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Simple reversed-phase liquid chromatography method for determination of tocopherols in edible plant oils

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Abstract

A simple and rapid reversed-phase high-performance liquid chromatography method for determination of α-, (β + γ), and δ-tocopherols in edible plant oils has been developed. Oils are diluted in 2-propanol and injected directly onto Symmetry C_{18} column. Methanol and acetonitrile (1:1) are used as a mobile phase. Tocopherols are detected using fluorescence detector set at excitation and emission wavelength 295 nm and 325 nm, respectively. The method is precise (R.S.D. not higher than 2.24%) and sensitive–detection limits (DL) are 8 ng/ml for γ- and δ-tocopherols and 28 ng/ml for α-tocopherol; quantification limits (QL) were calculated as three times higher than DL.

Keywords: Tocopherols; Oils; Vitamins; Food analysis.

1. Introduction

Vitamin E is a general term used for designation of tocopherols and tocotrienols (α-, β-, γ-, δ-). They function as the most effective lipid-soluble antioxidants, protecting cell membranes from peroxyl radicals and mutagenic nitrogen oxide species [1–2]. More recently, alternative non-antioxidant functions of vitamin E have been proposed, in particular, role as a "gene regulator" at the level of mRNA or protein. It could be consequent to regulation of gene transcription, mRNA stability, protein translation, protein stability and posttranslational events [3–5]. Moreover, tocopherols provide immunoprotection [6], antiproliferative [7] and anticlotting effects [8], reduce LDL oxidation, platelet adhesives and thrombosis [9–12]. Increased vitamin E intake has been inversely associated with lower risk of cardiovascular and coronary heart diseases [13–15]. α-Tocopherol exhibits the greatest activity in the prevention of vitamin E deficiency abnormalities, whereas γ-tocopherol was found to be potent NO(X) radical scavenger [2] with major implications in chronic inflammation [16,17] and steroid hormone [18] associated carcinogenesis. The main sources of vitamin E in human diet are vegetable oils, which may contain 70–1900 mg of tocopherols per kilogram of oil.

A variety of methods have been described for determination of vitamin E homologues and isomers. Normal-phase (NP) or reversed-phase (RP) high-performance liquid chromatography (HPLC) separations with UV and/or fluorescence detections have been mostly used. The NP columns provide separation of all tocopherols, while RP columns, which are usually octadecylsilane modified silica (C_{18}), do not completely resolve β- and γ-tocopherols, although recently some satisfactory separations were achieved with polymeric octadecyl polyvinyl alcohol [19], ODS-2 [20] and a C_{30} column [21]. On the other hand, the main advantages of RP-chromatography with comparison to NP-chromatography are fast equilibration time and better reproducibility of retention times [22]. When separation of β- and γ-tocopherols is not the point of analysis, RP-columns are preferred. In some HPLC methods, higher temperatures than ambient have to be used to obtain satisfactory separation of tocopherols [20,23].

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The sample preparation is generally one of the most tedious and time-consuming steps, which may also have effect on error propagation. In vitamin E analysis of oils using NP-HPLC, the sample is usually diluted in hexane or hexane with a small amount of a more polar modifier such as 2-propanol [24,25] and the sample is filtered or directly injected onto NP-column. If chromatography is performed by RP-HPLC, sample of oil in hexane is significantly diluted with methanol, ethanol or 2-propanol, or oil is dissolved in acetonitrile–methanol–propanol mixture, 2-propanol or tetrahydrofuran [20,23,26–30].

RP-HPLC usually resolves most of the analytical problems related to tocopherols analysis in more simple and rapid way than other methods, thus the main aim of the analytical chemistry is development of new RP-HPLC methods, which can be used for the routine analysis of vitamin E homologues and isomers. Although some RP-HPLC methods for simultaneous determination of all tocopherols in vegetable oils are already published [20,23,26–31] but they use higher than ambient temperature for separation of tocopherols and/or additional treatment than simple dissolving of oil in one type of solvent. Thus, in the present study, a simple, rapid and precise RP-HPLC method performed at room temperature and using fluorescence detection is proposed for determination of α-, (β + γ)-, δ-tocopherols in edible plant oils.

