

## Stability Indicating Hptlc Method for the Estimation of Clobetasol Propionate in Presence of Alkali Induced Degradation Product.

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**Abstract:** Clobetasol Propionate is a corticosteroid of the glucocorticoid class used to treat various skin disorders like eczema and psoriasis. An approach for the stress degradation was successfully applied for the development of stability indicating HPTLC method for the determination of Clobetasol Propionate in the presence of its degradation product on the plates precoated with silica gel 60 F<sub>254</sub>. The mobile phase used was Toluene: Methanol in the ratio of 8:2 v/v. The drug showed considerable absorbance at 239nm. Stress testing of Clobetasol Propionate was carried out according to the international conference of harmonization (ICH) guideline Q1A (R2). The drug was subjected to acid, base, neutral hydrolysis, oxidation, thermal degradation and photolysis. There was no interference between the drug peak and peak of product of degradation; therefore the method was specific for the determination of Clobetasol Propionate in the presence of the degradation product. This system showed a peak for Clobetasol Propionate at R<sub>f</sub> value of 0.49 ± 0.02. The data of linear regression analysis indicated a good linear relationship over the range of 200–1200 ng/band concentrations. The method was validated for robustness, precision and accuracy. The LOD and LOQ were 14.69 and 44.52ng/band respectively. Among various stress conditions, Clobetasol Propionate showed two degradation products under alkali hydrolysis at R<sub>f</sub> value of 0.37, 0.51.

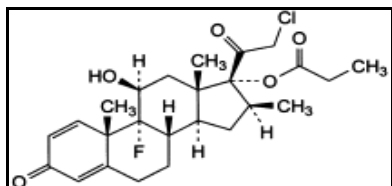
**Keywords:** Clobetasol Propionate, high-performance thin layer chromatographic (HPTLC) method, Stability-Indicating Method

### Introduction

Clobetasol Propionate, 21-Chloro-9-fluoro-11b-hydroxy-16b-methyl-3,dioxopregna-1,4-diene-17-yl propanoate a topical corticosteroid of the glucocorticoid class used to treat various skin disorders like eczema and psoriasis. It is also highly effective for contact dermatitis caused by exposure to poison ivy/oak. It has very high potency and typically should not be used with occlusive dressings. Different dosage forms for topical use are currently approved by United States Food and Drug administration (USFDA). It is available in dosage forms such as cream, gel, ointment, shampoo etc<sup>1-3</sup>.

Literature search reveals following methods reported viz. Validated RP-HPLC Method for the determination of Clobetasol Propionate in topical nanocapsule suspensions<sup>6</sup>, Validated HPLC method for determination of Clobetasol Propionate residues on the surface of manufacturing equipment<sup>7</sup>, Liquid chromatographic determination of Clobetasol-17-butyrate in ointment<sup>8</sup>, Liquid chromatography- tandem mass spectrometric assay for Clobetasol propionate in human serum from patients with acute dermatitis<sup>9</sup>, HPLC method for the determination of Clobetasol in rat plasma and its application to skin penetration<sup>10</sup>, Forced degradation studies of Clobetasol 17-propionate in methanol, propylene glycol, as bulk drug and cream formulations by RP-HPLC<sup>11</sup>, Simultaneous estimation of Clobetasol propionate and Fusidic acid in cream by

RP HPLC method<sup>12</sup>, Simultaneous estimation of Clobetasol propionate and Miconazole nitrate by HPTLC<sup>13</sup>, Simultaneous determination of Clobetasol Propionate and Calcipotriol in a novel fixed dose emulgel formulation by LC-UV<sup>14</sup>, HPLC assay for simultaneous determination of Everolimus and Clobetasol Propionate<sup>15</sup>, Simultaneous determination of Clobetasol Propionate and Chlorocresol in Cream by Stability Indicating RP-HPLC Method<sup>16</sup> However, there is no stability-indicating HPTLC method reported so far for the quantification of Clobetasol Propionate in the presence of its degraded products. In the current work, we have developed and validated stability indicating HPTLC method for estimation of Clobetasol Propionate as per ICH guidelines<sup>17</sup>. Intensive stress studies are carried out according to the international conference on harmonization (ICH) guidelines<sup>18, 19</sup> and the method could resolve degradation product from the response of Clobetasol Propionate.



**Fig.1: Structure of Clobetasol Propionate**

## Experimental

### Standards and chemicals

Standard Clobetasol Propionate was procured from Zydus Cadila Healthcare Ltd., Ahmedabad, Aluminum sheets precoated with silica gel (60 F<sub>254</sub>, 20 cm × 20 cm with 250 μm layer thickness) were purchased from E-Merck, Darmstadt, Merck (Germany). Methanol (AR grade), Chloroform (AR grade) were purchased from S. D. fine chemical Laboratories, Mumbai. Hydrochloric acid (HCl), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, 30% v/v) and sodium hydroxide (NaOH) were purchased from LOBA CHEMIE PVT. LTD. Mumbai.

