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Analytical Method Development for simultaneous estimation of Calcipotriene and Dipropionate in Pharmaceutical dosage form.

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ABSTRACT

A new method was established simultaneous estimation of calcipotriene and dipropionate by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of calcipotriene and dipropionate by using coloumn xterr C-18(4.6*250mm) 5µm, flow rate was 1.0ml/min mobile phase ratio Phosphate buffer (0.05M) pH 3.6: ACN (40:60%v/v) (pH was adjusted with ortho phosphoric acid), detection wave length 260nm.the instrument used was water HPLC auto sampler and PDA or detector. The analytical method was validated to ICH guidelines. The linearity range Dipropionate and Calcipotriene were found to be from 100-500 µg/ml of Dipropionate and 1-5µg/ml of Calcipotriene. Linear regression coefficient was not more than 0.999.

Keywords : calcipotriene, dipropionate, RP-HPLC, phosphate buffer and ACN.

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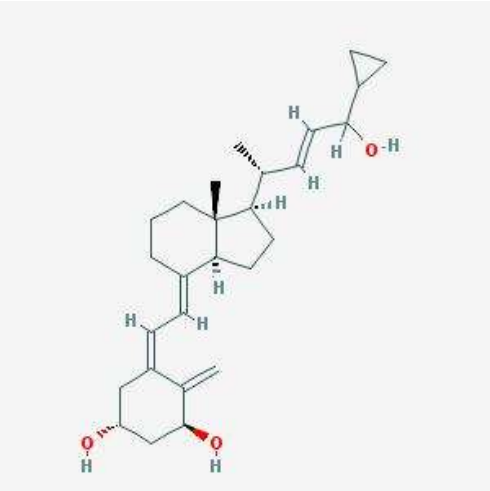
INTRODUCTION

Analytical chemistry is the science that seeks ever improved means of measuring the chemical composition of natural and artificial materials. Chemical composition is the entire picture (composition) of the material at the chemical scale and includes geometric features such as molecular morphologies and distributions of species within a sample as well as single dimensional features such as percent composition and species identity[1]

Drug Profile

Calcipotriene Calcipotriol (INN) or calcipotriene[2] (USAN) is a synthetic derivative of calcitriol, a form of vitamin D. It is used in the treatment of psoriasis, marketed under the trade name "Dovonex" in the United States, "Daivonex" outside of North America, and "Psorcutan" in Germany. This medication is safe for long-term application in psoriatic skin conditions.



IUPAC Name:(1R,3S,5Z)-5-[(2E)-2-[(1R,3aS,7aR)-1-[(E,2R)-5-cyclopropyl-5-hydroxypent-3-en-2-yl]-7a-methyl-2,3,3a,5,6,7-hexahydro-1H-inden-4-ylidene]ethylidene]-4-methylidenecyclohexane-1,3-diol

Chemical formula	$C_{27}H_{40}O_3$
Molecular weight	412.6047 g/mol
Description	This compound belongs to the class of organic compounds known as triterpenoids. These are terpene molecules containing 8 isoprene units.
Cas No	112965-21-6
Melting Point	305.11 °C
Category	Anti –retro Viral Activity.

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% as active as the calcitriol in regulating calcium metabolism. The Vitamin D

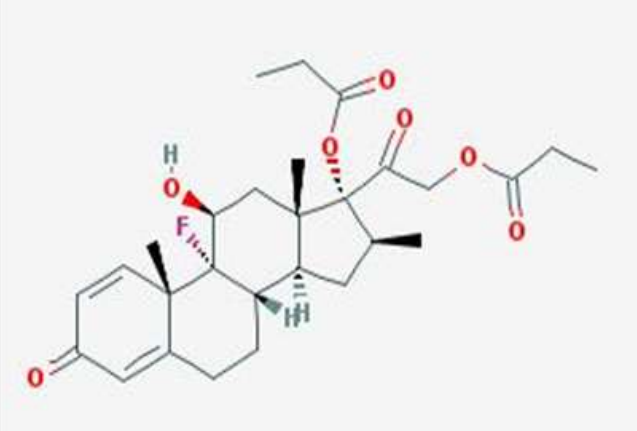
receptor (VDR)[3] belongs to the steroid/thyroid receptor superfamily, and is found on the cells of many different tissues including the thyroid, bone, kindney, and T cells of the immune system. T cells are known to play a role in psoriasis, and it is thought that the binding of calcipotriol to the VDR modulates the T cells gene transcription of cell differentiation and proliferation related genes.

