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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF DESONIDE IN PRESENCE OF SORBIC ACID

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ABSTRACT

A simple, rapid, economical, precise and accurate reverse phase high performance liquid chromatographic (RP-HPLC) method for simultaneous estimation of Desonide in presence of Sorbic acid for their combined dosage form has been developed. The separation was achieved by C_{18} Thermo (250 x 4.6 mm, 5 µm) column and Methanol: Acetonitrile: Water (70:08:22) at pH 3.0 as mobile phase, at a flow rate of 1 ml/min and the effluent was monitored at 240 nm using DAD detector. Retention time of Sorbic Acid and Desonide were found to be 3.471 min and 4.592 min, respectively. The method was validated in terms of linearity, precision, accuracy and specificity, limit of detection and limit of quantization. Linearity of the Desonide and Sorbic Acid were in range of 10 – 30 µg/ml and 35 - 105 µg/ml respectively. The percentage assays of two drugs were found to be 100.27% and 100.23% for Desonide and Sorbic Acid respectively from the cream formulation. Repeatability, Intra-day and Inter-day Precision obtained for Desonide and Sorbic Acid in the range of 0.25 – 1.12% and 0.68 – 0.88% RSD for intra-day and 0.35 – 1.13% and 0.23 – 0.76% RSD for inter day and 0.49 and 0.50% RSD for repeatability. The method was found to be precise, accurate and specific during the study. The proposed method enables rapid quantification and simultaneous analysis of two drugs from commercial formulations without any excipients interference. The method can be used for routine analysis of marketed products of Desonide and Sorbic Acid in combined cream formulation.

KEYWORDS: Reverse Phase High Performance Liquid Chromatography Method, Infra-Red Spectroscopy Method, Desonide, Sorbic Acid.

INTRODUCTION

Desonide is designated chemically as (1S,2S,4R,8S,9S,11S,12S,13R)-11-hydroxy-8-(2-hydroxyacetyl)-6,6,9,13-tetramethyl-5,7-dioxapentacyclo[10.8.0.0^{2},^{9}.0^{4},^{8}.0^{13},^{18}]icosa-14,17-dien-16-one is a synthetic nonfluorinated corticosteroid for topical dermatologic use. The corticosteroids constitute a class of primarily synthetic steroids used topically as anti-inflammatory and antipruritic agents.[1-3]

Like other topical corticosteroids, desonide has anti-inflammatory, antipruritic and vasoconstrictive properties. The drug binds to cytosolic glucocorticoid receptors. This complex migrates to the nucleus and binds to genetic elements on the DNA. This activates and represses various genes. However corticosteroids are thought to act by the induction of phospholipase A2 inhibitory proteins, collectively called lipocortins. It is postulated that these proteins control the biosynthesis of potent mediators of inflammation such as prostaglandins and leukotrienes by inhibiting the release of their common precursor arachidonic acid. Arachidonic acid is released from membrane phospholipids by phospholipase A2.[1-3]

Various analytical methods have been reported for the estimation of Desonide as alone and in the different dosage forms. They include RP-HPLC[4-6], HPTLC[7] and Stability indicating LC-UV[8] methods for the bulk formulations, Nanocapsule suspensions and Lotion formulations.

Sorbic Acid designated chemically as (2E,4E)-hexa-2,4-dienoic acid (Figure 2) is a a preservative for many foodstuffs. Mold and yeast inhibitor. Used as a fungistatic agent for foods, especially cheeses. Sorbic acid and its salts inhibit various bacteria, including sporeformers, at various stages of their life cycle (germination, outgrowth and cell division). This multiple action of sorbate may be responsible for its broad effectiveness compared to other antimicrobial agents. Inhibition of bacterial growth by sorbate may result from alteration of cell membranes, inhibition of transport systems and key enzymes, creation of a proton flux into the cell, or more than one of these actions.[9-14]

Various analytical methods have been reported for the estimation of Sorbic Acid as alone as well as in combination with other drugs. They include Titrimetry methods[15-18], RP-HPLC[19], HPLC[20] and Stability indicating HPLC[21] methods with Dehydroacetic acid,

benzoic acid and salicylic acid in cosmetic products and Sertaconazole nitrate, Sorbic acid and Methylparaben in cream dosage form.

