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Research Article

A STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF HALOBETASOL PROPIONATE AND P-CHLOROCRESOL IN CREAMS

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Abstract: A simple, selective, accurate high performance liquid chromatographic method was developed and validated for an analysis of Halobetasol Propionate and p-Chlorocresol in pharmaceutical formulations. Chromatographic separation achieved by gradient programme on a C18 column (Waters Novapak C-18, 250 x 4.6mm, 4µm column) utilizing a mobile phases of A (pH 2.5 phosphate buffer) and B (mixture of buffer, acetonitrile and methanol in the ratio of 20:70:10, *v/v/v* respectively) at a flow rate of 1.0 ml/min with UV detection at 240nm. The retention time of Halobetasol Propionate and p-Chlorocresol were6.3 and 8.9 min, respectively. The total run time is 15 minutes. The developed method was validated in terms of accuracy, precision, linearity, specificity, robustness and solution stability.This study aimed at developing and validating an HPLC method, being simple, accurate and selective and the proposed method can be used for the estimation of these two components in pharmaceutical preparations.

Key words: Halobetasol Propionate, p-Chlorocresol, RP-HPLC, Simultaneous determination, Method validation.

INTRODUCTION

Halobetasol Propionate (HP) cream contains Halobetasol Propionate, a synthetic corticosteroid for topical dermatological use. The corticosteroids constitute a class of primarily syntheticsteroids used topically as an anti-inflammatory and antipruritic agent.

Chemically HP is 21-chloro-6α,9-difluoro-11β,17-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione17-propionate, $C_{25}H_{31}ClF_2O_5$ .HP has the molecular weight of 485. It is a white crystalline powder insoluble in water.It has the following structural formula (Fig.1):

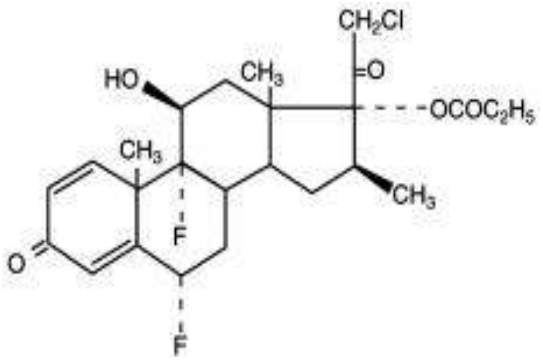

Figure 1: Chemical structure of Halobetasol Propionate

p-Chlorocresol (CC) is a chlorinated phenol which is used as an antiseptic and preservative.The IUPAC name, molecular formula and molecular weight of CC are 4-chloro-3-methylphenol, C_7H_7ClO and 142.58 $gmol^{-1}$ respectively.It forms colourless,dimorphous crystals at room temperature and is only slightly soluble in water. 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. It has the following structural formula (Fig.2):

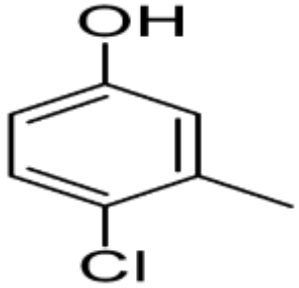

Figure.2: Chemical structure of p-Chlorocresol

Present drug stability test guidance Q1A (R2) issued by international conference on harmonization (ICH) [1] suggest that stress studies should be carried out on a drug product to establish its inherent stability characteristics, leading to identification of degradation products and hence supporting the suitability of the proposed analytical procedures. It also requires that analytical test procedures for stability samples should be stability indicating and they should be fully validated. Accordingly, the aim of the present study was to establish inherent stability of

Halobetasol Propionate and p-Chlorocresol through stress studies under a variety ofICH recommended test conditions [1] and to develop a rapid stability-indicating reverse phase assay method [2].

Literature survey reveals that a variety of spectrophotometric and chromatographicmethods have been reported [3-7].Whereas no liquid chromatography method has been reported for simultaneous quantitative determination of HP and CC increams (Tab.1).

