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UPLC method for the determination of vitamin E homologues and derivatives in vegetable oils, margarines and supplement capsules using pentafluorophenyl column

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ABSTRACT

A sensitive and rapid reversed-phase ultra performance liquid chromatographic (UPLC) method for the simultaneous determination of tocopherols (α-, β-, γ-, δ-), tocotrienols (α-, β-, γ-, δ-), α-tocopherol acetate and α-tocopherol nicotinate is described. The separation was achieved using a Kinetex pentafluorophenyl (PFP) column (150×2.1 mm, 2.6 μm) with both photodiode array (PDA) and fluorescence (FL) detectors that were connected in series. Column was thermostated at 42 °C. Under a gradient system consisting of methanol and water at a constant flow rate of 0.38 mL min^{-1}, all the ten analytes were well separated in less than 9.5 min. The method was validated in terms of linearity, limits of detection and quantitation, precision and recoveries. Calibration curves of the ten compounds were well correlated ($r^2 > 0.999$) within the range of 100 to 25,000 μg L^{-1} for α-tocopherol acetate and α-tocopherol nicotinate, 10 to 25,000 μg L^{-1} for α-tocotrienol and 5 to 25,000 μg L^{-1} for the other components. The method is simple and sensitive with detection limits (*S*/*N*, 3) of 1.0 to 3.0 μg L^{-1} (FL detection) and 30 to 74 μg L^{-1} (PDA detection). Relative standard deviations for intra- and inter-day retention times (< 1%) and peak areas (≤ 4%) were obtained. The method was successfully applied to the determination of vitamin E in vegetable oils (extra virgin olive, virgin olive, pomace olive, blended virgin and refined olive, sunflower, soybean, palm olein, carotino, crude palm, walnut, rice bran and grape seed), margarines and supplements.

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1. Introduction

Vitamin E is a term used to designate a group of essential fat-soluble compounds which share a common structure, i.e., a chromanol ring and either a phytyl tail (tocopherols, Ts) or isoprenoid chain (tocotrienols, T^3s) [1,2]. Both Ts and T^3s exist naturally in four of their corresponding homologues (α-, β-, γ-, δ-Ts and α-, β-, γ-, δ-T^3s), as shown in Fig. 1. Each homologue has its own distinct biological activity [3]. Vegetable oils and fat products of vegetal origin such as margarines are the main dietary source of vitamin E (mixture of Ts and T^3s) [4]. Each oil (sunflower, canola, soybean, olive, etc.) has different amounts of vitamin E, and the proportion of Ts or T^3s can be very different [5]. Approximately 70% of the vitamin E intake in the United States from food sources is in the form of γ-T, mainly due to the high intake of soybean and other vegetable oils rich in γ-T in the American diet [6]. Vitamin E in supplements is usually sold as α-tocopheryl acetate, a form of α-T that acts as a potent antioxidant.

During the last two decades, more than 90% of studies on vitamin E deal with the analysis of Ts, especially α-T [3]. However, as more beneficial effects of T^3s begin to be discovered (e.g. neuroprotective, anticancer, cholesterol-lowering properties) [7–10], scientists have shifted attention towards T^3s. The many beneficial effects of α-T^3, γ-T, and δ-T^3 to well-being and disease prevention are clearly distinct from that of α-T [8,10]. For these reasons, the profiling of vitamin E is of much interest. Therefore, practical and rapid analytical method to determine each analogue of Ts and T^3s is urgently needed.

The analysis of vitamin E is normally carried out either using high performance liquid chromatography (HPLC) or gas chromatography

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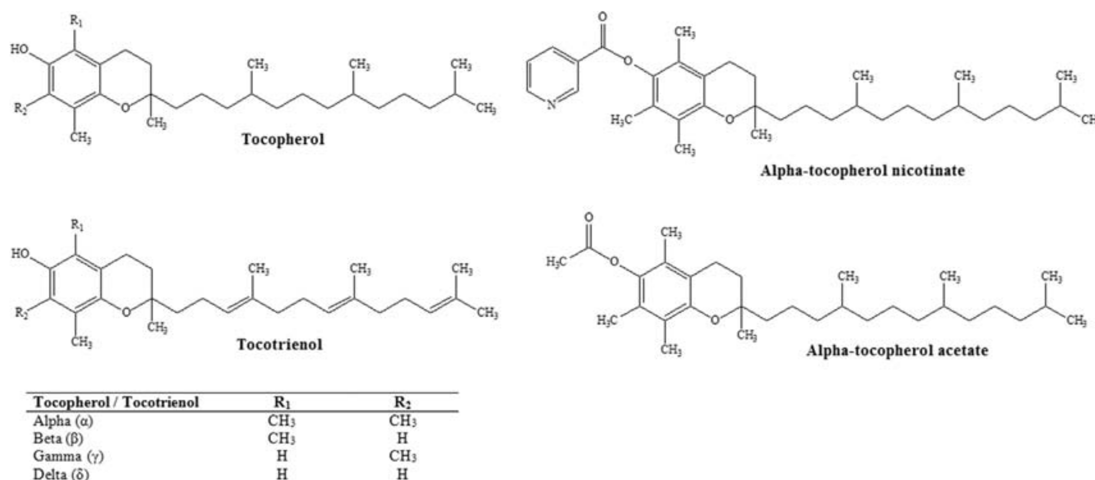

Tocopherol / Tocotrienol	R_1	R_2
Alpha (α)	CH_3	CH_3
Beta (β)	CH_3	H
Gamma (γ)	H	CH_3
Delta (δ)	H	H

Fig. 1. Chemical structures of the vitamin E components studied.

