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Development and Validation of a Novel Ultra Performance Liquid Chromatography Method for Dissolution of Paricalcitol in Paricalcitol Soft Gelatin Capsules

Bhaskara P.V Mantena[1,*], Sumathi V Rao[1], K.M.Ch. Appa Rao[2], K.Ramakrishna[2], Jayant Karajgi[1], Srikanth Reddy. R[1]

1.Aurobindo Pharma Limited (A division of APL Research centre), Survey No. 313, Bachupally, Quthubullapur Mandal, Ranga reddy District, Hyderabad-500072, Andhra Pradesh, India.
2.GITAM University, Gandhi Nagar, Rushikonda, Visakhapatnam-530 045. Andhra Pradesh, India

ABSTRACT

A new gradient reverse phase Ultra Performance Liquid Chromatography (UPLC) method was developed for the analysis of Dissolution profile samples of Paricalcitol in Paricalcitol Soft Gelatin capsules. The aim of the new method was to achieve proper accuracy and precision for the highly potent low dose drug product formulations. The normal injection loop allows upto 10µL of the sample in normal condition in UPLC systems. For the current method the loop was modified to handle 50µL of injection volume in order to achieve quantifiable area counts. Efficient separation is achieved on Acquity UPLC HSS T3 (100 mm length × 2.1 mm ID with, 1.8 µ particle size. Validation parameters such as specificity, linearity, precision, accuracy, and robustness were evaluated as per ICH guidelines. The validated RP-UPLC method was successfully applied to the Dissolution of Paricalcitol Soft Gelatin Capsules dosage forms.

Keywords: Paricalcitol, Soft Gelatin Capsules, Validation, Acquity UPLC, 50µL injector loop, dissolution profile tests

*Corresponding Author Email: praveenvarma@aurobindo.com

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INTRODUCTION

Paricalcitol is a white, crystalline powder with a molecular formula of $C_{27}H_{44}O_3$. Molecular weight is found to be 416.64. The chemical name of Paricalcitol is 19nor-1α, 3β, 25-trihydroxy-9,10-secoergosta-5(Z),7(E),22(E)-triene. Zemplar is available as soft gelatin capsules for oral administration containing 1 microgram, 2 micrograms or 4 micrograms of paricalcitol. The active form of vitamin D, calcitriol and paricalcitol indicated for the prevention and treatment of secondary hyperparathyroidism (SHPT) in chronic kidney disease and sometimes which leads to bone disease. Both calcitriol and paricalcitol may have different effects on blood calcium when administered. SHPT is very common in people in Stages 4 and 5 of chronic kidney disease (CKD)[1-3]. SHPT can also cause parathyroid glands to become permanently enlarged.

Literature review indicates that very few methods are available for quantification of Paricalcitol from the dissolution test medium[4-6]. The challenge is due to the extremely low dose and interference of the components of formulation matrix which co elute along with drug peak. The final concentration of the drug of dissolution testing is in the range of 0.002 ppm (for 1mcg) to 0.008 ppm (for 4mcg). To attain specificity and selectivity for quantification of Paricalcitol, UPLC method was developed using 50µL injector loop. The method was validated as per International Conference on Harmonization (ICH) guidelines[7-15] and applied for analysis of dissolution profile testing of Paricalcitol soft gelatin capsules 1mcg, 2mcg and 4mcg strengths using following dissolution conditions as mentioned in FDA/ Center for Drug Evaluation and Research Office of Generic Drugs (OGD). 4mg/mL (0.4%) Lauryldimethylamine N-oxide (LDAO) used as dissolution media, USP Apparatus type I (Basket) with stirring speed of 100 rpm, proposed dissolution volume is 500 mL, specified time points are 20, 30, 45 and 60 minutes respectively.