Present study involves development and validation of a stability indicating liquid chromatographic method for the determination of HP and CC in pharmaceutical cream formulations.

Table.1: Comparison of the performance characteristics of the present method with the published methods.

S.No.	Method	Estimated compounds	Test	Product	References
1.	RP-HPLC	Halobetasol Propionate &Fusidic Acid	Impurities	Cream	[3]
2.	RP-HPLC	Chlorocresol, MometasoneFuroate&Fusidic Acid	Assay	Cream	[4]
3.	RP-HPLC	Betamethasone Dipropionate&Chlorocresol,	Assay & impurities	Cream & Ointment	[5]
4.	Spectrophotometric method	Clobetasol propionate, Halobetasol propionate, Quinagolide HCl	Assay	Formulation	[6]
5.	MS,NMR & XRD	Halobetasol Propionate	Impurities	API	[7]
6.	RP-HPLC	Halobetasolpropionate &Fusidic acid	Assay	Ointment	[8]
7.	RP-HPLC	Halobetasol propionate &Chlorocresol	Assay	Cream	present work

EXPERIMENTAL

Chemicals and reagents

Standards (Halobetasol Propionate and p-Chlorocresol) and cream samples (Halobetasol Propionate 0.05% cream) were supplied by Dr. Reddy's laboratories limited, Hyderabad, India. The HPLC grade acetonitrile, methanol, tetrahydrofuranand analytical grade potassium dihydrogen phosphate, ortho phosphoric acid, hydrochloric acid, hydrogen peroxide, sodium hydroxide pellets were purchased from Merck, India. Water was prepared by using Millipore Milli-Q Plus water purification system.

Instrumentation

Waters Alliance HPLC system with photodiode array detector (Waters, USA) used consisting of a quaternary solvent manager, a sample manager and a UV detector. The out- put signal was monitored and processed using Empower Software, water bath equipped with MV controller (Julabo, Seelbach, Germany) was used for hydrolysis studies. Photo stability studies were carried out in a photo stability chamber (Sanyo, Leicestershire, UK). Thermal stability studies were performed in a dry air oven (MACK Pharmatech, Hyderabad, India).

Chromatographic conditions

The chromatographic column used was Waters Novapak C-18, 250 x 4.6mm, 4µm particle size column. The mobile phase A contains phosphate buffer with pH 2.5 (0.012M) and mobile phase B contains a mixture of phosphate buffer pH 2.5(0.012M): acetonitrile: methanol (20:70:10, v/v/v). The gradient programme carried out (%B) as follows time 0.01 min (55%), 2 min (60%), 5 min (90%), 8 min (80%), 12 min (55%), 15 min (55%). The flow rate was 1.0 mL min^{-1} and the injection volume was 100µL. The temperatures of column and sample were maintained at 40°C and 20°C, respectively. The total run time was 15minutes. The detection wavelength was 240 nm for Halobetasol Propionate and p-Chlorocresol. The diluent is tetrahydrofuran followed by a mixture of water and acetonitrile in the ratio 50:50, v/v respectively.

Preparation of standard solutions

Standard stock solutions of Halobetasol Propionate and p-Chlorocresol (0.025 mg/mL of HP, 0.25 mg/mL of CC) were prepared by dissolving an appropriate amount of the compounds in tetrahydrofuran. Working solutions HP and CC (0.0025 mg/mL of HP and 0.005 mg/ mL of CC)

were prepared from above stock solution in diluent for assay determination.

Preparation of sample solution

Transferred 2.5g of cream sample into a 100 mL volumetric flask, added 50 mL of tetrahydrofuran and extracted drug materials into tetrahydrofuran by using ultrasonicssonicator apparatus with intermediate shaking. Cooled at room temperature and dilute to volume with diluent and mix well. Transferred 5mL of above solution into 25 mL flask and made up to volume with diluent (0.0025 mg/mL of HP and 0.005 mg/mL of CC). Filter a portion a sample solution through 0.45µm membrane PVDF filter, use clear solution discarding few mL of the filtrate.

