RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF CLOBETASOL PROPIONATE IN BULK AND GEL FORMULATION

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Abstract :

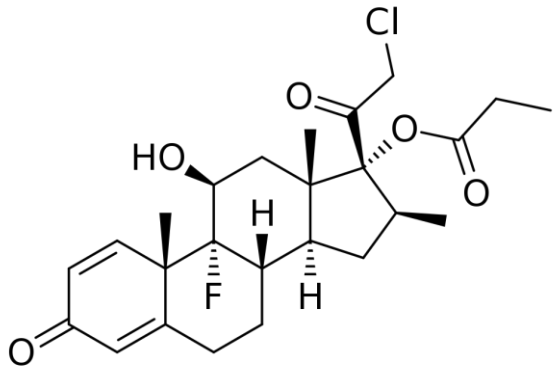
Reverse phase high performance liquid chromatographic was developed for the estimation of Clobetasol Propionate in bulk & Formulation and to validate the developed methods according to ICH Q2 (R1) guidelines. Analytical method development was started with preliminary studies of the Clobetasol Propionate according to USP 2010. Being freely soluble in Acetonitrile, stock solutions of the drugs were prepared in acetonitrile (100%). The RP-HPLC method for estimation of Clobetasol Propionate dosage form was developed. The quantification was carried out by using Phenomenex Luna C18 column (150 mm × 4.6 mm, 5 µm) as stationary phase and Acetonitrile: Water (51:49) as mobile phase. Mobile phase was maintained at a flow rate of 0.8 ml/min. The UV detector was operated at 242 nm at 30ºC and Clobetasol propionate eluted at RT 15.7 min.

Keywords-Clobetasol propionate,hplc,validation

Introduction

Clobetasol propionate

Clobetasol propionate is used to treat moderate to severe plaque psoriasis . as well as inflammatory and pruritic manifestations of corticosteroid responsive dermatoses. Clobetasol propionate is the 17-O-propionate ester of clobetasol. Clobetasol propionate is potent corticosteroid , it is used to treat various skin disorders, including exzema and psoriasis. It has a role as an anti-inflammatory drug. Clobetasol propionate is chemically (8S,9R,10S,11S,13S,14S,16S,17R)-17-(2-chloroacetyl)-9-fluoro-11-hydroxy-10,13,16-trimethyl3-oxo-6,7,8,11,12,14,15,16 octahydrocyclopenta [a]phenanthren -17-yl] propanoate clobetasol



BRAND NAMES- Clobex, Clodan, Cormax, Clobevate, Embeline, Impoyz, Olux, Olux-E, Temovate, Temovate E, Embeline E

This method can be successfully used for analysis of clobetasol propionate as it is rapid,simple ,selective and sensitive method for determination using HPLC technique.

MATERIALS AND METHODS

Materials

Pure standards of Clobetasol Propionate was obtained as a gift sample from Aadhaar Life Sciences Pvt. Ltd , and Clobetasol Propionate Gel 0.05% were purchased from market.

Instrument

HPLC-Agilent 1260 Infinity II, Software-Openlab Ezchrom , Column-Phenomenex, USA , Nylon membrane 0.45µm 15mm Syringe Filters-Quallisil , Melting Point Apparatus , UV-VIS Double Beam Spectrophotometer 1900-Labman Scientific Instruments , Infrared Spectrophotometer-Bruker

Method

Preparation of required solutions for estimation of clobetasol propionate by RP HPLC

I. Preparation of Standard Stock Solution
   a. Preparation of Clobetasol Propionate Standard Stock Solution (SSS-1)
   Accurately weigh 5 mg of Clobetasol Propionate in 10 mi volumetric flask and make up volume with diluent and lable it as standard stock solution (SSS) Conc of clobetasol propionate -500 µg/ml

II. Preparation of Working Standard
Pipette out 1ml of SSS into 10ml volumetric flask. Add diluent and then make up the volume with diluent. (Conc. of Clobetasol Propionate – 50 µg/ml). Label this solution as 'Working Standard'.

