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Research Article

Levels of Vitamin D in Some Vitamin D Veterinary Preparations Kept in Different Storage Conditions

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Abstract

In this study, it was aimed to determine suitable storage conditions for stability of vitamin D_3 in injectable preparations. Changes of vitamin D_3 to time under different storage conditions were determined in injectable vitamin D preparations. Vitamin D analyses in unsealed (seal of preparation was opened on day 0 of analysis, and the same preparation was used for analyses in other days) and sealed preparations (on each analysis day, seal of new preparation was opened and analysis was made) were conducted on days 0, 7, 14, 28 and 56 with a modified and validated method of the current US Pharmacopeia. The vitamin D_3 changes were found to be higher under storage conditions at 4 °C than 25 °C. The rate of vitamin D_3 changes significantly increased in unsealed preparations compared to sealed preparations under different storage conditions. In conclusion, after injectable vitamin preparations were unsealed, they can be suitably stored in environmental conditions with no light exposure and under room temperature.

Keywords

Vitamin D3, stability, storage conditions

INTRODUCTION

Vitamin D required for bone and mineral homeostasis is present in two forms as cholecalciferol or vitamin D_3. Vitamin D is obtained by the conversion of 7-dehydrocholesterol in the skin via sunlight or ingestion of nutrients rich in vitamin D (Latic and Erben, 2021). 25 (OH) D is converted to 1,25 $(OH)_2D$ by 1α-hydroxylase present in renal proximal tubular epithelium (Brunette et al., 1978). Vitamin D have functions such as calcium and phosphate metabolism, skeletal muscle development, immunity modulation, modulation of gut mucosal immunity, intestinal barrier integrity (Vernia et a al., 2022; Yang and Ma, 2021; Rak and Bronkowska, 2018). There are reports for the actions of vitamin D signaling in immune function and its beneficial effects for the prevention of infections (Ismailova and White, 2022). Air, oxygen, temperature, light, and chemical substances affect the stability of vitamins (Dominick 1980; Kondepudi, 2016; Kuong et al., 2016). Several studies have reported the changes of vitamin stability in extruded fish feeds (Gadient and Fenster, 1994; Anderson and Sunderland 2002) and in broiler premix diet due to added minerals (Zhuge and Klopfenstein, 1986). Vitamin D supplementation is used for the prevention and treatment of diseases such as periparturient hypocalcemia (Hajikolaei et al., 2021), and mastitis (Eder and Grundmann, 2022), rickets (Holick, 2006), cardiovascular (Wang, 2016), and for improving intestinal health and microbiota in laying hens (Wang et al., 2021). Accordingly, successful treatment of diseases, maintaining health of animal or human at good level depends on vitamin stabilities in formulations. Veterinarians buy vitamin drugs in large packages due to economic concerns and use them over a period of time. Unsealed bottles are maintained to be used in different times. Because unsealed bottles may be stored under unsuitable conditions, vitamin stabilities can be changed. Furthermore, prospectus information related to vitamin preparations may be different and cause conflict on the suitable storage conditions. Thus, in this study, it was aimed to determine suitable storage conditions for the injectable vitamin D preparations.

MATERIAL and METHODS

Methanol (Merck Lot No: I748007 433), distilled water (Lot No: Z0331233 431 Merck), and Vitamin D3 (100.0%, 0.15 g, Lot No: 30909) were supplied. The injectable preparations obtained from 3 different firms were coded as A, B, and C firms (Ademin, Ceva Dif, İstanbul, Turkey; Adesol, Topkim, İstanbul, Turkey; Adevilin, Vilsan, Ankara, Turkey). These preparations contained 75.000 IU vitamin D_3 per 1 mL. In this study, D_3 vitamin was analyzed. The unsealed and sealed preparations were analyzed for vitamin D_3 changes on days 0, 7, 14, 28 and 56 under different storage conditions such as light (bottles taken in boxes (uncovered)) and dark conditions (bottles kept in boxes (covered) in cupboard) at 25 °C and dark conditions (bottles kept in boxes (covered)) at 4 °C. For sealed group, on each analyze day, new preparation was unsealed and vitamin D_3 was analyzed, and preparation was discarded. The vitamin D analyzes were done according to US Pharmacopeia (USP 32-NF 27, 2009) using high pressure liquid chromatography (HPLC) (Serial No: L20225116387). Statistical evaluations for vitamin D_3 were carried out according to statistical package program SPSS (Ver. 20). Data of different temperature and environment conditions to time for vitamin D_3 were assessed using generalized linear models. Variance analyses of repetitive measurements were used in independent groups. Results were presented as means ± standard deviation. P values smaller than 0.05 were considered significant.

