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Determination of Pesticides and PCBs in Virgin Olive Oil by Multicolumn Solid-Phase Extraction Cleanup Followed by GC-NPD/ECD and Confirmation by Ion-Trap GC−MS

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A multicolumn solid-phase extraction cleanup for the determination of organophosphorus (OP) and organochlorine (OC) pesticides plus PCB congeners in virgin olive oil is presented. The method involves dissolution of the olive oil in hexane, followed by a cleanup system using a diatomaceous earth column (Extrelut-QE) with reversed (C_{18}) and normal (alumina) phase SPE columns. Determination of OPs was by GC-NPD, while the OCs and PCBs were analyzed using GC-ECD. Recovery assays for OPs varied from 81.7% to 105.3%, for OCs ranged between 74.3% and 99.4%, while for PCBs were from 60.1% to 119.2%. Quantitation limits ranged from 10 to 25 μg/kg olive oil for OPs, and from 1 to 6 μg/kg olive oil for OCs and PCBs. In the case of positive samples, the confirmation of pesticide identity was performed by ion-trap GC−MS/MS. The applicability of the method was assayed with 19 virgin olive oil samples collected from different olive mills of Aragón (Spain). Only one OP pesticide (acephate) was detected in one sample at a concentration of 10 μg/kg. Organochlorine pesticides were found in 5−47% of samples at very low levels ranging from 1.5 to 5.2 μg/kg. PCBs were found in 20−90% of samples, showing concentrations between 2.3 and 17.3 μg/kg.

KEYWORDS: Virgin olive oil; organophosphorus pesticides; organochlorine pesticides; polychlorinated biphenyls; capillary gas chromatography−mass spectrometric detection

1. INTRODUCTION

Olive oil is of great economic importance for Spain, where the olive groves occupy an area of 2 400 000 ha, which puts Spain in the world's leading olive oil producing countries with a production of 962 000 tons in 2000 (*1*). Because of its sensorial and nutritional characteristics, olive oil is one of the most important components of the universally recognized Mediterranean diet (*2*). The mean consumption of olive oil in Spain is about 13 kg/person per year (*1*). The virgin olive oil is obtained from the fruit of the olive tree solely by mechanical or other physical means without any treatment, and it is the only vegetable oil that is consumed in its crude form (unrefined).

Olive trees are attacked by several pests, mainly the olive fruit fly *Bactocera* (*Dacus*) *oleae*, and receive treatments (usually aerial applications) with several pesticides. Those more extensively used in Spain belong to the class of organophosphorus (OP) insecticides and are mainly dimethoate, diazinon, and parathion-methyl; the OP chlorpyrifos was formerly used in combination with the organochlorine endosulfan, but it has recently been withdrawn in compliance with European Union regulations. Unlike other Mediterranean countries, the organophosphorus fenthion is not registered for use in olive groves in Spain. The use of pesticides can determine the presence of residues on olives and consequently in olive oil, which should be controlled regularly (*3*−*6*). In addition, the persistent organic pollutants such as the lipophilic organochlorine pesticides (OC) and polychlorinated biphenyls (PCBs) can occur in the environment and in the human food chain, including the olive oil (*4*, *7*).

Olive oil is a complex vegetable oil to analyze as compared to rapeseed oil, soybean oil, sunflower oil, corn germ oil, and linseed oil. This is due in part to the relatively high amount of lipids (sterolic, squalene, and triacylgliceride fractions) that elute from the cleanup and the potential for interfering co-extractives (i.e., pigments) at the GC determination step (*8*).

Many multiresidue procedures employing different cleanup techniques and a variety of detection methods have been reported for the determination of pesticide residues in olive oil. Some cleanup techniques are based on liquid−liquid partitioning (*9*, *10*), gel permeation chromatography (*11*), or solid-phase extraction with different sorbents such as florisil (*12*), neutral alumina (*7*), or C_{18} (*13*). Other authors have analyzed olive oil without cleanup (*14*), or by using a low-temperature cleanup

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method (*15*). Many of the more recent methods reported a combination of two or more steps for the cleanup (*16*, *17*). A more sophisticated and automated method by on-line reversed-phase liquid chromatography-gas chromatography using the through oven transfer adsorption−desorption interface has been recently published (*8*).

