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Simultaneous Determination of Clobetasol (as Propionate) and Chlorocresol in Cream by Stability Indicating RP-HPLC Method

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ABSTRACT

A stability-indicating reverse phase-HPLC method has been developed and validated for the simultaneous determination of Clobetasol propionate (CLOB) and Chlorocresol (CHLOR) in pharmaceutical dosage form (cream). Beclomethasone dipropionate monohydrate (BEC) was used as internal standard. The column (Luna) C_{18} (250 mm × 4.6 mm, 5µm particle size) was used for chromatographic separation. The gradient elution system was performed using mobile phase A: Ammonium dihydrogen phosphate pH 4.5 and methanol in the ratio of 80:30 respectively, and Acetonitrile as mobile phase B. The flow rate was adjusted to 1.5 ml/min. The column oven was set at 25°C and the detection wavelength at 240nm. The retention times of CHLOR, CLOB and BEC were found to be 8.86 min, 13.36 min and 15.6 min respectively. The linearity was performed in the concentration ranges of 64.0-96.0µg/ml (CHLOR), 32.0-48.0µg/ml (CLOB), and 80µg/ml (BEC) with squared correlation coefficient of 0.9995 and 0.9996 for CHLOR and CLOB respectively. The percentage purity of CHLOR and CLOB was found to be > 99.5 %. The analytical method has been validated for specificity, linearity, precision, accuracy, ruggedness and robustness which were found to be within the acceptance limit according to ICH guidelines. The developed method was successfully employed for routine and stability analysis of CHLOR and CLOB in pharmaceutical cream.

Keywords: Chlorocresol, Clobetasol propionate, Beclomethasone dipropionate, Stability-Indicating, RP-HPLC, Validation.

INTRODUCTION

Chlorocresol (CHLOR), 4-Chloro-3-methylphenol [Fig. 1] is a preservative. Its molecular weight is 142.6g/mol with an empirical formula C_7H_7ClO. [1] CHLOR is a bactericide, closely related to carbolic acid used in the formulation of creams and ointments. [2] It is used as preservative in a large number of commercially available products and preparations such as metal working fluids, construction materials and glue. [3-4] It forms colorless, dimorphous crystals at room temperature and is only slightly soluble in water. [5] Very few spectrophotometer methods were reported for determination of chlorocresol. [6] Also few HPLC methods were found for the determination of chlorocresol. [2-5, 7-8] Clobetasol propionate (CLOB), Chemically described as 21-Chloro-9-fluoro-11β-hydroxy-16 β-methyl-3,20-dioxopregna-1,4-dien-17-yl propanoate [Fig. 2] is glucocorticoid. Its molecular weight is 467.0g/mol, with empirical formula $C_{25}H_{32}ClFO_5$. [1] Clobetasol propionate is a potent topical glucocorticoid, white to almost white crystalline powder that is practically insoluble in water, freely soluble in acetone and in dichloromethane and sparingly soluble in ethanol. [9] It is about 1000 times more potent than hydrocortisone. [10] Few HPLC methods have been reported for the estimation of clobetasol. [9-14] In the present work we are focused on to achieve the optimum chromatographic conditions for the simultaneous determination of CHLOR and CLOB in the topical preparations. Beclomethasone dipropionate (BEC), 9-Chloro-11β-hydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-17,21-diyl dipropanoate [Fig. 3] is glucocorticid. Its molecular weight is 521.1 g/mol, and its empirical formula is $C_{28}H_{37}ClO_7$. [1] The developed method can be applied successfully to quality control and stability testing purposes. To access the reproducibility and wide applicability of the developed method, it was validated as per ICH guidelines, which is mandatory also. [12-13, 15]

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MATERIALS AND METHODS

Reagents and Chemicals

Chlorocresol was purchased from SIGMA-ALDRICH, Clobetasol propionate from SYMBIOTICA and BEC Was purchased from USP. All chemicals used of HPLC grade: Acetonitrile and Methanol were purchased from J.T. Baker, Ammonium dihydrogen phosphate and phosphoric acid was purchased from Merck. Water used was freshly prepared by Sama Pharmaceuticals Manufacturing Co.

