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Journal of Chromatography A, 881 (2000) 251–254

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Rapid determination of vitamin E in vegetable oils by reversed-phase high-performance liquid chromatography

E. Gimeno, A.I. Castellote, R.M. Lamuela-Raventós, M.C. de la Torre, M.C. López-Sabater*

Dpt. Nutrició i Bromatologia, Centre de Referència en Tecnologia dels Aliments (CeRTA), Facultat de Farmàcia, Universitat de Barcelona, Avda. Joan XXIII s/n, 08028 Barcelona, Spain

Abstract

A quick and direct method for measuring tocopherols (α, β+γ and δ) in vegetable oils has been developed using RP-HPLC with UV detection. Previous extraction of tocopherols is not required. The oil is diluted in hexane and an aliquot is mixed with ethanol containing an internal standard (α-tocopherol acetate). The chromatographic system consists of an ODS-2 column with a methanol–water mobile phase. Tocopherols are detected at 292 nm in less than 5 min after injection. The method is precise (RSD=2.69%) and has a high mean recovery (98.14%).

Keywords: Oils; Food analysis; Vitamins; Tocopherols

1. Introduction

Vegetable oils are the main dietary sources of vitamin E, which decreases the risk of cardiovascular diseases and cancer [1]. Vitamin E is a collective term for tocopherols and tocotrienols, which are natural antioxidants that prevent the rancidity of oils during storage and thus delay its shelf-life [2–4]. Whereas α-tocopherol (5,7,8-trimethyltocol) is the most active form of vitamin E in vivo, γ-tocopherol (7,8-dimethyltocol) is the most active in vitro [5–7].

Several reversed-phase high-performance liquid chromatography (RP-HPLC) studies for the determination of tocopherols in oils have been reported [1,8,9]. However, they involve saponification, which implies multiple solvent extraction, drying and concentration steps. Tocopherols are sensitive to light and air. Thus, procedures requiring many manipulations can result in the partial degradation of these antioxidants and in considerable quantification errors [9]. On the other hand, long exposure to alkaline conditions significantly decreases α-tocopherol [7,10]. Therefore, sample preparation is the key step of the analysis. For these reason, in order to measure tocopherols, some studies have performed direct analysis after only diluting the oil in an organic solvent, but normal-phase (NP) HPLC was used [11–15]. RP versus NP provides higher column stability, reproducibility of retention times, quicker equilibration and shorter analysis time [9,16]. Moreover, RP-HPLC solvent systems preserve the environment more than those used in NP-HPLC [17]. In contrast, NP, unlike RP, resolves β- and γ-tocopherols [9,18]. However, one procedure using RP has been reported to allow an acceptable sepa-

*Corresponding author. Tel.: +34-93-4024-512; fax: +34-93-4021-896.

E-mail address: mclop@farmacia.far.ub.es (M.C. López-Sabater)

0021-9673/00/$ – see front matter
PII: S0021-9673(00)00219-3

ration of all the tocopherols [19]. In this study the oil was diluted in hexane and injected to an immiscible and incompatible mobile phase, which leads to a shorter column life [18].

We aimed to design a quick and simple method, suitable for routine analyses of large amounts of samples, that avoids saponification, and to simplify and shorten the time of analysis.

2. Experimental

2.1. Equipment

Separation by HPLC was carried out using a Hewlett-Packard liquid chromatographic system (Waldbronn, Germany) with an HP-1050 pump system and a Rheodyne Model 7125 injector (Cotati, CA, USA) with a final volume loop of 500 μl. The detector was an HP-1040 M photodiode-array detection (DAD) system. The data were stored and processed by an HPLC Chemstation (Dos Series) (Hewlett-Packard). The column was a Tracer Extrasil ODS-2 (150×4.4 mm I.D., 5 μm particle size) (Tracer Analítica, Barcelona, Spain) protected with a guard cartridge (Tracer, C_{18}, 5 μm) system.

2.2. Reagents and standards

All the solvents, of HPLC-grade, were from SDS (Peypin, France). Ultrapure water was generated by the Milli-Q system (Millipore, Bedford, MA, USA). Vitamin standards were from Sigma (St. Louis, MO, USA). Stock standard solutions of tocopherols and α-tocopherol acetate were prepared in ethanol and stored at −20°C in dark bottles for up to a month. Working standard solutions were prepared from the stock standard solutions every 2 weeks. Since standards are highly susceptible to oxidation and degradation, the purity and the standard concentrations were analysed by HPLC–DAD every week.

