

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

### **ABSTRACT:**

This work has been done with a motto to develop a simple, accurate, precise, reproducible and economic reverse phase HPLC method for Chlorocresol (CRC) as preservative and Betamethasone dipropionate (BTD) in bulk drug as well as in semisolid dosage formulations. In the current developed method Discovery HS, C18, (150 x 4.6mm), 3 $\mu$  was used as stationary phase and Mixture of Water and Isopropyl Alcohol in the ratio 80:20 as Mobile phase A and Mixture of Acetonitrile and Tetrahydrofuran in the ratio 70:30 Mobile phase B in a gradient mode as mobile phase. It is pumped through the chromatographic system at a flow rate of 1.00 ml min<sup>-1</sup>. The UV detector is operated at 240 nm. The validation study is carried out fulfilling the ICH guidelines Q2 (R1) to prove that the new analytical method, meets the reliability characteristics, and these characteristics show the capacity of an analytical method to keep, throughout the time, the fundamental criteria for validation: selectivity, linearity, precision, accuracy and specificity. The stability indicating method is applied during the working day for the quality control of commercial Betametasone Dipropionate semisolid dosage form to quantify the drug and its degradation products and to check the Tube uniformity test.

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

## **INTRODUCTION:**

Psoriasis is a chronic inflammatory skin disease-with increased epidermal proliferation related to dysregulation of immune system estimated to affect around 2-3% of the world population. This disease has different types: psoriasis vulgaris, guttate psoriasis, erythrodermic psoriasis, pustular psoriasis and nail psoriasis. The first type is the most common form of psoriasis, which is characterised by red, scaly and raised plaques [1-3]. Plaque psoriasis is a chronic, immune-mediated, inflammatory disease characterized by patches of raised, reddish skin covered by silvery-white scales. It affects about 7.4 million adults (3.2%) in the United States. Psoriatic plaques are most commonly found on the scalp, knees, elbows, and lower back, but they can occur anywhere on the body. The plaques are often itchy and painful and can crack and bleed [4-5].

Topical steroids such as betamethasone dipropionate (BTD) have been used as an evidence-based treatment for psoriasis and other steroid-responsive dermatoses for more than 40 years. It has been widely used as soothing anti-inflammatory or immunosuppressant for topical as well as systemic use. Topical application of corticosteroids, including betamethasone dipropionate, often produces inhibition of skin conditions in which inflammation is a major feature, such as eczema, seborrhoeic dermatitis, and some forms of psoriasis [6]. Chlorocresol(4-chloro-3-methyphenol) is also known as Chlorocresolum and mainly used as an antimicrobial preservative in cosmetics and pharmaceutical formulations [7-8]. Chlorocresol has disinfectant, antiseptic and bactericidal properties. It kills bacteria, and prevents microbial growth. It is also used in various pharmaceutical products to increase shelf life. People use it as a disinfectant such as hand wash; it is commonly dissolved in alcohol combined with other phenols. It is a moderate allergen for sensitive skin [9-10].

To perform batch release testing and to conduct stability studies for cream and ointment in pharmaceutical products, a stability-indicating analytical method is required to separate the active pharmaceutical ingredient (API) peak from the peaks of all potential degradation products, process related impurities, potential packaging leachable, excipients, and also separate these compounds from each other. Frequently, antimicrobial agents (e.g., chlorocresol) are also included in the topical formulations and need to be monitored at the product release and over the product shelf-life. Analytical method validation ensures that various HPLC analytical techniques shall give reliable and repeatable results; it is a crucial step in developing new dosage as it

provides information about accuracy, linearity, precision, detection, and quantitation limits. According to the ICH guideline, “the objective of validation of an analytical procedure is to demonstrate that; it is suitable for its intended purposes.” It is now obligatory in the process of drug development to supply the validation data to the responsible authorities. Guidelines for analysis method validation include ICH and USP guidelines [11–14].