(GC). Generally, GC methods require derivatization procedure to increase the volatility. These derivatization reactions often produce incomplete conversion of the compounds and undesirable interfering side products [2,11]. Hence, HPLC is the most commonly used technique for the analysis of T and T^3. Several reversed-phase (RP) and normal-phase (NP) HPLC methods are available. The NP-HPLC method enabled the separation of all homologues of T and T^3, while separations using RP-HPLC, which usually employed C18 column, were less successful. However, NP-HPLC methods are plagued by the lengthy analysis time, less compatible with aqueous biological sample matrices and consume more hazardous solvents [12,13]. Chromatographic separation of up to a maximum of six T and T^3 has been achieved by gradient elution RP-HPLC. Although a variety of stationary phases such as different types of C18 or two columns arranged in series were employed, the β- and γ-homologues, however, remained unresolved [13–16].

Various HPLC methods have been reported for the determination of Ts, T^3s and α-T-acetate in cereals [1,15], vegetable oils [2,5,13,17] human plasma [14], plant seed oils [16], human colostrum and milk [18], rice [19], vegetables, eggs, flour, herbs [17] and cosmetic products [20]. However, most of the described methods were incapable of separating either β- and γ-T or β- and γ-T^3. Grebenstein and Frank reported a breakthrough in the separation of vitamin E where all the eight homologues were separated using RP-HPLC [12]. This method was achieved using a solid-core pentafluorophenyl (PFP) column operated in the isocratic mode. The homologues were separated in about 14 min. Recently, Viñas et al. reported the separation of seven vitamin E homologues using dimethyl pentafluorophenyl-propyl column with fluorescence and mass spectrometry detection [21]. In their work, good separation was achieved (run time ~ 17 min), however, the separation of β-T^3 was not included. Our experience with the fluorinated column was the rapid built-up of back pressure that was encountered over time especially when gradient elution is used, causing the column lifetime to be significantly reduced. We rationalized that these shortcomings can be overcome by migrating to UPLC system. This report, therefore, describes the development of UPLC method for the gradient separation of the eight homologues of vitamin E together with two important derivatives i.e., α-T-acetate and α-T-nicotinate using PFP column. The method will be validated and its analytical practicality demonstrated in the determination of vitamin E in a few types of vegetable oils, margarines and supplement samples. The chemical structures of the compounds studied are shown in Fig. 1.

2. Experimental

2.1. Chemicals and reagents

Primary (P) reference vitamin E (α-, β-, γ-, and δ-tocopherols and α-, β-, γ-, and δ-tocotrienols) standards were purchased from ChromaDex (CA, USA). α-Tocopherol acetate (≥96%) and α-tocopherol nicotinate were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade methanol (MeOH) and acetonitrile (ACN) were purchased from Merck (Darmstadt, Germany). Ethanol (HPLC grade) was purchased from Fisher Scientific (Hampton, New Hampshire, USA). HPLC grade 2-propanol was obtained from Q-Rec (Auckland, New Zealand). Ultra pure water (18.2 $M\Omega\,cm^{-1}$) was produced by a Milli-Q system (Millipore, MA, USA).

2.2. Vegetable oil, margarine and supplement samples

Virgin olive, extra-virgin olive, pomace olive, blended virgin and refined olive, sunflower, soybean, walnut, grape seed, palm olein, carotino, and rice bran oils as well as margarines and supplements (α-T, α-T^3, salmon oil and omega-3 fish oil capsules) were purchased from a local supermarket. Crude palm oil sample was kindly donated by Carotino Sdn. Bhd. (Johor Bahru, Malaysia).

2.3. Ultra performance liquid chromatographic system

Separations were conducted on a Waters Acquity Ultra Performance Liquid Chromatographic System (UPLC) (Milford, MA, USA) equipped with a binary solvent delivery module, an auto sampler cooler, a column heater and a 2996 Photodiode Array (PDA) detector connected in series with an HPLC 2475 multi wavelength fluorescence detector (FL). The chromatographic separation was performed on a Kinetex PFP column (150 × 2.1 mm, 2.6 μm) (Phenomenex, CA, USA) operated at 42 °C. Optimum separation was achieved with a binary mobile phase which consisted of methanol and water under gradient conditions at a constant flow rate of 0.38 mL min^{-1} with injection volume of 2 μL. All solvents, standards and samples solutions were filtered through 0.22 μm nylon filter membranes (Agilent Technologies, Waldbronn, Germany). Fluorescence detection was performed at excitation and emission wavelengths of 297 and 328 nm, respectively with a sampling rate of 10 points per second. UV detection wavelength was programmed at 292 nm with sampling rate of 20 points per second.

2.4. Method development

The initial mobile phase composition (% methanol) and holding time of the gradient program were studied over the range 80–85% and 0.5–1.5 min, respectively. The first ramping composition (80–87%) and time (7.0–8.0 min) were investigated. The second ramping composition and time over the range 95–100% and 8.0–8.5 min, respectively, were examined. Holding time (8.2–8.5 min) and third ramping time (9.0–9.5 min) were also investigated. Column temperatures (38–46 °C) were tested. The identities of the tested compounds were confirmed by retention time and UV-scan over the range 200–500 nm.

2.5. Stock and standard solutions

Stock solutions (500 μg mL^{-1}) of each vitamin E homologue, α-T acetate and α-T nicotinate were prepared in ethanol by sonication for 1 min and then diluted to the desired concentrations. Calibration standards were prepared using serial dilution of the stock solution in methanol. All the solutions were stored refrigerated (4 °C) in the dark when not in use. All analytical procedures were performed in a dark room and amber vials were used throughout the work.

2.6. Preparation of samples

Prior to the UPLC analysis, oil samples were accurately weighed (0.4 g) and dissolved in 2-propanol (5 mL). The samples were then vortexed for 2 min with a LMS Mixer UZUSIO (Bunkyo-Ku, Tokyo, Japan). Margarine sample (0.5 g) was transferred to volumetric flask (5 mL), followed by the addition of 2-propanol (2 mL). The mixture was then placed in a mild water bath (50 °C) for 1 min to dissolve the margarine. The mixture was top-up to the mark using 2-propanol and then vortexed for 2 min. Supplement samples were prepared by transferring the content of the capsule to volumetric flask (5 mL) containing 2-propanol (3 mL). The mixture was diluted with 2-propanol to the mark and vortexed (2 min). Serial dilutions were carried out by diluting 0.1 mL to 5 mL using 2-propanol. All the samples were prepared in triplicates, filtered and injected (each preparation 3 times) directly to the UPLC system. All the preparations were carried out under dim light and at room temperature (25 °C).

