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Determination of Calcipotriene, its forced degradation and impurity analysis by HPLC

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Abstract

A simple, specific, sensitive, precise stability-indicating high-performance liquid chromatography method for determination of Calcipotriene was developed and validated. A Zorbax 300 SB-C_{18} column (250*4.6 mm, 3.5 µ) in isocratic mode, with mobile phase consisting of a mixture of solution methanol: water (70:30) was used. The quantitation performed at flow rate of 1.0 mL/min at 264 nm and run time was 7.5 min. The analytical method was validated as per ICH guideline for linearity, accuracy, precision, specificity, limit of detection, limit of quantification, and stability and method can be extended to the analysis of Calcipotriene in semis olid formulations. The relative standard deviation values for precision was less than 2%, and % recovery was greater than 98% for Calcipotriene. The drug is photo and thermal sensitive, so the analysis should be done in moderate conditions only.

Key-Words: Calcipotriene, RP-HPLC, Validation, Specificity

Introduction[1-12]

Calcipotriene is belonging to the chemical class of vitamin D derivatives and chemically, it is (5Z,7E,22E,24S)-24-Cyclopropyl 9,10-secochola-5,7,10(19),22 tetraene-1a,3b,24-triol. with molecular formula $C_{27}H_{40}O_3$ and CAS number 112828-00-9 (1); Calcipotriene is official in BP 2007[13-16]. The precise mechanism of calcipotriol in remitting psoriasis is not well-understood. However, it has been shown to have comparable affinity with calcitriol (Vit D) for the Vitamin D receptor, while being less than 1% as active as the calcitriol 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, kidney, 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. Its metabolism is still not so clear[17].

Literature review for Calcipotriene analysis revealed very few analytical methods. However, there is no method reported for quantification of Calcipotriene forms in the literature[18-25].

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Stress testing carried out to elucidate the inherent stability characteristic of the active substances and forms an important part of the API and drug product development. It suggests that degradation products that are formed under a variety of conditions should be identified and degradation pathways be established. The purpose of stress testing is to provide evidence on how the quality of drug substance varies with time under the effect of varieties of environmental factors such as acid, alkali, temperature, humidity, light and presence of oxygen. An ideal stability-indicating method is one that quantifies the drug and also resolves its degradation products[26]. The aim of present work is to develop a simple, specific, sensitive, accurate and stability indicating HPLC analytical procedure for the analysis of Calcipotriene in the presence of its degradation products and related impurities as per ICH guideline.

Material and Methods

Reagent and Materials

Calcipotriene working standard and sample cream was provided by ZYG Pharma Pvt. Ltd., Pithampur (Label claim: 0.005%; Calcipotriene cream; and manufacturer: ZYG Pharma Pvt. Ltd., Pithampur). Methanol, and water (HPLC grade), were used. Instrument used was Agilent Technologies 1200 series[27].

Preparation of Mobile Phase

Mobile phase was prepared by mixing 750.0 mL of methanol with 250.0 mL of water. (75:25 v/v).

Preparation of Standard Solution

About 10.0 mg of Calcipotriene reference standard was weighed out accurately, and transferred into a 200.0 mL volumetric flask. 100.0 mL of methanol was added into it, sonicated for 5 minutes or until dissolved, if necessary. Volume was make up with methanol, and mixed well **(Stock Std)**. This solution contained about 0.05 mg/mL of Calcipotriene.

Further 2.0 mL of stock standard was diluted to 100.0 mL volumetric flask with sample solvent, and mixed well. A portion was filtered through the sample filtration kit into auto sampler vials. Discard minimum 2.0 mL of filtrate prior to collecting for analysis **(Std)**. This solution contained about 1.0 µg/mL of Calcipotriene.

