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Analysis of vitamin E in commercial cosmetic preparations by HPLC

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Synopsis

A specific HPLC method, with an RP–C-18 column and a UV detector, for simultaneous determination of vitamin E (tochopherol, T)/T acetate (TA) in four commercial and two experimental cosmetic products is described. Three solvent systems for extraction of T/TA were assessed: isopropyl alcohol; 10:90 v/v hexane-methanol mixture (method 1); and methanol alone (method 2). The procedure was accurate, as indicated by high recovery (97.8–101.8% and 100.1–102.5% for T and TA, respectively) and precise (RSD was only 0.9–3.26% and 0.73–3.35% for T and TA, respectively). The limits of detection for T and TA were 200 and 300 ng/ml, respectively, while the limits of quantitation were 250 and 400 ng/ml, respectively. The range of reliable quantification was 5–50 μg/ml. Isopropanol as solvent resulted in a turbid extract. Method 1 and method 2 of extraction showed high recovery (98.5–99.9% and 97.2–97.9% for T and TA, respectively). After a few weeks of analysis, method 1 resulted in retention time drift, peak broadening, non-reproducible results, and progressive loss of HPLC-column integrity. Methanol alone (method 2) was equally as efficient as that of the mixture of methanol with 10% hexane (method 1) for extraction. The described analytical procedure proved to be accurate, precise, and suitable for simultaneous determination of T and TA in real commercial cosmetic products.

INTRODUCTION

Cosmetic changes associated with aging, especially in the face, are particularly concerning to the patient/consumer population that wishes to remain looking youthful all the time. Inclusion of botanical extracts such as vitamins and anti-microbials to cosmetics has become an important marketing advantage. These ingredients have the appeal of appearing wholesome and "organic." Although scientific evidence shows that some of these ingredients do have some *in vitro* anti-aging activity, the question remains as to whether it is possible to deliver adequate doses to the skin *in vivo* (1). Vitamin E is one of the best established ingredients in OTC products for skin aging. It is a lipid-soluble antioxidant that plays key roles in protecting cell membranes from lipid peroxidation by free radicals and in reducing photocarcinogesis (2–4). Thiele *et al*. concluded that α-tocopherol is the

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major antioxidant in the human epidermis, and that its depletion is an early and sensitive marker of environmental oxidative damage (5).

Vitamin E is available as the free alcohol or its esters, and the beneficial effects of vitamin E-containing cosmetic products depend on the concentration of added T and/or TA as well as their stability. Several analytical methods have been used to estimate the concentration of T and TA in pharmaceutical/cosmetic products and food supplements (6–8). Ruperes *et al*. reviewed chromatographic analysis of T and related compounds in food, pharmaceuticals, plants, animal tissues, etc., and pointed out the complications of the matrix in the analytical method (9). The authors indicated that sample preparation is a critical step, is time-consuming and expensive, and is the main source of errors in the analytical method. Moreover, it is worthwhile to mention that working with cosmetic products represents additional difficulties due to the presence of many excipients/vehicles, e.g., fats, oils, and waxes, that possess a similar lipophylic nature as the analytes. Such materials are usually present in very high concentrations relative to the vitamins, which further complicates the analytical process. Although numerous methods for determination of T derivatives in pharmaceuticals such as tablets and capsules are described in the literature, no typical analytical methods for determination of T and TA in actual commercial cosmetic products have been published (9). Guaratini *et al*. (8) reported on the stability of experimental cosmetic gel-cream formulations containing vitamin E acetate and vitamin A palmitate. Recently, Almeida *et al*. (10) documented an HPLC method for determination of tocopheyl acetate and ascorbyl tetraisopalmitate in an experimental cosmetic formulation. However, both studies (8,10) applied the proposed analytical methods on lab gel formulation, which in fact does not resemble the complex lipid matrices commonly encountered in commercial cosmetics. In addition, Dingler *et al*. (11) reported on an HPLC method for determination of T in a dermal product; however, the authors applied only cetyl palmitate as the lipid carrier, which does not simulate the actual complex nature of commercial products.

Hence, the present study was carried out to develop a specific, precise, and accurate HPLC method for simultaneous determination of both T and TA in real commercial cosmetic products. The investigation was focused on the development of an extraction procedure to selectively pick up the vitamin/ester from such cosmetics with good extraction efficiency and to leave the commonly associated lipophylic materials. The results of the proposed HPLC method were validated using two experimental cosmetic creams simulating the complex cosmetic bases commonly experienced in real commercial products, each containing about 0.5% of T or TA, as well as four marketed cosmetic cream products containing TA.