2.7. Validation of analytical method

The linearity of the calibration plots was studied using standard mixtures of α-T, β-T, γ-T, δ-T, α-T^3, β-T^3, γ-T^3, δ-T^3, α-T-acetate and α-T-nicotinate at thirteen concentration levels (5, 10, 20, 25, 50, 100, 200, 250, 500, 1000, 5000, 10,000 and 25,000 μg L^{-1}). Calibration curve was constructed by plotting the peak area (y) as a function of analyte concentration (x) in μg mL^{-1}. The limits of detection (LODs) were calculated at signal to noise ratio of 3. Intra-day precision was assessed by introducing three different preparations of the standard mixture of the vitamin E at four concentration levels (50, 200, 10,000 and 20,000 μg L^{-1}) on the same day ($n=9$), while the inter-day precision was assessed by using the same concentrations for six consecutive days ($n=54$). Accuracy studies were performed in three replicates, by spiking the vegetable oil (0.4 g) and margarine (0.5 g) with known amounts of standard (final concentration of 50, 200, 10,000 and 20,000 μg L^{-1}) and each sample was analyzed in triplicates.

3. Results and discussion

3.1. Optimization of chromatographic conditions

A major challenge in the analysis of vitamin E components is the separation of the β- and γ- homologues of T and T^3. β- and γ-homologues differ in structure merely in the position of the methyl group on the chromanol ring. The isomeric pair can be separated by adsorptive differentiation in the NP-HPLC mode but difficult to be separated by hydrophobic interaction in the RP-mode [22].

In the present work, a PFP column which has been reported to provide satisfactory separation for the β- and γ- homologues [12] was tried. The PFP column provides different selectivities for the analyzed compounds compared to the traditional C18 column. Due to the unique properties (high electronegativity, low polarizability and strong lipo- and hydrophobicity) of organofluorines, the fluorinated phases offered many possibilities that could not be accomplished by conventional C8 and C18 reverse phases [23–26]. The PFP column offers dispersive, dipole–dipole, π–π, charge transfer, and ion exchange interactions to enable the retention of different types of compounds [23,26]. Additionally, PFP phase also provides larger capacity factors for aromatics and polycyclic aromatic hydrocarbons due to the formation of donor–acceptor complex. Yamamoto and Rokushika also suggested that fluorinated phase also display some shape and size selectivity properties [27]. However, the exact retention mechanism on PFP columns are complex and remains largely unexplained [28].

The unique selectivity of the PFP stationary phases towards aromatic ring is exploited in the current work. The electron donor-acceptor interaction between the π-electrons of the solute and the stationary phase is the key to enable the separation of the homologues of vitamin E. This interaction (charge transfer), known as arene–perfluoroarene interaction (π–π interaction between two aromatic rings, one ring is substituted with many fluorine) occurs due to the presence of electron rich (solute) and electron poor (stationary phase) sites [29–31].

3.1.1. Selection of chromatographic parameters

Preliminary experiments were carried out to achieve the best chromatographic conditions for the simultaneous determination of all homologues (α-, β-, γ-, and δ-) of T and T^3, α-T-acetate, α-T-nicotinate. By referring to the chromatographic conditions described in a previous work by Grebenstein and Frank [12], MeOH and water were used as mobile phase. Different compositions

Table 1
Adopted UPLC operating conditions.

Variable	Optimum value
Flow rate (mL min^{-1})	0.38 (backpressure ≈ 7200 psi)
Temperature (°C)	42
Initial mobile phase composition (%)	MeOH:Water (81:19)
Gradient programming (Methanol composition, %)	0–1.0 min (81%)
	1.0–7.5 min (84%)
	7.5–8.0 min (100%)
	8.0–8.2 min (100%)
	8.2–9.0 min (81%)
	9.0–10.0 min (81%)
Injection volume	2 μL
PDA configuration	λ: 290 nm, resolution: 1.2 nm
	Scan rate: 20 points/s
FL configuration	$\lambda_{excitation}$: 297 nm, $\lambda_{emission}$: 328 nm
	PMT gain: 1.00
	Scan rate: 10 points/s

