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DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING RP-HPLC METHOD FOR THE ESTIMATION OF CLOBETASOL PROPIONATE IN BULK AND OINTMENT DOSAGE FORM

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ABSTRACT

A simple, gradient RP- HPLC method has been developed and validated for Clobetasol propionate in bulk and ointment formulation. The successful estimation was carried out of the drug product is developed on C(18) column reversed-phase using Acetonitrile: Methanol: Phosphate buffer (16:20:80 v/v) as mobile phase composition. The flow rate was adjusted to 1.5mL/minute and the absorption maxima were observed at 250 nm utilizing Shimadzu SPD-20A Prominence UV-Vis detector. Clobetasol propionate showed a good and precise linearity in the range 20-100µg/mL. The HPLC, assay shows the purity ranging 99.95 to 102.27% for ointment formulation. The mean percentage purity is 101.11%. The chromatographic retention time of Clobetasol propionate was found to be 5.12 minutes. The statistical analysis shows the method accuracy. Various forced degradation studies was conducted on Clobetasol propionate ointment to examine the stability of the drug. The developed method validated according to the ICH guidelines.

KEYWORDS: Clobetasol propionate, RP-HPLC, UV, validation and forced degradation.

INTRODUCTION

Clobetasol propionate is a potent topical glucocorticoid for topical use.[1] Clobetasol propionate chemicall called as (21-Chloro-9-fluoro-11β-hydroxy-16β- methyl-3, 20-dioxopregna-1,4-dien-17-yl propanoate) is derivative of prednisolone with high glucocorticoid activity and low mineralocorticoid activity.[2] Clobetasol propionate is used for short-term relief of anti-inflammatory, Pruritic manifestations of moderate to severe corticosteroid responsive dermatoses and in Psoriasis.[2-4] Clobetasol propionate is about 1000 times more potent than hydrocortisone.[5] Clobetasol propionate comes in shampoo, mousse, ointment and emollient cream presentations.[5] Clobetasol propionate has very high potency and typically should not be used with occlusive dressings, or for extended continuous use beyond two weeks.[3] It is also used to treat several autoimmune diseases including alopecia areata, vitiligo, lichen sclerosus, and lichen planus.[4] According to the literature review several methods has been developed for Clobetasol propionate, like UV spectroscopy, fluorescence spectroscopy capillary electrophoresis, HPTLC, HPLC and voltammetry method.[6-11] The thorough literature survey revealed that a few stability-indicating normal and RP-HPLC methods for Clobetasol propionate in respective dosage forms are available but all of these methods are specific to the bulk drugs. The proposed aim of the study was to develop simple, accurate, specific and precise RP-HPLC method for the estimation of Clobetasol propionate in the bulk and pharmaceutical formulation.

MATERIALS AND METHODS

Chemicals

The Clobetasol propionate reference standard (RS) was purchased from Sigma, Germany. The Gamavate ointment 0.05% w/w marketed drug of Clobetasol propionate, manufactured and marketed by Julphar**,** U.A.E, purchased from Jazan local Pharmacy, Saudi Arabia. The HPLC grade Acetonitrile and Methanol was purchased from Merck.

RP-HPLC instrumentation

Shimadzu LC-20 AT HPLC system, using SPD-10 detector (SPD- M20A, Japan). A Zorbax Eclipse Plus, Agilent Technology column (150mm x 4.6mm, 5µm) with Pore size 95Å. The column temoerature was maintained at a 29ºC and the flow rate 1.5 ml/min. The injection volume is 20µl, 250nm was set as a wavelength and the HPLC run time was seted for 15 minutes. Phosphate buffer was prepared

Preparation of Mobile phase

Accreatly weighed 1.35g of KH_2PO_4 transfered in to 1 liter volumaetric flask and dissolved by 500 ml of HPLC grade water and the pH was adjusted to 6 by gradual

adding of phosphoric acid, the resulting solution was filtered with 0.45µ membrane filter. The final mobile phase was prepared by adding the ratio of (16:20:64 v/v) Acetonitrile, Methanol and phosphate buffer.

