

## Effect of Light and Storage Time on Vitamin E in Pharmaceutical Products

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**Abstract:** Vitamin E is an important oxidant for both plant and animal and a lipid soluble vitamin which occurs naturally in edible plant oils and able to be synthesized by oxygenic bacteria. Besides, cooking oils and edible oil containing seeds, human can intake vitamin E via pharmaceutical products. With simply spectral method, vitamin E contents in tablets were quantified easily during storage. Vitamin E levels in medical products sold on Vietnamese market varied among producers ranging from 15 mg up to about 360 mg/tablet. Vitamin E in eight selected products was degraded gradually with prolonged storage time but much more rapidly in scatter light exposure condition than in the dark. The more vitamin E in the tablets, the quicker degradation occurred. Tablets exposed to natural sun light or UV source depleted vitamin E significantly within 5 h, approximately 50% for both lighted conditions. There was a relatively high tight correlation ( $R^2 = 0.8266$ ) between tablet price and its vitamin E content. Customer should buy the higher price products rather than the cheaper ones to get sufficient amount of vitamin E. Pharmaceutical vitamin E products should be used up soon after being bought or kept away any light sources.

**Keywords:**  $\alpha$ -tocopherol, dark, tablet, UV light

### INTRODUCTION

Vitamin E, a lipid soluble vitamin and an essential nutrient for reproduction, was discovered at the University of California at Berkeley in the early of twentieth century (Evans and Bishop, 1922) but we now still far from understand completely its biological functions in detail mechanisms (Brigelius-Flohé and Traber, 1999). Packer (1994) named vitamin E as a nature's master antioxidant. Vitamin E is a collective term covering wide range of naturally antioxidant molecules including four isoforms of tocopherols ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ) and four analogs of tocotrienols. Both tocopherols and tocotrienols are synthesized in higher plant plastids where  $\alpha$ -tocopherol is the main form in leaves and  $\gamma$ -tocopherol is the main form in seeds (Smirnov, 2005). Among them,  $\alpha$ -tocopherol is the most abundant and biologically active compound (Sheppard *et al.*, 1993; Ricciarelli *et al.*, 2001). Not only widely distributing in variously edible plant oils, a number of vitamin E forms also occur in oxygenic cyanobacteria (Cheng *et al.*, 2003; Maeda *et al.*, 2005; Nhan, 2007). In plants, tocopherols play an important role in adaptation to low temperature condition and are essential for preventing non-enzymatic lipid oxidation during seed germination and dormancy (Sattler *et al.*, 2004, 2006; DellaPenna and Maeda, 2007). Besides having protective function during reproductive phase in human and animals, unlike

many other vitamins, vitamin E has not been reported to be directly related to any enzyme activities (Sokol, 1996). In human, the most understanding mechanism of vitamin E function is its action as a scavenger of free radicals preventing cell membrane from peroxidation and disturbance (Burton, 1994; Traber and Packer, 1995; Hathcock, 2004; Schneider, 2005; Traber and Atkinson, 2007). It also has a protective function against heart disease and cancer (Stephens *et al.*, 1996; Dickinson, 2002; Jialal and Deveraj, 2003). Human can absorb all forms of vitamin E but keeping only  $\alpha$ -tocopherol in the body; therefore, vitamin E requirement for human nutrition based on the  $\alpha$ -tocopherol content in consumable products (International Institute of Medicine, 2000; Packer *et al.*, 2001). In human daily food consumption, vitamin E is provided via oils from lipid rich seeds such as soybean, olive, sunflower, peanut, walnut, sesame (Heinonen and Piironen, 1991; Packer *et al.*, 2001). Vitamin E presents as free molecule or in the esterified form, e.g.,  $\alpha$ -tocopherol acetate (Eitenmiller *et al.*, 2008); however, the natural  $\alpha$ -tocopherol has a higher biological activity than that of the synthetic counterpart (Brigelius-Flohé and Traber, 1999; Brigelius-Flohé *et al.*, 2002). Both natural and synthetic tocopherols are important in cosmetic and pharmaceutical industry but their stability in commercial products is recently not much monitored. It is well known that almost all vitamins are sensitive to

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Table 1: Basic information of pharmaceutical vitamin E products

Product name	Expiry date	Shelf life (months)	Form of vitamin E 400 IU
A	09.20.2013	36	$\alpha$ -tocopheryl acetate
B	04.01.2013	36	d- $\alpha$ -tocopheryl acetate
C	06.15.2013	36	d- $\alpha$ -tocopheryl acetate
D	11.07.2011	24	dl- $\alpha$ -tocopheryl acetate
E	09.17.2011	36	d- $\alpha$ -tocopherol
F	04.12.2012	24	d- $\alpha$ -tocopheryl acetate
G	07.22.2011	24	d- $\alpha$ -tocopheryl acetate
H	2012	36	d- $\alpha$ -tocopherol*

\*: Together with the mixture of  $\beta$ -tocopherol,  $\gamma$ -tocopherol and  $\delta$ -tocopherol

light, high temperatures and moisture. In this study, the stability of vitamin E in some pharmaceutical brands sold on Vietnamese medical market was investigated under different lighted conditions during storage.