2. Experimental

2.1. Materials

Bottles of olive, grapeseed, rapeseed, peanut, sunflower, soybean and corn oils were purchased from local markets. Standard of α-tocopherol was purchased from Aldrich (Steinheim, Germany), γ- and δ-tocopherols were from Sigma (St. Louis, USA). All solvents were of HPLC grade.

2.2. Sample and standard preparation

Samples of olive, corn, peanut, grapeseed, rapeseed, sunflower and soybean oils were weighted (0.0400–0.1200 g) and dissolved in 1 ml of 2-propanol. 2-Propanol was chosen because it allows solubility of oils and it is miscible with all solvents used in chromatography. Thus, no additional sample treatment is necessary. Vortex-mixed samples were directly injected onto HPLC column. Stock and working solutions of tocopherols were also prepared in 2-propanol. Sample and standard solutions were prepared directly before analysis. Care was taken to exclude air and light exposure of sample and standard solutions throughout the analytical procedure.

2.3. HPLC analysis of tocopherols

All HPLC analyses of tocopherols were performed at room temperature on Waters 600 high performance liquid chromatograph (Waters, Millford, MA, USA) equipped with Symmetry C_{18} (150 mm × 3.9 mm, 5 μm, Waters, Millford, MA, USA) fitted with μBondapak C_{18} cartridge guard column (Waters, Millford, MA, USA). For determination of tocopherols in oils, a mobile phase consisting of 50% of acetonitrile (solvent A) and 50% of methanol (solvent B) was used with the flow rate 1 ml/min. Injection volume was 20 μl; rheodyne type injector was used. The eluate was detected using a Waters 474 scanning fluorescence detector set at emission wavelength of 325 nm with an excitation at 295 nm. Emission slit width was 10 nm, fluorometer gain 100, and attenuation 1. Tocopherols were identified by comparing their retention times with those of corresponding standards and by spiking of samples with appropriate standard. Additionally, the Waters 996 photodiode-array detector was used to identify the compounds on the basis of their absorption spectra.

3. Results and discussion

3.1. Determination of oil concentration in sample

In order to determine the most suitable concentration of oil in sample used in HPLC analysis, different amounts of sunflower and soybean oils were dissolved in 2-propanol. A linearity of fluorescence signals for α-, (β + γ)- and δ-tocopherols with increasing amount of oil was observed to at least 150 mg/ml for sunflower oil and 100 mg/ml for soybean oil. The difference was related to the higher concentration of tocopherols in soybean oil than in sunflower one. To be sure that relationship between the amount of sample used and fluorescence signals is linear for all oils analysed, the concentration of oil in all samples was not higher than 120 mg/ml.

Table 1
Values of the retention factor (k') and the separation factor (α) for tocopherols

Compound	k'	α
δ-Tocopherol	4.13	1.17
γ-Tocopherol	5.03	1.22
α-Tocopherol	5.94	1.18

Table 2
Method validation parameters for determination of tocopherols in plant oils

	α-Tocopherol	γ-Tocopherol	δ-Tocopherol	Total
Standard linearity				
Range (μg/ml)	0.5–100	0.5–25	0.5–25	–
r	0.999	0.999	0.999	–
Precision (n = 6)				
Mean (mg/kg)	159.47	521.13	190.12	870.71
R.S.D. (%)	2.45	1.69	0.51	1.76
Sensitivity				
DL[a] (ng/ml)	28	8	8	–
QL[b] (ng/ml)	84	24	24	–

[a] Calculated based on a S/N ratio of three.
[b] Calculated as 3 × DL.