Preparation of Clobetasol propionate Stock solution
Accurately 2mg Clobetasol propionate reference standard was taken in 100 ml volumetric flasks and mixed with 100 ml of mobile phase solution. For 5 minutes the resulting solution is kept in the sonicator. The concentration of 20-100 µg/ml was achived by diluting the standard stock solution with mobile phase. Clobetasol propionate powder freely soluble in methanol.

Preparation of sample solution
1gm of marketed sample of Gamavate 0.05% w/w ointment weighed accurately and equivalent of 20 mg of Clobetasol propionate transferred into 25ml volumetric flasks and dissolved with 25 ml mobile phase and filtered through Whatman 1 filter paper. Further dilutions were made based on the required concentrations.

Solution stability
The prepared drug solution stability was analysed during the time of analysis and also repeated the same analysis method on same day with different time intervels. The same analysis is repeated after 24 hrs by keeping the drug solution under laboratory temperature (35 ± 1°C) and in refrigeration (6 ± 1°C).

Forced degradation study
Clobetasol propionate ointment was put into different stress conditions to perform degradation studies. The degradation study was conducted to examine the proposed assay technique. Clobetasol propionate is freely soluble in Methanol and also methonol is the portion of mobile phase, so methol was used as solvent. In all the experiments Clobetasol propionate ointment contents equivalent to 5mg Clobetasol propionate was weighed. Accordig to the stock solution procedure the solutions was prepared. 60 µg/ml of Clobetasol propionate is taken for every analysis. The drug solution was treated with acid, base, oxidative, dry and wet heat and direct sun light (photolytic stress). According to the proposed method the resulting drug samples were examined.

Acid degradation study
The stability of Clobetasol propionate ointment in acidic state was examined by treating with different strength of Hydrochloric acid 0.1N to 4N HCL. Solution of 60µg/ml Clobetasol propionate is taken for this study was treated with 4N hydrochloric acid in presence of methanol. The treated drug solution was kept in dark chamber at 35^{o}C for 12 hours.

Alkali degradation study
The stability of the Clobetasol propionate ointment in alkaline condition was examined by treating with different strength of sodium hydroxide 0.1N to 4N NaOH. Solution of 60 µg/ml Clobetasol propionate is taken for this study was treated with 4N sodium hydroxide in presence of methanol. The treated drug solution was kept in dark chamber at 35^{o}C for 12 hours.

Oxidation study
The stability of Clobetasol propionate ointment under oxidative condition using hydrogen peroxide was examined. Solution of 60µg/ml Clobetasol propionate is taken for this study was treated using 20 % H_2O_2 in methanol. The treated drug solution was incubated at 35ºC for 12 hours.

Wet heat study
Solution of 60µg/ml Clobetasol propionate is taken for this study treated with HPLC grade water and the resulting solution was kept in dark chamber at 35^{o}C for 12 hours.

Dry heat study
To conduct a dry heat analysis the drug solution was prepared by 2gm of Clobetasol propionate ointment approximately in a clean aluminium foil and kept in an oven at 35^{o}C for 12. The resulting Clobetasol propionate was weighed and solutions were prepared same as the preparation of stock solution procedure, 60µg/ml of Clobetasol is taken for analysis.

Photo stability study (Sun light)
2gm of Clobetasol propionate ointment on a glass dish and exposed to direct sunlight. Exposing Clobetasol propionate ointment over a period of 4 hours carried the testing. The resulting Clobetasol propionate ointment was weighed and solutions were prepared same as the preparation of stock solution procedure, 50µg/ml of Clobetasol propionate is taken for analysis.

RESULTS AND DISCUSSION

Method optimization
Chromatogram with good shape peaks and good retention time shows good resolution for Clobetasol propionate and forced degradation products. The proposed method was to identify the number of degradation products formed during the stressed conditions. The typical RP-HPLC conditions are presented in Table 1. The good separation of Clobetasol propionate and the products degraded peak under stressed conditions shows the success of the method. The HPLC chromatogram of Clobetasol propionate standard and Clobetasol propionate ointment is presented in figure 1 and 2.

Method validation
The method proceeded to achive sencitive, easy and economical for degermination and estimation by HPLC from ointment formulation. Based on the ICH recommended guidelines the experimental was validated.