EXPERIMENTAL

MATERIALS

The methanol, acetonitrile, hexane, and ethanol used in the study were of HPLC grade (Merck, Darmstadt, Germany). Vitamin E acetate was obtained from BASF, Ludwigshafen, Germany. Vitamin E, soybean oil, and corn oil were procured from Sigma Aldrich Chemie GmbH, Steinheim, Germany. Propylene glycol (Generico Medical Practice, AB Almere, Holland), stearic acid, white soft paraffin, potassium hydroxide (Loba CHEMIE-India), lanoline, glycerol, sorbitol (Gainland Chemical Company, UK), Captex SBE, and Acconon S-35 (ABITEC Corporation, Janesville, USA) were of analytical grade.

METHODS

Preparation of stock and working solutions. Stock solutions of T and TA in methanol (100–500 μg/ml) were prepared in amber glass vials and kept refrigerated. The stock solutions were used to prepare standard solutions of T and TA in methanol, covering a concentration range of 1–50 μg/ml.

HPLC method. The proposed method involved a Waters 2690 HPLC (Waters 2690 Seprations Module, Milford, MA) with a variable wavelength PDA detector, a disposable C-18 guard column, and an RP Waters Symmetry C-18 column (4.6 ratio × 150-mm, 5-μm particle size). The column temperature was maintained at 25°C. A series of drug solutions with a concentration range of 1–25 μg/ml, prepared as described above, was filled into sample tubes and loaded into the auto-injection chamber of the HPLC system. The system was programmed to inject each of the samples (50 μl) at a flow rate of 1.5 ml/min. The eluents were monitored at 290 nm and 283 nm for T and TA, respectively. The peak areas for T and TA were recorded, and analyzed using the Millennium Software Empower from Waters. The peak areas of T and TA were subjected to regression analysis against their concentrations.

Method development. Initial trial experiments were conducted, in a view to select a suitable solvent system for the accurate estimation of the vitamin and to achieve good resolution between T and TA. The suitability of the mobile phase was decided on the basis of the sensitivity of the assay, the suitability for stability studies, the time required for the analysis, ease of preparation, and the use of readily available cost-effective solvents. These included acetonitrile-methanol (95:5 %v/v), acetonitrile (100 %v/v), methanol-water (70:30 %v/v), acetonitrile-water (90:10 %v/v), and methanol-water (97:3 %v/v). A mobile phase system comprising of methanol-water (97:3 %v/v) was found to be optimum, based on retention time, peak symmetry, and resolution.

Method validation

(a) Linearity. A series of standard curves was prepared over a concentration range of 5–50 μg/ml from a stock solution (500 μg/ml) in methanol. Dilutions were prepared in methanol too. The data of peak area versus drug concentration were treated by linear least-square regression analysis. The standard curves were evaluated for intraday and interday reproducibility.

(b) Precision. The three components of precision, i.e., repeatability, intermediate precision, and reproducibility, in accordance with ICH recommendations (11), were determined as follows:

- *Repeatability.* Five injections containing a mixture of T and TA (10 μg/ml each) were analyzed and %RSD was calculated for injection repeatability.
- *Intraday variation.* Measurement of intraday variation of T and TA at three different concentrations (5, 10, and 25 μg/ml) was done by injecting the samples on the same day at different time intervals.
- *Intermediate precision (interday variation).* Measurement of interday variation of T and TA mix solutions at three different concentrations (5, 10, and 25 μg/ml) on five consecutive days determined the intermediate precision.

(c) Accuracy. Accuracy is the measure of how close the experimental value is to the true value. Recovery studies by the standard addition method were performed in a

view to justify the accuracy of the proposed method. A standard solution of the mixture containing both ester and base (10 μg/ml each) were spiked with 50, 100, and 200% extra standard and the mixtures were analyzed by the proposed method. The experiment was performed in triplicate. Recovery values (%) were calculated for each concentration.

(d) Detection (LOD) and quantification (LOQ) limits. In order to estimate the limit of detection and limit of quantification, the blank sample was injected six times and the peak area of this blank was calculated (the noise level was determined). The limit of detection was calculated to be three times the noise level, and ten times the noise value gave the limit of quantification (250 and 400 ng/ml for T and TA, respectively).

