MS.ID-IJPBS-6778
International Journal of Pharmacy and Biological Sciences-IJPBS™ (2022) 12 (2): 55-60
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Pharmaceutical Sciences | OA Journal | MCI Approved | Index Copernicus

Analytical Method Development and Validation for Simultaneous Estimation of Dipropionate and Calcipotriene in Pharmaceutical Dosage Forms

Afrin[1]*, M. Mamata[2], G. Sravanthi[3], S. Divya[4] and Shaheensultana[5]
[1,2,3,4&5]Trinity College of Pharmaceutical Sciences, Peddapalli, Telangana - 505172.

Received: 20 Jan 2022 / Accepted: 18 March 2022/ Published online: 01 April 2022
***Corresponding Author Email:** mamatha12madireddy@gmail.com

Abstract

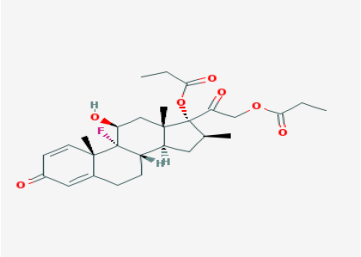
High performance liquid chromatography is at present one of the most sophisticated tools of the analysis. The estimation of Dipropionate and Calcipotriene was done by RP-HPLC. The Phosphate buffer was p^H 3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the 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. 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. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Dipropionate and Calcipotriene. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

Keywords
Inertsil C_{18} column Dipropionate and Calcipotriene, RP-HPLC.

*****

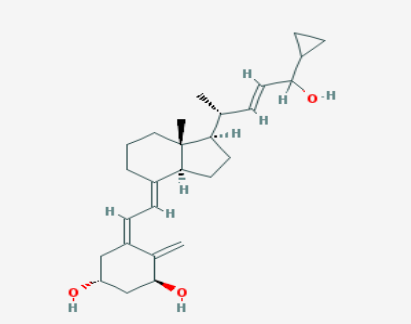
INTRODUCTION:

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.



Dipropionate

Calcipotriol, also known as calcipotriene, is a synthetic derivative of calcitriol, a form of vitamin D. It is used in the treatment of psoriasis. It is safe for long-term application in psoriatic skin conditions.



Calcipotriol

MATERIALS AND METHOD:

Instrumentation

System Alliance Waters 2690 separation module, Pump Analytical HPLC isocratic pump, Detector Photo diode array detector, Software Empower 2 software, Column Agilent (250×4.6mm, 5µ) C-18 RP-column, Sonicator Analytical Technologies Limited-Ultrasonic cleaner. U.V double beam spectrophotometer LABINDIA, UV 3000^{+}pH meter, Weighing machine

Chemicals

Dipropionate and Calcipotriene, Potassium dihydrogen orthophosphate, Water and Methanol for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid.

Trial 5: (Optimized)

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.

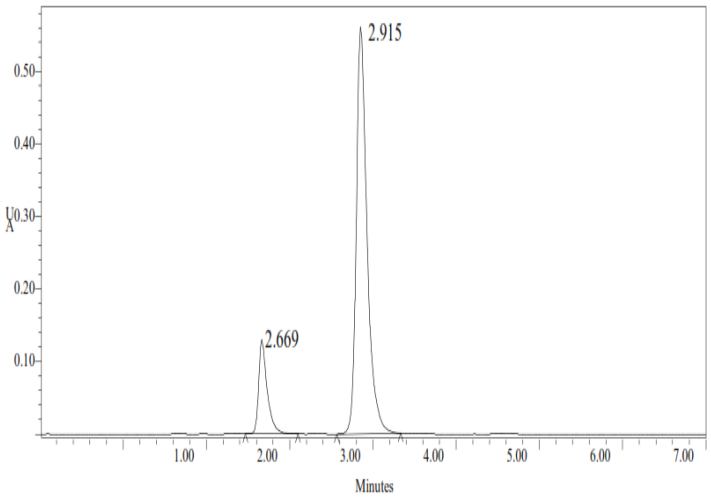


Fig. 1. Chromatogram of Trail-5

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.

