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Disparity in Vitamin D3 (Cholecalciferol) content among Pediatric Preparations in India.

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Abstract

Background: High prevalence of vitamin D deficiency (VDD) has been reported throughout India for children across all age groups. Several vitamin D3 preparations in different forms are available in the Indian market. However, the quality of these preparations remains questionable and there are several factors that may affect the quality. Considering that these preparations are for the Pediatric population, assessment of cholecalciferol content of commonly available and most prescribed vitamin D3 formulations is important.

Materials and Methods: We determined the cholecalciferol content of 4 commercial formulations in the form of oral drops prepared using nanotechnology and available in the Indian market. Lab analysis was carried out in Ramaiah Advanced Testing Laboratory by high-performance liquid chromatography. Two batches were evaluated for each formulation.

Results: The 4 formulations were analysed through validated assay method. Two batches of formulation A (96.53% and 94.91%) and one batch of formulation C Abstract Background: High prevalence of vitamin D deficiency (VDD) has been reported throughout India for children across all age groups. Several vitamin D3 preparations in different forms are available in the Indian market. However, the quality of these preparations remains questionable and there are several factors that may affect the quality. Considering that these preparations are for the Pediatric population, assessment of cholecalciferol content of commonly available and most prescribed vitamin D3 formulations is important. Materials and Methods: We determined the cholecalciferol content of 4 commercial formulations in the form of oral drops prepared using nanotechnology and available in the Indian market. Lab analysis was carried out in Ramaiah Advanced Testing Laboratory by high-performance liquid chromatography. Two batches

were evaluated for each formulation. Results: The 4 formulations were analysed through validated assay method. Two batches of formulation A (96.53% and 94.91%) and one batch of formulation.

Introduction

Vitamin D deficiency and supplementation

Vitamin D deficiency and insufficiency affect approximately 30% of children worldwide.[1] A recent study on children and adolescents in South Asia discovered that 25%-96.2% of subjects had VDD. Furthermore, VDD was most common in neonates (85%), preschool children (55%), and school-aged children (57%).[2] As per the Comprehensive National Nutrition Survey by the Ministry of Health and Family Welfare, Government of India, VDD was found to be prevalent among Indian children. Nearly, 14% of children aged 1-4 years, 18% of school-age children (5-9 years), and 24% of adolescents (10-19 years) were found to have VDD.[3]

Vitamin D is a secosteroid that exists in two forms, vitamin D2 and D3.[4] Although vitamin D2 (ergocalciferol) and D3 (cholecalciferol) have the same basic steroidal skeleton, their side chains and hydroxylated products differ.[5] However, vitamin D3 is the more active form of vitamin D and is either synthesized endogenously within the skin from 7-dehydrocholesterol or obtained from different dietary sources.[4,6,7] Once synthesized or absorbed, vitamin D molecules are transported to the liver and undergo a metabolic cycle, resulting in the formation of 1,25-dihydroxy vitamin D or calcitriol. Calcitriol is responsible for enhanced absorption of calcium and phosphate from the small intestine, maintaining blood calcium levels, and promoting skeletal mineralization.[8,9]

Severe VDD results in rickets and osteomalacia.[4] Both conditions weaken bones, making them soft and easily deformed.[8] Unlike osteomalacia, which affects both children and adults, rickets is only seen during early childhood and early puberty.[8,9] Vitamin D is thought to play a role in fatal skeletal development and may be the cause of skeletal deformities in infants.[4] Neonatal hypocalcaemia is related to VDD in mothers.[1] Neonatal hypocalcaemia can lead to seizures, craniotabes, or, less frequently, dilated cardiomyopathy.[4,8] Apart from these manifestations, VDD can also cause myalgia, myopathy, developmental delay, and immune system dysregulation.[8,9]

VDD can be prevented by adequate exposure to sunlight, dietary vitamin D3, or vitamin D-fortified foods. Although Indians receive adequate sunlight exposure, cutaneous synthesis of vitamin D is reduced due to several factors. Furthermore, the availability, acceptability, and cost of dietary and fortified food products limit their use. Therefore, vitamin D supplementation is an effective way of treating VDD.[10]

