Proposed Study to Quantify Vitamin E in Lotions Using HPLC

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ABSTRACT

Researchers have identified vitamin E as essential to one's diet and potentially beneficial for skincare. Vitamin E has been mainly reported to increase photoprotective properties and erythema. However, some studies researching scar formation and other effects were inconclusive and more research under randomized controlled settings should be pursued to better understand the role vitamin E plays in skin health. Vitamin E's increasing photoprotective properties benefited anti-aging and brightening of skin, but Vitamin E becoming more popular in cosmetics, but the amount of vitamin present in a product may not be clear. Therefore, scientists have developed various methods for measuring vitamin E in creams. The most widely adopted technique is high-performance liquid chromatography (HPLC). We propose a study to quantify the amount of alpha-tocopherol in commercially available lotions. This would be implemented by using an HPLC system to analyze commercially available lotions containing vitamin E. Pure Alpha- tocopherol and tocopherol acetate will be used as comparisons to the contents of the commercial lotions. .

INTRODUCTION

Vitamin E is an essential nutrient in our diet that is also beneficial for topical use on skin (*1*). Vitamin E is a necessity for growth and development and can help with damaged skin. The market for vitamin E is growing, as scientists discover more of its useful properties. The vitamin E market size is expected to increase from $2 billion (2019) by at least 4.5% in the next six years (*2*). Vitamin E is very common in skincare and hair care (*2*). Vitamin E is expected to have a 5% compound annual growth rate from 2020 through 2026 in cosmetic revenue, due to consumers' preference for more natural ingredients (*2*). However, there is a lack of information when it comes to the amount of vitamin E in cosmetic and skincare products. To measure vitamin E in products, researchers have established protocols using high-performance liquid chromatography (HPLC), which is a common analytical laboratory technique (*3*). In this proposed study, HPLC will be used to quantify the amount of vitamin E in popular lotion brands.

Vitamin E basics

The scientific name of Vitamin E is tocopherol, which is an entire family of compounds as shown in the appendix (Figure A1). There are three types of tocopherol: alpha, beta, and gamma. The difference is indicated by R_1 and R_2 functional groups (*3*). **α**-Tocopherol ($C_{29}H_{50}O_2$) is most commonly found in food and personal care products; its structure is given in Figure 1. Due to its primarily nonpolar structure, Vitamin E is fat-soluble and can accumulate in the body, unlike water-soluble vitamins that are quickly excreted.

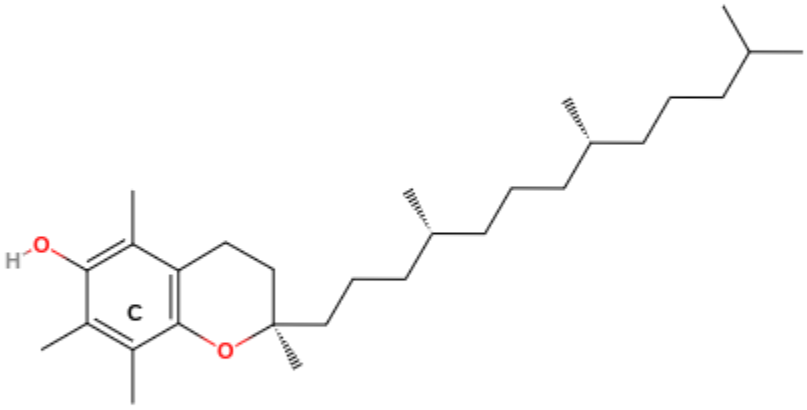

Figure 1. α-Tocopherol Generic structure of vitamin E (**α**-Tocopherol) (*4*)

Vitamin E is a nutrient and antioxidant that is found naturally in foods (*1*). A nutrient nourishes growth and maintains well being (*5*). An antioxidant fights off free radicals (*6*). Free radicals are chemical species with a highly reactive unpaired electron (*7*). Their presence at high levels can lead to a

cascade of broken bonds in important biological molecules, which may eventually be linked to illnesses, such as diabetes, heart disease, and cancer (*6*). Hence, vitamin E defends the body against such free radical attacks to prevent cell damage. Ingesting vitamin E can help with the metabolic process and build your immune system (*8*). Oral vitamin E is recommended for yellow nail, cancer prevention, and ulcers (*9*). Vitamin E is also a vital component necessary for reproduction (*10*).

