Talanta 119 (2014) 98–104

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Pressurized liquid extraction and dispersive liquid–liquid microextraction for determination of tocopherols and tocotrienols in plant foods by liquid chromatography with fluorescence and atmospheric pressure chemical ionization-mass spectrometry detection

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ARTICLE INFO

Article history:
Received 25 July 2013
Received in revised form
14 October 2013
Accepted 20 October 2013
Available online 30 October 2013

Keywords:
LC–APCI-MS
Pressurized liquid extraction
Dispersive liquid–liquid microextraction
Tocopherols
Tocotrienols
Foods

ABSTRACT

Pressurized liquid extraction (PLE) and dispersive liquid–liquid microextraction (DLLME) were used to isolate and preconcentrate tocopherols and tocotrienols from plant foods. The Taguchi experimental method was used to optimize the six factors (three levels for each factor), affecting DLLME, namely: carbon tetrachloride volume, methanol volume, aqueous sample volume, pH of sample, sodium chloride concentration and time of the centrifugation step. The influencing parameters selected were 2 mL of methanol:isopropanol (1:1) (disperser solvent), 150 μL carbon tetrachloride (extraction solvent) and 10 mL aqueous solution. The organic phase was injected into reversed-phase liquid chromatography (LC) with an isocratic mobile phase composed of an 85:15 (v/v) methanol:water mixture and a pentafluorophenyl stationary phase. Detection was carried out using both fluorescence and atmospheric pressure chemical ionization mass spectrometry (APCI-MS) in negative ion mode. Quantification was carried out by the standard addition method. Detection limits were in the range 0.2–0.3 ng mL^{-1} for the vitamers with base-line resolution. The recoveries obtained using the optimized DLLME were in the 90–108% range, with RSDs lower than 6.7%. The APCI-MS spectra, in combination with fluorescence spectra, permitted the correct identification of compounds in the vegetable and fruit samples. The method was validated according to international guidelines and using two certified reference materials.

1. Introduction

Pressurized liquid technology (PLE) is an emerging greener technique based on the use of liquid solvents at elevated temperature and pressure, thus improving the extraction performance of classical techniques [1]. The extraction of bioactive compounds, which are sensitive, thermolabile and found in low concentrations in foods, leads to low yields with traditional techniques. However, PLE enhances the extraction efficiency by increasing solubility and mass transfer properties [2–3].

The perspective of hyphenation and combination of different sample preparation techniques is one recent strategy in analytical chemistry. Thus, by combining PLE with miniaturized analytical techniques, it would be possible to extract compounds from samples and high preconcentration through chemical processes which use low quantities of solvents for dissolving or extracting analytes, in line with the priorities of green chemistry [4]. Dispersive liquid–liquid microextraction (DLLME) is a very simple and rapid technique [5] using a ternary component solvent system formed by an aqueous solution containing the analytes, a water-immiscible extraction solvent and a water miscible disperser solvent.

The vitamin E group includes eight liposoluble vitamers or tocols. Its structure is comprises two primary parts: a chromanol ring and a hydrophobic side chain [6], which are divided into two fundamental groups, four tocopherols (T), with saturated isoprenoid side chains, and four tocotrienols (T3) with isoprenyl side chains with three double bonds. Furthermore, each group includes four vitamers (α-, β-, γ- and δ), which differ in the number and position of the methyl substitutes in the chromanol ring [7]. The most widely distributed and biologically active as a vitamin is α-T, while the activity of β-T is 30% of that of α-T; γ-T has 15%; and δ-T only 3%. The activity of α-T3 is 25% of that of α-T.

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0039-9140/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.10.053

The vitamin E content in animal foods is very variable, depending on the animal diet. In contrast, it is mainly found in plant foods, especially in oilseeds, green parts of plants, and oils of wheat germen, sunflower, corn and olive. Because of its antioxidant role, the vitamin E content in foods also has a technological significance, since these compounds react with free radicals, and are reduced during the manufacture and storing of foods [8].

Liquid chromatography (LC) is the most widely used technique for vitamin E determination in foods [8,9]. It uses different detection systems, such as UV–vis [10–16], fluorescence [17–32], electrochemical [33–35] or mass spectrometry (MS), which combines the resolution of LC with the detection specificity of MS, mainly using atmospheric pressure chemical ionization (APCI) or electrospray ionization (ESI) [11,12,36–43].

The food sample preparation is the main source of error and the most commonly used methods to extract vitamin E are solvent extraction or alkaline hydrolysis [7–9]. Natural foods would not need to be hydrolyzed because the vitamers occur mainly as free compounds, but fortified foods should be saponified because they are generally added as esters [10]. As an alternative, tocols may be extracted by PLE techniques [33–35,40], thus achieving shorter extraction times and decreasing solvent volumes. The Standardized Method of analysis for vitamin E includes the determination of α-, β-, γ- and δ-tocopherol by LC separation and subsequent photometric (UV) or preferably fluorimetric detection. In most cases, a saponification of the material followed by an extraction is necessary [44].