According to the Indian Academy of Pediatrics 2021 guidelines, routine vitamin D supplementation in infancy (0-1 year of age) in doses of 400 IU/day is recommended. A dose of 400 IU/day is considered to be safe in preterm babies. A dose of 800 IU/day can be used to achieve faster levels, but the evidence and safety data for preterm babies are lacking. Routine vitamin D supplementation with a minimum of 400 IU/day is recommended in children with underlying high-risk conditions, such as cerebral palsy, neuromuscular disorders, chronic kidney disease, chronic liver disease, malabsorption syndromes, and disorders with extensive cutaneous involvement.[3] The US Endocrine committee recommended an intake of 400-1000 IU/day in children under 1 year of age and 600-1000 IU/day from 1 to 18 years of age.[11] Likewise, the Institute of Medicine (IOM, 2011) recommended a

daily intake of 400 IU in infants less than 1 year of age and 600 IU in children over 1 year of age.[11]

Vitamin D supplements are available in various forms, such as oral drops, syrups, soft gels, tablets, capsules, or granules. Oral drops are recommended for infants and children.[10] However, these solutions can become unstable and result in a decrease in vitamin D content. Furthermore, it is well known that several factors such as oxygen, temperature, humidity, and light exposure influence the stability of vitamin D, resulting in decreased vitamin D content.[12]

Nanotechnology for improved stability of vitamin D3

To prevent the degradation of vitamin D3, different nanotechnology-based approaches have been applied and formulations have been developed.[13-15] These include zein nanoparticles coated with carboxymethyl chitosan for protection from UV light exposure, protein-polysaccharide nanocomplexes for improved physical and chemical stability[14], nano emulsions for stability against different environmental stresses[14] and whey protein isolate nanoparticles for stability in air[15]. All these studies highlight the advantages that nanotechnology offers in dealing with stability-related issues of vitamin D3.

Preparations available and quality

According to a study conducted by Chugh and Dabas, the total number of vitamin D products available in the Indian market is 1115. Nearly, 41% of these are vitamin D3-based preparations and are available in different dosage forms. Around 92% of these contain only vitamin D3 or cholecalciferol. The number of cholecalciferol preparations available in the market has increased by 18 times in the last 7 years. Although several preparations are available, the quality and shelf stability of these preparations are questionable.[16] Shelf life is defined as a decrease in vitamin D3 content after opening to levels that are unacceptable by legislation (<90%).[12] Furthermore, Indian Pharmacopoeia mentions 85% to 120% as the acceptable limit for oral solutions.[17] Khadgwat et al. evaluated commercial vitamin D3 preparations available in India and found that only 28.57% of these were within the prescribed limits.[18] As supplementation with vitamin D3 is critical in the sensitive Pediatric population, it is important that the vitamin D3 content in these supplements meets the label claim and remains stable.

In view of these points, the objective of this study was to analyze vitamin D3 (cholecalciferol) content in the most prescribed drop preparations by paediatricians in India and compare it with their accuracy as per claims made on the label.

Materials and Methods

In the present study, four vitamin D preparations containing cholecalciferol available for Pediatric use in India were included.

Lab analysis

Lab analysis was carried out in Ramaiah Advanced Testing Laboratory (NABL accredited and ISO 9001 certified for biological and chemical testing).

Standard preparation

About 20 mg of Cholecalciferol working standard (1 mg = 40000 IU) was weighed and transferred into a dry 200 ml volumetric flask wrapped with aluminium foil. 160 ml of methanol was added to the flask and gently shaken to dissolve and diluted up to the mark with methanol. Further, 2 ml of the above solution was diluted to 100 ml with methanol and mixed well.

Test preparation

Weighed the test sample equivalent to 8000 IU of vitamin D3 (cholecalciferol) and transferred it into a dry 100 ml volumetric flask wrapped with aluminium foil, dissolved it in 30 ml of methanol and sonicated it for 20-

25 minutes. Volume was made up using methanol and filtered through a 0.45µ nylon syringe filter. The first 3 mL was discarded and a portion of the solution taken into an HPLC vial for analysis

High-performance liquid chromatography operating conditions

Waters Alliance HPLC was used with Methanol (HPLC grade) as the mobile phase and photodiode array (PDA) detector at 265 nm. C18 column (250 × 4.6 mm, 5 µm, Luna Phenomenex) was used at a flow rate of 1.5 ml/min and injected volume of 20 µl and kept the sample cooler and column temperature ambient. The column was saturated with the mobile phase for 30 minutes.