Linearity
The proposed method Linearity was examined for five concentrations. The concentration ranges from 20-100µg/ml. The Clobetasol propionate standard linearity was determined by the plotting graph concentration vs absorbance. By absorbance as a functional of analyte concentration linearity was evaluated for Clobetasol propionate. The linearity graphs presented in figure 3, and data presented in Table 2. The system suitability is demonstrated by the linearity analysis.

Accuracy
The recovery experiment shows the accuracy of the method. The good recovery shows the method was accurate. The analysis for recovery was performed by known amount of Clobetasol working standard added to pre-analysed solution of formulation in the test concentration range of (40%, 60% and 100 %). For each recovery level three samples was prepared and repeated for 3 consecutive days. The statistical results for recovery study are well within the range (S.D. < 2.0). The Clobetasol drug recoveries results are presented in Table 3

Precision
The proposed method precision (repeatability) experiment results of are shown in Table 4. In the proposed method intraday and interday precision was examined by analyzing the responeses of the sample on the same day for 4 repeatations and 3 alternate days for 20-80µg/ml concentration range of Clobetasol propionate. The obtained results are represented in % RSD. The % CV of the proposed method was precise as the values < 1.0 % for the repeatability study. The precision data are presented in Table 5.

Specificity
The standard reference and the drug formulation shows specificity of the method. The RP-HPLC chromatogram of Clobetasol propionate both bulk and the ointment formaulation are presented in figure 1, 2. The bulk and ointment formaulation retention time was found to be 5.1. For the ointment formulation there was no excipient interference was detected, which shows the specificity of the method. The proposed method shows the ability to determine the analyte in presence of excipients.

Limit of detection and quantitation
The limit of detection and quantification for Clobetasol propionate is presented in table 6.

System suitability
For the system suitability parameters five repeats of standards and two repeats of sample preparation are injected, the data is presented in table 7. The Assay data of Clobetasol propionate presented in table 8.

Statistical Parameters
The obtained assay results are subjected to the coefficient of variation, statistical analysis, regression equation and standard deviation are presented in table 9.

Force degradation of Clobetasol propionate in formulation
Clobetasol propionate showed slight and moderate degradation in dry heat, oxidative and Photo stability (Sun light) condition for a short period of time. Table 10 indicates the degradation of Clobetasol propionate under different stress conditions. According to the ICH guidelines of the forced degradation study of Clobetasol propionate was examined.

Alkali degradation
The stability of the Clobetasol propionate ointment in alkaline condition was examined. There was no degraded product was separated from Clobetasol propionate. The chromatogram of alkali-degraded result was compared with the formulation and standard chromatogram. The result shows around 1–2 % of the Clobetasol propionate drug is degraded and in alkaline condition the drug was highly stable.

Acid degradation
The stability of the Clobetasol propionate ointment in acid condition was examined. The chromatogram of acidic condition product was compared with the formulation and standard chromatogram. The result shows around 1–2 % of the Clobetasol propionate drug is degraded. The result shows that drug in acidic condition was highly stable.

Oxidation
The stability of the Clobetasol propionate ointment in oxidation condition was examined. It was observed around 2-4% degradation was taken place on exposure to 20 % H_2O_2 for 12 hrs. The chromatogram of oxidation product was compared with the formulation, standard chromatogram and blank H_2O_2. No degradation product peaks was observed. The 20 % H_2O_2 peak time observed at RT 10.25, and in the peak height and area no significant decrease with time presented in figure 4. The result shows that the drug was highly stable to oxidative conditions.

Wet heat (Hydrolysis)
The stability of the Clobetasol propionate ointment in neutral condition, 1–3 % drug degradation was observed after 12 hrs of incubation at 35ºC. The chromatogram of wet heat degraded product was compared with the formulation and standard chromatogram presented in figure 5. In the wet degraded chromatogram Clobetasol propionate peak area and height was decreased and no degradation peak was observed.

Dry heat
The chromatogram for Clobetasol propionate in dry heat shows the drug is slightly unstable as compare to acid,

base and wet degradation. Around 3-7% drug degradation was observed. The chromatogram of dry heat degraded product was compared with the formulation and standard chromatogram. In the dry degraded chromatogram the drug decomposed into minor degradation product. The result shows no significant decrease in the peak height and peak area with time presented in figure 6.

