

This article was downloaded by:

On: 23 January 2011

Access details: *Access Details: Free Access*

Publisher *Taylor & Francis*

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597273>

Development and Evaluation of a Normal Phase Liquid Chromatographic Method for the Determination of Tocopherols and Tocotrienols in Walnuts

Joana S. Amaral^{ab}; Susana Casal^c; Beatriz P. P. Oliveira^c; Rosa M. Seabra^a

^a REQUIMTE, Serviço de Farmacognosia Faculdade de Farmácia, Universidade do Porto, Porto, Portugal ^b Escola Superior de Tecnologia e de Gestão, Instituto Politécnico de Bragança, Quinta de Sta. Apolónia, Bragança, Portugal ^c REQUIMTE, Serviço de Bromatologia, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal

To cite this Article Amaral, Joana S. , Casal, Susana , Oliveira, Beatriz P. P. and Seabra, Rosa M.(2005) 'Development and Evaluation of a Normal Phase Liquid Chromatographic Method for the Determination of Tocopherols and Tocotrienols in Walnuts', *Journal of Liquid Chromatography & Related Technologies*, 28: 5, 785 – 795

To link to this Article: DOI: 10.1081/JLC-200048920

URL: <http://dx.doi.org/10.1081/JLC-200048920>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Development and Evaluation of a Normal Phase Liquid Chromatographic Method for the Determination of Tocopherols and Tocotrienols in Walnuts

Joana S. Amaral

REQUIMTE, Serviço de Farmacognosia, Faculdade de Farmácia,
Universidade do Porto, R. Aníbal Cunha, Porto, Portugal, and Escola
Superior de Tecnologia e de Gestão, Instituto Politécnico de Bragança,
Quinta de Sta. Apolónia, Apartado, Bragança, Portugal

Susana Casal and Beatriz P. P. Oliveira

REQUIMTE, Serviço de Bromatologia, Faculdade de Farmácia,
Universidade do Porto, R. Aníbal Cunha, Porto, Portugal

Rosa M. Seabra

REQUIMTE, Serviço de Farmacognosia Faculdade de Farmácia,
Universidade do Porto, R. Aníbal Cunha, Porto, Portugal

Abstract: A high performance liquid chromatographic (HPLC) method for the determination of tocopherols and tocotrienols in walnut samples is described. The compounds were extracted with *n*-hexane, using a simple solid-liquid extraction procedure. Tocol was used as internal standard and BHT as anti-oxidant. The chromatographic separation was achieved using an Inertsil 5 SI normal phase column operating with isocratic elution of *n*-hexane/1,4-dioxane (96.5 : 3.5, v/v), at a flow rate of 0.7 mL/min. The effluent was monitored by a series arrangement of a diode-array detector followed by a fluorescence detector. The detection limits were low, between 0.037 and 0.266 µg/mL. The method was precise (% CV less than

Address correspondence to Rosa M. Seabra, REQUIMTE, Serviço de Farmacognosia Faculdade de Farmácia, Universidade do Porto, R. Aníbal Cunha, 164, 4099-030 Porto, Portugal. E-mail: rseabra@ff.up.pt

2.8%), accurate (% CV less than 5.6%), and, as a general rule, the recovery values were high (mean values ranging from 93.4% to 104.0%).

Keywords: Tocopherol, tocotrienol, normal phase liquid chromatography, walnuts

INTRODUCTION

Tocopherols and tocotrienols are widely distributed in nature, and can be found in vegetable tissues such as seeds, nuts, cereals, and fruits, and also in foods of animal origin such as eggs and dairy products.^[1] The generic term Vitamin E is frequently used to describe tocopherols and tocotrienols since they are a group of structurally related compounds.^[1,2] They all share a common basic structural unit, based on a 6-chromanol ring with a side chain.^[1-3] In nature, four tocopherols can be found (α -, β -, γ - and δ -T) together with their four corresponding unsaturated tocotrienols (α -, β -, γ - and δ -TTR). As a consequence of their phenolic structure, tocopherols and tocotrienols are thought to possess strong antioxidant activity, conferring protection against lipid peroxidation in biological tissues and foods.^[1-5] Although in the past, α -tocopherol has been labelled as the most efficient antioxidant among this family of compounds, being, in consequence, the most studied member; there are many recent studies focusing on the biological activities and health effects of the other vitamin E isoforms.^[4-7]

