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A Stability Indicated Method for the Estimation of Clobetasol Propionate 0.05% Ointment and It's Related Impurities by RP-HPLC Method

D. China Babu[1], SK. Arshia tabassum*[1], MD. Ishaq[2], L. Siva Shankar Reddy[3], C. Madhusudhana Chetty[4]

Santhiram College of Pharmacy, Nandyal, Kurnool District, A.P. India.

Email Ids: Arshiatabassum27@gmail.com, shiva_s_rl@yahoo.co.in,drcmchetty@gmail.com
Mobile No: +918886967128.

Abstract : A sensitive, selective and efficient extraction method was developed for the estimation of Clobetasol and its impurities in 0.05% ointment by using RP-HPLC method. The impurities and Clobetasol was separated by using column Inert sustain C18 AQ column. The mobile phase was used mixture of water, acetonitrile and methanol in the ratio of 50:40:10 V/V (Solution-A) and acetonitrile (Solution-B), mixed the solution of A and B in the ratio of 96:04 V/V. The flow rate was maintained at 0.8 mL/min, column temperature was maintained at 45°C and the sample was scanned at 250 nm. The diluent was used as water and methanol in the ratio of 50: 50 V/V. The sensitivity of the method was proved with the limit of detection and limit of quantification values were 0.03 & 0.06 µg/mL for Clobetasol. The cumulative %RSD values for intraday and intermediate precision 1.9-4.1%. The accuracy of the method was studiedand obtained values for impurities were with in limit as per ICH guidelines.The correlation coefficient values of Clobetasol and its impurities in linearity study were obtained>0.999.The method was proved as robust after deliberate changing of parameters. The method was shown ability to different stress conditions and this is the one of the best method for the estimation ofsix related impurities of Clobetasol by using RP-HPLC.
Keywords : Clobetasol Propionate, RP-HPLC, Acetonitrile, Methanol.

1. Introduction

The Clobetasol propionate was used as a topical formulation for the treatment of psoriasis and common name is 21-chloro-9-fluoro-11-beta,17-dihydroxy-16-beta-methylpregna-1,4-diene-3,20-dione17-propionate [1-5].The multi involving dysergulated inflammation and genetic association occurs with psoriasis [6,7]. The koebner phenomenon was used for characterization of active inflammatory psoriasis and identified asnew lesions arises at the site of trauma or pressure [8]. The commercial available Clobetasol propionate in the form of cream, ointment, scalp solution, foam, gel and emollient, that contains 0.05% W/W of the steroid[9-12].

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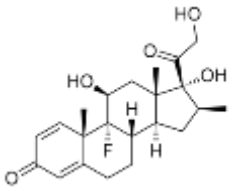
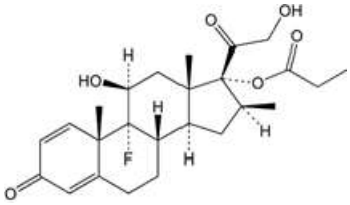
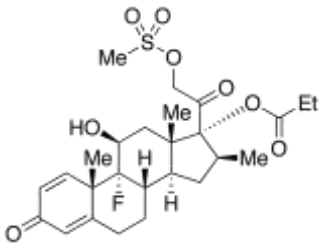
The Clobetasol propionate is a dihalogenated analog of prednisolone, it is more potent than hydrocortisone and more susceptible to acid and alcohol[13-17]. The Clobetasol propionate is official in USP, EP & Indian Pharmacopoeia and it contains 13 related substances[18-20]. To ensure the safety and efficacy of the drug must estimate the impurities or other substances in the product. Different methods were used for the study of the impurity profiling (Figure 1) in the product of Clobetasol. The method was validated as per ICH Q2 (R1) guidelines[21-28].