III. Preparation of Placebo Sample
Accurately weigh 1gm Placebo into 10 mL volumetric flask. Add diluent and make up the volume and mix. Vortex the solution

IV. Preparation of Drug Product Sample
Accurately weigh 1 gm Clobetasol Propionate Gel 0.05%, into 10 mL volumetric flask. Add diluent and make up the volume and mix. Label as 'Sample Solution' (Conc. Clobetasol Propionate - 50µg/ml)

V. Selection of Analytical Wavelength
CP, the solution in the diluent was scanned in the range of 200-400nm.

VI. Selection of Mobile Phase and its Strength
Solution of CP (CP-50 µg/mL) was prepared in diluent and filtered through 0.45 um Nylon syringe filter, then injected into the HPLC system, after the column saturated with mobile phase and constant back pressure. The solution was analyzed using different combinations of Acetonitrile: Water, at flow rate of 0.8mL/min for 40 min

VII. Selection of column (stationary phase)
To get well resolved, symmetric peak with highest no. of theoretical plates the solution of the CP was analyzed using C18 column as a stationary phase.

VIII. Preparation of Mobile Phase
Mix separately measured volumes: 490 mL of Water and 510 mL of Acetonitrile. Filter using 0.45µm nylon filter, for two times and sonicate for 15 mins and Degas

Validation of RP-HPLC Method

I. Specificity & Assay :-
The chromatogram of CP Gel 0.05% was compared with chromatogram of Blank & Placebo. Prepare Assay sample as per preparation for "Drug Product Sample".

II. Linearity
- Linearity Stock Solution (LSS): Accurately weigh about 25 mg of Clobetasol Propionate API into a 25 mL volumetric flask. Fill 2/3 full with diluent vortex with cap on and sonicate to dissolve. Allow the solution to equilibrate to room temperature then fill to volume with diluent and mix. (Approx. Conc. = 1000µg/mL).
- Linearity Stock Solution - 1 (LSS-1) : Pipette 5.0 mL of Linearity Stock Solution (LSS) into a 10 mL volumetric flask. Dilute to volume with diluent and mix. (Approx. Conc. = 500µg/mL).
- Linearity Stock Solution - 2 (LSS-2) : Pipette 0.05 mL of Linearity Stock Solution-1 (LSS-1) into a 10 mL volumetric flask. Dilute to volume with diluent and mix. (Approx. Conc. = 2.5µg/mL).
- Linearity Solutions : Transfer X.0 mL of the LSS-1 or LSS-2, into a 10mL volumetric flask as shown in Table given below. Fill to volume with diluent and mix.

Table no.1: Linearity

% Level wrt sample	Linearity Solution Name	Pipette (mL)	Stock Solution Name	Volumetric Flask (mL)	Final Concentration of Clobetasol Propionate (µg/mL)
125	Linearity – 1	1.25	LSS-1	10	62.5
100	Linearity - 2	1.0	LSS-1	10	50
75	Linearity – 3	0.75	LSS-1	10	37.5
50	Linearity – 4	0.5	LSS-1	10	25
25	Linearity – 5	0.25	LSS-1	10	12.5
12.5	Linearity – 6	0.125	LSS-1	10	6.25
6	Linearity – 7	1.2	LSS-2	10	3
3	Linearity – 8	0.6	LSS-2	10	1.5
1.5	Linearity – 9	0.3	LSS-2	10	0.75
0.75	Linearity – 10	0.15	LSS-2	10	0.375
0.4	Linearity – 11	0.08	LSS-2	10	0.2
0.2	Linearity – 12	0.04	LSS-2	10	0.1

III. Range

The range of analytical method was decided from the interval between upper and lower level of calibration curves by plotting the curve. The correlation coefficient (r2) of least square linear regression for CP was calculated

IV. Method Precision:

1gm of Clobetasol Propionate Gel 0.05% was weighed and transferred in 10mL volumetric flasks. made up volume to the mark with diluent. The solution was filtered through syringe filter and injected into the HPLC system and its chromatogram was recorded under the same chromatographic conditions after getting a stable baseline. Peak area was recorded. The procedure was repeated for five times.

V. Limit of Detection

LOD calculated by the following formulae.

LOD = 3.3(SD/S) - Where, SD- Standard deviation; S- Slope of Curve

VI. Limit of Quantitation

LOQ calculated by the following formulae.

LOQ = 10(SD/S)

VII. System Suitability-

Sample solutions of CP (CP-50 µg/mL) were prepared and analyzed five times. Chromatograms were studied for different parameters such as tailing factor, resolution and theoretical plates to see that whether they comply with the recommended limit or not.