However an extensive literature search didn't reveal any estimation method for both the drugs in their combined dosage form. Therefore, attempt was made to develop and validate simple, precise, accurate, sensitive and less time consuming RP-HPLC method for simultaneous determination of both the drugs in their combined dosage form. The parent guideline on drug analytical method validation FDA.[22]

MATERIALS AND METHODS

Reagents and Chemicals

Desonide and Sorbic Acid obtained as gift samples from Cadila Healthcare Ltd., Changodar. The Cream contains Desonide and Potassium Sorbate and Sorbic Acid combination equivalent to Sorbic Acid Manufactured by Galderma India Pvt. Ltd. were purchased from local market. HPLC grade Acetonitrile, Water, methanol and Phosphoric Acid of HPLC grade were obtained from FINAR Chemicals.

Instruments and Chromatographic Conditions

Young lin HPLC system was used for method development and validation. Data acquisition was performed on YL 9100 HPLC software. The separation were achieved on C_{18} Thermo (250 x 4.6 mm, 5 µm) column. The column was maintained at room temperature and the eluent was monitored at 240nm using DAD detector. The mixture of Methanol: Acetonitrile: Water in proportion of 70:08:22 %v/v at a flow rate of 1.0 ml/min was used as a mobile phase. The injection volume was 20 µl.

Preparation of Standard Solutions of Desonide and Sorbic Acid

Accurately weighed and transferred 25 mg of Desonide was weighed and transferred to a 50 ml volumetric flask. Volume was made up to the mark with diluent and used as the standard stock solution (Desonide - 500 µg / ml).

Accurately weighed and transferred 35 mg of Sorbic Acid was weighed and transferred to a 50 ml volumetric flask. Volume was made up to the mark with diluent and used as standard stock solution (Sorbic Acid - 700 µg / ml).

From the prepared stock solutions 2 ml from Desonide - 500 µg / ml and 5 ml from Sorbic Acid - 700 µg / ml was taken and transferred to a 50 ml volumetric flask. Volume was made

up to the mark with diluent get working standard solution comprising 20 µg/ml of Desonide and 70 µg/ml of Sorbic Acid.

Preparation of Sample Solutions of Desonide and Sorbic Acid:

4 gm of Cream (equivalent to 2mg of Desonide, 7 mg of Sorbic Acid) was transferred to a 100 ml volumetric flask. 60 ml Diluent was added and heated on water bath at 60° to 70°C for 10 minutes with intermittent shaking until the completely melt or dispersed and sonicate for 5 minutes.

Cooled at Room temperature and made up to the mark with diluent and mixed. Filtered through 0.45 µ MDI filter. (Desonide - 20 µg/ml and Sorbic Acid - 70 µg/ml.) The resulted test solution was then analyzed for assay determination.

System suitability parameters

System suitability tests were performed to verify that the resolution and repeatability of the system were adequate for the analysis intended. The parameters monitored for system suitability includes retention time, theoretical plate number, peak area, tailing factor and resolution. The repeatability of these parameters was checked by injecting three times the test solution of Desonide - 20 µg/ml and Sorbic Acid - 70 µg/ml. The results shown in Table 1 were within acceptable limits.

Method Validation

1) Specificity

Specificity of method can be termed as absence of any interference at retention times of samples. Specificity was performed by injecting blank and standard preparations. Chromatograms were recorded and retention times from sample and standard preparations were compared for identification of analytes.

2) Linearity and Range

A series of standard solutions 10 - 30 µg/ml of Desonide and 35 - 105 µg/ml of Sorbic Acid were prepared. An aliquot of 20µl of each solution was injected 3 times for each standard solutions and peak area was observed. Plot of average peak area versus the concentration is plotted and from this the correlation coefficient and regression equation were generated. The calibration data of Desonide and Sorbic Acid is given in Table 2, while Figure 4A and Figure 4B represents linearity graphs of two drugs respectively.

3) Precision

The method was validated in terms of intra-day inter-day precision. The solution containing Desonide - 20 µg/ml and Sorbic Acid - 70 µg/ml was injected six times for repeatability study. Inter-day and Intra-day study was performed by injecting 15, 20, 25 µg/ml of Desonide and 52.5, 70, 87.5 µg/ml of Sorbic Acid solutions three times for each aliquots. The %RSD for precision study was found less than 2% as shown in Table 3.