RESULTS AND DISCUSSION

Chromatographic method development

The wavelength has been finalized based on the spectra of UV spectrometry for Halobetasol Propionate and p-Chlorocresol. The selected final wavelength (λmax) was 240nm (Fig.3).

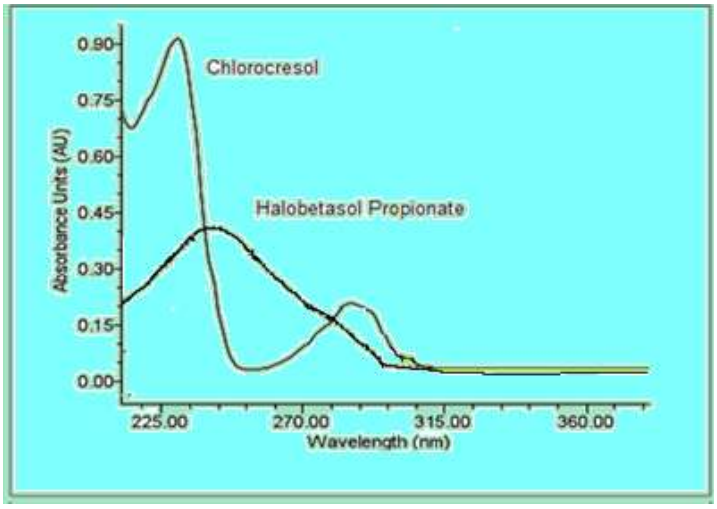

Figure.3: A typical UV spectrum ofHP and CC

The method was optimized to separate major degradation products formed under different stress conditions. The main target of the chromatographic methodis to get the separation for closely eluting degradation products, mainly the degradation product that is eluting between HP and CC. The degradation samples were run using different stationary phases like C18, C8, and Mobile phases containing buffers like sulphate, perchlorate and phosphate with different pH (2.3–7.0) and using organic modifiers like acetonitrile and methanol in the mobile phase. The isocratic method was not working since HP and CC peaks were not separated with a proper resolution and also thedegradents of HP were not separated from the actives. Hence the method is optimized with a gradient programme. As the concentration of HPis very less as compared to CC, so that to improve response, resolution and peak shapes the method was tried at different column temperatures. But the separation, response of peaks and peak shapes were satisfactory in the adopted chromatographic conditions only. It indicated that the gradient programme method with acetonitrile and methanolas organic modifiers in mobile phase Bwas successful in separating drugs and all chromatographic degradation products (Fig.4-6).

