Shelf life after opening of prescription medicines and supplements with vitamin D₃ for paediatric use

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ABSTRACT

Objectives The aim of this study was to evaluate the effect of storage conditions on the shelf life of liquid pharmaceuticals with vitamin D₃ in order to determine appropriate storage conditions and shelf life after the product has been opened. Manufacturers rarely specify the shelf life after products have been opened, which leads to a controversial debate among medical professionals regarding the safety of products after opening and optimal storage conditions and considerably different recommendations to the public.

Methods Effect of storage conditions on the shelf life was evaluated by comparing the isothermal stability of vitamin D₃ in three prescription and three non-prescription medicines at different temperatures (4°C, 25°C and 40°C) using stability-indicating high performance liquid chromatography assay.

Results Most of the examined preparations had a higher vitamin D₃ content than the label claim when the products were first opened, presumably to compensate for loss during production and storage. An association was observed between the initial vitamin D₃ content and its stability in the preparations. The vitamin D₃ overage in liquid prescription medicines guaranteed its content >90% of the amount claimed on the label for at least 1 year when stored at 25°C and for 4 months at 40°C. All tested preparations are consumed when used as recommended over this period. Since some manufacturers recommend storage below room temperature, redefining the storage conditions is of practical significance.

Conclusions The shelf life after opening examined prescription medicines containing vitamin D₃ as their main active ingredient is at least 1 year at room temperature.

INTRODUCTION

Vitamin D₃ (cholecalciferol), also known as the sunshine vitamin, can be obtained either from the diet or by endogenous synthesis in the skin following exposure to sunlight. The main physiological role of vitamin D is to maintain homeostasis of serum calcium and phosphate levels by promoting their absorption in the intestines as well as calcium reabsorption in the kidneys, which is essential for bone formation.¹ Genetic, nutritional and epidemiological evidence over the past decades has shown a link between the vitamin D endocrine system and the correct functioning of nearly every tissue in the body.^{2,3} Other functions of vitamin D include suppression of cell growth, regulation of apoptosis, modulation of immune responses, control of the renin-angiotensin and nervous systems as well as insulin secretion and muscle function.⁴

Fatty fish, specific fish oils and liver and fat from aquatic mammals are the only dietary sources naturally rich in vitamin D₃. Since the supply of these foods is limited, sunlight is the main source for humans.³ Growing concern about skin cancer has reduced sun exposure and increased the use of sunscreens; however, proper use of a sunscreen (2 mg/cm²) with a sun protection factor of 15 reduces previtamin D₃ synthesis in the skin by >99%,⁵ which is also suppressed by skin pigmentation, use of medications, body fat content, malabsorption and age.⁶ The combination of these factors has led to widespread vitamin D deficiency, which is estimated to affect around 1 billion people worldwide.⁷

Chronic severe vitamin D deficiency manifests as rickets and hypocalcaemia in infants and children and as osteomalacia in adults.^{3,8} Less severe deficiency is associated with long-term deleterious skeletal effects including secondary hyperparathyroidism, increased bone turnover and enhanced bone loss and fracture risk, which increases the risk of osteoporosis.^{9,10} The requirement for bone minerals (calcium and phosphorus) is highest during infancy when growth is most rapid; as such, infants are particularly vulnerable to vitamin D deficiency. Children begin to manifest clinical signs of rickets such as rachitic rosary, widened epiphyseal plates at the end of long bones and bowing deformities of the legs between the ages of 6 months and 1.5 years.⁴ Since infants are generally protected from the sun and breast milk does not contain adequate levels of vitamin D, daily supplementation in doses of 400 IU per day is mandatory for the prevention of rickets.¹¹ Babies born to vitamin D-deficient mothers are themselves vitamin D-deficient, as evidenced by the correlation in 25-hydroxyvitamin D levels between maternal and fetal metabolism.¹² These infants can rapidly develop hypocalcaemia, which can lead to seizures from birth onwards followed by dilated cardiomyopathy and/or rickets.¹² These can be treated by administration of higher vitamin D doses.

Vitamin D supplementation has gained increased acceptance due to the high prevalence of vitamin D deficiency. Vitamin D₃ is commercially available in various dosage forms as a prescription medicine or nutritional supplement including oil- and water-soluble capsules, tablets, vitamin D₃ concentrate (oily, powder and water-dispersible forms), injections and oral solutions. Prescription medicines containing vitamin D₃ are typically solutions, which is the only dosage form acceptable for infants. However, these can be unstable resulting in a decrease in vitamin D₃ content. A study of 16 commercially available samples containing vitamin

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D₃ obtained from a Jordanian market showed that more than two-thirds had a vitamin D content that was <90% of the amount specified on the label.¹³ Furthermore, it is hypothesised that the stability of vitamin D₃ decreases rapidly after the preparation has been opened because of the influence of oxygen, temperature, humidity and light exposure, leading to a significant decrease in vitamin D content. There are few reports on the effects of external factors on vitamin D₃ content in pharmaceutical products, and there is currently no information available about its stability once the product packaging has been opened.