The development of a stability-indicating method for steroid containing drug products such as BD is challenging due to the numerous, structurally, similar compounds that must be separated and monitored throughout the shelf-life of the product. In the past, numerous techniques for the simultaneous or single measurement of Betamethasone dipropionate and Chlorocresol have been developed, which have made use of a variety of equipment, such as the UV spectrophotometer, the high performance liquid chromatography, the HPLC-MS, and the UPLC [15-16]. In contrast, the current approach, which was developed via RP-HPLC for the determination of CRC and BTD in dosage forms, was found to be simple, exact, quick, and cost-effective to perform. Although multiple RP-HPLC approaches for measuring BTD were discovered in various works of literature; they were found to be complex and time-consuming. To promote green chemistry, the present research sought to create a new RP-HPLC technique for the measurement of CRC and BTD in dosage form that was accurate, sensitive, cost-effective, and stability-indicating, while employing the fewest amount of hazardous chemicals possible. According to ICH Q2 guidelines, the validation of the newly designed CRC and BTD, RP-HPLC technique was completed (R1). The accuracy, precision, linearity, specificity, limit of detection (LOD), and limit of quantification (LOQ) of the technique for CRC and BTD, as well as the quantification of CRC and BTD indicated Semisolid dosage form were evaluated throughout the development of the method for CRC and BTD [13-16].

## **MATERIALS AND METHODS**

### **Chemicals**

HPLC-grade solvents such as Methanol, Glacial acetic acid, Isopropyl Alcohol, Acetonitrile, and Tetrahydrofuran were obtained from Merck Ltd., Bangalore India. Water obtained from the Milli-Q water system. Chlorocresol was procured as a gift sample from Alkem Laboratories, Mumbai, Maharashtra and Betamethasone dipropionate was procured as a gift sample Marksans Pharma Ltd, Mumbai, Maharashtra.

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

40 mg of Betamethasone Dipropionate and 60 mg of Chlorocresol working standard was accurately weighed and transferred into a 100 mL volumetric flask and diluted with 60 ml of diluent and sonicated to dissolve, then diluted the above solution to volume with diluent and mix. 5.0 ml of the above solution was pipetted into a 100 mL volumetric flask and diluted to volume with diluents to obtain the standard concentration of Betamethasone Dipropionate 20 ppm & Standard concentration of Chlorocresol 30 ppm.

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

3.2 gm of sample (equivalent to 2 mg of Betamethasone Dipropionate) was accurately weighed and transferred into 100 ml volumetric flask. 60 mL of diluent was added and warmed on a water bath at 60°C for 30 minutes with intermittent vigorous shaking and diluted the volume. Then filtered through teflon membrane 0.45µ filter to obtain the sample preparation 20 ppm [17-18].

### **Chromatographic conditions for HPLC**

HPLC was performed using a Waters 2695 Alliance system with a 2996 photodiode array detector (PDA) and 2489 UV/Visible detector (UV). The standards as Betamethasone dipropionate and Chlorocresol were resolved on a reverse-phase column Discovery HS, C18, (150 x 4.6mm), 3µ (Mumbai, India). The mobile phase was in a gradient mode which mixed with water and isopropyl alcohol in the ratio 80:20 as mobile phase A. The mixture of Acetonitrile and Tetrahydrofuran was utilized in the ratio of 70:30 as mobile phase B. The mobile phase flow rate was kept at 1.0 ml/min. The selected diluent is 0.1 % Glacial Acetic acid in Methanol. Before the first injection, the column was saturated for 30 min with the initial mobile phase. The temperature was maintained at 50°C. Injection volume was decided to maintain at 20 µL. The PDA was set by optimizing wavelength to give the best response for two peaks at 240 nm to acquire the chromatogram. The standard Betamethasone dipropionate and Chlorocresol were identified by comparing the retention time and spectra obtained from the sample and standard solutions [19-20].

**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:**

Standard solution was injected five times into the HPLC and the chromatograms were recorded. The area counts of Betamethasone and Chlorocresol peaks were measured. The relative standard deviation of five replicate injections for both the peaks should not be more than 2.0%. Equal volumes of the blank (diluent) and sample solution (in duplicate) were injected into the HPLC, the chromatograms were recorded afterwards. The area counts of Betamethasone and Chlorocresol peaks were measured.

