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Development and Validation of Method for Determination of Clobetasol Propionate and Salicylic Acid from Pharmaceutical Dosage Form by HPLC

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MHUB and AFMAI designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors HAR and MIT managed the literature searches. Author AFMAI analyses of the study performed the chromatographic analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aim: A new reverse phase high performance liquid chromatography (RP-HPLC) method for the quantitative determination of Clobetasol Propionate and Salicylic Acid in ointment was developed and validated as per ICH (International Conference on Harmonization) guidelines.
Methodology: Separation of Clobetasol Propionate and Salicylic Acid was achieved on Zorbax Eclipse XDB-C18 (250 mm x 4.6 mm, 5μ) stationary phase by using mobile phase solution A, solution B and solution C (Solution A, B and C was composed of potassium dihydrogen phosphate, methanol [HPLC grade] and Acetonitrile [HPLC Grade]) with gradient flow rate. The injection volume and temperature of the column oven were 20µl and 30ºC± 2ºC respectively.

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Results: The developed method shows high specificity for Clobetasol Propionate and Salicylic Acid. The calibration curve for Clobetasol Propionate and Salicylic Acid was linear and correlation coefficient (r^2) for Clobetasol Propionate and Salicylic Acid was 1.0000 & 0.9999 respectively. The proposed method was adequate selective, reproducible and specific for the determination of Clobetasol Propionate and Salicylic Acid from ointment. Average percent of recovery for the drugs Clobetasol Propionate and Salicylic Acid were found 100.33% and 100.62% respectively. All of the results are within the acceptance criteria.

Conclusion: The proposed method was accurate and precise for the quantification of Clobetasol Propionate and Salicylic Acid in the ointment, also be used for routine analysis in quality control. The method parameters like selectivity, precision, intermediate precision, accuracy, linearity, recovery and stability was validated.

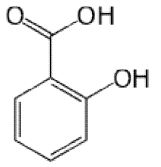
Keywords: Gluco-corticosteroid; HPLC column; NSAID; ICH; voltametry.

1. INTRODUCTION

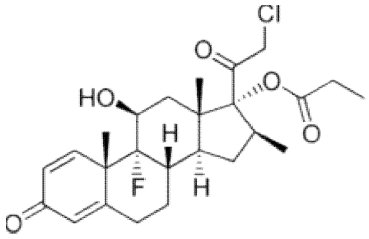
Clobetasol Propionate is the most potent Gluco-corticosteroids for topical use. It is used for short term relief of anti-inflammatory, Pruritic manifestations of moderate to severe corticosteroid responsive dermatoses and in Psoriasis. It is available in dosage forms such as cream, gel, ointment etc. [1-3]. The non steroid anti-inflammatory drugs (NSAID) are among the most prescribed because of their analgesic and anti-inflammatory properties [4]. In the literature, several analytical methods primarily based on separation were described. Therefore, quantitative methods to assay, for instance a drug substance or its impurities, have needed to be fully validated after development. Depending on the type of analytical method and its intended use, a check for linearity, precision, accuracy, specificity, range, limits of detection and quantification, and/or robustness, is required [5-9], gas chromatography coupled with the mass spectrometry were the most usually used methods for analytes separation [10].

From Literature survey it has been concluded that many methods have been developed for SAL and CLO in combination of drugs by using HPLC, UV Spectrophotometry, Colorimetry, GC, Voltammetry, HPCE [11-23].

Only few methods are available for determination of Clobetasol Propionate and Salicylic Acid from pharmaceutical dosage form, so present work was undertaken with the aim to develop and validate a rapid and consistent reversed phase high performance liquid chromatographic method for determination for Clobetasol Propionate and Salicylic Acid according to International Conference on Harmonization (ICH) guideline [24].