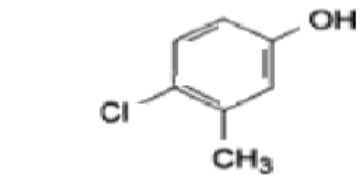

Fig. 1: Chemical Structure for Chlorocresol

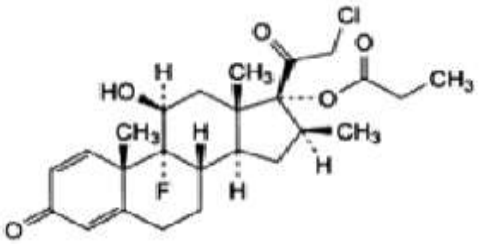

Fig. 2: Chemical Structure for Clobetasol propionate

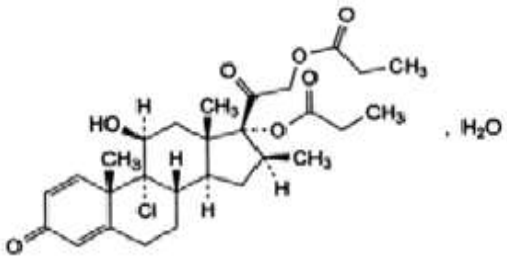

Fig. 3: Chemical Structure for Beclomethasone dipropionate monohydrate

Table 1: Gradient Elution System

Time(min)	Mobile phase A %	Mobile phase B %	Flow rate (ml/min)
0	80	20	1.5
10	40	60	1.5
18	40	60	1.5
20	80	20	1.5
22	80	20	1.5

Equipment and Chromatographic Conditions

A Dionex UltiMate 3000 HPLC system with Chromeleon software "version 6.8" and Photodiode Array Detector was used. It was manufactured by Dionex Corporation Company, USA. The column (Luna) C_{18} (250 mm × 4.6 mm, 5µm particle size) was used for analytical separation. The mobile phase consisted of mobile phase A: Ammonium dihydrogen phosphate buffer pH 4.5 and methanol in the ratio of 80:30, and mobile phase B: Acetonitrile. The gradient elution system used was as described in Table 1. The flow rate was adjusted to 1.5 ml/min, column oven was operated at 25°C, UV detection was achieved at 240nm and the injection volume was set to 20µL. Purity analysis was performed over a wavelength range of 200-400nm.

Preparation of Analytical Solutions

Preparation of Ammonium dihydrogen phosphate buffer

Prepared by dissolving 3.0 g of ammonium dihydrogen phosphate in 1000ml of distilled water, the pH was adjusted to 4.5 with 5% ammonium hydroxide solution or 5% phosphoric acid.

Preparation of mobile phase A

Mobile phase A was prepared by mixing 300 ml of Methanol and 800 ml of ammonium dihydrogen phosphate. Filtered through 0.45µ filter under vacuum filtration and degassed in ultrasonic water bath for 2 minutes.

Preparation of mobile phase B

Acetonitrile for HPLC filtered through 0.45µ filter under vacuum filtration and degassed in ultrasonic water bath for 2 minute.

Preparation of BEC internal standard solution

The BEC internal standard solution was prepared by dissolving 20 mg of USP BEC in 50 ml of methanol and filtered using 0.45µ filter to obtain a solution having a concentration of 0.4 mg/ml.

Preparation of system suitability solution

The system suitability solution was prepared by transferring an equivalent to 80 mg of CHLOR standard, an equivalent to 40 mg of CLOB and 1 mg of clobetasol related compound A to 100 ml volumetric flask, 10 ml of the resulting solution were diluted to 20 ml with methanol and mixed. 5 ml of the resulting solution and 5 ml of internal standard were transferred to 25 ml volumetric flask and diluted to volume with methanol. Mixed and filtered through 0.45µ filter.