### Chromatographic instrumentation

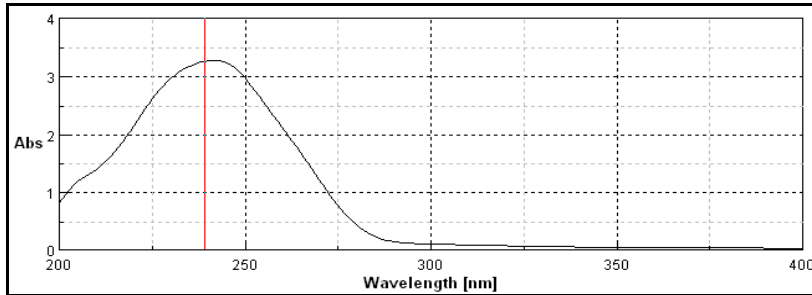
Chromatographic separation of drug was performed on Aluminum plates precoated with silica gel 60 F<sub>254</sub>, (10 cm × 10 cm with 250 μm layer thickness). Samples were applied on the plate as a band with 4 mm width using Camag 100 μL sample syringe (Hamilton, Switzerland) with a Linomat 5 applicator (Camag, Switzerland). The mobile phase was composed of Toluene: Methanol (8:2 v/v). 20 cm × 10 cm CAMAG twin trough glass chamber was used for linear ascending development of TLC plate under 15 min saturation conditions and 10 mL of organic solvent was used per run, migration distance was 90 mm. Densitometric scanning was performed using Camag TLC scanner 3 in the range of 400-200 nm, operated by winCATS software (Version 1.4.3, Camag), slit dimensions were 3.00 x 0.45 mm and Deuterium lamp was used as a radiation source.

### Preparation of Standard stock solution

Standard stock solution of Clobetasol Propionate was prepared by dissolving 10 mg of drug in 10 ml of methanol to get concentration of 1000μg/ml. From the standard stock solution, working standard solution was prepared to contain 100μg/ml of Clobetasol Propionate.

### Selection of detection wavelength

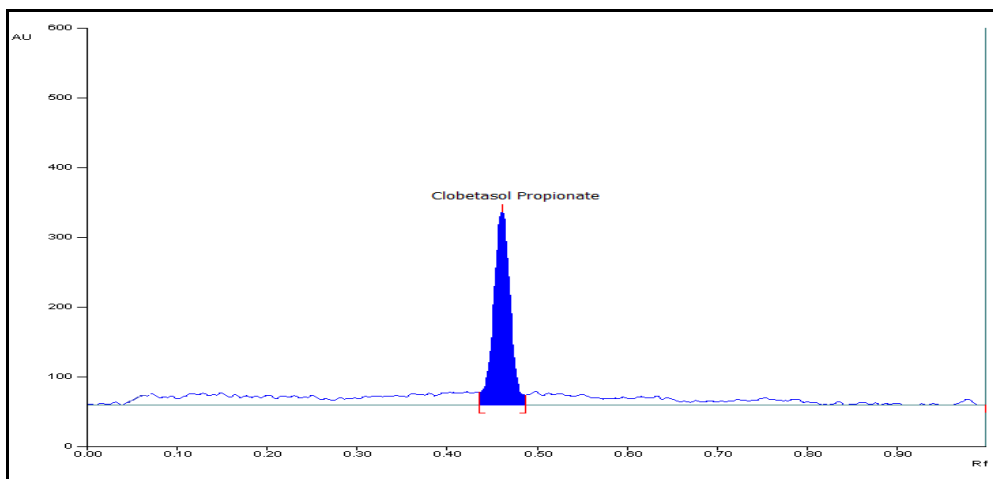
From the standard stock solution further dilutions were done using methanol and scanned over the range of 200 – 400 nm and the spectra was obtained. It was observed that the drug showed considerable absorbance at 239 nm.



**Fig.2: UV Spectrum Clobetasol Propionate (100µg/ml)**

### Densitogram:

Solution of Clobetasol Propionate (100µg/ml) was prepared. 6µl (600ng/band) of solution was applied on preactivated TLC plate with the help of Hamilton syringe (100µl), using Linomat5 sample applicator. The development chamber was saturated with mobile phase for 15 min. The spotted plate was placed in the saturated chamber and developed up to 90 mm distance. The plate was dried and was scanned over 90 mm distance at 239nm. The retention factor (Fig. 3) was found to be  $0.49 \pm 0.02$



**Fig.3: Densitogram of standard solution of Clobetasol Propionate (600ng/band)**

### Preparation of sample solution:

25ml of lotion (CLONATE lotion) containing 0.05% w/v of Clobetasol Propionate was purchased from local market, lotion equivalent to 10mg of Clobetasol Propionate was transferred to 100ml volumetric flask and diluted with methanol, shaken vigorously to disperse the lotion and centrifuged at about 3500rpm for about 10min. Filtered a portion of the supernatant through 0.45µm wattman filter paper. Further dilutions were made with methanol to get the final concentration of 100µg/ml (sample of Clobetasol Propionate).