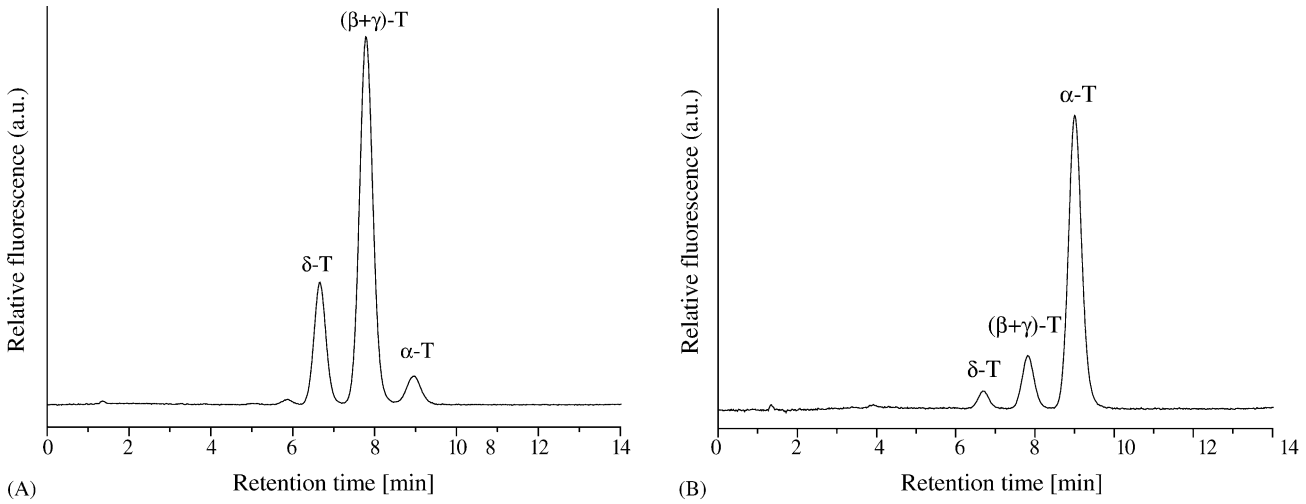

Fig. 1. Typical RP-HPLC chromatogram of: (A) soybean and (B) sunflower oils. The conditions were as follows: mobile phase acetonitrile–methanol (1:1) on a Symmetry C_{18} column; a scanning fluorescence detector with excitation and emission wavelengths of 295 nm and 325 nm, respectively; a.u.: arbitrary units.

3.2. Analytical characteristics

The RP-elution system was designed to achieve optimal separation of α-, (β + γ)-, δ-tocopherols within a reasonable time period. Fig. 1 shows the typical chromatograms obtained for oils tested. The retention times of δ-, γ- and α-tocopherol were about 6.7 min, 7.8 min and 9.0 min, respectively. Table 1 gives the values of the retention factor (k') and the separation factor (α) obtained for tocopherols. The k' values were within the optimum range ($1 \leq k' \leq 10$) for satisfactory chromatographic elution of tocopherols. All α values were higher than 1, what indicates good separation.

Reliability of method was tested for linearity, precision and sensitivity (Table 2). Quantification of tocopherols was performed using external standard method. The eight-point calibration curves were prepared with the standard solutions of α-, γ- and δ-tocopherols in 2-propanol at the levels similar to those present in assessed oils. All curves were linear to at least 25 μg/ml for γ- and δ-tocopherols and 100 μg/ml for α-tocopherol. The relative standard deviations (R.S.D.) of the standard curve slopes (n = 4), generated during a 4-month period, were 2.94%, 1.39% and 2.56% for α-, γ- and δ-tocopherol standard curve, respectively. Linear correlation coefficients (r) for all standard curves were not lower than 0.999 (r^2 = 99.9%). Instrumental precision was checked from six consecutive injections of soybean oil sample and the R.S.D. obtained was not higher than 2.45%. The detection limits (DL) and quantification limits (QL) (at signal to noise ratio S/N = 3) for tocopherols under the working conditions proposed were not higher than 28 ng/ml and 84 ng/ml, respectively (Table 2).

