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ORIGINAL PAPER

Quantification of tocochromanols in vitamin E dietary supplements by instrumental thin-layer chromatography

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Abstract

A variety of vitamin E dietary supplement capsules (DSC) based on different natural oils or synthesis products are currently found on the market whose vitamin contents need to be controlled before and after marketing. Here, we present an instrumental thin-layer chromatography (TLC) method which allows a direct determination of all tocopherols (T) and tocotrienols (T3) as well as α-tocopherol acetate simultaneously in one run with short analysis time. For this purpose, contents of the DSC were extracted, applied on silica gel 60 plates, and developed with *n*-hexane/ethyl acetate/acetic acid, 90:10:2 (*v/v/v*) as mobile phase. The UV scan of the plate at 293 nm was used for quantification based on the peak height. Following the scan, the plate was treated with 10% sulphuric acid in methanol which led to characteristic yellow-to-brown colouring of the tocochromanol spots which allowed to distinguish tocochromanols from matrix components with similar R_f values. In most cases, determined vitamin E contents matched well with the information listed on the label of the investigated DSC samples. The method is fast, easy to perform and gently treats the analytes as it requires no thermal treatment prior to quantification, which makes it suitable as a screening method.

Keywords Instrumental thin-layer chromatography · Vitamin E · Tocopherol · Tocochromanol · Dietary supplement

Introduction

For a century, α-tocopherol (α-T; vitamin E) is known as an indispensable food ingredient in human nutrition [1–3]. Gradually, different structurally related compounds which also feature a 6-chromanol backbone were discovered and summarized as tocochromanols (Fig. 1) [4, 5]. Although only α-T meets the criteria of preventing the human deficiency disease of vitamin E [6], these were also partly named vitamin E (compounds). Naturally occurring tocochromanols differ in the number and substitution pattern of methyl groups on the 6-chromanol ring. With the one on C-8 being obligatory due to the biosynthesis pathway, the optional presence of methyl groups on C-5 and/or C-7 gives rise to four variants, i.e., α-(5,7,8-trimethyl substituted), β-(5,8-dimethyl substituted), γ-(7,8-dimethyl substituted), and δ-tocochromanols (8-methyl substituted) (Fig. 1) [7]. In addition, an isoprenoid side chain consisting of 13 carbons in the longest chain along with four methyl groups is mandatory in naturally occurring tocochromanols, which is located on C-2 of the 6-chromanol unit. This side chain can be saturated (family of tocopherols, later on labelled T) or it can feature three double bonds, the so-called tocotrienols (T3) [7]. Additional tocochromanols with one or two double bonds were eventually discovered, but their contributions to the total tocochromanol content of food are low with the exception of α-T1 which has a higher share in palm oil [8].

Tocochromanols are one of the most important lipophilic antioxidants in the physiology of plants, animals, and humans [9], preventing unsaturated membrane lipids from peroxidation. Furthermore, a wide variety of health-promoting effects has been attributed to the dietary intake of vitamin E [5, 9]. These beneficial effects include prevention of arteriosclerosis (and cardiovascular diseases) along with anti-inflammatory, immune-supporting, and anti-angiogenic effects of these micronutrients [10–13]. Also, in cosmetics (creams, make-up) vitamin E is considered an important ingredient for skin protection [14]. The National Health and Nutrition Examination Survey (NHANES, 2017–2018) indicated mean vitamin E intakes of 10.5 mg/day and 8.6 mg/

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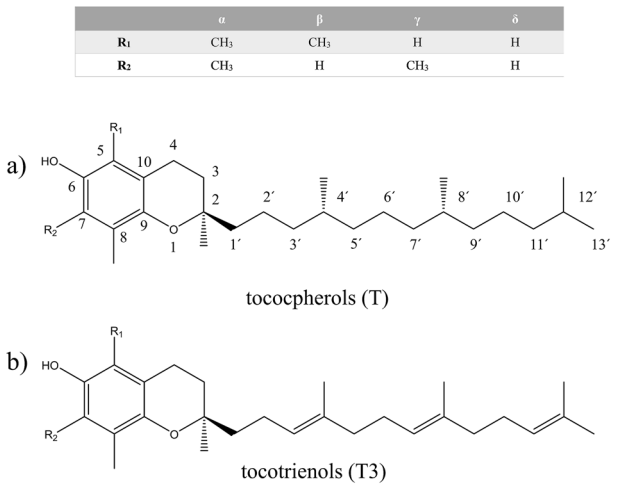

Fig. 1 General structure of (**a**) tocopherols and (**b**) tocotrienols including the substitution pattern on the 6-chromanol unit. Tocomonoenols feature one double bond in 11′-position

day, respectively, for adult males and females (> 19 y) from the U.S. [15]. Accordingly, recommended dietary allowances (RDAs) of 15 mg/day α-T for adults in the U.S. [16] and adequate intakes (AIs) of 13 mg α-T equivalents/day for men and 11 mg α-T equivalents/day for women in the EU [17] are not reached by consumers via conventional food. Therefore, it is not surprising that a large market exists for dietary supplements. According to the 2019 Council for Responsible Nutrition (CRN) consumer survey, 12% of adults in the U.S. supplied vitamin E and 58% of them used multivitamin supplements [18]. This demand is met by an extremely heterogeneous dietary supplement market (> 85,000 products [19]) valued at more than 130 billion $ per year [20]. Hence, it is important to screen and monitor vitamin E supplements to verify product quality and reported vitamin E levels in individual products.

