Iva Matijević,
Tanja Pušić*,
Sandra Bischof

Isolation of α-tocopherol from Cotton Cosmetotextiles

DOI: 10.5604/01.3001.0013.1818

University of Zagreb,
Faculty of Textile Technology,
Department of Textile Chemistry and Ecology,
Zagreb, Croatia
* e-mail: tpusic@ttf.hr

Abstract
Cotton fabrics were treated with cosmetic substances based on α-tocopherol and cyclodextrine. Isolation of α-tocopherol from cotton cosmetotextiles was performed using three different techniques: stirring, Soxhlet and microwave extraction. High performance liquid chromatography (HPLC) was optimised and applied for the quantification of α-tocopherols in the isolates. The results revealed that all techniques are applicable for the isolation of α-tocopherol from cotton cosmetotextiles. The HPLC method proved to be the most convenient for the quantification of α-tocopherol from cotton fabrics.

Key words: *cosmetotextiles, cotton, HPLC, isolation, α-tocopherol.*

Introduction

Cosmetotextiles are special materials where textile structures are the carriers of cosmetic substances or their mixtures. Cosmetic substances can be released in a specific time period to different surface areas of the human body, particularly to human skin. The purpose of applied substances can be skin cleaning, changing the appearance of the skin, correcting bodily scents or keeping skin in good health in general [1, 2].

It is necessary to ensure that cosmetic ingredients possess the ability to be released from the textile to the skin. Preparations that are not released to the skin are not considered cosmetic products, nor are the textiles with applied but not released active substances, classified as cosmetotextiles [1, 3, 4]. The cosmetic ingredient used in this research was α-tocopherol as a component carrying vitamin E, whose purpose is to improve skin moisture and elasticity, so as to reduce skin wrinkle and roughness. The slow release of α-tocopherol applied to textiles results in anti-ageing properties [1, 5, 6]. The structure and composition of α-tocopherol is presented in ***Figure 1***.

Qualitative analysis of α-tocopherol on cosmetotextiles can be done by specific reactions with iron and dipyridil. The reaction mechanism is presented in ***Figure 2***. The analysis is based on a redox reaction between α-tocopherol and iron (III)-chloride (solution A) where iron is reduced to iron (II) and α-tocopherol is oxidised to tocoquinone, shown in ***Figure 2.a***. After addition of the dipyridyl solution (solution B), the iron (II) ions form a red coloured metal organic chelate complex with dipyridyl, seen in ***Figure 2.b*** [7-10].

HPLC (*High Performance Liquid Chromatography*) is a key instrument to quantify α-tocopherol in different materials. European standard "Foodstuffs – Determination of vitamin E by high performance liquid chromatography – Measurement of α-, ß-, γ- and δ-tocopherol" (EN 12822:2014) was followed for the quantification of α-tocopherol from cosmetotextiles in this research [11, 12].

Sample preparation is the most time-consuming step in analysis, and also the main source of error, hence prior to performing the HPLC analysis, it is highly important to establish a procedure for α-tocopherol isolation from the cosmetotextiles. To reduce the efforts needed and avoid tocol losses, sample workup should be as simple as possible. Since tocols are lipid-soluble compounds they are readily soluble in organic solvents. Therefore, solvent extraction has been the most commonly used method to extract tocols from oil seeds, biological fluids and animal tissues. There are no universal extraction solvents that would yield optimal results in all materials. Instead, extraction parameters should be optimised for each purpose [13]. The aim of this research was to determine the effectiveness of three techniques of α-tocopherol isolation from cotton fabrics: stirring, Soxhlet and microwave extractions. Isolated α-tocopherol was quantified with HPLC, and a review of advantages and limitations of each method is given.

