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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF SALICYLIC ACID AND BECLOMETHASONE DIPROPIONATE IN THEIR BULK AND COMBINED DOSAGE FORM

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ABSTRACT
A simple, isocratic, rapid and accurate reversed-phase high performance liquid chromatography method was developed and validated for simultaneous estimation of Salicylic acid and Beclomethasone Dipropionate in bulk and ointment dosage form. Phenomenex C-18 (250 x 4.6 mm), 5 µ analytical column at ambient temperature and UV detection at 241nm was used. Water:Acetonitrile:Triethyamine (70:30:0.5) with pH set at 4.0 using o-phosphoric acid(1%) was used as mobile phase with flow rate of 1.0 ml/min, Injection volume 20µl. Calibration graph was found to be linear at range 50 – 150 µg/ml and 5 – 15 µg/ml for Salicylic acid and Beclomethasone dipropionate respectively. The regression coefficient (r^2) obtained was found to be 0.999 and 0.997 for Salicylic acid and Beclomethasone dipropionate respectively.The retention time were found to be 3.8 and 8.7 min for Salicylic acid and Beclomethasone dipropionate respectively.

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INTRODUCTION

Salicylic acid (SA) is chemically 2-Hydroxybenzoic Acid. SA is Keratolytic in nature.[1] Beclomethasone dipropionate (BD) is chemically 9α-chloro-11β-hydroxy-16β-methyl-3, 20-dioxopregna-1,4-diene-17,21-diyldipropionate. It is of category Anti-inflammatory agents and also adreno corticosteroid.[2-3] A formulation (ointment) containing SA 3% w/w in combination with BD 0.1% w/w is marketed by Cipla pharma Ltd. for the treatment of skin disorder like psoriasis, eczema, acne etc.

From literature survey it has been concluded that many methods have been done for SA and BD in combination with other drugs like RP-HPLC, UV Spevctrophotometry etc.[4 to 20] but there is no method developed for the combination of SA and BD.

MATERIALS AND METHOD

1.1 Chemicals and Reagents

SA and BD are obtained as a gratis sample from Intas pharma, Ahmedabad. Acetonitrile and Water (HPLC grade) were used for HPLC method. Triethylamine and o-phosphoris acid were used.

1.2 Instrumentation

HPLC (Shimadzu) LC-20 AT, Prominence solvent delivery module, a manual rheodyne injector with a 20-µl fixed loop,Detector SPD-20A Prominence UV–visible detector, **Phenomenex C18** column (Spinobiotech. Ahmedabad) (particle size 5µm; 250mm×4.6mm).**Software-Spinchrom Chromatographic Station® CFR Version 2.4.0.195** (Spinchrom Pvt. Ltd., Chennai, India).

EXPERIMENTAL

2.1 Selection of detection wavelength

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. In the present study, standard solution of Salicylic acid and Beclomethasone dipropionate were scanned over the range of 200–400 nm wavelengths. The both drugs showed same absorbance at 241nm. So the 241 nm wavelength was selected for simultaneous estimation of Salicylic acid and Beclomethasone dipropionate in ointment dosage form.

2.2 Selection of Chromatographic Conditions

Proper selection of the HPLC method depends upon the nature of the sample (ionic or ionizable or neutral molecule), its molecular weight and solubility. RP-HPLC was selected for the initial separation because of its simplicity and suitability. To optimize the chromatographic conditions the effect of chromatographic variables such as mobile phase, pH, flow rate and solvent ratio were studied. And the chromatographic parameters such as asymmetric factor, and resolution and column efficiency were calculated. The condition was chosen that gave the best resolution, symmetry and capacity factor was selected for estimation.

2.3 Selection of pH

The mixed standard solution containing 300ppm of Salicylic acid and 10ppm of Beclomethasone dipropionate were chromatographed with mobile phase containing different pH of buffer solution. For Salicylic acid and Beclomethasone dipropionate pH 4.0 was selected.

2.4 Chromatographic conditions:

- Column: Phenomenex C18 (250mm×4.6mm i.d.), 5µm
- Detector: 241 nm
- Injection Volume: 20 µl
- Flow Rate: 1.0 ml/min
- Temperature: Ambient
- Mobile Phase: Water-ACN-TEA (70-30-0.5) pH 4.0 with H_3Po_4
- Diluent: Methanol

METHODOLOGY

3.1 Preparation of Mobile Phase

Mix Double distilled water and Acetonitrile in proportion of 70:30 and set the pH 4.0 using Tri Ethy amine and 1% solution of o-phosphoric acid then sonicate it properly and filter the mobile phase through Whatman filter paper.