(e) Sample solution stability. The stability of T/TA in solution during analysis was determined by repeated analysis of the samples during the course of experimentation on the same day and also after storage of the drug solution for 72 h under laboratory bench conditions (25° ± 1°C) and refrigeration (8° ± 0.5°C). An accurately weighed quantity of vitamin E base and ester was dissolved in methanol to get a final concentration of 10 μg/ml. The solution was subjected to HPLC analysis immediately and after periods of 24, 48, and 72 hr.

(f) System suitability tests. The chromatographic systems used for analyses must pass the system suitability limits before sample analysis can commence. The capacity factor (K), injection repeatability (as mentioned under Precision), the tailing factor (t), the theoretical plate number (N) and the resolution (Rs) for the principal peak were tested on a 10-μg/ml sample of the T and TA mixture to assess the accuracy and precision of the developed HPLC system.

(g) Analysis of vitamin E in lab and marketed cosmetic creams. Two creams (formulated in the lab) each containing about 0.5% T or TA, as well as four commercial creams, viz., A, B, C, and D containing only TA, were analyzed after being extracted by the following method: Five hundred milligrams of each of the preparations was weighed out, and a small amount of methanol was added for extraction by sonication (J.P. Selecta s.a, Barcelona, Spain) for 15 min in order to dissolve the vitamin E base and ester. The mixture was transferred to a 50-ml standard flask and the volume was made up with methanol. The solution was centrifuged (Sorvall t-c-6, Newtown, CT) for 10 min at 3700 rpm and was filtered through a 0.45-μm filter and analyzed by HPLC.

(h) Preparation of vitamin E-containing laboratory products. Two cream–emulsion cosmetic formulations were prepared according to the method reported by Zaghloul *et al.* (12) to simulate the complex composition of commercial cosmetic preparations, each containing about 0.5% T/TA (Table I).

The cream was prepared by melting stearic acid in a porcelain dish over a water bath (75–80°C), then the semisolid ingredients (lanoline, white soft paraffin, and Captex SBE) were added until all the mixture was melted, and finally soybean oil and corn oil were added (oily phase). Potassium hydroxide was dissolved in water followed by Acconon S-35, and then glycerin and sorbitol were added and the mixture was heated to 75°–80°C (aqueous phase). The aqueous phase was added to the oily phase with trituration, and the mixture was then removed from the water bath and mixing was continued until a homogenous creamy liquid was obtained. Accurately weighed amounts of T and TA were added to the mixture, avoiding addition of the vitamin to the cream while hot to avoid possible

Table I
General Formula of Laboratory Formulations Containing 0.5% (w/w) of Vitamin E or Vitamin E Acetate

Ingredient	Percent (w/w)
Stearic acid	6
White soft paraffin	4
Lanoline	4
Soybean oil	8.4
Corn oil	8.3
Captex SBE (caprylic/capric/ stearic triglyceride)	4
Glycerol	11
Sorbitol	15
Acconon S-35 (PEG-35 soy glycerides)	5
Potassium hydroxide	0.28
Water	34.02

degradation. This resulted in nominal concentrations of 0.532% and 0.539% w/w, respectively. The final cream was filled in well-closed plastic jars.

(i) Commercial cosmetic products. Four commercial products (A, B, C, and D), obtained from retail pharmacies, were used to validate the proposed analytical HPLC method and the extraction method. The products contained only TA without declaration of its quantity.

The commercial and cosmetic preparations were tested for emulsion type by the dilution method, performed by mixing about 1-g samples of each with about 5 ml of distilled water and observing them for phase separation. Both experimental formulations and products A, B, and C were O/W emulsions, while product D was a W/O emulsion.

(j) Method of extraction of vitamin E/acetate from cosmetic products. Initially, isopropyl alcohol was utilized as the solvent for extraction of T/TA with reference to the method reported by Guaratini *et al*. (8). An approximately 500-mg sample of the formulation was taken in a 50-ml volumetric flask containing 20 ml of isopropyl alcohol. The sample was extracted by sonication for 20 min and centrifuged at 3700 rpm for 10 min after making up the volume to 50 ml with isopropyl alcohol. The supernatant samples were filtered through a 0.45-μm cellulose filter and were intended to be used for injection by the reported HPLC method.