Photo stability (Sun light)

The chromatogram for Clobetasol propionate under photo stablilty study was found to be unstable after drug exposure to direct sunlight for 4 hours. Almost 10-20% of the drug degraded in 4 hours. One minor drug degradation peak is observed between 12.54 minute and also there was a significant increase in the drug peak height and decrease in peak area was observed presented in figure 7.

Table: 1. HPLC conditions for estimation of Clobetasol propionate

Parameters	Description
Column	Agilent Technology column C_{18} (150mm x 4.6mm, 5µm)
Column temperature	29 ± 1°C
Mobile phase	Acetonitrile:Methanol:Phosphate buffer (16:20:80 v/v)
Detection	Photodiode array detection at 250 nm
Injection volume	20 µl
Flow rate	1.5 ml min^{-1}

Table 2. HPLC linearity data for Clobetasol propionate

Concentration (µg/ml)	Peak area
60	1723.47
80	2317.15
100	2949.79

Table 3: Recovery studies of Clobetasol propionate ointment formulation

S No	Drug	Amount of Drug present in preanalyzed Sample (µg/ml)	Amount of Standard drug (RS) added (µg/ml)	Amount of drug recovered (µg/ml)	% Recovery	Mean recovery in Percentage
1.	Clobetasol propionate	50	40.00 60.00 100.00	90.73 110.76 150.62	101.21 100.95 100.62	100.72

Table: 4. Method precision data of Clobetasol propionate by RP-HPLC method

Clobetasol propionate 50 µg/ml (n=4)	Retention time	Area
1	5.021	2174.67
2	5.191	2143.27
3	5.023	2274.49
4	5.141	2153.22
Mean	5.09	2118.21
S.D [a]	0.0221	113.441
% CV[b]	0.443	0.473

4 observations

Table: 5 Intermediate precision data of Clobetasol propionate by RP-HPLC method

Clobetasol propionate µg/ml	Inter-day measured mean area ± S.D.[a]	%CV[b] (n^c=4)	Intra-day measured mean area ± S.D.[a]	%CV[b] (n^c=4)
50	2157.85± 4.45	0.9942	2297.54± 4.75	1.075
100	4499.75±3.05	0.9864	4679.75±2.05	0.997
150	6173.22±5.56	1.0432	6376.22±4.16	0.976

n^c = 4 observations

Table: 6 Results of Limit of detection & limit of quantification

Parameters	Clobetasol propionate
LOD (µg/ml)	0.50
LOQ (µg/ml)	0.60

Table: 7 Results of system suitability parameters

SNo	Parameters	Clobetasol propionate
1.	Theoretical plates	3776
2.	Tailing factor	0.997
3.	Resolution factor	2.14
4.	Retention time	5.1± 0.2
5.	Calibration range or Linear dynamic range	20-100 µg/ml

Table: 8 Quantitative estimation (Assay) data of Clobetasol propionate

S No	Drug	Label claim (µg/Oin)	Amount found (µg/Oin)	Mean amount found (mg/ Oin)	Percentage purity (% w/w)	Mean percentage purity (% w/w)	% Deviation
1.	Clobetasol propionate	50	50.37 50.50 50.31 50.69 50.34	50.44	102.27 102.10 100.07 99.95 101.20	101.11	+ 0.6 +1.1 +0.5 -1.0 +0.4

Table 9: Results of statistical parameters Statistical parameters

SNo	Parameters	Clobetasol propionate
1.	Standard deviation (SD)	3.03
2.	Relative standard deviation (RSD)	0.0776
3.	% RSD	0.616
4.	Standard error (SE)	0.02177
5.	Correlation Coefficient (r)	0.9989
6.	Slope (a)	29.099
7.	Intercept (b)	16.167
8.	Regression equationY = (aX+b)	Y = 29.099 X + 16.167

n= 4 observations.