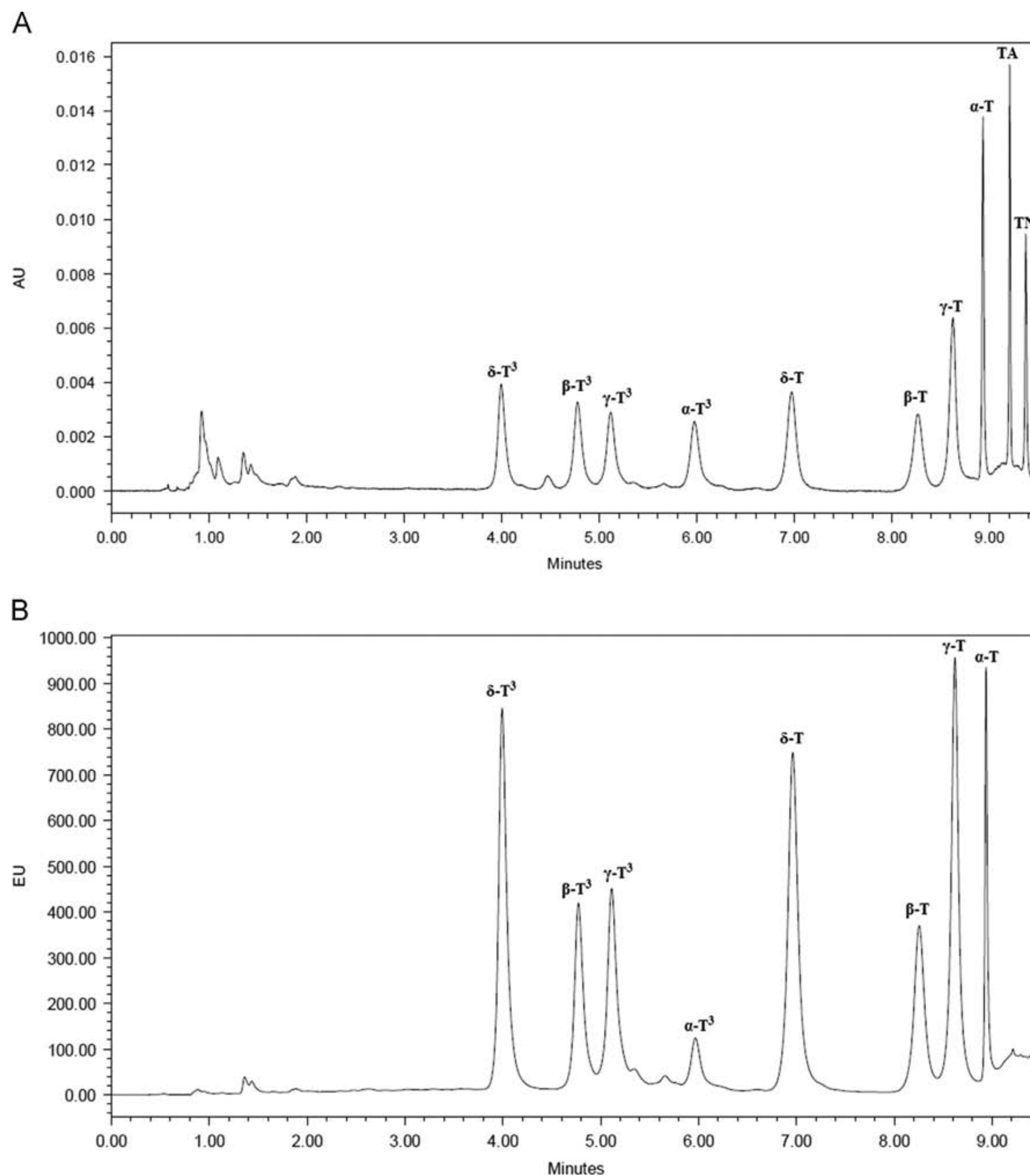

Fig. 2. Chromatograms obtained from the introduction of standard mixture containing T^3s, Ts, α-T-acetate and α-T-nicotinate (10 μg mL^{-1}) using UV (A) and FL (B) operated under the optimum chromatographic conditions (Table 1). TA, α-T-acetate and TN, α-T-nicotinate.

Table 2
Comparison of calibration curves and limits of detection for the vitamin E homologues and derivatives using UPLC-FL and UPLC-PDA[a] methods.

Vitamin E	Linear range (μg L^{-1})	Regression equation	r^2	LOD (μg L^{-1})
α-T	5–25,000 (200–25,000)	$Y=2E+06x+3736.5$ ($Y=2037.6x-104.4$)	0.9992 (0.9999)	0.2 (57)
β-T	5–25,000 (200–25,000)	$Y=2E+06x+109{,}998$ ($Y=1930.5x-187.6$)	0.9992 (0.9998)	0.2 (60)
γ-T	5–25,000 (200–25,000)	$Y=5E+06x-208{,}443$ ($Y=2925.0x-300.6$)	0.9996 (0.9991)	0.1 (40)
δ-T	5–25,000 (200–25,000)	$Y=6E+06x+217{,}649$ ($Y=2496.5x-309.9$)	0.9994 (0.9991)	0.1 (46)
α-T^3	10–25,000 (200–25,000)	$Y=653{,}264x-3251.9$ ($Y=1637.0x-77.16$)	0.9998 (0.9998)	0.6 (71)
β-T^3	5–25,000 (200–25,000)	$Y=2E+06x+92{,}477$ ($Y=1850.0x+103.3$)	0.9993 (0.9997)	0.2 (63)
γ-T^3	5–25,000 (200–25,000)	$Y=2E+06x+85{,}396$ ($Y=1564.4x+108.9$)	0.9995 (0.9999)	0.2 (74)
δ-T^3	5–25,000 (200–25,000)	$Y=5E+06x+140{,}416$ ($Y=2328.1x-257.6$)	0.9997 (0.9998)	0.1 (60)
α-T acetate	(100–25,000)	($Y=1378.4x-90.65$)	(0.9996)	(30)
α-T nicotinate	(100–25,000)	($Y=1079.0.0x-147.9$)	(0.9996)	(38)

[a] Figures in parenthesis refereeing to detection using PDA.

of this mobile phase were tested. It was observed that the small change in the composition of mobile phase had notable influence on the retention characteristics of the vitamin E components. Furthermore, the back pressure built up was gradual as the mobile phase was switched to different composition. 80–85% MeOH resulted in good peak characteristics and separation of the vitamin E homologues, especially for the positional isomers. The highest efficiency and shortest analysis time were attained when using the MeOH–water (85:15; v/v). However, the analysis time was rather long (~30 min) due to the late elution of the α-T-acetate, α-T-nicotinate. In order to shorten the analysis time, a gradient elution was developed. The gradient program was initiated using 81% MeOH, ramped to 100%, then returned to 81% MeOH and equilibrated for 1 min. By applying the gradient elution at flow rate of 0.38 mL min^{-1}, the back pressure increases abruptly up to ~7800 psi (54 MPa).

Column temperature was also investigated. It is well known that elevating the column temperature reduces the viscosity of the mobile phase and increases the mass transfer, thus reducing the back pressure. Rapid separation was obtained when 42 °C was used. The analysis time was markedly shortened without the loss of resolution under these conditions. By thermostating the column, the back pressure dropped to ~7200 psi (50 MPa) and remained steady thereafter. Injection volume more than 2 µL was not recommended due to the broadening and overlapping of the homologue peaks. Hence, injection volume of 2 µL was chosen.