Salicylic acid



Clobetasol propionate

2. MATERIALS AND METHODS

2.1 Reagents and Chemicals

The following chemicals were used for the process: Water [HPLC grade] Acetonitrile [HPLC grade] Methanol [HPLC grade], Potassium dihydrogen phosphate. All these chemicals were from Merck Germany. Clobetasol Propionate [Working Standard] and Salicylic acid [Working standard] were from Crystal Pharma S. A., Spain and Alta Laboratories Ltd., India.

2.2 Apparatus and Chromatographic Conditions

The equipment used for the method was Analytical balance Sartorious (model: TE214S). HPLC were Dionex Ultimate 3000(equipped with Auto sampler and UV-visible detector), HPLC Shimadzu Prominence (equipped with Auto sampler and PDA detector). The Column selected for the method was 250 mm x 4.6 mm,

5 μm (Zorbax Eclipse XDB-C18). The flow rate was monitored to separate the peak of methyl paraben and salicylic acid and minimize retention time of clobetasol propionate as per Table 1. The wavelength selected for the method was 240 nm and the injection volume was 20 µl. The temperature of the column oven was 30ºC±2ºC.

2.3 Method Development

2.3.1 Preparation of mobile phase

Solution A, Solution B and Solution C run at gradient elution program.

2.3.2 Preparation of solution A

Solution A was prepared 6.80 mg per ml of Potasium dihydrogen phosphate in water.

2.3.3 Solution B

Solution B was filtered and degassed Methanol.

2.3.4 Solution C

Solution C was mixture of Methanol and Acetonitrile in the ratio of 1:1.

All these solution was filtered through 0.45 µ filter under vacuum filtration and degassing in ultrasonic water bath for 5 minutes.

2.3.5 Preparation of diluent

The diluent consists of Solution A and Methanol in the ratio of 46:54.

2.3.6 Preparation of the clobetasol propionate and salicylic acid standard and sample solution

2.3.6.1 Standard solution preparation

The Standard solution was prepared by accurately weighing and transferring about 20 mg Clobetasol propionate working standard and about 60 mg of Salicylic Acid into a 100 ml clean and dry volumetric flask. About 70 ml of HPLC grade methanol was added and sonicated for 10 minutes with intermittent shaking. The sample was kept for few minutes to cool at room temperature and the volume was made up to mark with same solvent. 5 ml of the resulting solution was transferred into a 50 ml clean and dry volumetric flask and the volume was made up to the mark with diluting solvent. The content was filtered through 0.45 µm membrane filter before injection.

2.3.6.2 Sample solution preparation

The Sample solution was prepared by accurately weighing and transferring about 4.0 g ointment that contained about 2.0 mg of clobetasol propionate and 120 mg Salicylic Acid into a 50 ml clean and dry volumetric flask. About 20 ml of HPLC grade methanol was added and content was heated in a water bath at about 65ºC for 15 minutes with intermittent shaking. The sample was kept for few minutes to cool at room temperature and the volume was made up to mark with the same solvent. The content was shaking vigorously. The content was filtered through whatman filter paper No. 42 and filtrate was collected by discarding first few ml. From the filtrate 10 ml was pipette out into 20 ml clean and dry volumetric flask and diluted up to mark with diluting solvent (Sample for clobetasol Propionate). Further 5 ml was pipette out into 100 ml clean and dry volumetric flask and diluted up to mark with diluting solvent (Sample for Salicylic Acid). Then both Clobetasol propionate and Salicylic Acid solutions was filtered through 0.45 µm membrane filter before injection.

2.3.7 System suitability solution

The final standard solution is used as system suitability solution and inject 20 µl of six replicate injections of standard solution were injected. The chromatogram was recorded and the system suitability parameters for each of the injection were checked for % RSD of area within 1%. Tailing factor must not exceed 2.0 and resolution must be less than 15.

Table 1. Gradient program

Time (minutes)	Flow rate (ml/ minute)	Solution A (%)	Solution B (%)	Solution C (%)
0	1.0	46	54	0
3	1.0	46	54	0
5	1.5	37	0	63
24	1.5	37	0	63
25	1.0	46	54	0
30	1.0	46	54	0

3. METHOD VALIDATION

3.1 System Suitability

The system was deemed suitable if the following acceptance criteria were satisfied. The relative standard deviation (% RSD) of the peak area responses Clobetasol Propionate and Salicylic Acid from six replicate injections of standard solution is not more than 2.0%.Tailing factor must not exceed 2.0 and resolution must be less than 15.