RESULTS

The levels of vitamin D_3 in the unsealed preparations of A, B, and C firms were significantly decreased on 7, 14, 28 and 56 days compared to on 0 day in the light (uncovered) and dark (covered, in cupboard) conditions at 25 °C (Table 1). The levels of vitamin D_3 in the unsealed preparations of C firm were significantly different on day 7 between the light and dark conditions at 25 °C (P<0.05). Vitamin D_3 changes in the unsealed preparations of A, B, and C firms were 12.0, 9.0, 8.73% in the dark conditions, and 10.23, 11.67, 9.07% in the light conditions at 25 °C, respectively.

Table 1. Changes in vitamin D_3 levels (IU) of unsealed preparations kept in light and dark conditions at 25 °C to time

Firm	Temperature	Light or Dark	Day 0	Day 7	Day 14	Day 28	Day 56
			x ±sd	x ±sd	x ±sd	x ±sd	x ±sd
A	25 °C	Light	78705.55± 4440.35^{a}	74921.78± 3532.83^{b}	76467.78± 3834.31^{bc}	70652.29± 2645.62^{d}	70980.85± 2777.65^{ed}
		Dark	79629.03± 4471.17^{a}	75456.38± 2841.61^{b}	78228.11± 5314.28^{bc}	70068.13± 1895.50^{d}	72130.24± 2611.72^{ed}
B	25 °C	Light	73432.99± 1121.18^{a}	70208.04± 3009.67^{b}	68909.87± 2449.64^{c}	64865.73± 1523.95^{d}	69748.34± 1495.35^{e}
		Dark	74223.24± 922.03^{a}	72047.32± 2116.58^{b}	68415.24± 2857.10^{c}	67544.71± 2581.70^{d}	69756.99± 2485.86^{e}
C	25 °C	Light	76407.82± 3448.18^{a}	69474.74± 1971.52^{b*}	70620.47± 2511.93^{bc}	71027.34± 1740.41^{cd}	73277.45± 2474.21^{e}
		Dark	76955.50± 3292.72^{a}	72341.69± 3081.66^{b}	70234.90± 1211.18^{c}	70787.98± 1806.67^{cd}	74406.06± 1632.12^{e}

a, b, c, d, e: Different letters in the same line show statistical differences in changes of vitamin D_3 active ingredient to time (*P*<0.05)
* in the same time and in different storage conditions, vitamin D_3 changes are statistically significant (P<0.05)

The levels of vitamin D_3 in the unsealed preparations of A, B, and C firms were significantly decreased on 7, 14, 28 and 56 days compared to on 0 day in the dark conditions at 25 °C and 4 °C (Table 2). The levels of vitamin D_3 in the preparations of C firm were significantly different on day 14 in the dark conditions between 25 °C and 4 °C (P<0.01). The changes of vitamin D_3 in the unsealed preparations of A, B, and C firms were 12, 8.99, 8.73 % in the dark conditions at 25 °C and 12.60, 10.42, 6.89% in the dark conditions at 4 °C, respectively.