3.3. Quantification of tocopherols in edible plant oils

The distribution of individual tocopherols and their total content in assessed oils are reported in Table 3. This Table contains also precision data obtained using proposed method. All values are arithmetic mean of at least three separate determinations. In corn, soybean and rapeseed oils (β + γ)-tocopherols dominate. α-Tocopherol dominates in olive, grapeseed and sunflower oils. Peanut oil contains similar amounts of α- and (β + γ)-tocopherols, whereas soybean oil contains similar amounts of α-, and δ-tocopherol. The concentration of δ-tocopherol in all oils, with exception of soybean oil, is not higher than 30 mg/kg. The results obtained are generally in agreement with the literature data [25,29].

Table 3
Distribution of α-, (β + γ)-, δ-tocopherols in assessed oils, total tocopherol content and precision data for their determination

Oil	α-Tocopherol			(γ + β)-Tocopherol			δ-Tocopherol			Total tocopherols		
	Mean (mg/kg)	S.D. (mg/kg)	R.S.D. (%)	Mean (mg/kg)	S.D. (mg/kg)	R.S.D. (%)	Mean (mg/kg)	S.D. (mg/kg)	R.S.D. (%)	Mean (mg/kg)	S.D. (mg/kg)	R.S.D. (%)
Corn	203.78	1.37	0.67	582.66	8.00	1.37	29.36	0.58	1.98	815.80	8.11	0.99
Peanut	100.16	2.10	2.10	111.99	1.70	1.52	11.94	0.61	5.13	224.09	4.22	1.88
Grapeseed	100.55	2.67	2.66	17.14	0.56	3.26	3.89	0.18	4.73	121.58	2.80	2.30
Rapeseed	195.13	9.11	4.67	298.68	9.03	3.02	11.85	0.39	3.32	505.67	10.16	2.01
Sunflower	591.25	5.32	0.90	25.40	0.14	0.54	8.68	0.27	3.17	625.33	5.33	0.85
Soybean	153.43	1.84	1.20	504.01	2.17	0.43	188.53	0.72	0.38	845.97	4.35	0.51
Olive oil	160.78	1.01	0.63	12.47	0.16	1.25	1.32	0.14	10.77	174.58	1.16	0.67

Data are calculated from at least three separate samples.

Lack of separation of β- and γ-tocopherols in the case of edible plant oils introduces rather small error in quantification of these isomers. This is because, according to literature [32,33] plant oils do not contain β-tocopherol or contain it in relatively small quantities (0–2.80 mg/100 g depending of oil) as compared to γ-tocopherol. These quantities are comparable or even smaller than standard deviation values obtained for the concentration of (β + γ)-tocopherols in oils tested (Table 3). When necessary, other methods for determination of tocopherols in not only plant oils but also in other samples could be applied [30,31]. Moreover, if determination of tocotrienols is required, which in fact in plant oils are present in small quantities [33] the NP-HPLC methods could be recommended. The comprehensive reviews on vitamin E analysis using different chromatographic methods and techniques have been already published by Rupérez et al. [30] and Abidi [31].

4. Conclusions

In conclusion, this study presents simple, fast, precise and sensitive method for determination of tocopherols in edible plant oils. Plant oils contain relatively small quantities of β-tocopherol as compared to the other homologues and isomers [32,33], thus lack of separation of β- and γ-tocopherols did not introduce significant error in quantification of the latter one. Therefore, the method proposed can be useful for the routine analysis of α-, (β + γ)- and δ-tocopherols in plant oils. Separation and characterisation of vitamin E homologues and isomers in edible plant oils can be important in some studies. This is because tocopherols in crude vegetable oils are partially destroyed by refining treatments. The distribution and concentration of tocopherols is also changing during storage of oils. The assessment of the influence of genetic modification of oil seeds on the distribution of tocopherols is also of interest. Thus, the method proposed can be useful in the routine qualitative and quantitative analysis of tocopherols present in these products.

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