CC, 0.12% for MF, and 0.24% for FA.

Method precision or intra-assay precision was performed by preparing six different sample solutions involving different weights. Each solution was injected in triplicate under the same conditions and mean value of peak area response was taken for each solution. Corrections in area were made for each weight taken to prepare six sample solutions, and RSD of peak area response was calculated from the six solutions. RSD were 0.23% for CC, 0.46% for MF, and 0.62% for FA.

Intermediate precision was performed by two different analysts employing different instruments to analyze samples. Standard solution and six different samples at 100% target level were prepared by each analyst. RSD obtained from 12 assay results by two analysts were 0.52% for CC, 0.38% for MF, and 0.50% for FA.

Linearity and Range

The range of a method is defined as lower and higher concentrations for which the method has adequate accuracy, precision, and linearity. To demonstrate the range, six samples each of low concentration (50% of target level) and high concentration (150% of target level) similar to accuracy samples were prepared by spiking drug substance into blank matrix (placebo). Each sample was analyzed in duplicate. At low concentration, mean recovery of CC, MF, and FA was found to be 99.97%, 100.20%, and 99.99%, respectively. RSD obtained from these determinations were found to be 0.68% for CC, 0.98% for MF, and 0.86% for FA. At high concentration, mean recovery of CC, MF, and FA was found to be 100.20%, 100.10%, and 100.80%, respectively. RSD obtained at higher concentration level were found to be 0.40% for CC, 0.65% for MF, and 0.70% for FA.

Linearity

Peak areas versus concentrations in microgram per milliliter were plotted for CC, MF, and FA at the concentration range between 50% and 150% of target level. Five points were taken foreach linearity range. CC, MF, and FA showed linearity in the range 10–30, 10–30, and 200–600 μg/mL, respectively.

Selectivity

The Selectivity of an analytical method is its ability to measure the analyte of interest qualitatively.

Specificity

No interferences were observed due to the obvious presence of excipients like cetostearyl alcohol, cetomacrogal-1000, white petroleum jelly, propylene glycol, liquid paraffin, sodium dihydrogen phosphate, and 3-ketofusidic acid. The principal impurity 3-ketofusidic acid, which may have been present in the formulation, was separated from main peak of FA with a resolution factor of more than 3.0

Robustness

Robustness of the proposed method was performed by keeping chromatographic conditions constant with the following differences:

- •Changing mobile phase composition from buffer (55% v/v)–acetonitrile (45% v/v) to buffer (45% v/v)–acetonitrile (55% v/v)
- •Variation in the mobile phase pH from 3.8 to 4.1 • Changing flow rate from 1.0 mL/min to 1.2 mL/min.

Standard solution was injected six times in replicate for each minor change. System suitability parameters like resolution, peak asymmetry, theoretical plates, retention factor, and RSD were recorded for each peak and found to be within acceptable limits of validation criteria. Six test samples at target concentration levels were prepared and analyzed in duplicate for each change. Recoveries and RSD were calculated for each component during each change and found to be 99.99–100.20% and less than 1.0%, respectively.

Ruggedness

The Ruggedness of analytical procedure describes to its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness was performed by small variation in the chromatographic conditions and found to be unaffected by small variations like ± 1 % variation in volume of mobile phase and ± 0.1 mL/min. flow rate of mobile phase.

Limit of detection (LOD)

LOD were measured to evaluate the detection and quantitation limits of the method and to determine whether these were affected by the presence of impurities. They were calculated by using equations-
The LOD and LOQ were calculated using following formulae;
LOD = 3 × (standard deviation of y-intercept/slope of the calibration curve)
LOQ = 10 × (standard deviation of y-intercept/slope of the calibration curve)
SD = standard deviation of response and
S = average of the slope of the calibration curve

Limit of quantitation (LOQ)

Several approaches for determining the quantitation limit are possible, depending on whether the procedure is a non-instrumental or instrumental. Approaches other than those listed below may be acceptable.

METHODOLOGY

Materials

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 diluent. The solution was suitably made 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

Method

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 Mometasome Furoate and Mupirocin

For Mupirocin

An aliquot of 1, 2, 3, 4, 5 and 6 ml of stock solution of Mupirocin (100 µg/ml) were pipette out in si 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 aliquot 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.