Generic Name : Calcipotriol

Brand name : Dovonex , Calcitrene

Dipropionate

Betamethasone dipropionate is a glucocorticoid steroid with anti-inflammatory and immunosuppressive abilities. It is applied as a topical cream, ointment, lotion or gel to treat itching and other minor skin conditions such as eczema



IUPAC Name:[2-[(8S,9R,10S,11S,13S,14S,16S,17R)-9-fluoro-11-hydroxy-10,13,16- trimethyl-3-oxo-17-propanoyloxy-6,7,8,11,12,14,15,16-octahydrocyclopenta[a]phenanthren-17-yl]-2-oxoethyl] propanoate

Chemical formula	C28H37FO7
Molecular weight	504.587583 g/mol
Cas No	5534-09-8
Solubility	soluble in water, Methanol
Category	anti inflammatory

Mechanism of action

Unbound corticosteroids cross cell membranes and bind with high affinity to specific cytoplasmic receptors. The result includes inhibition of leukocyte infiltration at the site of inflammation, interference in the function of mediators of inflammatory response, suppression of humoral immune responses, and reduction in edema or scar tissue. The anti inflammatory actions of corticosteroids are thought to involve phospholipase A2 inhibitory proteins, lipocortins, which control the biosynthesis of potent mediators of inflammation such as prostaglandins and

leukotrienes[4]. For the investigated use in the treatment of GvHD or Crohn's[5], beclometasone acts by binding to interleukin-13 to inhibit cytokines, which in turn inhibits inflammatory chemicals downstream

Generic Name : Dipropionate

Brand Name : Diprolene, Diprolene AF

EXPERIMENTAL METHODOLOGY

HPLC METHOD DEVELOPMENT:

Mobile Phase Optimization:

Initially the mobile phase tried was methanol: Ammonium acetate buffer and Methanol: phosphate buffer with various combinations of pH as well as varying proportions[6]. Finally, the mobile phase was optimized to potassium dihydrogen phosphate with buffer (pH 3.0), Methanol in proportion 30: 70 v/v respectively.

Wave length selection:

UV spectrum of 10 µg / ml Dipropionate and Calcipotriene in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 260. At this wavelength both the drugs show good absorbance.

Optimization of Column:

The method was performed with various columns like C18 column, hypersil column, lichrosorb, and inertsil ODS column. Inertsil ODS[7](4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 0.8ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used	Waters HPLC with auto sampler and PDA or detector.
Temperature	Ambient
Column	Inertsil ODS (4.6 x 150mm, 5µm)
Buffer	6.8 grams of potassium dihydrogen ortho phosphate in 1000 ml water pH adjusted with ortho phasphoric acid.
pH	3.0
Mobile phase	30% buffer 70% Methanol
Flow rate	0.8 ml per min
Wavelength	260 nm
Injection volume	10 µl

Preparation of Buffer and Mobile Phase:

Preparation of Phosphate buffer:

Accurately weighed 6.8 grams of KH_2PO_4 was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 3.0 with Orthophosphoric acid.

Preparation of mobile phase:

Accurately measured 300 ml (30%) of above buffer and 700 ml of Methanol HPLC (70%) [8.] 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.

Preparation Of The Dipropionate & Calcipotriene

Standard & sample solution:

Standard Solution Preparation:

Accurately weigh and transfer 10 mg of Dipropionate and Calcipotriene 10mg of working standard into a 10mL& 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 3ml& 0.3ml of the above stock solutions[9] a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 10 mg of Dipropionate and Calcipotriene (marketed formulation) sample into a 10ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 3 ml of Dipropionate and Calcipotriene of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for Dipropionate and Calcipotriene peaks and calculate the %Assay by using the formulae.

System Suitability:

Tailing factor for the peaks due to Dipropionate and Calcipotriene[10] in Standard solution

Should not be more than 2.0.Theoretical plates for the Dipropionate and Calcipotriene peaks in Standard solution should not be less than 2000.

