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Multiresidue Method for Determination of 35 Pesticides in Virgin Olive Oil by Using Liquid−Liquid Extraction Techniques Coupled with Solid-Phase Extraction Clean Up and Gas Chromatography with Nitrogen Phosphorus Detection and Electron Capture Detection

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A method for the multiresidue determination of 35 pesticides (30 insecticides and five herbicides) in olive oil by gas chromatography (GC) is described. Three liquid−liquid extraction (LLE) procedures based on (i) partition of pesticides between acetonitrile (ACN) and oil solution in *n*-hexane, (ii) partition of pesticides between saturated ACN with *n*-hexane and oil solution in *n*-hexane saturated with ACN, and (iii) partition of pesticides between ACN and oil were tested for the optimization of the highest pesticide recoveries with the lowest oil residue in the final extracts. Experimental tests were preformed in order to study the efficiency of different clean up procedures with N-Alumina, Florisil, C18, and ENVI-Carb solid-phase extraction (SPE) cartridges for the compounds analyzed by GC-nitrogen phosphorus detection. A second step of clean up was also performed for the compounds analyzed by GC-electron capture detection (ECD), by using phenyl-bonded silica (Ph), diol-bonded silica (Diol), cyanopropyl-bonded silica (CN), and amino propyl-bonded silica (NH_2) SPE cartridges. LLE of the oil solution in hexane with ACN followed by an ENVI-Carb SPE clean up of the extract gave the best results for all target compounds. The ACN extract was additionally cleaned through a Diol-SPE cartridge for the determination of pesticides analyzed mainly by GC-ECD. Pesticide recoveries form virgin olive oil spiked with 20, 100, and 500 μg/kg concentrations of pesticides ranged from 70.9 to 107.4%. The proposed method featured good sensitivity, pesticide quantification limits were low enough, and the precision, expressed as relative standard deviation, ranged from 2.4 to 12.0%. The proposed method was applied successfully for the residue determination of the selected pesticides in commercial olive oil samples.

KEYWORDS: Pesticides; multiresidue analysis; olive oil; SPE clean up; GC-NPD; GC-ECD

INTRODUCTION

The presence of pesticide residues in olives and olive oil can negatively affect human health. Pesticide residues represent one of the most important factors of olive oil chemical contamination as the oil is obtained from olives by mild fermentations. In agriculture practice on olive groves, the use of insecticides and herbicides provides an unquestionable benefit for crop protection (*1*, *2*). The quantity of lipophilic pesticide residues in olives is expected to be concentrated in olive oil. One kilogram of olive oil could be produced in average from 4 kg of olives. There is therefore a need for rapid and reliable controls to ensure that the residual levels in olive oil are below the maximum residue limits (MRLs) permitted by different bodies of legislation (*3*, *4*).

Organophospates and pyrethroids are the major classes of insecticides used in olive trees, and endosulfan is the main organochlorine insecticide used with selective action on arthropod attacks. The determination of organophosphates is usually accomplished by gas chromatography (GC) using specific detectors (flame photometric, flame thermionic, nitrogen phosphorus, electron capture, and mass selective detectors), and many methods have been proposed (*5*−*9*) for their analysis; however, only few have been reported for endosulfan and pyrethroids (*10*−*12*) by using GC with ECD and MSD. However, for their determination, a further clean up step is generally required because of excessive interferences of fatty materials traces (*12*, *13*).

Herbicides consist of the other major class of pesticides applied in olive groves and could lead in oil contamination. Triazine herbicides such as simazine, atrazine, and prometryn are being applied and may contaminate olives mainly by

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Table 1. Main Characteristics of SPE Cartridges Used

SPE Cartridge	Symbol in Study	Sorbent Material	Retention Mechanism
LC-Alumina-N (60/325 mesh)	N-Alumina	Crystalline, chromatographic grade alumina for neutral pH (6.5)	Adsorption
LC-Florisil (100/120 mesh particles)	Florisil	Magnesium silicate	Adsorption
LC-18 (40μm particles ; 60Å pores)	C18	$-Si-(CH_2)_{17}CH_3$ Polymerically bonded, octadecyl (10%C), endcapped silica	Reversed Phase
ENVI-Carb (surface area 100m^2/g; 120/140 mesh)	ENVI-Carb	Graphitized Non-Porous Carbon Carbon surface comprised of hexagonal ring structures interconnected and layered into graphitic sheets	Reversed-Phase or Absorption
LC-Diol (40μm particles ; 60Å pores)	Diol	$-Si-(CH_2)_3CH_2-CH(OH)-CH_2OH$ Polymerically bonded, 2,3-Dihydroxypropoxypropyl (7%C), silica	Normal Phase
LC-CN (40μm particles ; 60Å pores)	CN	$-Si-(CH_2)_3CN$ Monomerically bonded, cyanopropyl (7%C), endcapped silica	Reversed Phase or Normal Phase
LC-Ph (40μm particles ; 60Å pores)	Ph	$-Si-C_6H_5$ Monomerically bonded, phenyl (7%C), endcapped silica	Reversed Phase
LC-NH_2 (40μm particles ; 60Å pores)	NH_2	$-Si-(CH_2)_3NH_2$ Polymerically bonded, aminopropyl phase (5%C) silica	Normal Phase or Weak Anion-Exchange

environmental pollution, e.g., airborne particulates and contamination from soil during harvest. These compounds are usually analyzed simultaneously with organophosphate insecticides (*7*, *14*, *15*). There are relatively fewer reported data for the analysis of other herbicides in olive oil (*15*−*17*).

The most common extraction technique used was liquid−liquid extraction (LLE) of pesticides from the oil followed by a liquid or solid-phase extraction (SPE) clean up (*5*, *6*, *10*, *12*, *18*, *19*). Clean up with gel permeation chromatography (*17*, *20*, *21*), size exclusion chromatography (*14*), other extraction methods involving solid-phase microextraction (*9*), and matrix solid-phase dispersion (*11*, *19*) have also been effectively developed. These methods are specific to well-studied pesticide groups and/or demand certain instrumentation. The main problem in the analysis of pesticides in olive oil remains the constitution of the fat matrix, including compounds with a wide range of polarities. The last explains the small number of reports on multiresidue methods for the determination of different classes of pesticides in olive oil with a single extraction method (*14*, *17*, *19*).

The objective of this work was the development and validation of a multiresidue method for the determination of 35 pesticides commonly used in olive groves in Greece and Mediterranean countries. Among them were included fenthion sulfoxide, omethoate, and endosulfan sulfate, which are the main metabolites of fenthion, dimethoate, and endosulfan, respectively. The latter insecticides have been found as residues in olive oils in many reported monitoring data from Mediterranean countries (*12*, *22*−*24*). The LLE technique was selected as the more suitable method for routine analysis of pesticide traces in oil with the advantage of low cost and nonspecific instrumentation demands. The determination of a wide range of analytes, including organophosphates, organochlorines, triazines, triadiazines, and pyrethroids, phthalic acid, and trifluoromethyl compounds was optimized by using a SPE clean up coupled to GC with NPD and ECD. The SPE sorbents N-Alumina, Florisil, C18, and ENVI-Carb, which have already been used for the isolation of certain classes of pesticides from oil extract solutions (*10*, *12*, *25*, *26*), and four silica-based sorbents Ph, Diol, CN, and NH_2 were used in order to have a wider range of packing sorbent materials available for the performance of the multiresidue determination with higher recoveries and lower oil levels transferred in the final extracts.

MATERIALS AND METHODS

Chemicals and Materials. All solvents, acetonitrile, acetone, methanol, ethyl acetate, toluene, and *n*-hexane were obtained from Labscan (Dublin, Ireland), and all were grade Pestiscan (pesticide residue analysis grade). SPE cartridges, used for the clean up experiments, were 500 mg of quantity and 6 mL of capacity (except those of LC-Alumina-N and LC-Florisil that were 1000 mg of quantity and 6 mL of capacity) and were all obtained from Supelco (Bellefonte, PA). A short description and the main characteristics of these materials are shown in **Table 1**.

