

**DEVELOPMENT AND VALIDATION OF THE RP-HPLC METHOD FOR THE ESTIMATION OF CHLOROCRESOL AND BETAMETHASONE DIPROPIONATE IN SEMISOLID DOSAGE FORMULATION**

**Running Title: RP-HPLC Method Validation for the Estimation of Betamethasone dipropionate and Chlorocresol**

**ABSTRACT:**

A simple, accurate, reproducible and cost-effective reverse phase HPLC technique for chlorocresol (CRC) as a preservative as well as betamethasone dipropionate (BTD) in bulk drug as well as semi-dose formulations. snakes were developed during this research. In this method, Discovery HS (150x4.6 mm), 3 was used as the stationary phase and an 80:20 mixture of water and isopropyl alcohol was used as the mobile phase A, and a mixture of acetonitrile plus tetrahydrofuran in the ratio. 70:30 ratio was used as mobile phase B in gradient mode to produce the final product. Approximately 1.00 ml min<sup>-1</sup> of the solution is passed through the chromatograph by pumping it through. The UV detector operates at a wavelength of 240 nm. A validation study in accordance with the ICH Q2 (R1) guidelines is required to demonstrate that the new analytical method meets the reliability characteristics and these characteristics demonstrate the ability of a new analytical method. analysis in maintaining, over time, the basic validation criteria: selectivity, linearity, precision, precision, and specificity. When this method is used for quality control of commercial semi-solid dosage forms of betamethasone dipropionate throughout the business day, the stability indicator technique can be used to measure the breakdown of the drug and its products it, as well as to evaluate the results of the tube homogeneity test.

**Keywords:** Chlorocresol, Betamethasone dipropionate, Assay, HPLC, Validation, Accuracy



## **INTRODUCTION:**

Characterized by inflammation, an inflammatory skin condition characterized by increased epidermal proliferation associated with dysregulation of the immune system, affects an estimated 2-3% of the world population. There are different types of psoriasis, including psoriasis vulgaris (common), psoriasis (guttural), skin psoriasis (red), pustular psoriasis (white), and nail (nail) psoriasis-colored hands). There are three types of psoriasis [1-3]. The former is the most common variety and is characterized by raised, scaly, red patches. Areas of red scaly skin covered with silvery-white scales define plaque psoriasis, an inflammatory disease that presents as a chronic immune-mediated disease. In the United States, it affects about 7.4 million people (3.2% of the population). The areas of the body affected where psoriasis is most common include the scalp, knees, elbows, and lower back, but the condition can affect any part of the body. Uncomfortable, itchy patches may develop, which may also fracture and bleed [4-5].

More than four decades have passed since topical steroids, including betamethasone dipropionate (BTD), were first used to treat psoriasis and other steroid-responsive dermatoses as a therapy. evidence for this disease. It has been widely used as a soothing anti-inflammatory and immunosuppressant for dermal and systemic applications for many years. The use of topical corticosteroids, such as betamethasone dipropionate, has been shown to be effective in the treatment of skin conditions in which inflammation is a prominent feature, such as eczema, seborrheic dermatitis, and a several types of psoriasis [6]. A chemical compound known as chlorocresol (4chloro3methylphenol) and also known as Chlorocresolum, chlorocresol (also known as Chlorocresolum) is primarily used as an antibacterial preservative in pharmaceutical and cosmetic formulations [7-8]. In addition to disinfectants and disinfectants, chlorocresol has bactericidal properties. It kills germs and inhibits microbial growth. It is used to extend the shelf life of many pharmaceutical drugs. For use as a disinfectant in products such as hand sanitizer, it is usually dissolved in alcohol and mixed with other phenols. It is a mild allergen for sensitive skin [9-10].

For pharmaceutical creams and ointments, an analytical method indicating stability is needed to separate the peak of the active pharmaceutical ingredient (API) from the peak of any potential by-products, impurities related to process, latent packing liquid and excipients, as well as for peak separation of these different compounds. Antimicrobial compounds (eg, chlorocresol) are commonly added to topical formulations and their presence should be checked during product

development and distribution, as well as during shelf life. To ensure that various HPLC analysis techniques produce reliable and reproducible results, analytical method validation is performed. This is an important step in the development of new dosage forms as it provides information on accuracy, linearity, accuracy, detectability and limit of quantitation, as well as other. According to the guidelines of the International Conference on Harmonization (ICH), “the objective of the validation of an analytical technique is to determine that it is fit for its intended use”. It is now imperative to provide validation data to the appropriate regulatory and regulatory authorities throughout the drug development process. The recommendations of the International Conference on Harmonization (ICH) and the United States Pharmacopoeia (USP) [11–14] are part of the validation standards for analytical techniques.