Further trials were run using n-hexane-methanol (1:9 v/v) as the extraction solvent (method 1) or 100% methanol (method 2). In each method, a 500-mg sample of the formulation was extracted similarly as that described above under the extraction method by isopropanol. The supernatant of the extracts was filtered through a 0.45-μm cellulose filter and was found to be clear; it was therefore used for injection. The extraction methods differ basically in the polarity of the extraction solvent system. The relative extraction efficiency of both methods was assessed in terms of the percent recovery before and after addition of a known quantity (2.5 μg) of T and TA to 500-mg samples of the products.

(k) Statistical analysis. The difference in the mean percent recovery of T and TA from the cosmetic products with the two methods of extraction was tested for statistical significance using a *t*-test and the non-parametric Mann-Whitney test.

RESULTS AND DISCUSSION

METHOD DEVELOPMENT

Methanol-water (97:3 % v/v) was selected as the optimum mobile phase. Under these conditions the retention time of the vitamin E base and ester were 9.870 ± 0.8 min and 13.240 ± 0.8 min, respectively (Figure 1). The tailing factors of T and TA were 0.93 and 1.0, respectively.

METHOD VALIDATION

Linearity. The peak area versus drug concentration was plotted to construct a standard curve of vitamin E base and ester (Figure 2). The polynomial regression for the calibration plots showed a good linear relationship with a coefficient of correlation of r = 0.9995. The range of reliable quantification was set at 5–50 μg/ml, as no significant difference was observed in the slopes of the standard curves in this range. The linear regression data for the calibration plot is indicative of a good linear relationship between the peak area and the concentration over a wide range. The correlation coefficient was indicative of high significance.

Precision. Precision was measured in accordance with ICH recommendations (13). Five consecutive injections of a 10-μg/ml solution of T and TA by the proposed method showed excellent injection repeatability, with RSDs of only 0.66% and 0.56%, respectively. The repeatability of the sample injection was determined as intraday variation, whereas intermediate precision was determined by measuring interday variation for three different concentrations. Results from the determination of repeatability, intermediate precision, and reproducibility are listed in Table II. The low RSD values indicate the repeatability and reproducibility of the method.

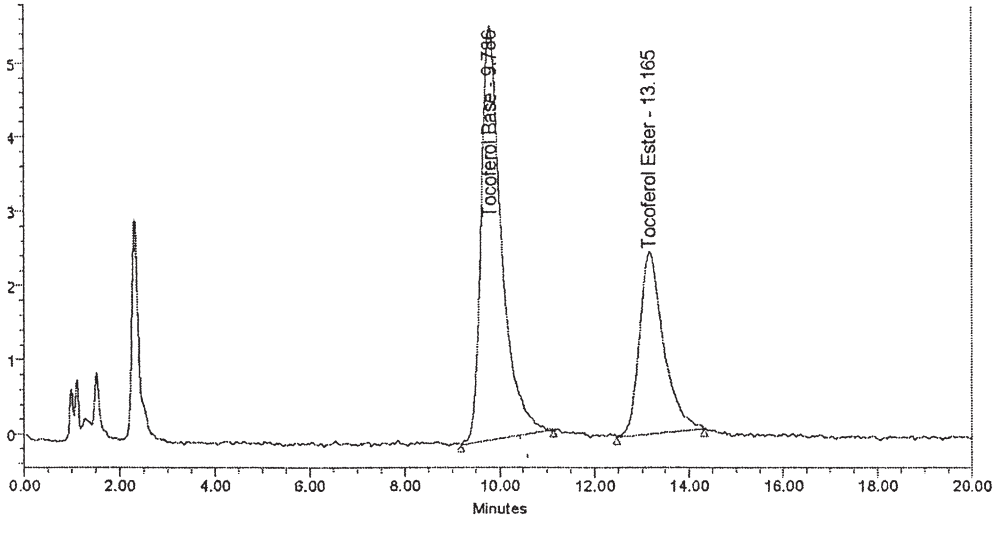

Figure 1. Sample chromatogram showing tocopherol and tocopherol acetate peaks after 9.8 and 13.2 minutes, respectively. RP Waters Symmetry C-18 column; mobile phase: methanol-water, 97:3 %v/v; UV detection at 290 nm and 283 nm for T and TA, respectively.