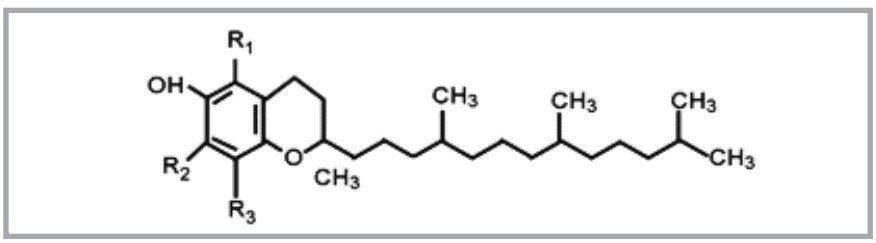

Figure 1. *Structure and composition of α -tocopherol (R_1=CH_3, R_2= CH_3, R_3= CH_3).*

Experimental

Materials and methods

Cotton fabric in plain weave, produced by Čateks, Croatia, with a surface mass of 175.62 g/m^2 and yarn density in the warp and weft directions of 25/25 yarns/cm was selected as textile material. Cyclodextrine with α-tocopherol complex was provided by Bezema-CHT [14]. Washing fastness of the cosmeto effect was tested with standard detergent ECE A at a concentration of 2.5 g/l through 5 cycles at 40 °C Linitest apparatus, Original Hanau, Germany. The bath ratio was 1:20 and the sample dimension 6x6 cm.

HPLC was chosen as the method for the quantification of α-tocopherol. The advantage of HPLC application is operating at ambient temperatures, thus there is relatively low risk for sensitive functional groups [11]. This is very important due to the fact that α-tocopherol is sensitive to light and high temperature [15]. Since tocols are easily oxidized, it is important to avoid conditions that would promote oxidation during sample preparation or storage. It is recommended to analyse fresh samples after grinding and homog-

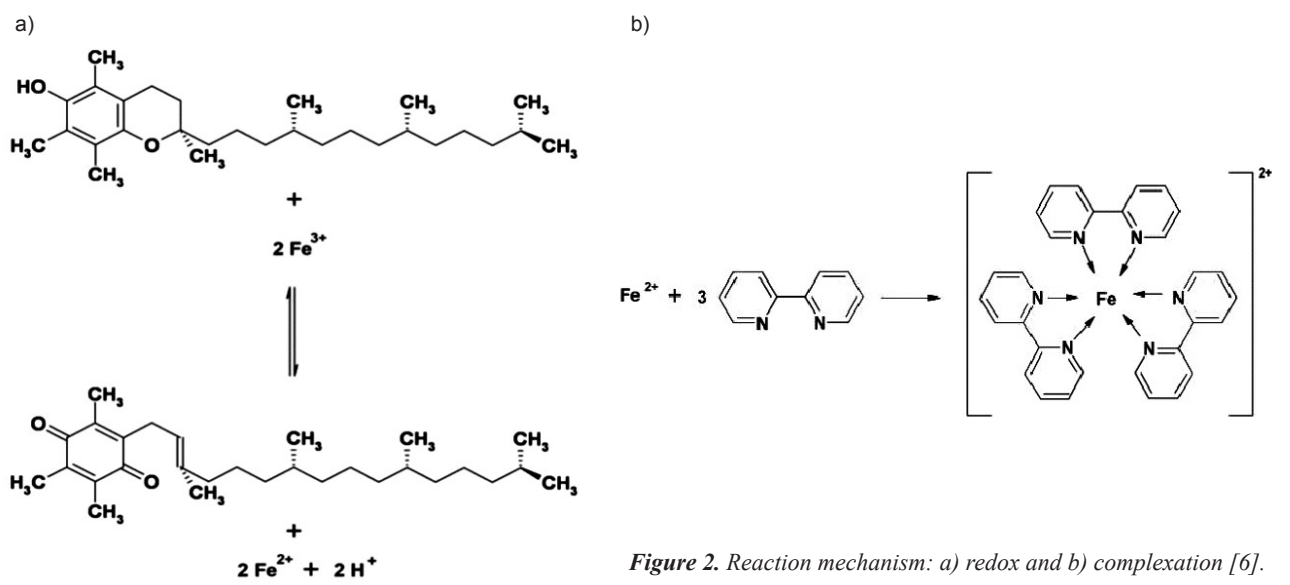

Figure 2. *Reaction mechanism: a) redox and b) complexation [6].*

enisation,store them at low temperatures in the dark, and work under subdued light [13]. Tocopherols absorb UV light at λ = 284-300 nm [11, 13, 16]. HPLC is used in various fields, including textile analysis. Some of the HPLC applications include the quantification of textile dyes, textile waste waters, cosmetotextiles, surfactants, antioxidants, and cosmetics [17-20].

