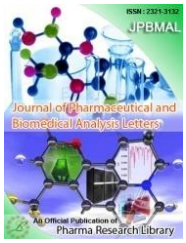

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New Validated Stability Indicating RP-HPLC Method Development for Simultaneously Estimation of Betamethasone and Calcipotriene in Its Bulk and Pharmaceutical Dosage Form

Naveen .S*[1], T. Praveena[2]
[1]Department of Pharmaceutical Analysis, Jagan's Institute of Pharmaceutical Science, Jangala Kandriga, SPSR Nellore –524 346. A.P. India.
[2]Associate Professor, Department of Pharmaceutical Analysis, Jagan's Institute of Pharmaceutical Science, SPSR Nellore –524346. A.P. India.

A B S T R A C T

This study focused on developing and characterizing floating microspheres of Propranolol HCl, aiming to enhance gastric retention and sustained drug release. A UV-Visible spectrophotometric method was validated for drug quantification in 0.1N HCl at 256 nm, exhibiting strong linearity over the concentration range of 2 to 10 µg/ml. Compatibility studies using FTIR spectroscopy confirmed no chemical interactions between the drug and polymers, ensuring formulation stability. The prepared microspheres showed high percentage yields between 81.58% and 95.50%, with drug entrapment efficiency improving as polymer concentration increased due to higher solution viscosity reducing drug diffusion during formation. Further evaluations revealed that the microspheres exhibited satisfactory swelling behavior and mucoadhesive properties essential for prolonged gastric residence. In vitro release experiments demonstrated efficient and controlled drug liberation over 12 hours, with release kinetics fitting diffusion-controlled models such as zero-order, first-order, Higuchi, and Korsmeyer-Peppas. Optimized formulations showed promising potential for sustained release and enhanced gastroretention, which could improve therapeutic effectiveness and patient adherence.

Keywords: Propranolol HCl, floating microspheres, UV-Visible spectrophotometry, sustained release, mucoadhesion, drug release kinetics, FTIR compatibility.

A R T I C L E I N F O

Corresponding Author
Naveen.S
Department of Pharmaceutical Analysis
Jagan's Institute of Pharmaceutical Science
Jangala Kandriga, SPSR Nellore –524 346. A.P. India

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C O N T E N T S

1. Introduction

Betamethasone is a systemic corticosteroid used to relieve inflammation in various conditions, including but not limited to allergic states, dermatologic disorders, gastrointestinal diseases, and hematological disorders.

IUPAC Name:
(1R,2S,3aS,3bS,9aS,9bR,10S,11aS)-9b-fluoro-1,10-dihydroxy-1-(2-hydroxyacetyl)-2,9a,11a trimethyl 1H,2H, 3H, 3aH, 3bH, 4H, 5H, 7H, 9aH, 9bH, 10H, 11, 11a H-cyclopenta [a] phenanthren-7-one
Chemical Formula: $C_{22}H_{29}FO_5$
Molecular weight : 392.4 g/mol
Melting point: 183-1840C

Solubility: The solubility in methanol, ethanol, 1-propanol, acetic acid, and N,N-dimethylacetamide has been measured between 278.15 and 323.15 K at atmospheric pressure.

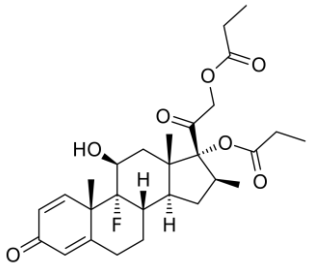

Fig.1: Betamethasone

Pharmacodynamics:

Corticosteroids bind to the glucocorticoid receptor inhibiting pro-inflammatory signals, while promoting anti-inflammatory signals.1 Corticosteroids have a wide therapeutic window as patients may require doses that are multiples of what the body naturally produces.1 Patients who require long-term treatment with a corticosteroid should be counselled regarding the risk of hypothalamic-pituitary-adrenal axis suppression and increased susceptibility to infections.

