International Journal of Pharma Research and Technology

Development and Validation of UV-Spectrophotometric and Stability Indicating RP-HPLC Method of Calcipotriene in Bulk Drug and Pharmaceutical Formulation

Tatiya Aayushi [1*] and Patil Javesh [2]

[1]Department of Quality Assurance, PSG Vidya Prasarak Mandal's College of Pharmacy, Shahada

[2]Department of Pharmacognosy and Phytochemistry, PSG Vidya Prasarak Mandal's College of Pharmacy, Shahada

aayushitatiya@gmail.com

Abstract

The objective of current study was to develop and validate stability indicating UV Spectrophotometric and RP-HPLC method for Calcipotriene in bulk drug and pharmaceutical formulation. UV Spectrophotometric method was developed utilizing Analytical Technologies limited. Detection was carried out at absorption maxima at 260nm using methanol as a solvent. Beer's law was followed in the concentration range of 5-25 µg/mL, and the drug was quantitated using A1% 1cm at 260 nm, which produced a correlation coefficient that was less than 1. The Chromatographic separation of analyte was achieved on Agilent C18 (4.6mm x 250mm, 5µm) column with mobile phase consisting of Methanol and 0.1 % Acetic Acid in the ratio of 65:35% v/v at flow rate of 1.0 ml/min. The retention time was found to be 5.273 min. Calcipotriene was subjected to forced degradation studies under different stress conditions like acid hydrolysis, alkaline hydrolysis, and hydrogen peroxide oxidation. The developed method was validated according to the guidelines of International Conference on Harmonization (ICH) for various parameters like linearity, precision, accuracy, robustness, limit of detection and limit of quantitation. The findings showed that the method performed well in accordance with the standards. The proposed method is simple, accurate, precise, economic, reproducible and stability indicating and hence suitable for routine quality control analysis of Calcipotriene in bulk drug as well as in formulations.

Keywords: Calcipotriene, RP-HPLC, Validation, Stability, Accuracy

Introduction

A chronic autoimmune inflammatory illness that affects 2-5% of the world's population, psoriasis is distinguished by macules and plaques on the skin as a result of hyperproliferation and aberrant keratinocytes differentiation.[1, 2] The first line therapy for

psoriasis is calcipotriene, a synthetic vitamin D analogue. Chemically it is (1R,3S,5E)-5-{2-[(1R,3aS,4Z,7aR)-1-[(2R,3E)-5-cyclopropyl-5-hydroxypent-3-en-2-yl]-7a-methyl-octahydro-1H-inden-4-ylidene]ethylidene}-4 methylidenecyclohexane-1,3-Diol;hydrate with molecular formula$C_{27}H_{42}O_4$ and molecular weight 430.6 g/mol. The structure of Calcipotriene is shown in Figure No.1. Although calcitriol and calcipotriene have similar affinities for the vitamin D receptor (VDR), calcipotriene has less than 1% of calcitriol's action in regulating calcium metabolism. Vitamin D analogues bind with vitamin D receptors on T cells and those on keratinocytes when administered topically, preventing keratinocyte proliferation and promoting keratinocyte differentiation.[3-6]

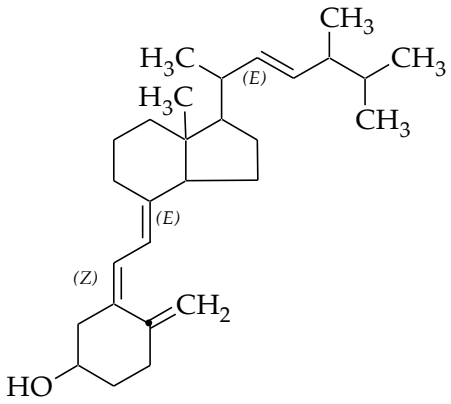

Figure No. 1. Structure of Calcipotriene

A drug substance's or product's ability to keep its identity, strength, quality, and purity over the course of the expiration dating period is known as stability. Forced degradation studies are carried out to develop and validate a stability indicating method.[7-8] To the best of our knowledge there is no stability indicating method for RP HPLC and UV was available having used solvent [9-10] The aim of current work was to develop and validate simple, rapid, reproducible and stability indicating method for Calcipotriene. Validation of the method was done in accordance with ICH, Q2 (R1) guidelines.[11]