VIII. Accuracy:

- Placebo-Prepare placebo according to "Preparation of Drug Product Sample" by using placebo instead of Finished Product.
- Stock Standard Solution-1 (SSS-1) -Accurately weigh about 25 mg of Clobetasol Propionate API on weighing paper and quantitatively transfer into a 25 mL volumetric flask. Fill 2/3 full with diluent, vortex with cap and sonicate to dissolve. Allow the solution to equilibrate to room temperature and then fill to volume with diluent. Mix well. (Approx. Conc. = 1000 µg/mL).
- Spiked Samples: Prepare in triplicate for each level. Weigh accurately about 1 grams of Placebo directly into a 10 mL volumetric flask. Transfer X.0 mL SSSS-1 into the same flask as shown in table below. Follow the sample preparation as per the Gel finished product method starting from: "Add diluent and mix…"

Table No 2 : Accuracy solution preparations (Assay)

% Accuracy Level wrt sample	Volume of SSSS-1 (mL)	Volumetric flask	Clobetasol Propionate Concentration (µg/mL)
80	0.4	10	40
100	0.5	10	50
120	0.6	10	60

Working Standard for Accuracy:

Transfer 0.25 mL of SSSS-1 into 10 mL volumetric flask. Dilute to volume with diluent.

RESULTS AND DISCUSSION:

Chromatographic Conditions

- ✓ **Analytical Column:** Phenomenex Luna C_{18} column (150 mm × 4.6 mm, 5 µm)
- ✓ **Mobile Phase:** Acetonitrile: Water (51:49)
- ✓ **Flow Rate:** 0.8 ml/min
- ✓ **Injection Volume:** 20 µl
- ✓ **Detection Wavelength:** 242 nm.
- ✓ **Runtime:** 40 min

Selection of wavelength and mobile phase

Clobetasol Propionate shows the maximum absorbance at 242 nm. Hence, the lambda max 242 nm for analysis was chosen.
(C18 Column, at 0.8ml/min flow rate, detection wavelength is 242 nm, mobile phase ratio containing 49:51 Water: Acetonitrile respectively)