### Stress degradation study of bulk drug

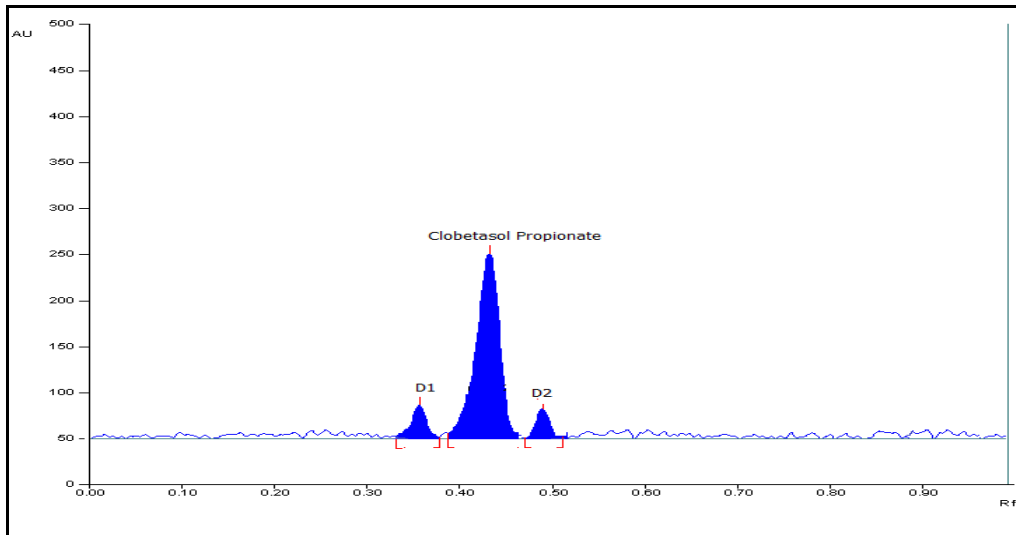
Stress degradation studies were carried under condition of acid/ base/ neutral hydrolysis, oxidation, dry heat and photolysis. For each study, samples were prepared as follows

1. The blank subjected to stress in the same manner as the drug solution
2. Working standard solution of Clobetasol Propionate subjected to stress condition.

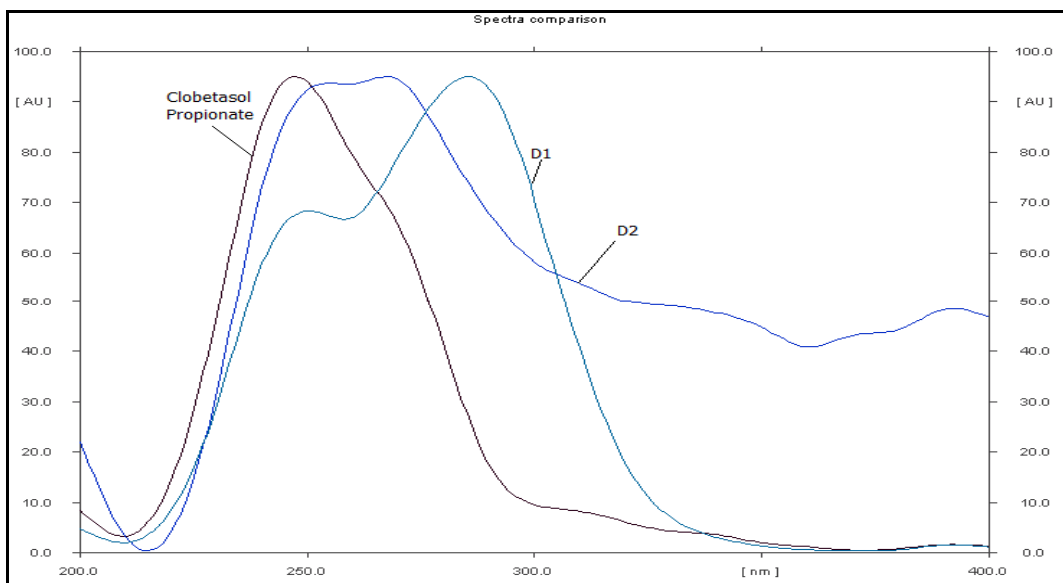
Dry heat and photolytic degradation were carried out in solid state. 6µL of the resultant solution was then applied at TLC plate and densitogram was developed.

### Degradation under alkali catalyzed hydrolytic condition

To 1 mL of 1000  $\mu\text{g}\cdot\text{mL}^{-1}$  solution of Clobetasol Propionate, 1 mL of 0.1 N NaOH was added. The volume was made up to 10 mL with methanol. The above solution was kept for 1 hour at room temperature.



**Fig.4: Representative Densitogram of alkali catalyzed degradation of Clobetasol Propionate 600ng/band, (D1, D2= alkali catalyzed degradation products)**



**Fig.5: Overlain UV spectra of Clobetasol Propionate and products formed after alkali catalyzed degradation (D1, D2)**

### Degradation under acid catalyzed hydrolytic condition

To 1 mL of 1000  $\mu\text{g}\cdot\text{mL}^{-1}$  solution of Clobetasol Propionate, 1 mL of 1N HCL was added. The volume was made up to 10 mL with methanol. The above solution was kept for 4 hrs at room temperature.

### Degradation under neutral hydrolytic condition

To 1 mL of 1000  $\mu\text{g}\cdot\text{mL}^{-1}$  solution of Clobetasol Propionate, 1 mL of distilled water was added. The volume was made up to 10 mL with methanol. The above solution was kept for overnight at room temperature.

### Degradation under oxidative condition

To 1 mL of 1000  $\mu\text{g}\cdot\text{mL}^{-1}$  solution of Clobetasol Propionate, 1 mL of 30%  $\text{H}_2\text{O}_2$  was added. The volume was made upto 10 mL with methanol. The above solution was kept for 2 hours at room temperature.

### Degradation under dry heat

Dry heat studies were performed by keeping drug sample in oven ( $80^\circ\text{C}$ ) for a period of 6 hours.