An Agilent chromatographic system, series 1220 Infinity LC (USA) with software "The Agilent Open LAB CDS ChemStation Edition" and with a selection of UV detectors was used. A column packed with 4 μm Poroshell 120, EC-C18 (4.6 x 250 mm) in a reverse phase was applied. α-tocopherol was analysed by HPLC, following the standard EN 12822:2014, which is intended for tocopherols in foodstuffs [12]. Response (peak area) was expressed in mili absorption units per minute (mAU*s). Due to specifiers of HPLC and conditions that could impact the results, some parameters (injection volume, detection wavelength and time of analysis) were tested on standard solution of α-tocopherol. The reference of α-tocopherol (Merck 613420 DL-α-tocopherol-CAS 10191-41-0 Calbiochem, purity ≥98%) was a standard substance diluted in methanol, HPLC grade, obtained from Lachner. Stock solution prepared by the dissolution of 30 mg of the α-tocopherol in 100 ml of methanol was stored at 5 °C and protected from light. Concentration and purity tests of the solution prepared were checked by UV-ViS Spectrophotometer, Carry 50 at 290 nm according to the European standard (EN 12822:2014) [12]. After the checkpoint of the solution, a HPLC calibration curve of α-tocopherol was made. The temperature, flow and ratio of mobile phases were constant in all the tests performed: temperature 35 °C ± 0.8°C, flow of 1.8 ml/min and mobile phases ratio: v/v = 97/3 ($MeOH/H_2O$).

Different volumes of the stock solution (0.8, 1.7, 2.5, 4.2, 5.0, 5.8, 6.7 and 7.5 ml) were pipetted into a 10 ml volumetric flask and diluted to the mark with methanol. The concentration of α-tocopherol in the standard solutions prepared varied from 24 to 225 μl/ml.

Three techniques of α-tocopherol isolation from cotton fabrics were applied: stirring (ST), Soxhlet extraction (SE) and microwave extraction (MWe). Methanol was chosen as a solvent for all isolation techniques as the most convenient, since it was also applied as a mobile phase in the HPLC analysis. All tests were done in triplicate. Cotton samples treated with α-tocopherol were cut int pieces of aprox. 10 mm^2 size, mass of 0.6000 g, and put in separate bottles with 10 ml of methanol. After stirring (speed 7 of Shaker Heidolph Unimax 1010 + Incubator 1000, Germany) for 1, 15 and 60 minutes (ST-1, ST-15, ST-60), aliquots (1.5 ml) were taken from each bottle and later analysed employing HPLC. The same procedure was applied 5 times on washed samples (ST-1-5x, ST-15-5x, ST-60-5x). The second technique of α-tocopherol isolation was Soxhlet extraction (SE). The cotton samples' mass was 0.6000 g, cut into pieces of 10 mm^2 and extracted in Soxhlet with methanol for 90 minutes (ISO/TR 5090:1977). Thevolume of MeOH was 120-140 ml – depending on the apparatus. The same procedure was applied to the samples after 5 washing cycles (SE-5x).

The last technique of α-tocopherol isolation applied was microwave extraction (MWe) using a Microwave Platform System – Multiwave 3000, Anton Paar GmbH. It was performed varying two parameters: the form and mass of samples. Selected masses included a = 0.5000 g and b = 0.0600 g of cotton fabric, which arecorrelated with the application manual for microwave extraction, which recommends a sample of maximum mass of 0.5 grams [21]. A previous investigation proved that extraction yielded from finely ground dry materials could improve the quality of isolation [13] and because of that, one group of samples were fibrils (MWeF) while the other was processed as the original sample – unit form (MWeU). Microwave extraction lasted for 5 min (plus 20 min of cooling the system) at 120 °C in all the analyses, and the volume of methanol was 10 ml. This procedure was also applied for washed samples (MWeF-a-5x, MWeF-b-5x, MWeU-a-5x and MWeU-b-5x).

