

## *To Understand the Chromatographic Technique to Perform Validation of Clobetasol Propionate*

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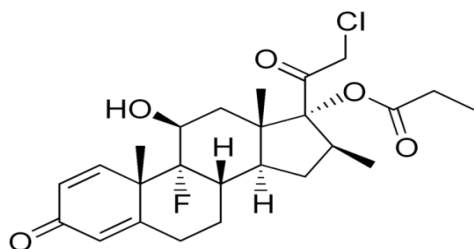
### **Abstract**

*Clobetasol propionate Samples were diluted with Sodium dihydrogen phosphate monohydrate: methanol: acetonitrile (30:20:50). The chromatographic conditions were set, at a flow rate of 1.0ml/min giving retention times of 4 to 8 minutes (at least 1.2 times of retention time of analyte) for Clobetasol Propionate. The sample injection volume was 20µL and the detection was set at a wavelength of 272nm (UV-Vis detector). Validation was carried out using pure standards of Clobetasol propionate to achieve good linearity at a concentration range of between 95% to 105.0% with a detection limit for Clobetasol Propionate. The internal standard method reported slight improvement of the confidence limit and the relative standard deviation relative to the external standard method. HPLC method has been used to analyse Clobetasol propionate.*

**Keywords:** *Analytical method development, Validation, Clobetasol propionate*

### **INTRODUCTION**

Clobetasol propionate is a topical (for the skin) steroid used to treat the inflammation and itching caused



by a number of skin conditions such as allergic reactions, eczema, and psoriasis. Clobetasol propionate is available in generic form. This medication is used to treat a variety of skin conditions (e.g., eczema, psoriasis, dermatitis, allergies, rash). Clobetasol reduces the swelling, itching, and redness that can occur in these

types of conditions. This medication is a very strong (super-high-potency) corticosteroid.

Clobetasol is a prescription drug. It's available as a topical lotion, shampoo, topical spray, and topical solution, and topical cream, topical gel. The topical cream is only available as a generic drug.

**Used:** This drug is used to treat inflammation & itching from various skin conditions, is used cosmetically by dark skin women for skin whitening. Clobetasol reduces the swelling, itching, and redness that can occur in these types of conditions.

**Side Effects:** Burning, irritation, & itching of the skin where you apply the drug.

Formula:  $C_{25}H_{32}ClFO_5$  Molar mass: 466.97 g/mole

## METHODOLOGY

### Chemicals and instrumentations

Sodium di hydrogen phosphate, monohydrate, methanol (HPLC grade) 1, acetonitrile Stock solutions of  $10000\mu\text{g mL}^{-1}$  of compound was prepared in methanol and were used to prepare the working solutions at different concentrations. The standard solutions were warmed to room temperature of  $20^\circ\text{C}$  prior to injection. The HPLC waters

system used comprised auto sampler, High pressure pump module number 313 & 515, analytical column C18,  $150 \times 4.6\text{mm}, 5\mu$  and a 272nm (Uv- Visible detector coupled with Empower 2 software with the use of digital balance and ultra-sonic bath sonicator use.

### Preparation Method

The mobile phase for HPLC analysis was prepared from Sodium dihydrogen phosphate monohydrate: methanol: acetonitrile (30:20:50) (v/v). The mobile phase was degassed. Stabilizing the temperature was controlled by insulating the mobile phase by placing the reservoir of the mobile phase in a polystyrene box. In addition, the mobile phase was prepared.

### Linearity of the Calibration Curve

The linearity of the calibration curve was tested at three ranges of concentrations (2, 2.25, 2.75, 2.5, 4.2  $\mu\text{g/mL}$ ) prepared stock solutions of a mixture of Clobetasol propionate in mobile phase. The different sets of standards were injected as duplicates into HPLC system. Linearity was evaluated according to the relationship between the peak area of the compound and its concentration. Excel software was employed to plot the calibration curve for each compound in the solution. From the

regression line, the coefficient of determination ( $R^2$ ) was obtained to statistically assess the linear relationship.