Because of the large number of structurally similar chemicals that must be isolated and monitored during the shelf-life of steroid drugs such as BD, the creation of a technique for specifying stability for This product is very difficult. Multiple methods of measuring betamethasone dipropionate and chlorocresol simultaneously or singly have been developed in the past, using a variety of instruments such as ultraviolet spectrometers, high performance liquid chromatography, high performance liquid chromatography with mass spectrometry and super performance liquid chromatography [15-16]. The results of this study, the current technique, established using RPHPLC to detect CRC and BTD in dosage form, is said to be easy, accurate, fast and cost-effective. Several RPHPLC techniques for measuring BTD have been identified in various literary works; however, they all proved difficult to implement and time consuming. A new RPHPLC approach to measuring CRC and BTD in dosage forms has been developed to promote green chemistry using as few hazardous chemicals as possible. The technique turned out to be very accurate, responsive, cost-effective, and stable. Tests of the new CRC and BTD process, RPHPLC, in accordance with the requirements of ICH Q2, have been successfully completed (R1). When developing this method for CRC and BTD [13-16], it was necessary to evaluate accuracy, precision, linearity, specificity, limit of detection (LOD), as well as limit of quantification (LOQ). of the technique, as well as quantification of CRC as well as BTD in the indicated semi-solid dosage form. These assessments were made at different stages of the method's development.

## **MATERIALS AND METHODS:**

### **Chemicals**

HPLC grade solvents used in this study were purchased from Merck Ltd. in Bangalore, India. These include methanol, glacial acetic acid, isopropyl alcohol, acetonitrile, and tetrahydrofuran. The water comes from the MilliQ water distribution system. To obtain these samples, Alkem Laboratories in Mumbai, Maharashtra sent us a gift sample of Chlorocresol, and Marksman's Pharmaceuticals in Mumbai, Maharashtra sent us a Betamethasone Dipropionate gift sample.

### **Preparation of the standard solution:**

After correctly weighing and shifting forty mg of Betamethasone Dipropionate and 60 mg of Chlorocresol operating general right into a a hundred-milliliter volumetric flask, the answer turned into diluted with 60 mL of diluent and sonicated to dissolve, and then it turned into diluted to quantity with diluent and mixed, the outcomes have been recorded. In a a hundred mL volumetric flask, 5. zero mL of the aforementioned answer turned into pipetted out and diluted into quantity with diluents to get the same old concentrations of Betamethasone Dipropionate 20 ppm and Chlorocresol 30 ppm, respectively.

### **Preparation of Sample Solution: (For 0.05% w/w Cream)**

To ensure the accuracy of the sample weighing (equivalent to 2 mg betamethasone dipropionate), the sample was placed in a 100 ml volumetric flask. It was diluted to the desired amount with the diluent after adding 60 mL of the diluent and heating over boiling water at 60°C for 30 min with intermittent vigorous stirring. Sample preparation was then filtered through a 0.45 micrometer Teflon filter to a concentration of 20 ppm [17-18].

### **Chromatographic conditions for HPLC**

Using a Waters 2695 Alliance system equipped with a photodiode array (PDA) detector 2996 and a UV/Visible 2489 detector, high performance liquid chromatography (UV) was performed. Tested on Discovery HS, C18, (150 x 4.6 mm) reverse phase column, 3 as betamethasone dipropionate and chlorocresol standards respectively (Mumbai, India). The mobile phase is used in gradient form, with mobile phase A consisting of a mixture of water and isopropyl alcohol in a ratio of 80:20 and mobile phase B consisting of a mixture of acetonitrile and tetrahydrofuran in a ratio of 70:30. The flow rate of the mobile phase is maintained at one milliliter per minute. Glacial acetic acid in methanol at a concentration of 0.1% was chosen as the diluent. Prior to initial injection, the column was saturated with the first mobile phase for 30 min before being

used. The temperature was maintained at 50 degrees Celsius. It was determined that the injection volume would remain at 20  $\mu$ L. For the chromatographic acquisition, the PDA was adjusted by adjusting the wavelength to provide an optimal response for two peaks at 240 nm for the best response. Using comparison of retention times and spectra obtained from samples as well as standard solutions [19-20], betamethasone dipropionate and chlorocresol standards were determined.