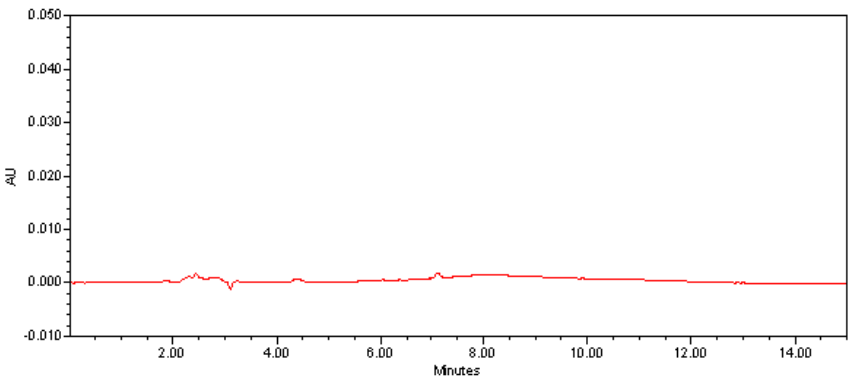

Figure.4: A typical chromatogram of diluent

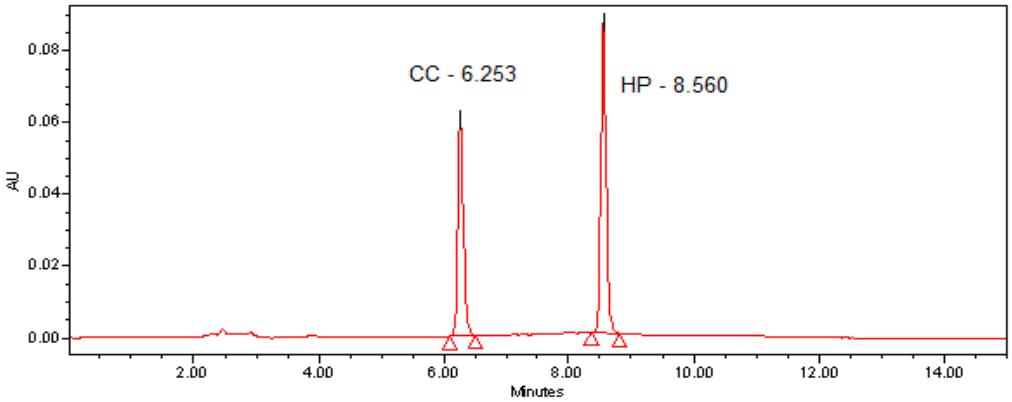

Figure.5: A typical chromatogram of standard

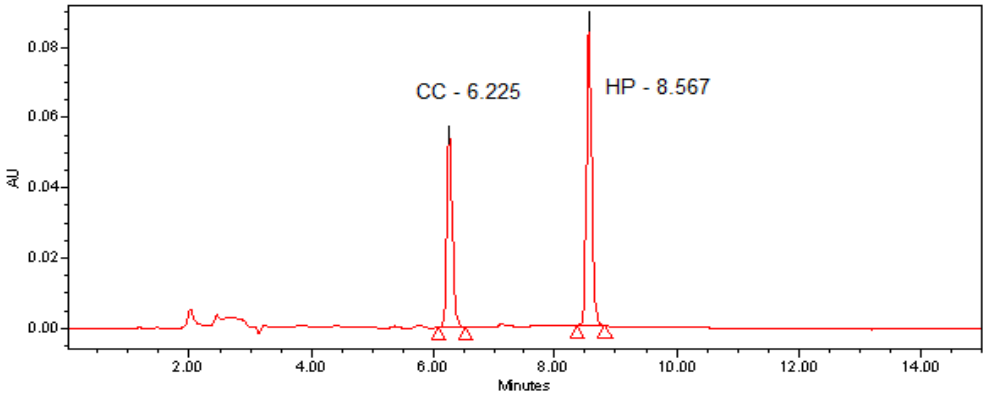

Figure.6: A typical chromatogram of finished product

Forced degradation studies

Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method. Stress testing of a drug substance can help to identify the likely degradation products, which can help to establish the degradation pathways and the intrinsic stability of the molecule.All solutions for use in stress studies were prepared at an initial concentrationand refluxed. All samples were then diluted in diluent to give a final concentration (0.0025mg/mL of HP, 0.005 mg/ mL of CC) and filtered before injection (Tab.2).

Table.2: The results of degradation study

Name of the study	Name of the condition	% Degradation	
		HP	CC
Acid	Refluxed with 0.5N HCl at 60°C for 30 min on water bath and neutralized with 0.5N NaoOH.	8.9	7.2
Alkali	Refluxed with 1N NaOH at 60°C for 15 min on water bath and and neutralized with 1N HCl.	26	0.5
Oxidation	Refluxed with10% H_2O_2 at 60°C for 20 min on water bath and neutralized with water.	1.6	5.2
Photo-Sun light	Exposed to 1.2 million lux hours.	9	13
Photo-UV light	Exposed to 200 watt-hours/square meters.	14.8	9.1
Thermal	Exposed to heat at 60ºC for 12 hours.	1.4	1.9
Humidity	Exposed to 90% RH-25ºC for 7 days.	4.1	26.6
Hydrolysis	Refluxed with water at 60°C for 60 min on water bath.	0.5	12.5

Acid and alkali degradation studies
Acid degradation study has been carried out with0.5N HCl at 60°C for 30 min on water bath and neutralized with 0.5N NaoOH. Similarly stress studies in alkaline conditions were conducted by refluxing with 1N NaOH at 60°C for 15 min on water bath and and neutralized with 1N HCl.