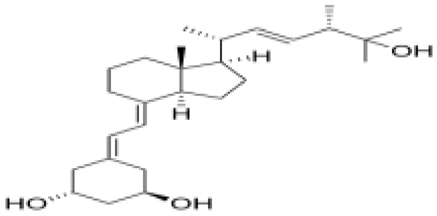


Figure 1: Paricalcitol chemical structure

MATERIALS AND METHODS

Instruments:

The LC system used for method development and method validation was Waters- Acquity UPLC equipped with separation module consisting of Binary gradient pump, Auto Sampler, thermostatted column compartment, Acquity UPLC Photo diode array detector, Computer with windows based Empower 3 Method validation manager software. The output signal was monitored and processed using Empower 3 software. Dissolution tests were performed using Electrolab® TDT-08L multi bath (n=8) dissolution test system. Column used was Acquity UPLC HSS T3 (100 mm x 2.1 mm id) with 1.8 µm particle size.

Chemicals:

Zemplar (Paricalcitol) Capsules from Abbott Labs were generously sponsored by Aurobindo pharma limited. Acetonitrile (UPLC grade, from Merck chemicals), Ethanol (AR/GR grade, from Honyon International, Inc, China), Lauryl dimethylamine N-Oxide (AR/GR grade, from Sigma Aldrich). Ultrapure water was prepared by using Millipore Milli-Q plus water purification system. All chemicals and reagents were used as such without further purification.

Method development and optimization for an UPLC method:

Paricalcitol structurally found to be non-polar in nature. Drug product is not official or cited in any pharmacopoeia and there is a limited analytical literature available on this drug product for Dissolution test. Considering the drug nature, it does not mandate to use any buffer for mobile phase preparation to maintain particular pH for chromatography. Dissolution media recommended by OGD is Lauryl dimethylamine N-Oxide (LDAO), which is amine oxide surfactant. It is one of the most frequently-used surfactants of this type and highly non polar in nature. Due to non polar nature of both Paricalcitol and LDAO, it is quiet difficult to flush the non polar matrix from the column using isocratic elution mode. Hence trials were initiated directly using gradient elution mode by using water and acetonitrile as mobile phase-A and Mobile phase-B.

Another difficult aspect in this chromatography was to get precise area counts for drug quantification purpose due to highly low potent drug. Generally peaks eluted at longer retention time show higher drug component area counts when compared to that of shorter retention times. Considering this key parameter, trails were taken using to retain Paricalcitol initially for longer run time followed by flushing of non polar eluents appeared from sample matrix and from LDAO. Accordingly a gradient programme was developed at a flow rate of 0.1 mL per minute to retain Paricalcitol peak followed by flushing of non polar eluents by increasing flow rate to 0.2 mL per minute as a flow gradient.

Different UPLC compatible columns were tried during initial trail purpose. Finally Acquity UPLC HSS T3 (100 mm x 2.1 mm id) with 1.8 µm particle size is found to be suitable with respective to specificity and peak sharpness. The same column was used for entire method development work. Spectral data for Paricalcitol has shown wavelength maxima at about 252nm, and hence the same wavelength has been chosen for quantification purpose. Sample analysis revealed that separation between the Paricalcitol and dissolution media peaks was found to be satisfactory in the above specified conditions with proper separation and area counts.

Finalized chromatographic conditions:

The chromatographic column used was Acquity UPLC HSS T3 (100 mm x 2.1 mm id) with 1.8 µm particle size. The mobile phase-A containing Milli-Q water, filtered through 0.22µ membrane filter. Acetonitrile was used as mobile phase-B. The column temperature is maintained at 35°C and the detection is monitored at wavelength of 252 nm. The injection volume is 50 µL. The flow rate of the mobile phase was 0.1 mL/min initially, followed by an increase to 0.2mL/min with a gradient program (Table 1). Optimum test concentration as per dose is found to be 0.002µg/mL for 1mcg and 0.008µg/mL for 4mcg capsules respectively. For general system suitability data refer Table 7.