Preparation of CHLOR and CLOB standard solution

The solution was prepared by dissolving an equivalent to 20 mg CLOB and equivalent to 40 mg of CHLOR in 100 ml of methanol. 5 ml of the resulting solution and 5ml of internal standard were transferred to 25 ml volumetric flask and completed to volume with methanol. Mixed and filtered through 0.45µ filter.

Preparation of sample solution

2.0 g of cream equivalent to 1.0 mg of CLOB and 2.0 mg of CHLOR were transferred to 50ml centrifuge tube. 10 ml of methanol were added and shook vigorously to dissolve the cream. Centrifuged at 6000 rpm for 10 minutes and the supernatant was decanted to 25 ml volumetric flask. The procedure was repeated with another 10 ml of methanol. 5 ml of internal standard were added and the volume was completed with methanol and mixed. The solution was passed through 0.45-µm filter to obtain a solution having a concentration of 0.04 mg/ml CLOB, 0.08 mg/ml of CHLOR and 0.08 mg/ml of BEC.

HPLC-Method Development and Validation of

The analytical variables parameters tested were including specificity, linearity, precision, accuracy, and system suitability.

Specificity of analytical method and peak purity

The specificity and peak purity were carried out to determine whether there were any interference due to presence of impurities, degradation products or other components that may be present in retention time of analytical peaks and affect the peak purity and specificity of the analytical method. Forced degradation studies were carried out by using 2M HCl, 0.5M NaOH, thermal degradation, Hydrogen peroxide and Photo degradation.

Linearity

The linearity of the method was established by spiking a series of sample mixtures of CHLOR and CLOB. The solutions of five different concentration levels 64.0-96.0µg/ml (CHLOR), 32.0-48.0µg/ml (CLOB), and 80µg/ml (BEC) were injected into the HPLC system. The calibration curve was constructed for the standard solutions by plotting CHLOR concentrations against their corresponding peak areas and CLOB concentrations against their corresponding CLOB to BEC peak area ratios. Linear regressions were applied and slope-a, intercept-b and correlation coefficient-R^2 were determined.

Precision and Ruggedness

Six independent sample preparations from a single lot of formulation 80.0µg/ml for CHLOR, 40µg/ml for CLOB and 80µg/ml for BEC were injected into HPLC system, the retention times and peak areas were determined and the mean

and % RSD were calculated while ruggedness was determined by injecting six independent preparations prepared by another analyst into another HPLC system. The retention times and peak areas were obtained and the mean and % RSD were calculated.

Accuracy (per cent recovery)

The accuracy study was performed on 80%, 100% and 120% of the analytical method target concentration of CHLOR and CLOB. Standard and sample solutions were injected into HPLC system in triplicate and percentage recoveries of CHLOR and CLOB were calculated. The areas of CHLOR and the ratio of peaks areas of CLOB to BEC at each level were used for calculation of % recovery.

Robustness

Robustness of the developed analytical method was tested by evaluating the effect of small variations in analytical method parameters such as changing in flow rate (± 6.6%), changing in column temperature (± 5°C), changing in wavelength (± 5nm) and the ratio of mobile phase B (±5%).

System suitability

System suitability test was carried out on freshly prepared standard solution of CHLOR, CLOB, Clobetasol related compound A and BEC and the values were recorded.

RESULTS AND DISCUSSION

The present analytical method is a new RP-HPLC method for the simultaneous determination of CHLOR and CLOB.