4) Accuracy

Accuracy was determined by calculating recovery of Desonide and Sorbic Acid by the standard addition method. Known amounts of standard solutions of Desonide (5, 10 and 15 µg/ml) and Sorbic Acid (17.5, 35 and 52.5 µg/ml) were added to a pre quantified test solutions of Desonide (10 µg/ml) and Sorbic Acid (35 µg/ml). Each solution was injected in triplicate and the recovery was calculated by measuring peak areas. Results obtained are shown in Table 4.

5) LOD and LOQ

LOD and LOQ for Desonide and Sorbic Acid were calculated as suggested by ICH guidelines using equations LOD = 3.3 σ/s and LOQ = 10 σ/s, respectively. Where, σ is the SD of the response and S is the slope of the calibration curve.

6) Robustness

The robustness study was performed to evaluate the influence of small but deliberate variation in the chromatographic condition. The robustness was checked by making two small changes. The mobile ration was changed by ± 0.2 ml and flow rate was changed by ± 0.02 ml/min and pH was changed by ± 0.2. After each changes sample solution was injected and system suitability parameters were observed. The results were shown in Table 5.

7) Ruggedness

The ruggedness study was performed to evaluate the influence of different conditions like different analysts and different instruments.

The Standard Solution was injected and percentage RSD was calculated for the Analyst 1 & 2 and Instrument 1 & 2. As per study sample solution was injected and system suitability parameters were observed. The results were shown in Table 6A and 6B.

RESULT AND DISCUSSION

System suitability study

The detection was carried out in the UV region at 240nm. The different composition of mobile phase was testing and the composition giving retention time of 3.471 min for Sorbic Acid and 4.592 min for Desonide with good resolution and theoretical plates was selected, that optimized mobile phase was Methanol : Acetonitrile : Water (70:08:22) % v/v at pH 3.0. A chromatogram of the mixture in optimized conditions is shown Figure 3 and the system suitability parameters are shown in Table 1.

Method Validation

A) Specificity

The method was found to be specific as there was no interference observed in any of the parameters under observation.

B) Linearity and Range

The linearity of Desonide and Sorbic Acid were found between 10-30 µg/ml and 35-105 µg/ml respectively. The results are shown in Table 2.

C) Precision

The %RSD for repeatability study for Desonide and Sorbic Acid were found 0.49 and 0.50 respectively. The Inter-day and Intra-day study also show %RSD value for Desonide and Sorbic Acid within the acceptable limit. Results for precision study are shown in Table 3.

D) Accuracy

Accuracy of the method was confirmed by recovery study at three levels (50%, 100 % and 150%) of standard addition. Percentage recovery for Desonide was found to be 99.72-100.69% while for Sorbic Acid it was found to be 99.62-99.76% as shown in Table 4.

E) LOD and LOQ

The LOD was found to be 0.39 µg/ml for Desonide and 2.27 µg/ml for Sorbic Acid, while the LOQ was found to be 1.17µg/ml for Desonide and 6.87 µg/ml for Sorbic Acid.

F) Robustness

The typical variations studied under this parameter were mobile phase composition, pH and Flow rate. Overall % RSD was found to be less than 2% for all the variations which indicates that the proposed method is robust. Robustness data are shown in Table 5.

G) Ruggedness

The typical variations studied under this parameter were different analysts and Different Instruments were used. Overall % RSD was found to be less than 2% for all the variations which indicates that the proposed method is Rugged. Ruggedness data are shown in Table 6A and 6B.

H) Analysis of marketed formulation by proposed method

Applicability of the proposed method was tested by analyzing the commercially available marketed formulation. The percentage of Desonide and Sorbic Acid were found to be 100.27 % for Desonide and 100.23% for Sorbic Acid. Results as % Assay are shown in Table 7.

I) Summary of Validation Parameters

The Summary of validation parameters performed during the research work is shown in Table 8.

FIGURES

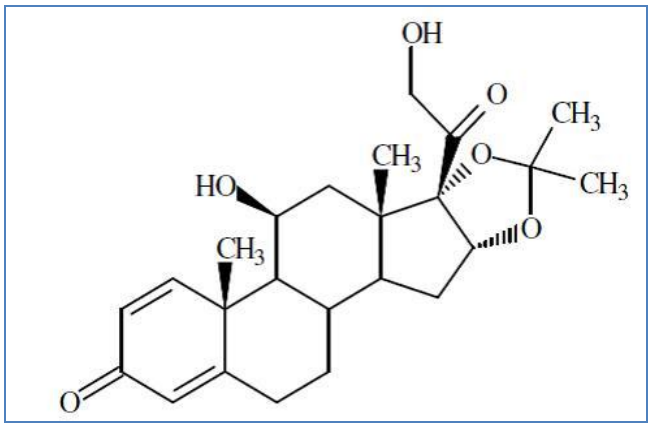

Figure 1: Chemical structure of Desonide.