Although vitamin E is naturally occurring, research indicates most Americans are vitamin-E deficient. In a survey, about 1000 individuals suggested that in the U.S. the majority of men and women do not meet the recommended serving of vitamin E (*11*). The most common foods containing vitamin E are vegetable oils, nuts, and green vegetables(*1*). The recommended serving of intake for those fourteen years old and older is 15mg/serving (*1*). To ensure a sufficient intake, vitamin E is also available as dietary supplements. For those who take blood thinners and other related medications, vitamin E supplements can be very harmful (*8*). This is harmful because it can increase the risk of bleeding. In addition to oral ingestion, vitamin E can also be applied topically to the skin and is sold as an oil or as a component of other skincare products.

Benefits of topical application of vitamin E

Vitamin E has many topical benefits (*9*) Many experiments have concluded topical use of vitamin E can be beneficial to different skin ailments. Several studies suggest topical use of vitamin E can reduce scar formation, but the information is inconclusive (*12*). Palmieri et al, tested the theory by adding Vitamin E to silicone gel sheets. Eighty patients between the ages 18-63 with hypertrophic scars were split into two groups (*13*). Hypertrophic scars are thick, wide scars caused by abnormal responses to trauma or injury (*14*). The first group received vitamin E-infused silicone gel sheets and the second group received only silicone gel sheets. Both sheets were wrapped on the scars for 10 hours overnight.After two months of treatment , 95% of patients who used Vitamin E infused gel sheets reported scar improvement up to 50%, while only 75% of the control group saw improvement up to 50% (*13*). Jenkins et al., reported that no beneficial effect of vitamin E could be demonstrated (*15*). In a randomized group of 159 patients who underwent burn contracture surgery, vitamin E was used as topical base cream, along with a topical steroid (Aristocort A) for 4 months. After a lack of significant effects, the patients were monitored and observed for scar thickness, change in graft size, range in motion,and cosmetic appearance for a duration year. 16.4% of those who were only treated with the active drug experienced adverse reactions compared to only 5.9% using the base cream experienced adverse reactions,yet the study still proposed no beneficial effect (*15*).

Vitamin E shows promise as a protective agent against sun damage; substances that reduce the damaging effects of exposure to ultraviolet (UV) light are classified as “photoprotective” (*9*). Ultraviolet

radiation exposure can increase small responses of the skin, but with the application of vitamin E those responses can be reduced. These skin responses consist of edema(swelling caused by excess fluid), sunburn, and erythema (i.e. skin reddening). These photoprotective effects have been studied in mice, rats, rabbits, and humans (*9*). In a review by Thiele, et al. twenty-nine studies were summarized and the vast majority showed that vitamin E application prior to exposure to UV radiation had positive effects on various indicators of damaged skin (*9*). Specifically, researchers tended to test for skin reddening (erythema) in human subjects and monitored a wider variety of biological responses in animal models (*9*). Dreher et al.(1999) focused on vitamin E´s role in minimizing erythema (i.e. skin color and skin blood flow) on human skin (*16*). Vitamin E was applied to the skin after UV-exposure for a single application, immediately or within 30 minutes. The study indicated vitamin E provides moderate protection when applied prior to UV-exposure, but no benefit was found if applied after UV-exposure. Montenegro et al.(1995) also studied the effect of vitamin E and vitamin E acetate on erythema(skin color) with humans as the test subjects (*17*). Two different formulations, an aqueous gels & oil-in-water emulsions, with active compounds of each tocopherol, tocopheryl acetate, and superoxide dismutase (SOD), glutathione, ascorbyl palmitate were tested on healthy humans after exposure to UV light. Vitamin E and vitamin E acetate suggested to have moderate protection when applied after UVR-exposure, however, there was no protection prior to UVR-exposure (*17*). Bissett et al.(1992) used mice to test the effects of vitamin E in skin wrinkling, skin tumor incidence, and histology (*18*). The mice receiving the treatment were split into two groups. Within those two groups, the mice were exposed to UV radiation five times or three times out of the week.The treatment occurred two hours prior to UV radiation exposure. The results suggest that vitamin E provides protection against skin wrinkling, skin tumor incidence, and histology. It also proposed additive protection when combined with other anti-inflammatories.