Routine vitamin E analysis is usually performed with reversed-phase high-performance liquid chromatography (RP-HPLC) and fluorescence or UV detection [21–24]. However, run times are comparably long and only one sample can be analyzed in parallel. Recent experience in enrichment of individual tocochromanols indicated unexpected good separation characteristics on silica gel [25]. This prompted us to explore the capabilities of thin-layer chromatography (TLC) for the separate quantification of different tocochromanols in dietary supplements. Modern instrumental TLC has been repeatedly shown to be a fast and reliable technique for the quantification of various food compounds [26–28]. However, existing TLC methods in the field of vitamin E were scarcely found in the literature. Moreover, these few TLC methods only aimed to quantify α-T or α-T acetate or only obtained one peak for the complete vitamin E fraction [29–33]. Only Ruggeri et al. succeeded in the quantification of α-T, γ-T, δ-T, and α-T3 in algal lipids [34]. However, it remained unclear if this method also enabled the separation of β- and γ-T, which are difficult to separate by LC methods. Similarly, α-T3 was the only tocotrienol that was analyzed in this approach [34].

The goal of this work was the simultaneous separation and quantification of tocochromanols in dietary supplements by instrumental TLC in one run. For this purpose, the separation was studied using four tocopherols, four tocotrienols, and α-tocomonoenol (11′-α-T1) as reference standards. The TLC method was applied to representative commercial dietary supplement products of varied tocochromanol composition and amounts to cover a wide range of products marketed worldwide.

Materials and methods

Organic solvents and chemicals

Diethyl ether, *n*-hexane, cyclohexane, and methanol were ordered from Chemsolute (Th. Geyer, Renningen, Germany). Diisopropyl ether was purchased from VWR Chemicals (Radnor, PA; USA). Ethyl acetate and *i*-octane were obtained from Honeywell Riedel-de Haën (Seelze, Germany). Ethanol, sodium chloride, hydrochloric acid (37%), pyridine (distilled before use), *n*-heptane, and silver nitrate were bought from Carl Roth (Karlsruhe, Germany). Acetic acid and acetic anhydride were ordered from Fluka (Buchs, Switzerland) and *n*-pentane was from Acros Organics (Geel, Belgium). Sulfuric acid came from Merck (Darmstadt, Germany).

Standards and reference substances

Cholesterol, palmitic acid methyl ester, and myristic acid methyl ester were bought from Merck (Darmstadt, Germany). Myristic acid, palmitic acid, squalene, tripalmitin, and triolein were from Fluka (Buchs, Switzerland). Oleic acid was purchased from Sigma-Aldrich (St. Louis, Missouri, USA). α-Tocopherol (97.0% purity) and γ-tocopherol (95.3% purity) were obtained from DSM Nutritional Products (Basel, Switzerland). Standard solutions of β-tocopherol (50 mg/mL *n*-hexane), β-tocotrienol (50 mg/mL *n*-hexane), and δ-tocopherol (100 mg/mL *n*-hexane) were ordered from Supleco Analytical (Bellefonte, PA, USA). Three tocotrienols (α-T3, γ-T3, and δ-T3) and 11′-α-T1 were isolated in high purity from palm oil DSCs [35, 36]. α-T acetate was generated from α-T by reaction with pyridine and acetic anhydride as shown by Hammann et al. [37]. This reaction should be carried out with care since pyridine is toxic at higher doses [38]. Tocopherols were combined in one standard mix (tocopherol mix) and tocotrienols in another

(tocotrienol mix), and diluted to concentrations of ~ 1 mg/mL for each tocochromanol.

Samples

Six DSCs were purchased in different online shops. The capsule-based products contained oily, viscous liquids. According to label, dietary supplement capsule 1 (DSC 1) consisted of a mixture of tocopherols from sunflower oil and tocotrienols from palm oil. DSC 2 was made from rice bran oil and DSC 3 from soybean oil with added α-T. DSC 4 was based on palm oil, DSC 5 on sunflower oil with additional tocopherol containing extracts of unreported origin, and DSC 6 on α-T acetate. Hence, these products were considered representative of a wide range of products marketed worldwide.