3.1.2. Detection method

As Ts and T^3s are chromophores and fluorophores, many of the previous studies used UV and FL as detection method [11–13]. Evaporative light scattering detector (ELSD) was also reported for vitamin E analysis [2]. Poor sensitivity and selectivity and the difficulty of quantitation in real samples was noted [22]. We choose to use both the FL and UV detections for our work as both detectors are readily available in most analytical laboratories. Although FL detector is more sensitive than UV, but the weak fluorescence properties of α-T acetate and α-T nicotinate required the use of UV detector. Moreover, the connection of the PDA and FL detectors in series can be more informative and helps to confirm the identity of Ts and T^3s. The detector configurations (excitation and emission wavelengths, scan rate, etc.) were optimized in order to obtain the maximum responses.

The adopted conditions are summarized in Table 1 while Fig. 2 shows typical chromatograms of the standards. The developed method achieved baseline-separation of all the eleven compounds of interest in less than 9.5 min. This is much shorter than some of the fastest NP-HPLC (~21 min) [2], nanoliquid chromatography (Nano-LC) (~18 min) [32] and RP-HPLC (~14 min [12] and ~16 min [21]) methods reported. In addition, separation time and mobile phase consumption (>65% for RP-HPLC and NP-HPLC) was also reduced by 80% compared to RP- and NP-HPLC methods. Additionally, the present method enables the separation of ten vitamin E components compared to the reported methods (8 components) [2,12,21].

3.2. Validation of analytical method

3.2.1. Linearity

alibration curves were found to be linear over the range 100–25,000 µg L^{-1} for α-T-acetate and α-T-nicotinate, 10–25,000 µg L^{-1} for α-T^3 and 5–25,000 µg L^{-1} for the other components. Correlation coefficients of 0.999 and above were observed, indicating the excellent linearity over the selected concentration range (Table 2).

Table 3
Intra- and inter-day precision (% RSD) of the studied vitamin E using UPLC-FL and UPLC-PDA methods[a].

Vitamin E	Intraday precision (n=9)							
	20,000 µg L^{-1}		10,000 µg L^{-1}		200 µg L^{-1}		50 µg L^{-1}	
	RT	Area	RT	Area	RT	Area	RT	Area
α-T	0.06 (0.03)	1.1 (0.62)	0.02 (0.08)	1.2 (0.53)	0.07 (0.03)	2.5 (1.4)	0.03 (–)	1.4 (–)
β-T	0.06 (0.07)	0.52 (0.84)	0.05 (0.04)	0.86 (1.7)	0.04 (0.20)	1.3 (1.6)	0.44 (–)	2.8 (–)
γ-T	0.04 (0.06)	0.85 (1.5)	0.07 (0.03)	0.84 (2.2)	0.03 (0.08)	2.3 (2.8)	0.30 (–)	2.4 (–)
δ-T	0.07 (0.06)	0.80 (1.6)	0.04 (0.04)	1.17 (2.3)	0.03 (0.2)	2.7 (2.1)	0.45 (–)	2.3 (–)
α-T^3	0.09 (0.09)	2.6 (0.64)	0.03 (0.04)	2.6 (1.4)	0.08 (0.23)	1.4 (2.3)	1.2 (–)	1.4 (–)
β-T^3	0.09 (0.09)	0.54 (1.4)	0.03 (0.03)	0.66 (0.89)	0.04 (0.58)	2.5 (1.8)	0.52 (–)	3.0 (–)
γ-T^3	0.09 (0.09)	0.54 (0.93)	0.03 (0.02)	1.12 (1.1)	0.04 (0.25)	0.04 (2.6)	2.0 (–)	2.1 (–)
δ-T^3	0.10 (0.09)	0.74 (2.4)	0.03 (0.03)	0.80 (2.1)	0.05 (0.23)	2.6 (2.9)	0.48 (–)	2.3 (–)
α-T acetate	(0.02)	(0.37)	(0.02)	(0.44)	(0.03)	(2.9)	–	–
α-T nicotinate	(0.02)	(0.8)	(0.02)	(1.6)	(0.04)	(1.7)	–	–

Vitamin E	Interday precision (n=54)							
	20,000 µg L^{-1}		10,000 µg L^{-1}		200 µg L^{-1}		50 µg L^{-1}	
	RT	Area	RT	Area	RT	Area	RT	Area
α-T	0.15 (0.16)	2.2 (2.2)	0.13 (0.15)	3.4 (3.4)	0.17 (0.15)	3.8 (2.5)	0.15 (–)	3.7 (–)
β-T	0.46 (0.44)	1.9 (1.9)	0.49 (0.46)	3.1 (2.1)	0.51 (0.57)	2.8 (2.5)	0.45 (–)	3.1 (–)
γ-T	0.32 (0.54)	3.2 (2.1)	0.37 (0.33)	3.5 (3.1)	0.64 (0.78)	3.7 (3.4)	0.31 (–)	2.7 (–)
δ-T	0.47 (0.44)	1.8 (1.7)	0.52 (0.46)	2.4 (2.6)	0.54 (0.53)	2.7 (2.3)	0.44 (–)	3.7 (–)
α-T^3	0.46 (0.44)	2.3 (1.7)	0.53 (0.46)	2.3 (2.4)	0.53 (0.59)	2.4 (2.2)	0.68 (–)	3.3 (–)
β-T^3	0.44 (0.43)	1.7 (1.8)	0.52 (0.47)	2.9 (2.1)	0.52 (0.54)	3.3 (2.5)	0.46 (–)	4.0 (–)
γ-T^3	0.48 (0.46)	2.6 (1.8)	0.55 (0.50)	2.5 (2.1)	0.57 (0.59)	2.0 (2.6)	0.52 (–)	4.0 (–)
δ-T^3	0.43 (0.43)	1.5 (2.4)	0.70 (0.45)	2.6 (2.3)	0.52 (0.52)	2.9 (2.4)	0.43 (–)	2.6 (–)
α-T acetate	(0.06)	(1.6)	(0.05)	(2.4)	(0.06)	(2.8)	–	–
α-T nicotinate	(0.05)	(1.8)	(0.06)	(2.5)	(0.12)	(2.2)	–	–

– Denote below the detection limit, not able to be determined.