Table 1: Gradient programme

Time (minutes)	Flow (mL)	Mobile phase A (%v/v)	Mobile phase B (%v/v)
0.0	0.1	25	75
9.0	0.1	25	75
9.1	0.2	5	95
16.0	0.2	5	95
16.1	0.1	25	75
22.0	0.1	25	75

PREPARATION OF SOLUTIONS

Preparation of standard solution

Initial Standard stock solution of Paricalcitol (0.08 mg/mL) was prepared by dissolving in 5 mL of ethanol followed by make up to the volume with acetonitrile. This stock solution was further diluted to obtain a concentration of 0.002µg/mL for 1mcg and 0.008µg/mL for 4mcg using dissolution medium.

Preparation of sample solution

Sample aliquots were directly analyzed after removal from dissolution vessels at specified time intervals by filtered with suitable filter.

Dissolution Test Conditions

The dissolution test profiling was performed for lower (1mcg) and higher strength (4mcg) capsules as per OGD recommended dissolution media of 4mg/mL (0.4%) Lauryldimethylamine

N-oxide (LDAO), using USP Apparatus type I (Basket) with stirring speed of 100 rpm with 500 mL dissolution medium maintained at 37°C (± 0.5°C) on dissolution bowls. Samples of 10 mL were withdrawn from the dissolution medium at specified time intervals of 20, 30, 45 and 60 minutes followed by immediate replacement with dissolution medium which was previously maintained at 37°C. Filtered the sample solutions using suitable filters. Six samples were analyzed to check for precision for both strengths of 1mcg and 4mcg.

ANALYTICAL METHOD VALIDATION

Paricalcitol Soft Gelatin Capsules are available in different strengths such as 1mcg, 2mcg and 4mcg per capsule. 1 mcg and 4 mcg strengths were considered for entire validation experimentation. The developed method validated for Specificity, Precision, Linearity, Range, Accuracy, Solution stability, Filter paper evaluation and Robustness as per ICH guideline requirement. Prior to injecting sample solutions, the column was conditioned for at least 22 minutes with the actual gradient programme using mobile phase-A and Mobile phase-B flowing through the system. System suitability tests were carried out by making five replicate injections of a standard solution containing 0.002µg/mL for 1mcg and 0.008µg/mL for 4mcg of Paricalcitol and analyzing the chromatograms for Paricalcitol peak area, theoretical plates and tailing factor.

Specificity

A placebo sample of the reference formulation of capsules in the usual concentration of excipients was prepared to demonstrate reproducibility and reliability of the method. The placebo sample was transferred to Dissolution vessels containing 500 mL of (0.4%) Lauryl dimethylamine N-oxide (LDAO), stirred at 37 °C for 60 minutes at 100 rpm using a Basket apparatus. Aliquots of the solutions were filtered through 0.22µ PVDF filter paper and analyzed by UPLC.

Precision

The precision of the method is checked by injecting six individual preparations of Paricalcitol Soft Gelatin capsules with the recommended dissolution media as mentioned in the OGD. The precision of the method was determined by measuring the intra-day precision and the inter-day precision, both expressed as % RSD.

Linearity

To assess the linearity, 50 % - 150 % level of concentrated solutions were prepared and standard curve of Paricalcitol were constructed, by plotting the concentration (µg/ml) against peak area. The calculation of regression line was employed by the method of least squares.

Accuracy

Paricalcitol reference substance was added to the dissolution vessels in known amounts at the 50%, 100%, and 150% levels. Accordingly 0.0005000, 0.0009999, and 0.0014999 mg of reference drug was added along with 1-mcg capsule placebo and 0.0019998, 0.0039996, and 0.0059994 mg of reference drug was added along with 4-mcg capsule placebo. The dissolution test was performed as per OGD recommended dissolution parameters. Each concentration was analyzed in triplicate.

Solution Stability:

In order to demonstrate the stability of both reference and sample solutions, the solution stability was established over a specified period of time, verifying the response of the sample solution stored at bench top condition (25°C) or cooler temperature (2-8°C). The chromatograms obtained by the RP-UPLC method from freshly prepared solution were compared.

Filter evaluation:

In order to demonstrate the effect of filters used for standard and sample solutions, these solutions were filtered using PVDF and Nylon membrane filters by initially discarding 2-3 mL of aliquots from the filters.