Calculation: (For Dipropionate)

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{\text{Label Claim}} \times 100$$

Where:

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 OF Dipropionate mg/ml.

Calculation: (For Calcipotriene)

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt.}}{\text{Label Claim}} \times 100$$

Where:

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 OF Calcipotriene mg/ml

S. No.	Samples
1	Dipropionate & Calcipotriene Tablets
2	Dipropionate & Calcipotriene working standards

RESULTS AND DISCUSSION

Optimized Chromatogram Is Obtained By Following Condition

Trial 1:

Mobile phase :Water: Methanol (50:50%v/v)

Column :Xterra C18 (4.6*250mm) 5µm

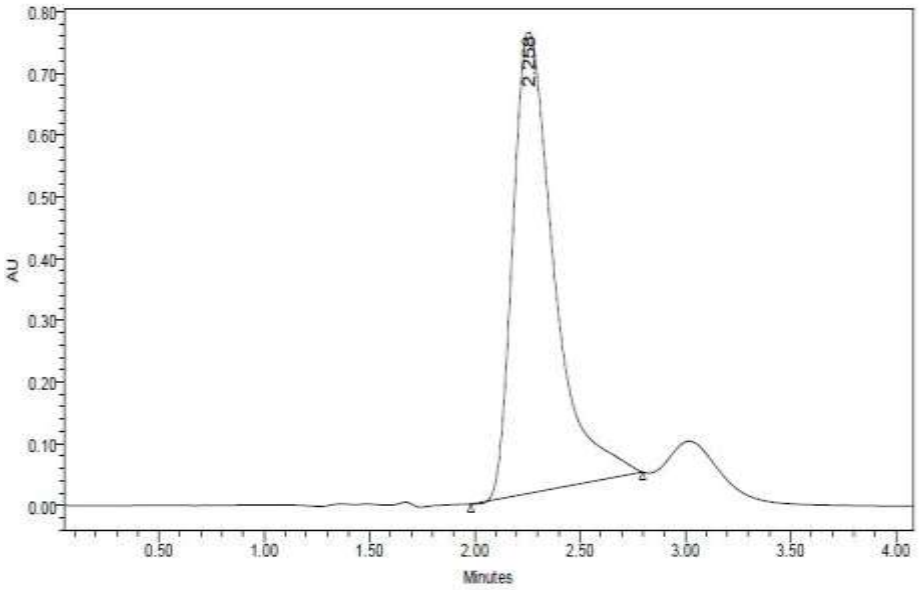
Flow rate :1.0 ml/min

Wavelength :260 nm

Column temp :Ambient

Sample Temp :Ambient

Injection Volume :10 µl



Trial chromatogram for dipropionate and Calcipotriene

From the above chromatogram it was observed that the dipropionate peak was splitted

Trial 2:

Mobile phase :Phosphate buffer (0.05m) pH 4.0: Methanol (40:60%v/v)