Table 2: Linearity results

CHLOR		(CLOB/BEC)	
CHLOR Conc. µg/ml	**peak area (mAu*min)**	**CLOB Conc. µg/ml**	**peak area ratio**
64	9.583	32	0.47350
72	10.637	36	0.53046
80	11.717	40	0.58180
88	12.844	44	0.64204
96	14.024	48	0.69624
R^2	0.9995	**R^2**	0.9996
Slope-a	0.1386	**Slope-a**	0.0139
y-intercept	0.672	**y-intercept**	0.0277

Table 3: System precision results

	CHLOR		(CLOB/BEC)	
	CHLOR retention time (min)	**peak area (mAu*min)**	**CLOB retention time (min)**	**peak area ratio**
Statistics	8.86	11.661	13.37	0.579483
	8.86	11.781	13.36	0.579843
	8.86	11.861	13.37	0.578804
	8.86	11.846	13.37	0.579701
	8.86	11.884	13.37	0.580200
	8.87	11.901	13.37	0.580375
Average	8.861667	11.82233	13.36833	0.579734
St. Dev.	0.004082	0.0892	0.004082	0.00056
% RSD	0.046	0.755	0.031	0.097

Table 4: Method precision results

	CHLOR		(CLOB/BEC)	
	CHLOR retention time (min)	**peak area (mAu*min)**	**CLOB retention time (min)**	**peak area ratio**
Statistics	8.87	11.671	13.36	0.578793
	8.86	11.821	13.36	0.578152
	8.87	11.801	13.37	0.581156
	8.86	11.846	13.37	0.578871
	8.86	11.794	13.37	0.579567
	8.86	11.891	13.37	0.581104
Average	8.863333	11.804	13.36667	0.579607
St. Dev.	0.005164	0.074054	0.005164	0.001262
% RSD	0.058	0.627	0.039	0.218

Table 5: Ruggedness results

	CHLOR		(CLOB/BEC)	
	CHLOR retention time (min)	**peak area (mAu*min)**	**CLOB retention time (min)**	**peak area ratio**
Statistics	8.87	11.907	13.38	0.582506
	8.88	11.937	13.39	0.579961
	8.88	11.952	13.39	0.581935
	8.88	11.904	13. 40	0.582296
	8.89	11.920	13.40	0.580998
	8.89	11.947	13.41	0.579757
Average	8.881667	11.92783	13.395	0.581242
St. Dev.	0.007528	0.020488	0.010488	0.001191
% RSD	0.085	0.172	0.078	0.205

Table 6: % Recovery for CHLOR

Concentration at specific level	Active drug added (mg)	Recovered amount (mg)	Mean recovered
80%	64	64.60	
100%	80	80.25	100.4%
120%	96	96.2	

Table 7: % Recovery for CLOB

Concentration at specific level	Active drug added (mg)	Recovered amount (mg)	Mean recovered
80%	32	32.08	
100%	40	39.9	100.15%
120%	48	48.2	

