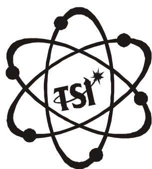

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Determination of mometasone furoate by HPLC in topical preparations: Validation

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ABSTRACT

A simple and rapid high performance liquid chromatographic (HPLC) separation method has been developed for determination of mometasone furoate in topical pharmaceutical preparations. The method was based on high performance liquid chromatographic separation of the mometasone furoate on reversed phase, Hypersil ODS column [C18(3μ,10cm×4.6mm, I.D)] at 30°C using mobile phase consisting of acetonitrile : water:trifluroacetic acid, 500:500:1, v/v. The flow rate was 1.0ml min^{-1} with an average operating pressure of 89.63 bar. The retention time (tR) was found to be 9.0±0.9 minutes. Extraction of the analyte from the sample is done with tetrahydrofuran. It was then diluted with mobile phase to achieve the concentration of 10μg ml^{-1}. Quantitation was achieved with UV detection at 254 nm based on peak area with linear calibration curves at concentration range 2.5-15μg ml^{-1}.When the method was applied successfully to topical preparations (Lotions, cream and ointment), no interference from the lotion, ointment and cream excipients were found. The method was validated as per ICH guidelines in terms of precision, robustness, recovery and linearity.

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KEYWORDS

Mometasone Furoate;
HPLC;
Tetrahydrofuran;
Topical preparations;
ICH guidelines;
Validation.

INTRODUCTION

Mometasone Furoate (MF) is a synthetic corticosteroid with anti-inflammatory activity.

Chemically, it is 9α,21-dichloro-11 β,17-dihydroxy-16 α-methyl pregna-1,4-diene-3,20-dione 17-(2-furoate), with the empirical formula $C_{27}H_{30}Cl_2O_6$, and a molecular weight of 521.4. MF is a white or almost white powder practically insoluble in water, slightly soluble in octanol, and moderately soluble in ethyl alcohol. MF is available as the sole active ingredient in different topical preparations like lotion, creams and ointments[1,2]. MF is a high potent chlorinated glucocorticoid with a favorable ratio between local and systemic side effects.

USP31[3] describes a HPLC method for assaying MF. A spectrophotometric method was described in BP 2007[4] for the determination of MF. USP 31[3] and BP 2007[4] described TLC methods for purity determination and quantitative determination of MF. European Pharmacopoeia[5] described the HPLC method for the related substance. Wulandari et al described the TLC densitometric determination of MF in topical preparations[6]. Xiao et al.[7] reported the simultaneous deter-

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mination of MF and its degradation products along with the degradation kinetics in human plasma. Earlier reported HPLC methods used an internal standard Beclomethasone dipropionate.

The present study reports a simple and accurate method suitable for routine determination of MF in topical preparations, without any internal standard. The method is unique in terms of chromatographic conditions, sample preparation and reproducibility.

EXPERIMENTAL

Chemicals and reagents

MF was provided by Symbiotic Pharmalab Ltd. Indore India.(Batch number MMF08001; Assay 99.79%; Conforms to USP 31 pharmaceutical grade). The substance was used for preparing laboratory-made pharmaceutical preparations and standard solutions.

Trifluoroacetic acid (TFA) AR grade (Merck), Tetrahydrofuran (THF), Acetonitrile HPLC grade Rankem RFCL.Filters 0.45μ Nylon and Teflon (Advance micro device (P) Ltd India). HPLC water (TKA pacific Water purification system). All excipients and materials used for topical preparation were of pharmaceutical grade.

Instrumentation and HPLC chromatographic conditions

An HPLC system Waters 2695 separation module, 2996 Photodiode Array Detector, 2487 Dual wavelength absorbance detector (Water corporation 34 Maple street Milford, Massachusetts, 01752-3696,USA) was used. The injection volume was 20μl. The chromatographic separation was carried out under isocratic reversed-phase conditions on Hypersil ODS column 100A, 100mm×4.6 mm column 3μ C18 Part No 30103 104630 (Thermo Electron corporation). The column oven temperature was 30°C.The detection wavelength was 254nm. The mobile phase was a mixture of water: acetonitrile (1:1)containing 0.1% v/v of TFA and the flow rate was 1.0ml min^{-1}. The mobile phase was filtered through a, 0.45μ membrane filter. Balance used was Mettler Toledo XS205 Dual range d- 0.01mg/0.1mg. Sonicator used was Bandelin Sonorex.