3.2 Preparation of Standard Solution of Salicylic acid and Beclomethasone dipropionate

The standard stock solution was prepared by transferring 100 mg of Salicylic acid and 10 mg of Beclomethasone dipropionate in a 100 ml volumetric flask. Dilute each upto 100ml using methanol. Take 3ml and 1ml solution from salicylic acid and beclomethasone dipropionate respectively in a 10ml volumetric flask and dilute this solution upto 10ml using methanol. Final standard concentration of Salicylic acid and Beclomethasone dipropionate is 300ppm and 10ppm respectively.

3.3 Estimation of Salicylic acid and Beclomethasone dipropionate in their ointment dosage form

Take ointment equivalent to 300mg of salicylic acid (10gm) into a 100ml volumetric flask. Add 20ml of methanol and place the volumetric flask on water bath previously adjusted temperature to 70ºC for 10 minute. Now allow to cool the solution and add

50ml of methanol. Shake the volumetric flask for 30 minute. Make up volume upto 100ml with methanol and filter through whatman filter paper. Take 1ml of filtrate into 10ml volumetric flask and make up volume using mobile phase.

Method Validation[21 to 23]

4.1. Linearity

The methods were validated according to International Conference on Harmonization Q2B guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for each analyte. Six point calibration curves were generated with appropriate volumes of working standard solutions for HPLC methods. The calibration range was 50-150 and 5-15 µg/ml for SA and BD, respectively in the HPLC methods of analysis for two drugs.

3.2. Precision and accuracy

Both precision and accuracy were determined with standard quality control samples (in addition to calibration standards) prepared in triplicates at different concentration levels covering the entire linearity range. Precision is the degree of repeatability of an analytical method under normal operational conditions. The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day) and reported as %R.S.D. for a statistically significant number of replicate measurements. The intermediate precision was studied by comparing the assays on 3 different days and the results documented as standard deviation and %R.S.D. Accuracy is the percent of analyte recovered by assay from a known added amount. Data from nine determinations over three concentration levels covering the specified range was determined .

3.3 LOD and LOQ

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from background levels. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with acceptable accuracy, precision and variability. The LOD and LOQ were calculated as

LOD = $3.3\sigma/S$, and LOQ = $10\sigma/S$

Where σ is the standard deviation of the lowest standard concentration

And S is the slope of the standard curve.

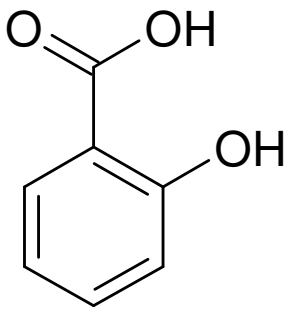
RESULTS AND DISCUSSION

The main objective of this work is to develop and validate RP- HPLC method for simultaneous estimation of Salicylic acid and Beclomethasone dipropionate in ointment dosage form. Pure drugs chromatogram was run in different mobile phases containing methanol, acetonitrile and different buffers in different ratios. The retention time and tailing factor was calculated for each drugs and for each chromatogram. Finally Water:Acetonitrile:Triethyamine (70:30:0.5) with pH set 4.0 using o-phosphoric acid(1%) and Phenomenex C-18 (250 x 4.6 mm), 5 µ analytical column was selected which gave a sharp and symmetrical peak with minimum tailing. This column has embedded polar groups. It has high carbon loads, which provide high peak purity and more retention to polar drugs. Calibration graph was found to be linear at range 50 – 150 µg/ml and 5 – 15 µg/ml for Salicylic acid and Beclomethasone dipropionate respectively. Six different concentrations of a mixture of two drugs in the range given above were prepared and 20 µl of each solution injected in HPLC. Regression analysis of the calibration data for Salicylic acid and Beclomethasone dipropionate showed that the dependent variable (peak area) and the independent variable (concentration) were represented by the equations: y = m x + c was found to y = 84.38 x + 41.14 and y = 63.44 x +6.654 for Salicylic acid and Beclomethasone dipropionate respectively. The regression coefficient (r^2) obtained was found to be 0.999 and 0.997 for Salicylic acid and Beclomethasone dipropionate respectively. It was observed that the concentration range showed a good relationship. The limit of detection was found to be 0.000667 µg/ml and 0.0201 µg/ml and the limit of quantification was found to be 0.00202 µg/ml and 0.0610 µg/ml for Salicylic acid and Beclomethasone dipropionate respectively. It proves the sensitivity