Extraction of dietary supplement capsules

One or two DSCs (depending on the expected tocochromanol content) including the shell, 10 mL bidest. water and three drops of concentrated hydrochloric acid were placed in a 100 mL centrifuge tube. After 1 h or more of standing under exclusion of light with vigorous shaking every 10 min, the centrifuge tube was supplemented with 10 mL *n*-hexane and 1 mL saturated sodium chloride solution. After centrifugation (5 min at 825 × *g*), aliquots of the *n*-hexane phase were transferred into 2 mL brown glass GC vials and the solvent was evaporated by means of a gentle stream of nitrogen. The residue was taken up in 1 mL ethanol and stored at 4 °C until further use. The anticipated concentration in the extract before application to the TLC plate was 1–3 mg vitamin E/mL ethanol based on the manufacturer`s information on the label.

Instrumental thin-layer chromatography (TLC)

The TLC instrument consisted of a Linomat 5 plate applicator, an immersion device, a TLC plate heater, a TLC scanner, a TLC visualizer and VisionCats, as well as WinCats software for evaluation (CAMAG, Berlin, Germany). The TLC method development started with the mobile phase suggested by Malins and Mangold for lipid class separation [39] except that *n*-hexane was used instead of petrol ether which resulted in more stable elution conditions. Following that, different solvent mixtures were studied. Separations were performed on glass plates coated with silica gel 60 (20 × 10 cm with 16 tracks or 10 × 10 cm with 8 tracks, Merck, Darmstadt, Germany). DSC extracts in ethanol and standard solutions in different concentrations (0.25–1 mg/mL) were applied in form of bands (application speed: 50 nL/s, band width 8 mm, distance between bands 9.4 mm). The application volume was restricted to 6 µL because higher volumes resulted in less sharp bands. Plates were developed to a height of 8 cm with chamber saturation using the mobile phase *n*-hexane/ethyl acetate/acetic acid, 90:10:2, *v/v/v*. After letting dry for 5 min, densitometric quantification of the tocochromanols was carried out by scanning at 293 nm in UV absorption mode (α-T, α-T3, β-T, γ-T, and γ-T3) or at an excitation wavelength of 276 nm with a K320-filter in fluorescence mode (δ-T and δ-T3).

Qualitative detection was performed under white light and at 366 nm. Bands were visualized by immersion of plates in 10% sulphuric acid in methanol (immersion speed 3.5 cm/s, immersion duration 0 s) followed by 2 min drying at 150 °C. Figure 2 shows a scheme of the instrumental TLC procedure. Qualitative evaluation was based on the comparison of retention factors (R_f, Eq. 1)

$$R_f = \frac{\Delta ST[\mathrm{cm}]}{\Delta SF[\mathrm{cm}]}, \tag{1}$$

with R_f—retention factor, ΔST—distance between start zone and tocochromanol band in cm, and ΔSF—distance between start zone and solvent front in cm.

Determination of limits of detection (LOD) and limits of quantification (LOQ) was based on DIN 32645:2008-1 [40], using the peak height of the UV scan at 293 nm. In practice, each tocochromanol standard was measured fivefold in five mass levels between ~ 0.35 µg and ~ 1.5 µg, respectively. Tocochromanol contents in the samples were calculated by recording external calibration curves reaching from ~ 0.5 µg to ~ 6 µg for each tocochromanol, respectively. For this purpose, each calibration point was applied three times on two TLC plates, which were scanned three times, respectively. Thus, 18 values were obtained for each measure point and the mean value was calculated from this. Overall, a 20 × 10 cm plate was assigned with 16 tracks which allowed to quantify tocochromanol extracts from four DSCs in triplicate along with two bands of the tocopherol mix and the tocotrienol mix, respectively. In parallel, a second plate was used for the calibration curves of the tocopherol mix and the tocotrienol mix which was aligned with the original calibration curve. Quantitative data were evaluated using VisionCats or WinCats software (CAMAG, Berlin, Germany).

R_f values of various lipid standards were determined to exclude interferences of tocochromanols by other lipid components that may be present in DSC samples. Lipid compounds [cholesterol, triacylglycerols (tripalmitin, triolein), free fatty acids (myristic acid, palmitic acid, oleic acid), fatty acid methyl esters (palmitic acid methyl ester, myristic acid methyl ester), lecithin, and squalene] were applied at ~ 4 µg (applied via 4 µL standard solution at ~ 1 mg/mL) on a TLC plate. After initial testing, triacylglycerols were combined in a separate standard mixture (triacylglycerol mix) and all other lipid components in another mix (lipid mix).