Pesticide standards were obtained from Riedel-de Haën (Seelze, Germany), and their purity ranged from 68.9 to 99.9%. Stock standard solutions of each pesticide were prepared in acetone at concentrations of 300−1000 μg/mL and stored in glass, tapered bottles at −20 °C. Working standard solutions were obtained by appropriate dilution with acetone. The main physicochemical properties of the pesticides studied, the detectors used for their determination, and the MRLs used for the pesticide levels estimation in olive oil are shown in **Table 2**.

GC. Analyses were performed on a Shimadzu GC-14B gas chromatograph equipped with a 63Ni ECD and on a Hewlett-Packard 5890 Series II gas chromatograph equipped with a NPD. Both were operated in the splitless mode (1 min, 1 μL injection).

Analyses on GC-ECD were performed on a fused silica capillary column Zebron ZB-5 (30 m long × 0.25 mm i.d. × 0.25 μm film thickness) and contained 5% phenyl−95% dimethylpolysiloxane (Phe-

Table 2. Chemical Groups (*27*), Physicochemical Properties (*28*), GC Detection, and MRLs of the Selected Pesticides[a]

peak number	pesticide	chemical group	vapor pressure (mmHg)	sol. in water (mg/L)	log K_{ow}	detection	MRL[b] (μg/kg)
1	dichlorvos	OP	0.0158 (20 °C)	8000 (20 °C)	1.47	NPD	100
2	omethoate	OP	2.48E-05 (20 °C)	1.0E-06	−0.74	NPD	200
3	dimethoate	OP	8.25E-06 (25 °C)	25000 (21 °C)	0.78	NPD	2000
4	simazine	TR	2.21E-08 (25 °C)	6.2 (22 °C)	2.18	NPD	NS
5	atrazine	TR	2.89E-07 (25 °C)	34.7 (26 °C)	2.61	NPD	100
6	diazinon	OP	9.01E-05 (25 °C)	40.0 (20 °C)	3.81	NPD	20
7	etrimfos	OP	8.00E-05 (25 °C)	40.0 (24 °C)	2.94	NPD	NS
8	parathion methyl	OP	3.50E-06 (25 °C)	37.7 (20 °C)	2.86	NPD	200
9	prometryn	TR	2.00E-06 (25 °C)	33.0 (25 °C)	3.51	NPD	NS
10	fenitrothion	OP	5.40E-05 (25 °C)	38 (25 °C)	3.30	NPD	500
11	pirimiphos methyl	OP	1.50E-05 (20 °C)	8.6 (20 °C)	4.20	NPD	50
12	malathion	OP	3.38E-06 (25 °C)	143 (20 °C)	2.36	NPD	500
13	fenthion	OP	1.05E-05 (25 °C)	7.5 (20 °C)	4.09	NPD	2000[c]
14	chlorpyrifos	OP, PYD	2.03E-05 (25 °C)	1.12 (24 °C)	4.96	NPD	50
15	mecarbam	OP	negligible (25 °C)	<1000 (25 °C)	2.29	NPD	50
16	quinalphos	OP	2.60E-06 (25 °C)	22 (24 °C)	4.44	NPD	50
17	methidathion	OP	3.37E-06 (25 °C)	187 (20 °C)	2.20	NPD	1000
18	buprofezin	TRDZ	9.40E-06 (25 °C)	0.9 (25 °C)	4.30	NPD	NS
19	fenthion sulfoxide	OP	5.51E-06 (25 °C)	3.72	1.92	NPD	2000[c]
20	ethion	OP	1.50E-06 (25 °C)	2.0 (25 °C)	5.07	NPD	100
21	phosmet	OP	4.90E-07 (25 °C)	24.4 (20 °C)	2.78	NPD	NS
22	azinphos methyl	OP, TR	1.60E-06 (25 °C)	20.9 (20 °C)	2.75	NPD	500
23	phosalone	OP	4.54E-08 (25 °C)	3.05 (25 °C)	4.38	NPD	100
24	azinphos ethyl	OP, TR	2.40E-06 (25 °C)	10.5 (25 °C)	3.40	NPD	50
25	chlorthal dimethyl	PA	2.50E-06 (25 °C)	0.5 (25 °C)	4.28	NPD	NS
26	α-endosulfan	Ocl	3.00E-06 (25 °C)	0.51 (20 °C)	3.83	ECD	50[d]
27	oxyfluorfen	TFM	2.48E-07 (25 °C)	0.116 (25 °C)	4.73	ECD	NS
28	β-endosulfan	Ocl	6.00E-07 (25 °C)	0.45 (20 °C)	3.83	ECD	50[d]
29	endosulfan sulfate	Ocl		0.48 (20 °C)	3.66	ECD	50[d]
30	λ-cyhalothrin	PYR	1.59E-09 (25 °C)	0.0008 (20 °C)	7.00	ECD	20
31	permethrin	PYR	2.18E-08 (25 °C)	0.006 (20 °C)	6.50	ECD	50
32	β-cyfluthrin	PYR	1.50E-10 (25 °C)	0.003 (25 °C)	5.95	ECD	20
33	α-cypermethrin	PYR	1.73E-07 (25 °C)	0.01	6.94	ECD	50
34	fenvalerate	PYR	1.50E-09 (25 °C)	0.024 (22 °C)	6.20	ECD	20
35	deltamethrin	PYR	1.50E-08 (25 °C)	0.002 (25 °C)	6.20	ECD	100

[a] OP, organophosphate; TR, triazine; PYR, pyrethroid; Ocl, organochlorine; PYD, pyridine; TRDZ, triadiazine; PA, phthalic acid; and TFM, trifluoromethyl. [b] MRLs present are those established by the European Union for the commodity of olives (*3*); NS, not specified. [c] The limit concerns all residues of fenthion, the sum of fenthion, its oxygen analogue, and their sulfoxides and sulfones, expressed as fenthion. [d] The limit is referred to the sum of α- and β-endosulfan and endosulfan sulfate.

nomenex). Helium (purity >99.5%) was used as the carrier at 1.5 mL/min, and nitrogen (99.999% purity) was used as makeup gas at 35 mL/min according to the optimization results of the instrument given by the manufacturer. The injector and detector were operated at 240 and 300 °C, respectively. The chromatographic temperature program was 100 °C for 1 min, raised to 210 °C (5 °C/min) and held for 16 min, and then raised to 285 °C (3 °C/min) and held for 10 min.

Analyses on GC-NPD were performed on a Zebron ZB-1 (30 m long × 0.32 mm i.d. column × 1.00 μm film thickness) and contained 100% methylpolysiloxane (Phenomenex). Helium (purity >99.5%) was used as the carrier gas at 1.0 mL/min. Gas flow rates used were hydrogen (ultrapure grade), 3 mL/min; air (ultrapure grade, 78% N_2, 22% O_2), 110 mL/min; and carrier plus makeup gas, 30 mL/min. The injector and detector were operated at 220 and 280 °C, respectively. The chromatographic temperature program was 100 °C for 1 min, raised to 190 °C (15 °C/min) and held for 3 min, and then raised to 270 °C (4 °C/min) and held for 15 min.

Olive Oil Samples. Twenty-one commercial virgin olive oil samples collected from Greek markets in November 2004 and two samples collected from olive mills of two different olive oil production areas in Greece (Preveza-Epirus and Iraklio-Crete) were used in this study. All samples were stored in 100 mL amber glass bottles capped with Teflon-lined screw caps and kept at 4 °C away from light before extraction.

The samples collected from olive mills and one of the commercials were organic virgin olive oils and were used in method studies in order to ensure the diversity of olive oils and the purity from pesticide residues. Appropriate amounts of a pesticide working solution were spiked in 5 g of organic virgin olive oil samples in order to have a range of pesticide concentrations between 5 and 500 μg/kg for recovery experiments and linearity studies. After agitation, the samples were allowed to equilibrate for 60 min prior to different extraction assays.

LLEs Studied. The extraction of pesticides from the oil sample using acetonitrile (ACN) as the extraction solvent was optimized by testing the following three LLE procedures.

LLE-1 Procedure (LLE-1). An aliquot of 5 ± 0.001 g of olive oil was weighted in a 40 mL screw-capped glass tube and dissolved in 5 mL of *n*-hexane. The solution was extracted twice with 10 mL of ACN.

LLE-2 Procedure (LLE-2). An aliquot of 5 ± 0.001 g of olive oil was weighted in a 40 mL screw-capped glass tube and dissolved in 5 mL of saturated *n*-hexane in ACN. The solution was extracted twice with 10 mL of ACN saturated in *n*-hexane.