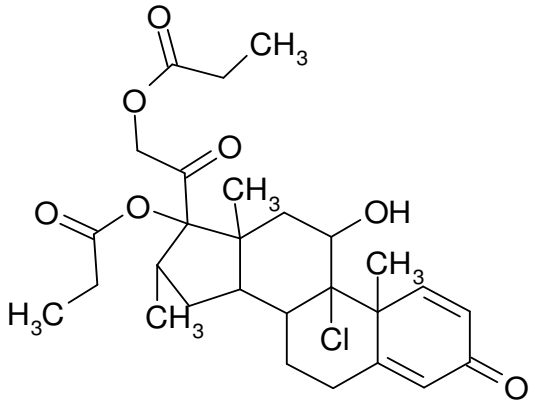
of method for the drugs. The average % recovery for Salicylic acid and Beclomethasone dipropionate was found to be 100.64% and 98.91% respectively which shows that method is free from interference from excipients present in the formulation. The low values of standard deviation and coefficient of variation at each level of the recovery experiment indicate high precision of the method.

CONCLUSION

The proposed RP-HPLC methods is simple, reliable and selective providing satisfactory accuracy and precision with lower limits of detection and quantification. The average % recovery for Salicylic acid and Beclomethasone dipropionate was found to be 100.64% and 98.91% respectively which shows that method is free from interference from excipients present in the formulation. The low values of standard deviation and coefficient of variation at each level of the recovery experiment indicate high precision of the method.



Structure of SA



Structure of BD

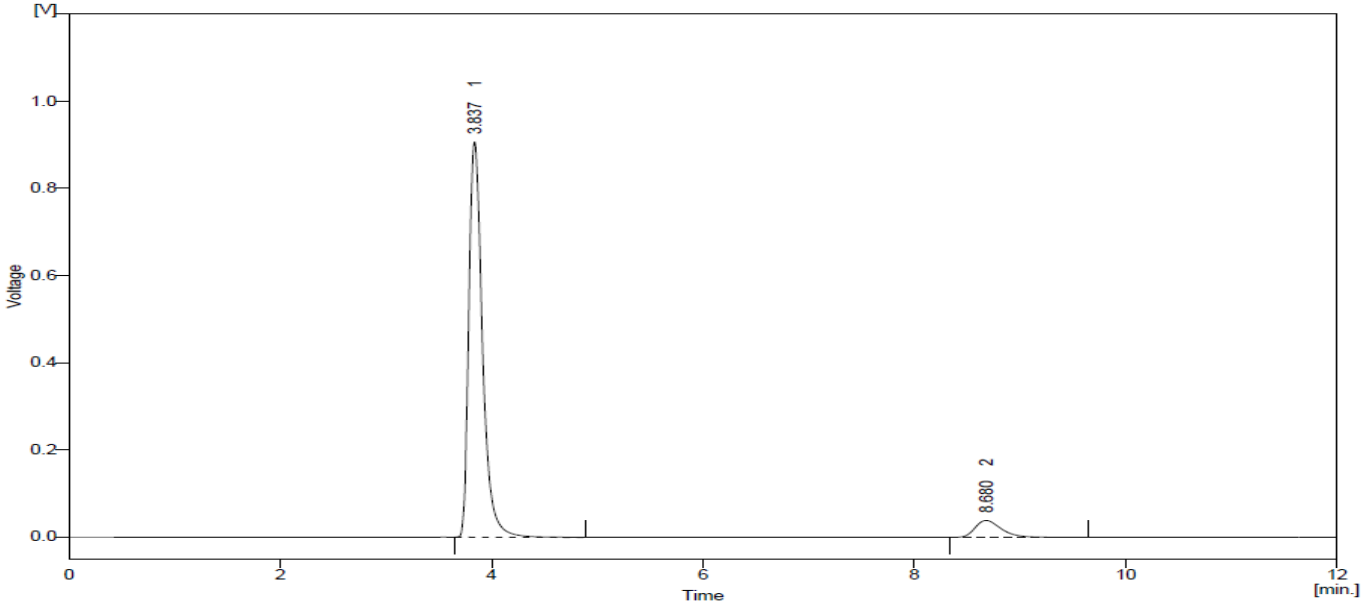


Figure 1: Chromatogram of Standard Salicylic acid and Beclomethasone dipropionate In Acetonitrile: water: triethyamine (40:70:0.5) with ph 4.0 using o-phosphoric acid