Oxidationdegradation study
Solutions for oxidative stress studies were prepared by reflexing with 10%H_2O_2 at 60°C for 20 min on water bath, neutralized with water and analysed.

Thermal degradation study
For thermal stress study, the finished product has been exposed to heat in vacuum hot air oven at 60ºC for 12 hours and analyzed.

Photo stabilitydegradation study
Photo degradation studies were carried out at according to option 2 of Q1B in ICH guidelines [2].The drug product was exposed to UV (200 watt-hours/square meters), fluorescent light (1.2 million lux hours.)and analyzed.

Humidity degradation study
The drug product exposed to 90% RH-25ºC for 7 days humidity, analyzed and injected into liquid chromatograph.

Hydrolysis degradation study
The drug product was refluxed with waterat 60°C for 60 min on water bathanalyzed and injected into liquid chromatograph.

Method validation
The method was validated for the following parameters: system suitability, precision, specificity,linearity, accuracy, robustness and solution stability [2].

System suitability
The system suitability solution was injected into liquid chromatograph.The system suitability test was performed to ensure that the complete testing system was suitable for the intended application. The parameters measured were peak area, retention time, tailing factor, and theoretical plates. In all measurements the peak area varied lesser than 2.0%, the average retention time was 6.3min and 8.6 min, relative standard deviation (%RSD) was 0.1, theoretical plates were less than 3000 and tailing factor was 1.1 (less than 1.5) for HP and CC peaks. The proposed method offers high sensitivity and both peaks can be detected accurately. In all the cases, the both peaks were well separated from the degradation products (Tab.3).

Table.3: The results of system suitability parameters

S.No.	System suitability parameter	Observed value	
		HP	CC
1.	Tailing factor	1.1	1.1
2.	Theoretical plates	38895	15424
3.	%RSD	0.1	0.1

Precision
The precision of an analytical method is the degree of agreement among the individual test results when the method is applied repeatedly to multiple samplings of a homogeneous sample [2].
The intra-day precision of the assay method was evaluated by carrying out 6 independent assays of a test sample of HP and CC. The % RSD obtained assay of assay from six preparations. The inter–day precision study was performed on two different days. (i.e. day 1 and day 2). The % RSD of six obtained assay values on two different days was calculated (Tab.4).

Table.4: The results of precision study

Sample No.	Intra - day %Assay		Inter - day %Assay	
	HP	CC	HP	CC
1.	101	103	99	102
2.	100	101	99	102
3.	101	101	100	103
4.	100	101	100	101
5.	102	102	99	101
6.	101	102	99	102
Average	101	102	99	102
%RSD	0.6	0.8	0.4	0.6

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present [2].

The placebo sample was prepared and injected into to liquid chromatograph. No, peak found at the retention time of the HP and CC. This indicates that excipients which are used in the formulation did not interfere with HP and CC. The chromatographic purity of the both components was passed and HP and CC peaks did not have any flag in purity results table (For Waters Empower software). The criteria of purity angle less than purity threshold was found to be satisfactory (Fig.7a-c).