The AOAC general multiresidue method for OP and OC pesticide residues in fat-containing foods (*9*) uses the classical separatory-funnel partition between immiscible solvents followed by cleanup with conventional Florisil columns to remove residual fat from sample extracts prior to GC determination. This technique is time-consuming and laborious and requires large volumes of organic solvents plus important use of glassware. Several workers have reported using disposable diatomaceous earth mini-columns for the on-column partitioning of OP and OC pesticides into acetonitrile from hexane solutions of vegetable oils and fats instead of the classical liquid−liquid partitioning (*18*).

In the present study, a multicolumn solid-phase extraction cleanup for the determination of 7 organophosphorus (OPs) and 10 organochlorine (OCs) pesticides plus 6 PCB congeners in virgin olive oil is presented. The method involves dissolution of the virgin olive oil in *n*-hexane, followed by on-column partitioning using a diatomaceous earth mini-column (Extrelut QE) and a cleanup system with reversed (C_{18}) and normal (neutral alumina) phase SPE columns. The target compounds studied, the organophosphorus acephate (and its metabolite methamidophos), chlorpyrifos, diazinon, dimethoate (and its metabolite omethoate), and parathion-methyl, the organochlorines HCB, α-HCH, *β*-HCH, lindane, α-endosulfan, *β*-endosulfan, endosulfan sulfate, *p,p′*-DDD, *p,p′*-DDE, *p,p′*-DDT, and the PCB congeners 28, 52, 101, 138, 153, and 180, were determined by capillary gas chromatography with nitrogen−phosphorus and electron-capture detection plus ion-trap GC−MS/MS confirmation.

2. EXPERIMENTAL PROCEDURES

2.1. Standards, Reagents, and Supplies. Pesticide and PCB standards (97% minimum purity) were obtained from Dr. Ehrenstorfer (Ausburg, Germany). Stock standard solutions (1 mg/mL) were prepared in pesticide-grade acetone, *n*-hexane, or dichloromethane according to their solubility. Standard working solutions of OP pesticides (100 *μ*g/mL acetone) suitable for GC-NPD, and of OC pesticides plus PCBs (10 *μ*g/mL acetone) amenable to ECD were prepared. These standard working solutions were used for spiking virgin olive oil blanks for recovery assays, for preparing neat calibration standards for the OCs and PCBs, and for the matrix-matched calibration standards in virgin olive oil blank used for the quantitation of the OPs.

Residue analysis grade solvents (acetone, acetonitrile, dichloromethane, isooctane, *n*-hexane) were supplied by Lab-Scan (Dublin, Ireland); ultrapure water was obtained from a Milli-Q Plus apparatus from Millipore (Milford, MA).

Extrelut-QE mini-columns (3 mL capacity, Part Number 901003-1) were from EMD Science, Gibbstown, NJ (USA). Extrelut-QE mini-columns are packed with a macroporous Kielselguhr-type material (diatomaceous earth). The C_{18} columns (500 mg sorbent mass, 6 mL reservoir volume, 10 mm bed height, 56 *μ*m average particle diameter) and Alumina-N columns (5 g sorbent mass, 25 mL reservoir volume, 21 mm bed height, 121 *μ*m average particle diameter) were obtained from International Sorbent Technology (Hengoed, UK). The neutral alumina columns were partially deactivated prior to use by passing 10 mL of acetonitrile containing 0.3 mL of water through the column.

2.2. Spiked Virgin Olive Oil Samples and Preparation of Calibration Standards. A residue-free virgin olive oil was obtained from a local organic grower with known spray history and environmental background. To certify as residue free, the sample was analyzed for organophosphorus and organochlorine pesticides plus PCB contamination by the AOAC multiresidue method (*9*). The olive oil sample (known as a virgin olive oil blank) did not contain detectable levels of the analytes.