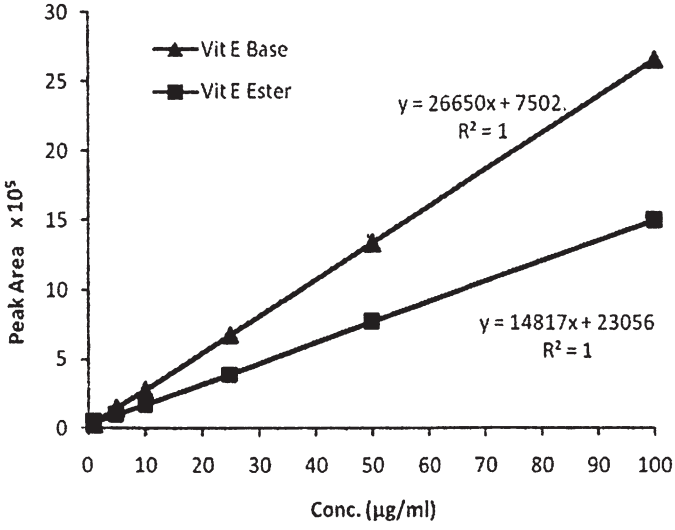

Figure 2. Calibration curve of vitamin E base (tocopherol) and ester (tocopherol acetate); RP Waters Symmetry C-18 column; mobile phase: methanol-water, 97:3 %v/v; UV detection at 290 nm and 283 nm for T and TA, respectively.

Table II
Intra- and Interday Precision Data of the Analytical Method for Tocopherol and Tocopherol Acetate Based on Prepared and Found Concentrations (mean ± %RSD) (n=5)

	Tocopherol				Tocopherol acetate			
	Intraday analysis		Interday analysis		Intraday analysis		Interday analysis	
Prepared concentration (µg/ml)	Found conc. (µg/ml), mean ± SD	RSD	Found conc. (µg/ml), mean ± SD	RSD	Found conc. (µg/ml), mean ± SD	RSD	Found conc. (µg/ml), mean ± SD	RSD
5	4.61 ± 0.07	1.54	5.18 ± 0.08	1.60	5.12 ± 0.14	2.77	5.12 ± 0.11	2.22
10	9.26 ± 0.24	2.61	10.16 ± 0.09	0.90	10.05 ± 0.07	0.73	10.75 ± 0.35	3.24
25	23.7 ± 0.39	1.64	26.16 ± 0.85	3.26	25.00 ± 0.26	1.05	25.62 ± 0.86	3.35

Accuracy, as recovery. The recovery of the method, determined by spiking a previously analyzed test solution with additional drug standard solution, was found to be in the range of 97.8–101.8% for T and 100.1–102.5% for TA. The high recovery (%) and low RSD (%) values presented in Table III indicate that the method is accurate.

Detection (LOD) and quantification (LOQ) limits. The limits of detection of T and TA were found to be 200 and 300 ng/ml respectively, where the drug could be detected without any noise. The limits of quantification of T and TA were 250 and 400 ng/ml, respectively. This indicates that the method can be used for detection and quantification of the vitamin E base and ester over a very wide range of concentrations.

Stability of vitamin solution. There was no significant change in analyte composition (sample concentration = 10 µg/ml) until the end of a period of 72 hr. The mean RSD between peak areas for the samples stored under refrigeration (8° ± 1°C) was found to be 0.90%

Table III
Intraday Accuracy of Tocopherol and Tocopherol Acetate (mean ± %,RSD) (n = 5)

			Tocopherol		Tocopherol acetate	
Standard conc. (μg/ml)	Excess added (μg/ml)	Theoretical content (μg/ml)	Conc. obtained (μg/ml) ±RSD	Recovery (%)	Conc. obtained (μg/ml) ±RSD	Recovery (%)
10	5	15	14.68 ± 1.8	97.8	15.3 ± 0.5	102.5
10	10	20	19.54 ± 0.5	97.7	20.3 ± 0.8	101.6
10	20	25	25.49 ± 1.4	101.8	25.04 ± 0.8	100.17

and 3.24% for T and TA, respectively. At laboratory temperature (25° ± 1°C), the corresponding values were 1.7% and 1.29% for T and TA, respectively, suggesting that the drug solution can be stored without any degradation for the time interval studied.