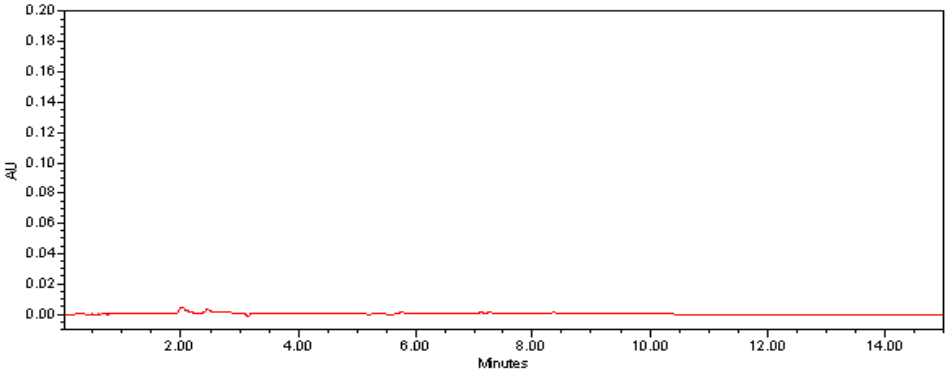

Figure.7a: A typical chromatogram of placebo

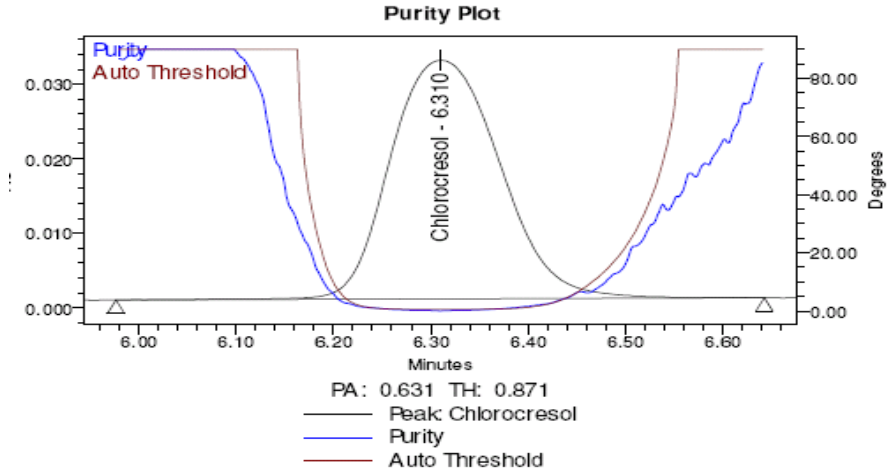

Figure.7b:A typical peak purity chromatogram of CC

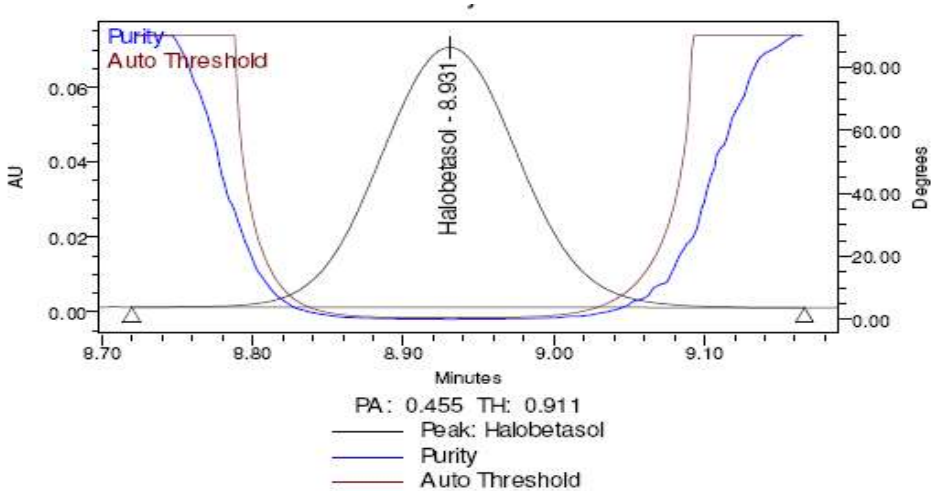

Figure.7c:A typical peak purity chromatogram of HP

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analytein the sample [2].Linearity test solutions for the assay method were prepared from a stock solution at five different concentration levels (50%, 75%, 100% 125% and 150%) of the target assay concentration of HP and CC (1.26, 1.89, 2.52, 3.15 and 3.78 mg/mL for HP and 2.45, 3.68, 4.92,6.14 and 7.36mg/mL). 100 µLof each solution was injected into the HPLC system and the peak area of the chromatogram obtained was noted (Tab.5a-b and Fig.8a-b).