Analysis

Blank (methanol), vitamin D3 standard solution, and test solution were injected individually, and chromatograms were recorded. The response of the peak corresponding to vitamin D3 was measured and the content of vitamin D3 in the sample was estimated.

Results

Four marketed formulations of vitamin D3 (800 IU/ml) available for Pediatric use were evaluated in the present study and the results are given in Table 1.

Ten months aged, batches 1 and 2 of formulation A deviated by only 3.46% and 5.08% from the label claim, respectively. Batches 1 and 2 of formulation B showed a 27% and 30.25% decrease in vitamin D3 content at 10 and 7 months aged, respectively, indicating less stability than formulation A.

In the case of formulation C, the vitamin D3 content decreased by 3.25% in a 6-month-aged sample. At eight months aged, batch 1 of formulation C showed a greater decline (19.75%) in vitamin D3 content. Lastly, at 6 months aged, formulation D also showed a larger decrease (21.5%).

Discussion

VDD results in rickets and other metabolic imbalances in infants and children.[4] For prevention and treatment of VDD, vitamin D supplements are recommended in infants and children.[19] The optimal level for 25-hydroxyvitamin D is considered to be level for 25-hydroxyvitamin D is considered to be >30 ng/dl[11].

Table 1: Vitamin D3 contents of different marketed formulations and their label claim

Formulation	Batch Number 1 with Manufacturing date	Batch Number 2 With Manufacturing date	Label Claim (IU/ML)	Obtained Value (IU/ml) for batch Number 1	Obtained Value (IU/ml) for batch Number 2	Sample age when the analysis was performed
A	KID21036, 08/2021-1/2023	KID21036, 08/2021-1/2023	800	772.3 (96.53%)	759.3 (94.91%)	10 months
B	B9CWT040- 12/2020	B9CWU005- 03/2021	800	584 (73%)	558 (69.75%)	10 and 7 months respectively
C	CHD21003- 02/2021	CHD21008- 04/2021	800	642 (80.25%)	774 (96.75%)	8 and 6 months respectively
D	K42113- 04/2021	K42113- 04/2021	800	628 (78.5%)	Not Detected	6 months

In order to achieve optimal levels of 25-hydroxyvitamin D, it is important that the vitamin D3 content in the formulations is accurate and meets the label claims.

Considering the stability-related aspects of vitamin D3, several marketed formulations are now available for Pediatric use that are formulated using nanotechnology for bioavailability. The present study evaluated the difference in vitamin D3 content between the label claim and the actual content in four such nanotechnology-based oral drops available in India. The results indicated variability in cholecalciferol content between the label claim and the actual content. Both batches of formulation A and the second batch of formulation C met the specifications of Indian Pharmacopoeia (85% to 120%)[17]. As mentioned earlier, the decline in formulation C occurred earlier than in formulation A. Thus, these results indicated that formulation A had better reproducibility and stability, resulting in greater content retention, and indicating better quality. Formulations B and D also showed a larger decrease than formulation A did at 10 months.

Such variations in the vitamin D3 content in the liquid preparations have been reported in other studies. Temova Ž and Roškar R evaluated the effect of different storage temperatures on vitamin D3 stability and shelf life after the product had been opened.[12] Vitamin D3 content in liquid prescription medicines remained >90% and <90% in the liquid nutritional supplement after 1 year of storage at 4°C after opening. The results for both batches of formulation A were comparable to those reported by Temova Ž and Roškar R.[12]

Both polar and nonpolar solvent-based preparations of cholecalciferol are available. Another recent study by Zane Temova Rakusa et al. evaluated the stability of vitamin D3 in commercial oil-based and water-based formulations, which were all in the form of oral drops. After 6 months of storage at ambient temperature, the content of approximately half of the tested formulations remained unaltered, whereas it steadily declined by up to 100% in the other half. Vitamin D3 was found to be stable in all oil-based formulations with <10% degradation. On the contrary, it was less stable in water-based formulations. However, the results from our study were different. None of the formulations in our study showed a decrease of 100% in content.[19]