System suitability tests. The results of the system suitability tests mentioned in Table IV, ensure the adequacy of the proposed HPLC method for routine analysis of TA and T. The precision test and the tailing factor studies show good injection repeatability and peak symmetry, respectively. The values of capacity factor for ester and base ($k \geq 2$) indicate that the peaks are well resolved with respect to the void volume. Also, the theoretical plate numbers ($N \geq 2000$) reflect good column efficiency. The resolution value confirms good separation of the two forms of vitamin E.

Analysis of vitamin E/acetate in lab and marketed creams. A single sharp peak of T/TA was observed at its respective retention time when a suitably diluted solution of the cream was injected following extraction. No interaction was observed between T/TA and the excipients present in the formulae. The vitamin content determined with RSD values less than 5 % (Table V) indicates the suitability of this method for routine analysis of T and TA (individually and in combination) in pharmaceutical and cosmetic preparations.

Table IV
HPLC System Suitability Tests for Tocopherol (T) and Tocopherol Acetate (TA)

Test	Tocopherol (T)	Tocopherol acetate (TA)
Precision (injection repeatability)	0.66	0.56
Tailing factor (asymmetry)	0.93	1
Capacity factor (k)	2.72	4.0
Theoretical plate count (N)	2151.68	2765.7
Resolution	4.47 (between T and TA)	

Table V
Concentration of Vitamin E (base) and Vitamin E Acetate (ester) in the Laboratory and Commercial Cosmetic Products

Product	Ester/base	Concentration (%w/w) ± SD	%RSD
Lab preparation 1	Base	0.57 ± 0.02	3.54
Lab preparation 2	Ester	0.66 ± 0.03	1.54
Commercial cream A	Ester	0.107 ± 0.001	0.78
Commercial cream B	Ester	0.670 ± 0.019	2.83
Commercial cream C	Ester	0.591 ± 0.084	14.25
Commercial cream D	Ester	0.579 ± 0.034	5.80

Comparison of extraction methods of vitamin E/acetate. Unlike oils, which can be diluted and injected directly into the HPLC system, vitamin E must be extracted from the sample matrix (9,14). It was observed during the extraction procedure with isopropanol that the marketed creams selected in this study showed inevitable precipitation and gave turbid samples even after filtration. Therefore, the use of isopropyl alcohol as the extraction solvent was discontinued. In addition, it is worthy to mention here that the formulations studied by Guaratini *et al*. (8), who applied ispropanol as extraction solvent, were simple lab gel creams. In contrast, commercial products in general, like those tested in the present study, are highly hydrophobic fatty/waxy matrix-based formulations. It was therefore considered of interest to develop a simple, fast, reproducible, and economical extraction method for these kinds of hydrophobic preparations, which are very widely available in the market. The objective was also to have a clear final sample for injection into the HPLC system as well as to have good extraction efficiency. Therefore, another extraction solvent system was tried consisting of a hexane-methanol mixture (1:9) (method 1), which was applied previously by Wielinski and Olszanowski (7) for simultaneous determination of fat-soluble vitamins in capsules. Initially, a C-18 column (Agilent; Hypersil-ODS; 125 × 4-mm; 5-µm particle size) was used for HPLC analysis by the proposed HPLC method. The chromatograms obtained for the standards and samples showed sharp peaks of T at 3.5 min and of TA at 4.75 min (Figure 3). However, it was observed that after a period of approximately four weeks of continuous analysis, the samples injected after extraction with hexane-methanol frequently blocked the column, as evident by high pressure beyond 4000 psi. Also, the retention time started drifting from the originally observed values of 3.5 min (T) and 4.75 min (TA) to 7 min (T) and 9 min (TA) (Figure 4), and it was never reproducible. There was peak broadening, and integration and quantification became difficult, resulting in irreproducible results. It was assumed that the use of hexane in the extraction solvent would have dissolved some proportions of the hydrophobic waxy