2.3. Sample preparation

To prevent the loss of vitamins, amber-coloured material and infrared light were used. We analysed olive oil, virgin olive oil, sunflower oil, corn oil and soybean oil samples. The oil sample was diluted in hexane (1:10). Thereafter, 200 μl was transferred to a screw-capped tube, where 600 μl of methanol and 200 μl of the internal standard solution (300 μg/ml of α-tocopherol acetate in ethanol) were added. After being vortex-mixed and centrifuged (3000 *g*, 5 min), the samples were filtered through a 0.45 μm pore size filter and an aliquot of the overlay was directly injected into the chromatograph. Samples could be kept in dark tubes at −20°C before injection, but only for up to a week.

2.4. Chromatographic analysis and quantification

The injection volume was 50 μl. The mobile phase was methanol–water (96:4, v/v) and the elution was performed at a flow-rate of 2 ml/min. The analytical column was kept at 45°C. To determine the compounds in the samples, the working standard solutions were always analysed together with the samples and peak-area ratios were used for calculations following the internal standard method. Detection was performed at 292 nm and each run lasted 6 min.

3. Results and discussion

3.1. Sample preparation

Fig. 1 shows the chromatograms of a virgin olive oil sample and a soybean oil sample.

Here, previous extraction of tocopherols from the oils was unnecessary, since a preliminary test showed that other minor constituents of the oils, such as carotenoids and phytosterols, did not elute at the same retention times as tocopherols. Dilution of the oil in hexane in a 1:10 ratio resulted in chromatograms with all the tocopherols diluting separately. The selection of a 1:10 dilution was based on trial runs which showed the detector response within the range of the prevailing tocopherols, while the least abundant tocopherols were still detectable.

Many studies published have used direct analysis of tocopherols after diluting the oil in an organic solvent [11,12,14,19]. Saponification causes pronounced losses of tocopherols even in protective conditions such as darkness and high nitrogen [7]. However, direct analysis after dilution, unlike