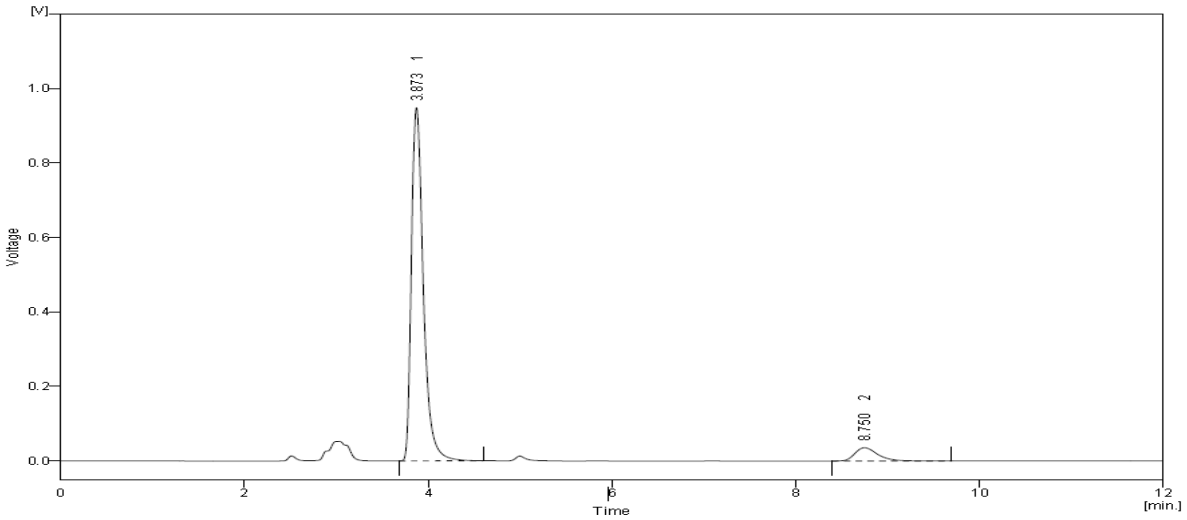


Figure 2:% Assay of marketed preparation (0.1% w/w Beclomethasone dipropionate and 3.0%w/w Salicylic acid)

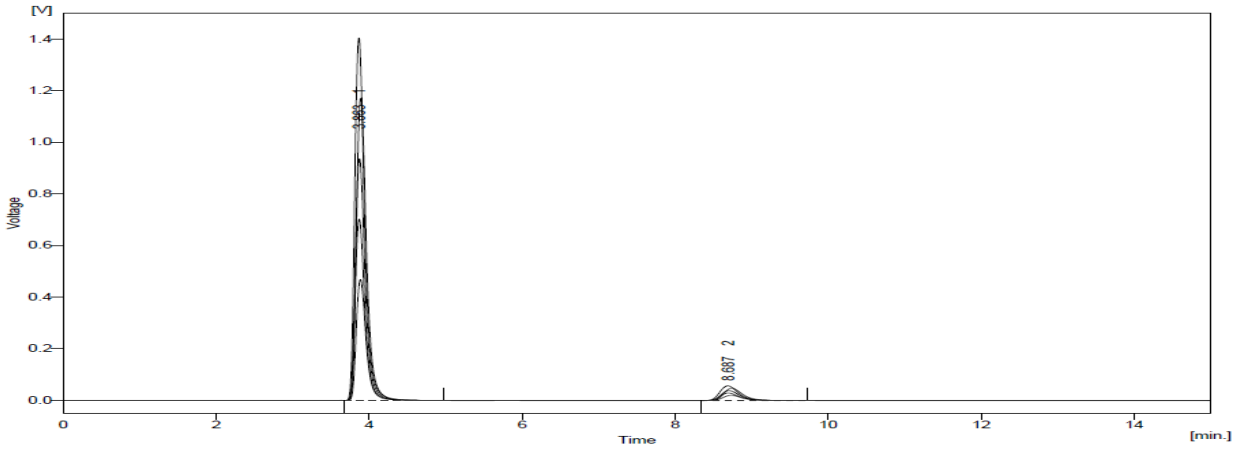


Figure 3: Overlain Chromatograms of Salicylic acid and Beclomethasone dipropionate