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

- **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.

- **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.

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

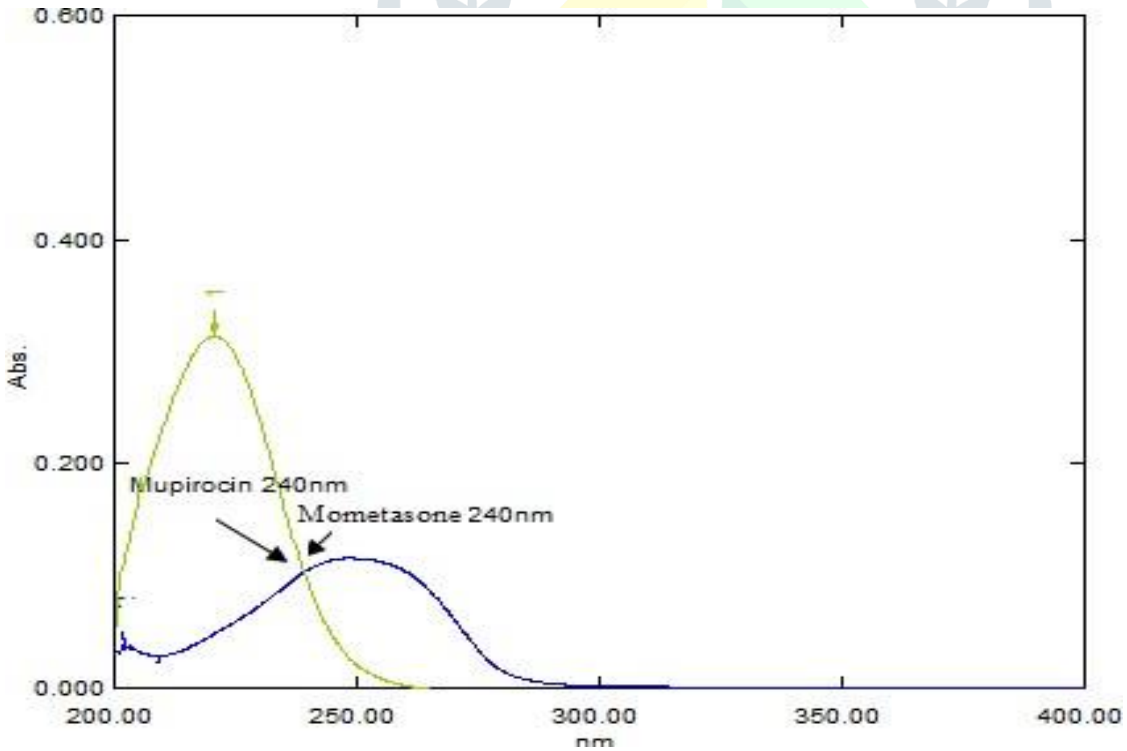
Limit of Quantification (LOQ)

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.

Selection of Wavelength

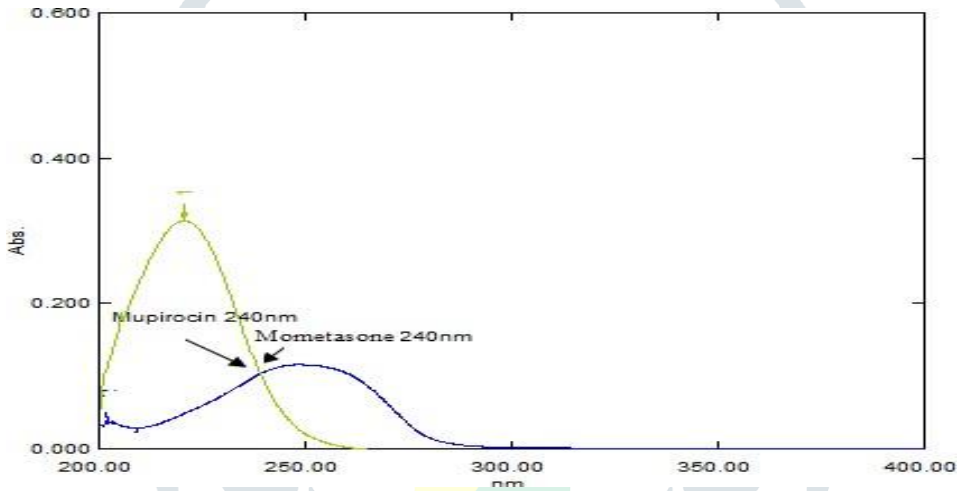


Fig. 3: Selection of analytical Overlain Spectra of Mupirocin (20 µg/ml) and Mometasone Furoate (1 µg/ml)

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

Calibration data for (n=3) Mometasone Furoate

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 MF.