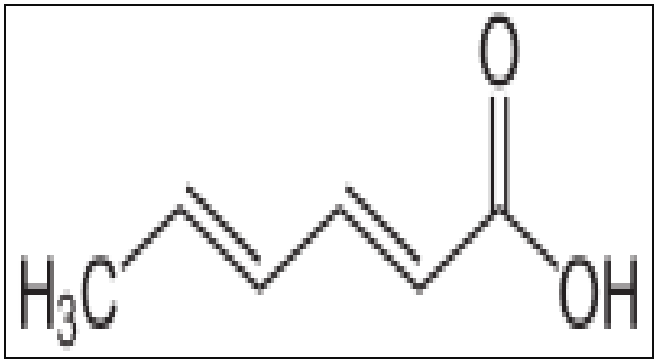

Figure 2: Chemical structure of Sorbic Acid.

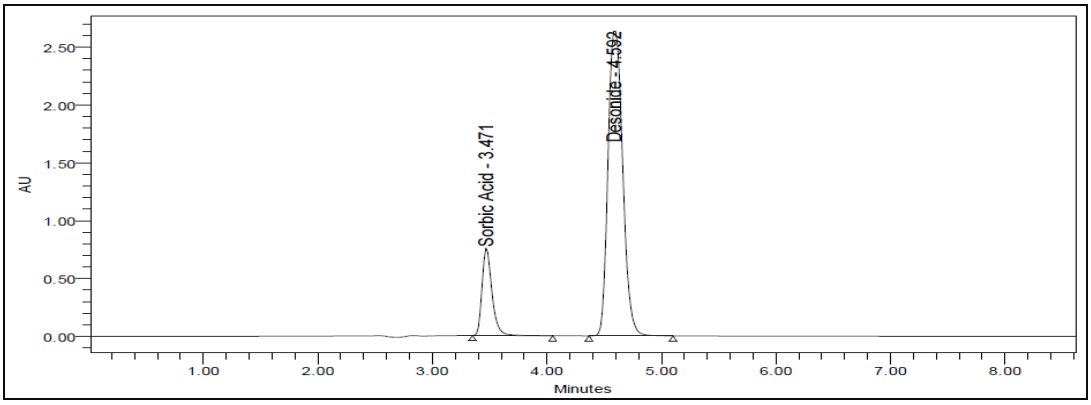

Figure 3: Optimized condition chromatogram of Desonide and Sorbic Acid.

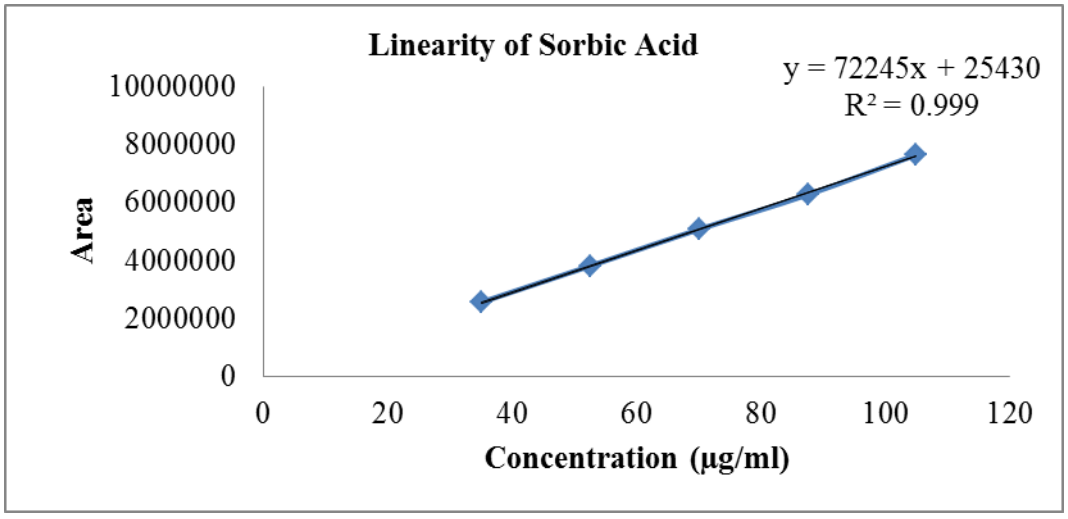

Figure 4A: Linearity graph for Sorbic Acid.