LLE Procedure 3 (LLE-3). An aliquot of 5 ± 0.001 g of olive oil was extracted twice with 10 mL of ACN in a 40 mL screw-capped glass tube.

Each extraction test was performed by agitation in a rotary shaker for 5 min. The combined extracts from each procedure were brought to dryness by the use of a rotary evaporator apparatus (water bath temperature <40 °C), and the coextracted oil was weighted in order to estimate the oil transferred in the extract after the different sample extraction treatments. In further recovery studies, the extracts of each LLE procedure were subjected to the selected SPE clean up.

SPE Clean up Studies. The combined extracts of the LLE procedure that were found to have the less olive oil residue were subjected to clean up procedures through different SPE cartridges based on different sorbents in order to find a material or materials combination that would allow the determination of the 35 pesticides by GC-NPD and GC-ECD. Four different SPE cartridges packed with N-Alumina, Florisil, C18, and ENVI-Carb were tested. A second step of clean up was tested by using Diol, CN, Ph, and NH_2 SPE cartridges in the cases of high oil

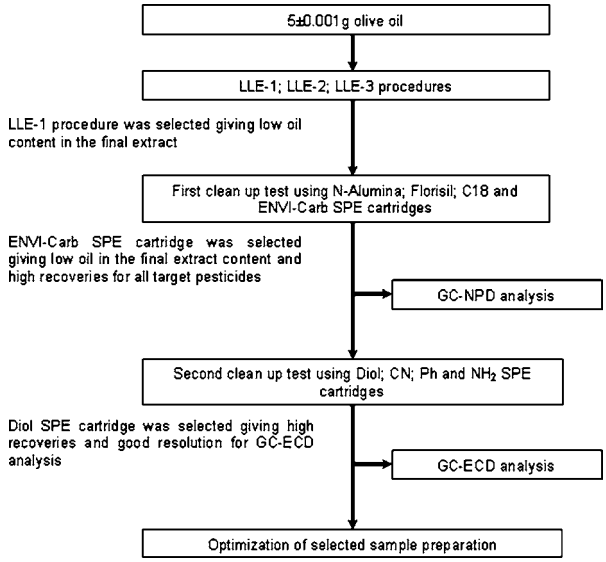

Figure 1. Experimental steps followed for the development and optimization of the analytical method.

transferred to the eluents or in cases of low chromatographic resolution. Two different operating procedures were followed in these studies depending on the polarity of the solid-phase matrix used

(i) N-Alumina, Florisil, C18, and ENVI-Carb SPE cartridges were conditioned with 6 mL of ACN, and 6 mL of the combined ACN extracts from LLE procedures was passed through the cartridge by avoiding the dry column. The elution was performed with 12 mL of ACN. The eluents of each clean up procedure tested were brought to dryness, and the residue was weighted in order to determine the oil residue passed through the eluant. After all, the residue was dissolved in 0.5 mL of a 200 μg/L standard solution of internal standard (IS) in acetone for the analysis on GC-NPD or in 1 mL of a 100 μg/L standard solution of IS in acetone for the analysis on GC-ECD. Bromophos-Ethyl and Endrin were used as IS in GC-NPD and GC-ECD, respectively.

(ii) Diol, CN, Ph, and NH_2 SPE cartridges were used in an additional clean up step for the compounds analyzed by GC-ECD where a lot of interfering peaks appeared or in the case when a high amount of transferred oil was observed in the final extract. Cartridges were conditioned with 6 mL of methanol and 6 mL of *n*-hexane. The ACN eluants from the previous first clean up procedure were brought to dryness in a rotary evaporator (water bath temperature <40 °C), and the residue was redissolved in 2 mL of *n*-hexane. After the 2 mL extract in *n*-hexane had passed through the conditioned column, the cartridge was eluted with 12 mL of *n*-hexane. The solvent was evaporated to dryness in a rotary evaporator, and the residue was dissolved in 1 mL of a 100 μg/L standard solution of Endrin (IS) and analyzed by GC-ECD.

The experimental extraction and clean up procedures followed are shown in **Figure 1**. Because the gas chromatographic response for many pesticides is known to be matrix-dependent (*29*, *30*), quantification was carried out by the IS method using standards in nonspiked residue-free olive oil extracts obtained by the same sample preparation followed each time.

RESULTS AND DISCUSSION

LLE Preliminary Study. LLE was chosen for the first isolation of pesticides from the complex oil matrix. Three of the most widespread LLE procedures in the determination of pesticides in olive oil, selected to be studied, are as described in the experimental session: (i) LLE-1 includes the extraction of pesticides from the olive oil solutions in *n*-hexane with ACN, (ii) LLE-2 includes the extraction of pesticides from the olive oil solutions in saturated *n*-hexane with ACN by using ACN saturated with *n*-hexane as the extraction solvent, and (iii) LLE-3 includes pesticides extracted straight from the olive oil by using ACN. LLE-1 and LLE-3 have already been evaluated for their

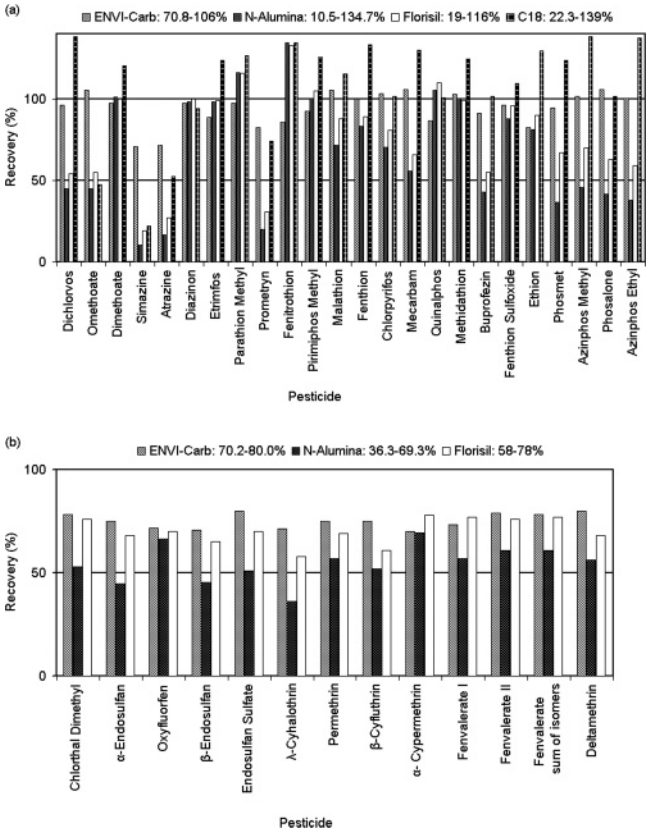

Figure 2. Overall mean percent recoveries ($n = 9$) of selected pesticides spiked at 20, 100, and 500 μg/kg in three different olive oil samples, as determined after the Envi-Carb, N-Alumina, Florisil, and C18 clean up of the extracts obtained by LLE-1. (**a**) Pesticides determined by GC-NPD and (**b**) pesticides determined by GC-ECD.

efficiency only for organophosphate pesticides determination in olive oil (*7*), but no comparative data were found in the literature on the efficiency of the three above procedures in such a wide range of analytes. The selection of the appropriate LLE procedure was primarily made by weighting the oil residue remaining in the extract, since the determination of pesticides recoveries was not possible without first optimizing the clean up procedure of the high molecular weight interferences. Three olive oil samples were extracted with the three different extraction procedures, and the oil residue was weighted. The mean value ($n = 9$) of the transferred oil in the extract, expressed as mg/g of olive oil extracted, was found to be 11.43 ± 1.24 mg/g for LLE-1, 16.12 ± 5.34 mg/g for LLE-2, and 22.82 ± 9.25 mg/g for LLE-3. The oil residue in the extract as calculated by LLE-1 confirms the results reported by other authors (*6*, *7*, *12*). The LLE-1 extraction procedure shows the lower oil residues transferred in the final extracts that were subjected to the following clean up studies through SPE cartridges.