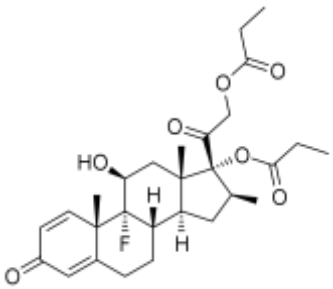
Betamethasone-17- Propionate (Impurity-A)



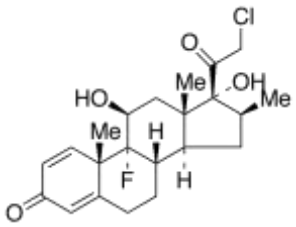
Betamethasone Propionate (Impurity-B)



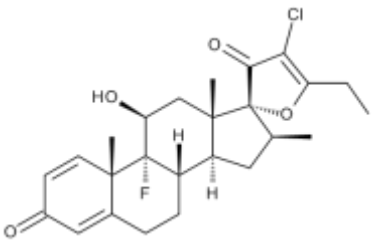
Betamethasone-17-Propionate-21-Mesylate (Impurity-I)



Betamethasone- 21- Propionate (Impurity-K)



ClobetasolPropionate (Impurity G)



Clobetasol propionate related compounds (Impurity J)

Figure: 1 Structures of Clobetasol and its impurities

2. Materials and Methods

Chemicals and Reagents

The methanol, acetonitrile were HPLC grade purchased from Merck, Mumbai, India. The hydrochloric acid, sodium hydroxide, hydrogen peroxide and n-hexane were of AR grade purchased form ChemFine Chemical Co.,Ltd. The Milli Q water was used for the preparation of mobile phase and diluent. Clobetasol propionate and its impurities (A, B, I, K, G & J) were procured from Dr. Reddys Laboratory, Hyderabad, India.

Instrumentation

The method development, validation and stability studies were performed with column Inert sustain C18 AQ (250 mm X 4.6 mm X5 µm) HPLC model was (Waters corporation 2695, USA) and equipped with

PDA detector (model AQC-PDA). The sonicator was Remi (model-RLR 400), Oven (Cintex model-CIC 63), Micro balance (Radwag model-MYA 2) and photo stability chamber was Newtronics (Model- NEC08RSDS).

Chromatographic Conditions

The sample temperature was maintained at 25℃ and column oven temperature maintained at 45℃ and column was used Inert sustain C18 AQ (250 mm X 4.6 mm X5 µm). The flow rate was maintained at 0.8 mL/min and detection wavelength was kept at 250 nm. The injection volume was 20 µL and run time 65 min for standard drug and 75 min for blank. The elution mode was isocratic and diluent used as equal proportions of methanol and water. The mobile phase consisted mixture of solution A (Methanol: Water: Acetonitrile) and solution B (Acetonitrile).

Standard Solution

Accurately weighed and transferred about 25 mg of Clobetasol propionate in to 200 mL volumetric flask, added few quantities of diluent and sonicated the solution for 5 min. The volume was made up to mark withdiluent (125µg/mL). Pipetted out 2mL of the above solution and transferred into 50 mL volumetric flask, the volume was made up to mark with diluent. The final concentration of the solution was attained 5µg/mL.

Sample Solution

Accurately weighed 4g of Clobetasol propionate and transferred into a 250 mL volumetric flask. To this added 35 mL of n-hexane and vortexed until a homogeneous dispersion was obtained. To this, added 20 mL of diluent and vortexed again for 5 minute with an intermittent hand shaking after every 1 minute. The total sample solution was transferred into separating funnel and allowed the solution for layer solution. The solution was centrifuged at 5,000 rpm for 5 minutes. The clear solution was collected (Expected some turbidity on the surface of the solution due to traces of carryover of n-hexane in the diluent) cautiously using needle of syringe and transferred into sample vial. The clear solution was injected into the chromatographic system of HPLC and chromatograms were recorded.

3. Results & Discussion

Method Development

The method was developed with different compositions of mobile phase, columns and diluents. The optimised method was obtained with mobile phase composition of Water: Methanol: Acetonitrile (Solution-A) in the ratio of (50:40:10) and Acetonitrile (Solution-B). The final composition of mobile phase was attained solution-A and solution-B in the ratio of 96:04% V/V. For the dilution of the sample used equal proportions of Methanol: Water. The good resolution of the drug and its impurities achieved with Inert sustain C18 AQ column, the column temperature was kept at 45℃ and injection volume was 20 µL. The drug and its impurities shown more response at 250 nm and it was selected as a wavelength and detected the samples with PDA detector.

Method Validation[29]

Method was validated according to ICH guidelines. Different validation parameters were studied selectivity & specificity, linearity, precision, accuracy, robustness and stability studies were performed. The results were achieved within limits as per ICH norms.

System Suitability

The system suitability of the method was studied for Clobetasol and its impurities with the above optimised HPLC conditions. The critical parameters were studied such as tailing factor, theoretical plate count and resolution efficiency. The tailing factor is less than 1.2 for all the respective peaks of interest and resolution between any closely paired components were eluted more than 2.0%.