Identification of Peaks

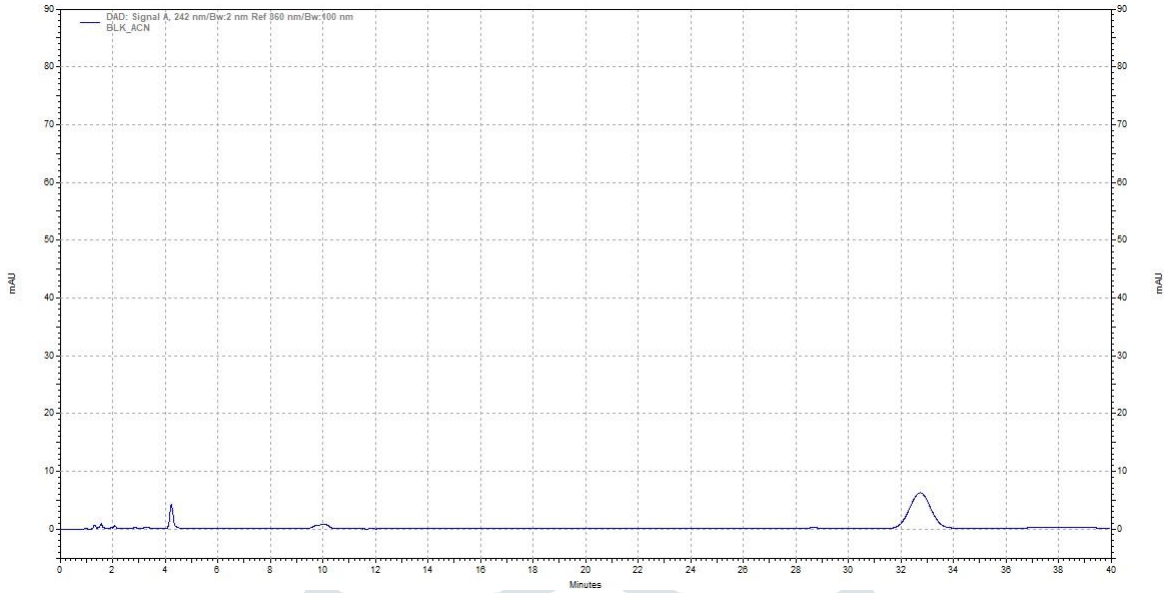


Fig no 1 : Chromatogram of Blank in optimized chromatographic conditions

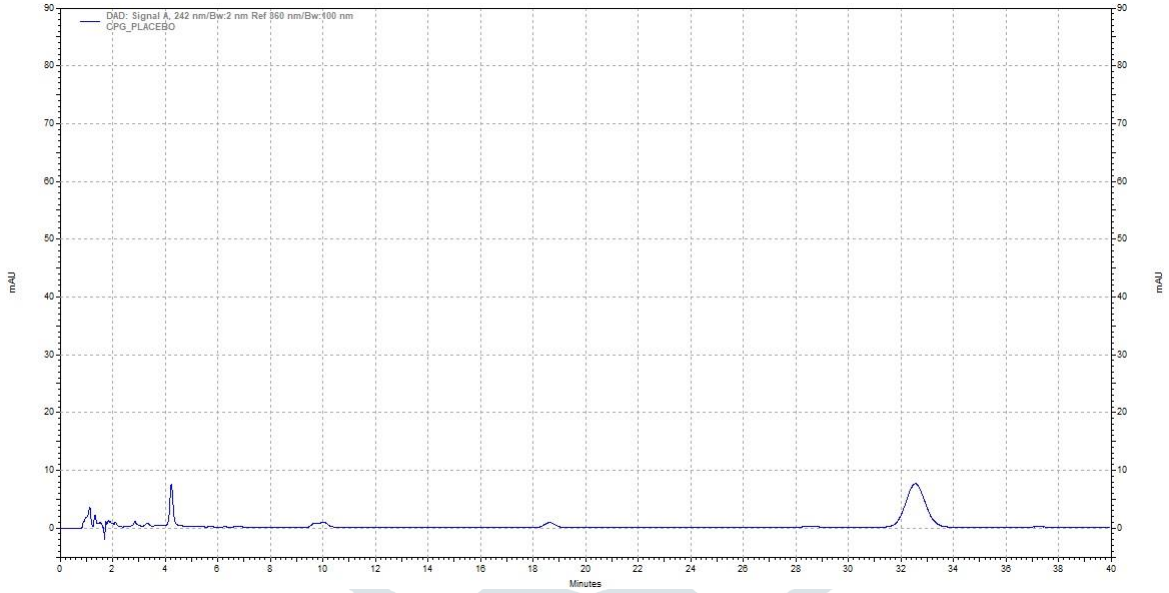


Fig no 2 : Chromatogram of Placebo in optimized chromatographic conditions

