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Appraisal of Vitamin D3 Concentration in Dietary Supplement Marketed in Bangladesh using HPLC

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ABSTRACT

This research work provides information about the concentration level of fat soluble vitamin D3 in different types of dietary supplements marketed in Bangladesh. Selected twenty-five samples were taken for analysis because physicians are prescribing randomly and available in local market. Vitamin D3 concentration levels were quantified using HPLC with C18 column and diode array detector(DAD). The mobile phase was isocratic mode with ACN and methanol (60:40) and detection wavelength was (λ) 265nm. Quantification level of D3 was ranging from 1. 80(μg.mL^{-1}) to 24.91 (μg.mL^{-1}) in analyzed samples. Quantification of vitamin D3 was conducted basis on the standard solution retention time (Rt) and peak area. The analyzed method provides excellent results with linearity, correlation co-efficient ($r^2 \geq 0.9995$), LOD (0.0282, μg. mL^{-1}), LOQ (0. 0941, μg. mL^{-1}) and reproducibility with analyte recovery.

Keywords: Vitamin D3, Dietary supplement, HPLC-DAD, ACN(acetonitrile).

INTRODUCTION

Fat soluble Vitamin D3 chemically known as (*3β,5Z,7E*)-9,10 seccocholesta-5,7,10 (19)-trien-3-ol ($C_{27}H_{44}O$, MW-384.64 g/mol) belongs to secosteroids group[1]. This vitamin is synthesized from 7-dehydrocholestrol endogenously after UV-irradiation or from diet[2]. Potentially it helps bone development and hemostasis of phosphorus and calcium. Severe deficiency of D-vitamin is connected with rickets and osteomalacia in children and young age people[3]. Similarly, insufficiency of D-vitamin is related with wide variety of disease like cardio vascular disease, insulin resistance, allergic disease and macular degeneration, rheumatoid arthritis, multiple sclerosis etc[4]. Generally in medical practice,

D3 prescribed as 400IU to 1000 IU/day where 1IU equivalent to 0.025µg[5]. Due to the lipophilic nature and the presence of stabilizing agent in nutraceuticals supplement that might interfere in quantification of this vitamin[6]. Similarly, low concentration and photo-degradation is also associated with poor quantification. RP-HPLC technique can be a right approach to identify the accurate concentration of vitamin D3 in nutraceuticals supplement along with repeatability and reproducibility[7]. Now HPLC and UHPLC-MS technique is mostly used for estimation of D3 for its higher resolution and sensitivity, lower solvent usage and short run time[8,9]. This RP-HPLC method is specially designed for simultaneous quantification of D3 in different matrices like nutraceuticals and pharma formulations[10]. Besides, the classical classical spectrophotometric published methods have some limitations regarding lower sensitivity capabilities[9], higher solvent consumption, costly chemicals and time consuming sample preparation[11,12].

The objective of this research work was to establish a stable and simple method for detection vitamin D3 in nutraceuticals using RP-HPLC with DAD detection. Following the International conference on Harmonization(ICH) this method was successfully validated to determine D3 in different matrices[13]. To maintain the uniformity and the adequacy of this method tablet and drops type dietary supplements were analyzed with high resolutated identical peaks and lower solvent consumption.

EXPERIMENTAL

Instrumentation

HITACHI chromaster HPLC was used to analyze D3 vitamin in dietary supplement. The Chromaster (HITACHI, Japan) series were equipped with diode array detector (Chromaster-5430), column oven (Chromaster-5310), auto sampler (Chromaster-5210) pump module (Chromaster-5110) and column LaChrom C18 column (5µm,4.6mm I.D. x250mm L.).

Chemicals

Standard D3(Purity ≥ 99.0%, HPLC grade) was purchased from Sigma-Aldrich, Germany. Hydrogen peroxide (H_2O_2), n-Hexane, ascorbic acid, ethyl acetate ($CH_3COOC_2H_5$) and phosphoric acid (H_3PO_4) were purchased from knato chemicals, Japan. HPLC grade acetonitrile(ACN) and methanol were purchased from Honeywell Chemicals, India. Ultra-pure water was collected through a Milli-Q water purify system EVOQUA ultra water technology (IONPURE, USA).