Selectivity & Specificity

The selectivity & specificity was proved by interference of drug, its impurities,placebo and degradation impurities. The peak similarity was studied for all the anticipated peaks using PDA detector. The blank and impurities were prepared into a solution and injected into chromatographic system. The interference due to blank or placebo with the retention times of Clobetasol and its impurities were verified. There was no interference was observed between all the peaks of impurities and Clobetasol propionate. Hence the method was proved as specific and chromatograms were shown in figure no 2& 3.

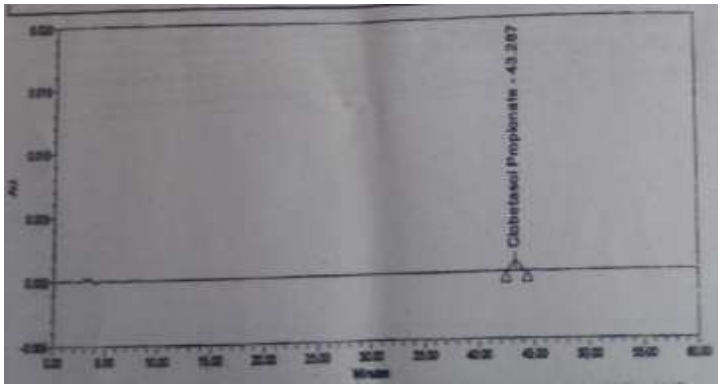


Figure: 2 Standard chromatogram of Clobetasol Propionate

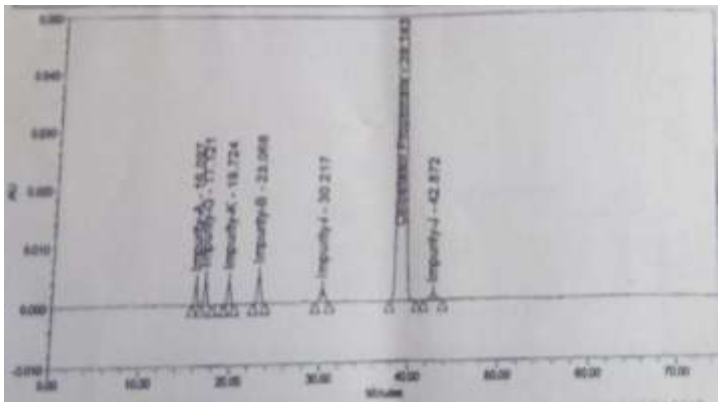


Figure: 3 Chromatogram of Clobetasol Propionate & its impurities

Linearity

The linearity was determined for impurities and Clobetasol propionate, a series of solutions were prepared from concentration ranging 0.2-0.96 µg/mL for Clobetasol propionate, 0.4-1.92 µg/mL for impurity-A, 0.4-1.98 µg/mL for impurity-G, 0.4-1.96 µg/mL for impurity-K, 0.4-1.84 µg/mL for impurity-B, 0.4-1.96 µg/mL for impurity-I and 1.1-4.69 µg/mL for impurity-J and correlation coefficient values were obtained above 0.999 for all impurities by plotted concentration of impurities on X-axis, peak area on Y-axis. The limit of quantification (LOQ) values were obtained 0.075 µg/mL for Clobetasol propionate 0.06 µg/mL for impurity-A, 0.061 µg/mL for impurity-G & K, 0.057 µg/mL for impurity-B, 0.076 µg/mL for impurity-I and 0.14 µg/mL for impurity-J.

Precision

Precision of the method was established by system precision and method precision. System precision was determined by injecting the six replicated injections of standard solution in to HPLC system and recorded the chromatograms. The %RSD value was obtained 0.3. Method precision for related substance was verified by preparing six replicates of Clobetasol propionate by spiking the impurities at specified level. As per the method

the samples were analysed and calculated content of percentage impurity. The %RSD was calculated from the attained results of six replicated injection and values were in between 2.6-3.3 for all the impurities.

Accuracy

The accuracy of the method was determination of related substances by recovery method. The recovery study was carried out by spiking the known amounts of impurities in the placebo matrix at three different levels of concentration, such as 50%, 100%, 150% and 200% specification. The recovery study (Table 1) of each impurity was calculated by using relative response factor of impurity and diluted standard area ofClobetasol propionate. From the established data, it is evident that the accuracy values were found in between 97.2%-109.2%. The accuracy results were proved the accurately recovery of the all desired impurities of Clobetasol propionate.