HPLC was chosen as an indirect method for the quantification of α-tocopherol isolated from treated cotton fabrics. All the solutions (isolates) were filtered through a polytetrafluoroethylene (PTFE) filter (average pore size = 0.45 m) before injection into the HPLC system. It was important to find out proper conditions for this application, hence the optimisa-

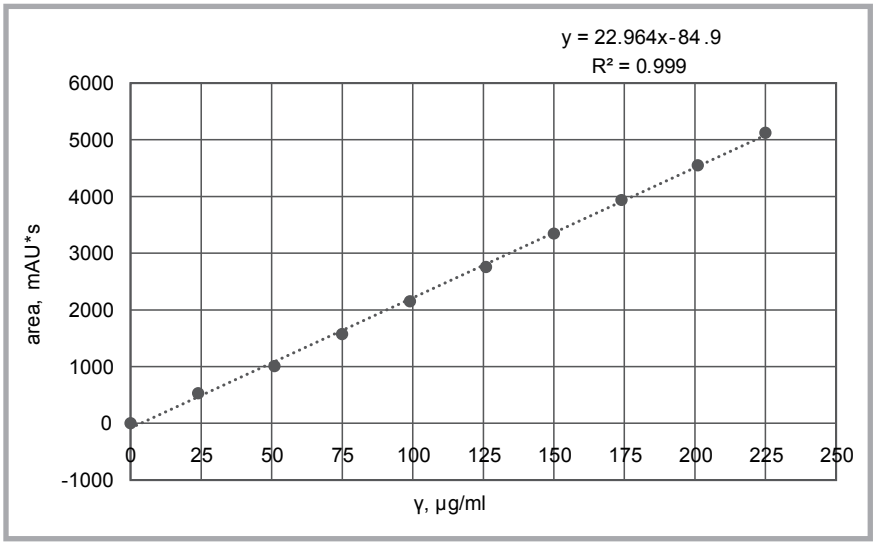

Figure 3. *Calibration curve of α-tocopherol.*

Table 1. *Parameters for HPLC analysis of α-tocopherol in solutions.*

Protocol	Injection volume, µl	Detection wavelength, nm	Time, min	Response, mAU*s	Retention time, min
1	1.8	284	20	20.7	10.16
2	20.0	284	20	261.5	10.30
3	20.0	290	20	297.6	9.86
4	20.0	292	15	300.7	9.92
5	100.0	292	15	2120.0	9.97

Table 2. *Concentrations of α-tocopherol isolated by stirring from cotton fabrics (before and after five washing cycles).*

Sample	Stirring time, min	m_{sample}, g	Response, mAU*s	Retention time, min	γ, µg/ml
ST-1	1	0.6000	3695	9.698	157.2069
ST-15	**15**	**0.6000**	**4102**	**9.681**	**191.1033**
ST-60	60	0.6000	4075	9.955	173.7546
ST-1-5x	1	0.6000	216	8.990	5.7128
ST-15-5x	**15**	**0.6000**	**534**	**9.107**	**19.5540**
ST-60-5x	60	0.6000	510	9.002	18.5061

Table 3. *Concentrations of α-tocopherol after isolation by Soxhlet (before and after 5 washing cycles).*

Sample	m_{sample}, g	Response, mAU*s	Retention time, min	γ, µg/ml
SE	0.6000	4662	9.925	199.3163
SE-5x	0.6000	720	9.642	27.6563

Table 4. *Concentrations of α-tocopherol after isolation from cotton fabrics in fibril (MWeF) and unit form (MWeU) in two different masses (a = 0.5000 g; b = 0.0600 g), before and after 5 washing cycles by microwave extraction.* ***Footnotes:*** *[1] peak is not visible.*

Sample name	Sample form	m_{sample}, g	Response, mAU*s	Retention time, min	γ, µg/ml
MWeF-a	Fibrils	0.5000	3407	9.611	144.6656
MWeF-a-5x	Fibrils	0.5000	907	9.623	35.7995
MWeU-a	Unit	0.5000	3717	9.595	158.1650
MWeU-a-5x	Unit	0.5000	935	9.608	37.0188
MWeF-b	Fibrils	0.0600	389	9.581	13.2425
MWeF-b-5x	Fibrils	0.0600	–[1)]	–	–
MWeU-b	Unit	0.0600	372	9.495	12.5022
MWeU-b-5x	Unit	0.0600	210	9.554	5.4477

tion of HPLC parameters was performed through variation of the injection volume, detection wavelength and time of analyses.

Results and discussion

The UV-ViS method was used for analysis of the stock solution purity. The measurement showed an absorbance peak of α-tocopherol at a wavelength of 292 nm, which was in accordance with the standard protocol.