Standard and working solution preparation

For preparing 100 (µg. mL^{-1}) of D3 stock solution 10 mg of D3 standard chemical was taken in 100 mL mobile phase solvent. D3 working standard solutions were prepared from stock solution in a linear range of 5(µg. mL^{-1}), 10(µg. mL^{-1}), 20(µg. mL^{-1}), 50(µg. mL^{-1}) by serial dilution for drawing a linearity in calibration graphs.

Sample preparation

The dietary supplement samples were prepared following some steps with modifications. Samples (tablets and drops) were extracted for analysis D3. Tablets were grounded with mortar and pestle. Accurately 5 g weighed sample taken in 50 mL centrifuge tube. 5 mL methanol was taken in the tube. A 10 mL mixture of hexane: ethyl acetate (7:3) was added in tube and vortex it for ten minutes. Then ten milliliters hexane was added in the tube and vortex for 5 minutes. The suspension was centrifuged for 10 minutes. The supernatant solution was taken with pasture pipette and filter through 0.45µm membrane filter. Again the solution filtered through 0.20µm membrane filter for HPLC injection and analysis.

Chromatographic condition

Chromatographic separation was experimented with the mobile phase ACN: Methanol (60:40) at 1.0 (mL.min^{-1}) flow rate. Injection volume was 10µL for single analysis. The separation was performed using C18 column and the oven temperature was 30°C. The detection wavelength was (λ) 265nm and the detector was diode array detector(DAD). Vitamin D3 was identified against the responsive peaks of standards with identical retention time.

Statistical analysis

SPSS software (version 22) and Excel 2016 software were used for statistical analysis. The analyzed results are represented as Mean, SD and relative standard deviation (RSD) after statistical analysis.

RESULT AND DISCUSSION

Deficiency of D3 vitamin is a threatening matter for growing children and adult also. Cause it helps to absorb calcium and accelerate the human metabolic process[14]. Supplementation of vitamin D3 is important for improving immune system and sound health for children and adult[15]. Now it is the routine issue to quantify the amount of added D3 vitamin in dietary supplement especially formulated for children. So, the quantification of fortified D3 using high performance liquid chromatography to make the comprehensive awareness among mass people regarding the real amount of this active nutrient in dietary supplement. This study represents an easy and simple extraction method for quantification of vitamin D3 in dietary supplement samples by HPLC-DAD. This developed HPLC method has a good resolution with a short analysis[16]. As the detection wavelength was (λ) 265nm for D3 and detected distinctively. The analysis time of chromatographic separation for vitamin D3 was 16.23 mins and showed in Figure 1.

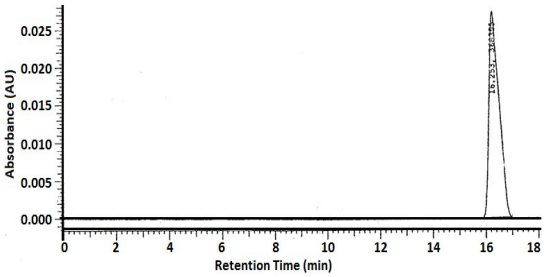

Fig. 1. Chromatogram of D3 standard

The system suitability requirements were designed after the development and validation of the method performed. The system suitability test results are showed in Table 1.

Table 1: System suitability parameters of the proposed HPLC method

Parameters	HPLC system suitability
Retention time(mins)	16.2±0.20
Theoretical plates(EP)(N)	30483±278
Tailing factor	1.08±0.01

A set of vitamin D3 standards were analyzed to validate this method based on the parameters (linearity range, detection limit, quantification limit)[17]. Five different concentration 5(μg. mL^{-1}), 10(μg. mL^{-1}), 20(μg.mL^{-1}) and 50(μg.mL^{-1}) of vitamin D3 were prepared respectively. The linearity was estimated by drawing the concentration vs peak area of D3. The calibration graph was obtained from the linearity of five concentrations. This method showed a good linearity where correlation coefficient was (r^2) ≥ 0. 9995. The LOD was 0.0282(μg. mL^{-1}) and LOQ was 0.0941(μg. mL^{-1})[18]. The validation data were exposed in Table 2.