High performance liquid chromatography (HPLC) is, currently, the technique of choice in the determination of tocopherols in foodstuffs.^[1,3] Although there are several reports referring the determination of tocopherols by reversed phase (RP) HPLC, normal phase (NP) HPLC has the advantage of allowing the resolution of the four isomers (α , β , γ and δ).^[1,3] RP-HPLC does not allow the complete resolution of β and γ isomers, and consequently, in RP-HPLC these two vitamers are quantified together.^[8-14] Although several detectors have been used in the analysis of these compounds,^[3,15,16] the more common include the ultra-violet and the fluorescence detectors.

Tocopherols have been extensively analysed in food products such as vegetable oils,^[9,10,17,18] margarines,^[19] milk and milk products,^[11,15,20,21] seeds,^[22] and also in tissues and biological fluids,^[8,23,24] but there are limited data concerning the determination of tocotrienols.^[25-28] Walnuts are a food product characterized by high quantities of unsaturated lipids, therefore expected to be particularly sensible to oxidation processes. Nevertheless, this kind of food is usually rich in Vitamin E, which functions protectively,^[1] both for the matrix itself and for those who consume it. However, bibliographic data regarding the determination of these compounds in walnuts is scarce and just refers to tocopherols composition.^[29] Lavendrine and co-workers^[29] reported a RP-HPLC method for the determination of

tocopherols in walnuts, but it only allowed the good resolution of three isomers. As far as we know, there are no reports concerning the simultaneous determination of tocopherols and tocotrienols in walnuts. Due to the wide variation of biological activities exhibited by tocopherols and tocotrienols vitamers, the determination and quantification of the individual compounds is becoming more important than the evaluation of the vitamin E global content. Since walnuts are widely consumed by the population in general, and perhaps even more in vegetarian diets, the aim of this study was to simultaneously quantify tocopherols and tocotrienols individual vitamers in walnuts, by a rapid, reproducible, and accurate NP-HPLC method. Despite the structural similarity of these compounds, the technique was validated for each of the compounds under analysis.

EXPERIMENTAL

Equipment

The chromatographic analysis was carried out in an analytical HPLC unit equipped with a PU-980 pump, an AS-950 auto-sampler, a MD-910 multiwavelength diode array detector (DAD), and a FP-920 programmable fluorescence detector (Jasco, Japan). Data was analyzed using a Borwin-PDA Controller Software (JMBS, France). The chromatographic separation was achieved with an Inertsil 5 SI (250 × 3 mm) normal phase column from Varian (Middelburg, Netherlands) operating at ambient temperature.

Standards and Reagents

Standard Solutions

Tocopherols (α , β , γ and δ) and tocotrienols (α , β , γ and δ) were purchased from Calbiochem (La Jolla, CA, USA). Individual stock solutions (5 mg/mL) of the 8 isomers were prepared in *n*-hexane, flushed with nitrogen and stored protected from light, at -20°C . A stock standard mixture, with the different isomers in relative proportions similar to those presented by the samples analyzed, was prepared by diluting appropriate volumes of stock solutions with *n*-hexane: α -tocopherol (75 $\mu\text{g}/\text{mL}$), β -tocopherol (30 $\mu\text{g}/\text{mL}$), γ -tocopherol (150 $\mu\text{g}/\text{mL}$), δ -tocopherol (75 $\mu\text{g}/\text{mL}$), α -tocotrienol (30 $\mu\text{g}/\text{mL}$), β -tocotrienol (4 $\mu\text{g}/\text{mL}$), γ -tocotrienol (10 $\mu\text{g}/\text{mL}$) and δ -tocotrienol (30 $\mu\text{g}/\text{mL}$). Working standard mixtures, with concentrations in the expected samples range, were prepared from the stock standard mixture.