**Preparation of Calibration Graph**

For establishing the linearity for Betamethasone Dipropionate & Chlorocresol, a series of standard preparation of Betamethasone Dipropionate & Chlorocresol were prepared to cover a range of 50 % to 150 % of sample concentration i.e, 20 ppm for Betamethasone Dipropionate, based on this the range proposed for Linearity determination is 50% to 150% test concentration (i.e.10 ppm to 30 ppm) for Betamethasone Dipropionate and 30 ppm for Chlorocresol, based on this the range proposed for Linearity determination is 50% to 150% test concentration (i.e. 15 ppm to 45 ppm) for Chlorocresol. A detail of dilutions is given in table 2. The Linearity graph should be plotted from 50% to 150%.

**Preparation of Linearity Stock Solution**

40 mg of Betamethasone Dipropionate 60 mg Chlorocresol working standard was accurately weighed and transferred into two different 100 mL volumetric flask. Add 70 mL of diluent to each and sonicate to dissolve, cool and make up volume with diluent and mix properly to get the stock solution of both.

**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**

The proposed HPLC method was validated in terms of specificity, precision, accuracy, the limit of detection (LOD), the limit of quantification (LOQ), standard solution stability, sample solution stability, and robustness as per the International Conference on Harmonization (ICH Q2 (R1)) guidelines [19-23].

#### **Specificity**

The specificity of the method was studied by assessment of peak purity of Betamethasone dipropionate and Chlorocresol using the Water empower software and diode array detector and represented in terms of purity angle, purity threshold, and purity flag. In which blank solution, standard, sample, spike sample & placebo of Betamethasone Dipropionate were injected, and Percent interference will be determined by comparing the response for any peak detected at the retention time for Betamethasone Dipropionate.

#### **Accuracy**

The accuracy of the method was determined from recovery studies by adding a known amount of each standard at the 80%, 100%, and 120% levels to the pre-analyzed sample

followed by replicate quantitative analyses by the proposed method. Recovery samples were prepared by spiking placebo preparations with known amounts of Betamethasone Dipropionate & Chlorocresol standard in triplicate at three levels (total nine determinations). Each test sample will be prepared as 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**

Precision was studied in terms of system precision, method precision, and intermediate precision.

### **System precision**

System precision was carried out by six replicate injections from the same vial of standard and was expressed in terms of percent relative standard deviation (% RSD) tailing, plate count, and resolution.

### **Method precision**

The sample was analyzed six times by mentioned procedure. One analyst was independently prepared six sample preparations of Betamethasone Dipropionate and analyze as per the method. The % assay for each analyte was expressed in terms of % RSD.

### Intermediate precision

Intermediate precision was performed on different systems, one the Waters e2695 Alliance system with a 2996 PDA and the other a 2489 ultraviolet (UV) detector by different analysts by analyzing six different samples of semisolid dosage form and was expressed in terms of % RSD.

Six standard solutions & six *sample* solutions of Betamethasone Dipropionate of the same lot using a different HPLC system, a different column on a different day will be analyzed.

### Robustness

The robustness of the method was determined by a slight deviation in the method parameters. The parameters were selected based on deviation in column chemistry, wavelength, column temperature, flow rate, and mobile phase gradient. The retention time of Betamethasone dipropionate and Chlorocresol was determined and % RSD using system suitability parameters was observed.

Semisolid formulation was analyzed to determine the contents of Betamethasone dipropionate and Chlorocresol per the method described under chromatographic conditions by HPLC. All analysis was repeated three times and results were expressed in mean  $\pm$  SD.

