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DEGRADATION STUDY OF CHOLECALCIFEROL IN API BY SPECTROSCOPIC METHOD

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ABSTRACT
A simple, sensitive, precise, accurate, economic and rapid visible spectroscopic method has been Developed for estimation of Cholecalciferol. It was a subjected to different stress condition as per ICH guideline Q1A (R2). A stability- indicating UV Spectroscopic method has been developed for analysis of the drug in the presence of the degradation products. Degradation of Cholecalciferol was studied in acid, alkaline, hydrogen peroxide, photolytic and thermal conditions. The amount of degraded drug was calculated by taking absorbance at 258 nm. The drug was found to be more liable to decompositions in acidic, alkaline, oxidative medium than in photolytic and thermal conditions.

KEYWORDS: Cholecalciferol, UV- Spectroscopy, API, International Conference on Harmonization.

INTRODUCTION[1-3]
Vitamin D3 is very important fat soluble vitamins for human and animal diets. For this reason, they take part in many pharmaceutical preparations, foods, and feed formulation. Due to chemical activity of the vitamins, their concentrations in the preparation are always very low. Cholecalciferol is one of the five forms of vitamin D. Cholecalciferol is a secosteroid, that is a steroid molecule with one ring open. Cholecalciferol also known as vitamin D_3 and Cholecalciferol is a type of vitamin D which is made by the skin when exposed to sunlight; it is also found in some foods and can be taken as a dietary supplement. It is used to treat and prevent vitamin D deficiency and associated diseases, including rickets. It is also used for familial hypophosphatemia, hyperparathyroidism that is causing low blood calcium, and Fanconi syndrome. Vitamin D is very important fat soluble vitamin in human and animal diet. It exists in two forms "viz", Vitamin D2 and D3. Vitamin D plays an important role in the maintenance of normal levels of calcium and phosphorus in the blood stream and is essential for the proper development and maintenance of bone. Scientific evidence revealed that, it is not only associated with skeletal disorder but also plays an important role in cancer, cardiovascular disease, autoimmune disease, hypertension, diabetes mellitus etc. Vitamin D is not a single compound but is a family of compounds that exhibit Vit D activity. Its measurement is important as a clinical indicator of nutritional vitamin D deficiency, which is one of the causes of osteoporosis.

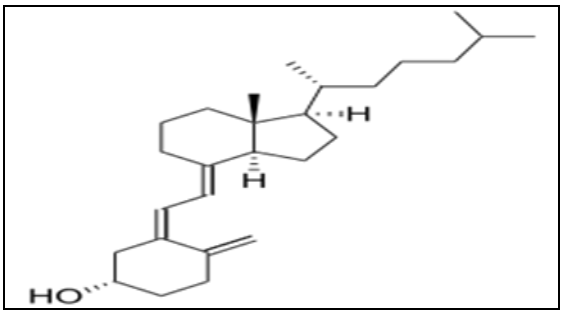

Fig: Chemical structure of Cholecalciferol.

MATERIAL AND METHODS
Materials & Reagents
- Pharmaceutical grade Cholecalciferol standard was obtained as generous gift from Zim Laboratories Limited Kalmeshwar, Nagpur, and Maharashtra, India. All the chemicals and solvents used were of analytical grade.
- The solution of 0.1 N Naoh, 0.1 N HCL, 5% hydrogen peroxide was prepared in double distilled water as per IP 1996 procedure.
- Methanol and other chemicals used which were of analytical grade and were procured from local market.

INSTRUMENTS
- Shimadzu UV-1800, double beam spectrophotometer with matching pair of 1cm quartz cuvettes with a fixed slit width 2 nm was used for all spectral measurements.

- Analytical balance (Acculab ALC-2014, Huntigdon Valley, PA)

PREPARATION OF STANDARD STOCK SOLUTION[4, 5]

The standard stock solution was prepared by dissolving 1.5 mg of Cholecalciferol in 10.0 ml of methanol to acquire a concentration of 150µg/mL. (Fig 1).