Materials and Methods

Reagents and Chemical

Calcipotriene working standard was procured from Swapnroop drug and pharmaceutical. Acetonitrile, methanol, water, 0.1% OPA, 0.1% Acetic Acid were from Merck Ltd., India. All solvents utilized were of HPLC grade and all chemicals were of analytical grade. The marketed preparation (ointment) was obtained from local market.

Instrumentation

Drug analysis was carried out employing Agilent Tech Gradient System with Auto injector Equipped with Reverse Phase (Agilent) C_{18} column (4.6mm x 250mm; 5µm), a Quaternary Gradient (G130A) S.NO.DE9180834) pump, a 20µl injection

loop and UV (DAD) G13148 S.NO. DE71365875 Absorbance detector and running CHEMSTATION 10.1software. Analytical Technologies®Limited UV-VIS Spectrophotometer was used having Quartz curette with path length 1cm.

Selection of Wavelength

Accurately weight and transfer 5mg Calcipotriene working standard into 10 mL volumetric flask with water to get 1000µg/mL standard solution and Sonicate 15min to dissolve it then from the resulting solution 0.4 mL was transferred to a 10 mL volumetric flask and the remaining volume was filled with water to the specified level.

Preparation of Standard Solution and Sample for HPLC

Accurately weight and transfer 10mg Calcipotriene working standard into 10 mL volumetric flask as about diluents methanol completely and make volume up to the mark with the same solvent to get 1000 µg/mL standard (stock solution) and Sonicate for 15min to dissolve it and then from the resulting stock solution 0.1mL was transferred to 10 mL volumetric flask and the volume was made up to the mark with mobile phase methanol: (0.1% Acetic acid)Water, prepared in 6.5mLmethanol: 3.5 mL (0.1%Acetic acid water v/v)solvent.

Assay Sample Solution

To determine the content of Calcipotriene in marketed Ointment, 20 Ointment were weighed, and average weight was calculated. It was sonicated for 15 minutes to achieve thorough extraction.0.3 mL of supernatant was then diluted up to 10 mL with mobile phase.

Preparation of Standard Solutionand Sample for UV

Accurately weight and transfer 5 mg of Calcipotriene working standard into 10 mL volumetric flask with distilled water and sonicate for 15 min then from the resulting solution 0.1-0.5ml solution was pipette out in 10 mL of volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration of 5-25 µg/ mL.

Results and Discussion Selection of Wavelength

The working standard solution of Calcipotriene was prepared and scanned in range of 200-400nm. The spectrum recorded is shown in Figure No.2.