Table 2: Statistical evaluation of the calibration data of vitamin D3 by HPLC-DAD method

Parameters	Vitamin D3
Linearity Range(μg.mL^{-1})	5-50 (n=5)
Correlation co-efficient(r^2)	0.9995
Limit of detection(LOD) (μg.mL^{-1})	0.0282
Limit of quantification(LOQ) (μg.mL^{-1})	0.0941

This method was conducted to appraise the D3 content in several dietary supplement marketed in Bangladesh. In this study 25 dietary samples were analyzed and obtained results were showed in Table 3. The analyzed compound was identified by HPLC using the retention time that matched with standard. The concentration was calculated using the peak area of standard and analyzed sample[19]. From the analyzed sample, the highest concentration of D3 was 24.91μg which recovery percentage was 99.61%. The lowest concentration of D3 was 2.15μg which recovery percentage was 86.0%.

Table 3: Statistical summary of data for HPLC Analysis of D3

Sample	Claimed amount,μg	Mean	SD	RSD%	Recovery%
S1	2.5	2.15	0.05	2.32	86.0
S2	10.0	9.10	0.82	9.01	91.00
S3	5.0	4.66	0.09	1.93	93.20
S4	10.0	8.46	0.11	1.30	84.60
S5	10.0	8.89	0.17	1.91	88.90
S6	15.0	14.64	0.12	0.81	97.60
S7	25.0	23.58	0.14	0.95	86.40
S8	10.0	8.64	0.18	2.08	86.40
S9	20.0	18.94	0.74	3.90	94.70
S10	25.0	23.95	0.16	0.66	95.80
S11	20.0	18.76	0.14	0.74	93.80
S12	10.0	9.35	0.23	2.45	93.50
S13	20.0	17.87	0.94	5.26	89.35
S14	25.0	23.33	1.30	5.57	93.32
S15	15.0	14.30	0.22	1.46	95.33
S16	25.0	24.91	0.41	1.64	99.64
S17	15.0	13.82	0.34	2.46	92.13
S18	10.0	8.07	0.62	7.68	80.70
S19	20.0	19.00	0.29	1.45	95.00
S20	15.0	14.48	0.22	1.51	96.53
S21	2.5	2.36	0.06	2.54	94.40
S22	20.0	19.39	0.20	1.03	96.95
S23	15.0	12.07	0.17	1.40	80.46
S24	25.0	22.92	0.16	0.69	91.68
S25	25.0	24.79	0.06	0.24	99.16

The analyzed chromatogram of samples using HPLC-DAD were shown in Figure 2.

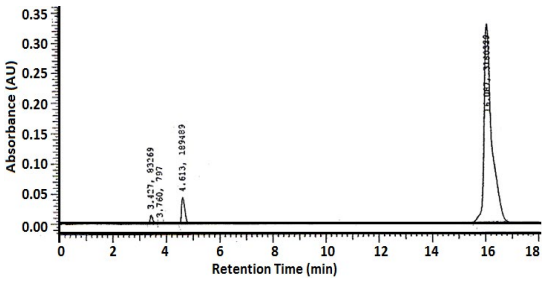

Fig. 2. Chromatogram of sample

To identify the accuracy of this method, known amount of D3 was spiked to this supplement sample and quantified by this developed method. Estimated test concentration was 100IU to 1000IU. Test sample spiking percentage was estimated (n=25) depending on spiking level[20]. Each sample was tested triplicate time to ensure the real concentration. A good recovery values showed that this HPLC method could avoid the stabilizing effects in sample analysis.

CONCLUSION

In this study D3 concentration level was analyzed in different dietary supplement which were claimed in its label. This study showed that some sample were in good quality and some were in excellent quality. The obtained results from this study were compared with WHO recommendation level. This study was done using HPLC with diode array detection cause its fast detection, excellent accuracy and easy extraction method. It is required to control and pledge the quality of these types of dietary supplement. Supplement sample analyzed results showed that a good accuracy and precision could be achieved from this developed method.

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