Column :Make; Xterra C18 (4.6*250mm) 5µm

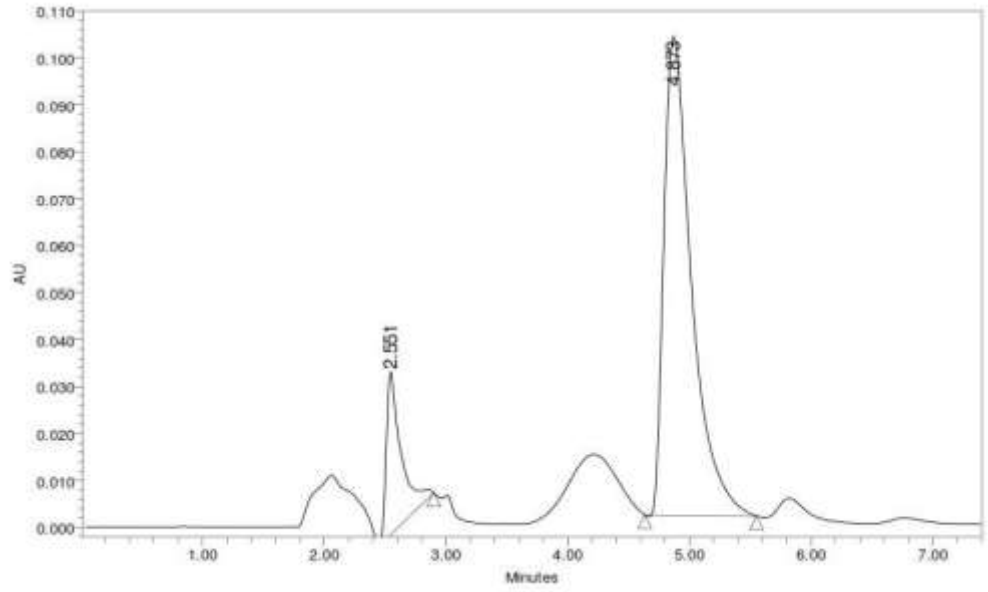
Flow rate :1.0 ml/min

Wavelength :260 nm

Column temp :Ambient

Sample Temp :Ambient

Injection Volume: 10 µl

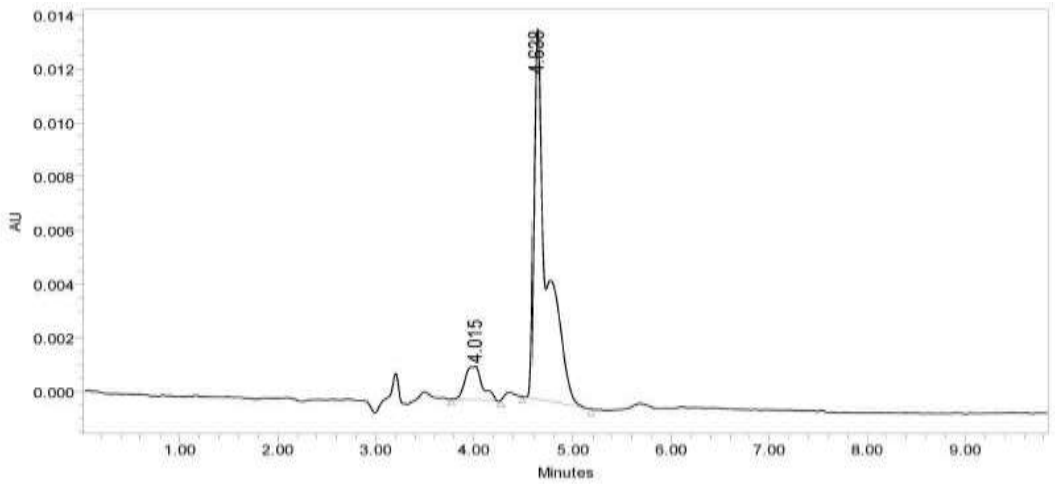


Trial chromatogram for dipropionate and Calcipotriene

From the above chromatogram it was observed that the dipropionate and Calcipotriene peaks are splitted

Trial 3:

Mobile phase	:	Phosphate buffer (0.05m) pH 4.0: Methanol (40:60%v/v)
Column	:	Symmetry C18 5µm (4.6*250mm) Make; water
Flow rate	:	0.8 ml/min
Wavelength	:	260 nm
Column temp	:	Ambient
Sample Temp	:	Ambient
Injection Volume	:	10 µl



Trial chromatogram for dipropionate and Calcipotriene

From the above chromatogram it was observed that the dipropionate and Calcipotriene peaks are splitted

Trial 4:

Mobile phase :Phosphate buffer (0.05M) pH 3.6: ACN (40:60%v/v)