## MATERIALS AND METHODS

All eight vitamin E products were bought randomly at only one locally pharmaceutical retailer without knowing of expiry date on December 12<sup>th</sup>, 2010. Some important details of each pharmaceutical vitamin E are listed in Table 1. To avoid any conflict, the real name of each product has been coded into letter randomly.

According to the producers, all selected products were contained 400 IU of Vitamin E for each tablet. Vitamin E tablets were stored in the desk for dark condition and on a shelf for light exposure at room temperature in the biochemistry lab. Vitamin E quantification in each product was carried out by the spectral method on a spectrophotometer (HELIOS  $\alpha$ , ThermoSpectronic, England) based on the absorbance of known concentration of  $\alpha$ -tocopherol (Merck, Germany). For each product, 60 tablets were used for the whole investigation. Each vitamin E tablet was diluted with 10 mL of methanol in a glass tube. After being shaken well, 100  $\mu$ L of the solution were loaded parallelly with 10  $\mu$ L of  $\alpha$ -tocopherol standard solution on a silica gel plate of 10 $\times$ 5 cm which was resized from the 20 $\times$ 20 cm TLC Silicagel 60 F<sub>254</sub> plate (Merk, Germany). The plate was developed with the running mixture of benzene/ethyl acetate of 96/4 (v/v) for 30 min. Afterwards, the plate was left to dryness under room temperature. Under UV light condition, both standard  $\alpha$ -tocopherol and pharmaceutical vitamin E were appeared in purple color on the plate. The silica gel area carrying vitamin E on the thin layer plate was removed and mixed with 3 mL of methanol. The mixture was shaken well for 30 sec and centrifuged at 5000 rpm for 5 min. The upper solution was transferred to a glass cuvette and the absorbance was measured at 294 nm.

To evaluate the loss of vitamin E under different storage conditions of light and time, vitamin E content in each product was quantified at the interval time period of 45 days starting from the date of products bought. The time span of this experiment was lasted for 135 days of storage. To compare the degradation of vitamin E in pharmaceutical tablets when exposed to

Table 2: Vitamin E contents in selected pharmaceutical products quantified by spectral method

Product name	Vitamin E content (mg/tablet)
A	14.46 $\pm$ 0.46g
B	67.23 $\pm$ 1.89de
C	57.74 $\pm$ 1.23f
D	88.76 $\pm$ 1.00c
E	160.96 $\pm$ 3.45b
F	63.52 $\pm$ 0.96e
G	68.48 $\pm$ 1.09d
H	361.21 $\pm$ 1.25a

Data presented are the means of triple quantifications with corresponding standard deviations

UV light condition and natural sun light, the Toconat was selected. For each treatment, 3 tablets were lighted under UV lamp of 40 W with the distance from the lamp of 20 cm for 3 and 5 h. For natural sun light exposure, vitamin E tablets were directly lighted by the natural condition at 10 $^{\circ}$ 7'21''N and 105 $^{\circ}$ 34'48''E. The vitamin E quantification of each pharmaceutical product was done with 3 replicates, one tablet for each replicate at each light condition and each interval time of storage. For data analysis, SPSS 106.0 was used.

## RESULTS AND DISCUSSION

At the concentration of 88  $\mu$ g/mL in methanol, standard  $\alpha$ -tocopherol was sensitive enough to recognize the maximal absorbance peak at 294 nm where the absorbance intensity reached the value of 0.538. When the vitamin E in pharmaceutical product presented in the form of  $\alpha$ -tocopherol as the E sample, the absorbance pattern and the maximum peak of absorbance intensity were identical with the standard  $\alpha$ -tocopherol. However, the maximum absorbance peak has been shifted to the left if the vitamin E in medical product existed in the conjugated form of  $\alpha$ -tocopheryl acetate (Fig. 1). This result approved that spectral method could be reliable enough to quantify vitamin E easily in other materials as well.