To obtain spiked virgin olive oil samples, the appropriate volume of standard working solutions was added to 15 g of virgin olive oil blank in an Erlenmeyer flask at three spiking levels (10, 50, and 100 *μ*g/kg olive oil for OPs, and 1, 5, and 10 *μ*g/kg olive oil for OCs and PCBs). The spiked virgin olive oil samples were set aside for 60 min at room temperature to let the acetone evaporate before sample preparation and solid-phase extraction cleanup.

The response of GC-NPD to the analytes sought was affected by the presence of interfering co-extractives from the virgin olive oil samples (known as a matrix effect). The matrix effect was observed as increased detector responses, as compared to those produced by simple solvent solutions of the analytes. A matrix-matched calibration was then required for recovery assays and quantitative analysis of the organophosphorus residues in virgin olive oil samples to reduce the matrix effect. On the contrary, the organochlorines and PCBs did not show the matrix effect at the GC-ECD determination system, allowing for external calibration with neat standards in solvent.

To obtain matrix-matched calibration standards for the GC-NPD determination system, a virgin olive oil blank was analyzed and one-half of the final eluate (25 mL) from the C_{18} SPE column was evaporated to dryness. The residue was then dissolved with appropriate volumes of standard working solutions at three different levels (10, 50, and 100 *μ*g/L).

2.3. Sample Preparation and Cleanup. A 15-g sample of virgin olive oil or spiked virgin olive oil was mixed with 25 mL of *n*-hexane (ca. 0.6 g fat/mL). Of the above, a 3 mL fat solution was loaded onto the Extrelut-QE mini-column allowing for even distribution of the fat solution during 10 min. The Extrelut-QE column was placed on top of a 500 mg C_{18} column by means of a small polypropylene connector in a SPE apparatus (SPE-Visiprep DL, Supelco, Bellefonte, PA). The lipid extract was eluted under gravity with 20 mL of acetonitrile saturated with hexane, and then only the C_{18} column was eluted with another 20 mL of acetonitrile saturated with hexane using gravity flow. The eluates were collected in a 50 mL Erlenmeyer flask, and the solution was diluted to volume (50 mL) with acetone, mixed well, then split in half (25 mL each), with each half transferred to 200 mL evaporation tubes.

The tube containing the solution for OP determination was evaporated to dryness at 40 °C under a gentle stream of nitrogen in a concentration workstation (Turbo Vap II, Hopkinton, MA), and the residue was redissolved in acetone (3 mL) for the GC-NPD analysis.

The second portion containing the solution for OC and PCB determination was evaporated to nearly 5 mL at 40 °C under a gentle stream of nitrogen, and then 20 mL isooctane was added and the solution was evaporated to 0.5 mL, and was redissolved in 20% dichloromethane in hexane (8 mL). The sample was transferred onto a 5 g neutral alumina column and eluted with 20 mL of 20% dichloromethane in hexane using gravity flow. The eluate was evaporated to ca. 0.5 mL at 40 °C under a gentle stream of nitrogen, and the residue was redissolved in hexane (3 mL) for the GC-ECD analysis.

2.4. Identification and Quantitation by GC-NPD/ECD. For the organophosphorus pesticides (OPs), a Hewlett-Packard model 5890 gas chromatograph with HP7673 autosampler and nitrogen−phosphorus detector (NPD) was used. The injection port and detector temperatures were 200 and 250 °C, respectively. The sample (2 *μ*L) was injected in the splitless mode. A fused-silica capillary column HP-101 (12 m × 0.20 mm, with 0.25 *μ*m film thickness) supplied by Agilent (Madrid, Spain) was used. The initial column temperature was 60 °C for 1 min, increased to 120 °C at a rate of 30 °C/min, then to the final temperature of 250 °C at 10 °C/min and hold for 5 min. The carrier gas was nitrogen at 35 cm/s, and the constant flow rates of the makeup gas (nitrogen), the hydrogen, and air were, respectively, 30, 3.5, and 100 mL/min. The GC was connected to a personal computer with HP 3365 Chemstation software.