Mechanism of action:

Glucocorticoids inhibit neutrophil apoptosis and demargination, and inhibit NF-Kappa B and other inflammatory transcription factors.1 They also inhibit phospholipase A2, leading to decreased formation of arachidonic acid derivatives.1 In addition, glucocorticoids promote anti-inflammatory genes like interleukin-10.1 Corticosteroids like betamethasone can act through nongenomic and genomic pathways. The genomic pathway is slower and occurs when glucocorticoids activate glucocorticoid receptors and initiate downstream effects that promote transcription of anti-inflammatory genes including phosphoenolpyruvate carboxykinase (PEPCK), IL-1-receptor antagonist, and tyrosine amino transferase (TAT). On the other hand, the nongenomic pathway is able to elicit a quicker response by modulating T-cell, platelet and monocyte activity through the use of existing membrane-bound receptors and second messengers.

Calcipotriol (INN) or calcipotriene (USAN) is a sythetic derivative of calcitriol or Vitamin D.

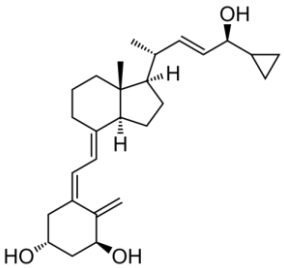

Fig.2: Calcipotriene

IUPAC Name:

3-ethyl-4-methyl-N{2[4({[(4methylcyclohexyl) carbamoyl] amino} sulfonyl) phenyl] ethyl}-2-oxo-2,5-dihydro-1H-pyrrole-1-carboxamide

Molecular Formula : $C27H_{40}O_3$

Mol. mass : 412.604 g/mol

Melting Point : 212-215^{o}C

Solubility : Soluble in water, DMSO and ethanol

Pharmacodynamics:

Calcipotriene is a synthetic analog of vitamin D. In humans, the natural supply of vitamin D depends mainly on exposure to the ultraviolet rays of the sun for conversion of 7-dehydrocholesterol to vitamin D3 (cholecalciferol) in the skin.

Mechanism of action:

The precise mechanism of calcipotriol in remitting psoriasis is not well-understood, however, it has been shown to have comparable affinity with calcitriol for the Vitamin D receptor while being less than 1% the activity in regulating calcium metabolism. The Vitamin D receptor (VDR) belongs to the steroid/thyroid receptor superfamily, and is found on the cells of many different tissues including the thyroid, bone, kindney, and T cells of the immune system. T cells are known to play a role in psoriasis and are believed to undergo modulation of gene expression with binding of calcipotriol to the VDR. This modulation is thought to affect gene products related to cell differentiation and proliferation.

2. Materials and Methods

Table 1: Instruments used

S.No	Instrument	Model
1	HPLC	WATERS, software: Empower, 2695 separation module.2487 UV detector.
2	UV/VIS spectrophotometer	LABINDIA UV 3000^{+}
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil

Table 2: Chemicals used

S.No	Chemical	Brand
1	Betamethasone	Supplied by KP LBAS
2	Calcipotriene	Supplied by KP LBAS
3	Ortho phosphoric acid	FINAR chemical LTD
4	Water and Methanol for HPLC	Standard solutions Ltd
5	Acetonitrile for HPLC	Standard solutions Ltd
6	HCl, H_2O_2, NaOH	MERCK

Optimized chromatographic conditions:

Instrument used : Waters HPLC 2695 Model with auto sampler and 2996 PDA detector.

Temperature : Ambient

Column: Dikma Endeversil C_{18} ODS (2.1x150mm, 3.0 µm)

Buffer : 3.4g of KH_2PO_4 in 1000 ml of HPLC water Ph was adjusted with OPA up to 3.0.

pH : 3.0

Mobile phase : 45% buffer 55% ACN

Flow rate : 1.0 ml per min

Wavelength : 254 nm

Injection volume : 20 µl

Run time : 12 min.

Preparation of buffer and mobile phase:

Preparation of Phosphate buffer:

3.4g of KH_2PO_4 in 1000 ml of HPLC water Ph was adjusted with OPA up to 3.0.final solution was filtered through 0.44 µm Membrane filter and sonicate it for 10 mins.

Preparation of mobile phase:

Accurately measured 450 ml (45%) of above buffer and 550 ml of ACN HPLC (100%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

Standard Solution Preparation

500 ppm of Betamethasone, 50 ppm of Calcipotriene): Accurately weigh and transfer 100 mg of Betamethasone and 10 mg of Calcipotriene working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 0.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation

500 ppm of Betamethasone & 50 ppm of Calcipotriene: Weigh accurately about 1000 mg of sample from top, middle and bottom position of the tube into a 10 ml clean dry volumetric flask add about 7mL diluents sonicate for 20 minutes with intermediate shaking. Cool to room temperature. Make up to the volume with diluents and mix well. Filter through 0.45µ PVDF filter and Inject.