Robustness

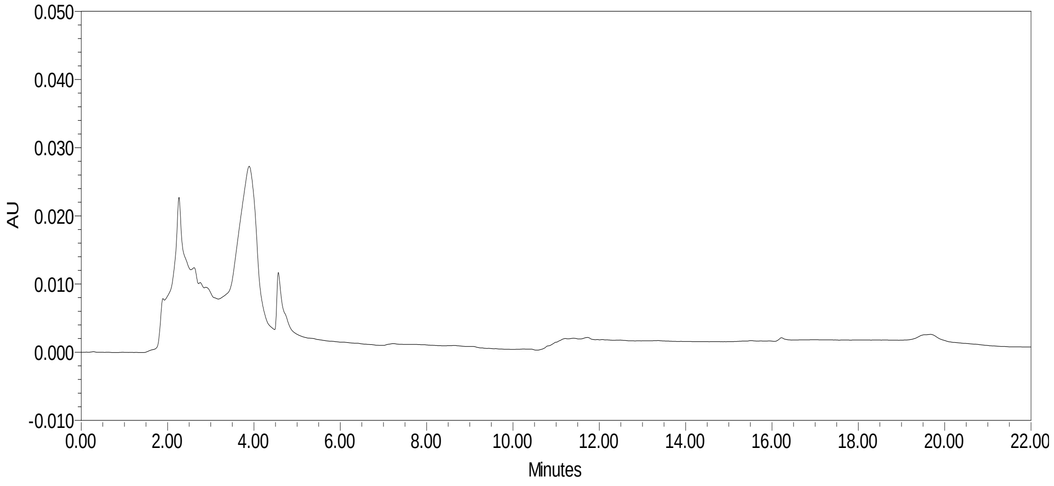
To determine the robustness of the developed method, experimental conditions are deliberately changed and the impact of the variation on drug was evaluated. The initial flow rate of the mobile phase is 0.1 mL/min and later it is 0.2 mL/min. To study the effect of flow rate ± 0.01 unit was changed i.e., 0.09 and 0.11 mL/min for initial flow and 0.19 and 0.21 mL/min for later flow gradient. The effect of column temperature (actual 35°C) is studied at 30°C and 40°C. The effect of pH of mobile phase is not studied, since there is no pH involved for mobile phase preparation. For gradient programme variation, the composition of mobile phase-B was changed by ± 2 absolute. For wavelength variation, ± 5 nm was changed from the working wavelength i.e., 252nm. In all the robustness conditions, only one parameter changed by keeping all remaining conditions unchanged.

RESULTS AND DISCUSSION

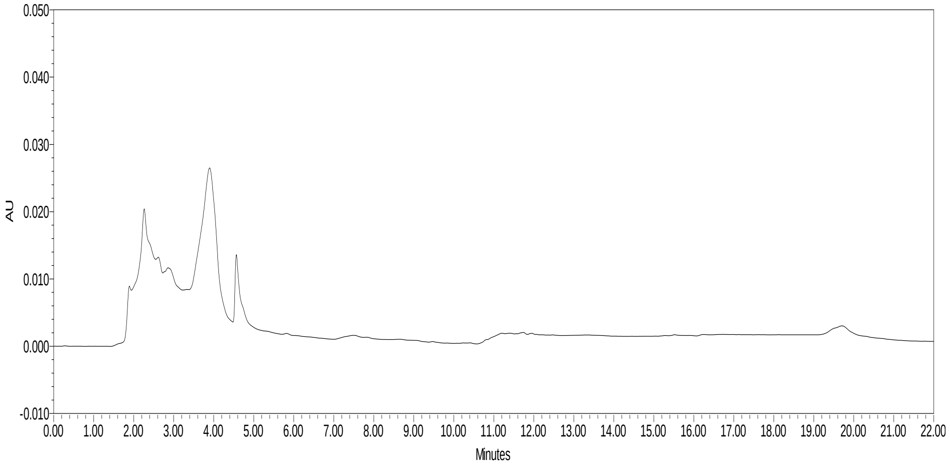
Specificity

The specificity of the dissolution test was evaluated through the analysis of placebo capsules from a dissolution test using the UPLC method (Figure. 2). The specificity test by UPLC demonstrated that the excipients from capsules do not interfere in the drug peak. Thus, the UPLC method is useful to quantify Paricalcitol in pharmaceutical formulation by comparing standard drug with sample (Figure 2).