### Photo-degradation studies

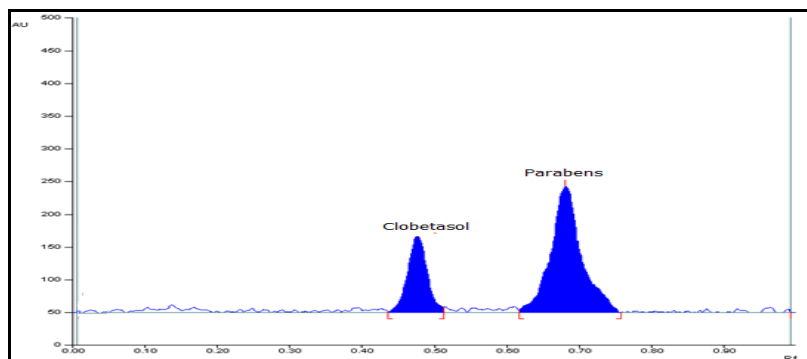
The photo degradation study of the drug was carried out by exposing the drug to UV light providing illumination of NLT 200 watt  $\text{hr}/\text{m}^2$ , followed by exposure to cool white fluorescence light of NLT 1.2million Lux-Hrs.

**Table 1: Summary of stress degradation of Clobetasol Propionate**

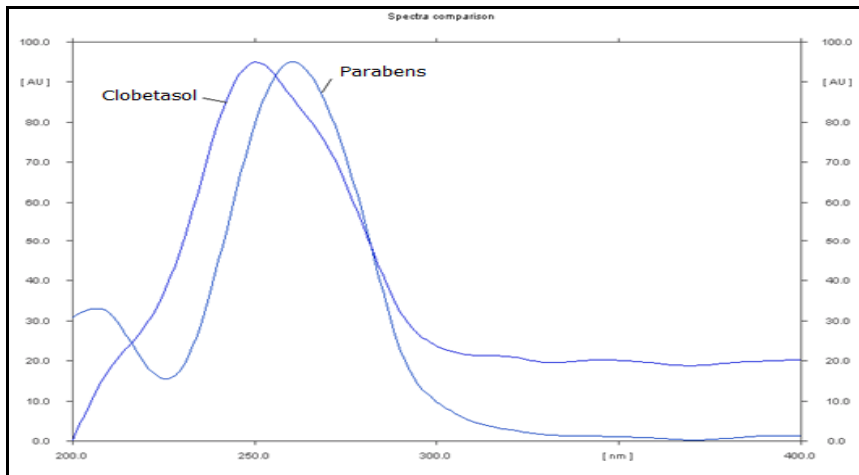
Stress Degradation conditions	Percent recovery (%)	Percent degraded (%)	Rf of degradation product	Peak purity	
				r(s,m)	r(m,e)
Initial	100	-	-		
Base (0.1 N NaOH, kept for 1 hr)	72.45	27.55	0.37 0.51	0.9971	0.9976
Acid (1 N HCl, Kept for 4Hrs.)	90.87	9.13	-	0.9986	0.9988
$\text{H}_2\text{O}_2$ 30% v/v (kept for 2hrs)	74.69	25.31	-	0.9995	0.9974
Water (Kept for overnight)	83.25	16.75	-	0.9976	0.9984
Dry Heat ( $80^\circ\text{C}$ , 6 hrs)	94.65	5.35	-	0.9980	0.9971
Photo stability UV(200 watt hrs/square meter), Florescence (1.2 million Lux. Hrs)	83.68	16.32	-	0.9972	0.9981
	66.67	33.33	-	0.9998	0.9992

### Assay of Marketed Formulation (CLONATE CREAM)

Formulation analysis was carried out as mentioned under section preparation of sample solution Procedure was repeated for six times. Sample solution was spotted and area was recorded. % assay was determined from linearity equation.



**Fig.6: Densitogram of Marketed Formulation of Clobetasol Propionate (Rf 0.71= Parabens)**



**Fig.7: Overlain UV Spectrum of Clobetasol Propionate and Parabens in marketed Formulation**

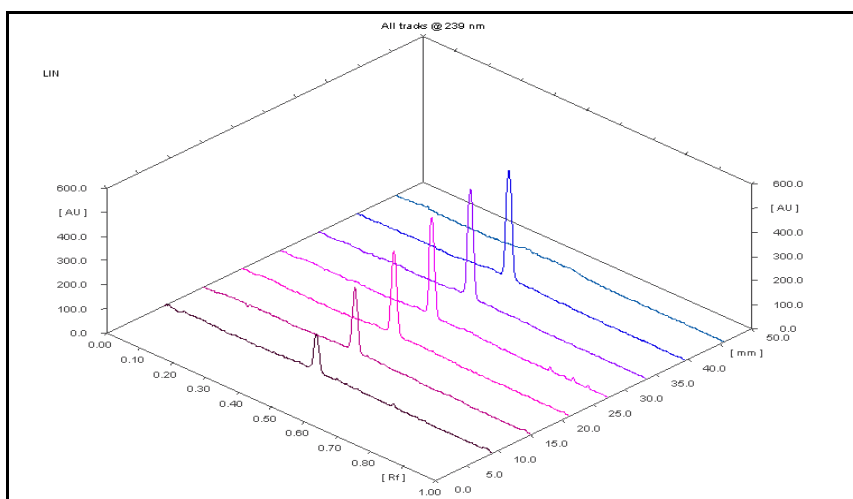
## Result

### Validation of method

The method validation was done as per the ICH guidelines.