Procedure:

Inject 4 µl of the standard, sample into the chromatographic system and measure the areas for Betamethasone and Calcipotriene peaks and calculate the %Assay by using the formulae.

- Standard and Sample solutions are stable for 48 hours at room temperature.
- 0.45µ Nylon and 0.45µ PTFE syringe filters can be used as alternative to 0.45µ PVDF syringe filter.

System suitability:

Tailing factor for the peaks due to Betamethasone and Calcipotriene in Standard solution should not be more than 2.0. Theoretical plates for the Betamethasone and Calcipotriene peaks in Standard solution should not be less than 2000. Resolution for the Betamethasonee and Calcipotriene peaks in standard solution should not be less than 2.

Calculation: (For Betamethasone)

$$\%\ Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\ weight}{Label\ Claim} * \frac{P}{100} * 100$$

AT = average area counts of sample preparation.
AS = average area counts of standard preparation.
WS = Weight of working standard taken in mg.
P = Percentage purity of working standard
LC = Label Claim mg/ml.

2. Linearity

Preparation of stock solution:

Accurately weigh and transfer 100mg of Betamethasone and 10 mg of Calcipotriene working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I: (250 ppm of Betamethasone and 25 ppm of Calcipotriene): 0.25 ml of above stock solutions has taken in 10ml of volumetric flask, dilute up to the mark with diluent.

Preparation of Level – II: (375 ppm of Betamethasone and 37.5 ppm of Calcipotriene): 0.375 ml of above stock solutions has taken in 10ml of volumetric flask, dilute up to the mark with diluent.

Preparation of Level – III: (500 ppm of Betamethasone and 50 ppm of Calcipotriene): 0.5ml of above stock solutions has taken in 10ml of volumetric flask, dilute up to the mark with diluent.

Preparation of Level – IV: (625 ppm of Betamethasone and 62.5 ppm of Calcipotriene): 0.625 ml of above stock solutions has taken in 10ml of volumetric flask, dilute up to the mark with diluent

Preparation of Level – V : (750 ppm of Betamethasone and 75 ppm of Calcipotriene): 0.75 ml of above stock solutions has taken in 10ml of volumetric flask, dilute up to the mark with diluent

Procedure:

Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient

3. Precision:

Preparation of stock solution:

Accurately weigh and transfer 100 mg of Betamethasone and 10 mg of Calcipotriene working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

The standard solution was injected for six times and measured the area for all six. Injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits

4. Intermediate Precision/Ruggedness

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day.

Preparation of stock solution:

Accurately weigh and transfer 100 mg of Betamethasone and 10 mg of Calcipotriene working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

The standard solutions prepared in the precision was injected on the other day, for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

5. Accuracy:

Preparation of Standard stock solution:

Accurately weigh and transfer 100 mg of Betamethasone and 10 mg of Calcipotriene working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration): Accurately weigh and transfer 50 mg of Betamethasone and 5 mg of Calcipotriene working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 0.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

For preparation of 100% solution (With respect to target Assay concentration):

Accurately weigh and transfer 100 mg of Betamethasone and 10 mg of Calcipotriene working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

For preparation of 150% solution (With respect to target Assay concentration):

Accurately weigh and transfer 100 mg of Betamethasone and 15 mg of Calcipotriene working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 0.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure: Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Betamethasone & Calcipotriene and calculate the individual recovery and mean recovery values.

6. Limit of Detection: (for Betamethasone)

Preparation of 500µg/ml solution:

Accurately weigh and transfer 100 mg of Betamethasone working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 1.39 µg/ml solution:

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Further pipette 0.28ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Acceptance Criteria:

S/N Ratio value shall be 3 for LOD solution.

Limit of quantification:

Preparation of 500 µg/ml solution:

Accurately weigh and transfer 100 mg of Betamethasone working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 4.63 µg/ml solution: Further pipette 1 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 0.925 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Acceptance Criteria:

S/N Ratio value shall be 10 for LOQ solution.