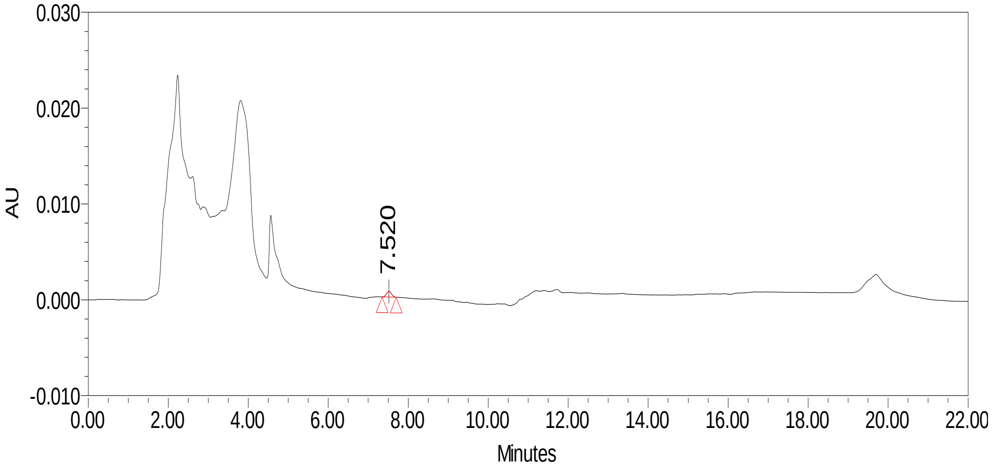
(a) Diluent chromatogram



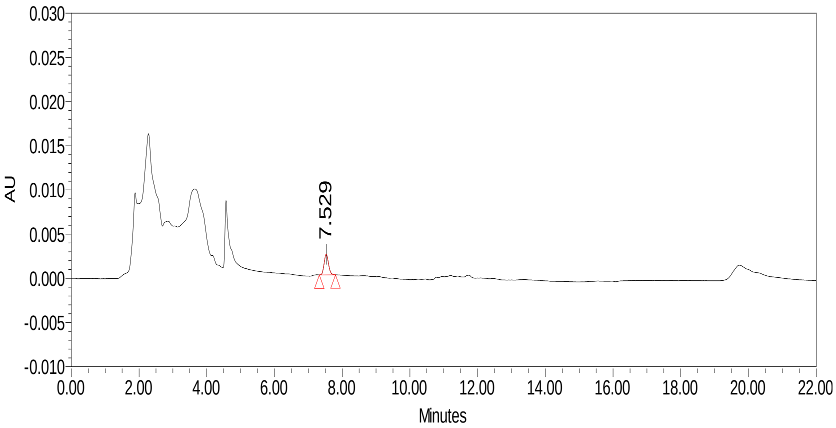
(b) Placebo chromatogram



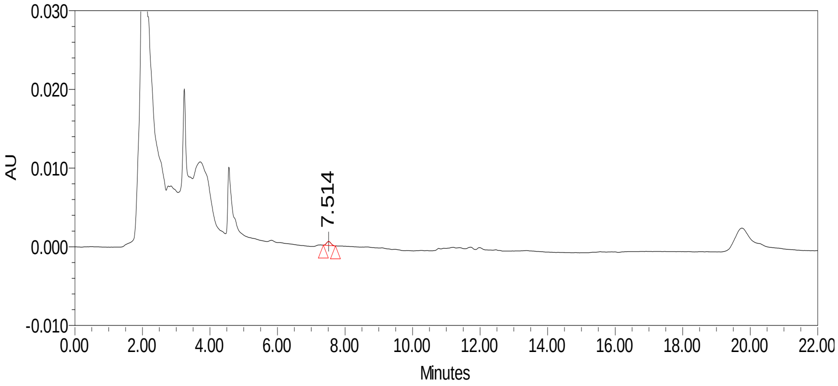
(c) Standard Chromatogram for 1mcg



(d) Standard Chromatogram for 4mcg



(e) Sample Chromatogram for 1mcg



(f) Sample Chromatogram for 4mcg

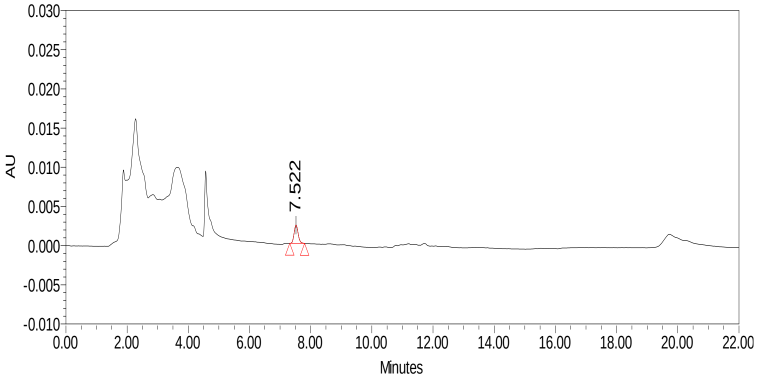


Figure 2: Typical chromatogram of Diluent, Placebo, Standard chromatogram for 1mcg, Standard chromatogram for 4mcg, Sample chromatogram for 1mcg and Sample chromatogram for 4mcg.

Method Validation

Precision

The precision results of the dissolution method were evaluated. The percent RSD did not exceed 5% for the Intra-Day and Inter-Day precision at 60 minutes dissolution time point, demonstrating suitable precision (Table 2, Table 3, Table 4).

Table 2 Dissolution Release Rate of Paricalcitol soft gelatin capsules 1mcg in Lauryl dimethylamine N-Oxide (LDAO), USP Apparatus I (Basket), 100 rpm (Inter-day precision)