Table.5a: Linearity results of Halobetasol Propionate

Sr. No.	Concentration in %	Concentration in µg/mL	Area
1	50	1.26115	278031
2	75	1.89173	418576
3	100	2.52231	557737
4	125	3.15288	698502
5	150	3.78346	833117
Correlation coefficient			0.9999
Slope			220448.573
Y-Intercept			1153.8422
Bias at 100% level			0.2

Table.5b: Linearity results of Chlorocresol

Sr. No.	Concentration in %	Concentration in µg/mL	Area
1	50	2.45492	157710
2	75	3.68238	235275
3	100	4.90984	313253
4	125	6.1373	391086
5	150	7.36476	468092
Correlation coefficient		0.9999	
Slope		63266.8274	
Y-Intercept		2453.2	
Bias at 100% level		0.8	

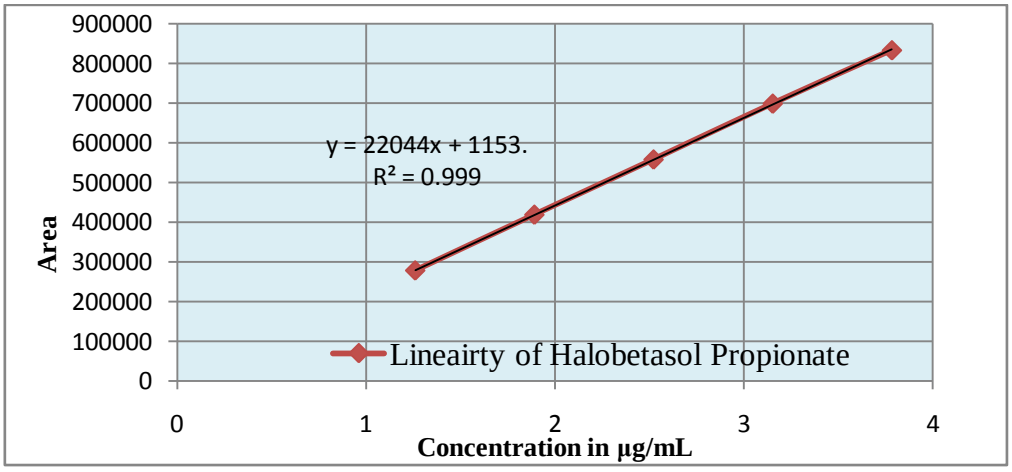

Figure.8a: A linearity graph of Halobetasol Propionate

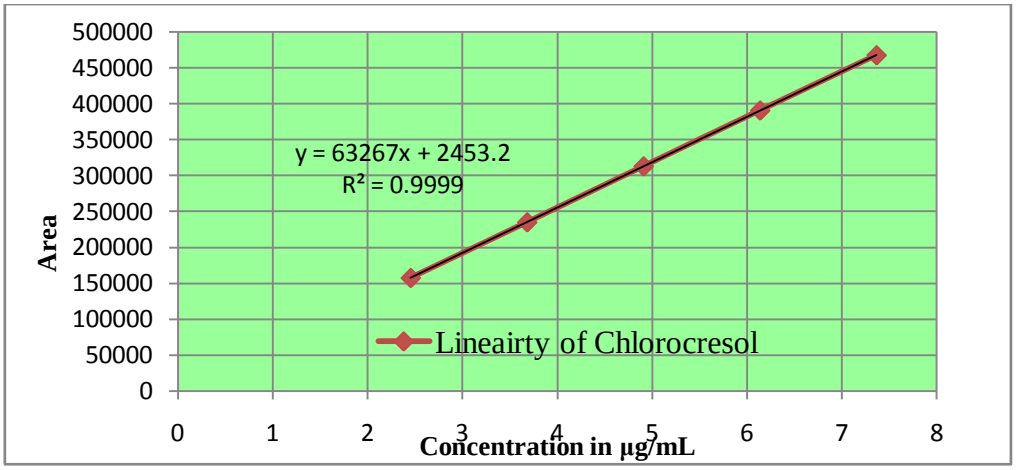

Figure.8b: A linearity graph of Chlorocresol

The solutions extracted from the marketed formulations were injected into the HPLC system and the peak area of the chromatograms was noted. The peak area versus concentration data was analyzed with least squares linear regression. The slope and y-intercept of the calibration curve was reported.

Accuracy

The accuracy of an analytical method is closeness of test results obtained by that method to the true value. The accuracy of an analytical method should be established across its range [2].The accuracy of the assay method was evaluated in triplicate at five concentration levels (50%, 75%, 100% 125% and 150%) and the percentage recoveries were calculated. The recovery experiments were conducted to determine the accuracy of the method for the quantification of HP and CC in the drug product. The study was carried out in triplicate at 1.26, 1.90, 2.53, 3.16, 1nd 3.79 mg/mL and 2.59, 3.88, 5.18, 6.47 and 7.77 mg/mL. The percentage recovery in each case was calculated(Tab.6a-b).

Table.6a: Accuracy results of Halobetasol Propionate

Spike level	Mean ‘µg/mL’ added	Mean ‘µg/mL’ found	Mean Recovery
50%	1.26	1.24	98.0
75%	1.90	1.90	100.0
100%	2.53	2.5	98.9
125%	3.16	3.23	102.4
150%	3.79	3.82	100.7

Table.6b: Accuracy results of Chlorocresol

Spike level	Mean ‘µg/mL’ added	Mean ‘µg/mL’ found	Mean Recovery
50%	2.59	2.62	101.4
75%	3.88	3.84	98.9
100%	5.18	5.21	100.6
125%	6.47	6.39	98.8