### Calibration curve of Clobetasol Propionate

Stock solution of Clobetasol Propionate (1 mg mL<sup>-1</sup>) was prepared in methanol. This solution as further used to prepare range of solutions containing six different concentrations. Five replicates per concentration were spotted. The linearity (relationship between peak area and concentration) was determined by analyzing six solutions over the concentration range of 200-1200 ng/band for Clobetasol Propionate. Prepared solutions were stored at 2 to 8<sup>o</sup>C until use. The spotted plate was developed as mentioned in previous section. Linearity equation and regression coefficient was found to be  $y = 6.652x + 1180$  and  $r^2 = 0.996$  respectively.



**Fig.8: Densitogram of linearity of Clobetasol Propionate (200-1200 ng/band)**

The method sensitivity was estimated with respect to limit of detection (LOD), limit of quantification (LOQ) and correlation coefficient. In order to evaluate LOD and LOQ, calibration curve was used and were evaluated by using equation:  $LOD = 3.3 \delta / S$ ,  $LOQ = 10 \delta / S$  respectively, where, S = the slope of the calibration curve,  $\delta$  = standard deviation. The Intra- and inter-day variation for the estimation of Clobetasol Propionate was evaluated for method precision. It was achieved by using concentration level of 600ng spot<sup>-1</sup>. Repeated analyses were carried out in a same day for intra-day analysis while the same practice was repeated next day for inter-day analysis. Intra- and inter-day analyses were performed to check the repeatability and reproducibility of

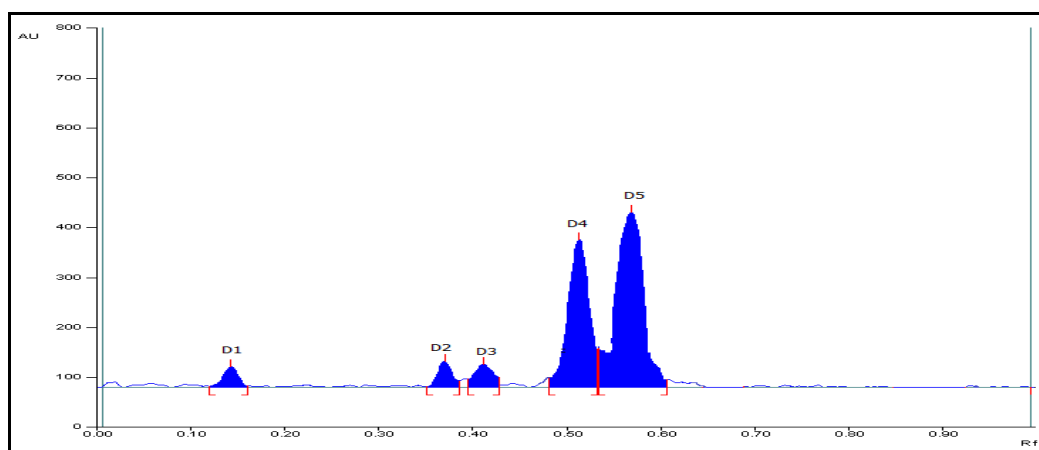
the method, respectively and results were statistically evaluated in terms of % R.S.D. In order to check the robustness, following parameters were intentionally changed within the range of  $\pm 2\%$  at 200, 400 and 600 ng/spot concentration level; mobile phase composition, chamber saturation time, time from spotting to development, time from development to scanning. The accuracy of the method was assessed by adding standard drug to sample at three different levels 80, 100 and 120 %. Basic concentration of sample chosen was 600ng/band of Clobetasol Propionate standard solution.

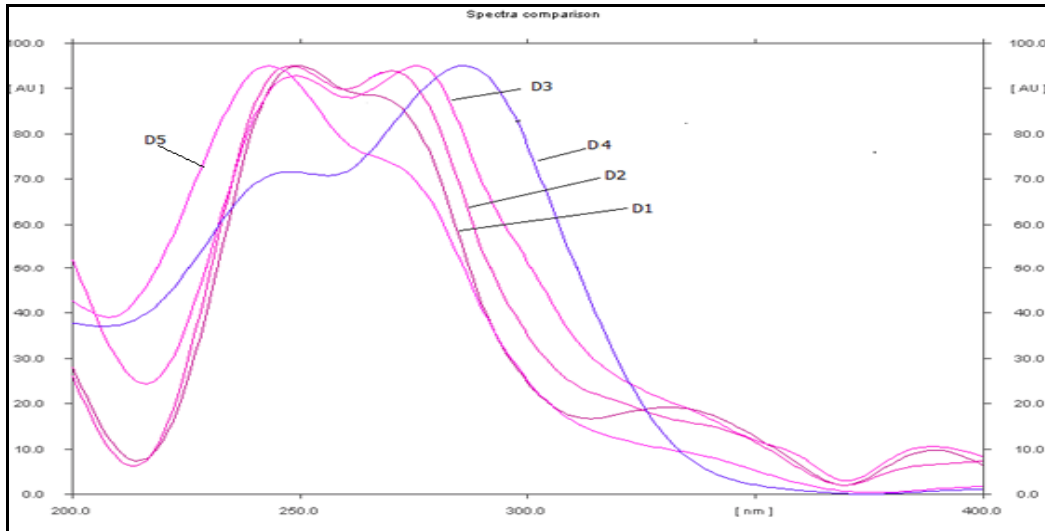
**Table 2: Summary of validation study**