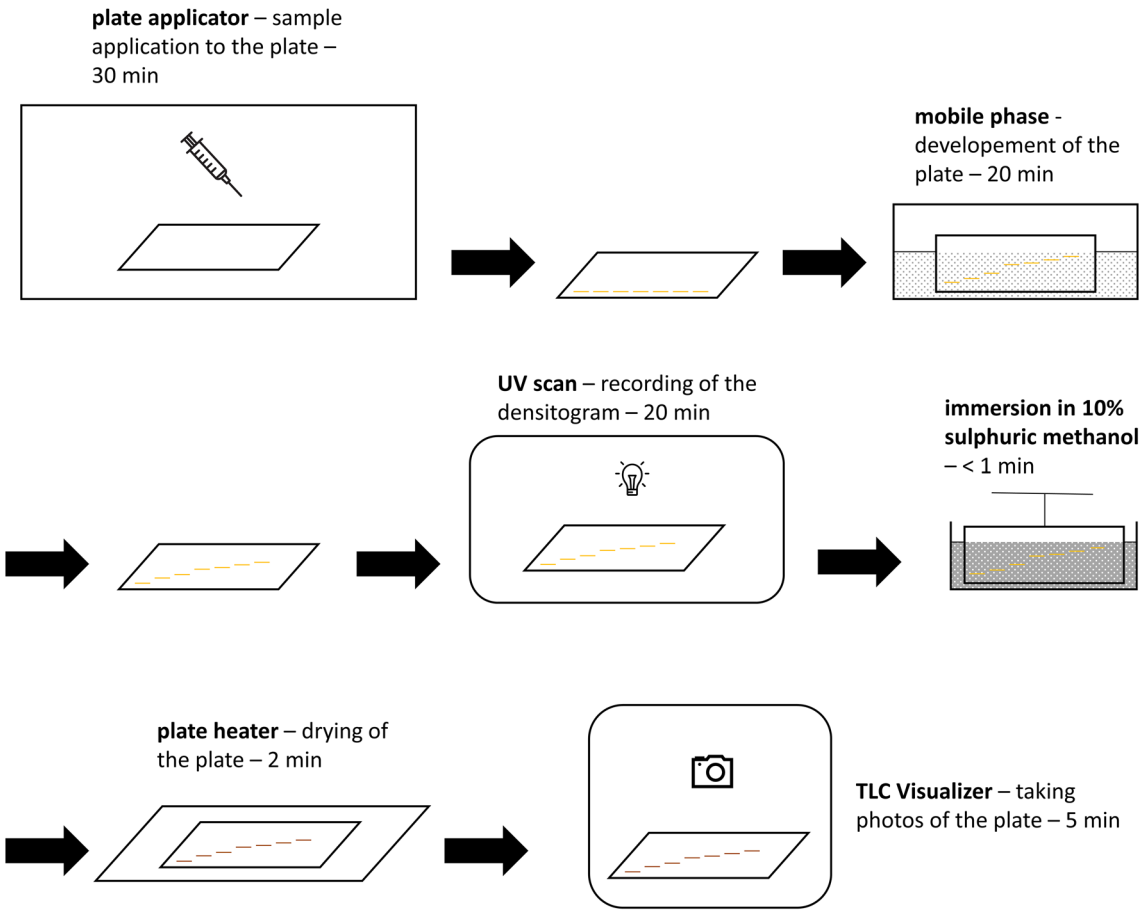

Fig. 2 Scheme of the instrumental TLC procedure from sample application to the plate to taking photos of the final developed plate with time indications for each step

Results and discussion

Development of the quantification conditions for classic tocochromanols

The starting point for mobile phase development (#1) (*n*-hexane/diethyl ether/acetic acid, 90/10/1 (*v/v/v*)) resulted in low R_f values of 0.08 to 0.18 for tocochromanols (Table 1). Higher shares of acetic acid (#2) increased the R_f values (R_f range 0.24–0.29), but gaps between the tocochromanols were insufficiently small. Replacement of diethyl ether with diisopropyl ether according to Rugeri et al. [34] (#3) resulted in similar R_f values as in #2, but indicated a better distribution of individual tocochromanols, albeit at the cost of band sharpness. Band sharpness could be slightly improved by adding small amounts acetic acid (#4) without affecting R_f values.

Substituting diisopropyl ether with ethyl acetate (#5) in the mobile phase resulted in higher R_f values and a better dispersion of individual tocochromanol bands, but band sharpness was decreased compared to #5. This drawback could be compensated by increasing the share of acetic acid content from 1% (#5) to 2% (#6). In general, such an increase in acetic acid led to higher R_f values and produced sharper bands but decreased the distance between the bands. Accordingly, mobile phase #6 (*n*-hexane/ethyl acetate/acetic acid, 90/10/2, *v/v/v*) represented a good compromise between sufficiently high R_f values and separation of tocochromanols paired with good band sharpness. Additional attempts using toluene or mixtures of toluene with ethyl acetate as polar organic component (#7) and substitution of acetic acid with trichloroacetic acid (#8) resulted in lower R_f values. Hence, mobile phase #6 was chosen for analysis of the samples.