**Table 1:Details of Gradient program**

Time (minute)	Flow (mL/minute)	% Solvent A	% Solvent B
0	1.0	80	20
22	1.0	80	20
25	1.0	40	60
30	1.0	80	20
35	1.0	80	20

**Procedure:**

The standard was injected into the HPLC five times and the chromatogram was recorded for each injection. The number of areas of the betamethasone and chlorocresol peaks is determined by counting the number of peaks. Both peaks should have a relative standard deviation of less than 2.0% when five replicate injections are performed. In HPLC, equal amounts of blank (diluent) and sample solution (in triplicates) are injected, and chromatograms are recorded as they are produced. The number of areas of the betamethasone and chlorocresol peaks is determined by counting the number of peaks.

**Preparation of Calibration Graph**

A series of standard preparations of betamethasone dipropionate and chlorocresol were prepared to cover the range from 50% to 150% of the sample concentration, i.e., 20 ppm for betamethasone dipropionate, and on this basis, the range proposed. to determine the linearity of 50 % to 150% of the test concentration. (i.e., 10 ppm to 30 ppm) for betamethasone dipropionate and 30 ppm for Chlorocre in Table 2, there is a detailed description of the dilutions. Between 50% and 150%, a linear histogram should be plotted.

### Preparation of Linearity Stock Solution

Working standards of 40 mg betamethasone dipropionate and 60 mg chlorocresol were carefully weighed and placed in two separate 100 ml volumetric flasks. To obtain a stock solution of both, add 70ml of diluent for all and sonic to dissolve. Cool and fill the volume with diluent and mix well.

**Table 2: Dilutions for Linearity Betamethasone Dipropionate and Chlorocresol**

% Conc of Sample	Betamethasone Dipropionate			Chlorocresol		
	Amount of stock solution to be transferred (ml)	Final volume with diluent (ml)	Concentration (ppm)	Amount of stock solution to be transferred (ml)	Final volume with diluent (ml)	Concentration (ppm)
50%	2.5	100	10	2.5	100	15
80%	4.0	100	16	4.0	100	24
90%	4.5	100	18	4.5	100	27
100%	5.0	100	20	5.0	100	30
110%	5.5	100	22	5.5	100	33
120%	6.0	100	24	6.0	100	36
150%	7.5	100	30	7.5	100	45

### VALIDATION OF HPLC METHOD

According to the guidelines of the International Conference on Harmonization (ICH Q2 (R1)) [18-24], the proposed HPLC method was validated for specificity, precision, accuracy, and limit of detection (LOD), limit of quantitation (LOQ), standard solution stability, sample solution consistency and stability.

#### Specificity

The specificity of the method was investigated by measuring the pure peaks of betamethasone dipropionate and chlorocresol with Waters empower software and a diode array detector, and plotting the performance in terms of purity, purity threshold and substance. purity indicator.

Betamethasone dipropionate blank, standard samples, test samples, peak samples, and mock samples were injected, and percent of noise was calculated by comparing the response of each found peak with the retention time of betamethasone dipropionate.

### Accuracy

Recovery experiments were used to evaluate the accuracy of the technique, which consisted of adding a known amount of each standard to the sample being analyzed first at the 80%, 100%, and 120% levels, and then are repeated analyzes of quantitative tests using the proposed method. . In triplicate at three levels, recovery samples were prepared with spike placebo preparations with known concentrations of betamethasone dipropionate and standard chlorocresol (9 determinations in total). Each specimen will be processed according to the steps described below.

**Table 3: Sample preparation for Accuracy**

Accuracy Level	Conc. Of spiked sample of Betamethasone Dipropionate	Conc. Of spiked sample of Chlorocresol
80%_sample_1	16 ppm	24 ppm
80%_sample_2	16 ppm	24 ppm
80%_sample_3	16 ppm	24 ppm
100%_sample_1	20 ppm	30 ppm
100%_sample_2	20 ppm	30 ppm
100%_sample_3	20 ppm	30 ppm
120%_sample_1	24 ppm	36 ppm
120%_sample_2	24 ppm	36 ppm
120%_sample_3	24 ppm	36 ppm

### Precision

System accuracy, engineering accuracy, and intermediate precision have all been studied.

### **System precision**

System accuracy was achieved by six repeated injections from the same vial of the standard and expressed as a percentage of relative standard deviation (%RSD), plaque count, and resolution.

### **Method precision**

The sample was subjected to this method six times. Six sample formulations of betamethasone dipropionate were produced separately by the analyzer and analyzed according to the technique. The percent test of each analyte is expressed as a percentage RSD.