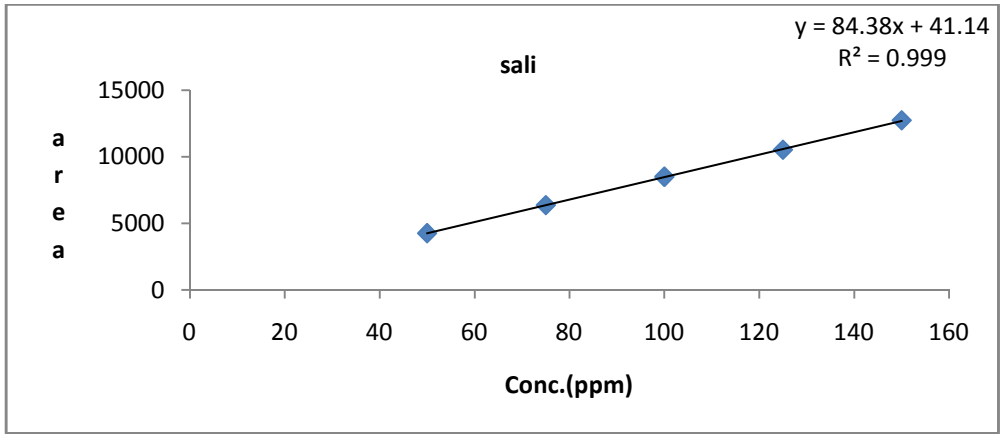


Figure 4: Linearity Calibration Curve of Salicylic acid

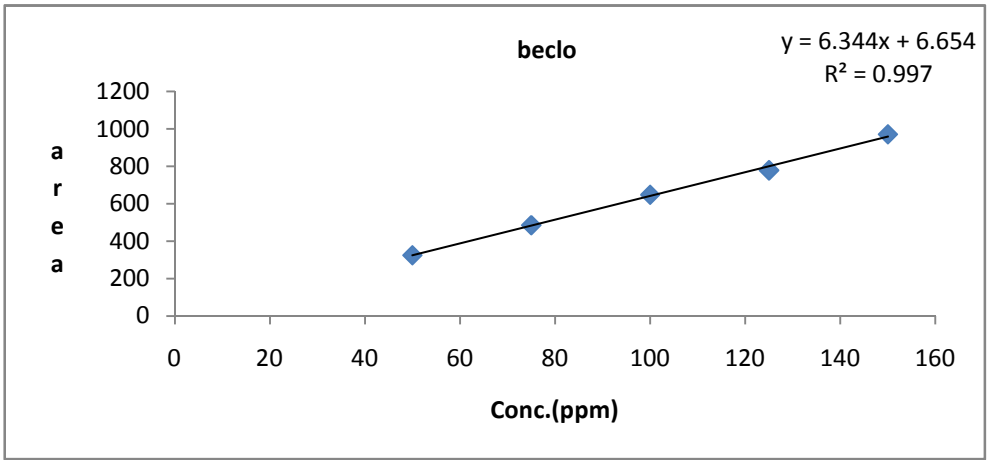


Figure 5: Linearity Calibration Curve of Beclomethasone dipropionate

Stock solution to be taken in ml	Dilute to volume with diluent	Final conc. in µg/ml Salicylic acid	Area
0.5	10	50	4262.72
0.75	10	75	6370.20
1.0	10	100	8503.24
1.25	10	125	10528.7
1.5	10	150	12730.9

Table 1: Linearity Data of Salicylic acid

Stock solution to be taken in ml	Dilute to volume with diluents	Final conc. in µg/ml Beclomethasone dipropionate	Area
0.5	10	5	324.23
0.75	10	7.5	484.89
1.0	10	10	647.56
1.25	10	12.5	778.42
1.5	10	15	970.58

Table 2: Linearity Data of Beclomethasone dipropionate

Parameters (n=5)	Salicylic acid	Beclomethasone dipropionate
Linearity Range	50 – 150 µg/ml	5 – 15 µg/ml
Linearity equation	y = 84.38 x + 41.42	y = 63.43 x +6.689
Regression coefficient	0.999	0.997
LOD	0.000667 µg/ml	0.020162 µg/ml
LOQ	0.00202 µg/ml	0.061097 µg/ml