Preparation of Sample preparation

1.0 g of the Calcipotriene Cream was weighed out accurately into a 50.0 mL volumetric flask. 45.0 mL of sample solvent was added and vortex to disperse the cream. Sonicated for 30 minutes at room temperature. Diluted to volume with diluting solvent. Mixed well by shaking and vortexing. Using a disposable pipette, 10.0 mL of the top solution was discarded and placed the sample at 2–8°C for 2 hrs to settle the solution. The supernatant clear solution was filtered through 0.2 µm nylon filtered discarding the first few mL. This solution contained about 1.0 µg/ml of Calcipotriene.

Chromatographic condition

Chromatographic separation was achieved at 25°C on a reverse phase Zorbax SB-300 column[28] using mobile phase consisting of methanol and water in the ratio of 70:30 (v/v). The flow rate was kept at 1.0 mL/min and detection was carried out at 264 nm. The sample was injected using 50 µl fixed loop, and the total run time was 7.5 min.

System suitability parameters

Depending on the theoretical plate count, and other parameters as pressure etc system suitability parameters were studied.

Calibration curve

The linearity of the response for Calcipotriene assay method was determined by preparing and injecting standard solutions with concentrations of 0.8-1.4 PPM Calcipotriene (Figure 2).

Analysis of the marketed formulation

The developed method was applied to the analysis of Calcipotriene from marketed product named as Calcipotriene Cream (Label claim 0.005% w/w)[29]. The contents of marketed dosage form were found to be in the range of 98-102% with % RSD less than 2% which indicate suitability for routine analysis of Calcipotriene in dosage form.

% ASSAY = Aspl/Astd x mg Std x f /200 x 2/100 x 50/ g Spl x 100/LC

Where,

f = correction factor for potency

Expressed as % of Calcipotriene reference standard x 10^{-2} = 98.6 x 10^{-2} = 0.986

LC = Label claim of Calcipotriene in Calcipotriene cream 0.005% = 0.05 mg/g

Recovery Studies

Recovery studies were performed by standard deviation method to validate the accuracy of developed method to different concentrations of preanalyzed sample solution of cream, varying concentration of standard drug was added and then its recovery was analyzed.

Preparation of Reference Standard: Calcipotriene (10 ppm): 50.0 mg of Calcipotriene reference standard was dissolved in 50.0 mL of methanol. 1.0 mL of above solution was taken and diluted to 100.0 mL with sample solvent (stock std solution)

Preparation of solution: About 1.0 g of placebo was weighed accurately in a stoppered test tube and added in it 2.5 mL Calcipotriene stock std solution and heated on a water bath and cooled to room temperature and the volume was make up to 50.0 mL with sample solvent, mixed and filtered**(50 %).**

About 1.0 g of placebo was weighed accurately in a stoppered test tube and added in it 5.0 mL Calcipotriene Stock std solution and heated on a water bath and cooled to room temperature and the volume was make up to 50.0 mL with sample solvent and mixed and filtered**(100 %).**

About 1.0 g of placebo was weighed accurately in a stoppered test tube and added in it 7.5 mL Calcipotriene Stock std solution and heated on a water bath and cooled to room temperature and the volume was make up to 50.0 mL with sample solvent and mixed and filtered**(150%).**

$$\% \text{ Recovery} = \frac{\% \text{ of Calcipotriene}}{\% \text{ of Calcipotriene added}} \times 100$$

Validation of the method

Validation of the developed method was done according to ICH Q2B guideline 1996[30-35].

Accuracy

The accuracy of the method was determined by adding working standard into the solution. The recovery studies were performed by standard addition method, at 50%, 100%, 150% level. The resulting solutions were assayed in triplicate and the results obtained were compared with expected results and expressed as percentage.

Precision

The precision of analytical method is determined by assaying a sufficient number of aliquots of homogenous sample to be able to calculate statistically valid estimate of % RSD (Relative Standard deviation). Precision of a standard sample were carried out using three replicates of three different solutions (0.8, 1, 1.2 PPM).