### **Intermediate precision**

Separate analysts analyzed six separate semi-solid dose samples on two different systems, the Waters e2695 Alliance system with 2996 PDAs and 2489 ultraviolet (UV) detectors, and average accuracy was measured. expressed as a percentage of RSD.

Six controls and samples of betamethasone dipropionate from the same lot was evaluated using separate HPLC systems, different columns, and different dates.

### **Robustness**

A slight difference in the parameters of the technique used to evaluate the robustness of the method. Column chemistry deviation, wavelength, column temperature, flow rate and mobile phase gradient were the factors used. Percent RSD was calculated using system suitability parameters and the retention time of betamethasone dipropionate plus chlorocresol was measured. The concentrations of betamethasone dipropionate plus chlorocresol in the semi-solid formulation were determined by HPLC using the technique described under the chromatographic conditions. All analyzes were performed in triplicate and the results are presented as mean  $\pm$  SD. The following changes to the test parameters will be evaluated:

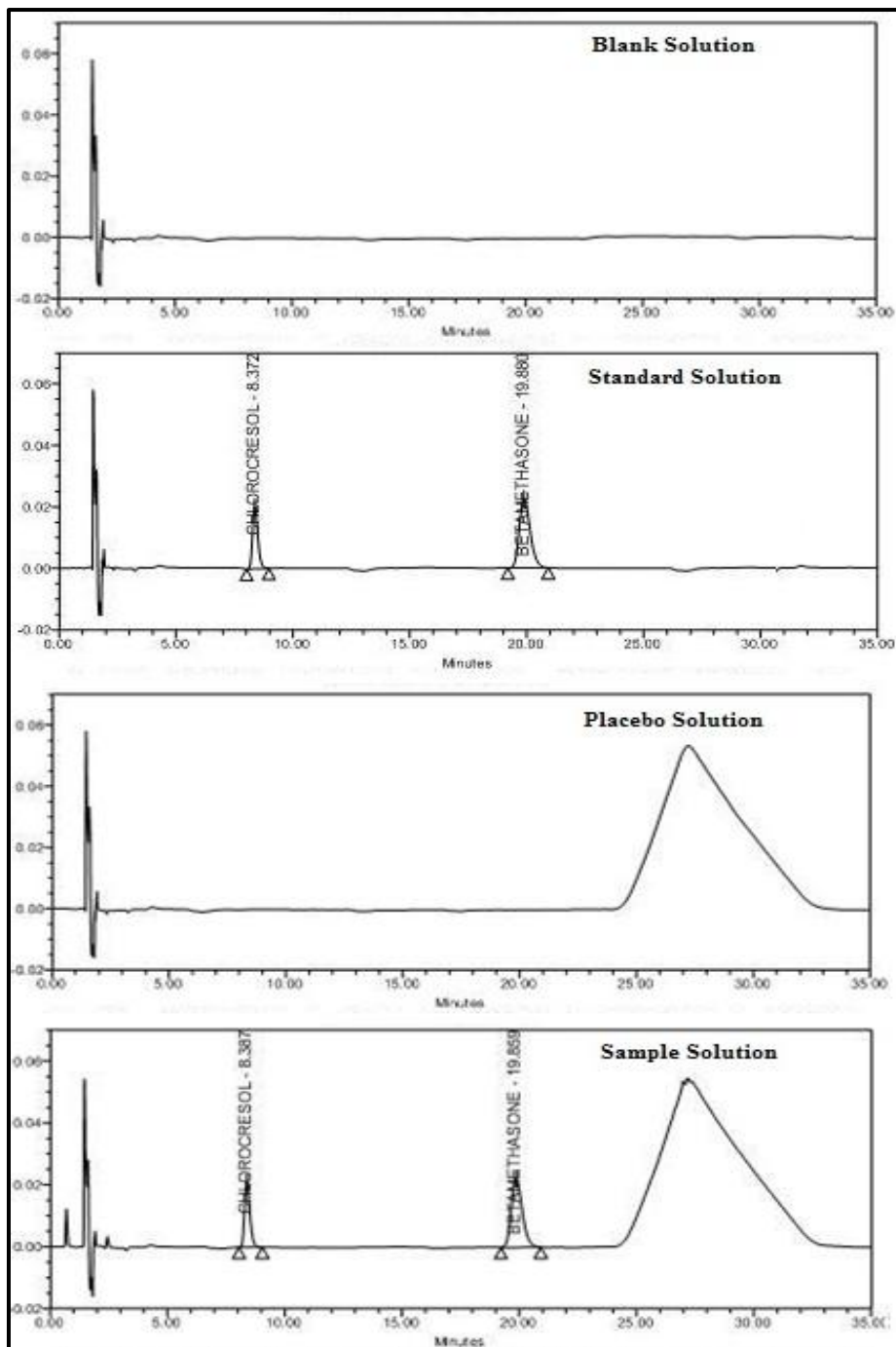
- Change in temperature (five degrees Celsius)
- Change in wavelength (five nanometers)
- Change in flow rate (0.1 milliliters per minute) a 10% modification