An HP model 5890, Series II gas chromatograph (Wilmington, DE) equipped with an HP7673 autosampler, a ^{63}Ni electron-capture detector (ECD), and a fused-silica capillary column Quadrex 007-2 for identification and quantitation (50 m × 0.25 mm, with 0.25 *μ*m film thickness, New Haven, CT) was used for the organochlorine residues

Table 1. Parent Ions Selected and Product Ions Observed for Each Organochlorine Pesticide and PCB Congener (Saturn 2000 Ion-Trap Mass Spectrometer, MS/MS Mode)

compound	parent ions (*m*/*z*)	product ions (*m*/*z*)
hexachlorobenzene (HCB)	284	177
α-hexachlorocyclohexane (HCH)	219	181:183
β-hexachlorocyclohexane (HCH)	219	181:183
lindane (γ-HCH)	219	181:183
α-endosulfan	340	267
β-endosulfan	340	233
endosulfan sulfate	387	325
p,p′-DDD	235	165
p,p′-DDE	246	176
p,p′-DDT	235	165
PCB 28	258	186:188
PCB 52	292	220:222
PCB 101	326	254:256
PCB 138	360	288:290
PCB 153	360	288:290
PCB 180	394	324

(OCs and PCBs). The operating conditions were: carrier gas, nitrogen C-55, 34 cm/s; makeup gas, nitrogen C-55, 55 mL/min; injector temperature, 210 °C; detector temperature, 300 °C; injection volume, 2 μL; splitless injection. Column oven temperatures were as follows: initial temperature, 125 °C for 1 min; from 125 to 205 °C at 20 °C/min; from 205 to 290 °C at 2 °C/min, then hold at 290 °C for 5 min. Data acquisition and processing were performed with an HP Vectra 486/33U computer with HP 3365 Chemstation software.

2.5. Confirmation by Ion-Trap GC−MS/MS. The identity of OC pesticides and PCB residues in the samples was confirmed by ion-trap GC−MS/MS with a Varian 3800 gas chromatograph equipped with a Varian 8200 autosampler and a split/splitless programmed temperature Varian 1079 injector, and fitted with a Saturn 2000 ion-trap mass spectrometer (Varian Instruments, Sunnyvale, CA). The fused-silica capillary column used was CP-Sil-8 (30 m × 0.25 mm, with 0.25 μm film thickness) supplied by Chrompack (Middelburg, The Netherlands). GC conditions were as follows: initial column temperature 70 °C (3.5 min), increased at 25 °C/min to 200 °C (10 min) and finally increased at 4 °C/min to 300 °C (held for 10 min). Pure GC grade Helium (99.999%) was used as carrier gas at a constant flow-rate of 57 cm/s; manifold, transfer-line, and trap temperatures were 60, 280, and 220 °C, respectively. A 2 μL volume of sample was injected in the splitless mode with a purge time of 0.01 min and injection rate 0.5 μL/s. The injection port temperature program was: initial temperature 70 °C (0.5 min) and increased at 100 °C/min to 300 °C and held for 10 min.

The MS was operated in electron impact (EI) ionization mode, and typical conditions (autotune calibration was performed before each injection sequence) were: emission current 30 μA and scanned-mass range 90−450 *m*/*z*. **Table 1** shows parent ions selected and product ions observed for each organochlorine pesticide and PCB congener in GC-MS/MS mode. The system was controlled by a Saturn GC/MS Workstation System software 5.4 on a PC personal computer.

2.6. Study of Linearity (Linear Dynamic Range of the Detectors). The standard working solutions of pesticides and PCBs were diluted by mass, covering a concentration range of 5−1000 μg/L for the OPs (linear range of NPD response) and between 0.1 and 200 μg/L for the PCBs and OCs (measurement of the linear range of ECD response). From each dilution, a 1 mL sample was taken, and 2 μL was injected three times, starting at the lowest concentrations.