Table: 10. Summary of Force degradation of Clobetasol propionate by RP-HPLC method

SNo	Stress condition/ state	Time	% Assay ± S.D. ^{a}(n^{b}=5)
1	Acidic 4N HCL(35 ^{o}C)/ solution	12 hrs	98.272 ± 1.632
2	Alkali 4N NaOH (35 ^{o}C)/ solution	12 hrs	99.123± 0.7143
3	Wet heat (35 ^{o}C)/ solution	12 hrs	98.377±1.323
4	Dry heat (35 ^{o}C)/ solid	12 hrs	95.771±1.243
5	Oxidative 20 % H_2O_2 (35 ^{o}C)/ solution	12 hrs	98.167±1.132
6	Photo stability/ solid	4 hrs	83.764±1.343

S.D.^{a} is standard deviation for n^{b} = 5 observations.

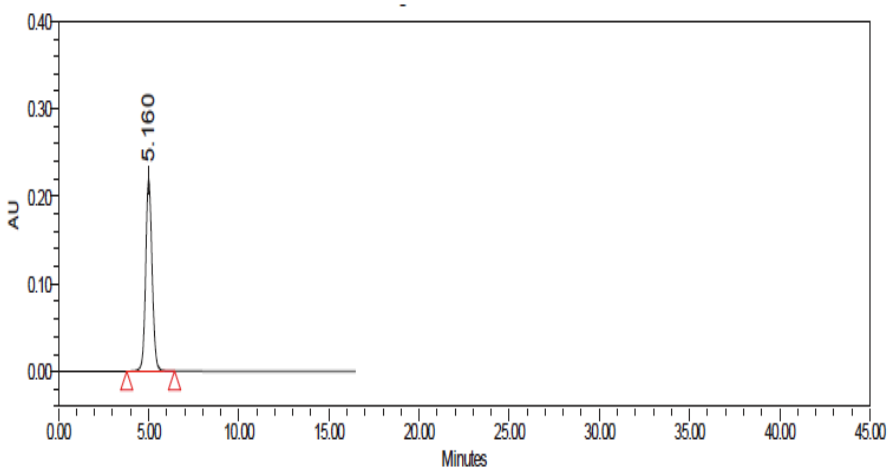

Fig.1: A Typical Chromatogram of Clobetasol propionate Standard