Sr. No	Time (min)	% Release 1	2	3	4	5	6	Average % Release	% RSD
1	20	66	66	70	77	76	71	71	6.67
2	30	88	79	91	91	93	90	89	5.63
3	45	92	84	95	98	94	95	93	5.18
4	60	94	94	98	97	100	95	96	2.52

Table 3 Dissolution Release Rate of Paricalcitol soft gelatin capsules 4mcg in Lauryl dimethylamine N-Oxide (LDAO), USP Apparatus I (Basket), 100 rpm (Inter-day precision)

Sr. No	Time (min)	% Release 1	2	3	4	5	6	Average % Release	% RSD
1	20	73	78	70	76	77	78	75	4.27
2	30	73	85	81	85	89	88	84	6.97
3	45	80	90	90	90	91	90	89	4.70
4	60	85	92	92	92	94	94	92	3.62

Table 4: Dissolution Release Rate of Paricalcitol soft gelatin capsules 4mcg in Lauryl dimethylamine N-Oxide (LDAO), USP Apparatus I (Basket), 100 rpm (Intra-day precision)

Sr. No	Time (min)	% Release 1	2	3	4	5	6	Average % Release	% RSD
1	20	65	79	74	74	73	74	73	6.21
2	30	80	91	89	88	89	89	88	4.41
3	45	90	92	93	93	93	92	92	1.27
4	60	90	94	94	95	95	93	94	1.99