**To prepare solution for linearity parameter per from:**

**To prepare stock solution:**

Take 100 mg of clobetasol propionate in a 100 ml of volumetric flask. Add 10 ml of mobile phase. Sonicate to dissolve. Make up the volume of the 100ml with mobile phase. (1000ppm solution)

**Further dilution:**

**RESULTS AND DISCUSSION**

**Assessment of the Linearity of the Calibration Curve**

Linearity was demonstrated by constructing a calibration curve using five concentration levels of standard solutions for each of the three ranges of concentration. Three calibration curves were plotted for each compound in this test. The coefficient of determination ( $R^2$ ) was employed to evaluate the linearity of the regression line.

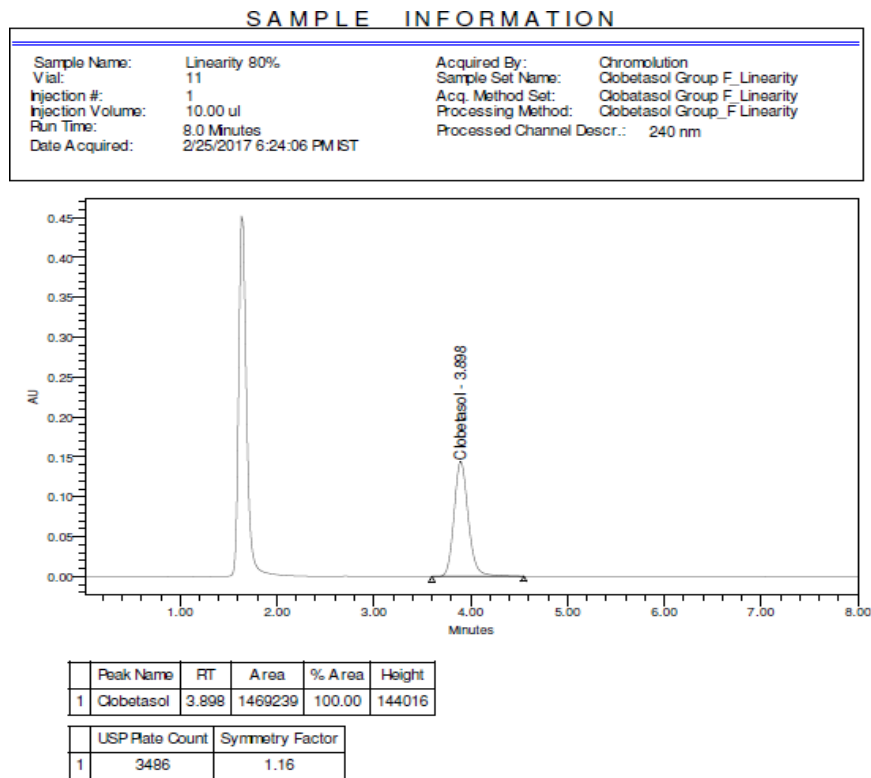
*Table: 1*

Percentage	MI	Makeup to (volumetric flask)
80%	2	25
90%	2.25	25
100%	2.5	25
110%	2.75	25
150%	4.2	25

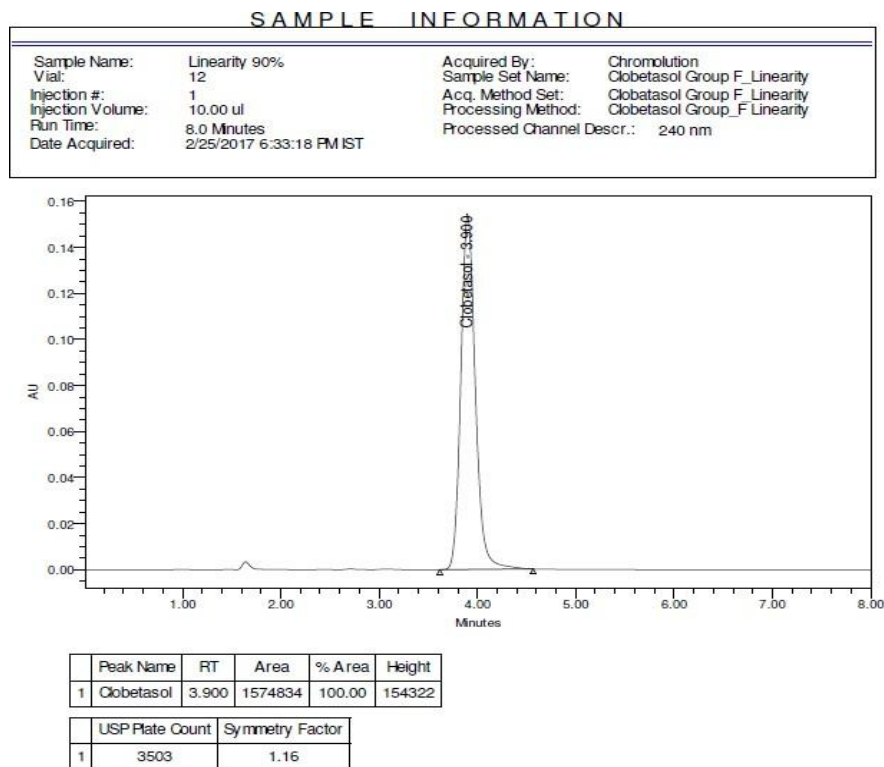
The results presented in Table 1 show  $R^2$  values were  $>0.990$  with the exception at the lowest concentration range (0.02–0.1  $\mu\text{g/mL}$ ) where the  $R^2$  was 0.983. This slightly lower value for the coefficient of