Sr.No.	Validation parameters	Clobetasol Propionate
1.	Linearity Equation ( $r^2$ ) Range	$Y=6.652x + 1180$ $r^2 = 0.996$ 200- 1200ng/band
2.	Precision (% RSD) Interday Intraday	0.96% 0.93%
3.	Accuracy	% Recovery
	80% level	100.38
	100% level	100.15
	120% level	99.13
4.	Limit of Detection	14.69ng/band
5.	Limit of Quantitation	44.52ng/band
6.	Specificity	Specific
7.	Robustness	Robust

### Characterization of alkali induced degradation product

During stress degradation studies, since a well resolved product peaks were observed under base induced degradation conditions, the objective of the next part of the study was to optimize the conditions so as to obtain complete conversion of Clobetasol Propionate to degradation products which may be characterised. The next steps planned were isolation, purification and characterization of the product. Alkali induced degradation was observed to commence within 1 hr and HPTLC studies over a prolonged period indicated absence of Clobetasol and presence of five products of degradation after about 24 hrs.





**Fig.9: Representative Densitogram of alkali induced degradation of Clobetasol Propionate (24 hrs exposure), 600ng/band and Overlain UV spectra of alkali induced degradation products of Clobetasol Propionate respectively (D1, D2, D3, D4, D5)**

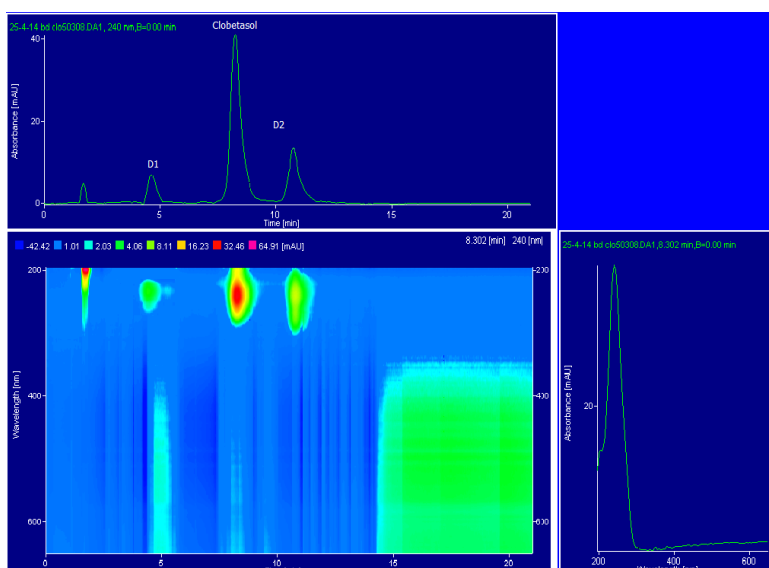
Since no single product was obtained, it became necessary to resolve the products on HPLC-MS system with a view to monitor the mass of the product which would help in characterizing it. Hence chromatographic conditions were developed using HPLC system with Diode array detector.

#### Chromatographic Conditions:

The mobile phase consisting of Methanol: water in the ratio of 60:40 v/v, was filtered through 0.45 $\mu$  membrane filter, sonicated and was pumped from the solvent reservoir. Separation was achieved on C<sub>18</sub> column. The flow rate of mobile phase was maintained at 1ml/min and the response was monitored between 200 to 400 nm with a run time of 20 min. The volume of injection loop was 20 $\mu$ l. The column was kept at ambient temperature.

#### Instrument:

Jasco HPLC system comprising: Model PU 2080 Plus pump, Rheodyne sample injection port, Grace C<sub>18</sub> Column 150 x 4.6 mm, MD 2010 PDA detector, Borwin- PDA software (version 1.5)