Column : Symmetry C18 5µm (4.6*250mm) Make; waters

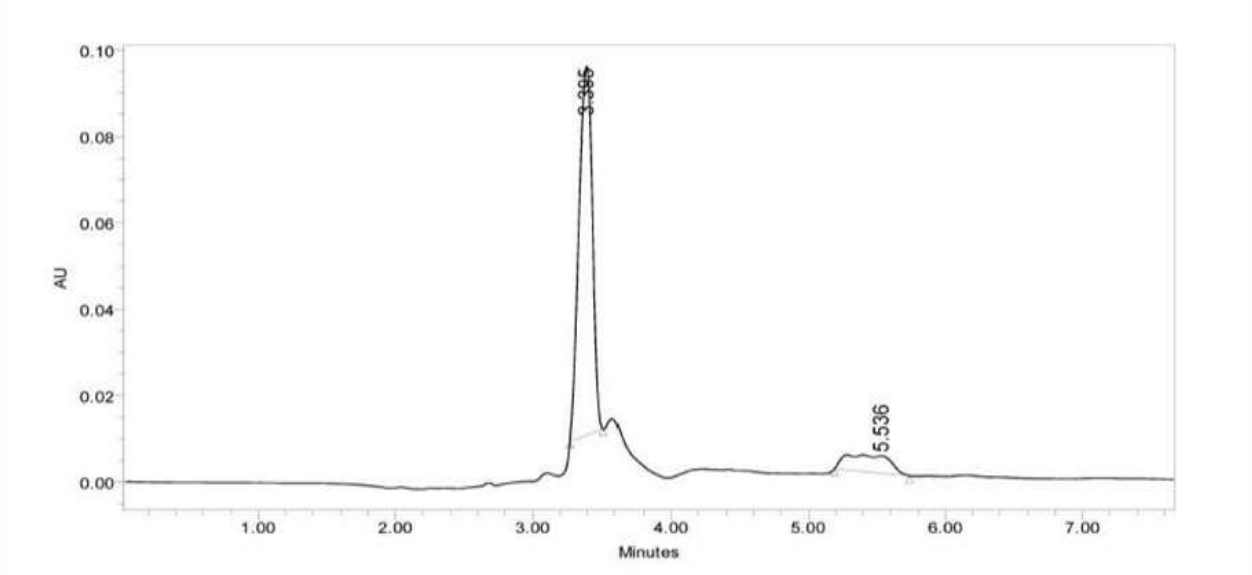
Flow rate :0.8 ml/min

Wavelength :260 nm

Column temp :Ambient

Sample Temp :Ambient

Injection Volume :20 µl



Trial chromatogram for dipropionate and Calcipotriene

From the above chromatogram it was observed that the dipropionate and Calcipotriene peaks are splitted

Trial 5:

Mobile phase :Phosphate buffer pH 3.0: Methanol (30:70%v/v)

Column : Inertsil C18 5µm (4.6*250mm)