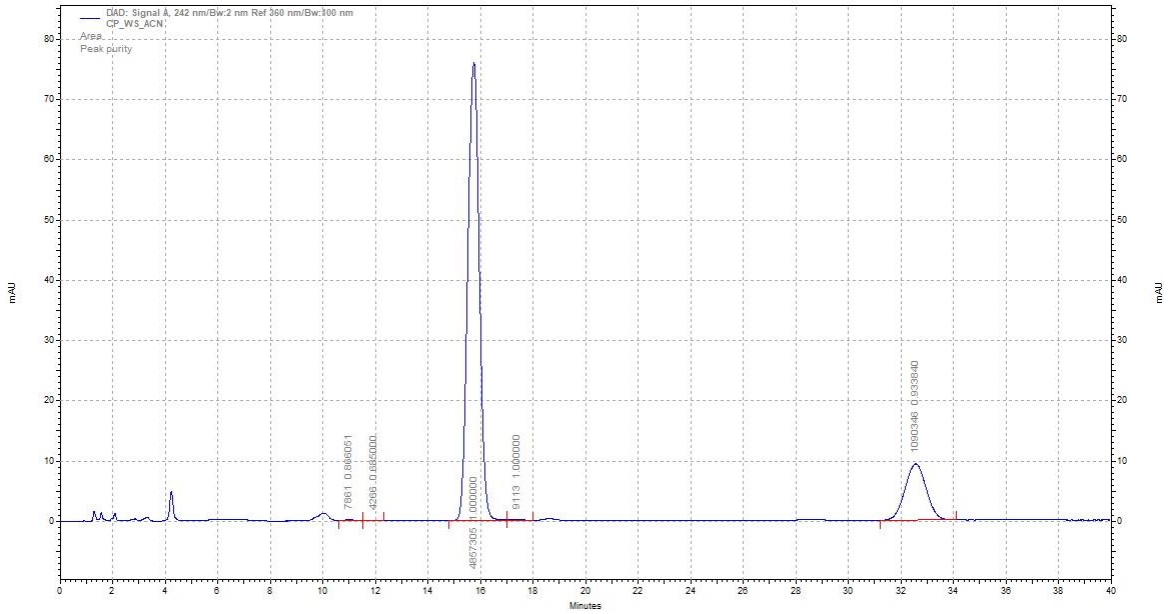


Fig no 3 : Chromatogram of CP in Bulk in optimized chromatographic conditions

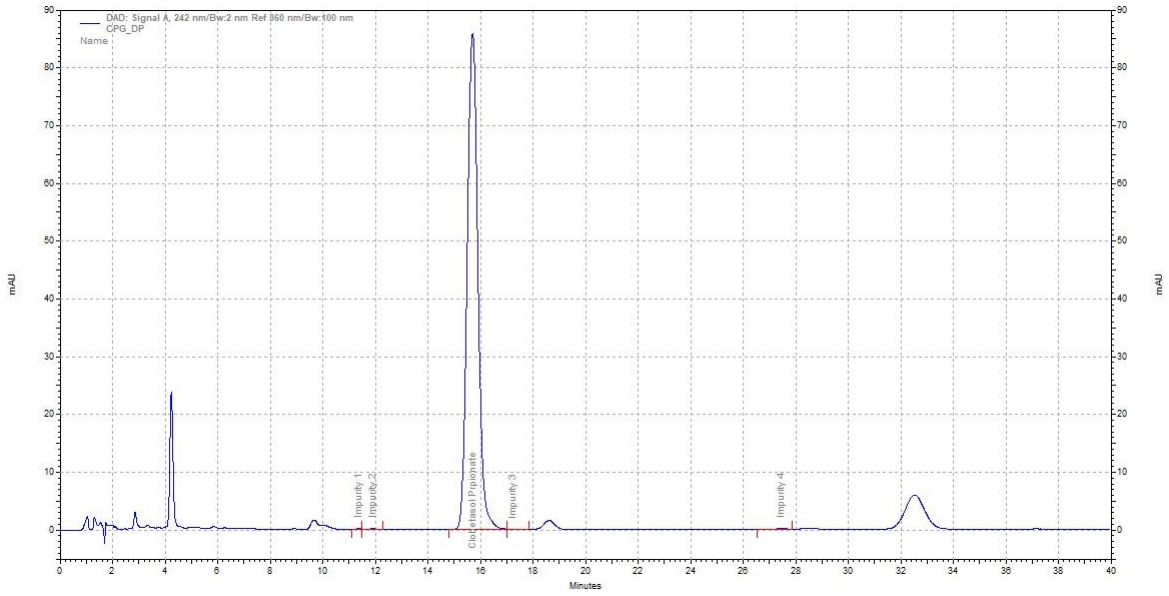


Fig no 4 : Chromatogram of CP in Finished Product in optimized chromatographic conditions(gel)