Internal Standard

Rac-Tocopherol (Tocol) (Matreya Inc., PA, USA) was used as internal standard (IS). A stock solution (10 mg/mL) prepared in *n*-hexane was kept at -4°C , protected from light, and diluted to working solutions (500 $\mu\text{g}/\text{mL}$) as necessary.

Anti-oxidant

2,6-Ditertbutyl-4-methylphenol (BHT) was obtained from Aldrich (Madrid, Spain). A working solution was prepared in *n*-hexane at a concentration of 10 mg/mL.

Other

n-Hexane was HPLC-grade from Merck (Darmstadt, Germany) and 1,4-dioxane was from Fluka (Madrid, Spain). All other reagents were of analytical grade.

Sample Preparation

Walnut samples were collected in Coimbra, Portugal. The samples were manually cracked, shelled, and chopped in a coffee mill and then immediately extracted using a simple solid-liquid extraction procedure. Then, a sample (approximately 600 mg) of finely chopped walnuts was accurately weighted in glass screw cap test tubes (Supelco, Bellefonte, Pa., USA) and, subsequently, the internal standard (150 μL of tocol solution) and the antioxidant (100 μL of BHT solution) were added. The sample was homogenized for 1 min by vortex mixing, after the addition of each of the following reagents: ethanol (2 mL), extracting solvent (*n*-hexane, 4 mL), and NaCl saturated solution (2 mL). Subsequently, the sample was centrifuged (2 min, 5000 rpm) and the clear upper layer removed to another screw cap test tube. The sample was re-extracted twice. The combined extracts were taken to dryness under a nitrogen steam on a Reacti-Therm module (Pierce, Rockford, IL, USA) and the residue reconstituted to a volume of 1.5 mL with *n*-hexane. The extract was dried with anhydrous sodium sulphate, centrifuged (10000g, 20 seconds), transferred into a dark 2.0 mL vial, and loaded into the HPLC programmable auto-sampler.

Chromatography

The best resolution was obtained with a mixture of *n*-hexane and 1,4-dioxane 96.5 : 3.5, v/v, at a flow rate of 0.7 mL/min. The injection volume was 10 μL . The effluent was monitored with a diode-array detector, connected in series

with a fluorescence detector programmed for excitation at 290 nm and emission at 330 nm. The compounds were identified by comparing their retention time and UV spectra with authentic standards. Quantification was made by fluorescence detection based on the internal standard method.

RESULTS AND DISCUSSION

Since the compounds under study are sensitive to light, heat, and oxygen,^[1,2] all standards and samples preparation was performed in a dark room with subdued red light and, as possible, during all the procedures the samples were kept on ice. In order to avoid the oxidation of the vitamins, several authors suggest the use of an antioxidant. Since BHT is often described for this purpose, and under the conditions of this method it gives no interferences with any eluting compound, it was used as antioxidant.

Eluent

Hexane, alone or with small quantities of more polar solvents, is the most frequently used mobile phase in the analysis of tocopherols using NP-HPLC,^[12] In the present work, the used mobile phase was a mixture of *n*-hexane and 1,4-dioxane, being the organic polar modifier (1,4-dioxane) tested in different proportions (2.5%, 3%, 3.5%, 4% and 5%). Better results were achieved using 3.5% (v/v) of 1,4-dioxane, allowing a good separation of all isomers in a short period of time.

Fluorescence Detector Settings

The fluorescence detector was selected for quantification purposes since it provided a higher sensitivity than the DAD detector. Since γ -tocopherol is reported to be the major isomer in walnuts^[18,29] and the other isomers were expected to occur in much lower quantities, different gains settled in the fluorescence detector were tested, in order to detect all the 8 isomers in the same run, and at the same time, allow their quantification. The better conditions achieved were: 0 min: gain 10; 11.5 min: gain 100; 14.3 min: gain 10 (Figure 1A). Although the proposed conditions allow the quantification of the 8 isomers in the same run, only 5 isomers were detected in the studied samples (Figure 1B).