Medical professionals—especially paediatricians and pharmacists—disagree in their recommendations of the shelf life and usability of these medicines after opening. Since infants and young children are a particularly sensitive population, the vitamin D₃ content and shelf life after opening are of great importance. These were evaluated in the present study in accordance with the well-established International Conference on Harmonization (ICH) guidelines at different storage temperatures (4°C, 25°C and 40°C).

METHODS

Chemicals and supplies

Vitamin D₃ (98%, CAS no. 67-97-0) as well as acetonitrile and methanol (both high performance liquid chromatography (HPLC) grade) were purchased from Sigma-Aldrich (Steinheim, Germany). H₃PO₄ (85%) was purchased from Merck (Darmstadt, Germany). High purity water was obtained using a Milli-Q A10 Advantage water purification system (Millipore Corporation, Bedford, Massachusetts, USA).

Samples

Three different prescription medicines (Plivit D₃, Pliva; Vigantol, Merck; and AD₃ drops, Krka) and three nutritional supplements (A+D₃ drops, Iva Farm; Vitamin D₃, Natural Wealth; and Vitamin D₃, Jamieson) that are commercially available in south-eastern Europe were analysed (table 1). All oral drops were originally packed in amber glass containers; tablets by both manufacturers were provided in plastic containers.

Liquid samples

Vitamin D₃ content was analysed in three different batches of each sample. After opening, every sample was prepared in triplicate and directly injected into HPLC system with ultraviolet detection to obtain the initial vitamin D₃ content. Three samples of each product were transferred to nine amber vials (approximately 1 mL per vial) and stored in the refrigerator at 4°C and in Vötsch VC 4034 climatic chambers (Reiskirchen-Lindenstruth, Germany) at 25°C and 40°C, and were analysed at different time points over a 1-year period to assess long-term stability.

Tablets

Samples were prepared according to a published method.¹⁴ In brief, one tablet of each sample (preparations E and F) was weighed, placed in a centrifuge tube and dissolved in 2.0 mL of 0.1% H₃PO₄, followed by vortexing for 2 min. Methanol (8.0 mL) was added to the samples, which were then sonicated for 10 min and vortexed for another 2 min. The solutions were centrifuged for 10 min at 4°C and 4130×g. The supernatant from each tube was passed through a 0.45 µm filter before HPLC analysis. Tablets processed after sample opening were used to quantify vitamin D₃ content. Three tablets from each batch were tested. One portion of the tablets was stored at

ambient temperature and another portion at 40°C in the opened original container. Both were further analysed (in triplicate) at different time points.

HPLC-UV analysis

Vitamin D₃ content in the preparations was measured according to a published method using an Agilent 1100/1200 Series instrument (Agilent Technologies, Santa Clara, California, USA) equipped with a diode array detector and ChemStation data acquisition system.¹⁴

RESULTS

Liquid prescription medicines (preparations A, B and C) used as vitamin D₃ supplementation in infants and young children and a liquid nutritional supplement (preparation D) were examined for initial vitamin D₃ content, stability and shelf life once the container had been opened. The initial content relative to the value claimed on the label expressed as mean±SE of three different batches was 115.9±0.6%, 100.0±1.0%, 128.5±1.0% and 100.0±0.8% for preparations A, B, C and D, respectively. The stability of the four liquid preparations when stored at three different temperatures (4°C, 25°C and 40°C) is shown in figures 1–3.

In addition, nutrition supplements in tablet form from two manufacturers were evaluated in terms of initial vitamin D₃ content and stability when stored at 25°C and 40°C. The initial content relative to the amount claimed on the label expressed as mean±SE of three different batches was 153.2±3.4% and 120.7±3.3% for preparations E and F, respectively. After 1 year of storage at 25°C, the content ranged from 135% in preparation E to 100% in F. When stored at 40°C, the content reached the critical value of 90% in preparations E and F after 5 and 3 months of storage, respectively.

DISCUSSION

The extent to which environmental storage conditions affect the stability of vitamin D₃ in pharmaceutical preparations was examined in order to determine appropriate storage conditions and shelf life after the product has been opened. An evidence-based approach would help to resolve disagreements among medical professionals regarding the maximum shelf life after opening, and the data are applicable to real-life situations (temperature fluctuations inside the home and during transport). Furthermore, the manufacturer's storage temperature recommendations may be less than ideal and must be verified, since infants and young children are a sensitive population.