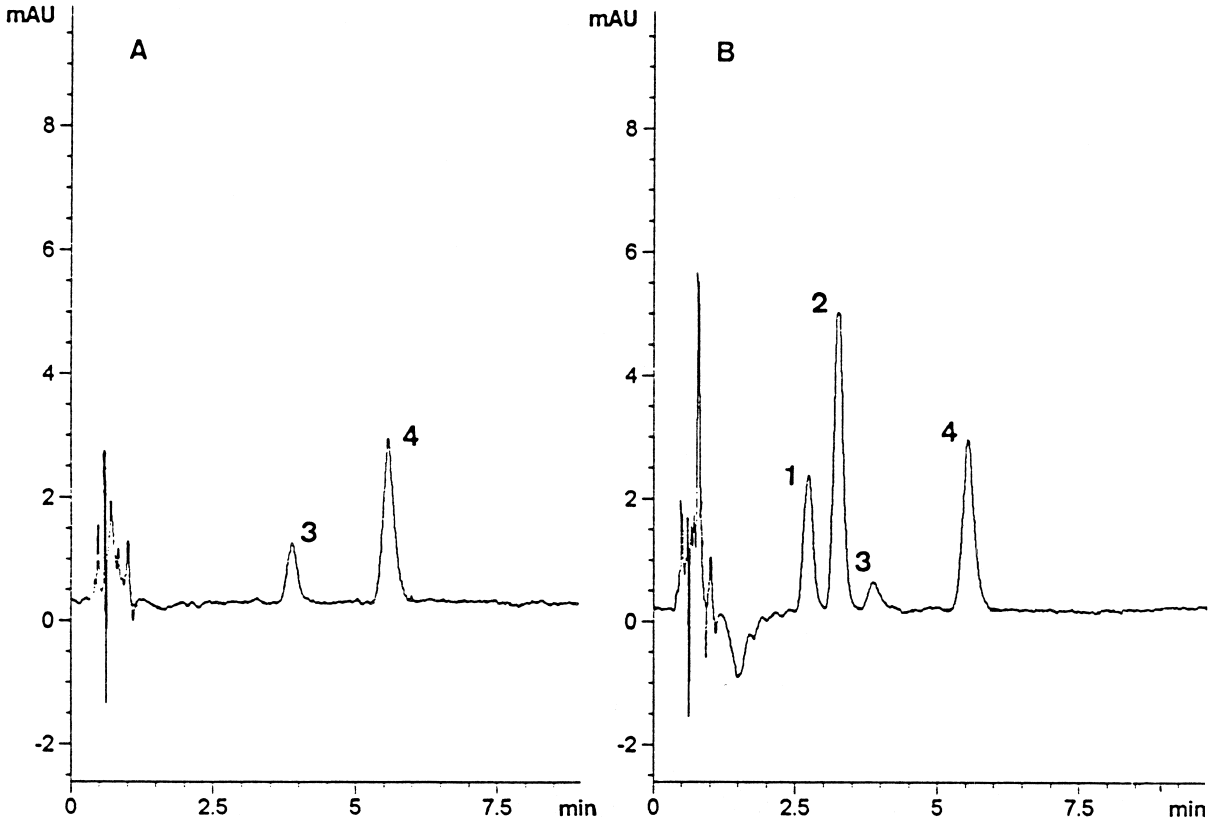

Fig. 1. Typical chromatograms of virgin olive oil (A) and soybean oil (B). Peak identities: 1=δ-tocopherol; 2=γ+β-tocopherols; 3=α-tocopherol; 4=α-tocopherol acetate.

saponification and extraction, simplifies the procedure and shortens the analysis. Moreover, many oil samples can be analysed several times without altering the chromatographic efficiency or the column efficiency, which remains high.

3.2. Method validation

Validation results are summarised in Table 1.

α-Tocopherol acetate was selected as the internal standard owing to its availability and its structural similarity to the compounds assayed. Standard linearity was tested in each case using linear regression. The peak-area ratio between α-tocopherol and α-tocopherol acetate (y) versus the standard mass of α-tocopherol (x) under these conditions was linear in the range tested. δ-Tocopherol also showed an acceptable linearity, with a correlation coefficient over 0.999, in the range studied.

The within-run precision of α-tocopherol and δ-

Table 1
Validation parameters of tocopherol measurements in vegetable oils

	α-Tocopherol	δ-Tocopherol
Standard linearity		
Intercept	−0.011	−1.051
Slope	1.048	2.303
r	0.999	0.999
Range (μg/ml)	1–25	1–25
Precision (n=10)		
Mean (mg/kg)	213.29	8.65
RSD (%)	2.96	2.43
Accuracy		
Mean recovery (%)	102.98	93.37

tocopherol was measured using the relative standard deviation (RSD) of α-tocopherol and δ-tocopherol in 10 replicates of olive oil, and 10 replicates of soybean oil, respectively. In both cases, the results were acceptable, in agreement with Horwitz [20] for analyte concentrations of about μmol/l.

The standard addition method was used for testing the accuracy of the method. Standards of α-tocopherol and δ-tocopherol were added to three aliquots of a sample at three concentration levels in order to calculate the recovery rates.

Each analysis was carried out in triplicate. Recovery rates were close to 100%: 107.05%, 102.02% and 104.09% for α-tocopherol and 91.95%, 94.50% and 94.40% for δ-tocopherol.

To test the sensitivity of the method in the conditions proposed, the detection limit (DL) and quantification limit (QL) were studied. The sensitivity results obtained in accordance with the USP criteria [21] were similar for both tocopherols. The DL was 11.5 ng for α-tocopherol and 12 ng for δ-tocopherol and the QL was 23 ng for α-tocopherol and 25 ng for δ-tocopherol.

Thus, the method proposed is sensitive, quick, precise and suitable for the routine determination of tocopherols in edible vegetable oils. Our results provide a cheap procedure to optimise the identification and quantification of tocopherols in different edible oils.

This analysis can be useful to asses the effect of several tocopherols on the oxidative stability of vegetable oils and, in some cases, to determine mixtures of oils based on the basis of the tocopherol distribution found.

Acknowledgements

This study was funded by the ''Comisión Interministerial de Ciencia y Tecnología'' (ALI97-1607-CO2-O2) of the ''Ministerio de Educación y Ciencia'' (Spain) and the ''Comissió Interdepartamental de Recerca i Innovació Tecnològica'' (CIRIT) of the ''Generalitat the Catalunya'' (Spain). We thank Mr. Robin Rycroft for assistance in the English revision.

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