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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF CHOLECALCIFEROL IN ORAL SOLID DOSAGE FORMS BY RP-HPLC

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Abstract

To develop a sensitive, simple, accurate, precise and linear Reverse Phase High-Performance Liquid Chromatographic (RP-HPLC) Method and verify for the quantitative estimation (Assay) of Cholecalciferol in tablets. The optimized method uses a reverse phase column, Waters X-Bridge C18 (150X4.6mm; 5µm), a mobile phase of Methanol: Acetonitrile in the proportion of 80:20 v/v flow rate of 1.0ml/min, injection volume of100µl, and detection wavelength of 268nm using a UV/PDA detector. The developed method gave Cholecalciferol eluting at about 3.378min. Cholecalciferol exhibited linearity in the range 5.80-40.43µg/ml. The precision is exemplified by a relative standard deviation of 0.92%. Percentage of individual recovery was found to be in the range of 98.20 and 101.28 during accuracy studies. A sensitive, simple, accurate, precise and linear RP-HPLC method was developed and verified for the quantitative estimation (Assay) of Cholecalciferol intabletsandhencethismethodcanbeexploredfortheanalysisofCholecalciferolintablets/variousdosageformsinvarious pharmaceutical industries.

Keywords RP-HPLC, Cholecalciferol, Analytical Method development, Tablets

INTRODUCTION

Cholecalciferol, also known as Vitamin D3, is widely prescribed for the treatment osteomalacia and osteoporosis [1]. It also plays a key role in calcium and phosphorus homeostasis and skeletal mineralization [2]. IUPAC name of Cholecalciferol is (3β, 5Z, 7E)-9, 10-secocholesta-5, 7, 10(19)-trien-3-ol, whose molecular weight is 384.64g/mol and its molecular formula is C27H44O. The chemical structure of Cholecalciferol is given in fig. 1.

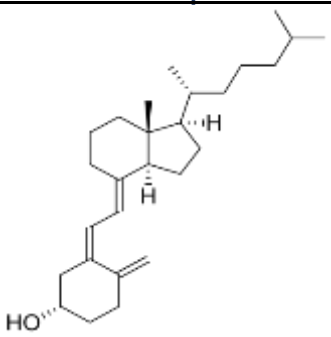

Fig.1: Structure of cholecalciferol

Various analytical methods are reported in literature by UV or HPLC, either in single or in combination with other drugs in various dosage forms [3-8]. In this review work, we focused on using a simple isocratic elution technique against gradient mode, mobile phase and diluents in a better combination and a simple sample extraction procedure for achieving reproducible assay results [9-11]. In this review article, we present purely only the analytical work on developing and verifying a simple and sensitive RP-HPLC method for the quantitative estimation (Assay) of Cholecalciferol in tablets, whose label claim is 595.31mg.

MATERIALS AND METHOD

Chemicals and reagents

Analytically pure sample of Cholecalciferol with purities greater than 95%was obtained from Sigma Aldrich and tablet formulation was prepared in our Formulation R&D laboratory, with a labeled amount 595.31mg of Cholecalciferol. Acetonitrile and Methanol (HPLC grade of Standard make) for the analysis.

Instrument

HPLC analysis was performed on Agilent and Waters makes HPLC shaving UV detector capable of setting a detection wavelength of 268 nm. A reverse phase C18 column, Waters X-Bridge (150X4.6 mm; 5µm), part number186003055was used. The HPLC system was controlled with "EMPOWER" software. An electronic analytical weighing balance (0.001 mg sensitivity, Sartorius make, ME5 model), and a sonicator (Hwashin Make, Powersonic 420model) were used for the analysis.

Selection of wavelength

Suitable wavelength for the HPLC analysis for Cholecalciferol was determined by recording UV spectrum in the range of 200-400nm. Suitable wavelength selected was268nm, considering the maximum absorbance at this wavelength.

Chromatographic conditions

The developed method uses a reverse phase C18column of Waters make, X-Bridge C18 (150X4.6mm.5µm) bearing a Part number of 186003055, mobile phase of Methanol: Acetonitrile in the proportion of 80:20 v/v respectively setting a flow rate of 1.0ml/min, injection volume as 100µl, detection wavelength as 268nm, setting column temperature and sample compartment temperature of 30°C and5°C respectively and run time as 15mins.

Mobile phase preparation

The mobile phase was prepared by mixing Methanol: Acetonitrile in the proportion of 80:20v/v/v respectively, followed by degassing in a sonicatorfor10min.

Diluents preparation

Based up on the solubility of the drugs, diluents was selected, Methanol and Mobile phase used as diluents.