Specificity

Preparation of Reference Standard: 50.0 mg of Calcipotriene reference standard was dissolved in 50.0 mL of methanol. 1.0 mL of above solution was taken and diluted to 100.0 mL with sample solvent. (stock std solution)**.** 5.0 mL of stock std solution was taken and diluted to 50.0 mL with sample solvent. (1ppm)

Sample preparation: A quantity of the cream containing 0.05 mg of Calcipotriene was transfered (about 1.0 g) in a stoppered test tube, heated on a water bath and cooled to room temperature and the volume was make up 50.0 mL with sample solvent and mixed.

Linearity

The linearity of detector response was studied for Calcipotriene by HPLC at the concentration of 80%, 90%, 100%, 110%, 120% and 140% by one analyst on the same day.

Preparation of reference standard: 50.0 mg of Calcipotriene reference standard was dissolved in 50.0 mL of methanol. 1.0 mL of above solution was taken and diluted to 100.0 mL with sample solvent. (stock std solution)

Limit of Detection and Limit of Quantitation

Preparation of reference standard (10 ppm): 50.0 mg of Calcipotriene reference standard was dissolved in 50.0 mL of methanol. 1.0 mL of above solution was taken and diluted to 100.0 mL sample solvent. (stock std solution)

The determination of LOD and LOQ is done as per the formula below:

$$\text{S/N Ratio} = \frac{\text{Height of signal}}{\text{Height of noise}} \times 100$$

Stress Degradation of Calcipotriene

(i) Acid and base-induced degradation

To the standard preparation, 10 ml of 5N hydrochloric acid and 10 ml 5N sodium hydroxide was added separately and finally volume were made up to 100ml. Sample was refluxed on water bath for 10 hrs.; neutralized by alkali and acid respectively, and diluted 10 times with same solvent before injection and sample was analyzed with respect to unstressed sample.

(ii) Hydrogen peroxide-induced degradation

The method described above (i) was followed by except that 3.5 mL of 3% H_2O_2 was added in place of HCl/NaOH. The sample was kept at ambient temperature for 5 min. and analyzed with respect to unstressed sample.

(iii) Thermal degradation

The sample was kept at 30°C and 40°C for 1 month of three consecutive batches and method described in (i) followed here and analyzed with respect to unstressed sample.

(iv) Photolytic degradation

The sample was kept in a photolytic chamber at 1.2 x 10^6 Lux/hour, followed by analysis as per proposed method.

Impurity Analysis of Calcipotriene

The determination of impurities was carried out by first performing calibration, in this study twelve injections of standard were given and then the deviation was found to be within the limit. The synthetic impurities were injected along with Placebo and system suitability injections to detect the impurities by comparing with the retention time.

The percent assay was found by the formula showed in experimental and found within the range, not more than 10%.

The sample was kept at 40°C and 50°C of the three consecutive batches RDC 02, RDC 03, RDC 04 respectively. By comparing the retention time % degradation of known and unknown product were found out.

Results and Discussion

The chromatographic conditions were optimized with a view to develop a stability-indicating assay method for Calcipotriene in semisolid dosage forms. Different mobile phases were tried as under chromatographic conditions. The column Zorbax SB-300 15 cm x 4.6 mm, gave good peak shape with response at affordable retention time with peak purity of Calcipotriene in presence its degradation products. The isocratic profile, methanol and water in mobile phase varied from 30-70% (v/v), was also altered to give the best separation of the peaks and Calcipotriene peak amongst degradation products peaks were found resolved. The final chromatographic system comprising reverse-phase C8 column (250 × 4.6 mm, 3.5µ) with a mobile phase consisting of a mixture of solution methanol and water in a ratio of 70:30 at a flow rate 1.0 mL/min was found optimum. Detection was performed at 264 nm (Table 1). Typical UV-Visible chromatograms are shown in Figure 1.

As per USP, system suitability tests were carried out at before performing each of validation parameters for Calcipotriene by the proposed HPLC method summarized in Table 4. "RP-HPLC estimation of Calcipotriene in the dosage form" suggested only

estimation of Calcipotriene in the dosage form with methanol: water-70:30, as a mobile phase; difference in ratio for establishing stability indicating method of Calcipotriene.