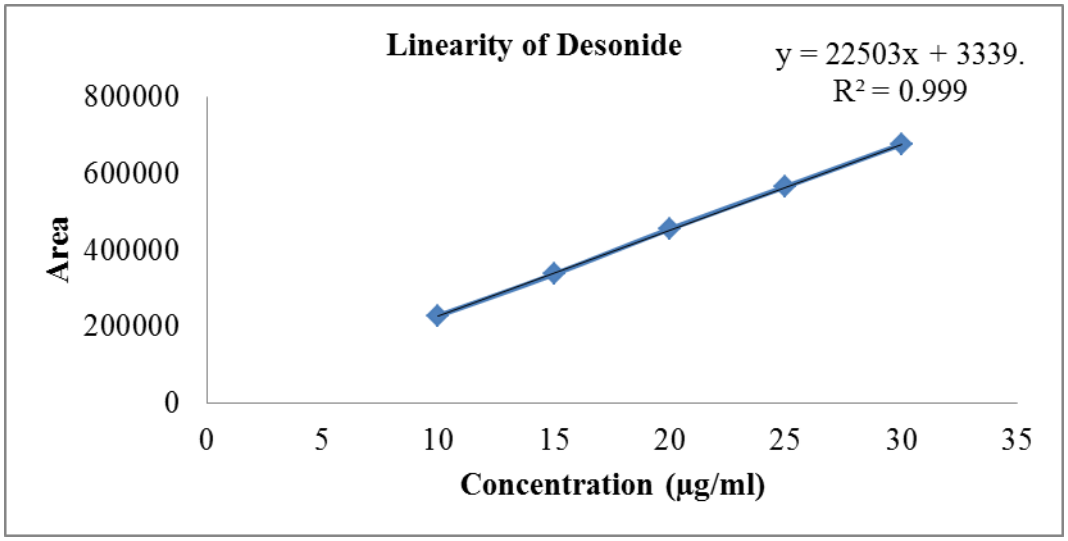

Figure 4B: Linearity graph for Desonide.

TABLES

Table 1: Results for System suitability parameters

Parameters	Data Observed	
	Desonide (Mean ± SD) (n=3)	Sorbic Acid (Mean ± SD)(n=3)
Theoretical plates per column	6776 ± 6.557	3.471 ± 0.001
Retention time	4.592 ± 0.003	8116 ± 13.503
Tailing factor	1.19 ± 0.015	1.32 ± 0.012
Resolution	5.78 ± 0.01	

Table 2: Linearity study for Desonide and Sorbic Acid

Sr. No	Desonide			Sorbic Acid		
	Conc. (µg/ml)	Average area *	% RSD	Conc. (µg/ml)	Average area *	% RSD
1	10	229221	0.30	35	2567573	0.68
2	15	337639	0.71	52.5	3821654	0.74
3	20	456227	0.72	70	5084842	0.23
4	25	566542	0.69	87.5	6278123	0.36
5	30	677337	0.83	105	7660798	0.15
*= average of three determinations, RSD=Relative standard deviation						

Table 3: Precision study results for Desonide and Sorbic Acid.

Parameters	Concentration		% RSD	
	Desonide (µg/ml)	Sorbic Acid (µg/ml)	Desonide	Sorbic Acid
Intra-day* precision	15	52.5	1.12	0.74
	20	70	1.12	0.68
	25	87.5	0.25	0.88
Inter-day* precision	15	52.5	0.68	0.66
	20	70	1.13	0.76
	25	87.5	0.35	0.23
Repeatability**	20	70	0.49	0.50
*= average of three determinations, **= average of six determinations				

Table 4: The accuracy study results of Desonide and Sorbic Acid.