Table 2. Changes in vitamin D_3 levels (IU) of unsealed preparations kept in the dark conditions at 25 °C and 4 °C to time

			Day 0	Day 7	Day 14	Day 28	Day 56
Firm	**Condition**	**Temperature**	**x ±sd**	**x ± sd**	**x ±sd**	**x ± sd**	**x ± sd**
A	Dark	25 °C	79629.03± 4471.17^{a}	75456.38± 2841.61^{b}	78228.11± 5314.28^{c}	70068.13± 1895.50^{d}	72130.24± 2611.72^{e}
		4 °C	80419.29± 3145.76^{a}	77422.91± 4423.23^{b}	78875.00± 4382.02^{c}	70283.14± 1719.08^{d}	72529.13± 3444.17^{e}
B	Dark	25 °C	74223.24± 922.03^{a}	72047.32± 2116.5^{b}	68415.24± 2857.10^{bc}	67544.71± 2581.70^{d}	69756.99± 2485.86^{e}
		4 °C	72932.75± 2012.27^{a}	71224.73± 4140.15^{b}	71147.50± 2459.91^{c}	65327.90± 1342.24^{d}	69349.99± 1389.78^{e}
C	Dark	25 °C	76955.49± 3292.72^{a}	72341.69± 3081.67^{b}	70234.90± 1211.18^{bc}	70787.98± 1806.67^{cd}	74406.06± 1632.12^{e}
		4 °C	75923.48± 2996.89^{a}	70853.02± 2241.14^{b}	73254.72± 3551.65^{bc**}	70688.59± 2029.69^{cd}	72367.98± 2459.94^{e}

a, b, c, d, e: Different letters in the same line show statistical significance in changes of vitamin D_3 levels to time ($P<0.05$)

**in the same time and in different storage conditions, vitamin D_3 changes are statistically significant ($P<0.01$)

The levels of vitamin D_3 in the sealed preparations of B and C firms were significantly decreased on day 56 compared to day 0 in the light and dark conditions at 25 °C (Table 3). Vitamin D_3 changes in the preparations of A, B, and C firms were 1.5, 1.31, 1.56% in the dark conditions, and 0.84, 1.41, 1.62% in the light conditions at 25 °C, respectively.

Table 3. Changes in vitamin D3 levels (IU) of sealed preparations kept in light and dark conditions at 25 °C to time

			Day 0	Day 7	Day 14	Day 28	Day 56
Firm	**Temperature**	**Condition**	**x ±sd**	**x ± sd**	**x ± sd**	**x ± sd**	**x ± sd**
A	25 °C	Light	74578.96± 3149.92^{a**}	73975.33± 2183.81^{b}	73954.61± 2451.25^{b}	74847.95± 2884.03^{a}	75336.72± 3467.34^{d}
		Dark	75129.49± 1084.81^{a}	74152.00± 1343.05^{b*}	73998.93± 1156.60^{bc}	74285.83± 689.15^{d**}	75637.00± 1127.93 ^{e*}
B	25 °C	Light	72545.75 ±1006.00^{a}	71526.47 ±942.19^{b}	71885.25± 786.57^{c**}	71968.03± 782.5314^{c}	72374.21± 1113.37^{cd}
		Dark	72425.81± 701.51^{a}	71544.63± 1082.14^{b}	71477.79± 1022.28^{b}	72590.98± 1474.54^{a}	71729.88± 605.81^{bc}
C	25 °C	Light	73493.52± 1589.83^{a}	73359.71± 1850.69^{b}	72302.16± 1925.18^{b}	72909.34± 1663.20^{c}	73037.94± 1773.22^{d}
		Dark	75514.05± 1958.87^{a}	75103.41± 2049.50^{b}	74337.09± 1824.40^{c}	75600.77± 1513.24^{a}	74778.58± 1816.22^{d}

a, b, c, d, e: Different letters in the same line show statistical significance in changes of vitamin D_3 levels to time ($P<0.05$)

* and **in the same time and in different storage conditions, vitamin D_3 changes are statistically significant ($P<0.05$) and ($P<0.01$), respectively

The levels of vitamin D_3 in the sealed preparations of B and C firms were significantly decreased on day 56 compared to day 0 in the dark conditions at 25 °C and 4 °C (Table 4). Vitamin D_3 changes in the preparations of A, B, and C firms were 1.5, 1.31, 1.56% in the dark conditions at 25 °C, and 1.55, 1.35, 1.1% in the dark conditions at 4 °C, respectively.

