Development and Validation of Stability Indicating Method of Mometasone Furoate by HPTLC

Suraj Pardeshi*[1],Dr. Madhuri Shelar[1],Jyoti Kadam[1], Dr.Ganesh Andhale[1], Dr. Nalanda Rangari[1]

Alard College of Pharmacy, Survey No. 50, Marunji, Near Rajiv Gandhi IT ParkHinjewadi,Pune 411028, Maharashtra India.

*Corresponding author
Mr. Suraj Pardeshi
Alard College of Pharmacy, Survey No. 50 Marunji,Near Rajiv Gandhi IT Park Hinjewadi, Pune 411028, Maharashtra India.
E-mail: surajpardeshi1000@gmail.com

ABSTRACT

Background: There is a need for the simple, fast, reliable, economical, specific and sensitivity stability-indicating high performance thin layer chromatographic (HPTLC) method for the estimation of Mometasone furoate (MF) in semi-solid dosage form.

Results: The chromatographic separation achieved by using ethyl acetate: acetonitrile: toluene: triethylamine (1.5: 1.5: 7: 0.2 v/v/v/v) as the mobile phase and Rf value was found to be 0.63 at 250nm in UV detection. The developed method is validated according to the ICH guidelines for simplicity, accuracy, precision, detection range, range of magnitude and consistency. The method described is linear with a correlation coefficient (R^2)> 0.9992 with a concentration range of 200–1000 ng / band. The recovery was found to be 98.08%.The LOD and LOQ were found to be 36.69 ng / band and 111.18 ng / band respectively. Drug is subject to stress conditions of acid hydrolysis, alkaline hydrolysis, oxidative hydrolysis, neutral hydrolysis, photolysis, thermal degradation.

Conclusion: The proposed stability index method can be used to determine the group samples of Mometasone furoate API and pharmaceutical Dosage form.

Key-words: Mometasone Furoate, HPTLC, ICH Guidelines, Validation, Stability Indicating.

1.0 BACKGROUND

Mometasone furoate is a topical corticosteroid; It has anti-inflammatory. Chemically, it is 9α-21-dichloro-11β-17-dihydroxy-16α-methyl pregna-1-4-dine-3-20-dione-17-(2-furoate)with the empirical formula $C_{27}H_{30}Cl_2O_6$and a molecular weight of 521.4 g/mol. Mometasone inhibits the action of allergic reactions, eczema and psoriasis, which can cause swelling, redness and inflammation.[1-4]Mometasone furoate is a high potent chlorinated gluco-corticoid with a favorable ratio between local and systemic side effects.[5,6]

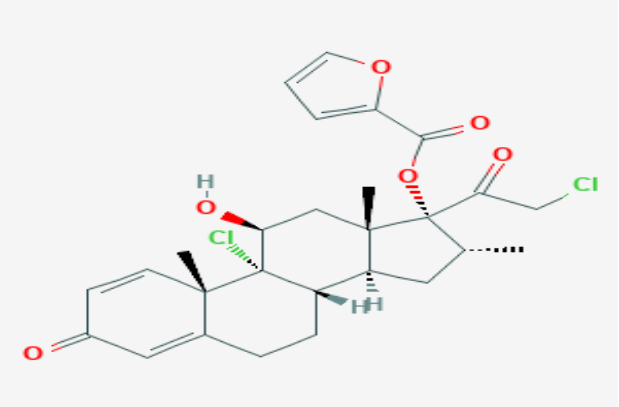

Fig no. 01: Chemical Structure of Mometasone furoate

It is a white to off-white powder, practically insoluble in water, soluble in organic solvents such as ethanol, DMSO and DMF, and soluble in aqueous buffers. Mometasone furoate only active ingredient in various topical preparations such as lotions, creams and ointments.[7,8] These molecules are officially reported in IP, EP and USP.

The literature survey suggests that only certain analytical methods are being developed for the determination of Mometasone furoate. HPLC methods for formulations, UV detection of Mometasone furoate[9], HPLC[10]and HPTLC methods have been reported for quantitative determination of MF in combination with other drugs.[11]Stability indicating HPTLC method for Mometasone furoate is not repeated yet. Hence, The main objective of the study was to developed a simple, fast, reliable, economical, specific and sensitive method developed for detection of mometasone furoateand in semisolid dosage form.