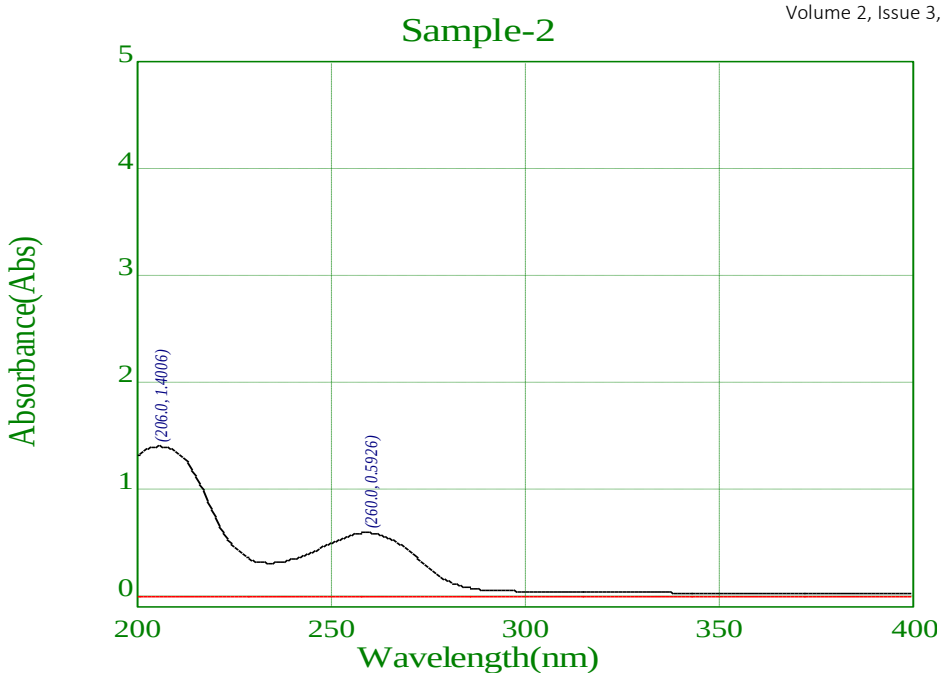

Figure No. 2. UV Spectrum of Calcipotriene

Optimized Chromatographic Conditions

The Chromatographic separation of analyte was carried out using Agilent Tech. Gradient System with Agilent C18 (4.6mm x 250mm, 5µm) column. The mobile phase consist of Methanol and 0.1 % Acetic Acid in the ratio of 65 : 35% v/v and pH was adjusted using 0.1%Orthophosphoric Acid, Buffer pH 3.2. The analyte was detected at 260nm. The run time was set at 15min at flow rate of 1.0 ml/min. The standard chromatogram of Calcipotriene is shown in Figure No.3.

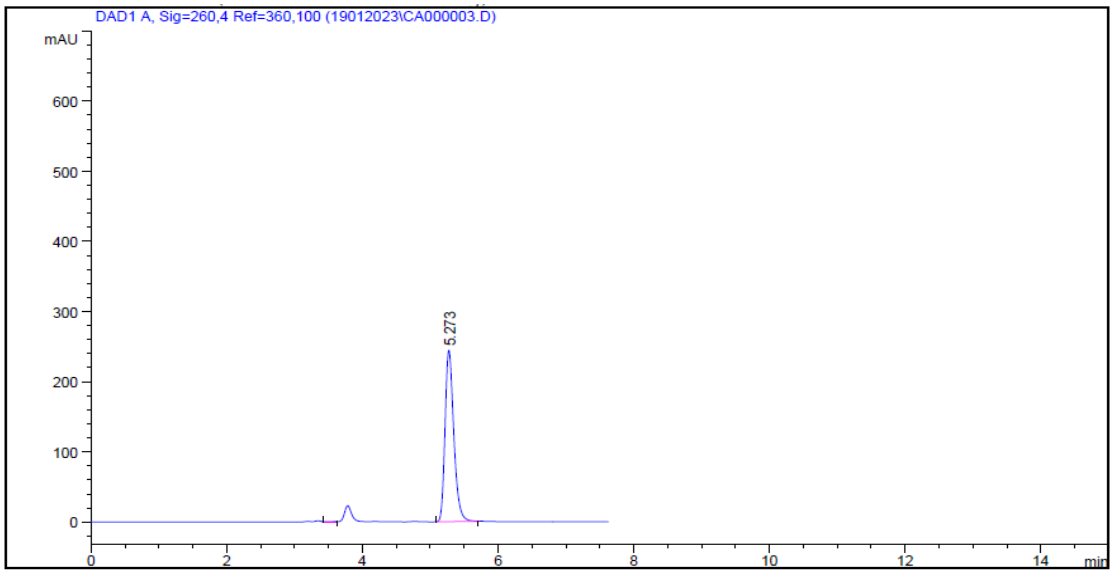

Figure No. 3. Standard Chromatogram of Calcipotriene

Method validation

Linearity

Different working standard solutions (10–50µg/ml for HPLC and 5–25 µg/ml for UV) were produced in the mobile phase from the Calcipotriene standard stock solution. A mixed volume loop injector was used to inject 20 µL of the sample solution into the chromatographic apparatus. The areaandabsorbance for each concentration were noted in Table 1.The Calibration curves are shown in Figure No. 4 and 5.