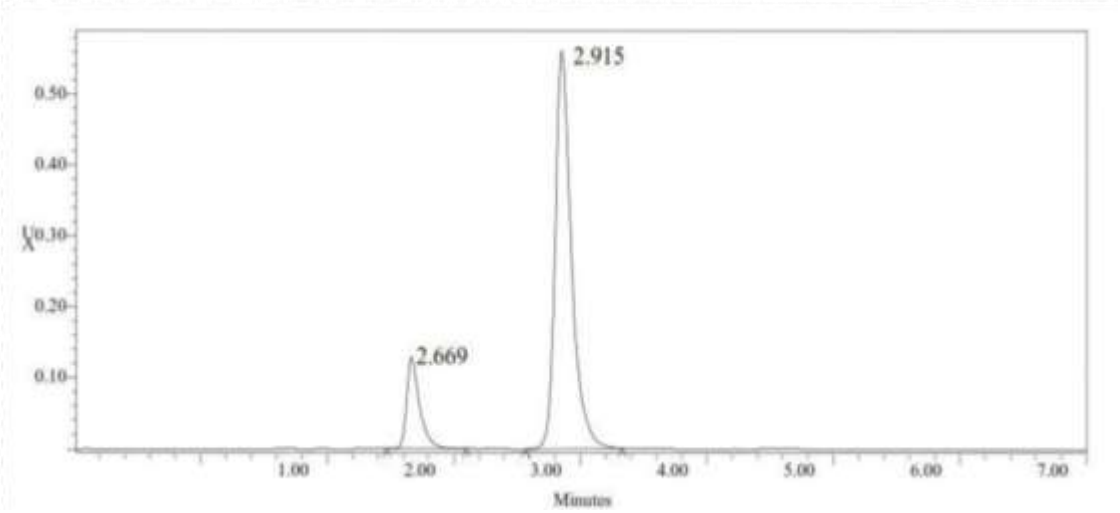
Flow rate :0.8 ml/min

Wavelength :260 nm

Column temp :Ambient

Sample Temp :Ambient

Injection Volume: 10 µl

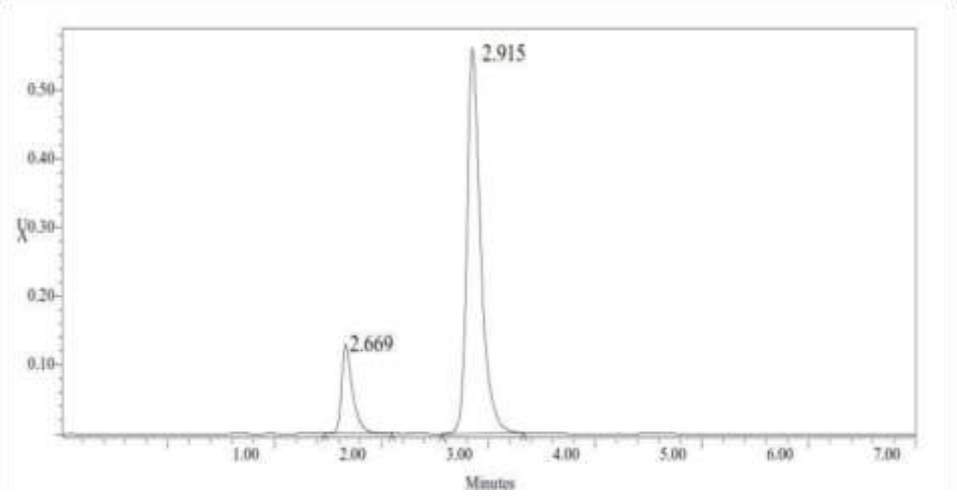


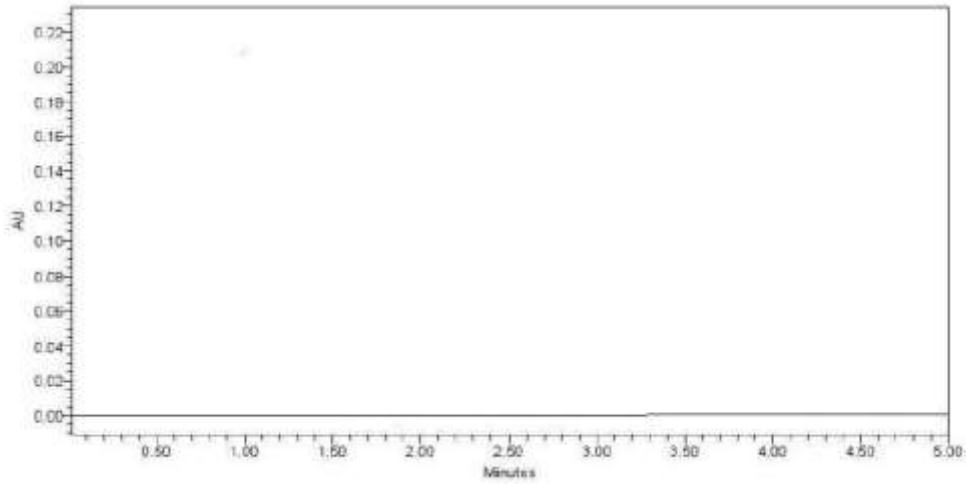
Trial chromatogram for dipropionate and Calcipotriene

From the above chromatogram it was observed that the dipropionate and Calcipotriene peaks are splitted.

Chromatogram for dipropionate and Calcipotriene

Column	:Inertsil C18 (4.6 x 250mm, 5µm)
Buffer pH	:3.0.
Mobile phase	:30% buffer 70% Methanol
Flow rate	:1.0ml per min
Wavelength	:260 nm
Temperature	:ambient.
Run time	:10min.