MATERIALS AND METHODS

Instrumentation and Chromatographic Conditions

HPTLC used with Camag (Muttenge, Switzerland) Linomat V applicator for sample application, Camag Twin-trough TLC Chamber was used for TLC plate development, Camag TLC Scanner 3 was used for detection, Camag Wincot Software was used and Hamilton (Reno, Nevada, USA) Syringe (100µL) was used for sample applicator.

Solvent and Chemicals

Mometasone furoate was purchase fromas a gift sample. Marketed formulations Elocon cream(By Mfg. Torrent Pharmaceuticals Ltd.) purchase from Local market, available in a dose of 10 grams from Pune. Ethyl acetate, acetonitrile, toluene, triethylamine,analytical grade solvent and TLC aluminum plates precoated with silica gel F254 used for this study were procured from Ostwald Laboratory, Pune.

Preparation Standard Stock Solution(API)

The 10mg mometasone furoate was accurately weighed and transfer into a 10ml volumetric flask, sonicated to dissolve, and the volume was makeup with methanol(1000µg/ml).

Preparation Sample Preparation

An accurately weighted sample (equivalent to 10mg mometasonefuroate) was taken into a 10ml volumetric flask, which was dissolved in approximately 5ml of methanol and Sonicated in an ultrasonic bath for 15 min with intermittent shaking, diluted to the volume with methanol. after that centrifuge for 5 min. Mix well (1000µg/ml).

MobilePhase

Ethyl acetate: acetonitrile: toluene: triethylamine (1.5: 1.5:7:0.2 v/v/v/v) is used as the mobile phase.

Chromatographic Condition

Chromatographic separation of the drug was done using precoated silica gelTLC plates F254,10x10cm in size. The specimens were mounted in bands on TLC plates, using a 6 mm wide Hamilton microliter syringe (100µl). A 10×10 cm twin-trough glass chamber (CAMAG) underwent a linear ascent, in which the ratio of ethyl acetate:acetonitrile: toluene: triethylamine was (1.5: 1.5: 7: 0.2 v/v/v/v). The slit dimension was kept as5×0.45mm.Optimized saturation time for mobile phase is used 20 minutes.Chromatogram was run till the distance of8cm. After development the plate was dried and densitometric analysis was performed atCamagTLC. Scanner with WinCats software at 250nm. The source of the radiation used is deuterium lamps.

Stability Study

Acid Degradation

From the 100µg/ml standard solution 1ml was pipette and to add 1ml of 0.1N HCl was added kept at room temperature for 6hrs. Standard solution of Mometasone Furoateand the degraded sample of Mometasone Furoate were spotted on TLC plate the plate was run with mobile phase consisting of Ethyl acetate : Acetonitrile: Toluene : Triethylamine (1.5:1.5:7:0.2 v/v/v/v). The plate was dried and scanned at 250nm. Densitogram was recorded and %degradation was calculated.

Alkaline Degradation

1ml of standard solution and 1ml of 0.1N NaOH, was kept at room temperature for 6hrs. Standard solution of Mometasone Furoate and thedegraded sample of Mometasone Furoate were spotted on TLC plate the plate was run with mobile phase consisting ofEthyl acetate : Acetonitrile: Toluene : Triethylamine (1.5:1.5:7:0.2 v/v/v/v). The plate was dried and scanned at 250nm. Densitogram was recorded and % degradation was calculated .

Oxidative Degradation

1ml standard solution and 1ml of 3% H_2O_2, was kept at room temperature for 12hrs. Standard solution of Mometasone Furoate and the degraded sample of Mometasone Furoate were spotted on TLC plate of size 3x10cm and the plate was run with mobile phase consisting of Ethyl acetate: Acetonitrile: Toluene : Triethylamine (1.5:1.5:7:0.2 v/v/v/v). The plate was dried and scanned at 250nm. Densitogram was recorded and % degradation was calculated.