Standard Calibration Curves and Detection Limits

In order to improve the accuracy, precision, and robustness of the method, quantification was done by the internal standard method. Tocol was used as

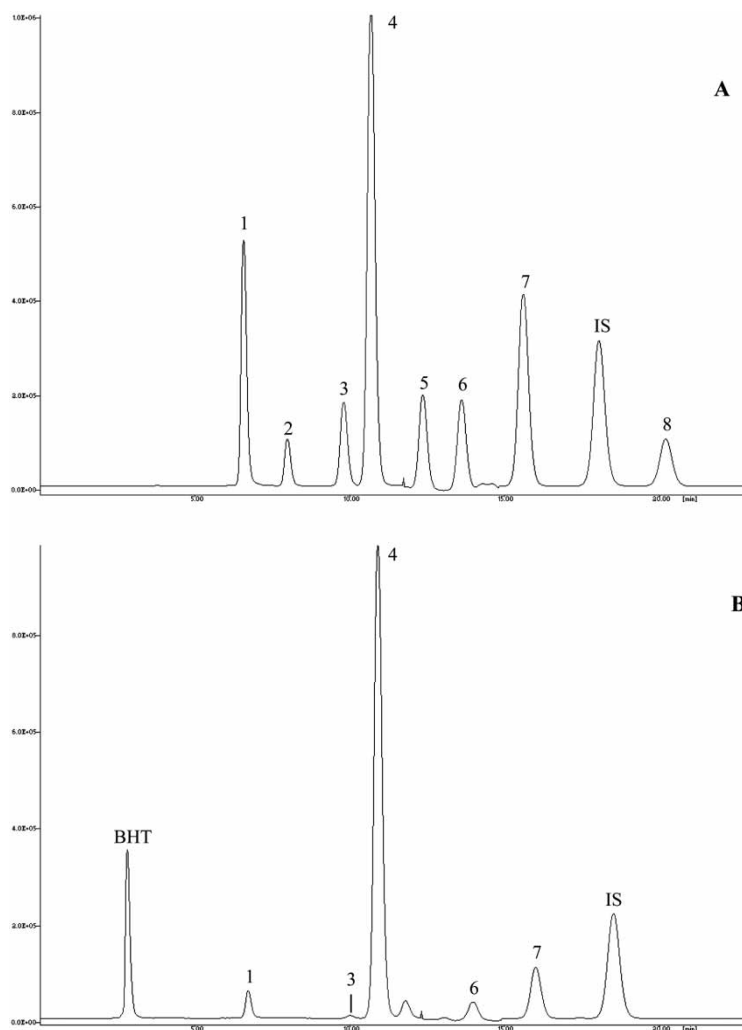


Figure 1. Chromatogram of a working standard mixture (A) and of a walnut sample (B). Peaks: I.S., internal standard (tocol); 1, α -tocopherol; 2, α -tocotrienol; 3, β -tocopherol; 4, γ -tocopherol; 5, β -tocotrienol; 6, γ -tocotrienol; 7, δ -tocopherol; 8, δ -tocotrienol. HPLC conditions as described in Experimental.

internal standard, since it is reported to be frequently employed for this purpose due to its chemical similarity with the analysed compounds.

A linear relationship between the concentration of the compounds and the fluorescence detector response was obtained under the assayed conditions (Table 1). A 6-level calibration curve was constructed for each compound,

Table 1. Analytical characteristics of the reported method and vitamin E isomers content of a walnut sample

Compound	Rt		Correlation coefficient (r ²)	Linearity range (µg/mL)	Detector gain	Detection limit (µg/mL)	Precision CV % (n = 6)	Reproducibility CV % (n = 6)	Walnut sample	
	min	CV % (n = 6)							Mean	± SD
α-tocopherol	6.67	0.12	0.9994	0.4–37.5	10	0.304	0.86	3.78	9.99	±0.09
α-tocotrienol	8.11	0.15	0.9994	0.3–15.0	10	0.221	—	—	nd	
β-tocopherol	10.19	0.21	0.9992	0.16–15.0	10	0.160	2.32	3.51	0.72	±0.02
γ-tocopherol	11.10	0.21	0.9994	0.8–100.0	10	0.110	0.52	2.36	190.05	±0.99
β-tocotrienol	12.87	0.22	0.9996	0.04–2.0	100	0.003	—	5.64	nd	
γ-tocotrienol	14.17	0.20	0.9995	0.1–5.0	100	0.026	2.84	3.28	2.53	±0.07
δ-tocopherol	16.24	0.21	0.9999	0.16–15.0	10	0.025	1.06	1.90	8.37	±0.09
I.S. (tocol)	18.96	0.26	—	—	10	—	—	—	—	
δ-tocotrienol	21.06	0.27	0.9993	0.3–8.0	10	0.224	—	—	nd	