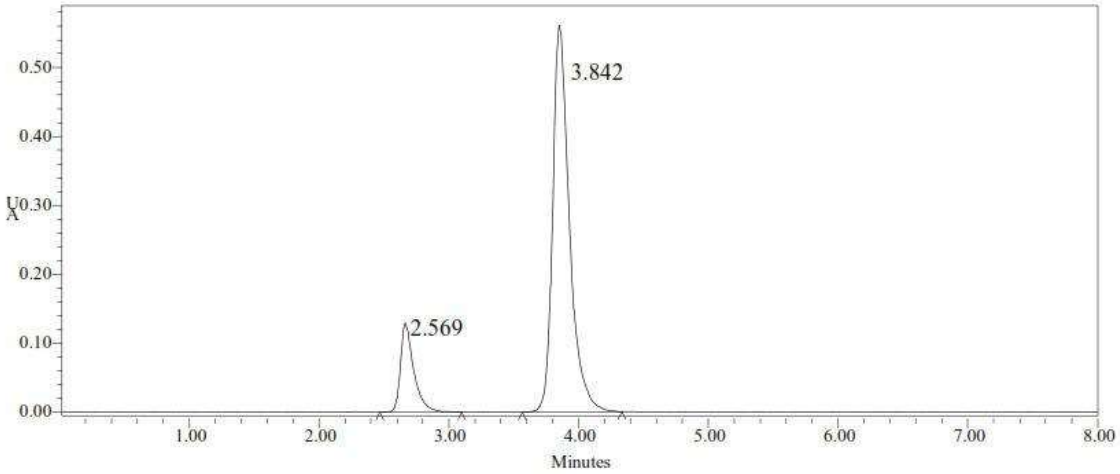


Chromatogram for blank

From the above chromatogram it was observed that there are no interferences.

Chromatogram for dipropionate and Calcipotriene sample Preparation

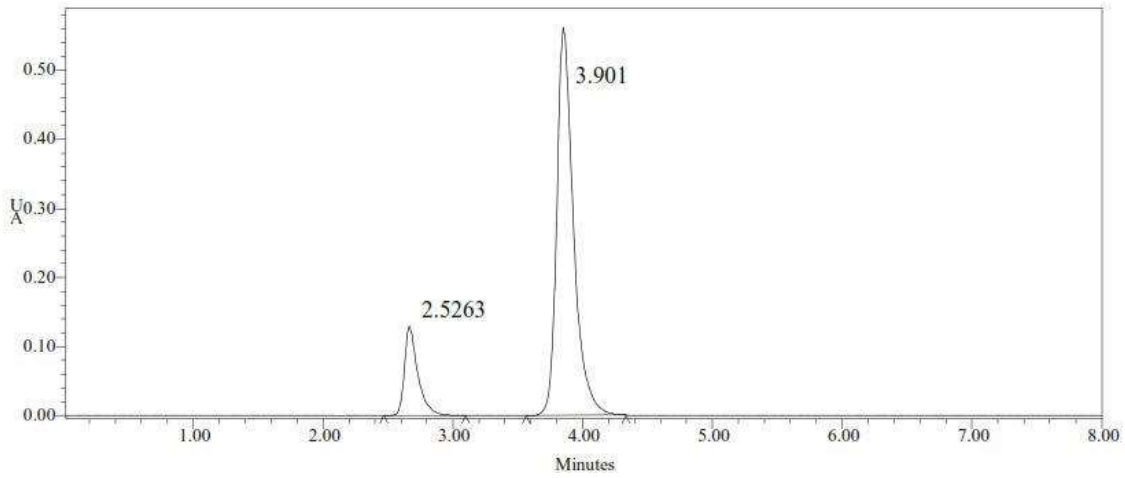
From the above chromatogram it was observed that the dipropionate and Calcipotriene peaks are well separated Retention time of dipropionate– 2.669min Retention time of Calcipotriene - 3.855 min



Chromatogram for dipropionate and Calcipotriene Standard Preparation

Retention time of dipropionate– 2.569 min, Retention time of Calcipotriene - 3.842 min.

SYSTEM SUITABILITY



Chromatogram for system suitability

Calculation: (For Dipropionate)

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{\text{Label Claim}} \times 100$$

Where:

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 OF dipropionatemg/ml.

RESULTS AND DISCUSSION

System Suitability Results:

1). Tailing factor Obtained from the standard injection is 1.3

2). Theoretical Plates Obtained from the standard injection is 4668.7

SUMMARY AND CONCLUSION

The estimation of Dipropionate and Calcipotriene was done by RP-HPLC. The Phosphate buffer was p^H 3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil C_{18} column C18 (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 260 nm. And the retention time was 2.5 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of Dipropionate and

Calcipotriene were found to be from 100-500 µg/ml of Dipropionate and 1-5µg/ml of Calcipotriene . Linear regression coefficient was not more than 0.999.

ACKNOWLEDGEMENTS

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