2.7. Samples. Nineteen virgin olive oil samples ("Empeltre" olive variety) were obtained directly from olive mills during oil extraction from different production areas of Aragón (Spain) in the harvest of year 2001. Olive oil samples were collected in dark glass bottles, transported to the laboratory, and kept for a short time at 4 °C until analysis.

3. RESULTS AND DISCUSSION

The proposed method to determine 23 pesticides and PCBs in virgin olive oil by GC-NPD/ECD is based on a previously published method for the analysis of six OCs and four OPs in vegetable oils and butterfat by GC with electron-capture and flame photometric detectors (*16*). The original method was modified by using acetonitrile saturated with hexane as eluting solvent for sample preparation and cleanup, allowing for the elution of the most currently used organophosphorus insecticides in olive tree treatment in Spain (dimethoate, diazinon, and parathion-methyl) and a wide range of organochlorine pesticides and polychlorinated biphenyls. Other modifications were necessary for the GC-NPD/ECD conditions to optimize the separation of all compounds. Additionally, the residues were confirmed by ion-trap GC−MS/MS.

Table 2. Linear Range, Recovery Percentage, and Relative Standard Deviation (RSD) for the Organophosphorus Insecticides in Spiked Samples (100 μg/kg) of Virgin Olive Oil ($n = 8$)

compound	linear range[a] (μg/L)	average recovery (%)	RSD(%)
acephate	5–800	103.1	16
chlorpyrifos	10–800	98.9	13
diazinon	5–800	102.6	12
dimethoate	5–800	105.3	13
methamidophos	15–600	89.6	15
parathion-methyl	5–800	81.7	14
omethoate	10–1000	86.0	20

[a] Linear range determined with standard solutions in pure solvent.

In this multiresidue method, the pesticides and PCBs are efficiently extracted by on-column partitioning on an Extrelut-QE mini-column in place of the usual liquid−liquid partitioning with solvents. Removal of fat from the extract is achieved by SPE columns containing reversed-phase C_{18} to provide adequate cleanup for OP pesticide quantitation using GC-NPD. Further cleanup of the lipid matrix by normal-phase SPE with neutral alumina is required to determine the OC pesticides and PCBs by GC-ECD. The confirmation of pesticide identity is performed by ion-trap GC−MS/MS.

3.1. Recovery Assays, Calibration, Precision, and Sensitivity of the Method. For recovery experiments, the appropriate volumes of standard working solutions were added to 15 g of virgin olive oil blank to obtain spiked virgin olive oil samples at 10, 50, and 100 μg/kg for the OPs, and at 1, 5, and 10 μg/kg for the OCs and PCBs. Spiked samples were shaken vigorously and were allowed to equilibrate for 60 min prior to extraction according to the described procedure. The recovery assays were replicated eight different times. The calibration was performed by the external standard method using neat standards in pure solvent for the OCs and PCBs, and matrix-matched calibration standards for the OPs to prevent errors caused by matrix effects, as described in the Experimental Procedures.

Tables **2** and **3** show the average recoveries and relative standard deviations (RSD) obtained by the described method for the OPs, and for the OCs plus the PCBs, respectively. Mean recoveries of OPs varied from 81.7% to 105.3%. Recovery results for OCs ranged between 74.3% and 99.4%, except for HCB where a slightly lower recovery was obtained (65.6%). Mean recoveries for PCBs were in the range of 60.1−119.2%. In general, the recovery of the analytes sought was within acceptable margins.