Clean up Efficiency. For the selection of the best clean up procedure of the olive oil extracts, four different SPE cartridges with N-Alumina, Florisil, C18, and ENVI-Carb were tested for their efficiency. Three olive oil samples, spiked at three different fortification levels (20, 100, and 500 μg/kg), were extracted with the chosen LLE-1 procedure, and the extracts were cleaned through the four SPE cartridges by the procedure described in the Materials and Methods. The mean recoveries ($n = 9$) of the 24 pesticides determined by GC-NPD are shown in **Figure 2a** and range from 70.8 to 106.0% for clean up on ENVI-Carb, from 10.5 to 134.7% for the clean up on N-Alumina, from 19.0 to 116.0% for Florisil, and from 22.3 to 139% for the clean up

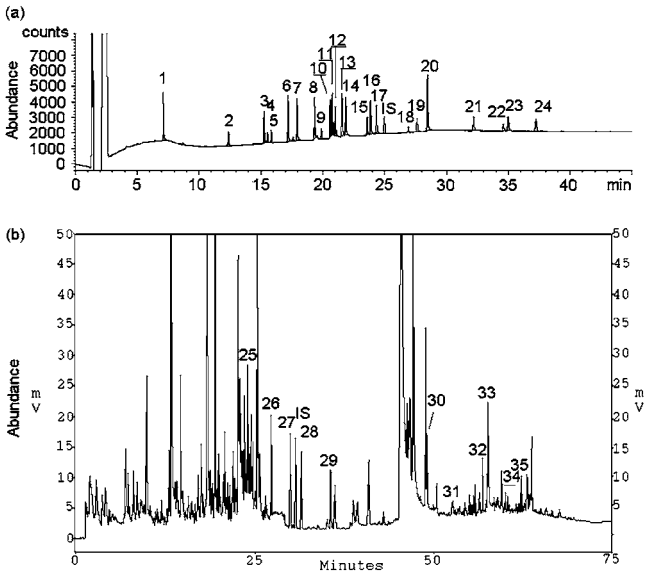

Figure 3. (**a**) Typical chromatogram of an olive oil extract (by LLE-1 procedure) cleaned through the ENVI-Carb cartridge and analyzed by GC-NPD. (**b**) Typical chromatogram of an olive oil extract (by LLE-1 procedure) cleaned through the ENVI-Carb cartridge and analyzed by GC-ECD. The fortification with pesticides of the olive oil sample was at 20 μg/kg except Mecarbam that was at 6 μg/kg. Peak numbers correspond to the compounds in **Table 2**.

on C18. The respective mean recoveries of the 11 pesticides determined by GC-ECD are shown in **Figure 2b**. The recoveries range from 70.2 to 80.0% for ENVI-Carb, from 36.3 to 69.3% for N-Alumina, and from 58.0 to 78.0% for Florisil. Clean up on the C18 cartridge was not evaluated by GC-ECD due to the high oil residues in the extract (8.4 $\pm$ 2.0 mg/g). Although the levels of oil residues in the eluants by using mainly N-Alumina and Florisil were found to be high (3.2 $\pm$ 1.1 and 4.3 $\pm$ 1.1 mg/g, respectively), these materials were more effective in interference removal from the extract according the GC-ECD chromatograms. Clean up through the ENVI-Carb cartridge gave less olive oil residue in the final extract (2.5 $\pm$ 0.8 mg/g) and the highest mean recoveries for all target pesticides from 70.2 to 106.0%, indicating a greater selectivity in the fractionation of target pesticides from high molecular mass coextracted components.

GC-NPD chromatograms after the ENVI-Carb clean up were shot of interferences (**Figure 3a**), but on GC-ECD analyses, there were a lot of interfering peaks (**Figure 3b**). To remove the interfering constituents of olive oil, the eluant from the ENVI-Carb cartridge was subjected in a second clean up test through normal-phase SPE clean up as the previous studies indicated that the interfering compounds were of medium to high polarity. The SPE cartridges tested were Ph, CN, NH_2, and Diol. All cartridges were conditioned with *n*-hexane and eluted with *n*-hexane as already described. The results of this comparative study are shown in **Figure 4**. Mean percentage recoveries ($n = 9$) ranged from 60.0 to 105.7% for clean up on the Ph cartridge, from 30.0 to 83.6% on CN, from 16.9 to 108.1% on NH_2, and from 77.6 to 96.4% on Diol. Exclusive of the clean up through the CN cartridge, the other normal-phase cartridges show great selectivity in interference removal from extracts, giving adequate recoveries for most of the analytes. The cleanest chromatograms were achieved by the purification of extracts through the NH_2 cartridge, but the recoveries of endosulfan remained in low levels. However, the results indicated that the clean up procedure by using the Diol cartridge

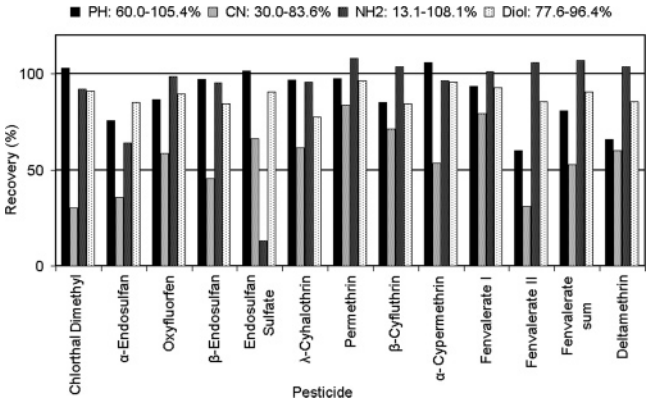

Figure 4. Overall mean percent recoveries ($n = 9$) of pesticides analyzed by GC-ECD and spiked at 20, 100, and 500 μg/kg in three different olive oil samples, as determined after the Ph, CN, NH_2, and Diol clean up of the extracts obtained by LLE-1 and previously purified with ENVI-Carb.

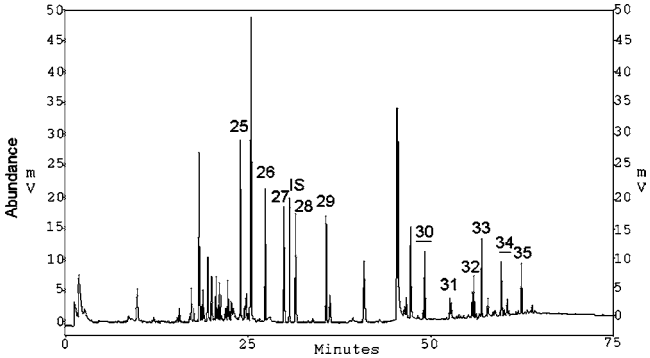

Figure 5. Typical chromatogram of an olive oil extract (by LLE-1 procedure) cleaned through both ENVI-Carb and Diol cartridges and analyzed by GC-ECD. The fortification with pesticides of the olive oil sample was at 20 μg/kg. Peak numbers correspond to the compounds in **Table 2**.

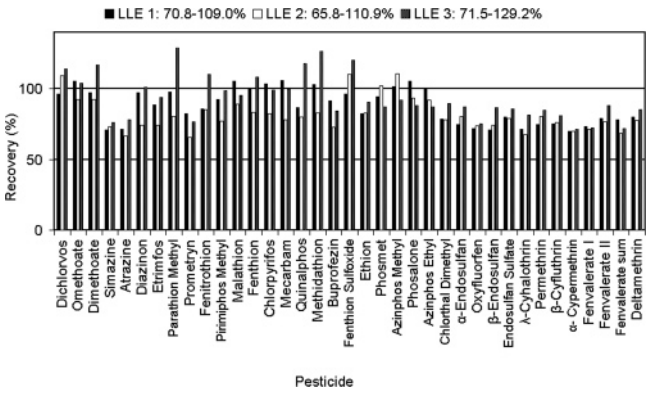

Figure 6. Overall mean percent recoveries ($n = 9$) of the 35 target pesticides spiked at 20, 100, and 500 μg/kg in three different olive oil samples, as determined by LLE-1, LLE-2, and LLE-3.

should be used as a good solution in multiresidue pesticide analysis giving the highest recoveries for all target compounds and the cleanest chromatograms according on their retention times (**Figure 5**).