Table No.1: Result for Linearity

HPLC			UV	
Sr No.	**Concentration µg/ml**	**Area**	**Concentration µg/ml**	**Absorbance**
1	10	430.83	5	0.1549
2	20	865.15	10	0.3254
3	30	1339.14	15	0.4751
4	40	1742.99	20	0.6414
5	50	2161.93	25	0.7733

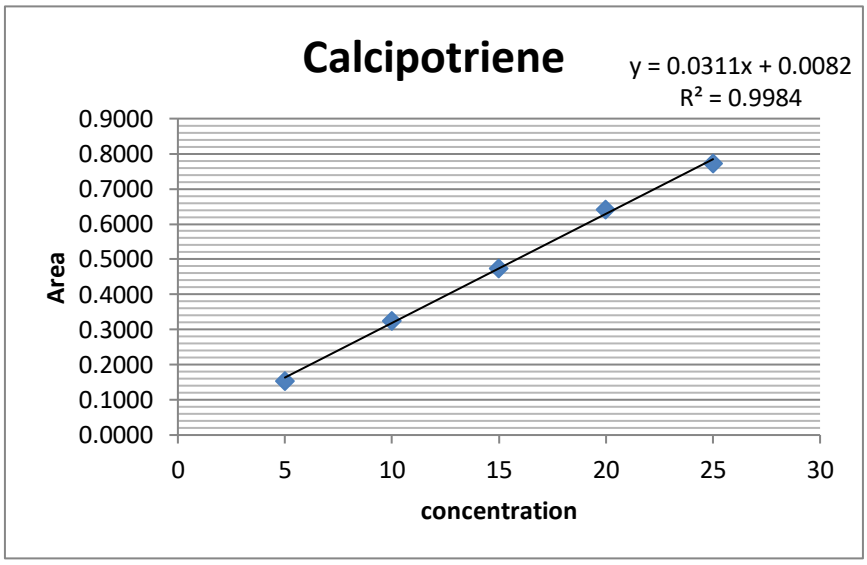

Figure No. 4. Calibration Curve UV

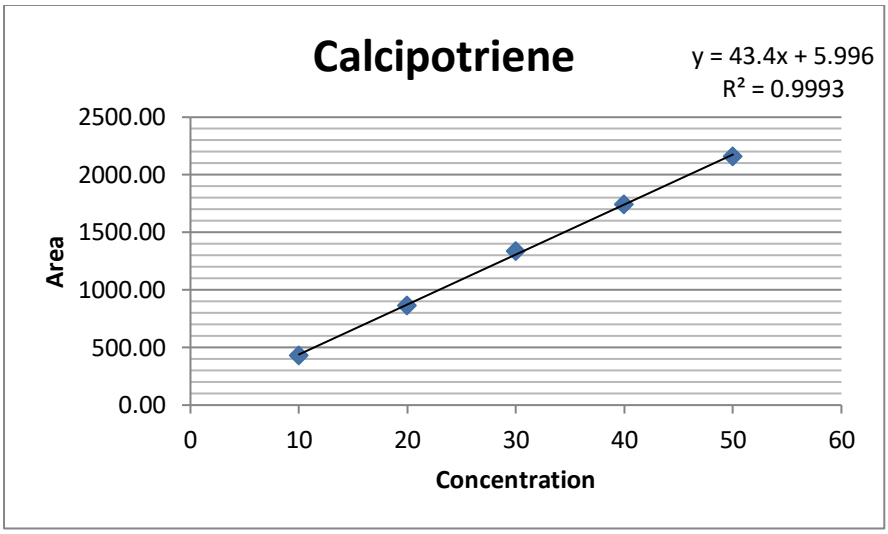

Figure No. 5. Calibration Curve HPLC

Accuracy

Studies on recovery were carried out to confirm the developed method's accuracy. An established standard drug concentration (80%, 100%, and 120%) was added to the previously examined ointment solution, and the recovery was then examined. Research on recovery that has been statistically validated is presented in Table 2.