R_f values of tocochromanols in mobile phase #6 decreased in the order $\alpha > \beta > \gamma > \delta$. Higher R_f values from α-T over γ-T to δ-T were expected due to the increase in polarity. However, the good separation of β- and γ-T isomers was unexpected, because the separation of both isomers can even be challenging in HPLC [41] and could not be reached by countercurrent chromatography (CCC) [36]. Moreover, R_f values of tocopherols were higher by ~ 0.05 than the corresponding tocotrienols. Also, tocochromanols produced

Table 1 Mobile phase compositions tested for the TLC separation of tocochromanols and corresponding R_f values

No	Elution	α-T	γ-T	δ-T	α-T3	γ-T3	δ-T3
#1	*n*-hexane/diethyl ether/acetic acid, 90/10/1 (*v/v/v*) (1)	0.18[a]	0.12[a]	0.09[a,I]	0.15[a]	0.10[a,I]	0.08[a,I]
#2	*n*-hexane/diethyl ether/acetic acid, 90/10/5 (*v/v/v*) (2)	0.29[a,I]	0.26[a,I]	0.25[a,I]	0.28[a,I]	0.26[a,I]	0.24[a,I]
#3	*n*-hexane/diisopropyl ether, 85/15 (*v/v*) [34] (3)	0.25[b]	0.17[b]	0.12[b,I]	0.20[b]	0.13[b,I]	0.09[b]
#4	*n*-hexane/diisopropyl ether/acetic acid, 85/15 /1 (*v/v/v*) (4)	0.25[c]	0.18[c]	0.13[c,I]	0.20[c]	0.13[c,I]	-
#5	*n*-hexane/ethyl acetate/acetic acid, 90/10/1 (*v/v/v*) (5)	0.35[c]	0.25[c]	0.19[c,I]	0.29[c]	0.20[c,I]	0.15[c]
#6	***n*-hexane/ethyl acetate/acetic acid, 90/10/2 (*v/v/v*) (6)**	**0.43[b]**	**0.33[b]**	**0.28[b,I]**	**0.39[b]**	**0.29[b,I]**	**0.24[b]**
#7	*n*-hexane/ethyl acetate/toluene/acetic acid, 84/6/10/2 (*v/v/v*) (7)	0.28[b]	0.18[b]	0.13[b]	0.23[b]	0.15[b]	0.10[b]
#8	*n*-hexane/ethyl acetate/trichloroacetic acid, 90/10/1 (*v/v/v*) (8)	0.30[b]	0.21[b]	0.17[b,I]	0.26[b]	0.18[b,I]	0.14[b]

The composition of the mobile phase that was finally used to carry out the experiments is highlighted in bold letters

[a]Good shape

[b]Medium shape

[c]Bad shape

[I]Partial coelution

characteristic yellow-to-brown spots (depending on the amount on the TLC plate) after destructive treatment with 10% sulphuric acid in methanol (Fig. 3). The fact that this treatment is destructive was the reason for performing the UV scan first and the treatment with 10% sulphuric acid in methanol afterwards. For the evaluation, however, the derivatized bands were first assigned to the tocochromanols on the basis of the R_f values and then the quantitative evaluation was started. Before the treatment, the spots only had a faint light orange colour at higher concentrations and were invisible at low-to-medium concentration. The resulting colours of the tocochromanol bands were quite similar and thus could be helpful for distinguishing tocochromanols from potential coeluting compounds in the samples.

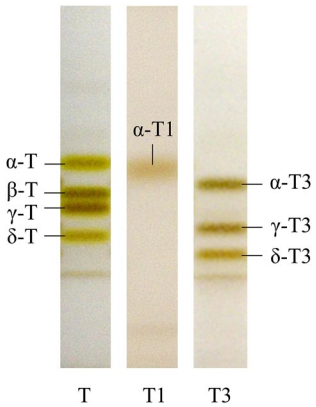

Fig. 3 TLC silica gel 60 plates (excerpts) of the tocopherol standard mix (T), the α-T1 standard (T1), and the tocotrienol standard mix (T3)

Increasing the application speed via plate applicator from 50 to 100 nL/s increased the relative standard deviation from < 1% to ~ 10% (Table 2). As a consequence, the slower application speed of 50 nL/s was chosen which was considered fast enough. Tests in UV absorption mode in the range of the absorption maxima of tocochromanols (280–310 nm) showed that 293 nm (absorption maximum of α-T) was well suited for the quantification of α-, β-, and γ-tocochromanols (Fig. 4). However, for δ-tocochromanols, the fluorescence mode (excitation wavelength 276 nm) resulted in ~ twofold higher peaks compared to UV mode. By contrast, peak heights in fluorescence mode of the other tocochromanols were smaller by a factor of 1.5. Based on these parameters, LOD of individual tocochromanols ranged between 0.10 and 0.22 µg and LOQ between 0.32 and 0.44 µg, respectively, except δ-T which showed a higher LOD (0.37 µg) and LOQ (1.2 µg, Table 3). This was surprising, since the sensitivity of δ-T3 was in the normal range of the other tocochromanols.