Recovery Efficiency of the LLE. In order to estimate the recovery yields of extraction, three olive oil samples spiked at 20, 100, and 500 μg/kg with the standard mixture of pesticides were extracted with the three different procedures, LLE-1, LLE-2, and LLE-3, as described previously. In **Figure 6** are shown the mean percentage recoveries ($n = 9$) of all pesticides studied as found by the three different extraction procedures. The

Table 3. Percent Mean Recoveries of Three Determinations of a Spiked Olive Oil Sample at 100 μg/kg That Was Extracted with LLE-1 and Purified with ENVI-Carb and Diol SPE Using the Initial Elution Tested and the Optimum Elution Found

pesticide	initial elution[a]	optimized elution[b]
chlorthal dimethyl	78.4 ± 8.7	102.0 ± 7.1
α-endosulfan	75.0 ± 9.5	96.0 ± 8.0
oxyfluorfen	71.8 ± 6.9	100.3 ± 7.2
β-endosulfan	70.9 ± 9.6	95.3 ± 9.0
endosulfan sulfate	79.9 ± 8.9	102.5 ± 7.6
λ-cyhalothrin	71.2 ± 13.5	86.3 ± 11.1
permethrin	75.0 ± 13.4	100.2 ± 10.8
β-cyfluthrin	75.2 ± 11.2	95.8 ± 9.0
α-cypermethrin	70.2 ± 10.2	107.8 ± 9.0
fenvalerate I	73.4 ± 10.3	103.7 ± 7.1
fenvalerate II	79.0 ± 10.9	97.1 ± 7.9
fenvalerate sum	78.4 ± 11.0	102.0 ± 8.0
deltamethrin	80.0 ± 11.9	96.4 ± 8.9

[a] ENVI-Carb cartridge eluted with 20 mL of ACN and Diol cartridge eluted with 12 mL of *n*-hexane. [b] ENVI-Carb cartridge eluted with 10 mL of ACN and 10 mL of ACN/toluene, 95:5, v/v, and Diol cartridge with 6 mL of *n*-hexane and 6 mL of *n*-hexane/ethyl acetate/methanol, 95:2.5:2.5, v/v/v.

Table 4. Relative Responses (R%, 100% = Response of Pesticide in Acetone) ± %RSD (n = 9) of Pesticides Studied in Olive Oil Extracts Spiked at 10, 25, and 100 μg/kg[a]

pesticide	R%	pesticide	R%
dichlorvos	*146.0 ± 2.1*	fenthion sulfoxide	*119.3 ± 3.5*
omethoate	*128 ± 2.5*	ethion	106.7 ± 3.3
dimethoate	*120.6 ± 0.8*	phosmet	*129.2 ± 0.8*
simazine	90.8 ± 7.7	azinphos methyl	*130.0 ± 0.4*
atrazine	83.0 ± 2.3	phosalone	109.0 ± 1.2
diazinon	94.6 ± 8.8	azinphos ethyl	*110.2 ± 1.4*
etrimfos	97.5 ± 9.6	chlorthal dimethyl	97.1 ± 6.9
parathion methyl	99.8 ± 10.0	α-endosulfan	102.7 ± 8.9
prometryn	81.4 ± 5.6	oxyfluorfen	95.9 ± 7.2
fenitrothion	101.7 ± 5.1	β-endosulfan	99.3 ± 4.7
pirimiphos methyl	104.8 ± 6.9	endosulfan sulfate	*117.6 ± 5.1*
malathion	*116.3 ± 1.9*	λ-cyhalothrin	*119.6 ± 10.8*
fenthion	103.4 ± 3.4	permethrin	*143.0 ± 5.4*
chlorpyrifos	113.7 ± 7.8	β-cyfluthrin	106.8 ± 10.6
mecarbam	106.6 ± 4.8	α-cypermethrin	108.4 ± 4.0
quinalphos	106.9 ± 7.2	fenvalerate I	*113.5 ± 8.3*
methidathion	*118.8 ± 8.6*	fenvalerate II	108.2 ± 9.2
buprofezin	95.4 ± 5.5	deltamethrin	*130.4 ± 1.3*

[a] Italicized values indicate relative responses exceeding 110%.

recoveries ranged from 70.8 to 109.0% for the LLE-1 procedure, from 65.8 to 110.9% for LLE-2, and from 71.5 to 129.2% for LLE-3. The extraction of pesticides by using LLE-1 (extraction of pesticides from the olive oil diluted in *n*-hexane with ACN) gave the highest recoveries for all target pesticides and the highest resolution in GC analyses.

Optimization of the Clean up Procedure. The optimization studies focused initially on the evaluation of the maximum amount of extract that could be applied to ENVI-Carb cartridge based on clean up procedure LLE-1 that was adequate for all target pesticides. In order to optimize this parameter, the clean up procedure on ENVI-Carb was applied to 6, 12, and 18 mL of ACN extract obtained by LLE-1. The oil residue transferred in ACN eluants was higher than expected by using an 18 mL volume sample (1.22 ± 1.21 mg/mL of ACN extract, n = 3) probably due to an overloading of the active sites of the sorbent. The cartridges tested as already reported were those packed with 500 mg of sorbent, and circumstantially, a cartridge of 1000 mg quantity should be utilized if all of the sample extract is to be used. However, in the present work, because the analyses were carried out in two GC instruments, we selected to split the ACN extracts in two fractions and perform the purification processes separately for each analysis. Six milliliters of ACN extracts was used for analyses on GC-NPD and 12 mL for those performed on GC-ECD.

Because the sample amount was defined, different elution solvents of ACN and toluene mixtures were tested in order to optimize the recoveries of the less polar pesticides analyzed by GC-ECD. An elution through ENVI-Carb cartridge with 10 mL of ACN and 10 mL of a ACN/toluene mixture (95:5, v/v) slightly increased the recoveries obtained by the elution with 20 mL of pure ACN without changing the oil residue in the final extract. Clean up through the Diol-SPE cartridge was optimized by testing different elution solvents of *n*-hexane, ethyl acetate, and methanol mixtures. The elution through the Diol cartridge by using 6 mL of *n*-hexane and 6 mL of a mixture of hexane/ethyl acetate/methanol, (95:2.5:2.5, v/v/v) was found to give higher recoveries than those obtained by the elution with 12 mL of pure *n*-hexane without changing the chromatographic profile. The mean recoveries of three determinations of a spiked olive oil sample at 100 μg/kg (**Table 3**) were estimated by the initial elution through the ENVI-Carb cartridge with 20 mL of pure ACN and the Diol cartridge with 12 mL of pure *n*-hexane and by the optimum found.

Finally, it should be noted that the SPE cartridges could be used multiple times, thereby lowering considerably the total cost of analysis. The elution of the ENVI-Carb cartridge with 20 mL of pure toluene completely removed the retained coextractives of the oil extract by allowing the reuse of the cartridge. After the purification process, the conditioning of the ENVI-Carb column was performed with 12 mL of ACN instead of the 6 mL proposed. The complete purification of the Diol cartridge was achieved with the elution with 10 mL of methanol. Both cartridges were reused five times, and the results were repeatable.

Matrix Effects. A comparison between calibration standards prepared in pure acetone and spiked matrix standards was performed in order to estimate matrix effects. The mean relative responses of three standards concentrations in solvent (10, 25, and 100 μg/kg) as compared, in triplicate, to those of spiked matrix standards are shown in **Table 4**. The pesticides responses from standard solutions in solvent were lower than those obtained from standards in olive oil extracts (positive matrix effect). Significantly higher responses to standards in solvent were observed only for triazines (negative matrix effect) probably due to interactions of the primary amino group they contain with nonvolatile sample components traces, which unavoidably remain in oil extracts. The highest positive matrix effects were observed for dichlorvos and permethrin, whereas significantly higher (R > 110%) relative responses were found for deltamethrin, azinphos methyl, phosmet, omethoate, dimethoate, λ-cyhalothrin, fenthion sulfoxide, methidathion, endosulfan sulfate, malathion, and fenvalerate I. It has been reported that the extent of positive matrix-induced effects is related to the high polarity of the analytes, the type of coextracts in the sample (most distinct detector response enhancement for matrices with a high content of essential oils and waxes), the analyte/matrix concentration, and the state (history) of the GC system (*24*). The matrix effects estimated by relative responses and introduced in **Table 4** could be attributed to the pesticides polarity and to the fat traces remaining in oil extracts. Therefore, matrix-matched standards in free residue olive extracts should be used in order to avoid quantitative errors.