The developed method was applied to the analysis of Calcipotriene in marketed as Calcipotriene cream (Label claim 0.005% strength, ZYG Pharma Pvt. Ltd.). The results of analysis are given in Table 6. The contents of marketed tablet dosage form were found to be in the range of 98.79±.89% with RSD less than 2% which indicate suitability for routine analysis of Calcipotriene.

Recovery of Calcipotriene in the range of 99.68-100.23% shows method may be used for routine analysis of Calcipotriene. The percent recovery indicates the accuracy of the developed method. Precision showed RSD of 0.580. This shows method is precise as relative standard deviation is below 2.0%. Validation parameters for analysis of Calcipotriene are presented in Table 3. The linear regression data for the calibration curves indicate that the response is linear over the concentration range studied with correlation coefficient, r^2 value as 0.9999. The value of slope was 1.24. The limit of detection was found to be 0.005ppm and limit of quantitation was found to be 0.02ppm.

Specificity was carried out to identify the retention time of the drug and the obtained degradation products was identified, which were formed due to isomerization. The identified product was pre-calcipotriol, since the relative retention time was found to be 0.9.

Accuracy for the developed method was studied and percentage recovery was found to be within the limit of 98 – 102% and % RSD was found to be not more than 2.

The values of assay, degradation (%), with stress condition are given in Table 6, 7. The stressed condition samples are evaluated relative to the control sample with respect to assay and degradation (%). The degradation (%) of Calcipotriene indicates susceptibility of drug to thermal and photo degradation only.

Table 1: Optimized chromatographic condition of Calcipotriene for the proposed method

Variable	Condition
Column	
Dimension	Zorbax SB-300 15 cm x 4.6 mm
Particle Size	3.5 µ
Bonded Phase	Octadecylsilane (C_{18})
Mobile Phase	
Methanol	750 ml
Water	250 ml
Chromatographic conditions	
Column Temperature	25° C
Flow rate	1.0 ml/min
Sample cooler Temperature	20° C
Injection volume	50 µl
Detection wavelength	264 nm
Total run time	10 min
Retention time	7.5 min

Table 2: Calibration of the standard for the proposed method.

Std Injection	RT	AUC
Std-1	7.597	125.534
Std-2	7.591	125.628
Std-3	7.593	125.946
Std-4	7.589	123.165
Std-5	7.592	125.335
Mean		125.100
S.D.		0.998
% R.S.D.		0.89

Table 3: Summary of validation parameters for the proposed method

VALIDATION PARAMETERS	CALCIPOTRIENE
Linearity (R)	0.9997
Accuracy by recovery study (% found)	100.23
Precision (% RSD)	
RT	**AUC**
0.03	0.57
Specificity	Implies as per the RT

Table 4: System suitability parameters for Calcipotriene by the proposed method
* mean of replicate determinations.

• Pressure at start: 179 bar, Pressure at end: 181 bar
• Left temp. at start: 25°C, Left temp. at end: 24.9°C
• Right temp. at start: 25°C, Right temp. at end: 25°C
• USP Tailing: 1.524
• Symmetry: 0.722
• Theoretical plates: Tangent method: 5766
Half width method: 5872
5 sigma method: 3182
Statistical: 3274
• Height: 7.56
• Width at half height: 0.233
5 sigma: 0.673
Tangent: 0.400
• Amount: 8.0904 ng/ul

Table 5: Analysis of marketed formulations

Std Injection	RT	AUC	% Assay
Std-1	7.589	125.877	97.81
Std-2	7.586	128.408	99.77
Average			98.79

