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Simplified pesticide multiresidue analysis in virgin olive oil by gas chromatography with thermoionic specific, electron-capture and mass spectrometric detection

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Abstract

In the present work, an analytical multiresidue method has been developed for the analysis of 32 organochlorine, organophosphorus and organonitrogen pesticides at $\mu g\,kg^{-1}$ levels in virgin olive oil. The method consists of the extraction of the pesticides with acetonitrile saturated in *n*-hexane followed by a clean-up process based on gel permeation chromatography (GPC) with ethyl acetate–ciclohexane (1:1) as mobile phase to separate the low-molecular mass pesticides from the high-molecular mass fat constituents of the oil. The target compounds were determined in the final extract by gas chromatography (GC) using thermoionic specific (TSD) and electron-capture (ECD) detection. In the case of positive samples, the amounts found were confirmed by GC–MS/MS, being the results in good agreement. Recoveries and RSDs ($n = 10$) values were 91–124% and 1–8% (GC-ECD), 82–100% and 9–20% (GC-TSD), and 89–105% and 4–14% (GC–MS/MS), respectively. The three proposed methods were applied to samples collected directly in two olive mills located in the Jaén province (Spain). Specifically, 24 samples of virgin olive oil were collected. The most frequently pesticide residues found were the herbicides terbuthylazine and diuron and endosulfan sulfate, a degradation product of the insecticide endosulfan. The herbicide concentration was higher in those oil samples obtained from olives which were collected from the ground after they had fallen down than in those oil samples from olives harvested directly from the tree. The GC–MS/MS developed method was also applied to the analysis of an olive oil sample from a proficiency test spiked with organochlorine pesticides and all the values obtained were within the specified "satisfactory" range.

Keywords: Pesticides; Olive oil; GPC; GC-TSD; GC-ECD; GC–MS/MS

1. Introduction

Virgin olive oil is a natural product obtained from the olive fruit (*Olea Europaea*) using solely mechanical or physical means. The fact that the oil extraction is solvent-free and natural antioxidants are maintained in the oil is reflected in the high nutritional and economic value of this product. Continuous effort is being made in order to preserve its high quality characteristics, being a low concentration or not detectable pesticide residues one of the most important quality criteria. In the case of olive cultivars, trees are attacked by a variety of insects and other pests which cause a reduction in the quality and quantity of the olives and oil produced. Some of the pesticides used to control these pests are lipophilic, with high *n*-octanol–water partition coefficients ($K_{O/W}$), which suggests that residues will be concentrated in the oil during extraction from olive fruits.

Because of the wide range of pesticides, their different characteristics complicate the trace-level determination of the whole range of these compounds. As a result, techniques which combine a short analysis time, sufficient selectivity and adequate sensitivity become necessary. The preferred method for the determination of volatile pesticides in oils is capillary gas chromatography (GC) due to its high separation efficiency and the variety of selective detection methods that can be used. Two of these detection methods are nitrogen-phosphorus detection (NPD), also called thermoionic specific detection (TSD), for N- or P-containing compounds [1–4] and flame photometric detection (FPD) [5,6], which is better suited for the detection of

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P-containing compounds [7]. Electron-capture detection (ECD) has also been widely used for organochlorine pesticide residues in this type of samples due to its high sensitivity and selectivity [8–11]. Nevertheless, analytical problems associated with the analysis of pesticides in fatty matrixes are well known and the detectors above mentioned do not provide unequivocal confirmation of identity and are often subject to matrix interferences. Mass spectrometry (MS), usually in the selected ion monitoring (SIM) mode, is the preferred method of choice [9,10,12–14] although confidence in the confirmation of identity may be reduced if one or more of the selected ions are affected by matrix interferences, giving poor spectral information. Alternatively, MS/MS with ion trap [15] or triple quadrupole [16] can be employed to achieve a high level of selectivity and low detection limits in dirty extracts [17].