[a] Figures in bracket refer to UPLC-PDA, RT; retention time.

3.2.2. Limits of detection

The limits of detection (LODs), with signal-to-noise ratio of 3 for the compounds are shown in Table 2. The developed UPLC-FL method exhibited significantly lower LOD values for the vitamin E homologues (1.0–3.0 μg L^{-1}) compared to the reported methods using RP-HPLC-FL (27–156 μg L^{-1}), Nano-LC-UV (70–160 μg L^{-1}), NP-HPLC-ELSD (250–5400 μg L^{-1}), but comparable to NP-HPLC-FL (0.1–3.2 μg L^{-1}) [2,12,32].

3.2.3. Precision

Good intra- and inter-day precision were obtained for retention times and peak areas. The RSD values for both retention time and peak area were less than 1% and 4%, respectively (Table 3).

3.2.4. Accuracy

The obtained accuracy results for margarine and oil samples are summarized in Table 4. Recoveries obtained ranged from 85.9 ± 2% to 109 ± 2% which is better than the method reported by Cunha, et al. (≥ 73.8%) for the determination of vitamin E in olive oils [2]. The good accuracy values obtained indicate the potential of this method for the determination of the vitamin E components in vegetable oils, margarines and supplements.

3.3. Analysis of vegetable oils

The validated method was applied to the determination of the vitamin E in several vegetable oils, results are shown in Table 5. Fig. 3 depicts typical chromatograms obtained for some of these samples. Ts and T^{3}s were found to be present in different amounts, depending on the type of the vegetable oil. The highest concentration of α-T was found in sunflower oil (300 ± 10 mg kg^{-1}) while the highest amount of γ-T (354 ± 10 mg kg^{-1}), and γ-T^3 (452 ± 20 mg kg^{-1}) were found in soybean and palm olein oil, respectively. α-, β-, γ- and δ- isomers of T^{3}s can be found in palm (carotino, olien and crude), rice bran, grape seed and walnut oils, but these isomers were not found in olive, sunflower or soybean oils.

Interestingly, one of the olive oils (sample no. 4) was found to contain relatively high concentration of α-T^3. Because T^{3}s do not occur naturally in olive oil [33], thus their presence in this sample clearly suggests that it has been adulterated with T^3-rich oil (e.g., palm oil). It should be pointed out that carotino oil is the only oil that contained all the homologues of vitamin E (Table 5). Carotino oil is a unique blend of palm and canola oils, produced from a process that involves deacidification and deodorization of red palm oil. Contrary to the normal processing methods, this process is able to retain as much as 80% of the original carotenoids which

Table 4
Accuracy of the UPLC- FL and UPLC-PDA methods ($n=9$)[a].

Level spiked (μg L^{-1})	Mean recovery (% ± SD)									
	α-T	β-T	γ-T	δ-T	δ-T^3	β-T^3	γ-T^3	α-T^3	α-T acetate	α-T nicotinate
Vegetable oil										
50	106 ± 1	92.2 ± 3	99.2 ± 4	86.3 ± 2	101 ± 1	104 ± 2	101 ± 1	98.4 ± 2	–	–
200	96.5 ± 4	97.0 ± 1	91.6 ± 3	86.8 ± 3	101 ± 1	98.9 ± 4	101 ± 2	103 ± 3	93.4 ± 3	98.4 ± 4
10,000	98.2 ± 3	85.9 ± 2	85.6 ± 2	101 ± 3	101 ± 2	88.3 ± 3	97.7 ± 2	103 ± 2	92.7 ± 3	90.1 ± 3
20,000	93.0 ± 2	88.4 ± 3	86.8 ± 2	93.8 ± 3	101 ± 3	89.9 ± 2	101 ± 2	101 ± 1	87.8 ± 1	85.9 ± 1
Margarine										
50	94.9 ± 2	93.7 ± 3	99.5 ± 3	96.3 ± 3	105 ± 3	97.0 ± 3	95.7 ± 3	92.8 ± 3	–	–
200	107 ± 2	101 ± 4	109 ± 2	104 ± 2	104 ± 2	99.0 ± 3	104 ± 2	94.9 ± 4	96.9 ± 2	91.2 ± 3
10,000	101 ± 1	97.7 ± 3	97.0 ± 2	94.0 ± 3	105 ± 3	97.1 ± 3	97.5 ± 1	96.7 ± 2	97.4 ± 1	101 ± 1
20,000	105 ± 2	102 ± 3	96.1 ± 3	103 ± 3	103 ± 3	95.9 ± 3	99.9 ± 3	97.2 ± 3	103 ± 1	98.7 ± 1

[a] All components were detected using FL, except α-T acetate and α-T nicotinate (PDA detection).

Table 5
Vitamin E compositions (mg kg^{-1}) of tested vegetable oils using the proposed UPLC method (FL detection).

