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New reversed phase dispersive liquid–liquid microextraction method for the determination of phenolic compounds in virgin olive oil by rapid resolution liquid chromathography with ultraviolet–visible and mass spectrometry detection

M.P. Godoy-Caballero, M.I. Acedo-Valenzuela, T. Galeano-Díaz *

University of Extremadura, Analytical Chemistry Department, Badajoz, Spain

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ABSTRACT

The determination of phenolic compounds in virgin olive oil using a new reversed phase dispersive liquid–liquid microextraction (RP-DLLME) procedure coupled with rapid resolution liquid chromatography-diode array and mass spectrometry detection (RRLC-DAD–MS) have been performed. A rapid resolution Zorbax Eclipse XDB-C18 column (4.6 mm × 50 mm, 1.8 μm particle size) has been employed and eighteen phenolic compounds belonging to different families have been identified and quantified spending a total time of 26 and 13 min with UV–visible and MS detection, respectively. Response surface methodology has been applied by means of a central composite design for the optimization of the variables affecting the extraction procedure searching for the best recovery. The validation of the methods was performed through the establishment of the external standard calibration curves and the analytical figures of merit. Limits of detection ranging from 10 to 400 ng mL^{-1} and 1 to 200 ng mL^{-1} were achieved using UV–visible and MS detection, respectively. The extraction of phenolic compounds from virgin olive oil was performed in a simple and rapid way by RP-DLLME with ethanol:water 60:40 (v/v) as extracting solvent and 1,4-dioxane as disperser solvent. The quantification of the phenolic compounds in virgin olive oils from different olive varieties was carried out by means of the standard addition method and, finally the procedure for the sample treatment was validated using the well established solid phase extraction procedure with Diol cartridges.

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1. Introduction

Virgin olive oil (VOO) is one of the fundamental ingredients in the Mediterranean diet, which has associated many benefits fundamentally due to its content in phenolic compounds [1]. Under this denomination there are more than 4000 compounds divided in 12 subclasses [2]. Currently, these compounds are receiving considerable attention, fundamentally due to its antioxidant activity, strongly related to cancer prevention, inflammatory disorders and cardiovascular diseases [3,4]. In addition, phenolic compounds and their strong natural antioxidant activity contribute to the stability of VOO against oxidation and influence in its organoleptic characteristics and nutritional qualities [5]. The composition of VOO in phenolic compounds is related to agronomic and technological aspects of production [6].

Many methods have been developed to analyze phenolic compounds in different types of samples being liquid chromatography (LC) the most used technique [2]. Specifying in the determination of those compounds in VOO, the traditional colorimetric methods [7] have been replaced by separation techniques, such as gas chromatography (GC), liquid chromatography (LC), capillary electrophoresis (CE) [8–11] and even microchips electrophoresis [12]. However, most of the proposed methodologies have been focused on the optimization of LC methods [13,14]. Reversed-phase columns (RP) are the most commonly used, mainly C_{18} conventional columns. Nevertheless, the use of C_{18} stationary phases results in high analysis time, as a consequence of its inherent non polarity. To reduce the total analysis time different alternatives have been used, such as ultra high performance liquid chromatography (UHPLC) [15,16], rapid resolution liquid chromatography (RRLC) [17–19], using more polar columns [20] and chemometric tools [21]. Most important advantages of the RRLC over conventional LC are improved resolution, shorter retention times, higher sensitivity and better performance.

* Corresponding author. Tel.: +34 924289375.
E-mail address: tgaleano@unex.es (T. Galeano-Díaz).

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Regarding the detection, UV–visible with diode array detection (DAD) is the standard method used for phenolic compounds which, together with MS, are the dominant systems nowadays [2]. Coupling RRLC with MS detection offers a powerful analytical alternative which has been recently applied to characterize food products [18]. Specifying in the analysis of phenolic compounds in VOO, interesting articles can be found in literature in which the MS detection is used [8,10,15,16,18,19,22–24].

Different procedures to isolate the polar phenolic fraction of the VOO have been employed [13,25], although liquid–liquid extraction (LLE) using methanol:water mixtures [26] and solid phase extraction (SPE) with Diol cartridges [27] have been fundamentally used. However, these procedures are generally expensive, time and organic toxic solvents consuming and also an intensive labor is required. In this sense, the main goal of the present work is to develop a simple and fast miniaturized extraction and preconcentration procedure, based in the dispersive liquid–liquid microextraction (DLLME).

DLLME was introduced by Rezaee et al. [28] in 2006 and it can be considered as a miniaturized version of conventional LLE which requires only microliter volumes of solvents. Conventional DLLME can be described as 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 use of only microliter volumes of extraction solvent, which makes the procedure environmentally friendly, the short extraction time and the high enrichment factor together with the simplicity of the operation, low sample volume and low cost are the main advantages of this procedure [29].

In the food analysis field, Asensio-Ramos et al. [30] published in 2010 an interesting review regarding the use of DLLME. Different approaches have been described and most of them comprise the use of DLLME in the conventional mode, in which analytes are initially presents in an aqueous phase. However, the complexity of certain food samples as well as their degradation capacity is a drawback that has greatly difficulted the conventional DLLME application and frequently requires a previous extraction or cleaning step, followed by a suitable reconstitution into an aqueous media in which the procedure is finally carried out [31]. Another alternative to the conventional DLLME consists in the use of low-density solvents as extractants being the floating drop collected after the centrifugation [30,32–35].