Degradation under Neutral Hydrolytic Condition

1ml of standard solution and 1ml of Distilled water was kept at room temperature for 12hrs. Standard solution of Mometasone Furoate and the degraded sample of Mometasone Furoate were spotted on TLC plate the plate was run with mobile phase consisting of Ethyl acetate : Acetonitrile: Toluene : Triethylamine (1.5:1.5:7:0.2 v/v/v/v). The plate was dried and scanned at 250nm. Densitogram was recorded and % degradation was calculated.

Thermal Degradation

Dry heat studies were performed by keeping drug sample separately in oven (60°C) for a period of 24hours. A sample were withdrawn after 2hr, 4hr, 6hr, 12hr,18hr,24 hours, dissolved in methanol to get solution of 1000µg/ml. The standard solution and degraded solution was applied on TLC plate and the plate was run with mobile phase consisting of Ethyl acetate: Acetonitrile: Toluene: Triethylamine (1.5:1.5:7:0.2 v/v/v/v). The plate was dried and scanned at 250nm. Densitogram was recorded and % degradation was calculated.

Photo-degradation Studies

The photo degradation study of the drug was studied by exposing the 10mg of drug under sunlight for 6hrs. After exposure the drug was transferred to 10ml volumetric flask; the volume was made up with methanol to obtain 1000µg/ml solution. The solution was applied on TLC plate and the plate was run with mobile phase consisting of Ethyl acetate: Acetonitrile: Toluene: Triethylamine (1.5:1.5:7:0.2

v/v/v/v). The plate was dried and scanned at 250nm. Densitogram was recorded and % degradation was calculated.

RESULTS AND DISCUSSION

The densitogram of the standard Mometasone furoate (200ng/spot) is measured at 250nm. Mobile phase ethyl acetate: acetonitrile: toluene: triethylamine (1.5:1.5:7:0.2 v/v/v/v) was selected because it gave high resolution, minimum tailing and Rf values of 0.63, respectively Figure no. 02.

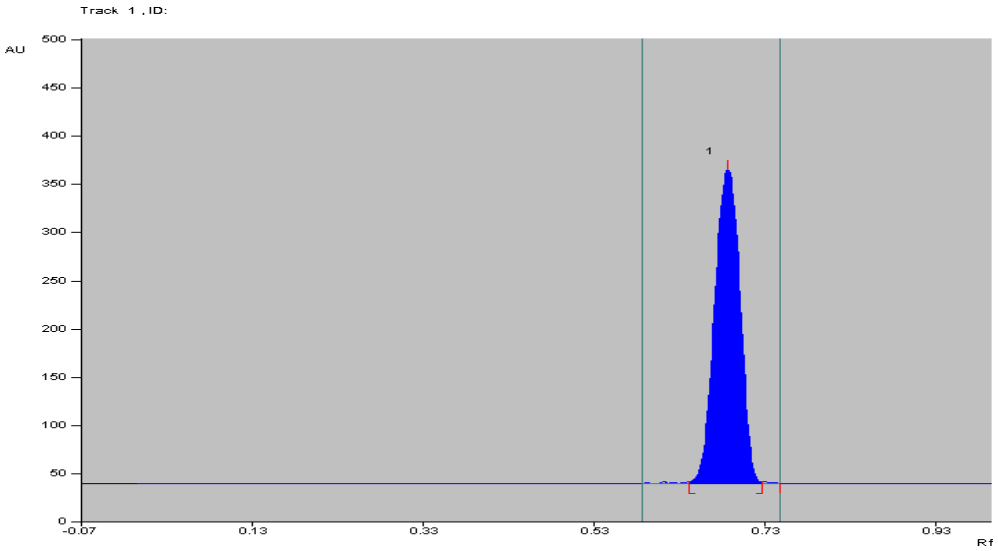

Fig.no.02: Typical Densitogram of MF (Rf= 0.63)

Method Validation [12-15]

Linearity

The calibration curve was plotted between peak areas versus concentration. The linear regression data for the calibration curves (n=5) showed good linear relationship over the concentration range of 200-1000ng/spot for Mometasone Furoate with R^2=0.9992. The slope and intercept of the regression equation were 1.5965 and 984.18 respectively for the Mometasone Furoate. The linearity was found to be satisfactory and reproducible. The calibration curve for Mometasone Furoate is shown in fig no.03