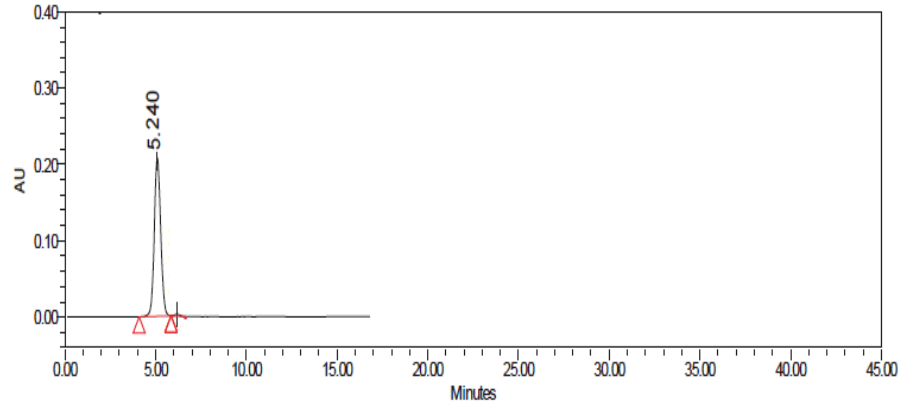

Fig. 2: A Typical Chromatogram of Clobetasol propionate ointment

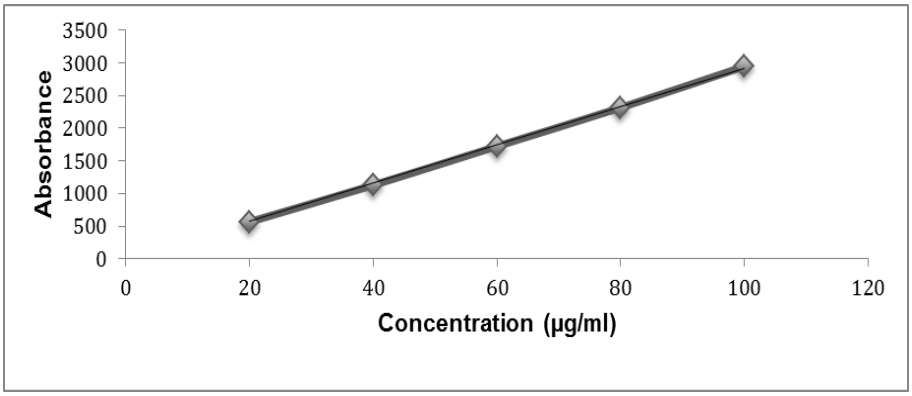

Fig. 3: Calibration graph of Clobetasol propionate 20-100µg/ml precision

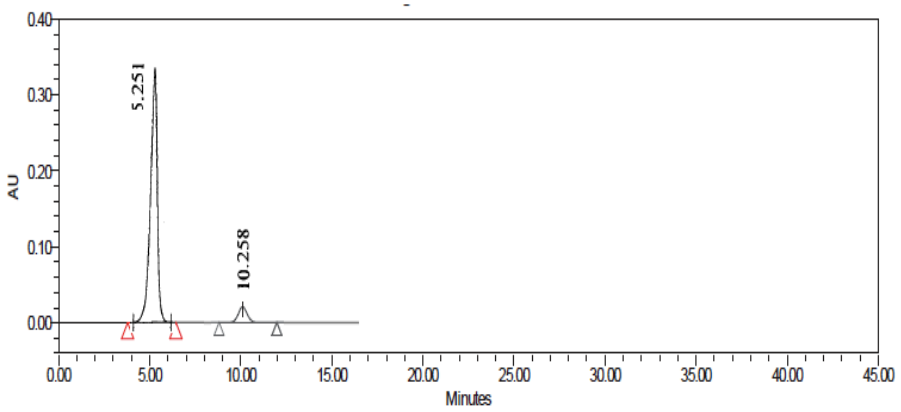

Fig. 4: Chromatogram of Clobetasol propionate under oxidation condition by RP-HPLC method

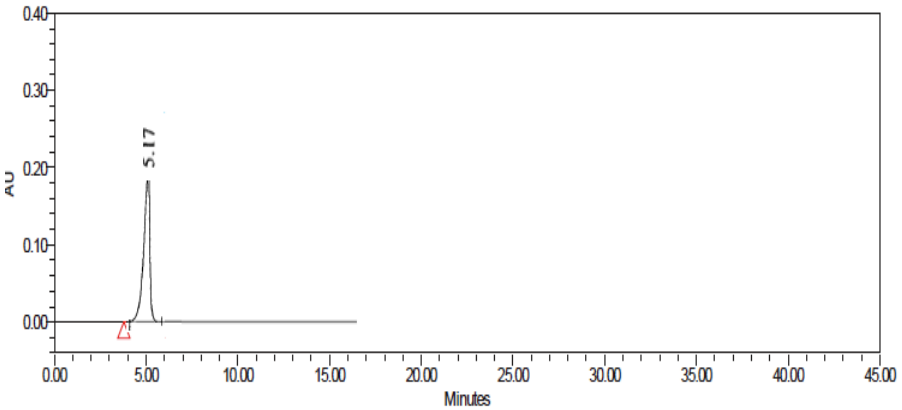

Fig.5: Chromatogram of Clobetasol propionate under wet heat condition by RP-HPLC method

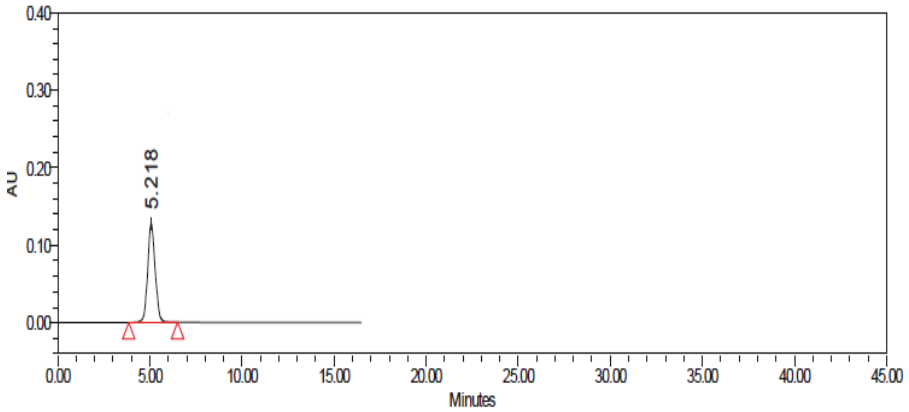

Fig.6: Chromatogram of Clobetasol propionate under dry heat condition by RP-HPLC method