Rt: Retention time; cv: coefficient of variation; SD: standard variation; nd: not detected.

using the peak-area ratio between the vitamin E isomer and tocol versus concentration of the standard ($\mu\text{g}/\text{mL}$). The calibration curves were obtained by triplicate determinations for each level used. The correlation coefficients invariably exceeded 0.999 for all the compounds (Table 1). The detection limits were calculated as the concentration corresponding to three times the standard deviation of the background noise, and the values ranged from 0.037 to 0.266 $\mu\text{g}/\text{mL}$ (Table 1).

A typical chromatogram of a standard mixture is shown in Figure 1. The retention times (RT) obtained for the studied compounds are present in Table 1.

Validation of the Method: Precision, Accuracy, and Recoveries

The precision of the chromatographic method was evaluated by measuring the peak chromatographic area of the compounds in the same sample extract injected 6 times. The chromatographic method proved to be precise, since the coefficients of variation were low (between 0.52% and 2.84%). For each compound detected in walnut samples, the reproducibility of the method was evaluated by determining the coefficient of variation of six consecutive extractions of the same hazelnut sample. The obtained coefficient of variation values were low (ranging from 1.90% to 5.64%). Results are shown in Table 1.

The effectiveness of the extraction procedure and the accuracy of the method were evaluated by the standard addition procedure (% of recovery) with three additional levels for each compound and duplicate determinations for each concentration level. Results of recovery studies are presented in Table 2 and demonstrate the good recovery for the compounds under study (mean values ranging from 93.4% to 104.0%). These results confirmed no interferences effects due to the complexity of the matrix.

Mean results obtained for the walnut cultivar under study are presented in Table 1. Figure 1B shows a typical chromatogram of a walnut sample.

CONCLUSIONS

The mobile phase composition and the fluorescence detector settings were optimized in order to separate all the isomers and allow the accurate quantification of the ones detected in walnut samples. The use of the fluorescence detector provided a higher sensitivity and selectivity, while the use of the DAD detector allowed the confirmation of the identity of the compounds by its spectrum analysis. The main advantages of this method are the simple sample preparation, and the simultaneous determination of the eight vitamin E naturally occurring isomers in less than 22 min. Although in walnut samples, besides the four tocopherols (α -, β -, γ -, δ -T) only one

Table 2. Recoveries of tocopherols and tocotrienols from a spiked walnut sample^a

	Present ($\mu\text{g/g}$)	Added ($\mu\text{g/g}$)	Found ($\mu\text{g/g}$)	Recovery	
				%	Average (%)
α -tocopherol	9.99	12.12	22.63	102.3	98.5
		24.17	33.01	96.6	
		48.53	56.42	96.5	
α -tocotrienol	0.00	4.85	4.87	100.5	95.7
		9.67	9.11	94.2	
		19.41	17.91	92.4	
β -tocopherol	0.72	4.85	5.26	94.4	93.4
		9.67	9.70	93.3	
		19.41	18.60	92.5	
γ -tocopherol	190.05	24.25	228.18	106.5	104.0
		48.34	242.79	101.8	
		97.05	297.83	103.8	
β -tocotrienol	0.00	0.65	0.63	98.1	95.5
		1.29	1.23	95.1	
		2.59	2.41	93.3	
γ -tocotrienol	2.53	1.62	4.19	101.2	97.0
		3.22	5.45	94.9	
		6.47	8.54	95.0	
δ -tocopherol	8.37	4.85	13.68	103.4	101.8
		9.67	18.31	101.5	
		19.41	27.89	100.5	
δ -tocotrienol	0.00	4.85	4.86	100.1	96.2
		9.67	9.20	95.1	
		19.41	18.08	93.3	

^aMean value found for duplicate assays for each studied concentration.

tocotrienol (γ -TTR) was detected, the method seems to be useful when applied to similar matrices having other tocotrienols in their composition, since the recoveries values were high for all the compounds, including the isomers not detected in the samples.