According to Landen *et al.* (2008) 1 mg of biological active  $\alpha$ -tocopherol is equal to 1.49 IU. All producers announced to their potential customers that each tablet contained 400 IU equivalent to about 268.5 mg which were much higher than those of quantified values presented in Table 2 except for the H product. The A brand contained least vitamin E among the commercially medical products, accounting for only

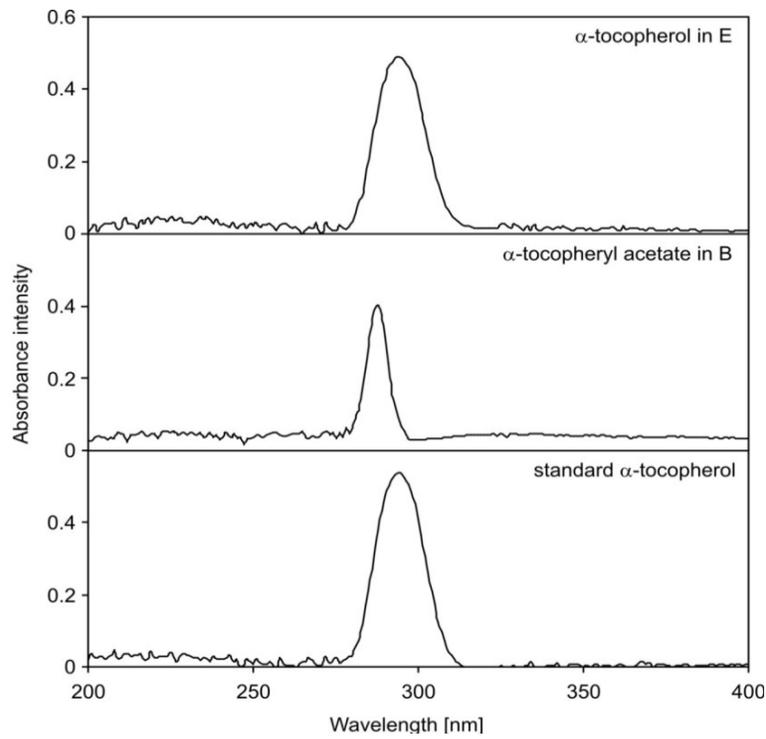


Fig. 1: Absorbance patterns of standard vitamin E and two pharmaceutical vitamin E products on the spectrophotometer

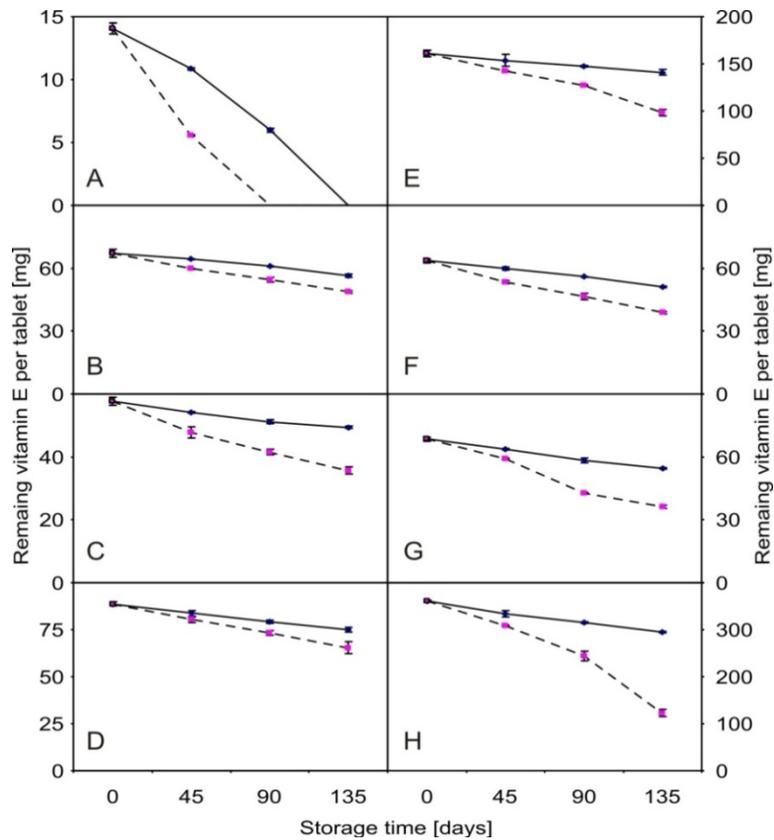


Fig. 2: The loss of vitamin E in some selected pharmaceutical products during storage in dark condition (the continuous lines) and in natural scatter light condition (the dashed lines)