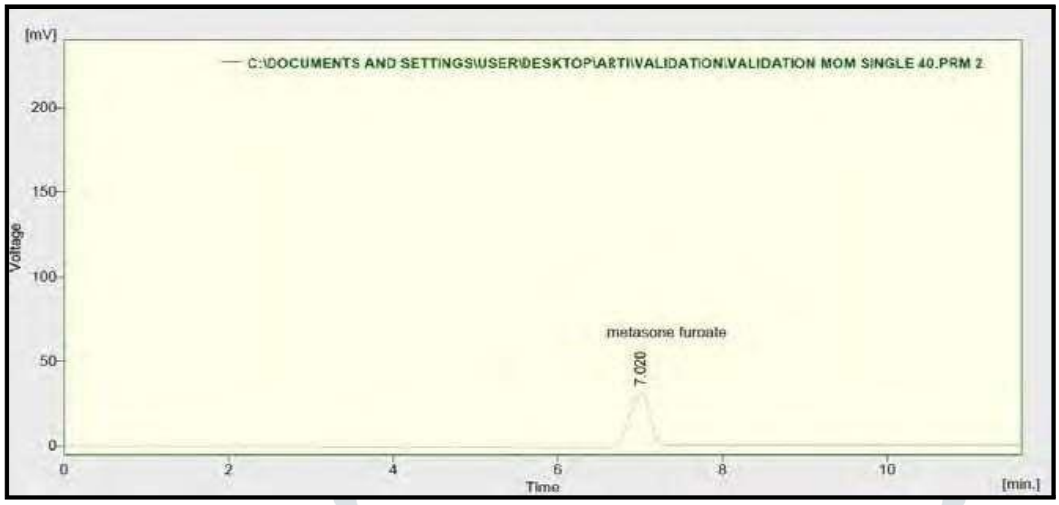


Fig 4: Chromatogram of Mometasone Furoate

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

LOD and LOQ data of Mometasone Furoate

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.

Drug name	Level of addition	Amount spiked (µg/ml)	Total amount (µg/ml)	Total amount obtained (n=3) ±SD	% Recovery±SD
Fusidic Acid (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

Accuracy Data

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)

Wavelength (nm)	Fusidic Acid (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

Robustness Data of Variation in Detection Wavelength

Change in Mobile Phase Ratio (ACN: Buffer)	Fusidic Acid (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	.98	9.68±1.034
70 : 30	100.14	100.14±0.897	.01	00.2±0.456
72 : 28	100.08	100.08±0.934	.99	9.80±1.108

Robustness Data for Variation in Mobile Phase (v/v)

Parameter	Fusidic Acid	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%

Optical Regression Characteristics and Summary of Validation Parameters.

Application to Pharmaceutical Dosage Form

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

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

Applicability to Pharmaceutical dosage form Data

Summary of Patients

A total of 175 patients were initially enrolled. Fifteen patients were excluded due either to violation of protocols or adverse reactions, and one patient was excluded due to a screening criteria violation. In total, 159 patients were analyzed (76 males and 83 females; age range, 5~79 years; mean age, 32.32±19.86, mean standard deviation years old). No clinically significant differences were observed in the PGA score, TEWL, or VAS between the mometasone furoate in MLE group and the methylprednisolone aceponate group. Basal demographic characteristics of the study groups and basal results of the PGA, TEWL, and VAS scores are summarized in Table 8 and 9.

Age	32.32 :19.86
Sex	
Male	76(47.80)
Female	83(52.20)
Past Skin Disease history	
Yes	25(15.72)
No	134(84.28)
Topical Drug Allergy history	
Yes	_-
No	159(100)

Values are presented as mean standard deviation of number (%)

	Mometasone	Fusidic Acid
PGA	7.46 :3.11	7.41 :3.18
TEWL	33.73 :22.47	33.47 :21.96
VAS	5.83 :2.31	5.99 :2.99

Baseline results of *PG*, TEWL, and VAS score Values are presented as mean ± standard deviation.