Table no. 01: Standard Calibration data for Mometasone Furoate

Sr. No	Concentration (ng/band)	Area
1	200	1318.3
2	400	1607.5
3	600	1928.1
4	800	2275.9
5	1000	2580.6

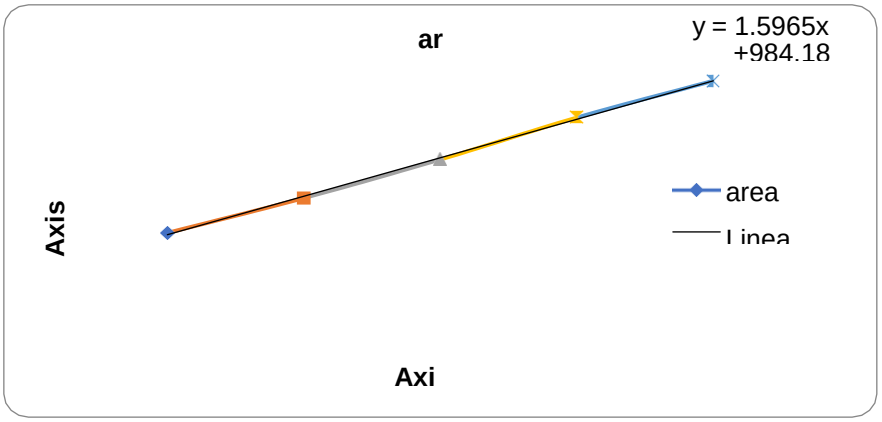

Fig no. 03 : Calibration Curve of Mometasone Furoate

Precision

Intraday and Interday precision of the method was assessed by developing the plate after application of 3 replicates of different concentration on TLC plate on the same day and the consecutive days respectively. Precision was reported in terms of %RSD. The %RSD values were found to be less than 2% as shown in table no.02.

Table no. 02 : Result of Intraday and Interday precision of Mometasone Furoate

Concentration (µg/ml)	Intraday Precision (Peak area)	Interday Precision	
		Day 1 (Peak area)	Day 2 (Peak area
200	419.6	690.02	472.21
200	422.12	670.27	468.46
200	421.4	684.16	459.54
200	429.7	664.66	474.31
200	433.5	680.84	479.33
200	430.6	685.9	482.63
Mean area	426.15	679.3083	474.41
SD	5.8016	9.8014	5.5811
% RSD	1.3614%	1.4428%	1.1764%

Accuracy

Accuracy is expressed as the nearness of agreement between the value found and values that are

already available. It can also be defined as the closeness between the true value and the observed value. It is sometimes called as trueness, and it could be determined by using at least 9 determinations over a minimum of 3 conc. (i.e.,80, 100,120%) of the drug. The mean percentage recovery is shown in table no. 03

Table no.03: Result of Recovery Study

Name of Drug	% Level Discovery	Amount of Drug estimated	% Recovery	% count(w/w)
ometasone Furoate	80%	350.54	97.37%	98.08%
	100%	392.05	98.01%	
	120%	435.04	98.87%	

Robustness

Robustness studies were done by making small, deliberate changes in optimized condition like mobile phase composition; developing time. The acceptance criteria for %RSD was found to be NMT 2% which indicates the reliability of method. The robustness of the method is shown in table4.

Table no. 4 : Result of Robustness study

Sr. No	Parameter	Robust Condition	Mean of Peak area	SD of Peak area	%RSD
1	velopingTime	20 min	1224.65	3.123316	0.255037
		40min	1273.52	5.66	0.44%
2	Mobile phase composition	Ethyl acetate + Acetonitrile + toluene + triethylamine (1.5:1.5:6.8:0.2)	1369.81	2.61	0.19%
		Ethyl acetate + Acetonitrile + toluene + triethylamine (1.5:1.5:7.2:0.2)	1568.67	3.98	0.25%

.1.5 Specificity

The peak purity of Mometasone Furoate was assessed by comparing spectra of standard and sample solution at the peak apex, peak start and end positions of the peak. A good correlation was obtained for standard and sample solution. Good correlation values and satisfactory peak purity suggest that there is no interference in the quantification of Mometasone Furoate in sample solutions. This verifies that the method is specific. Typical absorption overlain spectra of Mometasone Furoate are shown in Figure no. 04.