A co-delivery of vitamins may lead to better results compared to vitamin E alone. Mice treated with the codelivery of vitamin A & vitamin E showed reduced hyperkeratosis (*19*). Hyperkeratosis is the thickening of the skin due to rubbing and pressure, which can appear in the forms of corns and calluses (*20*). Vitamin E combined with Vitamin C can increase photoprotective properties (*9,21*).Vitamin E alone was suggested to be very effective in reducing hyperpigmentation (*21*). Hyperpigmentation is when patches of your skin are darker than other parts. The increase of photoprotective properties can connote beneficial effects to anti-aging and brightening of the skin. In a split face, random controlled trial of 50 female volunteers between the ages 30- 65, topical treatment was applied to one side of their face for two months, while still using a personal facial skin product on their face (*21*). Both sides of the face were monitored and tested after 4 and 8 weeks. The topical treatment was a serum containing vitamin E, vitamin C, and raspberry leaf cell culture extract. The study suggested significant improvement in skin

color, elasticity, and radiance. The skin appeared to be lighter in color and an increase of elasticity on the treated side. Researchers used skin parameters as evidence of improvement.

Many skincare products claim they contain vitamin E, but the specific form (e.g. ester) and concentration may not be clearly labeled. Beneficial results may depend on the identity of the "vitamin E" present, as well as its concentration. Research has suggested that **α**-tocopherol may increase skin's antioxidant defense when present in topical formulations in concentrations of 0.1%-1% (*9*). The FDA reported only 1072 out of the many existing cosmetic formulations actually contain alpha-tocopherol in 1998 (*9*). Other cosmetic formulations contained esters of alpha-tocopherol.There is an absence of data and studies quantifying vitamin E, as well as a lack of ways to measure oxidative stress to prove vitamin E theories or effectiveness. Hence, conscious consumers need more information about the concentration and type of vitamin E in commercially available lotions to make informed purchasing decisions

Quantifying Vitamin E

There are several reasons why scientists have developed methods for quantifying vitamin E, such as determining the nutritional value of food, maintaining quality control in the production of supplements, and measuring the amount of vitamin E in the body to monitor for deficiencies. Rupérez, et al. summarized the published analyses of **α**-tocopherol in a variety of samples in a *Journal of Chromatography A* review (*3*). The types of samples ranged from urine and blood to plants and food. The review stated the most common methods for the analysis of **α**-tocopherol; High-performance liquid chromatography (HPLC), and Gas chromatography-mass spectrometry (GCMS) (*3*). A less common method for analyzing vitamin E is SFC (*3*). SFC or supercritical fluid chromatography is a combination of GCMS and HPLC.

High-performance liquid chromatography (HPLC)

High performance liquid chromatography (HPLC) is used to separate a mixture of compounds which can be shown in **Figure 2**. The compounds are separated based on their relative affinities in both the mobile and stationary phases. Affinities are resemblances or similarities that are presented in structure and polarity. Polarity is the amount of electric charge distributed between bonded atoms. The most common method of HPLC used in analyzing vitamin E is reversed-phase chromatography. According to the "*Journal of Chromatography A* review", reversed-phase HPLC was claimed to be more efficient (*3*). In reversed-phase HPLC, the polarities of the column and mobile phases are reversed such that the stationary phase is nonpolar and the mobile phase is polar.. The mobile phase commonly is polar and mixed with methanol or acetronile. A UV detector is commonly used to record and determine the retention time and signal strength of the compound.