Relative to the applicability in olive oil, Daneshfar et al. [36] have performed the determination of cholesterol in food samples (milk, egg yolk and olive oil) using DLLME in the conventional mode.

On the other hand, and to the best of our knowledge only two papers have been published in relation to the applicability of the RP-DLLME procedure [37,38]. One of the most important advantages of this procedure in comparison to the conventional DLLME is that the polarity of the extraction solvent and the sample is reversed. This allows eliminating the evaporation to dryness and reconstitution of the sample in a polar solvent compatible with a RP-LC separation system (necessary steps in conventional DLLME), since the RP-DLLME is directly applied to organic samples with an adequate polar extraction solvent.

Those authors carried out the extraction of oleuropein from olive's processing wastewater and olive leaves extracts employing a mixture of ethyl acetate extract of sample and water (pH 5.0) which was rapidly injected into of cyclohexane. Also, they determined hydroxytyrosol and tyrosol from VOO samples using water (pH 11) as extraction and ethyl acetate as disperser solvents. In both cases, the determination was performed by conventional HPLC-UV with fixed wavelengths of 240 and 280 nm, respectively.

Taking into account the advantages of the RP-DLLME and its applicability to the VOO samples, the main goal of the present work is to develop a RP-DLLME procedure for the extraction and preconcentration as many as possible phenolic compounds from VOO. We pretend to simplify the work of the VOO researches taking into account the disadvantages of the currently most commonly used extraction procedures. On the other hand, the RRLC method transferred from a conventional HPLC one provides better performance with shorter analysis times. In this way, the separation of the phenolic compounds has been performed quickly in a simple way and both the DAD and MS detection have been used and compared.

2. Experimental

2.1. Chemicals, solutions and samples

For all experiments, analytical reagent grade chemicals and solvents were used. Ultrapure water was obtained from a Millipore Milli-QA10 System (Waters, Germany). Hydroxytyrosol (HYTY), oleuropein glucoside (OI), luteolin (LUT) and apigenin (APIG) were obtained from Extrasynthèse (Genay, France), gallic acid (GAL), 3,4-dihydroxybenzoic acid (DOPAC), tyrosol (TY), 4-hydroxybenzoic acid (4HB), 4-hydroxyphenylacetic acid (4HP), vanillic acid (VAN), caffeic acid (CAF), syringic acid (SY), *p*-coumaric acid (*p*-CUM), *m*-coumaric (*m*-CUM), *o*-coumaric acid (*o*-CUM), ferulic acid (FER) and cinnamic acid (CIN) from Sigma–Aldrich Chemie GmbH (Steinheim, Germany) and gentisic acid (GEN) from Aldrich (Gillingham-Dorset, England). All solvents employed were HPLC grade, except 1-butanol, isobutanol and 1,4-dioxane which were PA grade. Ethanol, methanol, acetonitrile, isopropanol, 1-butanol, isobutanol, ethyl acetate and 1,4-dioxane were provided by Panreac (Spain), acetone by Scharlab (Spain), tetrahydrofuran and isohexane by Merck (Germany), acetic acid by Romil Chemicals LTD (England) and 1-propanol by Sigma–Aldrich (USA).

1.00 mg mL^{-1} stock solutions of each compound were prepared in ethanol, except OI 2.00 mg mL^{-1}. These solutions were stored at 4 °C, avoiding exposure to direct light. Fresh solutions of lower concentrations were daily prepared by appropriate dilution of the stock solution with the selected solvent. VOO samples were acquired from the market and were kept at 4 °C avoiding exposure to direct light. Monovarietal olive oils analyzed were obtained from the olive varieties: M*anzanilla Cacereña, Cornicabra, Arbequina, Picual, Hojiblanca and Morisca*. All of them were obtained from ripe olive fruit, except *Arbequina* variety which was also obtained from green fruit.

2.2. Instrumentation and software

The chromatographic studies were performed with an Agilent Model 1100 LC instrument (Agilent Technologies, Palo Alto, CA, USA), equipped with degasser, quaternary pump, column oven, autosampler Agilent 1290 infinity thermostated at 15 °C, UV–visible diode-array detector (DAD) and the Chemstation software package to control the instrument, data acquisition, and data analysis. The analytical column employed was a rapid resolution Zorbax Eclipse XDB-C18 column (4.6 mm × 50 mm) and 1.8 μm particle size (Agilent Technologies). The components of the mobile phase were high-purity water with 0.5% acetic acid and 1% acetonitrile (phase A) and acetonitrile with 0.5% acetic acid (phase B) and were degassed by ultrasonication before use. The gradient program for the analysis of the phenolic compounds by DAD was as follows: 0–10 min, 0% B; 10–20 min, 25.6% B; 20–22 min, 27.8% B; 22–23 min, 40% B; 23–24 min, 98%; 24–30 min, 98% B. Finally, the B content was decreased to the initial conditions (0%) and the column re-equilibrated for 15 min. The flow rate was set constant at 0.6 mL min^{-1} and the injection volume was 10 μL. The separation temperature was 15 °C.

The mass spectrometry detection was performed with an Agilent Technologies single quadrupole 6120 mass spectrometer

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