Validation of CP Gel 0.05% by RP-HPLC Method

1. **Specificity & Assay**

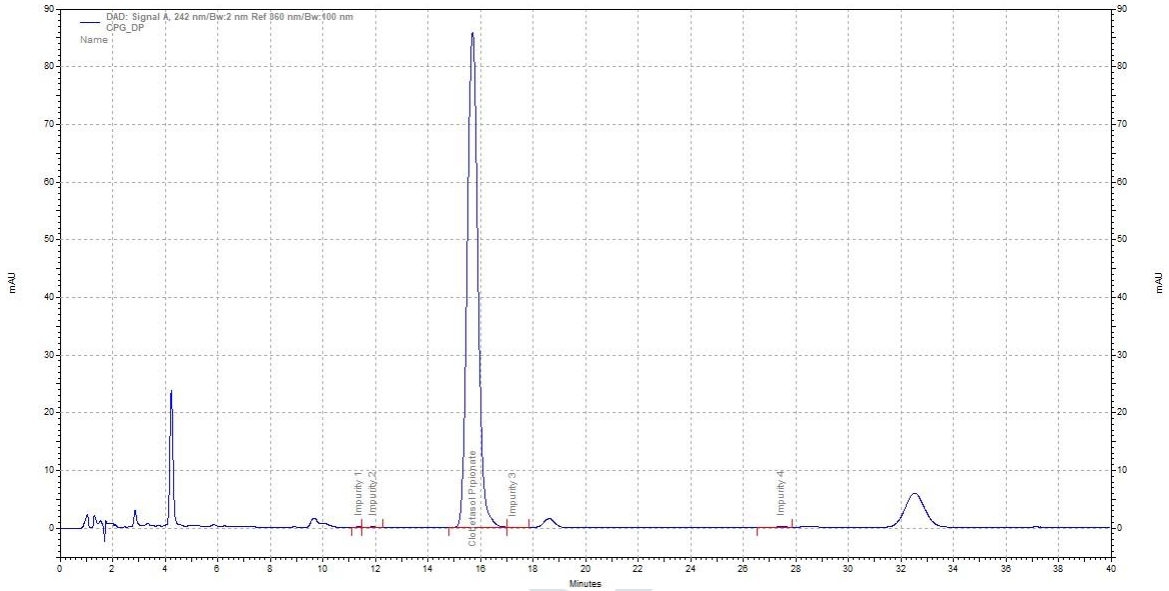


Fig no 5 : Chromatogram of Clobetasol Propionate Gel 0.05%

Table no 3 : Assay of Clobetasol Propionate Gel 0.05%

Sample	**Peak Area**	**Assay (%)**
Working Standard	4857024	-
CP Gel 0.05%	5096161	101.0

The assay of Clobetasol Propionate Gel 0.05% was found to be 101.0% based on the purity of standard.

2. Linearity

Table no 4 : Chromatographic Response on CP at various linearity levels

Sr.No	% Conc	Conc(ug/ml)	Area
1	0.2	0.1	4198
2	0.4	0.2	4804
3	0.75	0.375	6142
4	1.5	0.75	9047
5	3	1.5	15491
6	6	3	26041
7	12.5	6.25	564276
8	25	12.5	1121041
9	50	25	2305054
10	75	37.5	3404720
11	100	50	4422371
12	125	62.5	5756017