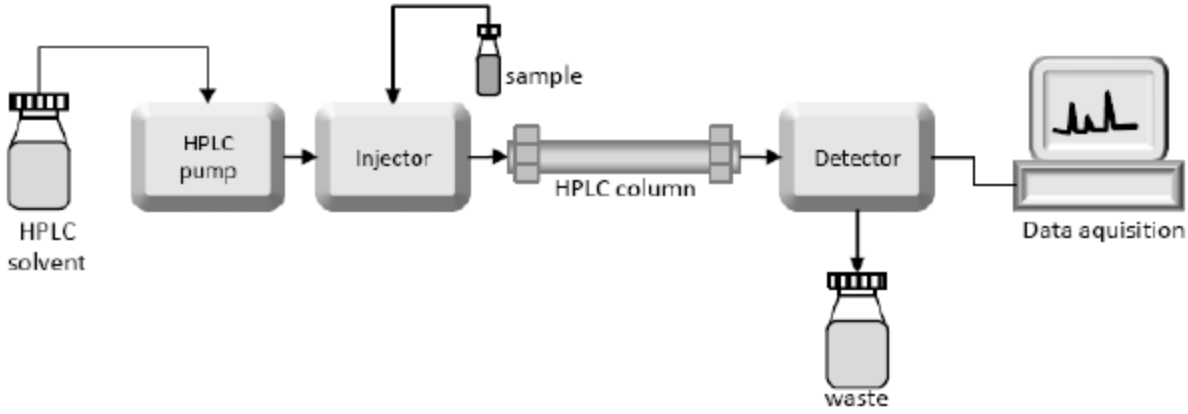

Figure 2. Schematic diagram of simple HPLC system (Image taken from ResearchGate) (*22*)

Prior studies measuring vitamin E in cosmetics and lotions

Relevant to the proposed study, vitamin E has also been measured in cosmetics. Although vitamin E is listed as an ingredient of many skin products, cosmetic products, and foods the actual quantity of vitamin E present may not be disclosed on the label. HPLC can be used to experimentally measure the content of vitamin E in these products. An initial HPLC method was developed by Almeida, et al. using gel-based laboratory formulations simulating cosmetics containing vitamin E (*23*). A 25:75 v/v mixture of methanol: isopropanol was used for the mobile phase with a C-18 reversed-phase HPLC column. Although this method showed promise for the analysis of commercial products, the complexity of creams required some modifications by other researchers. Subsequently, a valid and reliable method was developed by Nada et al.(2010) to analyze **α**-tocopherol and **α**-tocopherol acetate (*24)*. They tested four commercial cosmetic creams as well as two creams that they made in their lab to contain a known quantity of vitamin E (*24*).Of the three extraction solvent systems tested, a pure methanol extraction showed the highest recovery of **α**-tocopherol and **α**-tocopherol acetate compared to isopropyl alcohol and a 10:90 v/v mixture of hexane: methanol. A 97:3 v/v methanol: water mobile phase was found to be superior to alternative acetonitrile-based solutions and other ratios of methanol: water. Nada et al. used reverse-phase HPLC with a Waters Symmetry-ODS C-18 column and **α**-tocopherol and **α**-tocopherol acetate were eluted at 9.7 and 13.2 minutes, respectively (*24*). These compounds were detected using a UV-detector set at 290 nm (vitamin E) and 283 nm (vitamin E acetate). Concentrations (%w/w) of vitamin E acetate in the cosmetic creams ranged from 0.107 to 0.670; no vitamin E in the pure **α**-tocopherol form was detected in these commercially available creams.

Proposed Study

As summarized in the *Journal of Chromatography A* review, researchers have quantified vitamin E in a variety of samples, including urine, blood, plants, and food. Less research has focused on personal care products, though Nada, et al. measured vitamin E in commercial cosmetic creams. In our literature review, we did not find a study that investigated vitamin E in topical lotions. Though the RDA recommends vitamin E allowances, it does not appear as though the amount of vitamin E in skin care products is actually monitored.Many brands that contain vitamin E do not list the concentration or quantity that is present in the product. HPLC will be used to analyze the vitamin E content of various skin lotions and quantify the amount of Vitamin E present. These samples were chosen because of their popularity and affordability. "Up&Up Vitamin E Dietary Supplement Oil" is personally used as a topical oil for getting rid of dark marks and scars.

EXPERIMENTAL

Chemicals & Equipment

"Palmer's *with Vitamin E* Hemp Oil Calming Relief Body Lotion", " Up&Up Vitamin E Dietary Supplement Oil", and "Fruit of the Earth Vitamin E Skin Care Lotion" will be purchased from an online retailer. Alpha-tocopherol and tocopherol acetate will be purchased from Fisher Scientific. HPLC grade methanol (99%) was purchased from ACROS. HPLC analysis will be conducted using a Dionex system comprised of an ASI-100 automated sample injector, P680 HPLC pump, solvent rack SOR-100, and a UVD17OU UV detector. The column is a Hypersil BDS C18. The HPLC will be operated using Chromeleon software. Deionized water (18.5 MΩ) was generated using a Millipore Direct Q-5 system.

