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), 3µ 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^{-1}. 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, accuracyand 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.For use as a disinfectant such as a hand wash, it is commonly dissolved in alcohol in combination 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 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 leachables, 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 forms 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 purpose." It is now obligatory in the process of drug development to supply the validation data for 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 in indicated Semosolid 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 Tetrahydrofuranwere 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 diluentand sonicated to dissolve, then diluted the above solution to volume with diluent and mix. 5.0 ml of the above solution was pipetted outinto a 100 mL volumetric flask and diluted to volume with diluents to obtain the standard concentration of Betamethasone Dipropionate 20ppm & Standard concentration of Chlorocresol30ppm.

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

3.2 gm of sample (equivalent to 2mg of Betamethasone Dipropionate) was accurately weighed and transferred into 100ml 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 up the volume with diluent. Then filtered through teflon membrane 0.45µ filter to obtained the sample preparation 20ppm [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 dipropionateand Chlorocresolwere resolved on a reverse-phase columnDiscovery HS, C18, (150 x 4.6mm), 3µ (Mumbai, India). The mobile phase was in a gradient modewhich wasMixture 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. 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 theblank (diluents) andsample solution (in duplicate) were injected into the HPLC and the chromatograms were recorded. 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 20ppm for Betamethasone Dipropionate, based on this the range proposed for Linearitydetermination is50% 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 forLinearitydetermination is 50% to 150% test concentration (i.e. 15 ppm to 45 ppm) for Chlorocresol. A detail of dilutions is given in2. The Linearity graph should be plotted from 50% to 150%.

Preparation of Linearity Stock Solution

40 mg of Betamethasone Dipropionate 60mg Chlorocresol working standard was accurately weighed and transferred into two different 100mL volumetric flask. Add 70mL 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

	Betamethasone Dipropionate	**Chlorocresol**

% Conc of Sample	Amount of stock solution to be transferred (ml)	Final volume with diluent (ml)	Concent ration (ppm)	Amount of stock solution to be transferred (ml)	Final volume with diluent (ml)	Concentra tion (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 dipropionateand Chlorocresol using the Waters 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 selected were 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 ± SD.

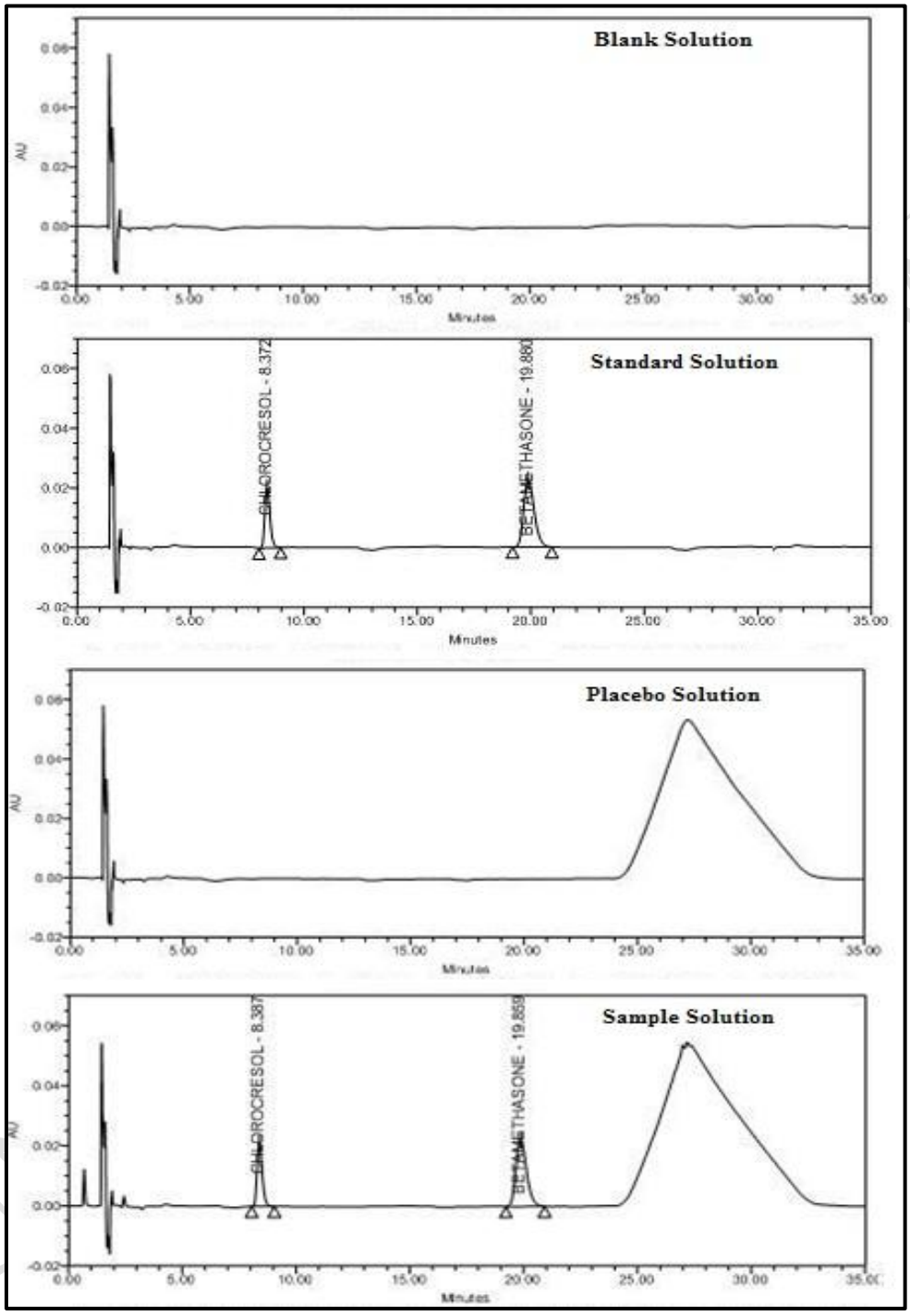
The following modifications to the Chromatographic conditions will be evaluated:

- Change in column Temperature (±5°C)
- Change in wavelength (±5 nm)
- Change in Flow rate (± 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 selected was 240 nm to provide comparable results and at this wavelength, all analytes showed an optimum response. Betamethasone dipropionateand Chlorocresol were satisfactorily resolved with retention times about 17 and 21 minutes respectively.

Figure 1: Chromatograms for Blank, Standard and Sample



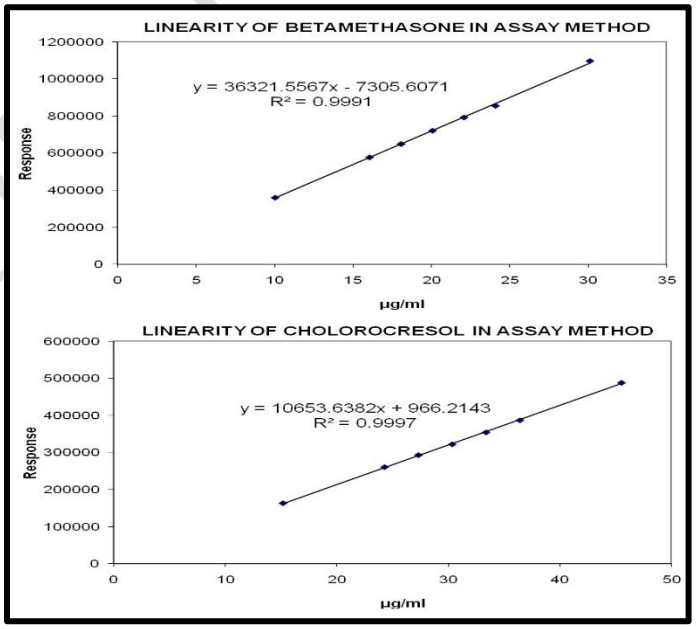
A series of Standard preparations of Chlorocresol and Betamethasone dipropionate Standard was prepared over a range of 50% to 150% of the working concentration of Betamethasone dipropionate and Chlorocresol in Betamethasone dipropionate augmented cream. (Minimum

Five points in the range 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

% Level	Conc. of BTD(ppm)	Average Peak area of BTD	Conc. of CRC (ppm)	Average Peak area of CRC
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
r^2	**0.99956**		**0.99984**	
Slope of Regression line	**36321.6**		**10653.6**	

Figure 2: Linearity graphs for standard



The values of system precision, method precision, and intermediate precision are given against sample application and scanning of peak area and are 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 dipropionateand 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**	
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	**100.5**	**100.2**	**100.7**	**100.0**
% RSD	**0.44**	**0.86**	**0.64**	**0.74**
Overall % RSD	**0.68**		**0.76**	

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**	**Purity**	**Purity**	**Peak**

	Time (Min)	Angle	Threshold	Purity
Blank (diluent)				
Betamethasone dipropionateand Chlorocresol	ND	NA	NA	NA
Standard solution				
Betamethasone dipropionate	19.880	0.222	1.242	Pass
Chlorocresol	8.372	0.310	1.239	Pass
Worst Case Placebo				
Betamethasone dipropionateand Chlorocresol	ND	NA	NA	NA
Sample solution				
Betamethasone dipropionate	19.859	0.242	1.251	Pass
Chlorocresol	8.387	0.241	1.257	Pass

The given method was optimized by doing 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 7indicate 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**	
SD	**0.283**		**0.443**	
% RSD	**0.28**		**0.44**	

CONCLUSION:

The present investigation resulted in the development of an 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 presented method in additionto its novelty for determination of two ingredients i.e. Betamethasone dipropionate and Chlorocresol at single wavelength is sufficiently rapid, simple, and sensitive as wellas precise and accurate that complies with ICH guidelines. The assay of the two active ingredients was not interfered by theexcipients in the Semisolid dosage form.Therefore, the proposed analytical method is recommendedfor the routine analysis of Betamethasone dipropionate and Chlorocresol as such, or in various dosageforms. In addition, the method can be

applied in many developingcountries or field stations where advanced analytical equipment are not available.

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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