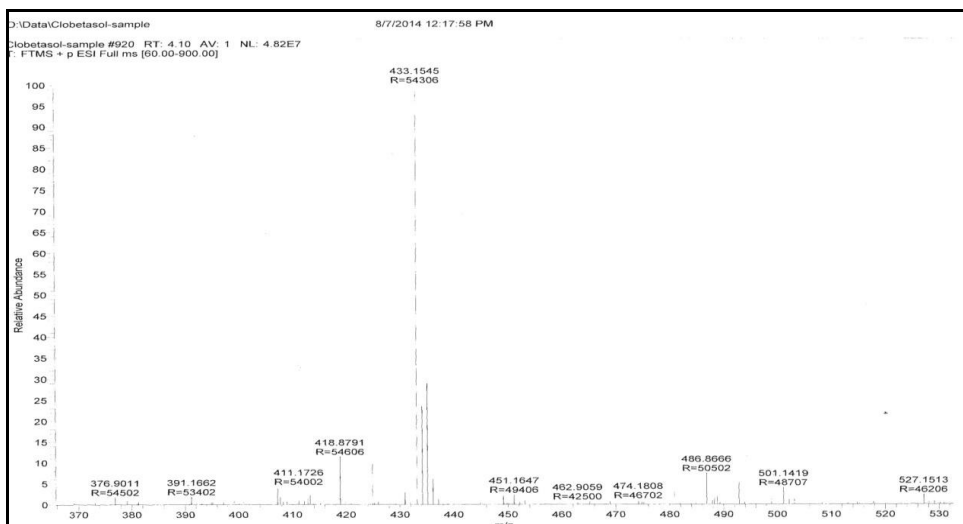
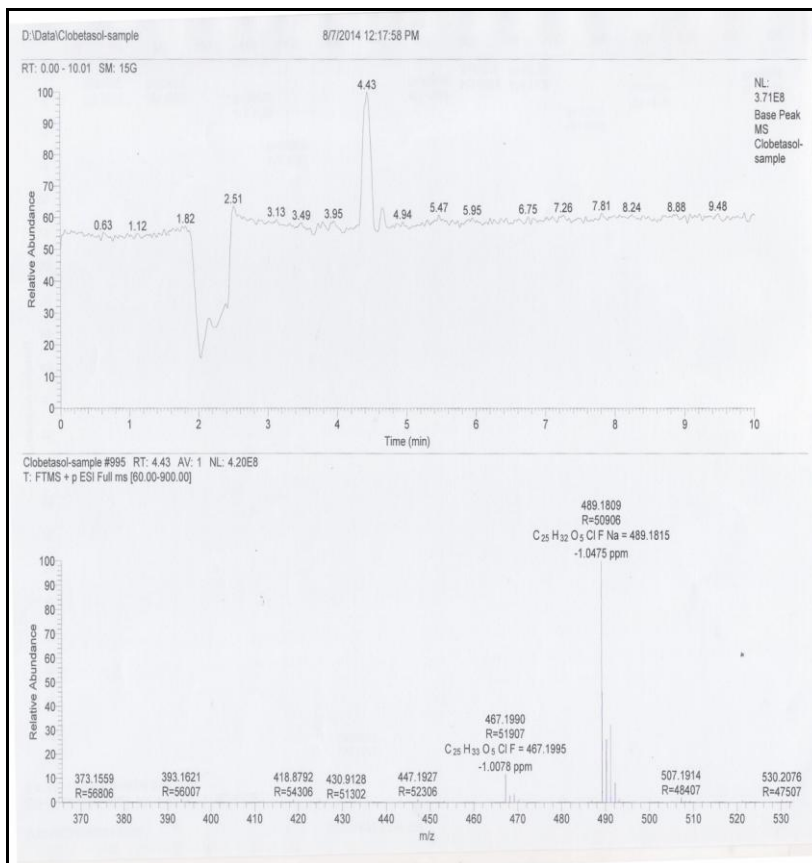
**Fig.10: Chromatogram of alkali induced degradation of Clobetasol Propionate 50 $\mu$ g/ml after 4hrs (D1, D2= Degradation Products)**

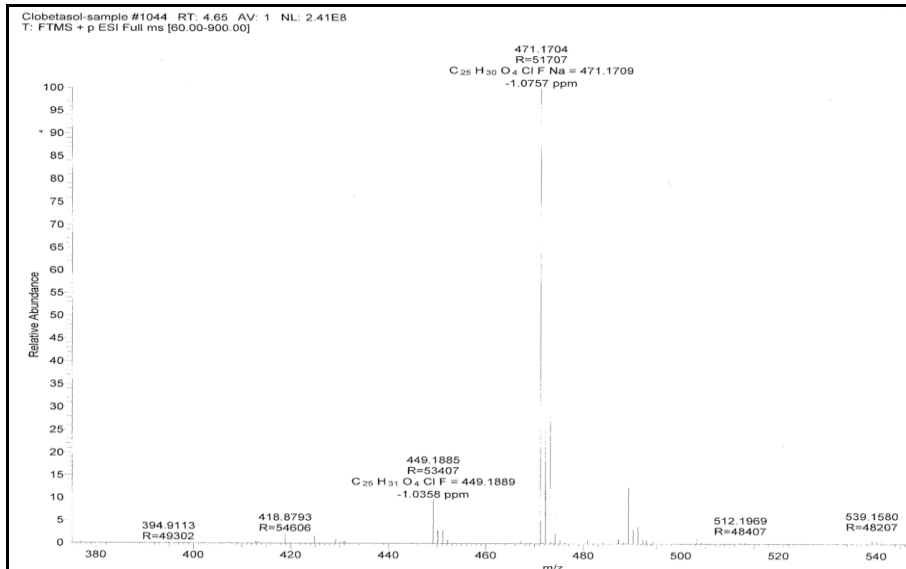


The peak area of Clobetasol Propionate was significantly reduced after 4 hrs. As is observed in the above chromatogram (Rt = 8.302 min). The peak for product of degradation is observed at 4.6min ( $\lambda = 239$ ) and 10.74min ( $\lambda = 239,267\text{nm}$ ).