Preparation of stock and working standard solution

Weighed accurately about 23mg of Cholecalciferol standard in to a clean and dried amber coloured 100ml volumetric flask containing 10ml of Methanol and then sonicated for10min to dissolve. Later the solution was made up to the mark using the diluents. This is considered as stock standard solution of a concentration of about 896.01 IU/mg. From the stock solution, 7.5ml was pipette out and diluted to100ml using the diluents to get a concentration of about 5µg/ml.

Preparation of system suitability solution

Transfer about 30ml of the above working standard solution in to a clean and dried100ml amber-coloured volumetric flask and then heat the solution at 60°Cfor1and half hour.

Preparation of stock and working sample solution

Not less than 20 doses of tablets were weighed, taken in a mortar and pestle and crushed to get a powder carefully to avoid any losses. Immediately transferred the complete powder into a clean and dried 500ml amber-colored volumetric flask. Added about350mlof diluents and then swirled the flask to ensure complete wetting of the tablets and later sonicated for about 5 min with intermittent shaking. Later, shaken the volumetric flask onarotaryshakerat200 rpmfor15minandthendiluted to volume with diluents and mixed well. Centrifuged a portion of the sample solution at 3000rpm for 10min and taken a liquor of the supernatant for analysis.

Method development

A Reverse phase HPLC method chromatographic conditions were developed keeping in mind the system suitability parameters *i.e.*, Tailing factor (T),%RSD from six replicate injections of standard and Blank interference along with simple extraction procedure, reproducible mobile phase and diluents, sensitivity and runtime. The optimized method developed resulted in the elution of Cholecalciferol atabout3.37min. Fig.2-6 represents specimen chromatograms of placebo, standard and sample solutions. Thetotalruntimeis15min.Systemsuitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system, whose details are summarized in tables1.