As regards new clean sample preparation techniques, solid-phase microextraction (SPME) has been used to the extraction of vitamins A, D_3 and E [45], microextraction of vitamin E with hexane and fluorescence detection [46]. A liquid-phase microextraction (LPME) procedure using solidification of a floating drop has been proposed for determination of fat-soluble vitamins [47], and DLLME has recently been applied in the determination of α-tocopherol [48] and tocopherols and tocotrienols using fluorescence detection [49].

When designing an optimization model, the multiple factors affecting DLLME can be considered together by a balanced orthogonal array design (OAD) based on the Taguchi method [50]. Depending on the number of parameters, the OAD approach made it possible to run experiments, analyze data, identify the optimum conditions and perform confirmation runs with the optimum levels of all the parameters.

This study proposes a procedure using LPE and DLLME such as green sample preparation techniques for the efficient determination of tocopherols and tocotrienols in plant foods with LC using fluorescence and APCI-MS detection. The Taguchi experimental method is applied to study the possible influence on the performance of the method of six factors. The method is validated according to international guidelines. The main contribution of this study is that is the first time that vitamin E forms are preconcentrated by PLE and DLLME and unequivocally identified by MS.

2. Experimental

2.1. Reagents and samples

Chromatographic quality acetonitrile, methanol and carbon tetrachloride were obtained from Sigma (St. Louis, MO, USA). The water used was previously purified in a Milli-Q system (Millipore, Bedford, MA, USA). DL-α-tocopherol, rac-β-tocopherol, γ-tocopherol and δ-tocopherol, were obtained from Sigma-Aldrich (St. Louis, MO, USA). Stock solutions (1000 μg mL^{-1}) were prepared in ethanol and stored in amber vials at −20 °C. The stock solutions were checked for concentration and purity by UV spectroscopy using the known absorption coefficient of each isomer [35]. Working standard solutions were prepared daily in ethanol and stored at 4 °C. Because tocotrienols were not available, refined palm oil was obtained from Fluka (Spain). The oil (2 g) was extracted by a PLE treatment [35] using methanol/isopropanol (50 mL, 1:1, v/v) as solvent at a temperature of 50 °C and a pressure of 1600 psi, with one cycle of extraction during a static time of 5 min. The extract contained α-, γ- and δ-tocotrienol and was used to provide reference retention times for tocotrienols. The calibration graphs of tocopherols were used to quantify both tocopherols and their corresponding tocotrienols, according to the literature [35]. Other reagents were hydromatrix celite (Agilent), sodium chloride, ascorbic acid and potassium hydroxide (Merck).

Samples of fruits and vegetables were commercially obtained and just analyzed. Samples were spiked with a mixture containing the standards and extracted after 30 min.

2.2. Instrumentation for LC-fluorescence

The LC-fluorescence system consisted of an Agilent 1100 (Agilent, Waldbronn, Germany) quaternary pump (G1311A) operating at room temperature. The solvents were degassed using an on-line membrane system (G1379A). The fluorescence detector was an Agilent FLD (G1321A) operating at an excitation wavelength of 298 nm and an emission wavelength of 345 nm.

The analytical column used for the reversed-phase technique was Ascentis® Express F5 filled with dimethylpentafluorophenylpropyl (15 cm × 0.46 cm × 5 μm) (Sigma). The mobile phase was a 85:15 methanol:water (v/v) mixture under isocratic conditions. The flow-rate was 1 mL min^{-1}. Aliquots of 20 μL were injected manually using a Model 7125-075 Rheodyne injection valve (Rheodyne, Berkeley, CA, USA). Solutions were stored in 2 or 10 mL amber glass vials. To filter the samples, PVDF filters (0.45 μm) (Teknokroma, Barcelona, Spain) were used. An EBA 20 (Hettich, Tuttlingen, Germany) centrifuge was used at a speed near to the maximum supported by the conical glass tubes, 3000 rpm. Vegetable samples were homogenized using an IKA A 11 homogenizer (Staufen, Germany).

Extractions were performed with a Dionex (Germany) 200 Accelerated solvent extractor system, equipped with 22 mL stainless steel extraction cells and 60 mL Dionex glass vials for extract collection.

2.3. LC–APCI-MS system

The LC system consisted of an Agilent 1200 (Agilent, Waldbronn, Germany) binary pump (G1312A) operating at a flow-rate of 1 mL min^{-1}. The solvents were degassed using an on-line membrane system (G1379A). The column was maintained in a thermostated compartment at room temperature (G1316A), and injection (20 μL) was performed using an autosampler (G1329A). The column and the mobile phase were the same as those optimized for fluorescence detection. The LC system was coupled to an ion-trap (IT) mass spectrometer (1036 model) equipped with an APCI interface operating in negative ion mode. The selected ion monitoring (SIM) mode was applied. The instrument parameters were: drying temperature 350 °C, APCI temperature 400 °C, drying gas flow 5 L min^{-1} and nebulizer gas pressure 60 psi.

2.4. PLE procedure for food samples

The fruit and vegetable foods consisted of different types, such as spinach, corn, cranberry, pomegranate and mango juice, all commercially obtained from street markets. The samples were cut