Table 4. Changes in vitamin D_3 levels (IU) of sealed preparations kept in dark conditions at 25 °C and 4 °C to time

			Day 0	Day 7	Day14	Day 28	Day 56
Firm	**Condition**	**Temperature**	**x ±sd**	**x ± sd**	**x ± sd**	**x ± sd**	**x ± sd**
A	Dark	25 °C	75129.49±1084.81^{a}	74152.00±1343.05^{b}	73998.93±1156.60^{c}	74285.83±689.15^{b}	75637.00±1127.93^{d}
		4 °C	74926.3±2525.4^{a}	73766.58±1912.03^{b}	75474.16±2588.37^{c*}	75131.73±2611.14^{d}	75064.74±2457.63^{d}
B	Dark	25 °C	72425.81±701.51^{a}	71544.63±1082.14^{b}	71477.79±1022.28^{b}	72590.98±1474.54^{a}	71729.88±605.81^{bd}
		4 °C	73026.52±1134.63^{a}	72528.58±1812.91^{b}	73231.83±1747.49^{ac*}	72688.61±1276.86^{b}	72042.99±698.51^{d}
C	Dark	25 °C	75514.05±1958.87^{a}	75103.41±2049.50^{b}	74337.09±1824.40^{c}	75600.77±1513.24^{ad}	74778.58±1816.22^{e}
		4 °C	75088.67±2435.16^{a}	74263.87±2481.29^{b}	74306.04±2195.32^{b}	74850.31±2830.58^{c}	74487.51±2395.05^{cd}

a, b, c, d, e: Different letters in the same line show statistical significance in changes of vitamin D_3 active ingredient to time ($P<0.05$)

* and **in the same time and in different storage conditions, vitamin D_3 changes are statistically significant ($P<0.05$) and ($P<0.01$), respectively

DISCUSSION

In this study, the changes of vitamin D were evaluated in preparations comprising vitamin D_3 frequently used in veterinary medicine and exposed to various storage conditions. Temperature, humidity, oxygen, light, oxidation and reduction substances and metal ions affect product stability (IADSA 2013). In this study, the vitamin D levels in injectable preparations of A, B and C firms were assessed due to temperature and the light conditions to time. The stability of drugs has been reported to be affected by exposure of high temperature and are protected better under room temperature than high temperature (Arshad et al., 2011). Lewis (2000) has reported that some anesthesia drugs are stored in refrigeration than room conditions to keep in a safety use. In this study, the changes of vitamin D_3 levels were found to be significant by storage under light and dark conditions at 25 °C and 4 °C to time. However, the rate of changes in vitamin D levels of unsealed preparations was not significantly different between at 25 °C and 4 °C to time. It was demonstrated that vitamins should be protected against oxygen, metal ions, and ultraviolet light (Combs 1992; Kondepudi 2016). The effect of light on vitamin D_3 is associated with oxidative reactions and the generation of free radicals (Temova Rakusa et al., 2021). It has been revealed that light decreases vitamin D stability (Combs, 2008; Fanali et al., 2017). In this study, vitamin D stabilities in unsealed preparations than sealed preparations were affected to various environmental conditions such as temperature and light and dark. D_3 vitamin is recommended to be stored in dark and cold conditions (Combs 1992). However, in this study, the rates of vitamin D_3 changes in formulations were found to be less at 25 °C than at 4 °C in the dark conditions. In conclusion, after injectable vitamin preparations were unsealed, they can be suitably stored in environmental conditions with no light exposure and under room temperature.

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