Determination of a low response of this compound at the detection wavelength used (272 nm). From the  $R^2$  values obtained, it can be concluded that the linearity was acceptable for compounds at

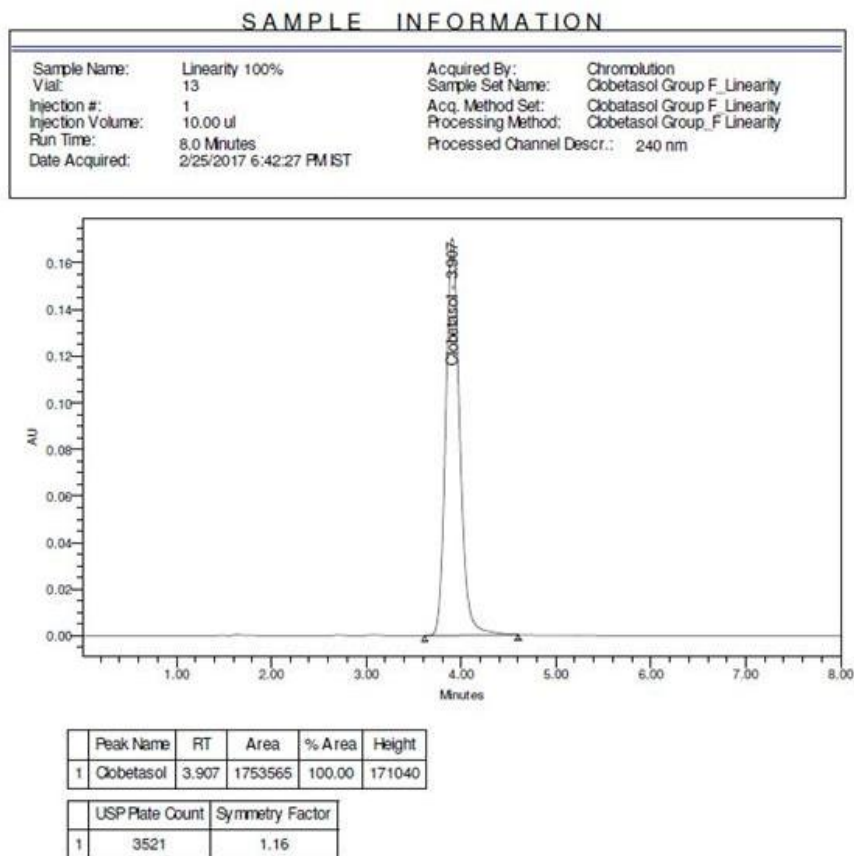
the five range of concentration tested.