Limit of Detection: (For Calcipotriene)

Preparation of 50 µg/ml solution:

Accurately weigh and transfer 10 mg of Calcipotriene working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 1.32 µg/ml solution:

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 2.63ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent

Acceptance Criteria:

S/N Ratio value shall be 3 for LOD solution.

Limit of quantification:

Preparation of 50 µg/ml solution:

Accurately weigh and transfer 10 mg of Calcipotriene working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 4.38 µg/ml solution:

Further pipette 2ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 4.38ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Acceptance Criteria: S/N Ratio value shall be 10 for LOQ solution.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

A. The flow rate was varied at 0.9ml/min to 1.1 ml/min

Standard solution 500 ppm of Betamethasone & 50 ppm of Calcipotriene was prepared and analyzed using the varied flow rates along with method flow rate. On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%.

B. The Organic composition in the Mobile phase was varied from ±10%

Standard solution 500 ppm of Betamethasone & 50 ppm of Calcipotriene was prepared and analyzed using the varied Mobile phase composition along with the actual mobile phase composition in the method. On evaluation of the above results, it can be concluded that the variation in 10%.Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the Mobile phase ±10.

Degradation Studies:

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Betamethasone and Calcipotriene using the proposed method.

Preparation of stock: Weigh accurately about 1000 mg of sample from top, middle and bottom position of the tube into a 10 ml clean dry volumetric flask add about 7mL diluents sonicate for 20 minutes with intermediate shaking. Cool to room temperature. Make up to the volume with diluents and mix well. Filter through 0.45µ PVDF filter and Inject

Hydrolytic degradation under acidic condition

Weigh accurately about 1000 mg of sample from top, middle and bottom position of the tube into a 50ml clean dry volumetric flask add about 7mL diluents sonicate for 20 minutes with intermediate shaking. Cool to room temperature volumetric flask and to this add 3 ml of 0.1N HCl was added. Then, the volumetric flask was kept at 60ºC for 24 hours and then neutralized with 0.1 N NaOH and Make up to the volume with diluents and mix well. Filter through 0.45µ PVDF filter and Inject

Hydrolytic degradation under alkaline condition

Weigh accurately about 1000 mg of sample from top, middle and bottom position of the tube into a 50 ml clean dry volumetric flask add about 7ml diluents sonicate for 20 minutes with intermediate shaking. Cool to room temperature volumetric flask and to this add 3 ml of 0.1N NaoH was added. Then, the volumetric flask was kept at 60ºC for 24 hours and then neutralized with 0.1 N HCL and Make up to the volume with diluents and mix well. Filter through 0.45µ PVDF filter and Inject

Thermal induced degradation

Betamethasone and Calcipotrienee sample was taken in petridish and kept in Hot air oven at 1100C for 3 hours. Then the sample was taken and diluted with diluents and injected into HPLC and analyzed.

Oxidative degradation

Weigh accurately about 1000 mg of sample from top, middle and bottom position of the tube into a 50 ml clean dry volumetric flask add about 7mL diluents sonicate for 20 minutes with intermediate shaking. Cool to room temperature volumetric flask and to this add 1 ml of 30% v/v H2O2 was added. Then, the volumetric flask was kept at 60ºC for 24 hours and then make up to the volume with diluents and mix well. Filter through 0.45µ PVDF filter and Inject

Photo degradation:

Weigh accurately about 1000 mg of sample from top, middle and bottom position of the tube into a 50 ml clean dry volumetric flask add about 7mL diluents sonicate for 20 minutes with intermediate shaking. Cool to room temperature volumetric flask then, the volumetric flask was kept at 60ºC for 24 hours in UV Chamber Make up to the volume with diluents and mix well. Filter through 0.45µ PVDF filter and Inject.