The next step of the analysis was the optimisation of HPLC parameters through five protocols. It was important to find out proper conditions for this application through variation of the injection volume, detection wavelength and time. Preliminary results of the analysis are presented in ***Table 1***. It can be noticed that the variation in the injection volume was proportional to the response. The results confirmed that peak responses for α-tocopherol were recorded in the interval 284-292 nm. Protocol 5 was selected as the most suitable for further analysis of isolated α-tocopherol from cotton samples. Parameters were the following: injection volume of 100 µl, retention time of 9.97 min and detection wavelength at 292 nm.

Therefore, the injection volume of 100 l was equal for all the samples in the analysis, and measurement was performed at the following conditional parameters: flow rate of 1.8 ml/min at 35 °C & 0.8 °C, and the duration of analyses was 15 min. The HPLC calibration curve of α-tocopherol, presented in ***Figure 3***, shows a fine linear relationship (y = 22.964x – 84.9) between the response and concentration range from 24 to 225 µl/ml with the regression and correlation coefficient R = 0.999.

The results of HPLC analysis of α-tocopherol isolated by the stirring of cotton fabrics in methanol for 1, 15 and 60 minutes are shown in ***Table 2***. Before using the stirring method for isolation of α-tocopherol from cotton textile, it is necessary to analyse the time needed for adequate analysis. Therefore, isolations were taken at three different intervals: 1, 15 and 60 min. A stirring duration of 15 min proved to be the best because after 1 min of stirring, the concentration of α-tocopherol was lower than after 15 min (***Table 2***), while 60 min was too long, resulting in desorption/adsorption phe-

nomena. ***Table 2*** also presents the rate of α-tocopherol isolated from cotton fabrics before and after the washing. The concentrations of α-tocopherol isolated from cotton fabrics before and after the washing for 15 minutes were in equilibrium, thus 60 minutes of stirring was unnecessary.

Concentrations of α-tocopherol after 15 min of stirring were compared before (191.1033 μg/ml) and after the 5 washing cycles (19.5540 μg/ml), and are presented in ***Table 2***. It can be concluded that 10% of α-tocopherol remained on cotton. According to the results obtained, stirring, as a cheap, simple and quick method, could be applicable for durability testing.

Soxhlet extraction of cotton fabrics before (SE) and after the 5 washing cycles (SE-5x) was performed with methanol for 90 minutes. The volume of the solvent and time required for the isolation of α-tocopherol were higher than with the stirring technique. α-tocopherol isolated by solvent extraction from the unwashed sample (SE) gave a value of 191 μg/ml. After the 5 washing cycles, 13.9% of α-tocopherol was still present on cotton (***Table 3***).

Two parameters were analysed during the microwave extraction of α-tocopherol from cotton: the preparatio (textile form) and mass of the sample. The textile formanalysed were fibrils (MWeF) and unit form (MWeU). Microwave extraction of α-tocopherol was performed varying the sample mass: a = 0.5000g (MWeF-a and MWeU-a) and b = 0.0600g (MWeF-b and MWeU-b). It also correlates with the application manual for microwave extraction, which recommends a sample of maximal mass of 0.5 g.

Concentrations of α-tocopherol isolated from cotton fabrics before and after the washing by MWe extraction are shown in ***Table 4***.

The sample in fibril form of mass a = 0.5000 g (MWeF-a) has a smaller concentration (13.4994 μg/ml = 8.5%) of α-tocopherol isolated from cotton than the sample with the same mass but in unit form (MWeU-a). The same tendency can be seen for the other sample mass: The sample in fibril form of mass b = 0.0600 g (MWeF-b) has a smaller concentration of α-tocopherol (0.7403 μg/ml = 5.6%) than that with the same mass but in unit form (MWeU-b). It is necessary to consider that the preparation of fibrils could bring possible risk of contaminating the sample. After the 5 washing cycles, textile in unit form (MWeU-a-5x and MWeU-b-5x) contains 23.4% and 43.57% of α-tocopherol, respectively. According to the results obtained , a mass of 0.5000 g was optimal for the quantification of α-tocopherol using the microwave isolation method.