Table 6: Thermal degradation data for Calcipotriene

Sample	% DP		% Assay
	Unknown		
	RT	AUC	
RDC 02, 30°C			
1	7.695	97.92353	2.8454
2	35.308	22.97404	0.6676
RDC 02, 40°C			
1	7.720	37.98305	1.1010
2	23.753	23.16610	0.6715
3	28.256	20.32342	0.5891
4	35.411	34.98650	1.0141
RDC 03, 30°C			
1	7.696	109.46489	3.1748
2	35.394	46.25597	1.3416
3	36.967	13.51587	0.3920
RDC 03, 40°C			
1	7.730	32.81137	0.9542
2	23.755	23.17108	0.6739
3	27.590	24.88893	0.7238
4	28.257	32.00032	0.9306
5	35.446	39.92484	1.1611
RDC 04, 30°C			
1	7.694	89.53648	2.5938

Table 7: Other stress degradation data for Calcipotriene

Degradation	% DP		% Assay
	Known		
	RT	AUC	
UV	30.775 28.891	3198.20532 44.80098	92.7426 1.2992
H_2O_2	16.672	144.43134	4.1883
HCl	12.862	133.02393	3.8575
NaOH – 1	7.993	124.09323	No degradation
NaOH - 2	9.962	144.44184	4.1886

Table 8: Results of calibration by standard injection for impurities

Std Injection	AUC
Std-1	39.76872
Std-2	40.64349
Std-3	40.50095
Std-4	40.77260
Std-5	41.06226
Std-6	41.00000
Std-7	40.95805
Std-8	40.70186
Std-9	41.43579
Std-10	41.36085
Std-11	42.75281
Std-12	43.40882
Mean	41.2
SD	0.7
% RSD	1.7

Table 9: Results of assay of sample for impurity detection of CAO RC3

Sample	% DP		% Assay
	Known		
	RT	AUC	
RDC 02, 30°C	33.198	72.11067	2.0954
RDC 02, 40°C	33.257	305.33801	8.8508
RDC 03, 30°C	33.265	70.66675	2.0495
RDC 03, 40°C	33.298	309.44608	8.9993
RDC 04, 30°C	33.169	69.43761	2.0116
RDC 04, 40°C	33.290	167.73039	4.8639

Table 10: Results of assay of sample for impurity detection of CAO RC1

Sample	% DP		% Assay
	Known		
	RT	AUC	
RDC 02, 30°C	28.860	41.55424	1.2050
RDC 02, 40°C	28.868	58.25610	1.6893
RDC 03, 30°C	28.891	44.80098	1.2992
RDC 03, 40°C	28.6848	78.24355	2.2689
RDC 04, 30°C	28.854	45.26620	1.3126
RDC 04, 40°C	28.904	53.63138	1.5552

Table 11: Results of assay of sample for impurity detection of CALCI

Sample	% DP		% Assay
	Known		
	RT	AUC	
RDC 02, 30°C	30.734	3402.89258	98.6782
RDC 02, 40°C	30.767	2660.93506	77.1627
RDC 03, 30°C	30.775	3198.20532	92.7426
RDC 03, 40°C	30.306	2747.15112	79.6628
RDC 04, 30°C	30.721	3306.60707	95.8861
RDC 04, 40°C	30.796	3050.78467	88.4677