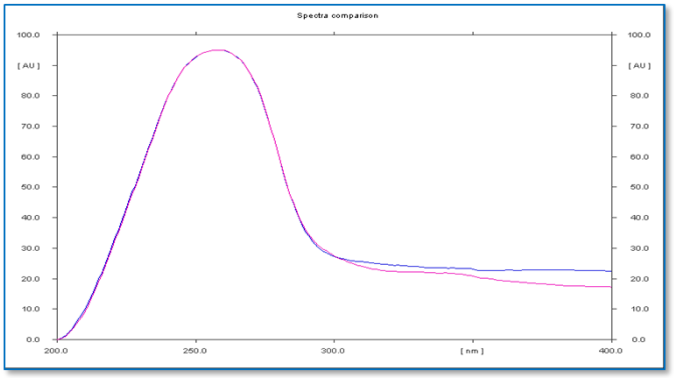

Figure no.04: Overlay Spectra of Mometasone Furoate

Sensitivity

Sensitivity of proposed method was estimated in terms of limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD and LOQ were determined separately through calibration curve. The residual standard deviation of a regression line or standard deviation of y-intercept of regression lines were used to calculate the LOD and LOQ.LOD & LOQ was found to be 36.69 and 111.18 ng/band respectively.

Stress Degradation Study

Stability studies were carried out to provide evidence on how the quality of drug varies under the influence of a variety of environmental conditions like hydrolysis, oxidation, temperature, etc. and to establish specific storage conditions, shelf-life and retest period. Dry heat and photolytic degradation were carried out in the solid state.