3.2 Specificity

Specificity and selectivity is defined as the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample. The standard solution was prepared and injected to the column and the retention time was checked. There were no interferences found. The method was found to be précised and specific.

3.3 Linearity

It is the relationship between instrument response and known concentrations of the analyte. The linearity was carried out by observing the correlation coefficient (r) of standard solution.

3.4 System Precision

System precision was carried out by performing six replicate injections of standard at 100% of the test concentration and calculating the % RSD of the measured area.

3.5 Method Precision

The precision of an analytical method describes the closeness of individual measures of an analyte when the procedure is applied repeatedly to multiple aliquots of a single homogeneous volume of sample. To demonstrate method precision, six replicate of sample against standard at 100% of test concentration was carried out and the precision of method was calculated by computing % RSD of six measurements.

3.6 Intermediate Precision (Ruggedness)

Intermadiate precision or ruggedness study of an analytical method is the degree of reproducibility of the test results obtain by the analysis of the same samples under a variety of normal test conditions. Test sample of representing single batch was analyzed by two different analysts on two different equipments on two different days. The ruggedness of the test method was calculated by measuring % RSD of six results and % RSD of results of two analysts.

3.7 Accuracy

The accuracy of an analytical method describes the closeness of mean test results obtained by the method to the true value (concentration) of the analyte. Study was carried out over a range of 80% - 120% (3 replicate each) of the test concentration. The % recovery and RSD of % recovery for each concentration was also measured.

3.8 Range

Data generated in linearity, precision and accuracy were considered for establishing the range of the analytical method.

3.9 Robustness

Robustness of the method was investigated by changing flow rate 0.80 ml/min and 1.30 and 1.20 ml/min and 1.70 ml/min, changing column temperature (±5ºC) and ratio of components of mobile phase.

3.10 Stability Study

The solution stability experiments were carried out under room temperature at intervals of 0 h 6 h 12 h 18 h 24 h 30 h and 48 h.

4. RESULTS AND DISCUSSION

4.1 System Suitability

System suitability is an integral part of analytical procedures. In optimized chromatographic conditions Relative standard Deviation (%RSD) of area of Clobetasol propionate and Salicylic Acid 0.223% (NMT 1.0%) and 0.074% (NMT 1.0%) respectively. Average tailing factor Clobetasol Propionate and Salicylic Acid were 1.14 and 1.10 respectively. Resolution was found 39 (Table 2).

4.2 Specificity

Specificity of an analytical method is its ability to assess unequivocally the analyte in the presence

of components that may be expected to be present. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedures. From the specificity study it is observed that the chromatogram for Clobetasol Propionate and Salicylic Acid sample with reference standard showed positive response and Placebo had no response, So the method was specific.

4.3 System Precision

System precision was carried out by performing six replicate injections at 100% of the test concentration and calculating the % RSD, Tailing factor, resolution and Theoretical plate count. From the data it is observed that the % Relative standard Deviation (% RSD) of area of Clobetasol propionate and Salicylic Acid 0.223% (NMT 1.0%) and 0.074% (NMT 1.0%) respectively. Average tailing factor Clobetasol Propionate and Salicylic Acid were found 1.14 and 1.10 respectively Theoretical plate count for Clobetasol Propionate and Salicylic Acid were 12800 and 5114 respectively. Resolution was found 39 (Table 2).

4.4 Intermediate Precision or Ruggedness

Assay results by two different analysts at different days have been found very much close to each other. The % RSD of two analysts (12 samples) was 0.22% and 0.15% for clobetasol propionate and salicylic acid respectively. This was within acceptance criteria. So the method can be considered to be rugged enough (Table 3).