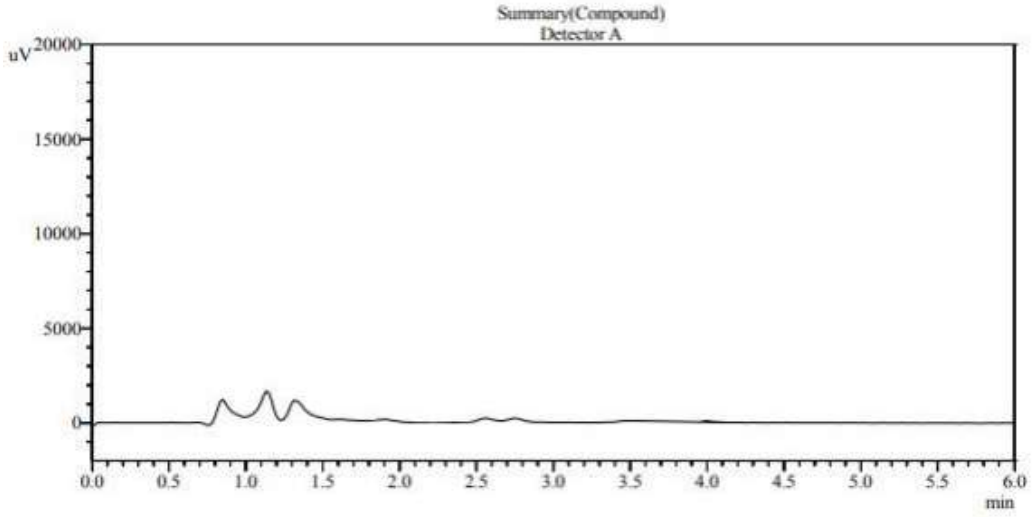

Fig.2: Typical chromatogram of the Placebo solution

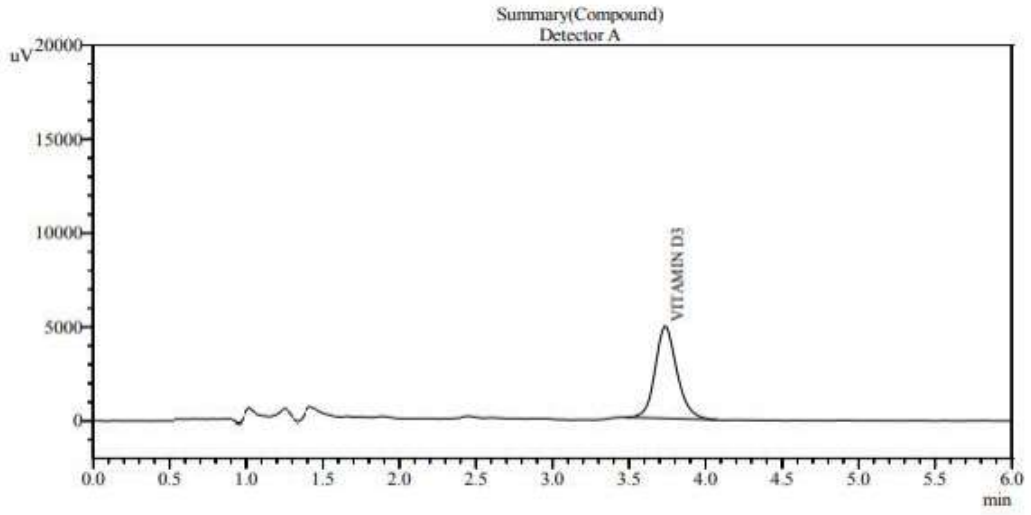

Fig.3: Typical chromatogram of the standard solution

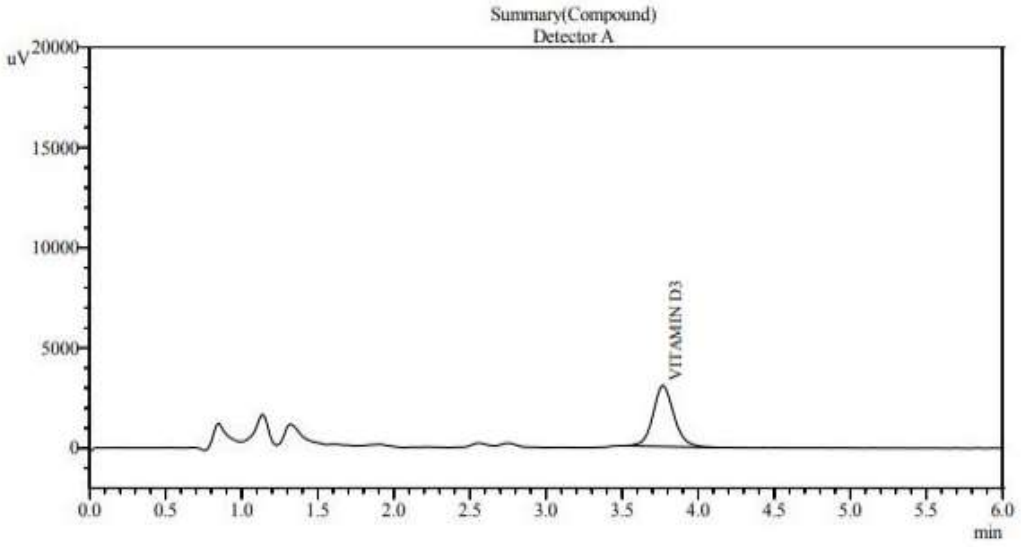

Fig.4: Typical chromatogram of the sample solution

1. **System suitability**

Table1: System suitability

Parameters	Acceptance criteria	Results
Blank interference	No peak shall be detectedintheblankattheretentiontimeofPre-CholecalciferolandCholecalciferolpeaks.	Nil
Tailing factor(T)	TheTailingfactorforCholecalciferolpeakshallnotbemorethan2.0fromfirstinjectionofStandardsolution.	1.17
%RSD(Relative Standard deviation)	Thepercentrelativestandarddeviationoftheaveragepeakarearesponsefrom6injectionsofCholecalciferol Shall be NMT2.0%.	1.65

In order to test the applicability of the developed method to formulation, Cholecalciferol tablets were chromatographed at a concentration of about 0.23µg/ml and it is shown in fig. 3. The sample peak was identified by comparing the retention time with the standard drug fig.4.To ensure the method developed meets the requirements of verification parameters, verification was employed whose details are mentioned in the below section.

Calculations

%Assay of Cholecalciferol in tablets/tablets blend was calculated by using the below formula.

$$\text{Tablets Assay (\% Percent Label claim)} = \frac{A_T \times Ws \times 5 \times 5x \times 500 \times P \times 100}{As \times 200 \times 250 \times 100 \times N \times 100 \times L}$$

Where,

(i)Peak area of Cholecalciferol =Peak area of Cholecalciferol + Peak area of pre- Cholecalciferol in the standard as well as sample chromatogram.

(ii)Relative retention time (RRT) for Pre-Cholecalciferol is about 1.08withrespecttoCholecalciferol.

AT is the peak area of Cholecalciferol in the sample solution.

AS is the average peak area of Cholecalciferol from 6 replicate injections of Standard solution A injected under system suitability.

WS is the weight of Cholecalciferol standard taken, in mg for the preparation of Standard solution

N is the number of doses 20 used during the sample solution preparation

P is the potency of Cholecalciferol standard

L is the label claim of Cholecalciferol in mg, **595.31mg**

Method verification

Assay method verification of the analytical method was done for the parameters like system suitability, specificity, linearity, accuracy and precision.

2. **Specificity**

To establish non-interference, blank and placebo solutions were prepared and injected into HPLC along with standard and sample solutions (Refer fig.2-4).

Acceptance criteria

No peak shall be detected in the blank and placebo chromatograms at the retention time of Cholecalciferol and Pre-cholecalciferol peaks.