## **RESULTS AND DISCUSSION**

The mobile phase composition in the HPLC method was optimized by testing different solvent compositions with different polarities, column chemistry, column temperature and mobile phase pH, and the best results were obtained by current method, produces highly symmetric peaks that show good resolution between each standard and the others [Figure 1). The scanning wavelength was chosen at 240 nm to provide comparable results, and at this wavelength all analytes showed

optimal responses. Betamethasone dipropionate and chlorocresol were well resolved with retention times of approximately 17 and 21 min, respectively.

**Figure 1: Chromatograms for Blank, Standard and Sample**



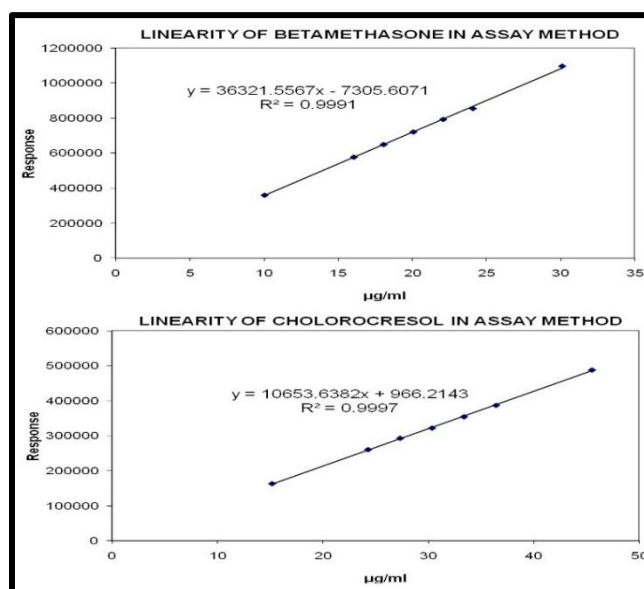
A series of standard preparations of chlorocresol dipropionate and betamethasone have been produced at concentrations ranging from 50% to 1500% of the working concentration of

betamethasone dipropionate plus chlorocresol in a betamethasone dipropionate booster cream. (For the test, a minimum of five points in the 80-20% range of standard/sample concentration) [Table 4]. Figure 2 shows the curve for each standard.

**Table 4:Linearity of Betamethasone dipropionate and Chlorocresol**

<b>% Level</b>	<b>Conc. of BTD (ppm)</b>	<b>Average Peak area of BTD</b>	<b>Conc. of CRC (ppm)</b>	<b>Average Peak area of CRC</b>
50%	10.034	360695	15.179	163386
80%	16.055	577523	24.286	260865
90%	18.062	649383	27.322	293332
100%	20.069	720764	30.358	322393
110%	22.075	792247	33.394	354744
120%	24.082	854761	36.430	387424
150%	30.103	1095931	45.537	488589
<b>r<sup>2</sup></b>	<b>0.99956</b>		<b>0.99984</b>	
Slope of Regression line	<b>36321.6</b>		<b>10653.6</b>	

**Figure 2: Linearity graphs for standard**



These intermediate precision, methodical, and systematic accuracy values are expressed as a percentage of RSD and are provided in relation to sample application and peak region scanning.

For betamethasone dipropionate as well as chlorocresol, the percentage RSD values were 0.38 and 0.06%, respectively, for system accuracy. The percentage RSD values for betamethasone dipropionate and chlorocresol, respectively, are 0.44% and 0.64%, depending on the accuracy of the method. Betamethasone dipropionate and chlorocresol had RSD values of 0.68% and 0.76%, respectively, for the mean between two analysts (Table 5). The percentage RSD results demonstrated that the proposed technique provides an acceptable level of systematic accuracy, method accuracy, and intermediate precision for system accuracy, precision, and accuracy. method accuracy and intermediate precision values.