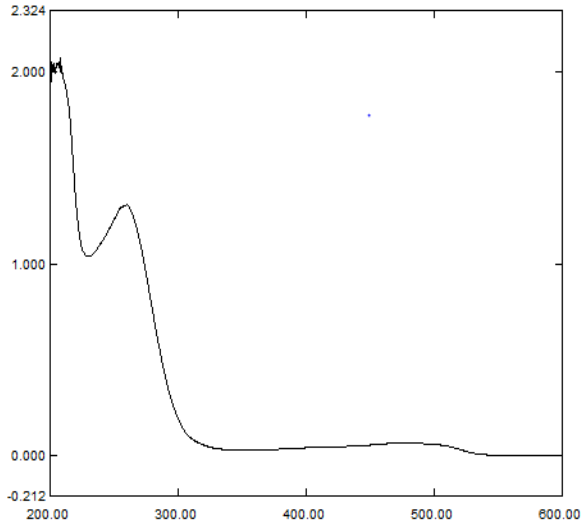

Fig 1: UV spectrum of Standard Cholecalciferol.

FORCED DEGRADATION STUDIES

To assess the stability indicating property of the developed HPLC method stress studies were carried out under ICH recommended conditions. Forced degradation of Cholecalciferol was carried carried out by exposing the bulk sample of acidic, alkaline, oxidative, photolytic, neutral conditions. The aim was to study the ability of the proposed method to, measure the analyte response in presence of its degradation products.[6, 7, 8, 9]

Acidic Degradation

Transferred 1 ml sample solution, 1 ml of 0.1 N HCL was added and refluxed at 60^0c for about 30 minutes. Then the resultant solution was sufficiently diluted to get 15ug/ml and then scan over a range of 400-200 nm by UV- Spectrophotometer. (fig:2)

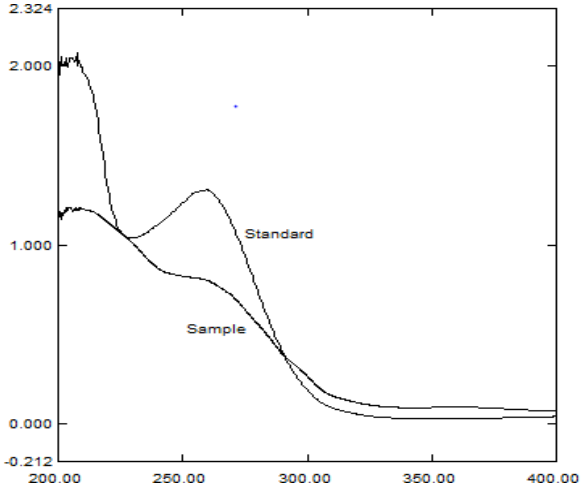

Fig 2: Degradation in acidic condition.

Alkali Degradation

Transferred 1 ml of sample solution, 1 ml of 0.1 N NaOH was added and refluxed at 60^0c for about 30 minutes. Then the resultant solution was sufficiently diluted to get 15 ug/ml and then scan over a range of 400-200 nm by UV-Spectrophotometer. (Fig: 3)

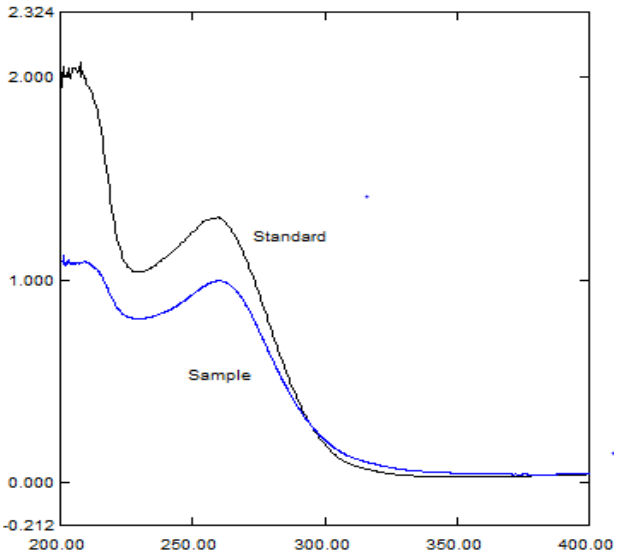

Fig 3: Degradation in alkaline condition.

Oxidative Degradation

Transferred 1 ml of sample solution, 1 ml of 3% H_2O_2 was added and refluxed at 60^0c for about 12 hrs. Then the resultant solution was sufficiently diluted to get 15 ug/ ml and then scan over a range 400-200 nm by UV-Spectrophotometer. (Fig: 4)