Precision was determined by repeatability studies, expressed by the relative standard deviation (RSD) of the recovery percentages from eight spiked virgin olive oil extracts run the same day by same operator. RSD values for OPs ranged from 12% to 16%, except for omethoate (a metabolite of dimethoate), which was 20% (**Table 2**). RSD values for OCs were less than

Table 3. Linear Range, Recovery Percentage, and Relative Standard Deviation (RSD) for the Organochlorine Pesticides and PCBs in Spiked Samples (10 μg/kg) of Virgin Olive Oil ($n = 8$)

compound	linear range[a] (μg/L)	average recovery (%)	RSD (%)
hexachlorobenzene (HCB)	0.1–145	65.6	6
α-hexachlorocyclohexane (HCH)	0.1–140	74.3	8
β-hexachlorocyclohexane (HCH)	0.4–145	88.2	13
lindane (γ-HCH)	0.1–150	89.2	21
α-endosulfan	0.1–140	83.2	8
β-endosulfan	0.4–140	99.1	7
endosulfan sulfate	0.7–145	99.4	13
p,p′-DDD	0.4–175	88.9	6
p,p′-DDE	0.4–140	95.1	8
p,p′-DDT	0.7–150	96.6	21
PCB 28	0.4–140	60.1	3
PCB 52	0.7–140	61.0	14
PCB 101	0.4–140	71.7	15
PCB 138	0.4–140	119.2	6
PCB 153	0.4–140	73.0	9
PCB 180	0.4–140	70.7	5

[a] Linear range determined with standard solutions in pure solvent.

15% except for lindane and *p,p′*-DDT (21%), while RSD figures for the PCBs fell at or below 15% (**Table 3**).

Method quantitation limits (LOQs) were calculated according to the definitions of the Royal Society of Chemistry (*19*). Thus, the LOQs were set as 6 times the standard deviation of the calculated concentration above the mean concentration determined in the analysis of eight representative virgin olive oil blanks. The study of sensitivity indicated that the quantitation limits ranged from 10 to 25 μg/kg olive oil for the OPs, and from 1 to 6 μg/kg olive oil for OCs and PCBs.

The linearity of a method is a measure of range within which detector response is directly proportional to the concentration of analyte in standard solutions or samples. Even though the GC-ECD and GC-NPD are fundamentally nonlinear over its entire dynamic response range, within smaller segments of this range, however, the detectors display acceptable linear response. Thus, linear dynamic range for OPs varied from 5 to 1000 μg/L (**Table 2**), while that for OCs and PCBs ranged from 0.1 to 175 μg/L (**Table 3**). Linearity was good for all analytes, with correlation coefficients in the order of 0.9800−0.9990. These results indicated the correct linearity of the calibration curves at the respective spiking levels (10, 50, and 100 μg/kg olive oil for OPs, and 1, 5, and 10 μg/kg olive oil for OCs and PCBs).

3.2. Application of the Method to Virgin Olive Oil Samples. The applicability of the method was assayed during a pilot study where 19 virgin olive oil samples of "Empeltre" olive variety, collected from different olive mills of Aragón (Spain), were analyzed. Reported results were not corrected for recoveries. Three parameters were used to report residue levels: the mean, the median, and the standard error of the mean, expressed as μg/kg. The mean and the median were calculated using one-half the LOQ for results lower than the LOQ. The maximum concentration value (μg/kg) was the highest amount of a given residue recorded in a sample.

The descriptive analysis of residues found in the samples is summarized in **Table 4**. The organophosphorus chlorpyrifos, diazinon, dimethoate, methamidophos, omethoate, and parathion-methyl were not detected in any sample. Acephate was detected in one sample at a concentration of 0.01 mg/kg. There are no maximum residue limit (MRL) established for acephate in virgin olive oil (Codex Alimentarius), and the EU MRL for acephate in olives is 0.02 mg/kg of olives. However, the use of acephate is not registered for olive groves in Spain, so the source of contamination is unknown.