3. Results and Discussion

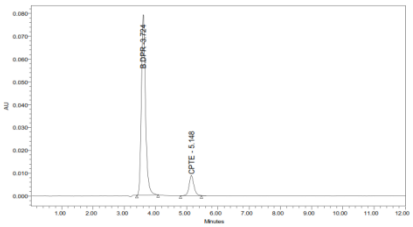

Fig.3: Chromatogram for system suitability

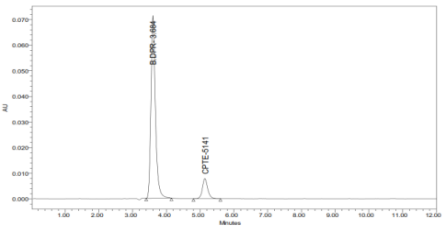

Fig.4: Chromatogram for Standard

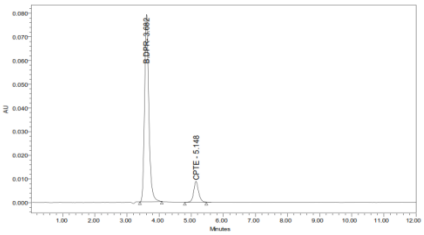

Fig.5: Chromatogram for Sample

Assay Results: (Betamethasone)

$$\frac{855999}{854516.7} * \frac{100}{10} * \frac{0.5}{10} * \frac{10}{1000} * \frac{1}{1} * \frac{100}{0.5} * \frac{99.8}{100} * 100 = 99.97\%$$

Assay Results: (For Calcipotriene)

$$\frac{115671}{114706} * \frac{10}{10} * \frac{0.5}{10} * \frac{10}{1000} * \frac{1}{1} * \frac{100}{0.05} * \frac{99.8}{100} * 100 = 100.64\%$$