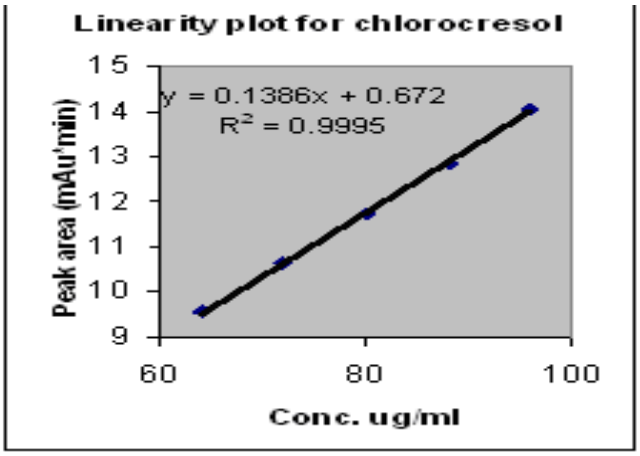

Fig. 4: Linerity plot for CHLOR

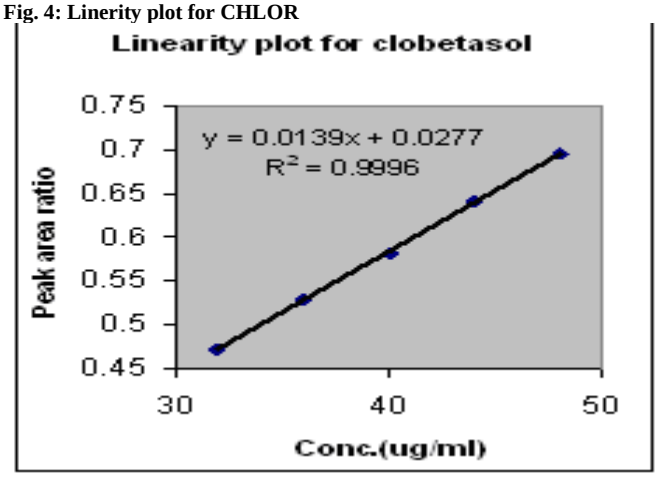

Fig. 5: Linerity plot for CLOB

Mobile phase A: Ammonium dihydrogen phosphate pH 4.5 and methanol in the ratio of 80:30 respectively), and Acetonitrile as mobile phase B in a gradient elution as described in Table 1. The column (Luna) C_{18} (250 mm × 4.6 mm, 5µm particle size) was used for chromatographic separation. The flow rate was adjusted to 1.5 ml/min, column oven was operated at 25°C and the injection volume was set

to 20µL. The linearity was determined as linearity regression of the claimed analyte concentrations of the ranges 64.0-96.0µg/ml (CHLOR), 32.0-48.0µg/ml (CLOB), and 80µg/ml (BEC). The calibration curves were obtained by plotting CHLOR concentrations against peak areas and CLOB concentrations against ratios of peaks areas for CLOB to BEC. Linearity results, exhibited in Table 2 were linear and the squared correlation coefficients were found to be of 0.9995 and 0.9996 for CHLOR and CLOB respectively. The purity analysis was performed over a wavelength range of 200-400nm.

The % Relative Standard Deviation for system precision exhibited in Table 3 was found to be 0.046 and 0.097, the % Relative Standard Deviation for method precision exhibited in Table 4 was found to be 0.058 and 0.218 and the % Relative Standard Deviation for ruggedness exhibited in Table 5 was found to be 0.172 and 0.205 for CHLOR and CLOB respectively. The accuracy study was performed on 80%, 100% and 120% of the target concentration. The percentage recoveries were found to be 100.4 % and 100.15% for CHLOR and CLOB respectively as exhibited in Tables 6 & 7.

Table 8: Robustness results

Analytical Parameter	Adjusted to	CHLOR		(CLOB/BEC)	
		CHLOR retention time	peak area (mAU.min)	CLOB retention time	peak area ratio
Column Temp. (ºC)	20	9.11	12.118	13.7	0.580335
	25	8.86	11.861	13.37	0.578804
	30	8.70	11.872	13.15	0.577139
Flow rate (ml/min)	1.4	9.20	12.835	13.83	0.57840
	1.5	8.86	11.861	13.37	0.578804
	1.6	8.56	11.216	13.04	0.580645
Wavelength	235	8.89	31.366	13.41	0.585273
	240	8.89	11.947	13.41	0.579757
	245	8.89	3.360	13.41	0.581194
Max. Mobile phase B (%)	55	9.36	11.936	15.02	0.577776
	60	8.86	11.861	13.37	0.578804
	65	8.52	12.001	12.39	0.580613

Table 9: System suitability values

CHLOR		CLOB		CLOB Rel. Comp A		BEC	
Theoretical plates	Tailing factor	Theoretical plates	Tailing factor	Theoretical plates	Tailing factor	Theoretical plates	Tailing factor
50388	1.16	76070	1.19	65081	1.02	54470	1.160
Resolution between CHLOR and CLOB						25.9	
Resolution between CLOB and CLOB Rel Comp A						2.8	
Resolution between CLOB Related Compound A and BEC						7.0	