Table 2 Effect of application speed on relative standard deviation (rsd) (%) for the peak height of α-tocotrienol (α-T3) and γ-tocotrienol (γ-T3) being applied on the same silica 60 plate

Tocochromanol	Measurement	Peak height (AU), $v = 50$ nL/s	Peak height (AU), $v = 100$ nL/s	rsd, $v = 50$ nL/s (%)	rsd, $v = 100$ nL/s (%)
α-T3	1	286	234	0.9	11.1
	2	285	216		
	3	281	197		
γ-T3	1	329	296	0.6	9.6
	2	326	274		
	3	325	244		

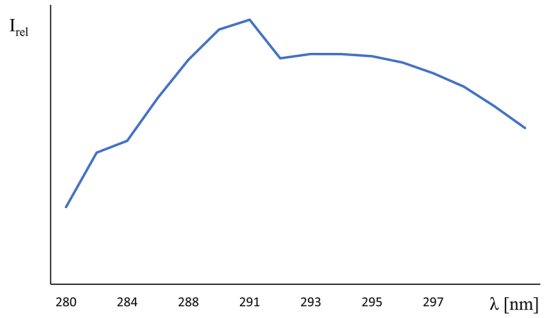

Fig. 4 UV absorption band of γ-tocotrienol in the wavelength range 280–300 nm

Relative standard deviations for all tocochromanols were 0.05–2.5% at the highest point of the calibration and 4.7–8.5% (exception δ-T3: 24.5%) at the lowest concentration of the calibration. Plate-to-plate variations based on mean values of different tracks showed up to 7.5% relative standard deviation. Inter-day reproducibility for the mean values of peak heights on different plates showed a relative standard deviation of only 1.8%.

Due to similar R_f values, a few pairs of tocochromanols could not be fully resolved (Fig. 5). The highest overlap was observed in the case of γ-T3 and δ-T. However, standard additions (2 µg each of γ-T3 and δ-T) showed that γ-T3 and δ-T could be determined without interference based on peak heights. Hence, γ-T3 and δ-T and other closely eluting tocochromanols (except of 11′-α-T1) could be individually quantified with this method.

Analysis of 11′-α-tocomonoenol (11′-α-T1), α-tocopherol acetate (α-T-Ac), and potentially interfering lipid components

Recently, 11′-α-T1 was isolated by CCC [36], and the R_f value of this rare tocochromanol could be determined, as well. As anticipated, the 11′-α-T1 band showed up between

Table 3 Limit of detection (LOD) (µg), limit of quantification (LOQ) (µg), and retention factors (R_f) of the tocochromanols being quantifiable with the TLC method

Tocochromanol	LOD	LOQ	R_f
α-T	0.22	0.42	0.43
α-T3	0.15	0.34	0.39
β-T	0.14	0.33	0.37
β-T3	0.10	0.32	0.34
γ-T	0.12	0.31	0.33
γ-T3	0.18	0.44	0.29
δ-T	0.37	1.20	0.28
δ-T3	0.17	0.42	0.24

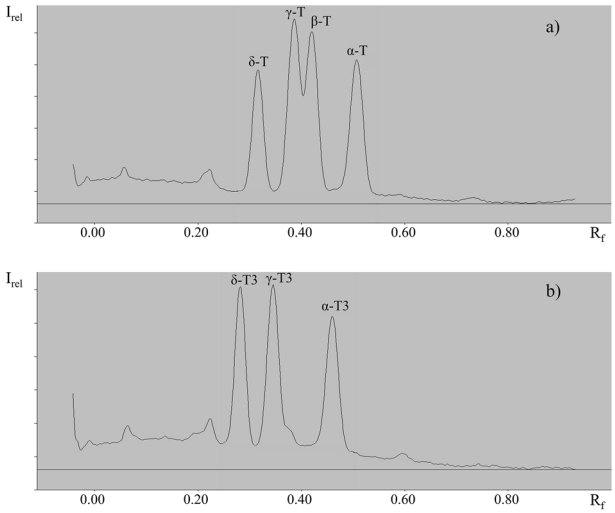

Fig. 5 TLC/UV densitograms of (**a**) the tocopherol standard mix and (**b**) the tocotrienol standard at 293 nm

α-T and α-T3. Unfortunately, ΔR_f between α-T and 11′-α-T1 was very small. When applied on the same track, not even a shoulder peak could be detected for 11′-α-T1. Hence, determinations of α-T in palm oil-based samples also included 11′-α-T1, which typically amounts to ~ 10 to 19% of α-T [8, 42]. Since palm oil is the only known relevant source of11′-α-T1, this disadvantage of the TLC method was deemed acceptable, also in the view that 11′-α-T1 is also difficult to determine by HPLC [43]. By contrast, the present method enabled the simultaneous determination of α-T-Ac because of its higher R_f value of 0.50 (Fig. 6). α-T-Ac is not naturally occurring but frequently added to dietary supplements due to its higher stability [44].