Linearity

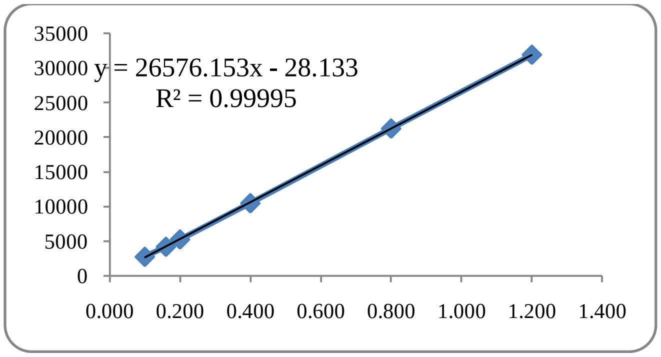


Figure 3: Typical Linearity chart for Paricalcitol

To assess linearity, a standard curve for Paricalcitol was constructed by plotting average absorbance versus concentration (Table 4). The curves depict good linearity in the range of 0.001–0.012 µg/mL. The line equation was y = 26576.153x – 28.133 with a slope of 26576.153 and R^2 of 0.99995. This data indicate that the method is linear for Paricalcitol within the specification limits. (Figure 3) Linearity Plot concentration (µg/mL) vs. area

Accuracy

The accuracy expresses the agreement between the accepted value and the observed value. According to ICH guidelines, the recovery results for a dissolution test must be in the range of 95–105%. The percent recovery was from 98.24% to 101.12% for 1mcg and 100.41% to 101.64% for 4mcg observed. The accuracy of the method is acceptable (Table 5 and Table 6).

Table 5: Dissolution test Accuracy results for Paricalcitol Soft gelatin capsules 1mcg

Concentration of spike level	Amount added (mg/mL)	Amount found (mg/mL)	% Recovery*	% RSD
50%	0.0005000	0.0004936	98.24%	0.451
	0.0005000	0.0004904		
	0.0005000	0.0004894		
100%	0.0009999	0.0010083	101.12%	0.836
	0.0009999	0.0010044		
	0.0009999	0.0010206		
150%	0.0014999	0.0015303	101.04%	0.934
	0.0014999	0.0015141		
	0.0014999	0.0015021		

* (N=3), mg/mL = milli gram/milli liter, % RSD = Percentage Relative Standard Deviation

Table 6: Dissolution test Accuracy results for Paricalcitol Soft gelatin capsules 4mcg

Concentration of spike level	Amount added (mg/mL)	Amount found (mg/mL)	% Recovery*	% RSD
50%	0.0019998	0.0020430	101.64%	0.517
	0.0019998	0.0020326		
	0.0019998	0.0020219		
100%	0.0039996	0.0040140	100.41%	1.415
	0.0039996	0.0040742		
	0.0039996	0.0039604		
150%	0.0059994	0.0061133	101.35%	0.498
	0.0059994	0.0060540		
	0.0059994	0.0060731		

* (N=3), mg/mL = milli gram/milli liter, % RSD = Percentage Relative Standard Deviation

Table 7. General system suitability data

Name of the Standard	USP Theoretical plates	USP Tailing factor	%RSD for replicate injections
Paricalcitol 1mcg standard	9409	1.23	1.15
Paricalcitol 4mcg standard	9998	1.25	0.93

Solution Stability:

No significant changes are observed in the area of Paricalcitol during solution stability experiment at 25°C. The solution stability experiment data confirms that standard and sample solutions are stable up to the study period of 28 hours. Since there is no issue observed at room temperature for solution stability, separate experiment was not conducted at 2-8°C temperature.

Filter evaluation:

The results of the filter evaluation reveal that the absolute differences between the concentrations of standard samples and filtered/centrifuged samples were within 98–102%. This demonstrates the absence of Paricalcitol adsorption by the filter and the suitability of PVDF or Nylon filter paper in the dissolution test.

Robustness

Close observation of analysis results for deliberately changed chromatographic conditions Flow rate, column temperature, change in pH, wave length and change of organic component in gradient programme which revealed that there is no significant change observed in the retention times of the Paricalcitol illustrating the robustness of the method.

CONCLUSION

The proposed UPLC method enables the quantitative determination of Paricalcitol in Paricalcitol soft Gelatin capsules. The developed method is validated as per ICH requirements. The studies indicated that method is selective and stability indicating. UV detection at 252nm was found to be suitable without any interference from excipients. Calibration curve obtained was found linear with values of correlation coefficients greater than 0.9999. Recovery tests confirmed the accuracy of the method. The proposed UPLC method is fast, precise, accurate, sensitive and efficient.

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