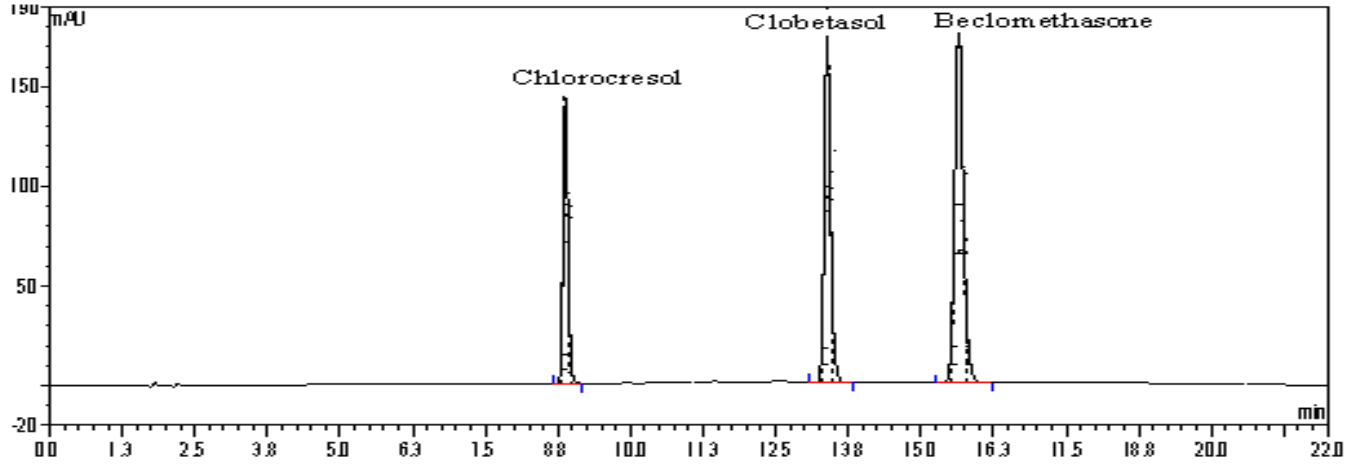

Fig. 7: Chromatogram for Standard Solution

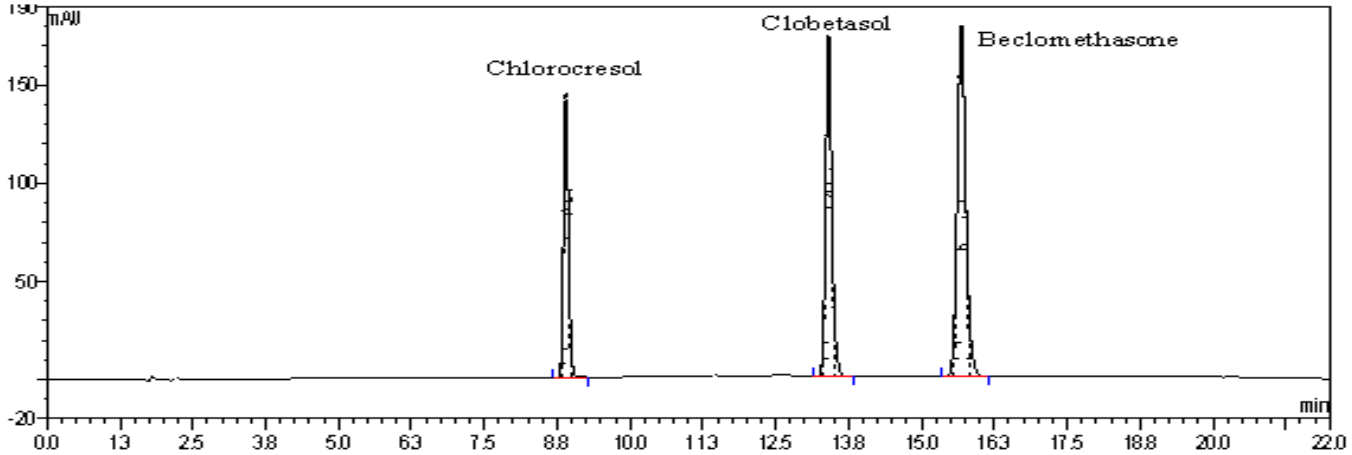

Fig. 8: Chromatogram for Test Solution

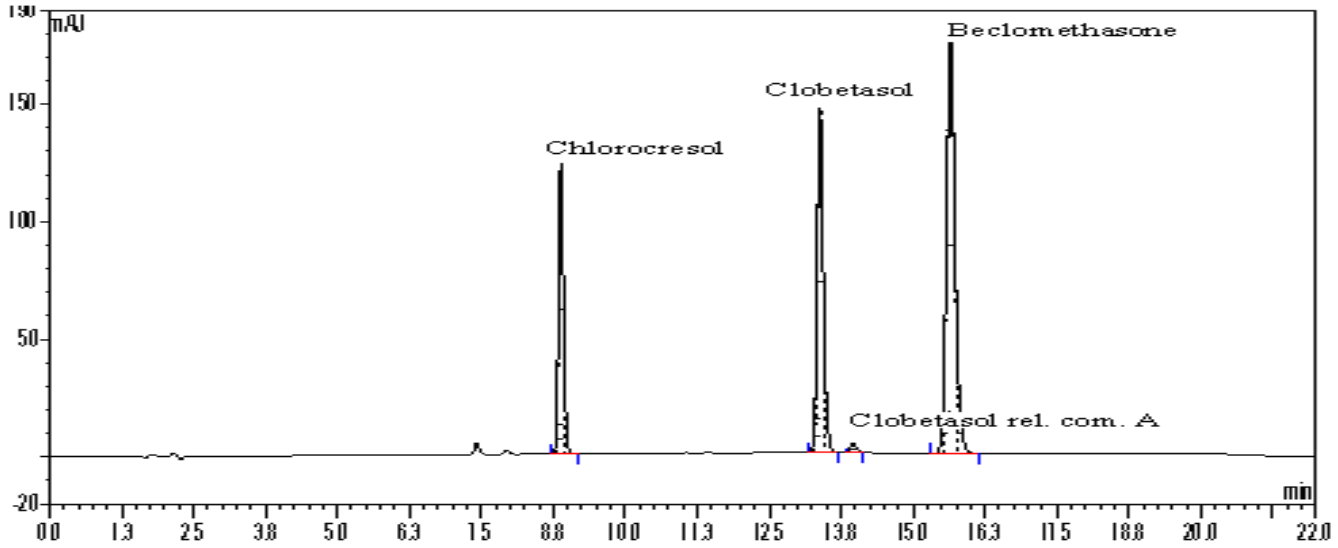

Fig. 9: Chromatogram for System Suitability

The robustness were carried out with minor but deliberate changes in parameters i.e., detection wavelength, column temperature, and flow rate as exhibited in Table 8. Theoretical plates and tailing factor were observed and were found to be 50388 and 76070 (theoretical plates) and 1.16 and 1.19 (tailing factor) for CHLOR and CLOB respectively. The resolution was found to be 25.9 between CHLOR and CLOB, 2.8 between CLOB and clobetasol related compound A and 7.0 between clobetasol related compound A and BEC exhibited in Table 9**.** And the Relative Standard Deviation in retention time were found to be 0.062% for CHLOR, 0.041 % for CLOB, 0.039 % for clobetasol related compound A and 0.035 % for BEC. Figures (7, 8 and 9) exhibit the chromatograms for Standard solution, Sample solution and System Suitability solution.

Advantages of the prescribed analytical method

It can be used for the simultaneous determination of CHLOR and CLOB in pharmaceutical cream which reduces the laboratory costs and its efficiency to separate the principal peaks from impurities and any other degradation products that may be present in exipients matrix.

In conclusion, the method can be used for the simultaneous determination of CHLOR and CLOB in pharmaceutical cream. The analytical method was developed and validated for system suitability linearity, specificity, accuracy, robustness and ruggedness. All parameters tested were found to be within limits. The study indicates that the method has significant advantages in term of good resolution between analytes, high purity of active drug peak and preservative peak, accuracy and precision.

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