Analytical Performance. In **Table 5** are shown the analytical characteristics of the method developed for the multiresidue

Table 5. Analytical Characteristics of the GC-NPD[a] and GC-ECD[b] Methods for the 35 Pesticides Studied[c]

		instrument		method				
pesticide	t_R (min)	LOD[d] (μg/L)	LOQ[d] (μg/L)	mLOD (μg/kg)	mLOQ (μg/kg)	linear range (μg/kg)	R^2	RSD[e] (%)
				GC-NPD method				
dichlorvos	7.00	5	15	2.6	8.8	10–200	0.9993	12.0
omethoate	12.05	10	30	2.9	9.6	10–200	0.9943	8.4
dimethoate	15.02	1	3	1.5	4.8	5–200	0.9980	8.9
simazine	15.31	10	30	6.4	19.8	20–500	0.9987	10.1
atrazine	15.61	10	30	6.5	22.2	20–500	0.9966	7.5
diazinon	16.96	1	3	1.3	2.9	5–200	0.9976	7.7
etrimfos	17.69	5	15	1.2	4.0	5–200	0.9934	5.7
parathion methyl	19.08	5	15	1.3	4.4	5–200	0.9975	8.0
prometryn	19.67	10	30	7.5	24.7	25–500	0.9937	5.7
fenitrothion	20.35	5	15	1.2	3.9	5–200	0.9976	5.3
pirimiphos methyl	20.55	5	15	1.3	4.2	5–200	0.9976	4.2
malathion	20.77	5	15	1.4	4.4	5–200	0.9954	8.6
fenthion	21.29	5	15	1.4	4.6	5–200	0.9982	2.4
chlorpyrifos	21.59	5	15	2.4	8.9	10–200	0.9990	11.4
mecarbam	23.35	2	6	0.6	1.9	5–150	0.9966	8.1
quinalphos	23.62	5	15	1.2	3.9	5–200	0.9979	8.0
methidathion	24.07	10	30	1.4	4.5	5–200	0.9984	5.6
buprofezin	26.65	50	150	12.6	41.6	50–500	0.9965	7.8
fenthion sulfoxide	27.58	5	15	2.5	8.4	10–500	0.9935	9.1
ethion	28.26	2	6	0.4	1.6	5–500	0.9964	9.1
phosmet	31.91	50	150	13.1	43.1	50–500	0.9964	9.3
azinphos methyl	34.25	50	150	14.0	46.1	50–500	0.9955	6.9
phosalone	34.64	50	150	14.5	47.8	50–500	0.9921	8.1
azinphos ethyl	36.89	50	150	13.8	45.6	50–500	0.9932	7.3
				GC-ECD method				
chlorthal dimethyl	23.87	0.2	0.6	0.8	2.6	5–500	0.9915	7.4
α-endosulfan	27.19	0.1	0.3	1.3	4.3	5–500	0.9974	8.5
oxyfluorfen	29.65	0.2	0.6	1.4	4.0	5–500	0.9968	7.7
β-endosulfan	31.57	0.5	1.5	1.7	5.7	5–500	0.9960	8.4
endosulfan sulfate	35.53	0.5	1.5	1.7	5.0	5–500	0.9972	7.3
λ-cyhalothrin	49.07	2.0	6.0	2.6	8.0	10–500	0.9997	10.9
permethrin	52.67	5.0	15.0	7.3	24.2	25–500	0.9946	10.4
β-cyfluthrin	55.72	2.0	6.0	6.1	20.0	20–500	0.9991	9.5
α-cypermethrin	56.73	2.0	6.0	2.5	9.6	10–500	0.9987	9.2
fenvalerate I	59.51	1.0	3.0	7.1	23.3	25–500	0.9997	7.2
fenvalerate II	60.30	5.0	15.0	9.1	30.0	10–500	0.9922	8.3
deltamethrin	62.25	10.0	30.0	13.1	43.3	15–500	0.9916	9.4

[a] Extraction of pesticides by LLE-1 procedure and cleanup by ENVI-Carb. [b] Extraction of pesticides by LLE-1 procedure and cleanup by ENVI-Carb and Diol. [c] t_R, retention time. [d] Instrument LODs and LOQs of standards in acetone. [e] Relative standard deviation ($n = 6$) for 100 μg/kg.

determination of pesticides by GC-NPD and GC-ECD. The limits of detection (mLOD) and quantification (mLOQ) of the method were calculated experimentally from a signal-to-noise ratio of 3.0 and 10.0, respectively, by spiking at low concentrations the olive oil samples and subjecting them to the sample preparation reported. Blank olive oil extracts were used for the estimation of the background noise of the chromatographic analysis. For the 24 pesticides analyzed by GC-NPD, the mLOD ranged from 0.4 to 14.5 μg/kg, and the mLOQ ranged from 1.6 to 47.8 μg/kg. The mLOD for the 11 pesticides determined by GC-ECD ranged from 0.8 to 13.1 μg/kg, and the mLOQ ranged from 2.6 to 43.3 μg/kg. In the same table are shown the instrument limits of detection (LOD) and quantification (LOQ) of the pesticides studied as determined by a signal-to-noise ratio of 3.0 and 10.0, respectively, by spiking pesticide solutions in acetone at low concentrations.

The linearity of the method was checked in the range 5−500 μg/kg by measuring the peak areas relative to that of the IS. Correlation coefficients were >0.99 in all cases, indicating a good linearity of both GC-NPD and GC-ECD methods for the quantification of target pesticides in the range studied. The precision of the method, expressed as repeatability (%RSD, $n = 6$), was evaluated on olive oils fortified at 100 μg/kg. As already reported, quantification was carried out by the IS method using matrix-matched standards in residue free olive extracts. Overall recovery and repeatability data are summarized in **Table 6**. The average recoveries of three determinations of five spiked concentrations of pesticides ranged from 71.4 to 107.4%, and the respective RSDs ranged from 2.4 to 12.0%.

Application to Olive Oil Samples. The proposed method was applied in analyses of 20 commercial virgin olive oil samples from Greek markets. A reagent blank, a standard prepared in acetone, and a blank sample were analyzed at the beginning of each set of samples, in order to control the cleanness of the instruments and to check the response of the detector. A matrix standard was analyzed twice with every set of samples in order to check the performance of the preparation of samples and to achieve accurate quantification. The results obtained with both detectors were transparent with the exception of some false positives introduced by GC-MS confirmation performed for dichlorvos and permethrin. Matrix-induced effects already reported for these two pesticides due to coeluted constituents of olive oil could not always be avoided by matrix-matched standards. An isotope standard addition should be used for further analyses whenever there is a positive determination of these pesticides in virgin olive oil samples.

The positive identifications of pesticides found are shown in **Table 7**. From the 35 pesticides included in the method, 15

Table 6. Percent Recoveries ± Standard Deviation ($n = 3$) of Pesticides Spiked to Olive Oil Samples at Different Concentrations