Fig. 1: Chromatogram of standard solution

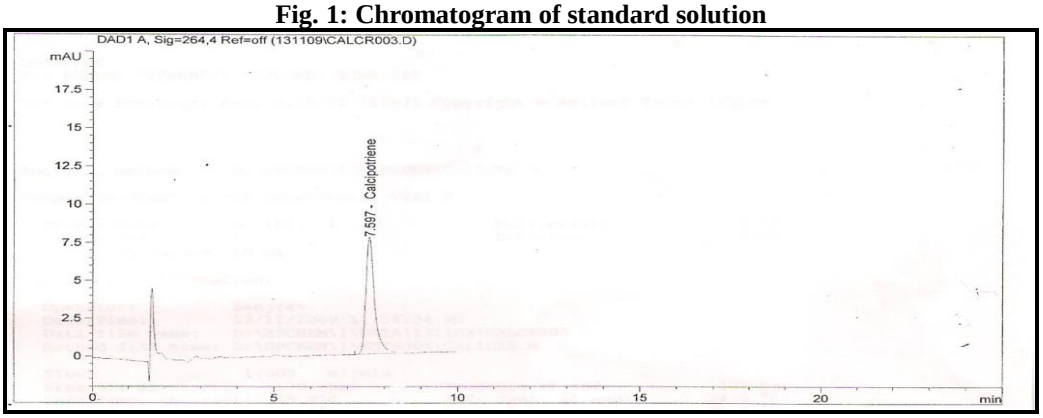


Fig. 2: Linearity curve for Calcipotriene by the proposed method

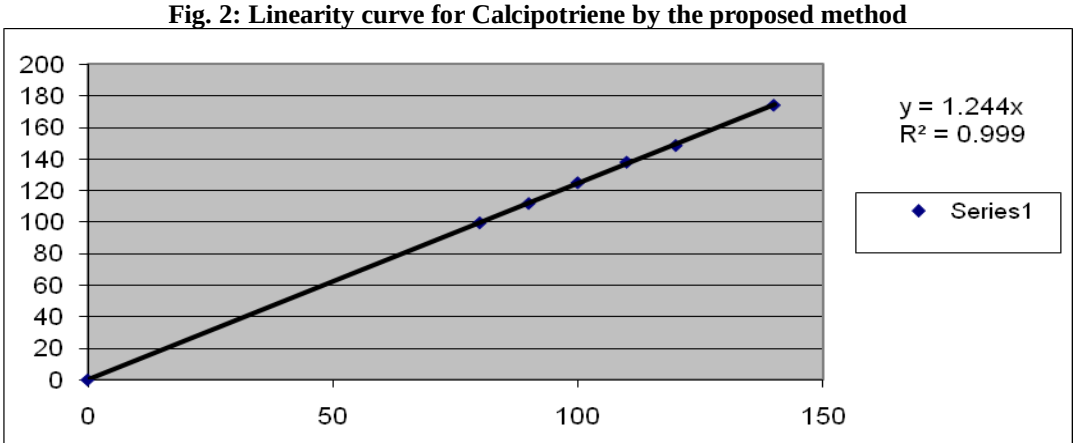
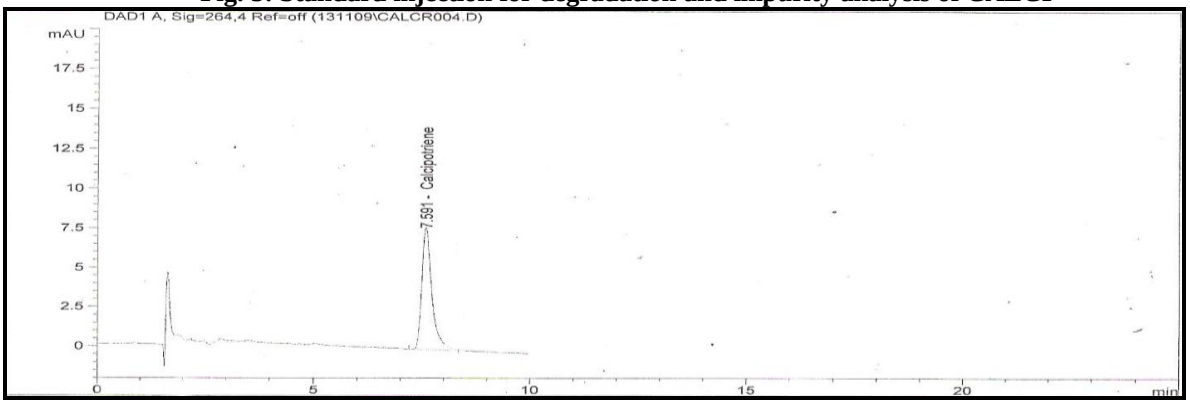


Fig. 3: Standard injection for degradation and impurity analysis of CALCI



Conclusion

The developed HPLC method is specific, accurate and stability indicating. Statistical analysis proves that method is precise, and selective for analysis of Calcipotriene. The developed method is suitable for the quality control analysis of Calcipotriene.

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