Variation in cholecalciferol content has been reported in some studies for other vitamin D3 or cholecalciferol formulations. Khadgwat R et al. evaluated 14 commercial cholecalciferol preparations available in the Indian market, including 12 sachets and 2 tablet forms. Four out of fourteen preparations had cholecalciferol content within the specified limits (90% to 125%) of the Indian Pharmacopoeia. Some preparations had higher values than the specified limits, while others had lower values. This variation from the label claim ranged from -91% to +65%. Variations from the label claim ranged between -3.46% and -30.25% in the present study. Our results indicate lesser variation from the label claim in comparison to those reported by Khadgwat R *et al.*[18]

LeBlanc et al. evaluated the potency of cholecalciferol pills from 14 manufacturers. They evaluated these pills using different sampling methods to evaluate the variation in cholecalciferol content. Analysis of pills from 5 bottles with different lot numbers demonstrated the content ranged from 9% to 140% of the stated dose. Such variability in the different batches was observed in the present study as well. The analysis of oral drops with different batch numbers in the present study demonstrated that the content ranged between 69.7% and 96.7%, which was different from that reported by Leblanc *et al.*[20] On applying the stringent USP limit (90% to 120%) followed in this study to our

formulations, it was evident that both batches of formulation A and the second batch of formulation C were within the specified limits.

Garg S et al. analyzed vitamin D3 formulations, including six tablet formulations, two hard gelatine capsules, six soft gelatine capsules, and one emulsion available in New Zealand. Among these, only two were registered as medicines. This study demonstrated that only 60% of these were within 100 ± 10% of the label claim. The two registered prescription formulations contained 90 ± 4% and 97 ± 2% of the label claim. A large variation was observed in the non-registered dietary supplements with content ranging from 8 ± 2% to 201 ± 29% of the label claim. Registered prescription medicines are required to follow regulations for maintaining the strength and quality of these formulations. This variability in the label claim among the non-registered dietary supplements may be due to a lack of strict regulation, indicating the need for the same to ensure the quality of these supplements. [21]

Deviation of active compound content from the label claim and variability among various compositions of a given dietary supplement with the same batch number may also be due to a lack of good manufacturing practices.[22] The need for quality control and good manufacturing practices to assure that the content meets the prescribed limits has been highlighted earlier in the Indian context.[18] Thus, good manufacturing practices are necessary for ensuring the acceptable quality and safety of these preparations.[17]

Apart from content accuracy, adequate solubility is also important for ensuring bioavailability.[23] Polar solvent-soluble forms have the advantage that they lead to increased absorption of vitamin D3, resulting in greater efficacy.[24] In this study, we observed that solubility, dispersion, and dissolution of formulation A in the polar medium were comparatively greater in comparison to other formulations. This finding highlights that formulation A has better solubility in a polar matrix which may lead to better bioavailability.

As discussed earlier, several factors[18] may affect vitamin D3 stability and these can be encountered during the processing, packaging, and storage of these formulations. Vitamin D is a dietary supplement and the quality of dietary supplements is primarily the manufacturer's responsibility.[25] The manufacturer of formulation A in this study most likely followed good manufacturing practices, which may account for the lowest deviation from the label claim and comparatively better solubility in a polar matrix.

Lastly, we discuss the strengths and limitations of this study. The strengths of this study were: (1) It involved the evaluation of two batches for each formulation. (2) Standard method of analysis was followed and the samples were evaluated at an accredited laboratory. (3) Solvent-based effects on the solubility of formulations were evaluated, and the impact of good manufacturing practices has been highlighted. This study had one limitation, which was batches of different ages were evaluated.

Conclusion

Good manufacturing practices and processes are critical for vitamin D3 nano solutions for their stability throughout the shelf life. Based on the analysis of vitamin D3 content in various marketed formulations, it was found that formulation A had the best match with the label claim and has batch-to-batch reproducibility. Formulation A is a suitable preparation for the treatment of VDD in the Pediatric population.

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