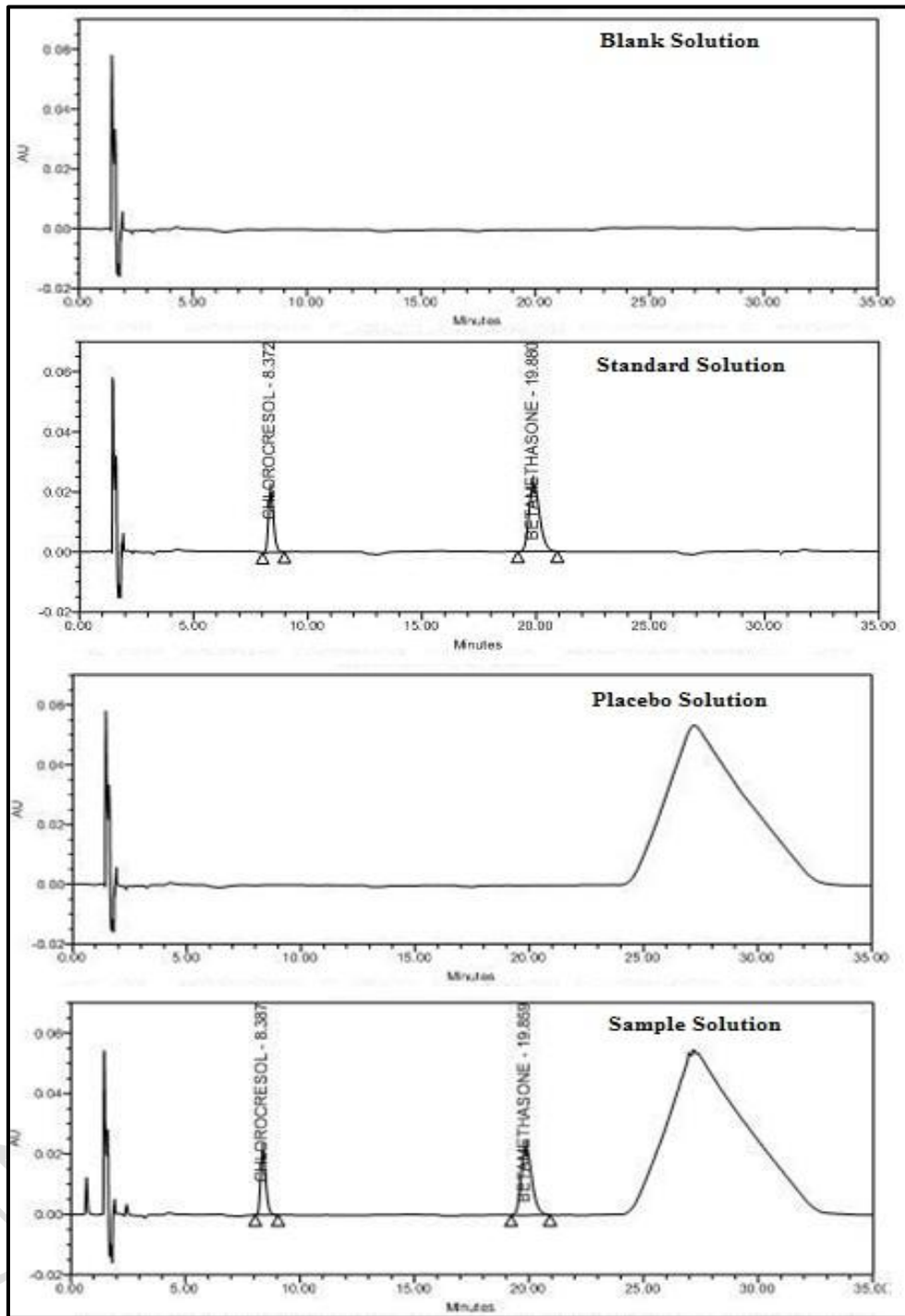
The following modifications to the Chromatographic conditions will be evaluated:

- Change in column Temperature ( $\pm 5^\circ\text{C}$ )
- Change in wavelength ( $\pm 5$  nm)
- Change in Flow rate ( $\pm 0.1$  ml/min) 10% change

### RESULTS AND DISCUSSION

The composition of the mobile phase in the HPLC method was optimized by testing different solvent compositions of varying polarity, column chemistry, column temperature, and pH of mobile phase, and the best results were obtained by using the present method, which produces highly symmetrical peaks showing good resolution between each standard and other peaks [Figure 1]. The scanning wavelength was selected 240 nm to provide comparable results at this wavelength, all analytes showed an optimum response. Betamethasone dipropionate and Chlorocresol were satisfactorily resolved with retention times about 17 and 21 minutes respectively.