4.5 Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The result shows that Average % recovery at different accuracy lavel is 100.33% and 100.62% for Salicylic Acid and Clobetasol propionate respectively (Table 4, Figs. 1 and 2).

4.6 Linearity

The linearity of an analytical method is its ability to elicit test results directly proportional to the concentration of the analyte in samples within given range. Linearity of the method was evaluated from the correlation coefficient of calibration curves that were constructed from mean peak area of Clobetasol Propionate and Salicylic Acid at different concentrations level (80%, 90%, 100%, 110% and 120%). Correlation coefficient of Clobetasol Propionate and Salicylic Acid was 1.0000 and 0.9999 respectively (Table 5, Figs. 5 and 6).

4.7 Range

The specified range is normally derived from linearity studies and depends on the intended application of the procedure. It is established by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy and precision when applied to samples containing amounts of the analyte within the extremes of the specified range of the analytical procedure. Based on the linearity, precision and accuracy results, the Range of the method was determined as 80% to 120% of the target concentration.

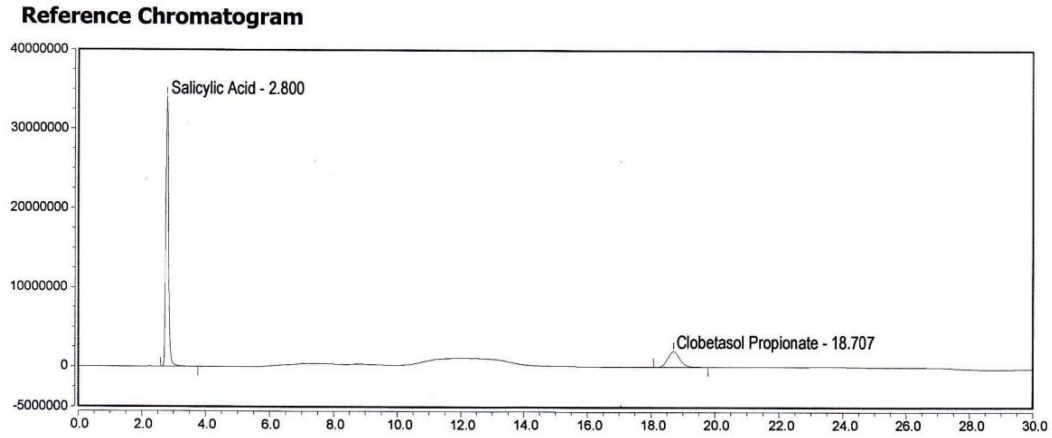


Fig. 1. Representation of chromatogram of clobetasol propionate and salicycle acid obtained from the standard solution

4.8 Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variation in method parameters and provides an indication of its reliability during normal usage. The robustness of this method was determined by analyzing the same batch of sample by deliberately changing the method parameters like machine, ratio of mobile phase and column temperature. From the results presented on table it is clear that the system suitability criteria meet with the acceptance limit. Hence the method is robust (Table 6).

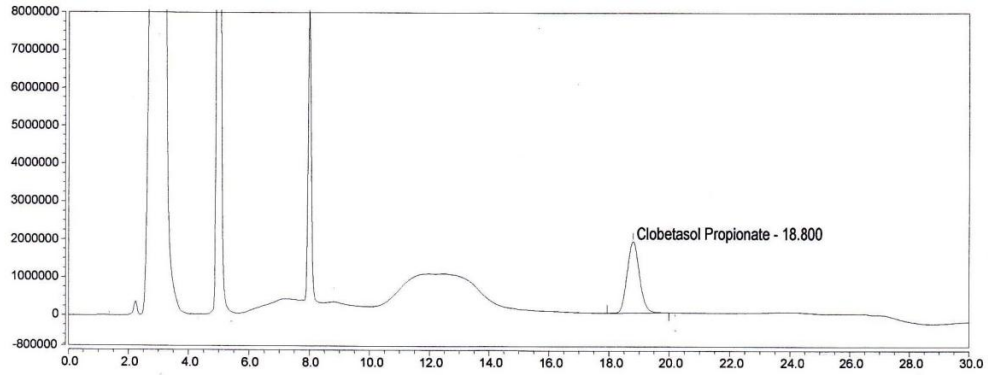


Fig. 2. Representation of chromatogram of clobetasol propionate obtained from the test solution