This sample (exposed to base conditions for 4 hrs) was also analyzed simultaneously on LC-MS system at, National Chemical Lab., Pune. The details of chromatographic conditions are as follows

1. Make of LC-MS INSTRUMENT : Thermo Fischer Scientific
2. Model : Q Exactive(LC-MS system)
3. Ionisation technique : Electrospray Ionization
4. Column used: Hypersil Gold 150x4.6mm, 8um





**Fig.11: Mass Spectra of Alkali Treated Clobetasol Propionate**

### Results of LCMS study

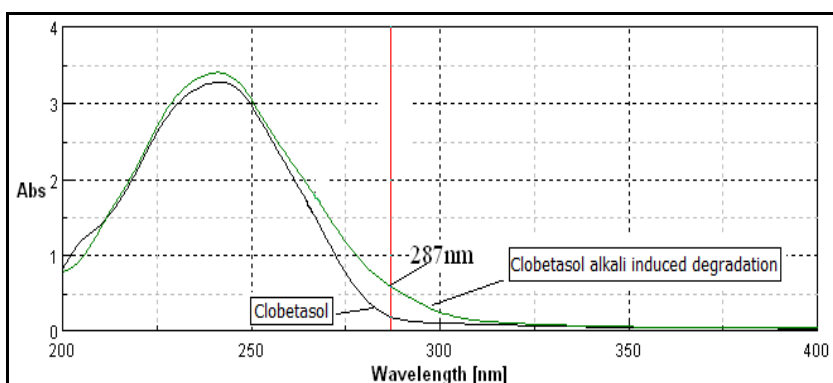
The mass difference of 56 units is obtained between Clobetasol Propionate [Rt 4.43min, Mass 489 (Adduct with Na)] and the product (Rt 4.10 min, Mass 433.15), indicating loss of  $\text{CH}_3\text{CH}_2\text{CO}$  by ester hydrolysis and the mass different of 18 unit is observed between Clobetasol Propionate and the product (Rt 4.65 min, Mass 471.17) indicating loss of water molecule by dehydration.

### Hydrolytic Degradation Monitoring of Clobetasol Propionate by UV Spectrophotometric and Derivative Spectrophotometric Method

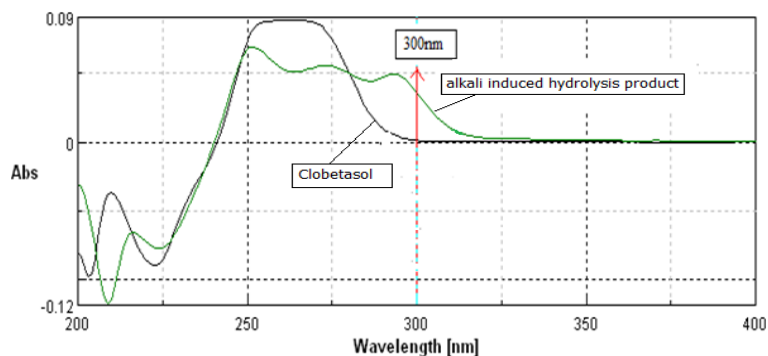
#### Selection of analytical wavelength

UV Spectrum of standard solution and alkali induced degraded solution was scanned over the range of 200-400nm.

- Overlain UV spectra of working standard solution of Clobetasol Propionate and alkali induced degraded solution of Clobetasol Propionate were obtained. Simple spectrophotometry allowed specific detection of alkali induced degradation solution of Clobetasol Propionate at 287nm with negligible contribution by Clobetasol Propionate.
- Simple UV Spectra was converted to First Order Derivative spectra and were overlain. First derivative spectrophotometry allowed specific detection of alkali induced degradation solution of Clobetasol Propionate at 300nm with no contribution by Clobetasol Propionate.



**Fig.12: Overlain UV Spectra of Clobetasol Working Standard (100µg/ml) and product of alkali catalyzed hydrolysis (100µg/ml)**



**Fig.13: Overlain First Derivative Spectra of Clobetasol Working Standard (100µg/ml) and product of alkali catalyzed hydrolysis (100µg/ml)**

The results indicate that if Clobetasol propionate undergoes degradation due to alkaline hydrolysis, a simple UV spectrum can be monitored at 287nm or first derivative spectrum at 300nm to detect degradation.

## Discussion

Stress degradation study of Clobetasol Propionate was carried out on HPTLC, HPLC and UV spectrophotometer. The drug showed considerable absorbance at 239nm. In case of HPTLC, mobile phase used was Toluene: Methanol (8:2v/v). R<sub>f</sub> was found to be 0.49±0.02 with acceptable peak parameters and peak purity greater than 0.995. The method was linear over the concentration range of 200-1200 ng/band. After stress degradation it was concluded that Clobetasol Propionate was very sensitive to alkaline, neutral hydrolysis, oxidation and photolytic degradation. Clobetasol showed degradation products only under alkaline hydrolysis at R<sub>f</sub> value of 0.37, 0.51 after 1 hr. The developed HPTLC method was found to be simple, sensitive, specific, accurate and precise for analysis of Clobetasol Propionate in market cream without any interference from the excipients. The method was successfully used for the determination of drug in a pharmaceutical formulation. The results indicated the suitability of the method to study stability of Clobetasol Propionate under various forced degradation conditions like acid, base, dry heat, neutral, oxidative and photolytic degradation.

Hydrolytic Degradation Monitoring was done using RP HPLC and UV spectrophotometer. In case of RP HPLC mobile phase used was Methanol: Water (60:40v/v), retention time was found to be 7.8 min for Clobetasol and 7.4 min, 8.3min for alkali induced degradation products, as the time period of exposure increases, the extent of degradation goes on increasing (area of drug decreases whereas the area of degradation product increases). When alkali degraded solution was tested after 24hrs, complete degradation was observed. The results of UV indicate that if Clobetasol propionate undergoes degradation due to alkaline hydrolysis, a simple UV spectrum can be monitored at 287nm or first derivative spectrum at 300nm to detect degradation.

## Acknowledgement

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