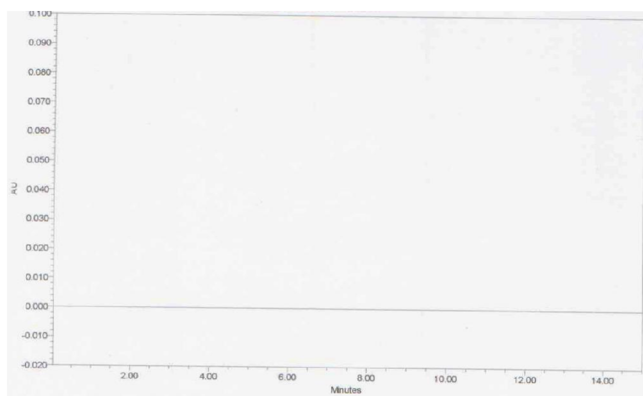

Figure 1a : Blank chromatogram

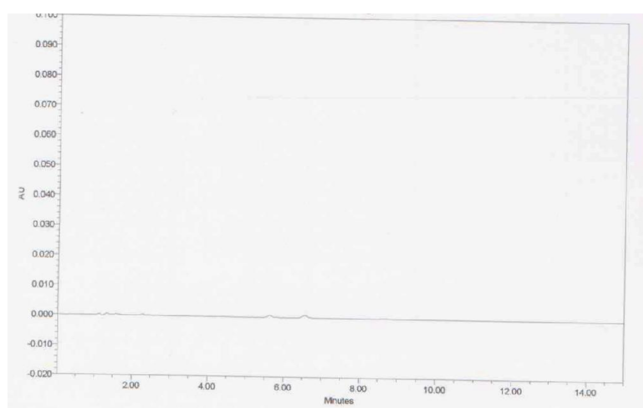

Figure 1b : Placebo chromatogram

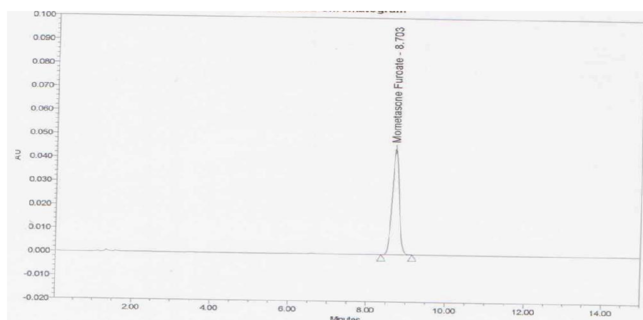

Figure 1c : Standard chromatogram

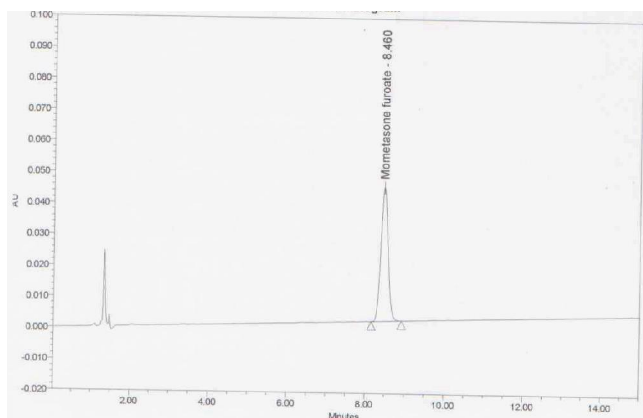

Figure 1d : Sample chromatogram

Sample preparation

Lotion, Cream and Ointment formulation containing 0.1% w/w MF were prepared by Dr.Reddy's Dermatology Department.

The quantification of MF in lotion, cream and ointment was done by external standard method. Standard Concentration was 10μg ml^{-1}.

A stock solution containing 200μg ml^{-1} of MF was prepared by dissolving 40 mg of drug in 20ml of THF and sonication for 5 minutes in 200ml volumetric flask. The volume was made up to the mark with diluent (mobile phase).

The stock solution was diluted to 10 μg ml^{-1} by pippeting 5ml of stock solution and 10ml of THF to 100 ml of volumetric flask and diluted to the mark with diluent and filtered through 0.45μ membrane filter and was injected directly in to the HPLC.