	μg/kg				
pesticide	500	250	100	50	20
dichlorvos	92.7 ± 10.8	93.9 ± 11.1	93.3 ± 12.0	99.4 ± 13.0	100.1 ± 13.0
omethoate	100.4 ± 7.6	105.8 ± 7.4	106.0 ± 7.9	106.3 ± 9.2	108.2 ± 10.0
dimethoate	92.2 ± 7.2	95.7 ± 7.3	96.9 ± 9.0	100.4 ± 11.0	101.0 ± 10.2
simazine	69.5 ± 10.3	69.9 ± 9.4	70.7 ± 7.9	72.0 ± 7.2	72.6 ± 15.8
atrazine	69.6 ± 6.1	71.0 ± 4.9	68.8 ± 8.1	73.3 ± 7.5	*[a]
diazinon	95.8 ± 4.6	93.8 ± 5.5	98.0 ± 9.2	99.1 ± 10.2	98.9 ± 9.9
etrimfos	87.6 ± 6.5	87.0 ± 5.6	89.2 ± 4.9	90.0 ± 5.4	90.4 ± 6.1
parathion methyl	96.8 ± 5.7	96.3 ± 5.4	97.6 ± 9.2	97.4 ± 10.3	100.2 ± 9.4
prometryn	73.0 ± 5.2	84.5 ± 6.1	85.6 ± 6.4	84.3 ± 4.9	*
fenitrothion	85.9 ± 6.9	85.2 ± 2.9	82.9 ± 7.3	86.4 ± 3.7	88.2 ± 5.4
pirimiphos methyl	90.9 ± 4.4	91.6 ± 4.5	92.5 ± 3.1	94.7 ± 3.9	94.6 ± 5.4
malathion	100.4 ± 7.2	106.2 ± 6.2	107.5 ± 8.0	104.8 ± 9.4	107.4 ± 11.4
fenthion	100.0 ± 2.6	100.4 ± 2.3	101.6 ± 2.1	100.8 ± 2.4	101.4 ± 2.8
chlorpyrifos	83.9 ± 11.2	102.3 ± 10.0	104.7 ± 11.9	102.8 ± 12.6	107.3 ± 11.1
mecarbam	106.3 ± 4.3	106.0 ± 6.4	106.8 ± 9.8	106.0 ± 10.8	106.9 ± 9.3
quinalphos	86.2 ± 7.3	86.7 ± 5.5	87.2 ± 9.1	86.6 ± 12.2	87.0 ± 5.9
methidathion	102.8 ± 4.7	102.3 ± 7.2	102.1 ± 6.5	102.9 ± 5.3	103.1 ± 4.1
buprofezin	91.6 ± 5.6	90.6 ± 7.0	91.7 ± 7.6	91.7 ± 9.1	*
fenthion sulfoxide	97.2 ± 4.5	96.4 ± 3.2	95.8 ± 11.1	96.5 ± 12.0	96.1 ± 14.8
ethion	82.5 ± 5.2	81.9 ± 5.6	82.6 ± 7.7	82.8 ± 12.4	83.6 ± 14.1
phosmet	94.4 ± 3.6	94.2 ± 10.0	94.6 ± 10.9	96.0 ± 10.1	*
azinphos methyl	101.3 ± 5.9	100.4 ± 5.6	100.7 ± 6.5	102.7 ± 7.9	*
phosalone	104.9 ± 6.3	105.7 ± 5.8	105.0 ± 5.6	104.1 ± 8.1	*
azinphos ethyl	99.8 ± 7.8	100.4 ± 7.0	100.7 ± 5.6	100.9 ± 7.5	*
chlorthal dimethyl	100.2 ± 7.3	101.7 ± 8.0	102.0 ± 7.1	103.5 ± 8.1	103.0 ± 6.5
α-endosulfan	93.0 ± 7.8	95.0 ± 8.0	96.0 ± 8.0	98.0 ± 9.1	99.0 ± 9.4
oxyfluorfen	100.2 ± 7.7	99.9 ± 7.4	100.3 ± 7.2	101.0 ± 8.1	101.0 ± 8.3
β-endosulfan	96.7 ± 9.4	96.1 ± 9.4	95.3 ± 8.0	95.4 ± 7.9	93.5 ± 7.3
endosulfan sulfate	101.3 ± 7.9	101.4 ± 5.3	102.5 ± 7.6	103.3 ± 7.6	99.5 ± 8.0
λ-cyhalothrin	88.6 ± 10.6	88.3 ± 11.9	86.3 ± 11.1	89.3 ± 12.7	90.5 ± 12.9
permethrin	104.8 ± 10.7	109.4 ± 8.0	108.2 ± 10.8	107.1 ± 10.7	*
β-cyfluthrin	93.3 ± 8.7	94.8 ± 8.3	95.8 ± 9.0	96.2 ± 10.3	*
α-cypermethrin	105.4 ± 9.7	106.3 ± 9.2	107.8 ± 9.3	106.8 ± 8.8	107.1 ± 9.0
fenvalerate I	103.4 ± 7.4	103.4 ± 7.5	103.7 ± 7.1	104.7 ± 7.0	*
fenvalerate II	96.1 ± 7.7	96.3 ± 7.3	97.1 ± 7.9	95.6 ± 8.7	*
deltamethrin	97.1 ± 8.7	93.4 ± 9.0	96.4 ± 8.9	98.5 ± 10.0	*

[a] *Recoveries were not estimated as the fortification level is below mLOQ of these pesticides (see **Table 5**).

Table 7. Pesticide Residues Found in the 20 Olive Oil Samples Analyzed by the Multiresidue Method

pesticide	mean value (μg/kg)	concn range (μg/kg)	positive samples[a] (no.)	samples exceed MRLs (no.)	MRLs[b] (μg/kg)
dimethoate	6.6	5.9–8.1	16 (13)	0	2000
diazinon	3.3	3.3	2 (1)	0	20
parathion methyl	10.0	10.0	4 (3)	0	200
fenthion	17.6	4.9–35.7	18 (1)	0	
fenthion sulfoxide	23.3	8.8–90.2	17 (2)	0	
total fenthion	39.9	7.3–113.8	18	0	2000
chlorpyrifos	10.4	10.4	7 (6)	0	50
methidathion	6.6	4.9–8.3	4 (2)	0	1000
ethion	24.1	24.1	2 (1)	0	100
α-endosulfan	6.7	6.2–7.2	4 (2)	0	
β-endosulfan	7.8	5.9–9.6	4 (2)		
endosulfan sulfate	28.1	12.6–52.7	6		
total endosulfan	32.9	12.6–56.4	6	2	50
λ-cyhalothrin	19.5	19.5	3 (2)	0	20
α-cypermethrin	48.9	48.9	1	0	50
fenvalerate I	BQL[c]	BQL	2 (2)		
fenvalerate II	BQL	BQL	2 (2)		
fenvalerate sum of isomers	BQL	BQL	2 (2)	0	20
deltamethrin	45.2	43.3–47.6	3	0	100

[a] Number (no.) of samples where the residue was detected. In parentheses are shown the number of samples that were positive and below method quantification limit. [b] MRLs established by European Union for olives (*3*). [c] BQL, below quantification limit.

compounds were determined. No residues were found only in one sample from the 20. Fenthion and its metabolite fenthion sulfoxide were found to be the most frequently determined pesticides in olive oil, in low concentrations according to the permitted MRL. Dimethoate was detected in 16 samples, but in three only was it in calculable quantities. Its metabolite omethoate was not determined in any of the samples analyzed. Endosulfan, chlorpyrifos, ethion, λ-cyhalothrin, α-cypermethrin,

and deltamethrin were present in the samples in substantial amounts. Two samples were found to contain endosulfan at concentrations slightly above the MRLs given by EU for olives (*3*), whereas the concentrations of λ-cyhalothrin and α-cypermethrin that were determined in two samples were very close to permitted MRLs. The method was applied with success to virgin olive oil samples.

Conclusions. The determination of 35 pesticide residues of a wide range of polarities (log K_{ow} from −0.74 to 7.0) in olive oil was developed. The method was based in the classic LLE of pesticides with ACN followed by clean up procedures based on the selectivity of different SPE cartridges (N-Alumina, Florisil, C18, ENVI-Carb, Ph, CN, NH_2, and Diol) in order to decrease the oil level in the final extracts, to remove olive oil interfering constituents, and to keep the pesticide recoveries in a high level by allowing the multiresidue determination of them. LLE of the oil solution in *n*-hexane by using ACN as the extraction solvent, followed by an ENVI-Carb SPE clean up of the extract, gave the highest recoveries of all of the pesticides studied with less oil residues in the sample. For the compounds analyzed by GC-ECD, pyrethroids, organochlorines, the phthalic acid (chlorthal dimethyl), and the trifluoromethyl (oxyfluorfen), after the first clean up through the ENVI-Carb cartridge, an additional clean up step through a Diol-SPE cartridge was used giving the highest recoveries and less interferences in chromatographic resolution.

Relative to existing methods, the proposed sample preparation lead to a higher preconcentration of the pesticide fraction by allowing the sensitive and selective determination of pesticides with widely different physicochemical properties in olive oil with similar or lower mLODs of the pesticides studied. This advantage together with the advantages of low cost, low solvent consumption, and nonspecific instrumentation demands in sample preparation are significant since a major task of the analytical discipline is to provide reliable and cost-effective methods. The survey of pesticide residues in commercial virgin olive oil samples performed by method application pointed to the urgent need for control analysis using multiresidue methods.