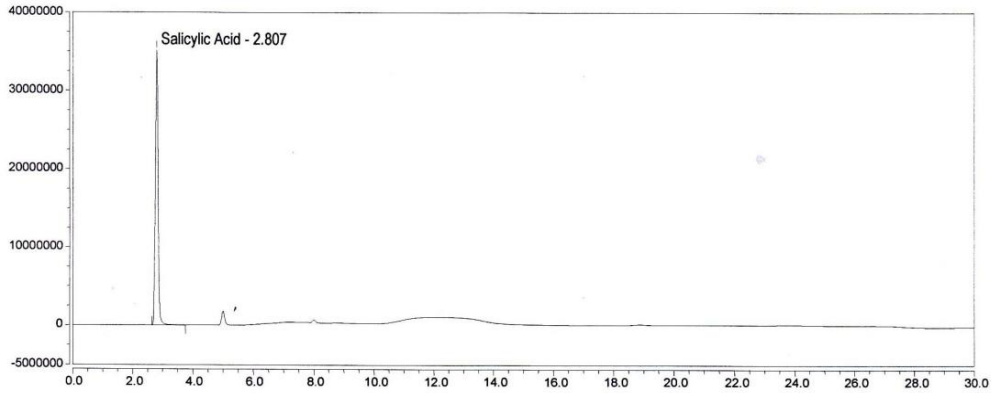


Fig. 3. Representation of chromatogram of salicycle acid obtained from the test solution

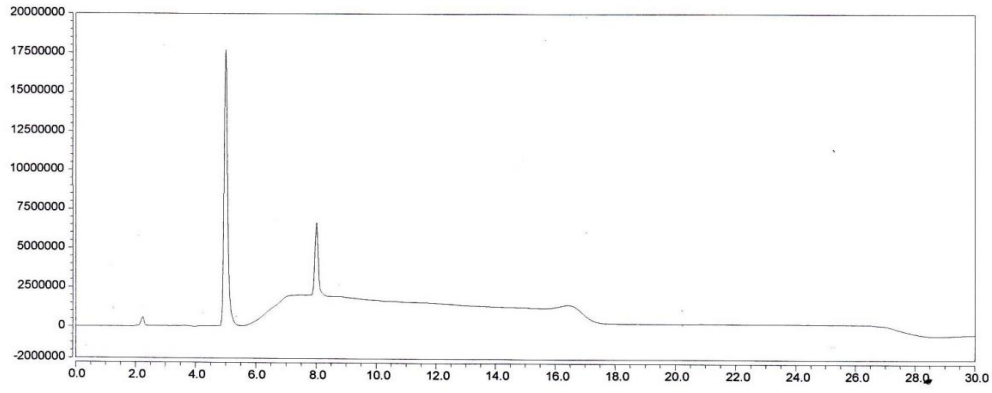


Fig. 4. Representation of chromatogram of placebo matrix obtained from the placebo solution

Table 2. Data for system precision

Sl. no.	Retention time		Peak area		Theoretical plate		Resolution	Asymmetry factor	
	Clobetasol propionate	Salicylic acid	Clobetasol propionate	Salicylic acid	Clobetasol propionate	Salicylic acid		Clobetasol propionate	Salicylic acid
01	18.750	2.800	873365	3502679	12851	5234	39	1.13	1.12
02	18.793	2.800	873495	3503150	12590	5192	39	1.13	1.09
03	18.707	2.797	873870	3498655	12974	5146	39	1.13	1.10
04	18.690	2.797	873232	3496389	13261	5113	40	1.15	1.10
05	18.720	2.800	871649	3501734	12694	5030	39	1.15	1.10
06	18.673	2.800	868736	3500553	12430	4970	38	1.15	1.11
Average	18.722	2.799	872391	3500527	12800	5114	39	1.14	1.10
Relative standard deviation	0.233%	0.062%	0.223%	0.074%	-----		--	---	