No.	Type of oil	Average concentration ± SD							
		α-T	**β-T**	**γ-T**	**δ-T**	**α-T^3**	**β-T^3**	**γ-T^3**	**δ-T^3**
1	Carotino	76.2 ± 3	[a]BLD	158 ± 5.6	6.4 ± 0.2	41.2 ± 2	2.5 ± 0.1	112 ± 4	14.9 ± 0.6
2	Palm olein	89.9 ± 3	[b]ND	3.93 ± 0.6	ND	218 ± 11	10.8 ± 0.7	452 ± 20	58.3 ± 2
3	Crude palm	57.4 ± 2	ND	1.2 ± 0.01	ND	141 ± 5	12 ± 0.4	421 ± 6	72.4 ± 2
4	Olive ((blended virgin and refined)	ND	ND	2.4 ± 0.09	37.4 ± 2	235 ± 10	ND	ND	ND
5	Olive (blended virgin and refined)	22.8 ± 0.8	0.5 ± 0.0	3.65 ± 0.1	ND	ND	ND	ND	ND
6	Olive (pomace)	93.9 ± 2	1.8 ± 0.0	2.5 ± 0.1	ND	ND	ND	ND	ND
7	Virgin olive	0.4 ± 0.0	0.4 ± 0.0	0.9 ± 0.02	ND	ND	ND	ND	ND
8	Extra virgin olive	10.6 ± 0.4	0.6 ± 0.0	3.97 ± 0.1	ND	ND	ND	ND	ND
9	Extra virgin olive (cold press)	26.2 ± 0.6	1.0 ± 0.0	0.94 ± 0.03	ND	ND	ND	ND	ND
10	Sunflower (cold press)	300 ± 10	20.6 ± 0.7	1 ± 0.04	ND	ND	ND	ND	ND
11	Sunflower	79.7 ± 3	5.0 ± 0.2	0.77 ± 0.02	ND	ND	ND	ND	ND
12	Soybean	16.8 ± 0.6	ND	353 ± 10	203 ± 3	ND	ND	ND	ND
13	Soybean	30.8 ± 1	ND	269 ± 7	213 ± 12	ND	ND	ND	ND
14	Rice bran	10.2 ± 0.4	ND	5.6 ± 0.2	0.64 ± 0.1	ND	ND	35.4 ± 0.1	7.5 ± 0.1
15	Rice bran	32.2 ± 1	ND	21.9 ± 0.8	1.13 ± 0.0	ND	ND	167 ± 1	ND
16	Grape seed	50.8 ± 2	1.3 ± 0.0	3.3 ± 0.1	ND	5.45 ± 0.4	ND	79.2 ± 3	0.23 ± 0.0
17	Walnut	8.38 ± 0.2	1.7 ± 0.1	143 ± 5.7	18.8 ± 0.7	51 ± 1	ND	85.6 ± 1	17.3 ± 0.5

[a] BLD, below limit of detection.
[b] ND, nondetectable.

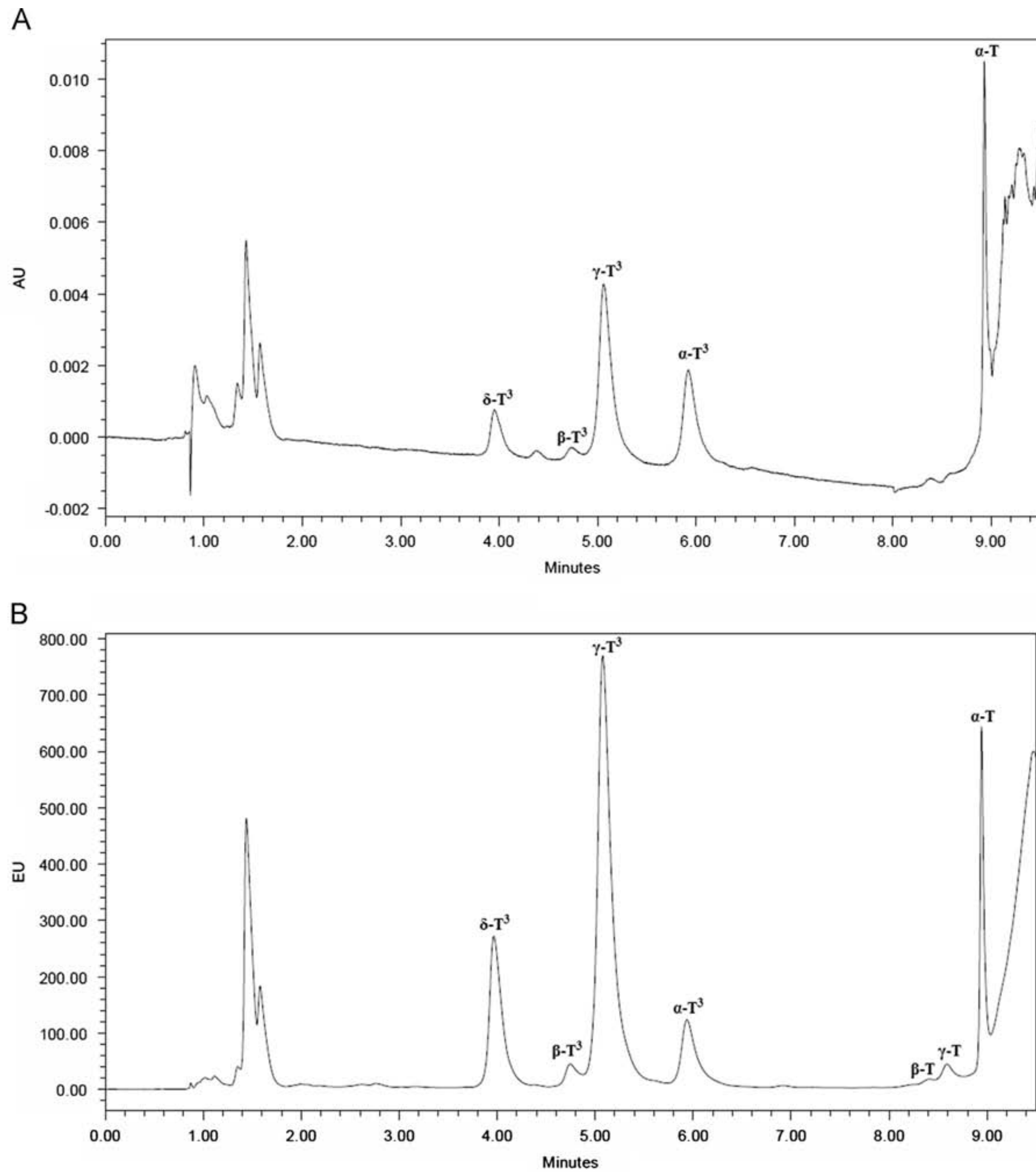

Fig. 3. Representative chromatograms of carotino oil sample using UV (A) and FL (B).