Table 4. Descriptive Analysis of Residues Found in 19 Virgin Olive Oil Samples (Percentage of Positive Samples, Mean ± Standard Error of the Mean, Median, and Maximum Concentration Detected)[a]

compound	% positives	mean ± std. error	median	maximum
α-HCH	26.3	1.5 ± 0.4	0.5	6
β-HCH	5.3	2.3 ± 0.3	2.0	8
lindane (γ-HCH)	47.4	3.1 ± 0.8	1.0	13
endosulfan sulfate	10.5	3.1 ± 0.1	3.0	4
p,p′-DDE	21.1	1.8 ± 0.2	1.5	5
p,p′-DDT	42.1	5.2 ± 0.8	3.0	13
PCB 28	21.1	2.6 ± 0.5	2.0	10
PCB 52	31.6	17.3 ± 5.4	3.0	70
PCB 138	26.3	2.3 ± 0.5	1.5	8
PCB 153	21.1	2.5 ± 0.3	2.0	7
PCB 180	89.5	12.4 ± 3.9	9.9	77
acephate	5.3	2.9 ± 0.4	2.5	10

[a] Results expressed as μg/kg virgin olive oil.

The organochlorine pesticides were found in few virgin olive oil samples and at very low levels ranging from 1.5 to 5.2 μg/kg. The fungicide HCB and the metabolites *p,p′*-DDD, α-endosulfan, and β-endosulfan were not detected in any sample. The insecticide of the organochlorine class detected most frequently (almost half of the samples), lindane, showed a maximum concentration of 13 μg/kg, and a mean concentration of 3.1 μg/kg. Endosulfan sulfate was detected in 2 out of 19 samples (10.5%), but at very low levels (less than 5 μg/kg), well below the European Union MRL of 1 mg/kg, set up for olives (the MRL refers to the sum of α-endosulfan, β-endosulfan, and endosulfan sulfate). PCBs were found in 20−90% of virgin olive oil samples, showing low concentrations between 2.3 and 17.3 μg/kg. Only PCB congener 180 was detected in more than one-half of the samples and showed a maximum value of 77 μg/kg.

The use of the very specific ion-trap GC−MS/MS allowed the confirmation of identity of the OC and PCB residues in the samples. Ion-trap GC−MS/MS confirmation of OC pesticides and PCB congeners was made according to these two criteria: (i) retention time for each analyte must be within 0.2 min of the retention time of the analyte in the standards, and (ii) the spectrum of each analyte must be within a satisfactory margin (match threshold 700) of the spectrum found for the analyte in the standards.

Other researchers of the Mediterranean area have recently reported residues of the organophosphorus insecticides fenthion and dimethoate in virgin olive oil from Greece (*10*, *13*, *15*) and Italy (*17*, *20*), at mean concentrations significantly lower than the MRLs. It has been reported that acephate and its metabolite methamidophos can occur in olives, but processing into olive oil removes both residues (*21*).

Reports of organochlorine residues in virgin olive oil are scarce. Endosulfan was detected in 22% of Greek virgin olive oil samples, and the residues occurred almost exclusively as the sulfate metabolite of endosulfan (*7*). In Spain, PCBs residues were detected in vegetable oils (*22*) and olive oil from Catalonia (*23*) at levels lower than 0.1 μg/kg.

3.3. Conclusions. The method proposed is based on a previous technique for OPs and OCs (*16*), which was modified by using acetonitrile saturated with hexane as eluting solvent for sample preparation and cleanup, allowing for the elution and subsequent determination of the most currently used organophosphorus insecticides in olive tree treatment in Spain (dimethoate, diazinon, and parathion-methyl), and a wide range

of organochlorine pesticides and polychlorinated biphenyls. Other modifications were necessary for the GC-NPD/ECD conditions to optimize the separation of all compounds. Additionally, the residues were confirmed by ion-trap GC−MS/MS. The application of these changes led to a novel analytical approach with all of the advantages mentioned in the original method, like short sample preparation times, low solvent consumption, little use of glassware, and increased sample throughput.

This adapted multiresidue GC method for the monitoring of OPs, OCs, and PCBs has been validated for 23 compounds, which can be found at trace levels in virgin olive oil. The recoveries were satisfactory for all OP and OC pesticides, with values for the most part being between 80% and 105% and relative standard deviations being below 15%. The PCBs showed somewhat lower recoveries on average, although the repeatability was fairly acceptable. The low solvent volume and laboratory equipment required per sample make this method economical. Also, its suitability for the most commonly used insecticides in olive groves in Spain makes it very useful for routine analysis.

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