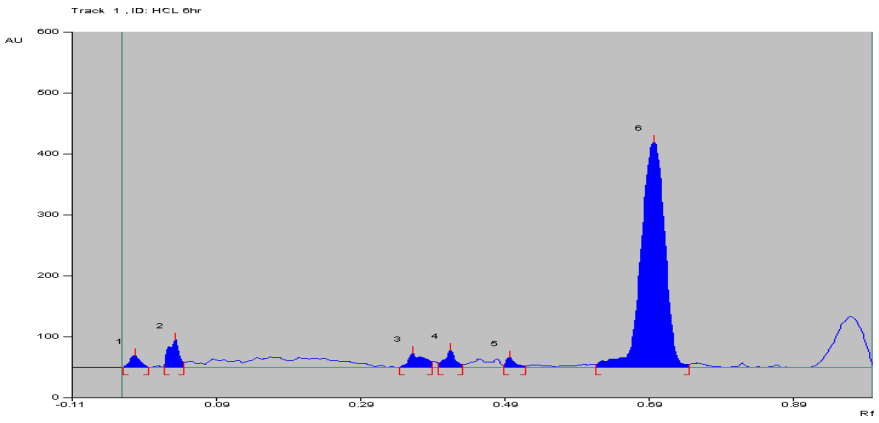

Figure no. 05: Acidic Degradation

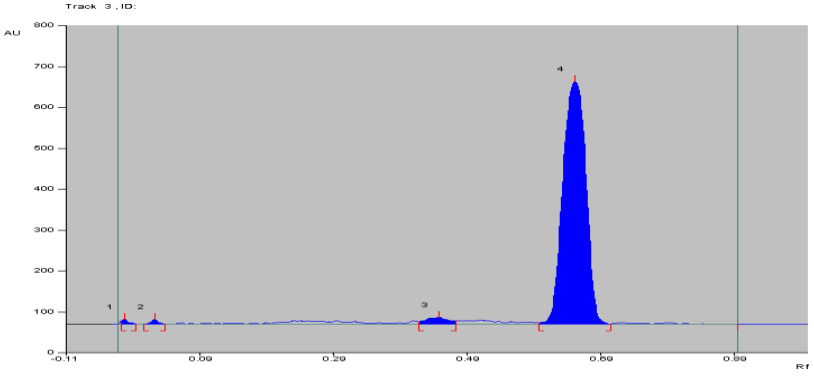

Figure no.06: Basic Degradation

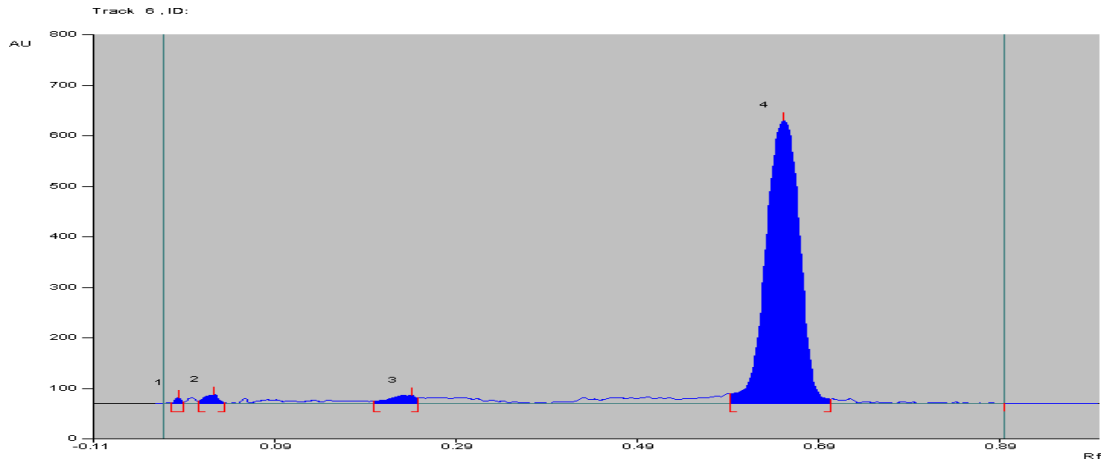

Figure no.0 7 : Densitogram of Oxidative Degradation

The chromatograms of the samples degraded with acid, base, hydrogen peroxide, neutral, Thermal, and Photolytic degradation showed well-separated spots of MF as well as some additional peaks at different Rf values. The spots of degraded product were well resolved from the drug spots as shown in Figs. 4-9. The percent degradation were calculated and listed in Table no. 5.