Table:1 Results of accuracy study of drug and its Impurities

Betamethasone 17 Propionate (impurity A) Accuracy results				
Sample Name	Avg. measured conc. In %	Theoretical conc. In %	Average % Recovery	% RSD
50% Preparation	0.51	0.48	108.1	1.7
100% preparation	1.02	0.96	107.2	1.2
150% preparation	1.50	1.44	104.8	0.8
200% preparation	1.97	1.92	103.0	2.0
Clobetasol (Impurity G) Accuracy results				
50% Preparation	0.53	0.49	107.2	1.1
100% preparation	1.06	0.99	107.6	1.5
150% preparation	1.55	1.48	105.1	0.6
200% preparation	2.08	1.98	105.4	0.9
Betamethasone 21 Propionate (Impurity K) Accuracy results				
50% Preparation	0.52	0.49	107.7	1.2
100% preparation	1.06	0.98	108.1	1.8
150% preparation	1.56	1.47	106.0	0.3
200% preparation	2.12	1.96	108.0	1.1
Betamethasone 16,21- Chloro (Impurity K) Accuracy results				
50% Preparation	0.50	0.46	109.2	1.2
100% preparation	1.00	0.92	109.0	1.5
150% preparation	1.48	1.38	107.2	0.6
200% preparation	1.98	1.84	107.8	0.8
Betamethasone 21 Mesylate 17 Propionate (Impurity I) Accuracy results				
50% Preparation	0.52	0.49	107.7	1.8
100% preparation	1.06	0.98	108.5	1.1
150% preparation	1.55	1.47	105.5	0.5
200% preparation	2.07	1.96	105.9	0.8

Limit of detection and Limit of quantification

LOD and LOQ recognised for the Clobetasol propionate and its related substances by diluting the standard solution of each related substance and Clobetasol propionate. It was calculated by signal to noise ratio, LOD values for Clobetasol propionate and its impurities were found to be in between 0.03-0.04 µg/mL and LOQ values were 0.06- 0.14 µg/mL for Clobetasol propionate and its impurities. The %RSD of Clobetasol

propionate acknowledged impurities from six replicate preparations have to be much less than 15 for LOQ stage and 10 for 200% level. At LOQ level the precision and accuracy was established for all the impurities, the % RSD of precision was found to be less than 6.3 and average recoveries at LOQ level were found to be in the range of 101.1%-107.9%.

Robustness

Robustness of the method was performed for confirm the suitability of the method after deliberate changes in method parameters, such as flow rate, column temperature and organic phase. The flow rate was changed to 0.6 mL/min (at low)-1.0 mL/min (at high), temperature changed (±5°C) 40°C-50°C, organic phase was changed to (±04 mL) 96.4:3.6% V/V-95.6:4.4% V/V. The results of robustness revealed the method was robust and it is useful for the formulation development of Clobetasol propionate and its related products and also product development and quality control in laboratories for regular analysis of Clobetasol propionate.

Stability studies

The spiked sample of formulation was used for the stability study. The study was conducted up to 2 days by storing the sample on bench top. The data was compared on day-0 and on day-2.

Degradation interference :

The degradation studies (Table 2) were organized with different stress conditions of acid, base, peroxide, thermal, photolytic and hydrolytic, placebo solutions, clean solutions are injected into the HPLC system (Figure 4). No interference was once located from degradation products. Purity perspective is much less than purity threshold for CLOB top and there are no purity flags. This suggests that the approach is unique for analysis.

Table: 2 Results of degradation product interference

Degradation Products Interference				
Sample Name	**Percentage Interference**			
	% Assay	Total Impurities	Assay + total impurities	Mass balance (% interference)
As in sample	94.3	0.40	94.70	NA
Acid/ 0.1N HCl/1ml/ Bench top/ 30 min	90.3	1.39	91.69	3.0
Base/0.1N NaOH/2ml/Bench top/ 15 min	74.7	16.34	91.04	3.7
As in sample	94.6	0.50	95.10	NA
UV/0.5 watts hour/ Sq. meter	90.6	0.49	91.09	4.0
Photolytic/3k lux hour	91.8	0.53	92.33	2.8
Thermal/70^0c/day 7	93.2	2.25	95.45	2.8
As in sample	93.1	0.40	93.50	NA
1% H_2O_2/1ml/bench top/30 min	91.9	0.50	92.40	1.1q
Water/1ml+methano/1ml/bench top/30 min	93.3	0.44	93.74	-0.2