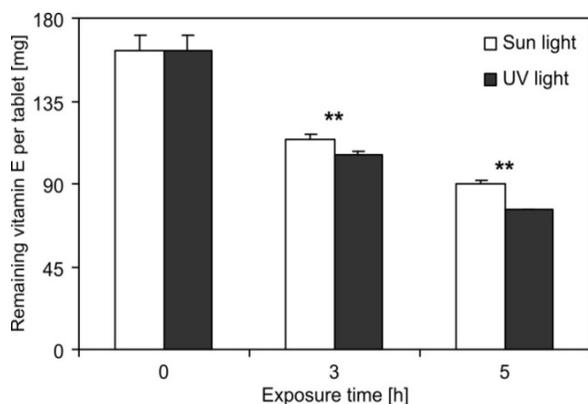


Fig. 3: Vitamin E loss from E brand when exposed to the natural sun light and the UV light  
The two asterisks showed the significant difference at 1% level by t-test between the two lighted conditions

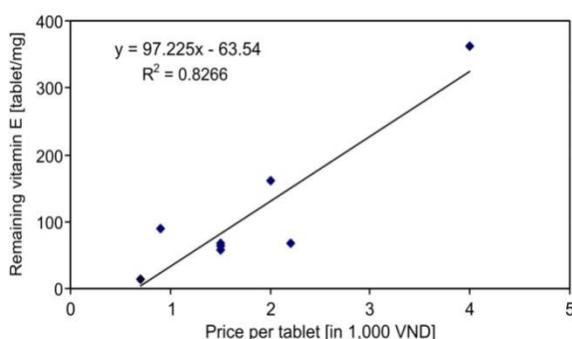


Fig. 4: The correlation between the vitamin E level in each tablet and the tablet price  
At the experimental time, the exchange rate was approximately 19,000 VND for one USD; VND: Vietnamese dong, the currency of Vietnam

less than one eighteenth the amount appeared on the product label. Vitamin E contents in most selected pharmaceutical products were 3 to 4.5 times lower than the levels announced on the brands valid for B, F, C, G, D. The E product had relatively sufficient vitamin E level in comparison with that printed on its label, about 60% in contrast with the actual amount. Only one among 8 selected products, the H brand assured the level of vitamin E, approximately 92.5 mg/tablet higher than that read on its brand.

Light had a powerfully degradable effect on vitamin E content in tablets of investigated medical products (Fig. 2). Vitamin E tablets from different producers kept in the dark condition lost their vitamin E contents at a slower speed than those exposed to the scatter light environment. The longer storage time, the more vitamin E loss occurred. This phenomenon happened to all chosen medical products in which vitamin E levels were extremely sensitive to the light, particularly for the products containing the lowest and the highest vitamin E contents like A and H. The

remaining level of vitamin E in A brand was under limit of detection at 90 and 135 days after storage for the light and dark condition, respectively. The product with highest vitamin E content, the H brand was tremendously sensitive to the light; it lost almost 3 times of vitamin E amount when compared with the dark condition after 135 days of storage. Therefore, vitamin E tablets should be taken as soon as possible after buying from the pharmaceutical shops.

To examine the sensitivity of vitamin E to the natural sun light and the UV light, the product with medium vitamin E level, E brand was chosen to investigate the degradation of vitamin E when exposed to these light conditions. The results presented in Fig. 3 showed that both natural sun light and UV light can destroyed strongly vitamin E in E brand although the exposure time was lasted limiting to only 5 h. The UV light could damage up to 55% of vitamin E in E product if the tablets were exposed to it continuously in 5 h. However, if the E tablets were lighted directly by the natural sun light which exhibited a powerfully destructive potential on their  $\alpha$ -tocopherol contents, about 46% of total vitamin E in each tablet. This might be a valuable and convincing evidence for any medical drug user to keep their vitamin E tablets away from any source of light.

An interesting analysis from this investigation was the strong correlation ( $R^2 = 0.8266$ ) between vitamin E content in each tablet and its price as shown in Fig. 4. The more vitamin E presented, the more tablet cost. It is also noticed from the Fig. 4 that the market competition occurred at the medium price, 3 out of 8 products had the price of 1,500 VND. The lowest price was recorded at 800 VND/tablet for A which contained the lowest level of vitamin E in contrast with the highest vitamin E tablet, H cost 4,000 VND.

These monitored results could surprise many potential drug users because the products were bought at relatively distance from expiry date but their quality appeared in unexpected data particularly for the cheapest product, the A brand. It is seemly that the poorer medical customers can be more vulnerable to the pharmaceutical quality than other groups in Vietnamese society. Most of investigated brands are disqualified vitamin E contents printed on their labels. For this antioxidant medicine, potential customers should buy the higher price product in order to get sufficient amount intake of vitamin E for their remedy.

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