In the present study, preparations with vitamin D₃ showed high variability in initial vitamin D₃ content (range 98.4–154.9%). All preparations examined, with the exception of preparations B and D, had higher content than that indicated on their respective labels. The average value in three of the prescription medicines (oral drops) was 114.8%, in the liquid nutritional supplement the value was 100.0% and the average value for the two solid nutritional supplements (tablets) was 136.9%. Vitamin D₃ content is regulated due to the risk of toxicity from excessive intake. Hypervitaminosis D can occur in patients who take vitamin D supplements and is associated with hypercalcaemia and other effects such as hypercalciuria, anorexia, weight loss, weakness, fatigue, disorientation, vomiting, constipation and even irreversible renal and cardiovascular damage.¹⁵ Acute toxicity and hypercalcaemia symptoms are not expected to emerge in intakes <10 000 IU/day. However, the tolerable upper intake level for vitamin D₃ is 4000 IU/day for children aged ≥9 years, but lower for young children and infants

Table 1 Examined preparations with vitamin D₃

Preparations		Dose	Recommended storage temperature	Recommended doses	Time* (months)
Liquid preparations					
A	Prescription medicine Plivit D ₃	4000 IU/mL	2–8°C	2–10 drops/day	1–5
B	Prescription medicine Vigantol	20 000 IU/mL	Room temperature	1–8 drops/day	1.3–10
C	Prescription medicine AD ₃ drops	2000 IU/mL	Below 25°C	2–10 drops/day	1.2–5.8
D	Supplement A+D ₃ drops	4000 IU/mL	Below 20°C	1 drop/day	10
Tablets					
E	Supplement Vitamin D ₃ (Natural Wealth)	400 IU/tbl	Room temperature	1–2 tbl/day	1.7–3.3
F	Supplement Vitamin D ₃ (Jamieson)	400 IU/tbl	Room temperature	2 tbl/day	1.5

*The time of consumption when used at the doses recommended for infants and young children.
tbl, tablet.

(1000 IU/day for ages 0–1 year, 2500 IU/day for ages 1–3 years and 3000 IU/day for ages 3–8 years).¹⁶ US Pharmacopeia has established limits for vitamin D₃ in prescription medicines (90–120%)¹⁷ and nutritional supplements (90–165%).¹⁸ As demonstrated by the quantitative analysis of vitamin D₃ content, all prescription medicines except for preparation C and nutritional supplements comply with the criteria.

According to the doses prescribed for infants and young children, all tested liquid preparations should be used up in less than 10 months (table 1). However, given that medical professionals—especially paediatricians and pharmacists—have different recommendations regarding the shelf life and usability of medicines after opening the packaging, we carried out our analysis based on ICH guidelines.¹⁹

All of the examined liquid dosage forms were packaged in dark glass containers that protected the product from light and oxygen. However, once the preparations are opened, they are exposed to both of these factors, making them further susceptible to oxidation and degradation of the vitamin. Shelf life was assessed under three different isothermal conditions (4°C, 25°C and 40°C) in temperature-programmable control cabinets. A temperature of 4°C was chosen as an approximation for storage in a refrigerator, which is recommended by some manufacturers (eg, preparation A; table 1), 25°C represents ambient temperature and 40°C is a temperature that can be reached under extreme conditions in homes without air conditioning in the summer. Samples from liquid preparations were evaluated at time 0 and at regular time intervals for a period of 1 year. During storage, the vitamin D₃ content gradually decreased at a rate that was dependent on formulation and storage

temperature; shelf life is defined as a decrease in the vitamin D₃ content after opening to levels that are unacceptable by legislation (<90%). The examined preparations had different shelf lives, indicating differences in vitamin D₃ stability.

A temperature of 25°C represents a typical ambient and storage temperature for most of the examined preparations. We observed a marked decrease in vitamin D₃ content in preparation C (figure 2), which also had the highest initial vitamin D₃ concentration (129.0%) of all the prescription medicines that were tested. After 1 year of storage at 25°C, preparation C had a vitamin D₃ content of 108%, representing a loss of 21%, with the most rapid decrease (around 10%) occurring in the first 10 days of storage (figure 2). This initial reduction at the beginning of the storage period can be ascribed to immediate reaction with available oxygen. It is likely that the vitamin D₃ overage accounts for its loss during manufacture and storage.

After 1 year of storage at 25°C, the vitamin D₃ content of preparation A was 107.0% of the value indicated on the label (figure 2), representing a loss of only 3% compared with the initial content. The initial vitamin D₃ content of preparation B was close to the value on the label (98.4%), and showed a negligible decline after 1 year of storage (<2%). As a nutritional supplement, preparation D is not regulated as strictly as prescription medicines. Although the initial vitamin D₃ content was 100.1%, it had low stability, reaching the critical level of 90% after only 2 months when stored at room temperature (figure 2).