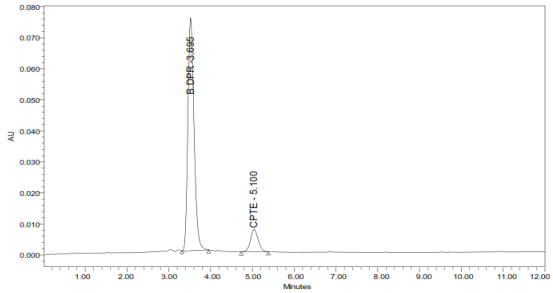

Fig.6: Chromatogram for linearity-5

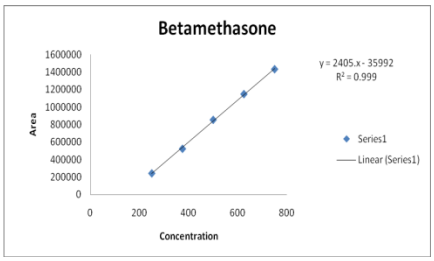

Fig.7: Calibration graph for Betamethasone

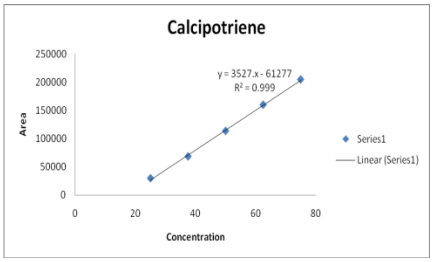

Fig.8: Calibration graph for Calcipotriene

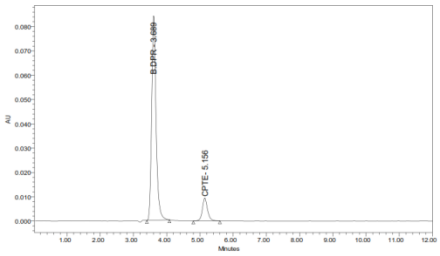

Fig.9: Chromatogram for Precision -6

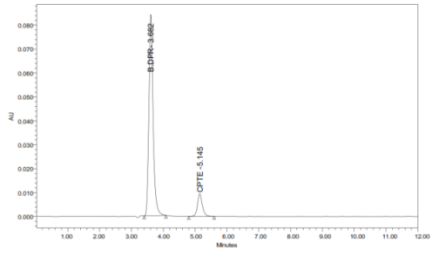

Fig.10: Chromatogram for ID Precision -6

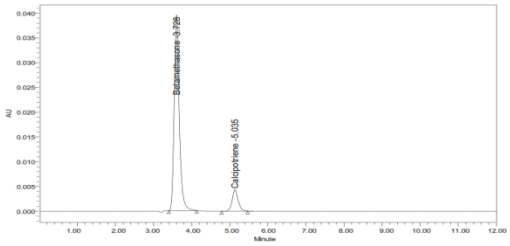

Fig.11:: Chromatogram for Accuracy 50%-3

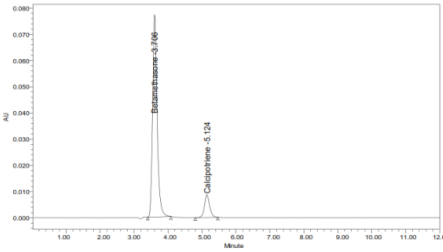

Fig.12: Chromatogram for Accuracy 100%-3

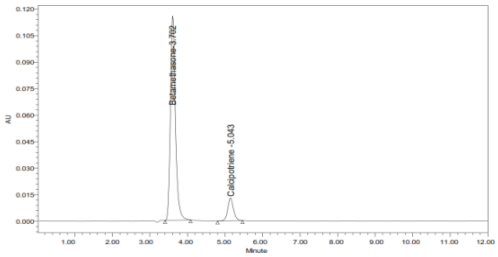

Fig.13: Chromatogram for Accuracy 150%-3

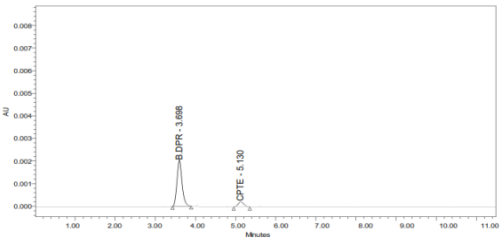

Fig.14: Betamethasone, Calcipotriene showing LOD

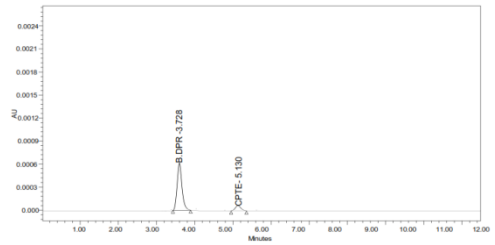

Fig.15: Betamethasone, Calcipotriene showing LOQ

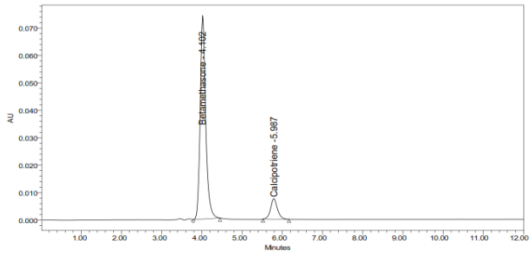

Fig.16: Chromatogram showing less flow

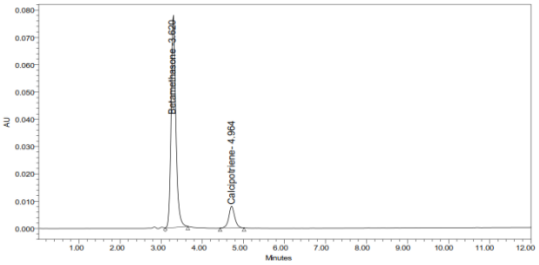

Fig.17: Chromatogram showing more flow

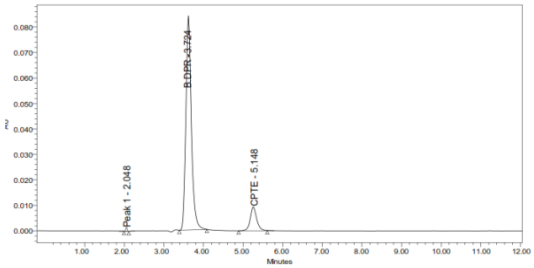

Fig.18: Acid degradation

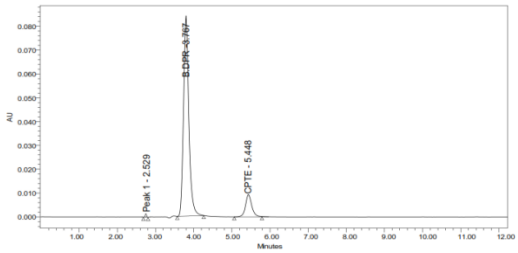

Fig.19: Base Degradation

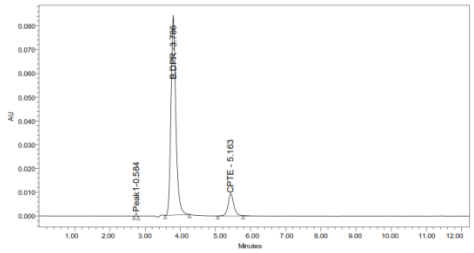

Fig.21: Thermal degradation

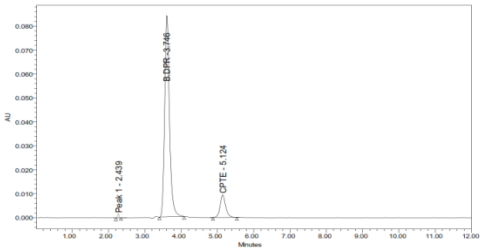

Fig.20: Peroxide degradation

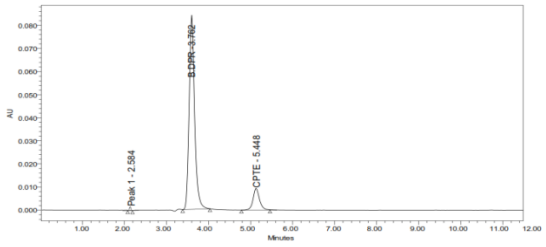

Fig.22: Photo degradation

Table 3: Area of different concentration of Betamethasone and Calcipotriene

S. No	Betamethasone		Calcipotriene	
	Concentration (µg/ml)	**Area**	**Concentration (µg/ml)**	**Area**
1	250	244841	25	29672
2	375	525756	37.5	68336
3	500	856654	50	113345
4	625	1150925	62.5	159680
5	750	1435608	75	204473

Table 4: The results are summarized for Betamethasone and Calcipotriene

Injection	Area for Calcipotriene	Area for Betamethasone
Injection-1	111368	852828
Injection-2	112717	852337
Injection-3	112655	858355
Injection-4	113939	852839
Injection-5	111576	858513
Injection-6	112282	857582
Average	112662.3	855409.0
Standard Deviation	845.7	12.524.5
%RSD	0.8	0.4

Table 5: The results are summarized for Betamethasone and Calcipotriene

Injection	Area for Betamethasone	Area for Calcipotriene
Injection-1	859453	112535
Injection-2	857162	111224
Injection-3	859458	112915
Injection-4	858377	113391