In conclusion, the technique herein presented proved to be suitable for the simultaneous and individual determination of all tocopherols and tocotrienols isomers in the proposed matrix (walnuts). This is of great importance, since it is known that the different vitamers present different biological potencies. The results obtained demonstrate that the method is sensitive, precise, accurate, and fast, being suitable for routine determinations.

ACKNOWLEDGMENT

J.S. Amaral is grateful to Programa para o Desenvolvimento Educativo para Portugal (PRODEP III) for financial support.

REFERENCES

1. Woollard, D.C.; Indyk, H.E. Tocopherols. In *Encyclopedia of Food Science and Nutrition*; Academic Press: London, 2003; 5789–5796.
2. Lopes, C.; Casal, S.; Oliveira, B.; Barros, H. Retinol, beta-carotene and alpha-tocopherol in heart disease. In *Nutrition and Heart Disease. Causation and Prevention*; Watson, R.R., Preedy, V.R., Eds.; CRC Press: Boca Raton, FL, 2004; 118–136.
3. Rupérez, F.J.; Martín, D.; Herrera, E.; Barbas, C. Chromatographic analysis of α -tocopherol and related compounds in various matrices. *J. Chromatogr. A* **2001**, *935*, 45–69.
4. Mishima, K.; Tanaka, T.; Pu, F.; Egashira, N.; Iwasaki, K.; Hidaka, R.; Matsunaga, K.; Takata, J.; Karube, Y.; Fujiwara, M. Vitamin E isoforms α -tocotrienol and γ -tocopherol prevent cerebral infarction in mice. *Neurosc. Lett.* **2003**, *337*, 56.
5. Theriault, A.; Chao, J.; Wang, Q.; Gapor, A.; Adeli, K. Tocotrienol: a review of its therapeutic potential. *Clin. Biochem.* **1999**, *32*, 309.
6. Campbell, S.; Stone, W.; Whaley, S.; Krishnan, K. Development of gamma (γ)-tocopherol as a colorectal cancer chemopreventive agent. *Crit. Rev. Oncol./Hematol.* **2003**, *47*, 249.
7. Khor, H.T.; Chieng, D.Y. Effect of dietary supplementation of tocotrienols and tocopherols on serum lipids in the hamster. *Nutr. Res.* **1996**, *16*, 1393.
8. Taibi, G.; Nicotra, C.M.A. Development and validation of a fast and sensitive chromatographic assay for all-*trans*-retinol and tocopherols in human serum and plasma using liquid-liquid extraction. *J. Chromatogr. B* **2002**, *780*, 261–267.
9. Gimeno, E.; Castellote, A.I.; Lamuela-Raventós, R.M.; Torre, M.C.; López-Sabater, M.C. Rapid determination of vitamin E in vegetable oils by reversed-phase high performance liquid chromatography. *J. Chromatogr. A* **2000**, *881*, 251–254.
10. Gliszczynka-Swiglo, A.; Sikorska, E. Simple reversed-phase liquid chromatography method for determination of tocopherols in edible plant oils. *J. Chromatogr. A* **2004**, *1048*, 195–198.
11. Mendoza, B.R.; Pons, S.M.; Bargalló, A.I.C.; López-Sabater, M.C. Rapid determination by reversed-phase high performance liquid chromatography of vitamins A and E in infant formulas. *J. Chromatogr. A* **2003**, *1018*, 197–202.
12. Kamal-Eldin, A.; Gorgen, S.; Petterson, J.; Lampi, A. Normal-phase high performance liquid chromatography of tocopherols and tocotrienols. Comparison of different chromatographic columns. *J. Chromatogr. A* **2000**, *881*, 217–227.
13. Abidi, S.L.; Mounts, T.L. Reversed-phase high performance liquid chromatographic separations of tocopherols. *J. Chromatogr. A* **1997**, *782*, 25–32.
14. Pyka, A.; Sliwiok, J. Chromatographic separation of tocopherols. *J. Chromatogr. A* **2001**, *935*, 71–76.
15. Rodrigo, N.; Alegría, A.; Barberá, R.; Farré, R. High performance liquid chromatographic determination of tocopherols in infant formulas. *J. Chromatogr. A* **2002**, *947*, 97–102.