LITERATURE CITED

(1) Montiel, B.; Jones, O. Alternative methods for controlling the olive fly, *Bactrocera oleae*, involving semiochemicals. *IOBC/WPRS Bul.* **2002**, *25*, 1−11.

(2) Haniotakis, E. G. Olive pest control: Present status and prospects. Proceedings in IOBC/WPRS Conference on Integrated Protection of Olive Crops, Chania, Crete, May 2003, pp 1−9.

(3) EC (European Communities). Council Directive 76/895. *Off. J. Eur. Commun.* **1976**, *November*, L340; 9.12.76 (modifications 90/642 of 27 November 1990, L350, 14.12.90; 93/58 of 29 June 1993, L211; 95/38 of 17 July 1995, L197, 22.8.95; and 96/32 of 21 May 1996, L144, 18.6.96).

(4) Codex Alimentarius Commission. *Codex Alimentarius Pesticide Residues in Food-Maximum Residue Limits*, 2nd ed.; FAO/WHO: Rome, 1996; Vol. 2B.

(5) Lentza-Rizos, Ch.; Avramides, E. J. Determination of residues of fenthion and its oxidative metabolites in olive oil. *Analyst* **1990**, *115*, 1037−1040.

(6) Cabras, P.; Angioni, A.; Melis, M.; Minelli, E.; Pirisi, F. Simplified multiresidue method for the determination of organophosphorus insecticides in olive oil. *J. Chromatogr. A* **1997**, *761*, 327−331.

(7) Lentza-Rizos, Ch.; Avramides, E. J.; Cherasco, F. Low-temperature clean-up method for the determination of organophosphorus insecticides in olive oil. *J. Chromatogr. A* **2001**, *912*, 135−142.

(8) Rastrelli, L.; Totaro, K.; De, Simone, F. Determination of organophosphorus pesticide residues in Cilento (Campania, Italy) virgin olive oil by capillary gas chromatography. *Food Chem.* **2002**, *79*, 303−305.

(9) Tsoutsi, Ch.; Albanis, T. Optimization of headspace solid-phase microextraction conditions for the determination of organophosphorus insecticides in olive oil. *Int. J. Environ. Anal. Chem.* **2004**, *84*, 3−13.

(10) Ramesh, A.; Balasubramanian, M. Rapid preconcentration method for the determination of pyrethroid insecticides in vegetable oils and butter fat and simultaneous determination by gas chromatography−electron capture detection and gas chromatography−mass spectrometry. *Analyst* **1998**, *123*, 1799−1802.

(11) Di Muccio, A.; Pelosi, P.; Bardini, D. A.; Generali, T.; Girolimetti, S.; Stefanelli, P.; Leonelli, A.; Amendola, G.; Vergori, L.; Fresquet, E. V. Determination of pyrethroid pesticide residues by solid-matrix dispersion partition, followed by mini-column size-exclusion chromatography. *J. Chromatogr. A* **1999**, *833*, 19−34.

(12) Lentza-Rizos, Ch.; Avramides, E. J.; Visi, E. Determination of residues of endosulfan and five pyrethroid insecticides in virgin olive oil using gas chromatography with electron-capture detection. *J. Chromatogr. A* **2001**, *921*, 297−304.

(13) Chen, Z.; Wang, Y. Chromatographic methods for the determination of pyrethrin and pyrethroid pesticide residues in crops, foods and environmental samples. *J. Chromatogr. A* **1996**, *754*, 367−395.

(14) Barrek, S.; Paisse, O.; Grenier-Loustalot, M. F. Determination of residual pesticides in olive oil by GC−MS and HPLC−MS after extraction by size-exclusion chromatography. *Anal. Bioanal. Chem.* **2003**, *376*, 355−359.

(15) Carabias, M. R.; Rodríguez, G. E.; Hernández, F. E.; Hernández, M. J. Membrane extraction-preconcentration cell coupled on-line to flow-injection and liquid chromatographic systems. Determination of triazines in oils. *Anal. Chim. Acta* **1995**, *304*, 323−332.

(16) Aramendía, A. M.; Borau, V.; Lafont, F.; Marinas, A.; Marinas, M. J.; Moreno, M. J.; Porras, M. J.; Urbano, J. F. Determination of diquat and paraquat in olive oil by ion-pair liquid chromatography-electrospray ionization mass spectrometry (MRM). *Food Chem.* **2006**, *97*, 181−188.

(17) Sánchez, A. G.; Martos, N. R.; Ballesteros, E. Multiresidue analysis of pesticides in olive oil by gel permeation chromatography followed by gas chromatography-tandem mass-spectrometric determination. *Anal. Chim. Acta* **2006**, *558*, 53−61.

(18) AOAC (Association of Official Analytical Chemists). *Official Methods of Analysis of the Association of Official Analytical Chemists*, 14th ed.; AOAC: Arlington, VA, 1984; p 533.

(19) Ferrer, C.; Gomez, M. J.; Garcia-Reyes, J. F.; Ferrer, I.; Thurman, E. M.; Fernandez-Alba, R. Determination of pesticide residues in olives and olive oil by matrix solid phase dispersion followed by gas chromatography/mass spectrometry and liquid chromatography/tandem mass spectrometry. *J. Chromatogr. A* **2005**, *1069*, 183−194.

(20) Vreuls, J. J.; Swen, R. J. J.; Goudriaan, V. P.; Kerkhoff, M. A. T.; Jongenotter, G. A.; Brinkman, U. A. T. Automated on-line gel permeation chromatography-gas chromatography for the determination of organophosphorus pesticides in olive oil. *J. Chromatogr. A* **1996**, *750*, 275−286.

(21) Jongenotter, G. A.; Kerkhoff, M. A. T.; Van der Knaap, H. C. M; Vandeginste, B. G. M. Automated on-line GPC-GC-FPD involving co-solvent trapping and the on-column interface for the determination of organophosphorus pesticides in olive oils. *J. High Resol. Chromatogr.* **1997**, *22*, 17−23.

(22) Hiskia, E. A.; Atmajidou, E. M.; Tsipi, F. D. Determination of organophosphorus pesticide residues in Greek virgin olive oil by gas chromatography. *J. Agric. Food Chem.* **1998**, *46*, 570−574.

(23) Tsatsakis, A. M.; Tsakiris, I. N.; Tzatzarakis, M. N.; Agourakis, Z. B.; Tutudaki, M.; Alegakis, A. K. Three year study of fenthion and dimethoate pesticides in olive oil from organic and conventional cultivation. *Food Addit. Contam.* **2003**, *20*, (6), 553−559.

(24) Botitsi, E.; Kormali, P.; Kontou, S.; Mourkojanni, A.; Stavrakaki, E.; Tsipi, D. Monitoring of pesticide residues in olive oil samples: Results and remarks between 1999 and 2002. *Int. J. Environ. Anal. Chem.* **2004**, *84*, 231−239.

(25) Method No. 970.52. In *Official Methods of Analysis of the AOAC International*, 16th ed.; Cuniff, P., Ed.; AOAC International: Arlington, VA, 1995; Vol. 1, pp 1.

(26) Barranco, A.; Alonso-Salces, R. M.; Bakkali, A.; Berrueta, L. A.; Gallo, B.; Vicente, F.; Sarobe, M. Solid-phase clean-up in the liquid chromatographic determination of polycyclic aromatic hydrocarbons in edible oils. *J. Chromatogr. A* **2003**, *988*, 33−40.

(27) Hartley, D., Kidd, H., Eds. *The Agrochemical Handbook*, 2nd ed.; Royal Society of Chemistry: United Kingdom, 1987.

(28) ChemIDplus Lite at http://chem.sis.nlm.nih.gov/chemidplus/chemidlite.jsp, National Library of Medicine, Specialized Information Services (SIS).

(29) Hajšlova, J.; Holadová, K.; Kocourek, V.; Poustka, J.; Godula, M.; Cuhra, P.; Kepný, M. Matrix-induced effects: a critical point in the gas chromatographic analysis of pesticide residues. *J. Chromatogr. A* **1998**, *800*, 283−295.

(30) González, F. J. E.; Torres, M. E. H.; López, E. A.; Rodríguez, L. C.; Vidal, J. L. M. Matrix-effects of vegetables commodities in electron-capture detection applied to pesticide multiresidue analysis. *J. Chromatogr. A* **2002**, *966*, 155−165.

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