METHOD VALIDATION:

- System Suitability
- Linearity
- Specificity
- Precision
- Intermediate Precision
- Accuracy
- Limit of Detection and Limit of Quantification
- Robustness

RESULTS AND DISCUSSION:

System suitability

Table 1 Results of system suitability parameters for Dipropionate and Calcipotriene

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	**Dipropionate**	2.5	124505	213642		1.2	4673.4
2	**Calcipotriene**	3.9	1308495	154566	6 0	1.3	6090.3

Precision

Table 2 Results of method precession for Dipropionate

Injection	Area
Injection-1	1302729
Injection-2	1302947
Injection-3	1303236
Injection-4	1303977
Injection-5	1309759
Average	1304529.8
Standard Deviation	2961.1
%RSD	0.2

Table 3 Results of method precession for Calcipotriene

Injection	Area
Injection-1	123149
Injection-2	123766
Injection-3	124271
Injection-4	124691
Injection-5	124956
Average	124162.7
Standard Deviation	725.6
%RSD	0.6

Intermediate Precession (Ruggedness):

Table 4 Results of Intermediate precision for Dipropionate

Injection	Area
Injection-1	1300148
Injection-2	1304520
Injection-3	1305937
Injection-4	1306476
Injection-5	130871
Average	1305070.2
Standard Deviation	3061.8
%RSD	0.2

Table 5 Results of Intermediate precision for Calcipotriene

Injection	Area
Injection-1	122487
Injection-2	122626
Injection-3	122632
Injection-4	122702
Injection-5	122962
Average	122681.8

Standard Deviation	174.8
%RSD	0.1

Accuracy

Table 6 Accuracy (recovery) data for Dipropionate

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	656659.5	5.0	5.036	100.7%	
100%	1304258	10.0	10.003	100.0%	99.84%
150%	1854608	14.4	14.224	98.780%	

Table 7 Accuracy (recovery) data for Calcipotriene

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	65800	5.3	5.34	100.8%	
100%	124353	10	10.10	100.01%	100.51%
150%	177940	14.2	14.45	99.68%	

Linearity

Table 8 Area of different concentration of Dipropionate

S.No.	Linearity Level	Concentration	Area
1	I	100ppm	668934
2	II	200ppm	956781
3	III	300ppm	1313873
4	IV	400ppm	1563458
5	V	500ppm	1867084
Correlation Coefficient			0.999

Table 9 Area of different concentration of Calcipotriene

S.No	Linearity Level	Concentration	Area
1	I	1ppm	66510
2	II	2ppm	94701
3	III	3ppm	124802
4	IV	4ppm	152731
5	V	5ppm	179732
Correlation Coefficient			0.999