Results and conclusions

No peak was observed in the blank and placebo chromatograms at the retention time of Cholecalciferol peak when compared to the standard and sample chromatograms. Accordingly, it is concluded that, the method developed is said to be specific.

3. **Method precision**

Label claim is 595.31mg of Cholecalciferol per tablet.

Method precision was determined by performing % Assay or % Label claim of the tablet blend, which is prepared by spiking API (at100%level) to the placebo equivalent to the tablet weight and extracting as

per the sample preparation procedure in triplicate. The percentage assay of each replicate, average of three replicates and% RSD were calculated.

Acceptance criteria

1. The %Assay shall be between 98.20and101.28.

The relative standard deviation of three replicate assay results shall be NMT 2.0%.

Results and conclusions:

The results were found to be within the acceptance criteria and summarized in table2. Hence, the method is precise.

Table2: Method precision results

Sample no.(n)	% Assay OR% label claim
1	99.21
2	100.47
3	100.85
Average	100.17
%RSD	1.50

4. **Accuracy**

Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of the sample at two different levels (80%and120%).At each level, three determinations were performed. Individual and percent mean recovery was calculated as shown in table2**.**

Acceptance criteria

The accepted limits of recovery are 98.20%-101.28%.

Results and conclusions

All observed data are within the required range which indicates good recovery values and hence the accuracy of the method developed.

5. **Linearity**

Standard solutions of Cholecalciferol at different concentrations level (25%, 50%, 80%, 100%, and125%) were prepared. Calibration curve was constructed by plotting the concentration level of the drug versus the corresponding peak area.

Acceptance criteria

1. The correlation coefficient shall be NLT0.9997.

2. Bias-Y-Intercept shall not be more than ±2% of the response at 100%level.

Results and conclusions

The results show an excellent correlation between peak area and concentration level of the drug within the concentration range (5.80-40.43µg/ml) for the drug and the results; linearity graph are given in table 4**.** The correlation coefficient of Cholecalciferol is 0.9997. The results reveal that method is linear for the quantification Cholecalciferol in the proposed range.

Table3: Results of accuracy studies

%Level	%Individual recovery	%Mean recovery
80	98.64	99.89
	99.75	
	101.28	
120	98.59	98.89
	99.06	
	99.02	

Table4: Linearity data and linearity graph

%Level	Concentration (µg/ml)	Peak area
25	0.052	10324
50	0.104	19343
80	0.156	29561
100	0.208	38706
125	0.260	48634
Regression/correlation coefficient	0.9997	
Regression equation	y=356318x-978.33	

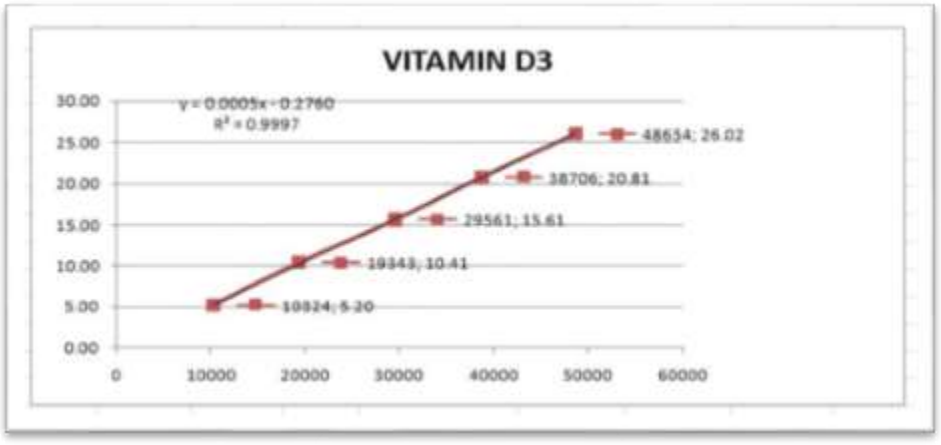

Fig 5 Linearity Curve for Vitamin D3

CONCLUSION

A reverse phase HPLC isocratic method developed has been verified in terms of specificity, accuracy, precision and linearity for the quantitative estimation (Assay) of Cholecalciferol in tablets. The precision is exemplified by a relative standard deviation of 1.50%.A good linear relationship was observed for the drug between concentration ranges of 5.80 and 40.43µg/ml. Accuracy studies revealed that mean recoveries were between 98.20and101.28%,an indicative of accurate method. Accordingly, it can be concluded that the developed reverse phase isocratic HPLC method is specific, accurate, precise and linear and therefore, the

method can be explored for the routine analysis of Cholecalciferol in tablets. In this study the use of RP-HPLC for evaluation and validation for the determination of Cholecalciferol in oral dosage form has been determined more precisely and accurately.

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