Table 3: Results of Linearity, LOD and LOQ Study

Std	Conc. (µg/ml)	Area	Mean	SD	% RSD
	50	4296.977	4257.9	37.30	0.87
	50	4222.66			
	50	4254.163			
	100	8588.647	8500.8	81.46	0.95

SA	100	8427.703			
	100	8486.204			
	150	12794.346	12685	120.3	0.94
	150	12556.195			
	150	12705.519			
BD	5	326.793	324.09	2.47	0.76
	5	321.925			
	5	323.56			
	10	654.059	647.56	5.95	0.91
	10	642.362			
	10	646.263			
	15	975.543	967.35	8.87	0.91
	15	957.92			
	15	968.616			

Table 4: Intra-day precision data for Salicylic acid and Beclomethasone dipropionate

Std	Conc. (µg/ml)	Area	Mean	SD	% RSD
SA	50	4207.39	4263.38	52.50	1.23
	50	4311.53			
	50	4271.22			
	100	8435.24	8520.71	85.71	1.00
	100	8606.67			
	100	8520.23			
	150	12572	12731.9	149.18	1.17
	150	12867.4			
	150	12756.4			
BD	5	320.02	324.53	4.36	1.34
	5	328.74			
	5	324.83			
	10	642.36	649.08	6.82	1.05
	10	656.01			
	10	648.89			
	15	958.49	970.839	11.58	1.19
	15	981.48			
	15	972.53			

Table 5: Inter-day precision data for Salicylic acid and Beclomethasone dipropionate

Std	Conc. (µg/ml)	Area	Mean	SD	% RSD
SA	100	8435.249	8526.69	59.05	0.69
	100	8606.674			
	100	8520.235			
	100	8498.364			
	100	8569.341			
	100	8530.284			
BD	10	656.014	647.50	5.63	0.86
	10	642.364			
	10	648.89			
	10	643.854			
	10	651.624			
	10	642.258			

Table 6: Repeatability data for Salicylic acid and Beclomethasone dipropionate

For Salicylic acid					
Level	Amount of Drug added (µg/ml)	Amount of Drug recovered (µg/ml)	Recovery (%)	Mean ± SD%	% RSD
80 %	240	239.43	99.84	100.14 ± 0.33	0.33
	240	241.22	100.50		
	240	240.20	100.08		
100 %	300	297.74	99.24	99.59 ± 0.40	0.40
	300	298.48	99.49		
	300	300.13	100.04		
120 %	360	355.64	98.78	99.66 ± 0.81	0.82
	360	359.30	99.80		
	360	361.45	100.40		

Table 7: Results of Accuracy Data of Salicylic acid

For Beclomethasone dipropionate					
Level	Amount of Drug added (mg)	Amount of Drug recovered(mg)	Recovery (%)	Mean ± SD (%)	% RSD
80 %	8	8.005	100.07	100.34 ±0.32	0.32
	8	8.056	100.70		
	8	8.020	100.25		
100 %	10	9.942	99.48	100.52 ±1.19	1.19
	10	10.183	101.83		
	10	10.026	100.26		
120 %	12	11.889	99.07	100.46 ±1.26	1.25
	12	12.185	101.54		
	12	12.090	100.75		

Table 8: Results of Accuracy Data of Beclomethasone dipropionate

Sr.No	Flow rate +2%	Flow rate -2%	M.P.+2%	M.P.-2%	pH+0.2	pH-0.2
1	8177.538	8934.701	8597.052	8401.437	8537.023	8452.178
2	8079.149	9070.284	8373.162	8577.762	8443.616	8607.779
3	8086.177	8954.929	8469.201	8503.138	8486.211	8520.117
%RSD	0.67	0.81	1.32	1.04	0.55	0.91
Overall %RSD	0.88					

Table 9: Results of Robustness Study of Salicylic acid

Sr.No	Flow +0.2	Flow -0.2	M.P.+2%	M.P.-2%	pH+0.2	pH-0.2
1	622.979	680.258	654.631	639.737	650.174	643.661
2	613.429	687.161	636.889	650.834	639.549	650.377
3	616.018	681.84	644.921	647.586	646.217	648.875
%RSD	0.79	0.52	1.37	0.88	0.83	0.54
Overall %RSD	0.82					

Table 10: Results of Robustness Study of Beclomethasone dipropionate

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