Table No.2: Statistical Validation of Recovery Studies

	Level of Recovery (%)	Mean % Recovery	SD*	% RSD
UV	80%	101.61	0.11	0.11
	100%	101.39	0.32	0.31
	120%	101.02	0.08	0.08
HPLC	80%	100.82	0.04	0.04
	100%	99.44	0.24	0.24
	120%	100.23	0.52	0.52

***Denotes average of three determinations.**

Precision

The method was established by analyzing various replicates standards of Calcipotriene. All the solution were analyzed thrice in order to record any intra-day & inter-day variation in the result. The results obtained for intraday and interday precision are shown in Table 3 respectively

Table No.3: Result of Intraday and Inter day Precision

Conc^n (µg/ml) HPLC	Intraday Precision			Interday Precision		
	Mean± SD	%Amt Found	%RSD	Mean± SD	%Amt Found	%RSD
10	437.21±1.07	99.36	0.24	432.20±6.01	98.20	1.39
30	1331.14±0.48	101.78	0.04	1332.68±3.54	101.90	0.27
50	2166.62±6.10	99.57	0.28	2164.23±1.30	99.46	0.06
UV method						
10	0.32±0.001	101.73	0.76	0.32±0.002	101.05	0.07
15	0.47±0.001	99.05	0.18	0.47±0.001	99.35	0.03
20	0.62±0.01	101.87	0.43	0.63±0.005	99.52	0.08

***Mean of each 3 reading**

Repeatability

Two replicates of sample solution i.e. 20 g/ml for UV and 40g/ml for HPLC were analyzed, the peak areas were measured and % RSD was calculated .The results are depicted in Table 4 and 5.

Table No.4: Result for repeatability (UV)

Sr No.	concentration	Peak Absorbance	Amount found	%Amount found	SD	%RSD
1	20	0.62200	19.81	99.07	0.0004	0.06
2	20	0.62250	19.81	99.07	0.004	0.06

Table No.5: Result for repeatability (HPLC)

Sr No.	concentration	Peak Area	Amount found	%amount found	SD	%RSD
1	40	4069.3820	40.10	100.26	0.04	0.001
2	40	4069.1000	40.09	100.24	0.04	0.001

Robustness

A method's robustness is its capacity to withstand minor changes in the parameters. To determine the robustness of the method small changes in chromatographic conditions were made such as the mobile phase composition was changed in (±1 ml/min) proportion, flow rate (±1ml/min) and wavelength change (±1nm). The results of robustness studies are shown in Table 6.

Table No.6: Result of Robustness

Parameters	Conc.(µg/ml)	(mean±SD)	%RSD
Mob-phase composition (64ml+36ml) Methanol + 0.1% (Acetic Acid)water	20	855.1±0.43	0.05
Mob-phase composition(66 ml+34ml) Methanol + 0.1% (Acetic Acid)water	20	855.10±3.93	0.46
Wavelength change 259nm	20	779.8±0.70	0.09
Wavelength Change 261 nm	20	785.54±1.11	0.14
Flow rate change(0.9ml)	20	951.32±1.48	0.16
Flow rate change(1.1 ml)	20	784.44±1.12	0.14

Limit of Detection

The LOD is defined as the lowest limit of an analyte that can be detected. Based on the standard deviation of the response and the slope,the limit of detection (LOD) may be expressed as:

$$LOD = 3.3\ (SD)/S$$
$$= 3.3 \times 3.91 / 43.4$$
$$= 0.2973$$

Where, SD = Standard deviation of Y intercept S = Slope

The result is shown in Table 7.

Limit of Quantification

The LOQ is the lowest concentration that can be quantitatively measured. The quantitation limit (LOQ) may be expressed as follow depending on the response's standard deviation and slope:

LOQ = 10 (SD)/ S

=10 X 3.91/ 43.4

= 0.9009

Where, SD = Standard deviation Y intercept

S = Slope

The result is shown in Table 7.