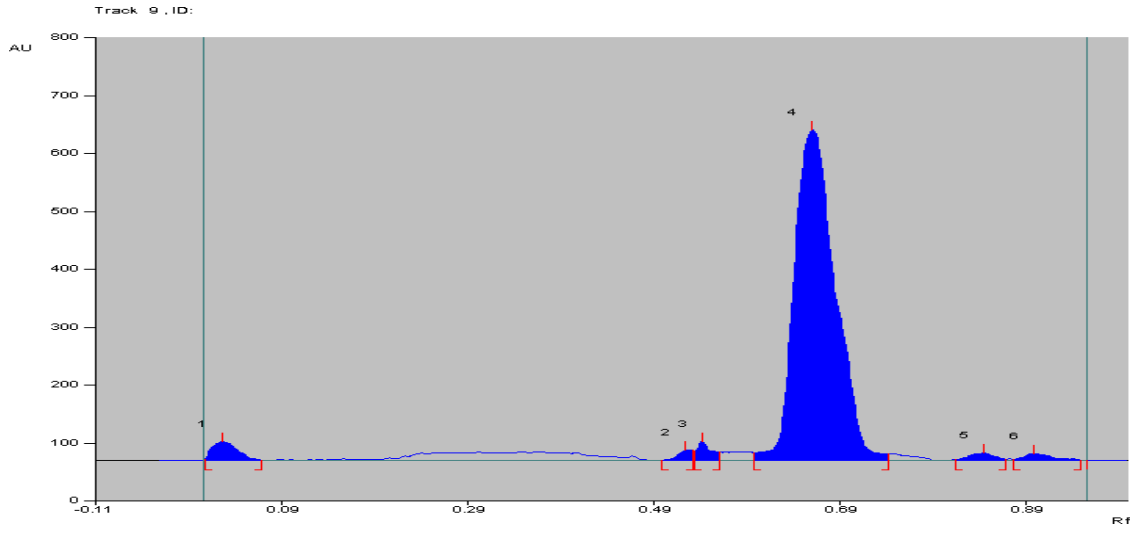

Figure no. 08: Degradation under Neutral Hydrolytic Condition

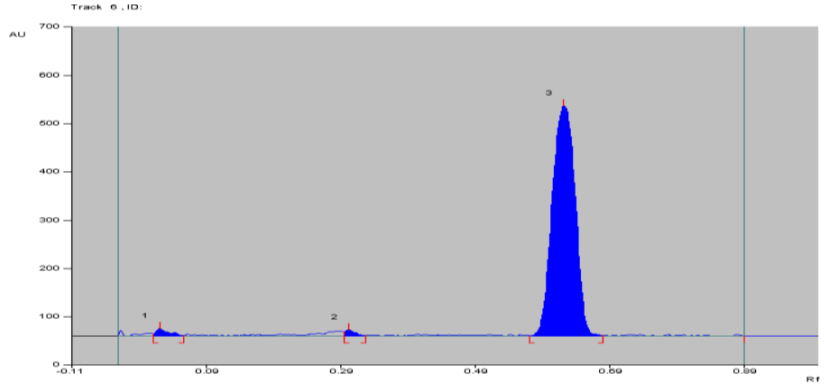

Figure no.09 : Thermal Degradation

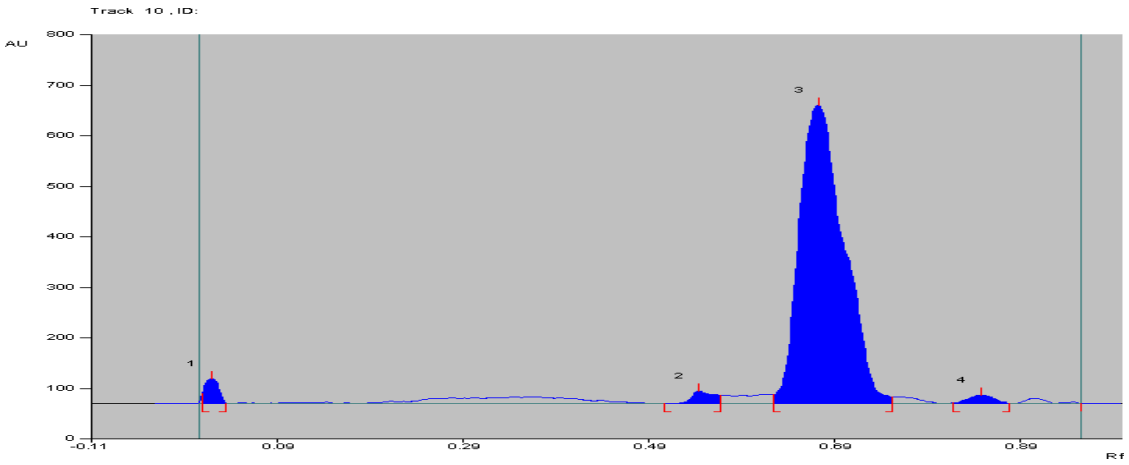

Figure no. 10 : Photolytic Degradation Table no.05 : Result of Stability Study

Stress Condition	Time (Hrs)	% Assay of active substance
Acid hydrolysis (0.1M HCl)	6	16.28%
Base hydrolysis (0.1M NaOH)	6	33.05%
Neutral (H2O)	12	59.03%
Oxidation(3%H2O2)	12	49.32%
Thermal degradation	24	35.70%
Photolytic	6	63.98%

4.0 CONCLUSION

The proposed stability-indicating method was simple, precise, accurate, reproducible, and sensitive and can be used for determination of Mometasone Furoate in bulk samples and in pharmaceutical dosage form. In that method, no interference from blank or excipient at Mometasone furoate peak retention were observed. This method can be adopted for regular quality control analysis of API and formulation. The developed method was validated as per the ICH guidelines.

5.0 CONFLICTS OF INTEREST

The authors does not have any conflict of interest.

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