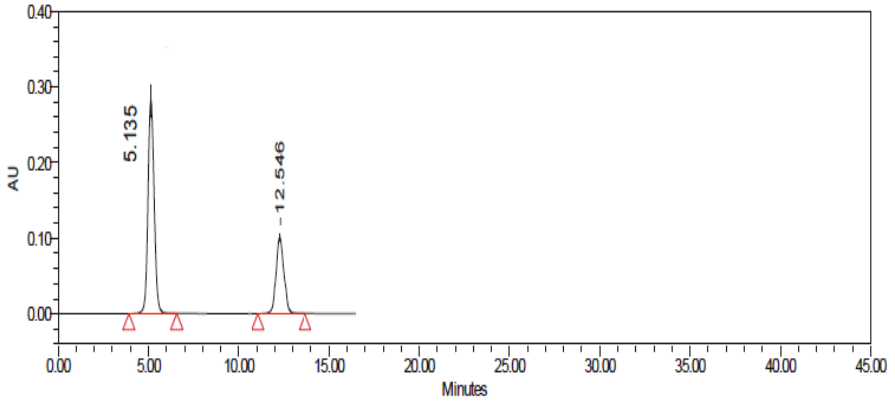

Fig.7: Chromatogram of Clobetasol propionate under Photo stability condition by RP-HPLC method

CONCLUSION

The force degradation study was performed according to the guidelines of International Conference on Harmonization (ICH), The developed RP-HPLC method shows the accuracey, sensitive and stability indicating. The developed method is rapid, reproducible. The developed method can be used for the routine analysis for Clobetasol propionate formulations.

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REFERENCES

1. British Pharmacopoeia, International edition, Vol. I & II, HMSO, London, 2009; 1271: 1445.
2. Elise A, Olsen MD, Roger C, Cornell MD. Topical clobetasol-17-propionate: Review of its clinical efficacy and safety, J. American Academy of Dermato, 1986; 15(2): 246-255.
3. Stanislaw D, Edmund G, Edyta S, Agnieszka M. Pharmaceutical availability of clobetasol- 17-propionate from cream and ointment. Acta Poloniae Pharmaceutica – Drug Research, 2001; 58(6): 435-438.
4. Carrascosa JM, Vanaclocha F. Update of the topical treatment of psoriasis, Actas Dermosifiliogr, 2009; 100: 190-200.
5. Tsai JC. Content and transdermal delivery of clobetasol 17-propionate from commercial cream and ointment. J of Food and Drug Analysis, 2002; 10: 7-12.
6. Konstantianos DG, Ioannou PC. Simultaneous determination of diflunisal and salicylic acid in human serum by second-derivative synchronous fluorescence spectroscopy. Eur. J. Pharma. Sci., 1994; 1(4): 209–217.
7. Gagliardi L, Orsi D, Giudice MR, Gatta F, Porra R, Chimenti P, Tonelli D. Development of a tandem thin-layer chromatography-highperformance liquid chromatography method for the identification and determination of corticosteroids in cosmetic products. Anal. Chem. Acta, 2002; 22: 187-198.
8. Yang Q, Wang Z, Xiao Y. Determination of clobetasol propionate by the method of linear sweep adsorptive stripping voltammetry. Chem. Resea, 1997; 2: 21-27.
9. Gagliardi L, Orsi DD, Manna F, Tonelli D. HPLC determination of clobetasol propionate in cosmetic products. J of Liquid Chromatography & Related Products, 2000; 23: 355-362.
10. Marika K, Katherine F, Jianmin L, Mike CG, Debra F. A sensitive high-throughput HPLC assay for simultaneous determination of Everolimus and Clobetasol propionate. J of Chromatographic Sciences, 2008; 46: 23-29.

11. Fontana MC, Bastos MO, Beck RCR. Development and validation of a fast RP-HPLC method for the determination of clobetasol propionate topical nanocapsule suspension. J of Chromatographic Sciences, 2010; 48: 637-640.