Table 3. Data for intermediate precision

Sample no.	% of label claim			
	Day-1		Day-2	
	Clobetasol propionate	Salicylic acid	Clobetasol propionate	Salicylic acid
01	100.20%	99.95%	99.88%	100.02%
02	99.85%	99.76%	99.78%	100.25%
03	100.15%	99.85%	100.30%	100.08%
04	100.12%	100.17%	99.95%	100.25%
05	99.93%	100.08%	99.81%	99.78%
06	99.87%	99.87%	100.25%	100.04%

% RSD (12 samples), Clobetasol propionate, 0.22% salicylic acid 0.14%

Table 4. Data for accuracy

Concentration level	Sample no.	Amount added in (µg/ml)		Amount recovered in (µg/ml)		% recovery	
		Salicylic acid	Clobetasol propionate	Salicylic acid	Clobetasol propionate	Salicylic acid	Clobetasol propionate
80%	Sample-1	48.8	16.3	49.09	16.40	100.59	100.61
	Sample-2	48.9	16.3	49.08	16.46	100.57	100.98
	Sample-3	48.6	16.4	48.95	16.40	100.72	100.61
	Sample-1	60.2	20.4	60.36	20.56	100.27	100.78
100%	Sample-2	60.2	20.5	60.38	20.60	100.30	100.49
	Sample-3	60.6	20.5	60.76	20.67	100.26	100.83
120%	Sample-1	72.2	24.3	72.20	24.41	100.00	100.45
	Sample-2	72.1	24.3	72.11	24.35	100.00	100.21
	Sample-3	72.9	24.6	73.11	24.76	100.29	100.65
Average						100.33	100.62

Table 5. Data for linearity

Concentration level	Concentration (µg/ml)		Peak area		Correlation co-efficient	
	Salicylic acid	Clobetasol propionate	Salicylic acid	Clobetasol propionate	Salicylic acid	Clobetasol propionate
80%	48.16	16.16	2829054	701140		
90%	54.18	18.18	3165420	786188		
100%	60.20	20.20	3500414	874565	0.9999	1.0000
110%	66.22	22.22	3866651	960699		
120%	72.24	24.24	4200993	1049951		