Drug	Accuracy Level %	Amount taken (µg/ml)	Amount added (µg/ml)	Total Amount found* (mg/ml) ± S.D.	Mean % Recovery ± SD
Desonide	50%	10	05	15.02 ± 0.70	100.69 ± 0.73
	100%	10	10	19.99 ± 0.34	99.84 ± 0.31
	150%	10	15	24.98 ± 0.20	99.72 ± 0.24
Sorbic Acid	50%	35	17.5	52.47 ± 1.85	99.76 ± 1.87
	100%	35	35	69.96 ± 0.46	99.76 ± 0.44
	150%	35	52.5	87.47 ± 0.67	99.62 ± 0.69
* average of three determinations					

Table 5: Robustness study results for Desonide and Sorbic Acid.

Parameters	Change Level	Area (n=3)	
		Desonide	Sorbic Acid
pH (± 0.2)	2.8	446942	6619855
	3.0#	452303	6509743
	3.2	444113	6590688
	Mean ± SD	447786 ± 4159.72	6573429 ± 57048.89
	% RSD	0.93	0.87
Flow Rate (± 0.02 ml/min)	0.98 ml/min	464230	5214203
	1.0 ml/min#	469772	5276224
	1.02 ml/min	458539	5334122
	Mean ± SD	464180.3 ± 5616.66	5274850 ± 59971.31
	% RSD	1.21	1.14
Mobile Phase Composition: - Methanol: Acetonitrile: Water (± 2 mL)	68:08:24	466512	5470276
	70:08:22#	471784	5299646
	72:08:20	475361	5374224
	Mean ± SD	471219 ± 4451.47	5381382 ± 85539.91
	% RSD	0.94	1.59
# actual parameter as control standard			

Table 6A: Ruggedness data for Desonide and Sorbic Acid (Analyst 1 & 2)

	Desonide (Area)	Sorbic Acid (Area)
Analyst 1	5295319	470421
Analyst 2	5315528	473069
Mean ± SD	5305423.5 ± 14289.92	471745 ± 1872.41
% RSD	0.27	0.40

Table 6B: Ruggedness data for Desonide and Sorbic Acid (Instrument 1 & 2).

	Desonide (Area)	Sorbic Acid (Area)
Instrument 1	5245263	467033
Instrument 2	5318382	471421
Mean ± SD	5281822.5 ± 51702.94	469227 ± 3102.78
% RSD	0.98	0.66

Table 7: Analysis of Marketed formulation (Cream Dosage form) of Desonide and Sorbic Acid by proposed Method.

Desonide (0.05 % w/w)			Sorbic Acid (1.75 % w/w)		
Labeled Amount mg	Amount Found (mg)	% Assay	Labeled Amount mg	Amount Found (mg)	% Assay
0.5 mg/g	0.502	100.40	1.75 mg/g	1.765	100.86
	0.497	99.40		1.745	99.71
	0.505	101.00		1.752	100.11
Mean ± SD	0.501 ± 0.0040	100.27 ± 0.8083	Mean ± SD	1.754 ± 0.0101	100.23 ± 0.5799
% RSD	0.81	0.81	% RSD	0.58	0.58

Table 8: Summary of Validation Parameters.

Sr. No.	Parameters		Desonide	Sorbic Acid
1	Specificity		No interference Observed	
2	Linearity /Range (µg/ml)		10-30	35-105
3	Regression Equation		y = 72245x + 25430	y = 22503x + 3339
4	Correlation co efficient (R^2)		0.999	0.999
5	LOD (µg/ml)		0.39	2.27
6	LOQ (µg/ml)		1.17	6.87
7	Precision (% RSD)	Repeatability	0.49	0.50
		Intraday	0.25 – 1.12	0.68 – 0.88
		Interday	0.35 – 1.13	0.23 – 0.76
8	Accuracy (%Recovery)	50%	100.69 ± 0.73	99.76 ± 1.87
		100%	99.84 ± 0.31	99.76 ± 0.44
		150%	99.72 ± 0.24	99.62 ± 0.69
9	Robustness		The method was found Robust.	
10	Ruggedness		The method was found Rugged.	
11	Assay		100.27 % w/w	100.23 % w/w

CONCLUSION

From the above discussion it can be concluded that the proposed method is simple, specific, precise, accurate, rapid and economical, as it separates component with good chromatographic criteria. Method has short run time and all degradants are well separated from drug. All results were found to be satisfactory. So, the developed and validated RP-HPLC method for the estimation of Desonide in presence of Sorbic Acid can be applied to the Cream dosage form.

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