Table No.7: Result for LOD and LOQ

	UV	HPLC
LOD	0.188182	0.2973
LOQ	0.570247	0.570247

Analysis of marketed formulation

Assay sample solutions were prepared as described in sample preparation for both UV and HPLC. Peak regions of common solutions were used to create the regression equation Calcipotriene levels in the sample were determined using the regression equation and peak area of the sample. According to the calibration curve regression equation presented in the formulation analysis, the amount of Calcipotriene is displayed in Table 8.

Table No.8: Analysis of marketed formulation

	Amount (mg)	Amount found	%Lable claim	SD	%RSD
HPLC	40	40.6779	101.69	0.038	0.094
	40	40.6245	101.56	0.093	0.093
UV	15	15.06129	100.41	0.007	0.046
	15	15.07097	100.47	0.045	0.045

Forced degradation studies

Stress degradation of the method was conducted toquantify the analyst response when the potential contaminants are present. The resolution factors of the drug peak from both its closest resolving peak and from all other peaks were measured during stress testing of the drug material. The drugs were subjected to acidic, alkaline, oxidizing and

photolytic conditions. The results are shown in Table 9.

Table No.9: Degradation of different stress condition

Sr No.	Degradation parameter	%Degradation
1	Alkali (0.1N NaOH- After 1hr)	100
2	Acid (0.1 N HCL- After 1hr	14.88
3	Oxidation (3% H_2O_2 – After 1hr)	10.15
4	Neutral After 1hr	6.48

Discussion

The UV spectrum of Calcipotriene was scanned in the range of 200-400nm and the selected wavelength was 260 nm. The retention time was found to be 5.273min. Adequate retention with good peak shape (Theoretical plates 8381) was observed using mobile phase of Methanol+0.1% (Acetic Acid)water,(65:35 % v/v) at flow rate of 1ml/min. Linearity of Calcipotriene was observed in the range of 5-25µg/mL (UV) and 10-50 µg/mL(HPLC) with good correlation coefficient (r2) 0.999. Accuracy of method is ascertained by recovery studies performed at different levels of concentrations (80%, 100% and 120%). The % recovery was found to be within 98-102% and %RSD was less than 2.All the results indicate that the method is highly accurate Intraday and Inter day Precision for developed method was validated and the results obtained were within the acceptable limit. The robustness of method was determined and % RSD was found to be less than 2.The limit of detection was found to be 0.188182 for UV and 0.2973 for HPLC. The limit of quantitation obtained was 0.570247(UV) and 0.570247 (HPLC). Assay for %Lable claim was found to be in range 98-102% with % RSD less than 2.The results for forced degradation studies are summarized in Table 9. High degradation was found in alkaline degradation. All the results obtained were satisfactory and within the acceptance criteria of ICH guidelines.

Conclusion

A Stability indicating UV Spectrophotometric and RP-HPLC method has been successfully developed and validated for determination of Calcipotriene in bulk drug and pharmaceutical formulation. All the method validation parameters were found within acceptance criteria. The established methods are accurate and precise as it indicates low relative standard deviation and high percent of recovery. The method is selective and stability suggesting, according to the results of experiments on forced degradation The established method has been found to be better because of its less retention time, isocratic mode and use of an economical and easily accessible mobile phase, readily available column, UV detection and better resolution of peaks. The method provides selective quantification of Calcipotriene. Hence the developed method is simple, accurate, precise, economic and reproducible.

List of Abbreviations

UV: Ultraviolet; RP-HPLC: Reversed- phase high performance liquid chromatography; HPLC:
High performance liquid chromatography; A (1%, 1cm): Absorptivity; ICH: International Conference on Harmonization; OPA: Ortho phosphoric acid; λ_{max}: Wavelength of maximum absorbance; SD.: Standard deviation; RSD: Relative standard deviation; LOD: Limit of Detection; LOQ: Limit of Quantification

Acknowledgements

Authors are thankful to management and principal of P.S.G.V.P.Mandal's College of Pharmacy, Shahada, District Nandurbar for their assistance and direction throughout the research work.

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