**Table 5: Method precision and Intermediate precision for Betamethasone dipropionate and Chlorocresol**

Name of Analyte	Betamethasone dipropionate		Chlorocresol	
Sr. No.	Assay (% w/w, Analysis-1) MP	Assay (% w/w, Analysis-2) IP	Assay (% w/w, Analysis-1) MP	Assay (% w/w, Analysis-2) IP
1	100.7	99.1	100.9	99.1
2	100.1	100.6	100.3	100.3
3	101.2	99.8	101.8	99.2
4	100.3	101.7	100.1	100.1
5	100.8	100.6	100.2	100.1
6	100.1	101.3	100.9	101.1
Average	<b>100.5</b>	<b>100.2</b>	<b>100.7</b>	<b>100.0</b>
% RSD	<b>0.44</b>	<b>0.86</b>	<b>0.64</b>	<b>0.74</b>
Overall % RSD	<b>0.68</b>		<b>0.76</b>	

The maximum purity of each analyte was determined by comparing the spectra of the trace from the standard as well as the extracts at the top of the peak, at the top of the peak, and at the end of the peak. Table [Table 6] shows purity angle values and purity thresholds.

**Table 6: Specificity of Betamethasone dipropionate and Chlorocresol**

Sample Name	Retention Time (Min)	Purity Angle	Purity Threshold	Peak Purity
<b>Blank (diluent)</b>				
Betamethasone dipropionate and Chlorocresol	ND	NA	NA	NA
<b>Standard solution</b>				
Betamethasone dipropionate	19.880	0.222	1.242	Pass
Chlorocresol	8.372	0.310	1.239	Pass
<b>Worst Case Placebo</b>				
Betamethasone dipropionate and Chlorocresol	ND	NA	NA	NA
<b>Sample solution</b>				
Betamethasone dipropionate	19.859	0.242	1.251	Pass
Chlorocresol	8.387	0.241	1.257	Pass

Robustness was used to improve the method provided. For each parameter, the peak of each analyte was determined and the percentage RSD was determined to be less than 2%. The RSD percentage figures in Table 7 imply that this approach is more flexible.

**Table 7: Robustness for Betamethasone dipropionate and Chlorocresol**

Robustness parameter		% RSD		Remark
		Betamethasone dipropionate	Chlorocresol	
Wavelength (nm)	235	0.53	0.62	Pass
	240	0.44	0.64	Pass
	245	0.58	0.59	Pass
Temperature (°C)	45	0.37	0.34	Pass
	50	0.44	0.64	Pass
	55	0.64	0.58	Pass
Flow (mL/min)	0.9	0.20	0.59	Pass
	1.0	0.44	0.64	Pass
	1.1	0.29	0.30	Pass

The addition of a known amount of standard in the mock solution at 80%, 100%, and 120% working concentrations was used in the recovery test. For betamethasone dipropionate as well as

for chlorocresol, the total recovery rates ranged from 98.0 to 102.0%, according to Table 8. [22-29]

**Table 8: Recovery for Betamethasone dipropionate and Chlorocresol**

Analyte	Betamethasone dipropionate		Chlorocresol	
	% Recovery	Average % Recovery	% Recovery	Average % Recovery
<b>80% - 1</b>	100.2	100.3	99.9	100.3
<b>80% - 2</b>	100.2		100.6	
<b>80% - 3</b>	100.4		100.3	
<b>100% - 1</b>	100.5	100.2	99.8	100.4
<b>100% - 2</b>	99.7		100.9	
<b>100% - 3</b>	100.5		100.6	
<b>120% - 1</b>	100.6	100.4	100.8	100.2
<b>120% - 2</b>	100.1		99.7	
<b>120% - 3</b>	100.5		100.2	
<b>Mean</b>	<b>100.3</b>		<b>100.3</b>	
<b>SD</b>	<b>0.283</b>		<b>0.443</b>	
<b>% RSD</b>	<b>0.28</b>		<b>0.44</b>	

**CONCLUSION:**

The present study led to the creation of a betamethasone dipropionate and chlorocresol measurement technique RPHPLCUVDAD that has been validated for linearity, accuracy, precision, specificity, adaptability, and robustness of the system. The method described is fast, simple and sensitive, as well as accurate and precise, to comply with the ICH requirements for the determination of two components, betamethasone dipropionate and chlorocresol, at a single wavelength. Excipients of the Semisolid dosage form do not affect the dosage of the two active ingredients. Therefore, the proposed analytical method is proposed for routine analysis of betamethasone dipropionate and chlorocresol in different dosage forms. Furthermore, the method can be used in many poor countries or in field stations that lack specialized analytical equipment.

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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