PGA: Physician global assessment of clinical response

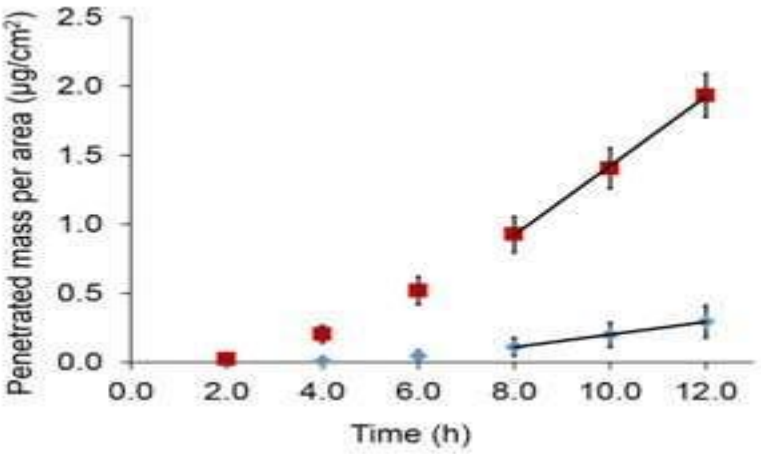
TEWL: Transepidermal water loss

VAS: Visual analog scale

PGA: Physician's global assessment of clinical response

TEWL: Trans epidermal water loss

VAS: Visual analog scale



Drug Depletion

Cream A	Cream B	Cream A	Cream B
Silicone			
J_{ss}(µg/cm²/h)	0.25 ± 0.02	0.046 ± 0.014	5.5
Amount of MF in membrane (µg/cm²)	2.70 ± 1.05	0.63 ± 0.12	4.3
Skin			
J_{ss}(µg/cm²/h)	0.34 ± 0.15	0.024 ± 0.017	13.1

Amount of MF in membrane (µg/cm^2)	.04 ± 0.48	.95 ± 0.53	.1
after/ebefore	.94 ± 1.38	.71 ± 0.31	.a.
R_{after}/R_{before}	.16 ± 0.12	.14 ± 0.93	.a.
Amount / J_{ss}	.0	0	.a.

hClinical and biochemical variables of individuals with overweight-obesity

Time (h) for		**Amount MF in Membrane (µg/cm^2)**	
Removal of Cream	**Extraction of MF**	**Cream A**	**Cream B**
6	6	1.74 ± 1.36	0.42 ± 0.35
6	12	0.93 ± 0.37	0.62 ± 0.19
12	12	2.04 ± 0.48	0.95 ± 0.53

Amount of MF accumulated in the skin membranes after application of Cream A and Cream B for 6 or 12 h (n = 4–6, error bars show SD).

SUMMARY AND CONCLUSION

Mometasone Furoate from the two creams absorbs to the same extent in excised skin, while Cream A generates an order of magnitude higher steady state drug flux through skin. In corresponding experiments with silicone sheets as membrane the ratios in absorbed amounts of MF and drug flux are similar, which is expected when neither of the creams affects the membrane. Cream A also caused a two-fold increase in the skin dielectric constant (e) and a decrease in resistance (R). The increase in e may be attributed to an increased fluidity of the extracellular lipid matrix in the stratum corneum corresponding to a higher skin permeability for lipophilic molecules like MF. No significant change in e was seen with cream B.

The ratio between absorbed amount of MF in skin (after 12h exposure) and drug flux over skin was 6 for Cream A and 40 for Cream B. The relative depletion of MF from skin after removing the formulations was also higher in skin treated with Cream A. To conclude, Cream B appears to be the safer alternative as it does not seem to perturb the skin and imposes less systemic burden without sacrificing clinical efficacy.

Thus, it can be determined that techniques in present research work were simple, sensitive and reproducible when checked for validation parameters like accuracy, precision and ruggedness for monotonous purpose of Mometasone Furoate in bulk along with pharmaceuticals (cream).

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