Each of the formulation cream, ointment and lotion were weighed about 2 grams in triplicate and transferred in separate 200 ml volumetric flasks. 20ml of THF was added to each flask and kept for sonication for 20 minutes. The samples were cooled to room temperature and made up to the mark with diluent (mobile phase). Samples were filtered through 0.45μ membrane filter. 20μl of each sample preparations were injected in to the HPLC.

System suitability

The relative standard deviation of peak area of MF peak in five injections of standard solution should not be more than 2.0 % and USP tailing factor for MF peak should not be more than 2.0. The USP plate count for the MF peak should not be less than 8000.

RESULTS AND DISCUSSION

Method validation

System suitability and system precision

The Standard solution prepared by using MF as per developed method was injected five times into the HPLC system. The system suitability parameters were evaluated and found to be within the limits. The % RSD for peak areas from five replicate injections of MF was found to be 0.05%, the Tailing factor for MF was found to be 1.08. The tangent for MF is 11570.8.

Specificity

Placebo interference

A study of Placebo (interference from excipients) was conducted. Placebo interference was checked by weighing about 2.0gm of placebo in triplicate (equivalent to about the weight of sample) and proceeded as per the sample preparation. There was no interference at retention time of MF peak.

Interference from degradation products

Experiments were conducted to study any interference from the degradation products in the process. Separate portions of lotions and Placebo were exposed to following stress conditions to induce degradation.

a. Acid degradation.
b. Base degradation.
c. Peroxide degradation.
d. Thermal degradation.
e. UV degradation.
f. Sun light degradation.
g. Humidity degradation.

Stressed samples were injected into the HPLC system with photo diode array detector as per test method conditions. All degradant peaks were resolved from MF peak in the chromatograms of all samples. The nearest degradation peaks in all degradation conditions were observed to have a resolution of 3.7 from the main peak. The major degradation was found in basic condition.The chromatograms of the stressed samples were evaluated for peak purity of MF using Waters Empower software. For all forced degradation samples, the purity angle was found to be less than Threshold angle. This indicates that there is no interference from degradants in quantitating the MFin the formulation. Thus, this method is considered to be "Stability Indicating".The Purity angle and Purity Threshold results are summarized in TABLE 1.

Precision

The precision of test procedure was evaluated for MF by performing the assay as per the test method for six times. The mean, standard deviation and % Relative standard deviation for the assay of MF for six samples was found to be 100%, 1.0 and 0.01 respectively.

Accuracy

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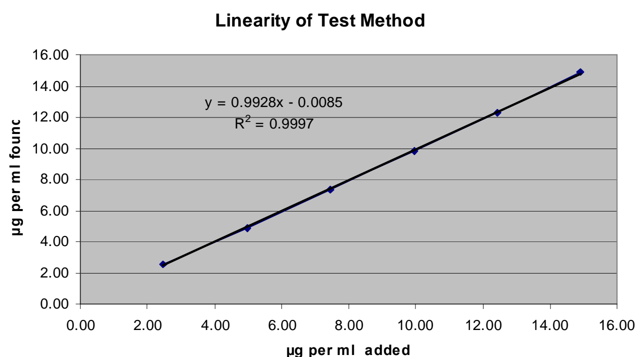

Figure 2 : Linearity of test method

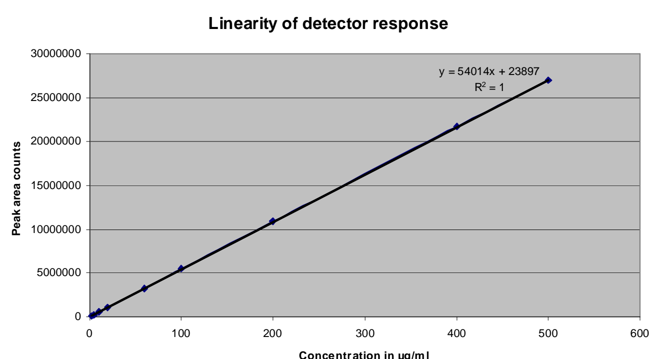

Figure 3 : Linearity of detector response

TABLE 1 : Force degradation study of samples under different stress conditions

S.No.	Degradation condition	Purity angle	Purity threshold
1	Control	0.305	0.472
2	Acid (1N HCl) 1hrs 60°C	0.249	0.452
3	Base (0.025N NaOH)	0.400	0.421
4	Peroxide 5% H_2O_2 1hrs 60°C	0.257	0.432
5	Thermal 105°C for 3hrs	0.32	0.462
6	Humidity 90%, 96hrs	0.454	0.503
7	UV 1.2 million lux hrs	0.311	0.501
8	Sunlight 24hrs	1.682	2.128