150%	7.77	7.65	98.6

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage [2].

The robustness of the assay method was established by introducing small changes in the HPLC conditions which included wavelength (240nm), percentage of methanol and acetonitrile in the mobile phase (±10%), flow rate (± 0.2mL/min) and pH (± 0.2). Robustness of the method was studied using five replicate injections of standard at a concentration level of 0.0025 mg/mL of HP and 0.005 mg/mL of CC.

Solution stability and mobile phase stability

The ruggedness test of analytical assay method is d efined as the degree of reproducibility of test result s obtained by the analysis of the same samples und er a variety of normal test conditions.

The solution stability of HP and CC in the assay method was carried out by leaving both the sample and reference standard solutions in tightly capped volumetric flasks at room temperature for 48 hours. The same sample solutions were assayed at 12 hours intervals over the study period. The mobile phase stability was also assessed by assaying the freshly prepared sample solutions against freshly prepared reference standard solutions at 12 hours intervals up to 48 hours. The prepared mobile phase remained constant during the study period. The %RSD of the HP and CC assay was calculated for the mobile phase and solution stability experiments. An additional study was carried out using the stock solution by storing it in a tightly capped volumetric flask at room temperature.

CONCLUSION

A stability-indicating HPLC method was developed, validated and applied for the simultaneous determination of Halobetasol Propionate and p-Chlorocresol in pharmaceutical creams. The developed method was validated as per ICH guidelines and was found to be accurate, precise, robust and specific. The chromatographic elution step is undertaken in a short time. No, interference from any components of pharmaceutical formulation or degradation products was observed and the method has been successfully used to perform long-term and accelerate stability studies of Halobetasol Propionate and p-Chlorocresol formulations.

Acknowledgement

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