Possible interferences caused by other lipid components that may be present in the DSC were studied by measuring

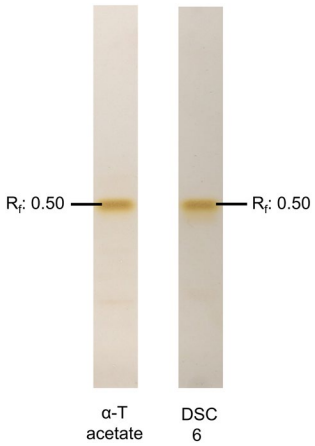

Fig. 6 TLC silica gel 60 plate (excerpt) of an α-tocopherol (α-T) acetate standard (left) and dietary supplement capsule DSC 6 (right) with R_f-values

several lipid standards (Table 4). However, none of the studied compounds interfered with the R_f range of tocochromanols. Namely, triacylglycerols showed higher R_f values, while free fatty acids, fatty acid methyl esters, lecithin, as well as cholesterol were moved less than tocochromanols. Of all tested compounds, R_f values of free fatty acids were closest to the one of δ-T3, but the resulting red spots could be easily distinguished from those of δ-T3 which was yellow/brown in colour. Squalene could not be detected, but was expected to have a higher R_f value. Apart from that, UV absorption and fluorescence signals of tocochromanols were quite unique, so that the selectivity was high.

Tocochromanol determination in dietary supplement capsules (DSCs)

DSC extracts were determined in triplicate and TLC plates were scanned thrice, and the mean value was used for quantification (Table 5). Visual inspection of the R_f range of tocochromanols indicated only one band interfering with δ-T and lying between δ-T3 and γ-T3 in DSC 4 (Fig. 7). However, this band could be distinguished by its violet colour from yellow to brown bands of the tocochromanols. This underlined the benefits of band visualization as a quality control measure as carried out in this study. Consequently,

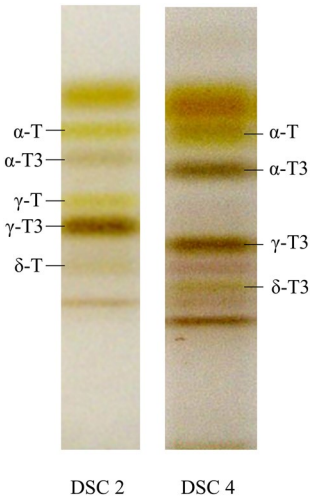

Fig. 7 Two silica gel 60 plates (excerpts) of the quantitative determination of tocochromanols in the dietary supplement capsules DSC 2 (left) and DSC 4 (right)

due to the violet colour, the band was not assigned to δ-T. In addition, all DSC samples showed an intense band directly above the fastest moving tocochromanol (α-T) whose identity could not be determined.

Tocochromanol amounts in the DSC samples ranged from 13 to 250 mg/capsule (Table 5). Amounts of α- and γ-tocochromanols matched well with labelled values. However, the total tocochromanol content in DSC 2 and the δ-T3 content in DSC 4 was higher than the one reported on the label. This could be due to general problems in the determination of δ-tocochromanols noted in other studies and matrices [45]. In view of this difference between labelled value and TLC result, we also determined the δ-T3 content in DSC 4 by HPLC-fluorescence according to the method of Grebenstein and Frank [46]. The values from HPLC-fluorescence (25.3 ± 1.3) and TLC (24.0 ± 1.6) agreed well indicating the correctness of our TLC measurement.

DSC 5 only featured α-T which indicated a non-natural source. Also, the high amount (250 mg α-T/capsule) in

Table 4 Retention factors (R_f) of fatty acid methyl esters, triacylglycerols, cholesterol, free fatty acid, and lecithin eluted with *n*-hexane/ethyl acetate/acetic acid, 90/10/2 (v/v/v) on a silica gel 60 plate

Lipid component	R_f
fatty acid methyl esters (myristic acid methyl ester, palmitic acid methyl ester)	0.62
triacylglycerols (tripalmitin, triolein)	0.53
cholesterol	0.27
free fatty acids (myristic acid, palmitic acid, oleic acid)	0.21
lecithin	0.05

Table 5 Tocochromanol content in dietary supplement capsule (DSC) samples 1–5 determined by thin-layer chromatography (TLC) ± standard deviation (mg/capsule) compared to the manufacturer's information (MI) (mg/capsule)