TABLE 2 : Robustness of HPLC Method

S.N.	Parameters	Retention time (in minutes)	%RSD Standard n=5	Tangent	Tailing
1	Control(As per test meth)	8.697	0.05	11570	1.08
2	Flow 0.9 ml min^{-1}	9.612	0.1	11607	1.09
3	Flow 1.1 ml min^{-1}	7.931	0.07	11208	1.09
4	Temperature 35°C	8.246	0.04	11435	1.09
5	Temperature 25°C	8.620	0.15	11452	1.08
6	Organic composition (Acetonitrile:Water) 525:475	5.165	0.08	10039	1.26
7	Organic composition (Acetonitrile:Water) 475:525	14.445	0.70	110457	1.14

A study of Accuracy was carried out by spiking (in triplicate). The equivalent amount of MF in lotion placebo into each volumetric flask to get the concentration of MF equivalent to 25%, 50%, 75%, 100%, 125% & 150% of the test concentration as per the test method. The average % recovery of MF was found to be within the limits.

A graph was plotted between µg $ml^{-1}$of MF added "versus µg $ml^{-1}$of MF found" for Accuracy. The correlation coefficient was found to be 0.9997.

Linearity of detector response

The Linearity of detector response of MF was evaluated by Injecting MF standard with concentration ranging from 2.5µg ml^{-1} to 500µg ml^{-1}. A graph was plotted between "concentration µg ml^{-1}" versus "Area counts of MF peak". The correlation coefficient was found to be 1.0.

Ruggedness

System to System/column to column/Analyst to Analyst variability study was conducted on MF using two different Systems by performing drug assay and analyzed under similar conditions as per the method.

The mean, standard deviation and % Relative standard deviation for the assay of MF for twelve samples was found to be 100.3 %, 1.1 and 1.1 respectively.

Robustness

To evaluate HPLC method robustness a few parameters were deliberately varied. The parameters included variations of flow rate, column temperature and percentage of acetonitrile. The different values for retention time, % RSD, Tangent, The tailing for all peaks for variable parameters was about 1.1 given in TABLE 2.

Suitability of the HPLC method

The validated method is tested for the Laboratory preparations and Marketed samples of MF in triplicate as illustrated in TABLE 3.

TABLE 3 : Suitability of the HPLC method for LP and MS

Results from determination of the accuracy of analysis of the laboratories preparation(LP) and marketed samples(MS)					
S.No.	Sample(n=3)	Amount found(μgml^{-1})(mean±SD)	Amount added(μgml^{-1})	%Recovered	±SD
1	Lotion LP	10.07±0.05	10.00 μgml^{-1}	100.7	±0.48
2	Lotion MS	10.28±0.07	-	100.3	±1.66
3	Cream LP	9.73±0.04	9.90 μgml^{-1}	98.3	±0.38
4	Cream MS	10.17±0.36	-	100.7	±0.98
5	Ointment LP	10.16±0.12	10.10 μgml^{-1}	100.6	±1.19
6	Ointment MS	9.78±0.06	-	99.96	±1.66

CONCLUSION

Reversed-phase HPLC method with UV detection was developed for determination of MF in topical dermatological creams, lotions and ointments. The great difficulty in separation of major degradation impurity was overcome with the addition of trifluroacetic acid and the separation is achieved. The optimized mobile phase is a mixture of water:acetonitrile (1:1) containing 0.1% of trifluoroacetic acid and the flow rate was 1.0ml min^{-1}. The retention time was approximately 9.0 minutes with no interference from other components of lotion ,cream and ointment formulation. The method is validated as per ICH guidelines and found suitable in terms of specificity, linearity, accuracy, robustness and ruggedness. The given method is sensitive towards the solvent composition in the mobile phase as the retention time of main peak changed by ±50% of the (tR) retention time, 9 minutes. The method is also suitable for the marketed samples as it gives 100% recovery of the label claim and there is no interference. In conclusion, the developed method is suitable for the routine analysis of products of similar compositions in pharmaceutical industry quality control laboratories. Experience of the given method shows simplicity in terms of sample preparation, precision and ruggedness, compared to the existing method of determination of MF in lotion, creams and ointments.

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