Table 6
Vitamin E compositions (mg kg^{-1}) of tested margarines using the proposed UPLC method (FL detection).

No.	Oil composition	Average concentration ± SD							
		α-T	β-T	γ-T	δ-T	α-T^3	β-T^3	γ-T^3	δ-T^3
1	Soybean, palm, coconut, rapeseed	54.8 ± 2	[a]BLD	90.3 ± 3	11.0 ± 0.4	6.20 ± 0.1	[b]ND	19.5 ± 0.8	1.11 ± 0.1
2	Soybean	41.5 ± 2	ND	43.5 ± 2	ND	ND	ND	ND	ND
3	Palm, palm kernel, sunflower, soybean	50.1 ± 1	ND	96.5 ± 2	0.97 ± 0.0	ND	ND	2.84 ± 0.1	ND
4	Not labelled	39.9 ± 1	1.90 ± 0.1	152 ± 4	1.58 ± 0.3	ND	ND	12.4 ± 0.4	ND
5	Rapeseed, sunflower, palm, soybean	82.2 ± 2	0.90 ± 0.1	164 ± 4	34.1 ± 1	4.16 ± 0.0	ND	8.11 ± 0.2	0.22 ± 0.0
6	Rapeseed, palm, sunflower, soybean	55.3 ± 1	0.54 ± 0.0	122 ± 3	35.0 ± 0.8	ND	ND	2.44 ± 0.0	ND
7	Palm, palm kernel	54.0 ± 1	1.30 ± 0.1	22.2 ± 0.8	ND	53.8 ± 2	ND	127 ± 4	17.2 ± 0.6
8	Canola	19.6 ± 0.3	ND	34.9 ± 0.8	ND	ND	ND	ND	ND
9	Palm, palm kernel	35.2 ± 0.4	ND	ND	ND	54.9 ± 0.2	ND	61.8 ± 0.3	ND

[a] BLD, below limit of detection.
[b] ND, nondetectable.

Table 7
Vitamin E compositions of tested supplement capsule samples using the proposed UPLC method.

No.	Type of supplement (content per capsule)	Average content per capsule (mg ± SD)										Assay (%)
		α-T	β-T	γ-T	δ-T	α-T³	β-T³	γ-T³	δ-T³	α-T acetate	Total Vitamin E	
1	**α-T (250 IU ~ 167 mg)**	129 ± 3	1.40 ± 0.3	2.00 ± 0.1	[a]ND	2.82 ± 0.4	ND	[b]BLD	ND	ND	135 ± 4	81.0
2	**α-T acetate (400 IU ~ 294 mg)**	2.96 ± 0.3	ND	1.13 ± 0.0	BLD	1.14 ± 0.2	ND	BLD	ND	211 ± 8	216 ± 8	73.4
3	**Salmon oil**	4.07 ± 0.1	0.05 ± 0.0	0.04 ± 0.0	ND	0.03 ± 0.0	ND	BLD	ND	ND	4.19 ± 0.1	–
4	**Omega-3 fish oil**	0.19 ± 0.0	2.32 ± 0.1	ND	ND	0.03 ± 0.0	ND	ND	ND	ND	2.54 ± 0.1	–

All components were detected using FL except α-T acetate (PDA detection).

[a] ND, Nondetectable.
[b] BLD, Below limit of detection.

impart a distinct red color to the oil [34,35]. Gan et al., reported that this highly colored oil is rich in unsaturated fatty acids (80.9%), which is markedly different compared to palm oil (~50%) [35]. As noted earlier, one of the unique features of palm oil is the vitamin E content, represented mainly by T³ (70%) rather than T (30%) [36]. Results obtained for the other oils are generally in good agreement with the literature [33,37].

3.4. Analysis of margarine samples

Nine margarine samples were analyzed. α-T (19.6 ± 0.3 to 82.2 ± 1.7 mg kg^{-1}) was found in all the analyzed samples, other vitamin E homologues (except β-T³) varied depending to the type of vegetable oils used in the manufacturing process. The fact that T³s was present in most of the analyzed samples supports the current trend of manufacturers in using inter-esterification of mixed oils to produce low- or trans-free margarines. As shown in Table 6, it can be seen that most of the margarines (samples 1, 3–7 and 9) contained inter-esterified palm oil. Bayard et al. also suggested that partially hydrogenated oils can be replaced by palm oils to produce virtually TFA-free margarines [38].

3.5. Analysis of supplements

Vitamin E was found in all of the tested supplement samples (range, 2.54–216 mg per capsule) (Table 7). Vitamin E content in capsules was less than the manufacturer's declared concentrations (% assay, 81.0 and 73.4% for α-T and α-T acetate capsules, respectively) were probably due to the long shelf-life, or alternative analytical methods such as UV spectroscopy were used. The UV method, widely used in the industry, approximates vitamin E content as α-T or α-T acetate.

4. Conclusion

A UPLC method for the simultaneous determination of ten vitamin E components had been for the first time reported. The developed method was successfully validated and applied to the determination of T and T³ homologues and derivatives in vegetable oils, margarines and supplement samples. The method exhibited considerable advantages as it is not only simpler, faster (≈ 9.5 min) and sensitive (especially with FL detection) but also managed to overcome the pressure build-ups that are inherent of the conventional RP-HPLC systems. The profiling of these vitamin E analogues can be very useful especially to distinguish palm oil from olive or soya bean oils. Hence, the method is useful not only for routine quality control of vegetable oils and margarines but also in clinical, dietary and nutrition studies.

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