16. Tasioula-Margari, M.; Okogeri, O. Simultaneous determination of phenolic compounds and tocopherols in virgin olive oil using HPLC and UV detection. *Food Chem.* **2001**, *74*, 377–383.
17. Lechner, M.; Reiter, B.; Lorbeer, E. Determination of tocopherols and sterols in vegetable oils by solid-phase and subsequent capillary gas chromatographic analysis. *J. Chromatogr. A* **1999**, *857*, 231–238.
18. Firestone, D.; Reina, R.J. Authenticity of vegetable oils. In *Food authentication*; Ashurst, P.R., Dennis, M.J., Eds.; Chapman & Hall: London, 1996; 198–285.
19. Rader, J.I.; Weaver, C.M.; Patruscu, L.; Ali, L.H.; Angyal, G. α -Tocopherol, total vitamin A and total fat in margarine-like products. *Food Chem.* **1997**, *58* (4), 373–379.
20. Escrivá, A.; Esteve, M.J.; Farré, R.; Frigola, A. Determination of liposoluble vitamins in cooked meals, milk, and milk products by liquid chromatography. *J. Chromatogr. A* **2002**, *947*, 313–318.
21. Panfili, G.; Manzi, P.; Pizzoferrato, L. High performance liquid chromatographic method for the simultaneous determination of tocopherols, carotenes, and retinol and its geometric isomers in Italian cheeses. *Analyst* **1994**, *119*, 1161–1165.
22. Bruni, R.; Medici, A.; Guerrini, A.; Scalia, S.; Poli, F.; Romagnoli, C.; Muzzoli, M.; Sacchetti, G. Tocopherol, fatty acids and sterol distributions in wild Ecuadorian *Theobroma subincanum* (Sterculiaceae) seeds. *Food Chem.* **2002**, *77*, 337–341.
23. Casal, S.; Macedo, B.; Oliveira, M.B.P.P. Simultaneous determination of retinol, β -carotene and α -tocopherol in adipose tissue by high performance liquid chromatography. *J. Chromatogr. B* **2001**, *763*, 1–8.
24. Chatzimichalakis, P.F.; Samanidou, V.F.; Papadoyannis, I. Development and validated liquid chromatographic method for the simultaneous determination of eight fat-soluble vitamins in biological fluids after solid-phase extraction. *J. Chromatogr.* **2004**, *B805*, 289–296.
25. Panfili, G.; Fratianni, A.; Irano, M. Normal phase high performance liquid chromatography method for the determination of tocopherols and tocotrienols in cereals. *J. Agric. Food Chem.* **2003**, *51*, 3940–3933.
26. Gama, P.; Casal, S.; Oliveira, B.; Ferreira, M.A. Development of an HPLC/diode-array/fluorimetric detector method for monitoring tocopherols and tocotrienols in edible oils. *J. Liq. Chromatogr. & Relat. Technol.* **2000**, *23*, 3011–3022.
27. Al-Saqer, J.M.; Sidhu, J.S.; Al-Hooti, S.N.; Al-Amiri, H.A.; Al-Othman, A.; Al-Haji, L.; Ahmed, N.; Mansour, I.B.; Minal, J. Developing functional foods using red palm olein. IV. Tocopherols and tocotrienols. *Food Chem.* **2004**, *85*, 579–583.
28. Hewavitharana, A.K.; Lanari, M.C.; Becu, C. Simultaneous determination of vitamin E homologs in chicken meat by liquid chromatography with fluorescence detection. *J. Chromatogr. A* **2004**, *1025*, 313–317.
29. Lavendrine, F.; Ravel, A.; Poupard, A.; Alary, J. Effect of geographic origin, variety and storage on tocopherol concentrations in walnuts by HPLC. *Food Chem.* **1997**, *58* (1–2), 135–140.

Received October 30, 2004

Accepted November 27, 2004

Manuscript 6519