	DSC 1 TLC	DSC 1 MI	DSC 2 TLC	DSC 2 MI	DSC 3 TLC	DSC 3 MI	DSC 4 TLC	DSC 4 MI	DSC 5 TLC	DSC 5 MI
α-T	90.0 ± 3.1	93.0	15.0 ± 1.0	–	13.0 ± 1.2	12.0	17.0 ± 0.68	14.0	250.0 ± 10.0	268.0
α-T3	9.3 ± 0.07	13.0	7.1 ± 0.42	–	–	–	18.0 ± 0.64	17.0	–	–
β-T	–	2.0	1.6 ± 0.13	–	–	–	–	–	–	–
γ-T	26.0 ± 2.4	25.0	6.2 ± 0.8	–	–	–	–	–	–	–
γ-T3	21.0 ± 1.2	19.0	40.0 ± 2.8	–	–	–	23.0 ± 0.94	26	–	–
δ-T	19.0 ± 0.3	13.0	-	–	–	–	–	–	–	–
δ-T3	7.3 ± 0.09	6.0	2.9 ± 0.14	–	–	–	24.0 ± 1.6	9.5	–	–
total	172.6	173.0	72.8	60.0	13.0	12.0	64.0	69.0	250.0	268.0

DSC 5 was ~ 17-fold higher than the RDA of α-T [16]. Overdosing of vitamin E may lead to increased risk of prostate cancer among men [47]. These health issues strongly suggest that higher doses of vitamin E should be avoided by men [47].

The tocochromanol profile of DSC 4 matched the one of palm oil from which it was produced according to label (Fig. 8). Apart from that, higher shares of δ-T3 and lower shares of α-T (presumably including 11′-α-T1, which was not mentioned on label) were present in DSC 4 compared to the pattern in crude palm oil [8]. The amounts of α-T3 and γ-T3 were almost on the same level. Also, the tocochromanol pattern of DSC 2 matched well with the pattern of rice bran oil [48] from which it was obtained according to label. Remarkably, our TLC method enabled the detection of β-T in DSC 2 which was not reported in rice bran oil by van Hoed et al. despite the use of HPLC [48].

High chloroparaffin levels in several vitamin E-based DSC gained from palm oil indicated that tocochromanols were gained from a fraction separated during oil refining [49]. Slight differences in the tocochromanol patterns in DSC 2 and DSC 4 compared with literature compositions of the corresponding oils (Fig. 8) indicated that such processing steps may slightly alter the tocochromanol pattern.

The vitamin E fraction of DSC 6 consisted of pure α-T-Ac according to the manufacturer. Notably, also the α-T-Ac content in DSC 6 could be determined without any modifications to this method. Hence, the TLC measurement verified the absence of free tocochromanols, while the α-T-Ac content of 260 mg agreed well with the information on the product label (268 mg, Table 5).

Conclusion

The TLC method enabled the quantification of all four tocopherols (α-T, β-T, γ-T, and δ-T) and four tocotrienols (α-T3, β-T3, γ-T3, and δ-T3) in DSCs. The method also enabled the detection α-T-Ac. Hence, the TLC method allowed to check for the absence of artificial tocochromanol acetates in natural extracts and may be used in quality control protocols (verifying the absence of free tocochromanols, e.g., by ester cleavage) without investing extra analysis time.

Most importantly, application of TLC benefits from the parallel development of samples at the same time. Namely, 16 tracks could be measured on one TLC plate in 100 min. Overall, 16 samples could be prepared (opening of capsules, extraction, and centrifugation) and quantitatively and qualitatively analyzed by TLC (application on the plate, development in the chamber, drying and immersion, scanning, and integration, Fig. 2) within ~ 5 h. Another benefit is the absence of any thermal treatment which may result in the partial degradation of the tocochromanols. Hence, this fast and easy to perform method is well suited for screening DSCs or otherwise enriched tocochromanols, as well as for quality assurance in producers' laboratories.

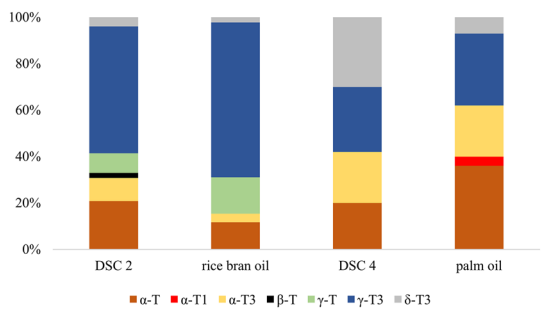

Fig. 8 Tocochromanol pattern of dietary supplement capsule DSC 2, rice bran oil, dietary supplement capsule DSC 4, and palm oil [7, 46]. Note that the share of α-T1 in DSC 4 is likely included in α-T because of their coelution

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Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

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