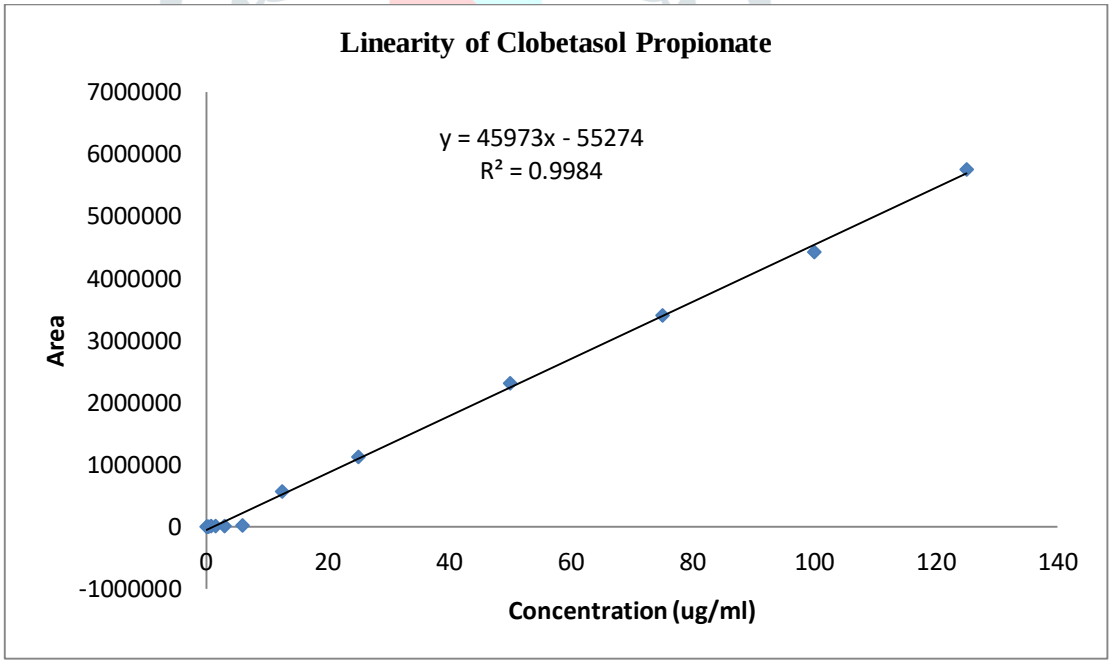


Fig no 6: Calibration curve of CP of RP-HPLC method.

Table no 5: Linear regression analysis of calibration curve for CP

Parameters	CP
Slope	45973x
Intercept	55274
Correlation Coefficient (r^2)	0.998

3. Range:

Table no 6 : Range for RP-HPLC Method

Parameter	***CP***
Linearity Range (µg/ml)	0.025-62.5

4. Method Precision

Table no 7 : Precision study for CP

Sr. No.	**Sample**	**Sample Wt (gm)**	**Area**	**Assay**
1	MP-1	1.0173	4871769	100.9
2	MP-2	1.0229	4899593	100.9
3	MP-3	1.0317	4913824	100.3
4	MP-4	1.0018	4844225	101.9
5	MP-5	1.0032	4826579	101.4
			AVG.	101.3
			STD DEV.	0.7537
			% RSD	0.7

5. Limit of Detection & Limit of Quantification

Table No 8 : ANOVA

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.999197155
R Square	0.998394955
Adjusted R Square	0.998249042
Standard Error	83011.32512
Observations	13

ANOVA

	Df	*SS*	*MS*	*F*	*Significance F*
Regression	1	4.71501E+13	4.72E+13	6842.39	1.0043E-16
Residual	11	75799681085	6.89E+09		
Total	12	4.72259E+13			

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	-55273.64334	28664.12409	-1.92832	0.080014
X Variable 1	91946.75195	1111.558869	82.71874	1E-16

LOD	1.03	ug/ml

LOQ	3.12	ug/ml

6. System Suitability Testing

Table no 9 : Results of System Suitability Parameters

Analyte	Retention Time (min)	Tailing Factor (T)	Theoretical Plates (N)	Resolution (R)
CP	15.70	1.09	7133	5.6
Required limits	--	T < 2	N > 2000	R >2

Study of resolution, tailing factor and capacity factor shows system is suitable for this method.

7. Accuracy

Table no 10 : Accuracy study for CP

% Level	Clobetasol Propionate						
	Spiked Amount (µg/mL)	Spiked Amount (wrt sample)	Peak Area	Amount Recovered (µg/mL)	% Recovery	Average % Recovery	% RSD of % Recovery
ACC_80_1	41.934	41.9	4014199	43.317	103.3	103.3	0.0
ACC_80_2	41.934	41.9	4014629	43.322	103.3		
ACC_80_3	41.934	41.9	4017584	43.354	103.4		
ACC_100_1	52.417	52.4	4990820	53.856	102.7	102.8	0.0
ACC_100_2	52.417	52.4	4994247	53.893	102.8		
ACC_100_3	52.417	52.4	4993867	53.889	102.8		
ACC_120_1	62.900	62.9	5988631	64.623	102.7	102.8	0.1
ACC_120_2	62.900	62.9	5997090	64.715	102.9		
ACC_120_3	62.900	62.9	5999123	64.736	102.9		

The recovery for CP 80% was found to be 103.3% with %RSD 0.0
The recovery for CP 100% was found to be 102.8% with %RSD 0.0
The recovery for CP 120% was found to be 102.8% with %RSD 0.1

CONCLUSION

In conclusion, the proposed HPLC and UV method is simple, accurate, reproducible method for estimation of Clobetasol Propionate in bulk and pharmaceutical Gel formulation. The short chromatographic time makes this method suitable for processing of multiple samples in short time. The method shows no interference by the excipients. The statistical parameters and recovery data reveals the good accuracy and precision of the proposed method. Finally, the proposed methods could be useful and suitable for the estimation of the Clobetasol Propionate in bulk & Gel formulation.

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