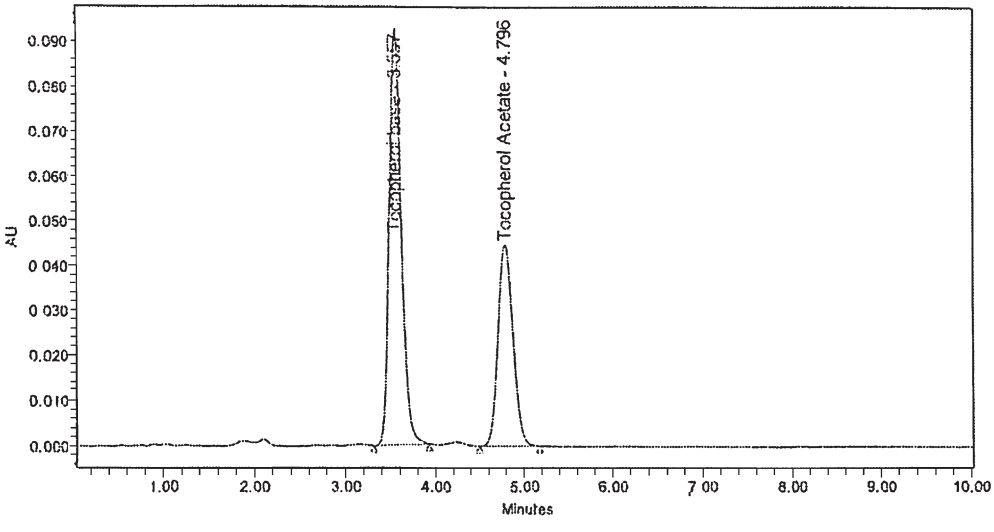

Figure 3. Initial sample chromatogram showing tocopherol and tocopherol acetate peaks after 3.5 and 4.8 minutes, respectively. Agilent RP–C-18 column; mobile phase: methanol-water, 97:3 %v/v; UV detection at 290 nm and 283 nm for T and TA, respectively.

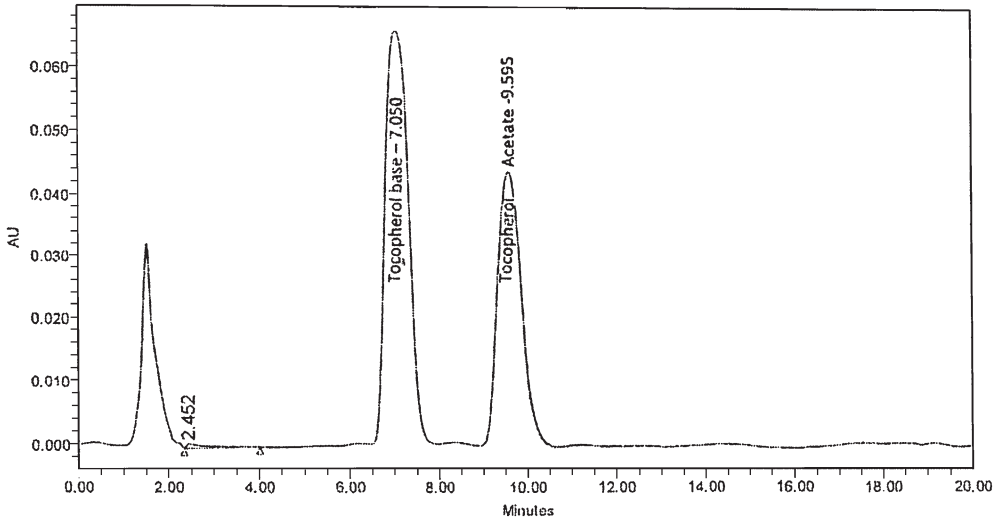

Figure 4. Late sample chromatogram, four weeks after starting analysis, showing tocopherol and tocopherol acetate peaks after 7.0 and 9.5 minutes, respectively. Agilent RP–C-18 column; mobile phase: methanol-water, 97:3 %v/v; UV detection at 290 nm and 283 nm for T and TA, respectively.

excipients present in the commercial creams, which were not eluted out, eventually causing irreparable damage to the column.

Hence, based on further literature survey, methanol was tried as the extraction solvent (method 2), which was previously applied by Huo *et al*. for extraction of tocopheryl acetate and tocopherols from aquatic organisms and fish feed (6). A new column (Waters; Symmetry-ODS, 150 × 4-mm; 5-μm particle size) was used, and based on the previous experience, it was considered necessary to introduce a guard column to prevent any possible damage to the column and to increase its longevity. Sharp reproducible peaks were obtained at 9.7 and 13.2 min. for T and TA, respectively (Figure 1). Therefore, all validation studies and analyses of lab and commercial products were carried out using the same extraction procedure (method 2) and using the new Waters column. No antioxidant was added, since the extraction procedure does not include the saponification process (9).