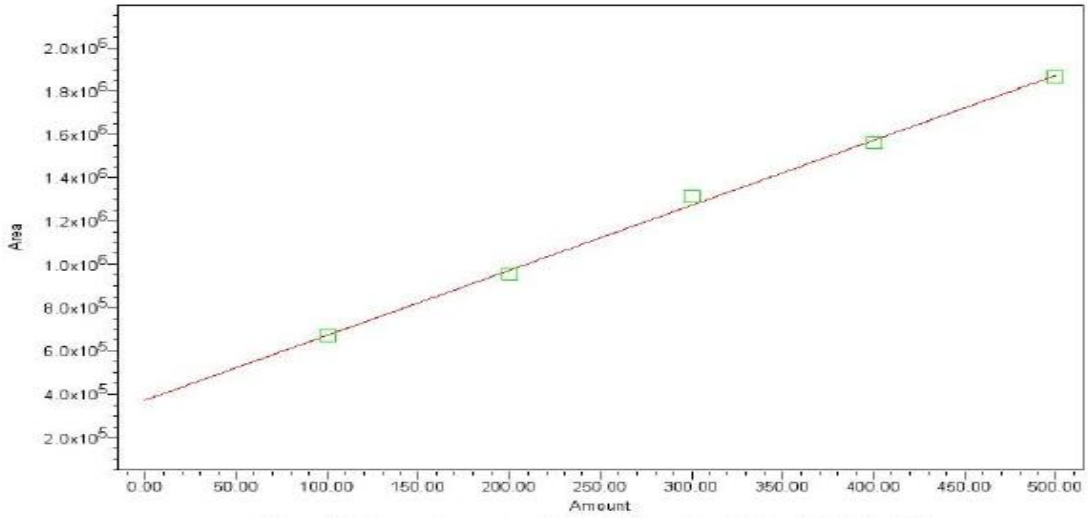


Figure 2 Calibration graph for Dipropionate

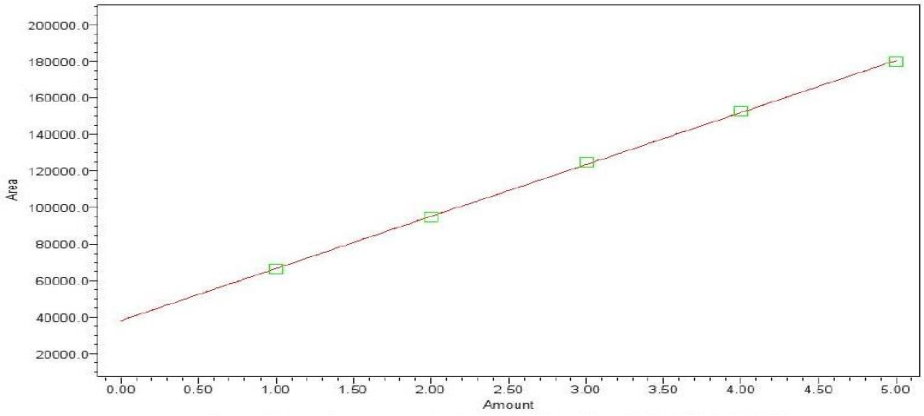


Figure 3 Calibration graph for Calcipotriene

Table 10 Analytical performance parameters of Dipropionate and Calcipotriene

Parameters	Dipropionate	Calcipotriene
Slope (m)	66574	12529
Intercept (c)	53592	50245
Correlation coefficient (R^2)	0.999	0.999

Limit of Detection for Dipropionate and Calcipotriene

Table 11 Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Dipropionate	52	152	2.9
Calcipotriene	52	156	3

Limit Of Quantification (LOQ):

Table no 12 Results of LOQ

Drug name	Signal obtained (µV)	S/N ratio
Dipropionate	522	10.03
Calcipotriene	524	10.1

Robustness:

Table 13 Flow Rate (ml/min) data for Dipropionate

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.6	5339.9	1.4
2	0.8	4673.4	1.3
3	1.0	5216.0	1.4

Table 14 Flow rate (ml/min) data for Calcipotriene

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	7063.3	1.3
2	1.0	6090.3	1.2
3	1.2	6998.0	1.3

Table 15 Change in Organic Composition in the Mobile Phase for Calcipotriene

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6387.7	1.2
2	*Actual	6090.3	1.2
3	10% more	6232.5	1.2

SUMMARY AND CONCLUSION:

The proposed method was found to be a simple, rapid, sensitive, selective and economic high performance thin layer chromatographic method for simultaneous determination of Dipropionate and Calcipotriene in pharmaceutical dosage form.

BIBLIOGRAPHY:

1. Jitamanyu Chakrabarty et al, Development and validation of a stability-indicating NP-HPLC method for simultaneous determination of betamethasone dipropionate and calcipotriene in topical dosage form Applied Science Research, 2013, 5 (2):15-24
2. N.Karnakar,et al. Analytical Method Development and validation for the Simultaneous Estimation of Ephidrine and Theophylline by RP-HPLC Method. A.J.Med.pharm, sci.,2021,9(1):23-26.
3. Efstratios Vakirlis et al, Calcipotriol/betamethasone dipropionate in the treatment of psoriasis vulgaris, Asian Journal of Chemistry; Vol. 26, No. 19 (2014), 6465-6468
4. shankar l et al, Development of Fast Reverse Phase Liquid Chromatographic Method for Simultaneous Analysis of Betamethasone Dipropionate and Calcipotriol in Ointments and Bulk Drug.
5. Paduri Amani, Narendar Malothu, Karnakar N, Ramya sri S, RP-HPLC Method for Estimation of Zanamivir in API and Pharmaceutical Formulation. (2022) Int. J. Life Sci. Pharma Res.12(1): P20-27.
6. Lene Simonsen et al, Development of a New Formulation Combining Calcipotriol and Betamethas one Dipropionate in an Ointment Vehicle Drug Development and Industrial Pharmacy (Impact Factor: 2.1). 02/2004; 30(10):1095-102.