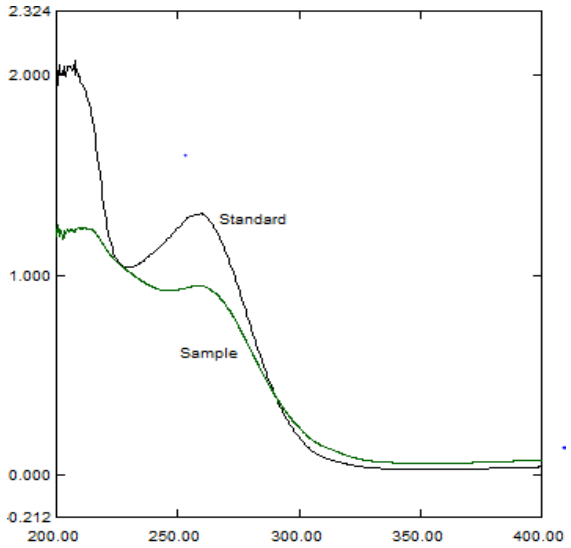

Fig 4: Degradation in Oxidative Condition.

Photolytic Degradation

Transferred about 1.5 mg of cholecalciferol standard in petri plate and exposed to the sunlight for 60 min, then weighed about 1.5 mg of exposed standard to 10 ml volumetric flask, add methanol shake to dissolve, made up the volume up to the mark, then transferred 1 ml of this solution to 10 ml volumetric flask and dissolved to get final concentration of about 15 µg/ml. (fig:5)

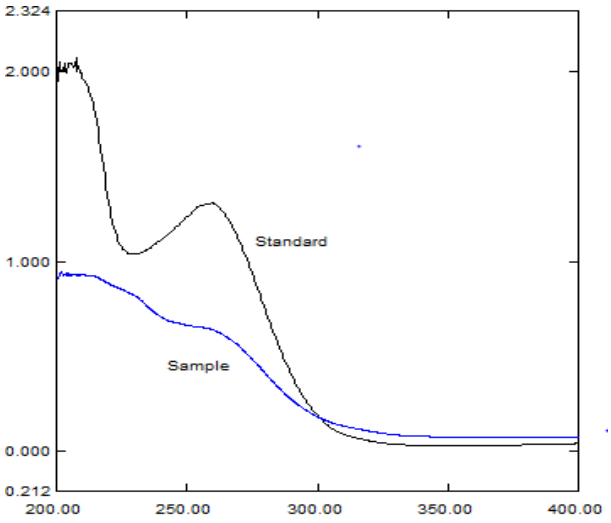

Fig 5: Degradation in photolytic condition.

Thermal degradation

Transferred about 1.5 mg of cholecalciferol standard in petri plate and exposed to the 45^0c for 30 min. in oven, then weighed about 1.5 mg of exposed standard to 10 ml volumetric flask, add methanol to dissolve , made up the volume up to the mark, then transferred 1 ml of this solution to 10 ml volumetric flask and dissolved to get final concentration of about 15 µg/ml..(Fig: 6)

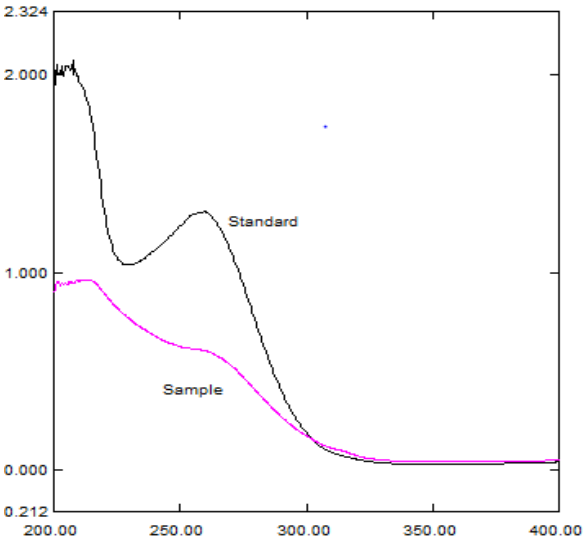

Fig 6: Degradation in Thermal Condition.

RESULT

Condition	Concentration Standard (µg/ml)	Absorbance	Absorbance after treatment (µg/ml)	% degradation
Acid hydrolysis	15	1.306	0.940	28.02
Alkaline hydrolysis	15	1.306	0.981	24.39
Oxidation	15	1.306	0.948	27.42
Photolytic	15	1.306	0.643	50.77
Thermal	15	1.306	0.616	52.84

CONCLUSION

On observing the assay results the method was found to be accurate, precise and specific. From the degradation studies it is proved that drug is susceptible to Acid hydrolysis, Alkaline hydrolysis, Oxidation, photolytic and Thermal degradation. Hence, the method can be

employed for quality control and routine analysis of estimation of Cholecalciferol in pharmaceutical dosage form in pharmaceutical formulations.

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