On the other hand, the preparation of oil samples for the determination of pesticides by GC requires the complete removal of the high-molecular mass fat from the sample to maintain the chromatographic system in working order. The sample preparation is a crucial step in the analytical procedure since even small amounts of lipids can harm columns, detectors or cause signal suppression. This procedure involves extractions of the analytes from its bulk matrix into an appropriate solvent and then removal of potentially interfering substances from the solvent extracts by various clean-up methodologies and concentrating the extracts to small volumes before analysis. Often the extraction and clean-up steps for the determination of pesticide residues in olive oil can be achieved simultaneously, in part or fully, by techniques such as: (1) solid-phase extraction (SPE) [4,8,9,18] using adsorbents such as florisil, alumina, silica gel, etc.; (2) solid-phase microextraction (SPME) [19,20] using fused-silica fibers coated with different phases, namely polydimethylsiloxane-divinylbenzene (PDMS-DVB), PDMS, Carboxen-polydimethylsiloxane (CAR-PDMS), DVB-CAR-PDMS, etc.; (3) matrix solid-phase dispersion (MSPD) [14] which is used, in general, employing the same adsorbents mentioned in SPE; and (4) supercritical fluid extraction (SFE) [21] which allows extraction and separation of the analytes by appropriately controlling the pressure, modifier or flow. In some occasions, these preparation steps could be replaced by a liquid chromatography (LC) step, by coupling LC to GC, where the specific components of a complex matrix are pre-fractionated by LC and then transferred on-line to the highly efficient and sensitive GC system for analytical separation [12,22,23]. However, the common and traditional approach is to carry out extractions and clean-up separately in multiple stages because, while the method of extraction usually depends on the sample matrix, the clean-up procedure may depend on the nature of the interfering substances that may be present in the extract. So, low temperature clean-up methods [2,11], which are based on the removal of the fat with the separation by low-temperature precipitation, have been applied after liquid–liquid partition [1,3,5]. But most of the clean-up steps currently applied are based on gel permeation chromatography (GPC), where macromolecules are separated according to size, in the usual "big first" order of elution. This methodology is relatively effective at removing fats and oils and is applicable to a wide range of both polar and non-polar pesticides within the same injection on a fully automated system [6,7,10,13,16].

The purpose of this work is two-fold. Firstly, to develop and validate a simple and rapid multiresidue method for the analysis of pesticide residues in olive oil by GC-ECD, GC-TSD and GC–MS/MS after a liquid–liquid extraction procedure and a GPC clean-up. Secondly, to apply this method to olive oil sampled from olive mills which carry out the olive processing in different ways depending on how the olives were harvested. To the best of the authors knowledge, no studies have been performed dealing with the influence of the olives origin in the presence of pesticide residues in virgin olive oil.

2. Experimental

2.1. Chemicals and reagents

All pesticides standards, ECD internal standard (pentachloronitrobenzene (quintozene)), and the recovery standard (triphenylphosphate (TPP)), were purchased from Riedel-de Häen (Seelze-Hannover, Germany), with purity of >99%. TSD internal standard, caffeine, was supplied by Fluka, with purity >99%. HPLC grade solvents: dichloromethane, acetone, ciclohexane, acetonitrile, *n*-hexane and ethyl acetate were from Panreac (Barcelona, Spain).

Individual stock standard solutions of pesticides, internal and recovery standard (200 $\mu g\,ml^{-1}$) were prepared by weighing and dissolving them in acetone. They were stored in a freezer at −18 °C. Working standard solutions were prepared by appropriate dilutions in ciclohexane and then stored in a refrigerator (4 °C). Matrix matched standards were prepared by adding working standard solutions to blank olive oil.

2.2. Instrumentation and apparatus

The GPC system was comprised of a L7110 LaChrom HPLC pump (Merck) and two Waters Envirogel GPC clean-up columns, a guard column (150 mm × 19 mm) and a main clean-up column (19 mm × 300 mm). A L-7490 LaChrom RI detector (Merck), a fraction collector and an autosampler (704 Varian ProStar) were also used. The flow-rate was set at 5.0 $ml\,min^{-1}$ and the mobile phase was ethyl acetate–ciclohexane (1:1). In between runs, the flow-rate was set at 0.5 $ml\,min^{-1}$ to clean the GPC system and to avoid the drying of the columns.

The GC-ECD/TSD consisted of a Varian (from Varian Instruments, Walnut Creek, CA, USA) CP-3800 gas chromatograph equipped with ^{63}Ni ECD and TSD systems and two 1177 split/splitless injectors with Electronic Flow Control (EFC) operated in the splitless mode with the split closed for 1 min. Both injector and detector temperatures were set at 270 and 300 °C, respectively. Carrier gas was N_2 at a flow-rate of 1 $ml\,min^{-1}$ for ECD column and 2 $ml\,min^{-1}$ for TSD column. The ECD make-up gas was N_2 at a flow-rate of 30 $ml\,min^{-1}$ and the TSD gas flows were hydrogen at 4 $ml\,min^{-1}$, air at 175 $ml\,min^{-1}$ and N_2 as the detector make-up at 30 $ml\,min^{-1}$. A Varian fused-silica capillary column, (30 m × 0.25 mm I.D.), coating CP-SIL 5CB and film thickness 0.25 μm and a Varian