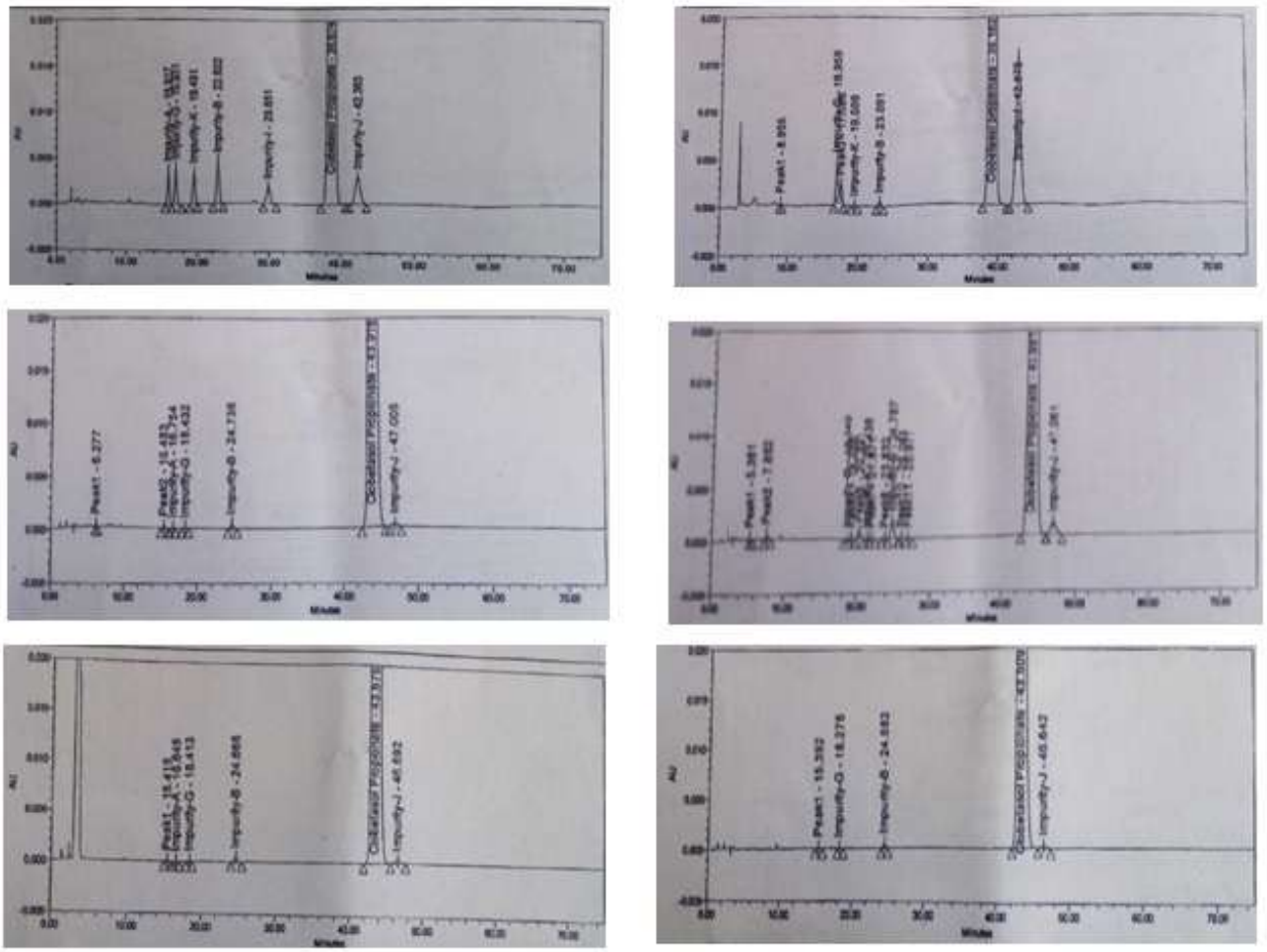


Figure: 4 Degradation chromatograms of acid, base, peroxide, thermal, photolytic and hydrolytic

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