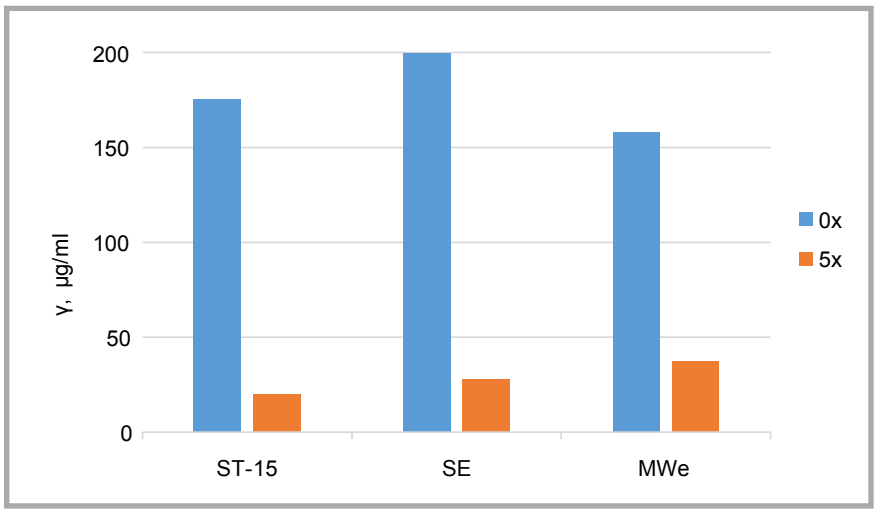

Figure 4. *Concentrations of α–tocopherol isolated from cotton samples by stirring after 15 minutes (ST-15), Soxhlet extraction (SE) and microwave extraction (MWe) before (0x) and after 5 cycles of washing (5x).*

The three techniques applied: stirring (ST-15), Soxhlet (SE) and microwave extraction (MWe) gave different concentrations of isolation of α-tocopherol from cotton samples, both treated (0x) and washed (5x). A comparison of the α-tocopherol concentrations obtained by HPLC is given in ***Figure 4***.

The highest concentration of α-tocopherol from the treated cotton samples was obtained by Soxhlet extraction. The next in order was stirring, and the last was microwave extraction. This was not confirmed by the range order at the concentration of α-tocopherol isolated from cotton samples after 5 washing cycles, shown in ***Figure 4***. Microwave extraction was the most convenient for this type of sample. ***Table 5*** presents some advantages/disadvantages and limitations of the isolation techniques tested. For easier comparison, the concentration of α-tocopherol isolated from the sample (MWeU-a) was calculated for 0.6000 g, presented in ***Table 5***.

Conclusions

The results obtained prove that all techniques used: stirring, Soxhlet and microwave extractions are applicable for the isolation of α-tocopherol from cotton cosmetotextiles. However, the techniques used for the isolation of α-tocopherol from cotton fabrics have advantages and limitations in this special application, hence the selection of the appropriate technique depends on the sample and experience of the analyst.

Table 5. *Key points for techniques applied for isolation of α-tocopherol from cotton fabrics.* ***Footnotes:*** *[2] time of starring was 15 min, [3] concentration of sample (MWeU-a) was calculated for 0.6000 g.*

	Stirring	Soxhlet	Microwave
Time, min	15	90	25
Solvent volume, ml	10	120-140	10
Controlled conditions (pressure and temperature)	–/+	–/+	+/+
Closed system	–	–	+
Safety (explosion) risks	–	–/+	+
Confidence	medium	low	high
Limitations	desorption/adsorption phenomena	unreliable equilibrium state	mass of sample
Sample form	piece size: ~10 mm^2	piece size: ~10 mm^2	unit form
γ, μg/ml	191.1033[2)]	199.3163	189.7980[3)]

The comparison of the percentages of α-tocopherol from cosmetotextiles before washing with the three extraction methods revealed similar concentrations of the isolated compound. Meanwhile, the comparison of the residual amount of α-tocopherol isolated from washed cosmetotextiles revealed more pronounced differences: MWE (23% of residual α-tocopherol), Soxhlet (13,8% of residual α-tocopherol) and stirring (10,2% of residual α-tocopherol). It can be concluded that the isolated concentration of α-tocopherol obtained with MWe extraction was higher and therefore a more convenient method as compared to the Soxlet and stirring extraction methods.

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Received 26.07.2018 Reviewed 26.02.2019