The shelf lives of preparations A and B were 3 and 5 years, respectively, indicating high vitamin D₃ stability; this is in accordance with results obtained in our stability analysis. The

Figure 1 Vitamin D₃ content in prescription medicines A–C and nutritional supplement D stored at 4°C after opening.

Figure 2 Vitamin D₃ content in prescription medicines A–C and nutritional supplement D stored at 25°C after opening.

Figure 3 Vitamin D₃ content in prescription medicines A–C and nutritional supplement D stored at 40°C after opening.

shelf life of preparation C was 1 year, suggesting that the vitamin D was less stable and that the manufacturer added an excess of vitamin D₃ to assure product quality.

Manufacturers generally do not specify shelf life after opening. One exception was preparation B, which specified a shelf life after opening of 6 months. Our results showed that the shelf life after opening of all tested preparations was at least 1 year, after which the product would be expected to be consumed if used at the recommended doses (table 1).

In addition to shelf life after opening, we also assessed the suitability of the storage conditions specified for individual products. We found that ambient temperature was adequate for all the examined prescription medicines. The most important conclusion was that preparation A can be stored at room temperature (25°C) over a long period, as evidenced by the negligible decrease in vitamin D₃ levels. Given that the manufacturer recommends storage in a refrigerator, redefining the storage conditions is of practical significance since the medicine is currently involved in a cold chain system.

The shelf life after opening liquid preparations was also evaluated at 4°C, with preparation A as a particular point of interest since its manufacturer recommends storage between 2°C and 8°C. As expected, all examined liquid preparations showed higher stability at lower temperature (figure 1). Nevertheless, storage under this condition was irrelevant since all liquid prescription medicines were stable for 1 year at 25°C (figure 2). However, the shelf life for the less stable nutritional supplement (preparation D) could be extended to almost 8 months by storing at 4°C after opening.

The preparations were also stored at elevated temperatures, which simulated conditions that may occur in real-life situations. The vitamin D₃ content of most of the liquid preparations was markedly diminished by storage at 40°C (figure 3). Preparation A was very stable even at this temperature, with a decline in vitamin D₃ content of only 3%. The vitamin D₃ content in preparations C and D decreased considerably. Since a temperature of 40°C is attained only under extreme conditions, the short-term impact is significant. After 4 months of storage, all examined liquid prescription medicines had a vitamin D₃ content that was above the specification limit, indicating that these medicines can be exposed to higher temperatures for a short period without deleterious effects.

Although the solid forms of vitamin D₃ are not appropriate for infants and young children, we nonetheless examined the stability of vitamin D₃ in these products at 25°C and 40°C. Active ingredients in solid dosage forms have certain advantages

over liquid formulations (eg, greater stability and consequently longer periods of usability), which was confirmed by our results.

Compared with the liquid nutritional supplement (preparation D, 100.0%), the measured vitamin D₃ content in solid nutritional supplements was higher than label claims (153.3% and 120.7% for preparations E and F, respectively). The vitamin D₃ content decreased more rapidly over time in the liquid nutritional supplement than in the solid nutritional supplement, confirming that solid pharmaceutical forms are more stable. However, the rate of degradation was slightly higher in these products than in liquid prescription medicines. When stored at ambient temperature, the vitamin D₃ content of solid nutritional supplements was >90% for a period of at least 1 year; at 40°C and 75% relative humidity, the critical value of 90% was reached after approximately 3 months. Higher decomposition at elevated temperatures may result from exposure to greater relative humidity.

CONCLUSION

All examined prescription medicines were stable under the manufacturer's recommended storage temperatures for the period of their assumed consumption or for at least 1 year. Based on stability analysis, prescription medicine A can be stored at ambient temperature without a significant decrease in vitamin D₃ content, even though the manufacturer recommends storage in a refrigerator (2–8°C). Products differ in terms of their shelf lives depending on the stability of vitamin D₃ in the formulations. Tested liquid prescription medicines showed significantly higher stability than the tested liquid nutritional supplement. Our results suggest a link between the initial vitamin D₃ content and its stability in a given preparations.

Key messages

What is already known on this subject

- ▶ Vitamin D₃ solutions can be problematic in terms of stability.
- ▶ Manufacturers usually do not specify the shelf life after opening pharmaceuticals containing vitamin D₃.
- ▶ Doctors and pharmacists disagree in their recommendations regarding the shelf life and usability of these medicines after opening the packaging.

What this study adds

- ▶ Vitamin D₃ content in pharmaceuticals is typically higher than the label claim to compensate for loss during manufacture and storage.
- ▶ All examined prescription medicines can be stored at room temperature if used within 1 year after opening despite different manufacturer recommendations and the tested pharmaceuticals can be safely stored at elevated temperatures over the short term.

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Competing interests None declared.

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