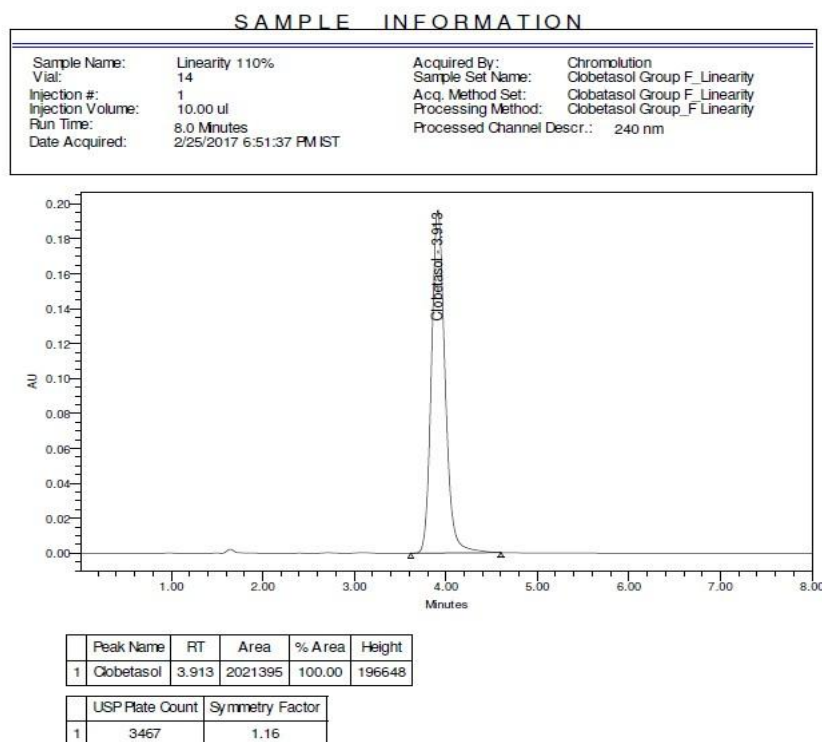
*Figure: 1*



*Figure: 2*



*Figure: 3*



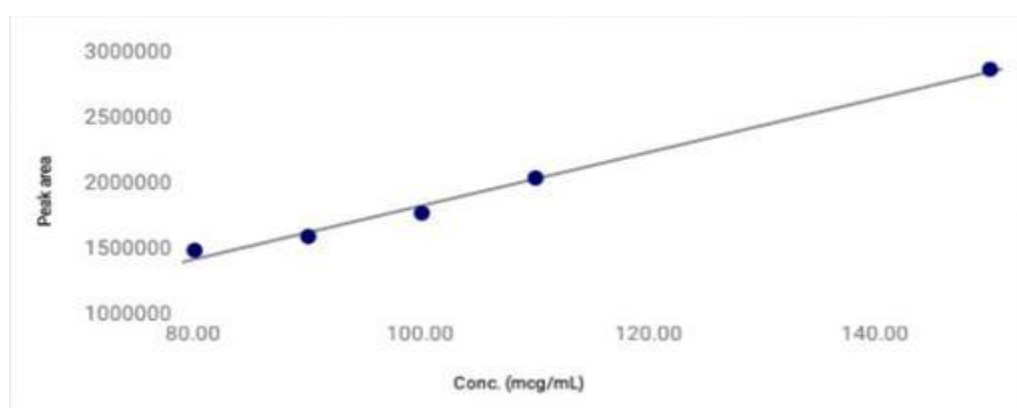
*Figure: 4*

**Table 1: For linearity stoke solution**

Stoke solution			
	mg	ml	ppm
	100	100	1000
	ml	Makeup to	
For 80%	2	25	80
For 90%	2.25	25	90
For 100%	2.5	25	100
For 110%	2.75	25	110
For 120%	3.4	25	136

**Table 2: For peak area of Clobetasol propionate**

Clobetasol Linearity	
Concentration in PPM	Peakarea
80	1469239
90	1574834
100	1753565
110	2021395
150	2851252



**Graph: 1**

**Correlation coefficient= 0.9925**

## CONCLUSION

Part of HPLC “pump, detector, injector” system calibration are successfully passed. Our assay result is 98%, 98.002%, 12 %.(limit 95% to 105.0%). So our assay process is completely acceptable.

Validation

Correlation coefficient=0.9925

Visual= Linear

A successful and rapid analytical method was developed and validated for the separation and quantification of these compounds using an internal standard. Furthermore, this project was undertaken during a global shortage of acetonitrile in 2008. It was therefore essential to develop and validate a method using an alternative solvent and methanol was selected for this purpose.

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