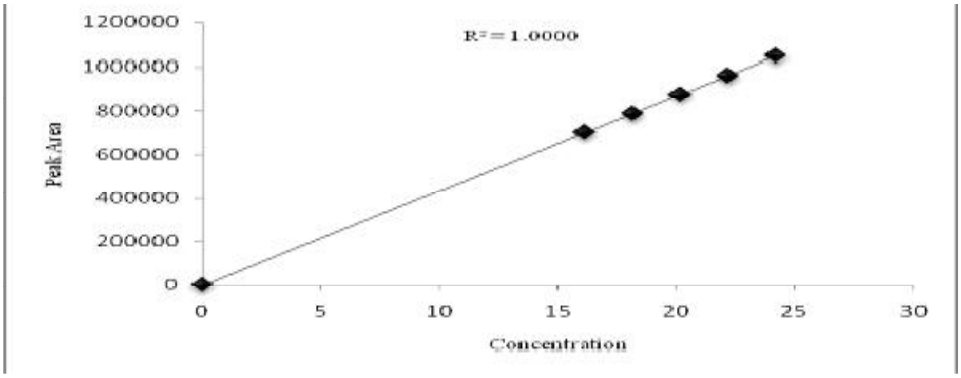


Fig. 5. Graphical representation for linearity of clobetasol propionate

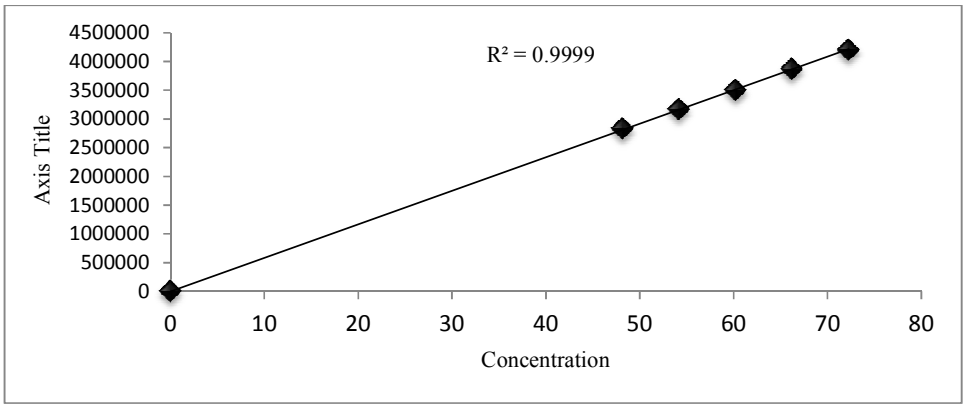


Fig. 6. Graphical representation for linearity of salicylic acid

Table 6. Data for robustness

Sl. no.	Changing parameters	Assay results (%)	
		Salicylic acid	Clobetasol propionate
01.	Flow rate actual.	100.10	99.60
	Flow rate change to 0.80 ml/min	99.87	100.20
	Flow rate change to 1.20 ml/min	100.04	100.15
	Flow rate change to 1.30 ml/min	100.41	99.60
	Flow rate change to 1.70 ml/min	100.34	99.85
02.	Ratio actual.	99.70	99.60
	Ratio of solution A decrease 2% and compensate with Solution C at each stage	99.92	100.25
	Ratio of solution A increase 2% compensate with Solution C at each stage.	99.86	99.60
03.	Column oven temperature actual.	100.20	99.85
	Column oven temperature change to 25ºC.	100.33	100.40
	Column oven temperature change to 35ºC.	100.39	99.80

4.9 Stability Study

From the stability study data, it was observed that the test sample solution is found to be stable up to 48 h at ambient condition.

4.10 Discussion

Reverse phase chromatography was chosen because of its recommended use for ionic and moderate to non-polar compounds. Reverse phase chromatography is not only simple,

convenient but also better in terms of efficiency, stability and reproducibility. C18 column was selected because less retentive and it is less polar compare to C4 and C8 columns. C18 column allows eluting non-polar compounds more quickly compare to polar compounds. In addition to this, UV detector is used, to optimize response of the components. At 240 nm Clobetasol Propionate gives maximum response where 270 nm for Salicylic Acid. At 240 nm peak height of Clobetasol Propionate shows maximum peak height and partially decline peak height of Salicylic Acid which allows easy detection of the compounds. A 250 x 4.6 mm column of 5 µm particles packing was preferred as a starting point for method development. Flow rate was changed to separate the peak of methyl paraben and salicylic acid and minimize retention time of clobetasol propionate. Gradient mode was chosen due to simplicity in application and robustness with respect to longer column stability. This configuration provides a large number of theoretical plate's values for most separation. Various solvent ratios of solution A, solution B and solution C were tried as mobile phase for proper separation, good peak shape and sharpness, more Theoretical plates, good resolution & less tailing of Clobetasol propionate and Salicylic Acid. All the results were found within acceptance criteria.

5. CONCLUTION

A simple RP-HPLC Method is developed to quantify Clobetasol Propionate and Salicylic Acid in pharmaceutical ointment. The validated method was covers the wide range of linearity. The method adopted for estimation of by RP-HPLC is precise, linear, accurate, rugged and robust enough. The sample solution is found to be stable up to 48 h at ambient condition. Hence this method can be considered validated for its intended purpose to establish the quality of the drug substance during routine analysis with consistent and reproducible results.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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