Injection-5	858482	113108
Injection-6	859771	112959
Average	858783.8	112688.7
Standard Deviation	976.1	769.7
%RSD	0.1	0.7

Table 6: The accuracy results for Betamethasone

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	427928	50	49.98	99.96	99.86
100%	854989	100	99.86	99.86	
150%	1281399	150	149.66	99.77	

Table 7: The accuracy results for Calcipotriene

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	57620	5	5.01	100.26	99.96
100%	114986	10	10.00	100.04	
150%	171648	15	14.93	99.56	

Table 8: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Betamethasone	61	181	2.97
Calcipotriene	61	182	2.98

Table 9: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Betamethasone	61	608	9.97
Calcipotriene	61	609	9.98

Table 10: System suitability results for Betamethasone:

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	4361.01	1.24
2	1.0	4281.91	1.14
3	1.1	4137.68	1.18

Table 11: System suitability results for Calcipotriene:

S. No	Flow Rate (ml/min)	System Suitability Results		
		USP Plate Count	USP Tailing	USP Resolution
1	0.9	9749.13	1.09	6.44
2	1.0	9959.43	1.13	5.66
3	1.1	9286.06	1.04	6.11

4. Conclusion

A simple, precise, and robust RP-HPLC method was successfully developed and validated for the simultaneous estimation of Betamethasone and Calcipotriene in bulk and pharmaceutical dosage forms, following ICH Q2(R1) guidelines. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.8 and 0.4 for Betamethasone and Calcipotriene which shows that the method is precise. The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.1 and 0.7 for Betamethasone and Calcipotriene which shows that the method is repeatable when performed in different days also. The acceptance criteria for LOD and LOQ is 3 and 10. The LOD and LOQ for Betamethasone was found to be 2.97 and 9.97 and LOD and LOQ for Calcipotriene was found to be 2.98 and 9.98. The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions. The Forced degradation also done for the proposed method and it is suitable for the stability Studies of betamethasone and Calcipotriene in its pure and Ointment Dosage form. Hence the Developed method is used for the routine analysis of the drugs in the quality control of the industry.

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