Figure 1: Chromatograms for Blank, Standard and Sample



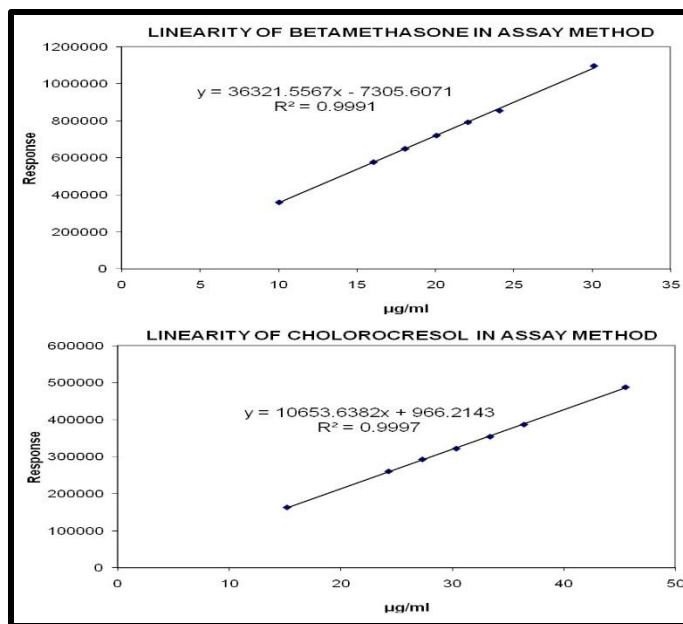
How many times did you run the experiment? Peak is quite wide. Please check the result. Check the software. It should not be like this.

A series of Standard preparations of Chlorocresol and Betamethasone dipropionate Standard was prepared over a range of 50% to 150% of the working concentration of Betamethasone dipropionate and Chlorocresol in Betamethasone dipropionate augmented cream (minimum five points in the range of 80-120% of standard / sample concentration for Assay) [Table 4]. The graph for each standard is given in Figure 2.

**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**



The values of system precision, method, and intermediate precision were given against samples and scanning of peak area expressed in terms of % RSD. For system precision % RSD values were found to be 0.38 and 0.06% for Betamethasone dipropionate and Chlorocresol respectively. Method precision was done and % RSD value was found to be 0.44% and 0.64% for Betamethasone dipropionate and Chlorocresol respectively. For intermediate precision % RSD values between the two analysts were found to be 0.68% and 0.76% for Betamethasone dipropionate and Chlorocresol respectively (Table 5). For the values of system precision, method precision, and intermediate precision, the % RSD values showed that the proposed method provides an acceptable level of system precision, method precision, and intermediate precision.

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

Name of Analyte	Betamethasone dipropionate		Chlorocresol	
	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 peak purity of each analyte was assessed by comparing their respective spectra at peak start, peak apex, and peak end positions of the spot from standard and extracts. The purity angle and purity threshold values are given in table [Table 6].

**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

The given method was optimized by observing robustness. The peak area for each analyte was calculated for each parameter and % RSD was found to be less than 2%. The values of % RSD as shown in Table 7 indicate better robustness of the method.

**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 recovery study was carried out by spiking a known amount of standards into placebo solution at 80%, 100%, and 120% of working concentration. Table 8 indicated that, overall recovery percent were found between 98.0% to 102.0% for Betamethasone dipropionate and Chlorocresol. [22-28].

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

Analyte	Betamethasone dipropionate		Chlorocresol	
Recovery level	% Recovery	Average % Recovery	% Recovery	Average % Recovery
80% - 1	100.2	100.3	99.9	100.3
80% - 2	100.2		100.6	
80% - 3	100.4		100.3	
100% - 1	100.5	100.2	99.8	100.4
100% - 2	99.7		100.9	
100% - 3	100.5		100.6	
120% - 1	100.6	100.4	100.8	100.2
120% - 2	100.1		99.7	
120% - 3	100.5		100.2	
Mean	100.3		100.3	

<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 investigation helped to develop a RP-HPLC-UV-DAD analysis method for Betamethasone dipropionate and Chlorocresol that was validated in terms of linearity, precision, accuracy, specificity, system suitability, and robustness. The described method in this study shows its novelty to determine two ingredients i.e., Betamethasone dipropionate and Chlorocresol at a single wavelength is sufficiently rapid, simple, and sensitive as well as precise and accurate that complies with ICH guidelines. The assay of the two active ingredients was not interfered by the excipients in the Semisolid dosage form. Therefore, the proposed analytical method is recommended for the routine analysis of Betamethasone dipropionate and Chlorocresol as such, or in various dosage forms. In addition, the method can be applied in many developing countries or field stations where advanced analytical equipments are not available.

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