Preparation of Standards

Tocopherol and Tocopheryl-acetate will be used as the standards of the experiment. These standards are prepared in the concentration ranges 1, 12, 24, 36, 48, and 50µl of methanol.

Preparation of Samples

Three products containing vitamin E ("Palmer's Hemp Oil Calming Relief Body Lotion", " Up&Up Vitamin E Dietary Supplement Oil", and "Fruit of the Earth Vitamin E Skin Care Cream")will be tested. Samples for HPLC injection will be prepared by extracting with 200µl of methanol and 100µl of each sample. The sample will be stored in ambered glass vials. Oil samples will be diluted in methanol and filtered through a 0.2 µm syringe filter. The lotion and cream samples will be centrifuged to separate the methanol-soluble components, prior to dilution and filtering.

HPLC Parameters

We will adapt the method parameters described by Nada, et al.HPLC will be used to investigate Specifically using Reversed-phase HPLC, we will inject a volume of 50µl of either standard or sample. It

was programmed at a flow rate of 1.5ml/m, a mobile phase of methanol-water (96:4,v/v), a column temperature of 25℃, and a wavelength of 290nm. (Nada, et al.) The standards will be individually run once and the data triplicated in collection for each sample and averaged.

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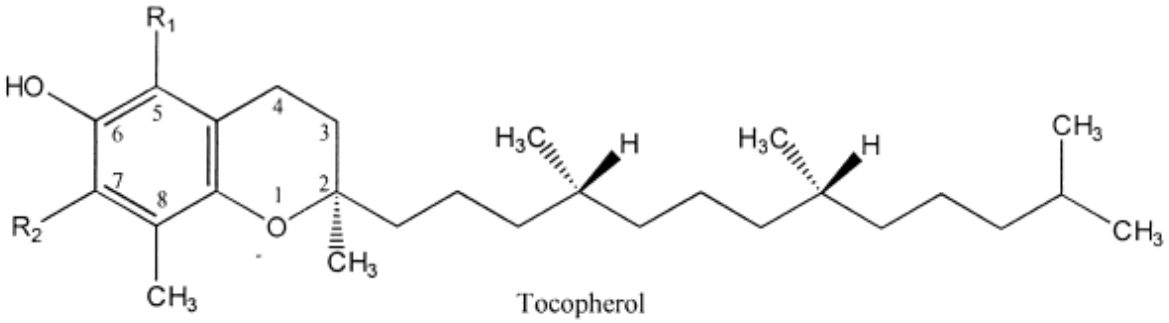
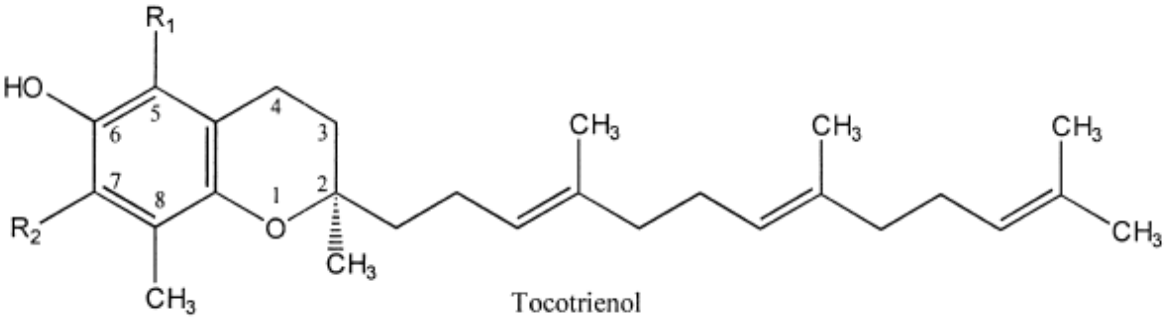
APPENDIX





R_1	R_2	Compound
CH_3	CH_3	α
CH_3	H	β
H	CH_3	γ
H	H	δ

Figure A1. The structure of tocopherol and tocotrienol family compounds.