Extraction efficiency with laboratory formulations. The adopted methods of extraction proved to be efficient for T and TA when laboratory formulations were subjected to HPLC analysis. This was indicated by high mean recovery values (Table VI). High extraction was achieved with mean values of 99.87 (method 1) and 97.24% (method 2). The corresponding mean values determined for TA were 98.54 (method 1) and 97.89 (method 2). This indicates that either methanol alone (method 2) or with 10% of hexane could selectively extract T and TA from the laboratory products without an interference from the additives.

Extraction efficiency with the commercial product (C). The commercial product contained only TA, while no mention of T was indicated under the ingredients. Therefore, the relative efficiency of both methods of extraction was based on comparison of the found TA concentration. The observed concentrations of TA were 0.586% and 0.562% with method 1 and method 2, respectively, with relative standard deviations less than 4.79% (n=4). This indicates that both methods are quite equivalent and that none of the additives in the commercial cosmetic product interfered with the estimation of TA. There was no significant

Table VI
Mean Extraction Efficiency of Tocopherol (T) and Tocopherol Acetate (TA) From Experimental Cream Formulations Based on Nominal and Found Concentrations (mean ± %RSD) (n=4)

	Method 1			Method 2		
Vitamin	Nominal conc. (%)	Found conc. (%)	Recovery (%)	Nominal conc. (%)	Found conc. (%)	Recovery (%)
T	0.5319	0.5312 (4.71)	99.87	0.5319	0.5172 (4.84)	97.24
TA	0.5394	0.5315 (1.803)	98.54	0.5394	0.5282 (0.809)	97.89

statistical difference between the two investigated methods of extraction as indicated by *t*-test (t=0.215 and p=0.834) and the non-parametric Mann-Whitney test (p=0.814). Furthermore, to further assess the extraction efficiency of method 2, known quantities (2.5 μg) of T and TA were added to a 500-mg sample of the commercial cosmetic product and the percent recovery of T/TA was estimated. The percent recoveries of T and TA with extraction method 2 were 99.05% and 96.01%, respectively (Table VII). Since both methods were of similar extraction efficiency, only method 2 was applied throughout the present study.

The negative effect of hexane may be avoided by evaporating the solvent mixture and reconstituting with methanol. However, method 2 remains simpler, needs less processing time, and maintains the column's integrity, ultimately with equal extraction efficiency.

In summary, the advantages of the present method in comparison with the previously described method by Guaratini *et al*. (8) include the following: (a) there is simultaneous determination of both T and TA, which was achieved by modifying the mobile phase (methanol-water, 97:3 %v/v) instead of pure methanol, (b) the previous method was tested in a gel–cream base that does not resemble the complex nature of commercial products, containing diverse oily, fatty, and waxy ingredients, (c) the use of isopropanol as extraction solvent in the reported method (8) was unsuccessful in extraction of T and TA from the preparations tested in the present study, and (d) other reported methods for T and TA analysis in dosage forms like tablets, capsules, injctions, etc. (9) were unsuccessful due to the different nature of the matrices compared with cosmetic products.

Table VII
Extraction Efficiency by Methanol (method 2) of Commercial Product (C) after Addition of Known Amount of Tocopherol (T) and Tocopherol Acetate (TA)

Tocopherol			Tocopherol acetate		
Nominal conc. (μg/ml)	Found conc., mean ± S.D (% CV), n = 3	Extraction efficiency (%)	Nominal conc. (μg/ml)	Found conc., mean ± S.D (% CV), n = 3	Extraction efficiency (%)
10	9.905 ± 0.054 (0.55)	99.05	20	19.201 ± 0.44 (2.34)	96.01

CONCLUSIONS

1. The described analytical procedure proved to be accurate, precise, and suitable for simultaneous determination of T and TA in real commercial cosmetic products.
2. Both tested methods of extraction proved to be accurate and precise and statistically indifferent for the extraction of T and TA in cosmetic preparations.
3. Methanol alone (method 2) is equally as efficient as a mixture of methanol with 10% hexane (method 1) in terms of extraction efficiency.
4. Method 2 is simpler and less time-consuming.
5. Method 2 maintains column integrity and thus is more suitable for routine determination of T and TA in cosmetic preparations.
6. The use of highly non-polar solvents, e.g., hexane, should be avoided unless the extract is evaporated, reconstituted with methanol, and centrifuged before injection into the HPLC column.

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