JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

A Multiresidue Method for the Determination of Insecticides and Triazine Herbicides in Fresh and Processed Olives

ELIZABETH J. AVRAMIDES*,† AND SOTIRIS GKATSOS‡

National Agricultural Research Foundation (N.AG.RE.F.), 1 South Venizelou Street, 14123 Lycovrissi, Athens, Greece, and School of Chemical Engineering, National Technical University of Athens, 9 Heroon Polytechniou Street, Zografou Campus, 15773 Athens, Greece

A simple, rapid, and low-cost gas chromatographic multiresidue method has been developed for the analysis of pesticide residues in raw and processed olives. This has been validated for 19 insecticides and triazine herbicides, covering a wide range of polarities. The method uses low-temperature precipitation to remove lipids and gives good cleanup for gas chromatography analysis with nitrogen phosphorus and electron capture detection. Recoveries are between 71 and 99%, with relative standard deviation values of 5−15%.

KEYWORDS: Olives; organophosphorus insecticides; pyrethroid insecticides; triazines; residues

INTRODUCTION

A variety of pests may attack olive trees, leading to a reduction in the quality and quantity of the olives and oil produced. Control of these pests is carried out with various pesticides, and it is therefore necessary to analyze olives and oil for residues of these products. Problems associated with the analysis of fatty substrates are well-known (*1*, *2*), and in recent years, a considerable amount of effort has gone into the development of improved analytical methods for olive oil that provide the necessary cleanup to protect chromatographic columns from the detrimental effects of lipids, while keeping the time and cost of analysis to a minimum. These methods are based on various techniques such as low-temperature fat precipitation (*3*), direct injection of olive oil into the gas chromatograph (GC) (*4*), simplified hexane−acetonitrile partitioning (*5*), matrix solid-phase dispersion (*6*), and on-line reversed-phase liquid chromatography (LC)−GC (*7*), although not all of them provide the adequate cleanup and sensitivity necessary for routine monitoring purposes. In contrast, analysis of the olives themselves has received little attention, and available methods based on techniques such as liquid−liquid partitioning are laborious and time-consuming (*8*). Although processing procedures for the production of table olives may be expected to reduce pesticide residues in the final product, it is still important to control pesticide residues in olives after processing and in fresh olives destined for both oil extraction and the production of table olives. A study on the effects of processing and storage on residues of dimethoate and omethoate in olives found that they were reduced in sterilized canning (processing factors 0.21 and 0.12, respectively) but not in nonsterilized canning. The residues fell substantially during storage to give processing factors after 6 months of 0.06 and 0.02, respectively, for sterilized canning and of 0.30 and 0.06, respectively, for nonsterilized canning (*9*).

The aim of this work was to apply the principle of low-temperature fat precipitation, which has previously been applied in our laboratory for the analysis of insecticide and triazine herbicide residues in olive oil (*3*), to the development of a simple, low-cost, rapid, and efficient method suitable for routine determination of pesticide residues in fresh and processed olives.

MATERIALS AND METHODS

Matrices. Several different varieties of both fresh and processed olives were tested, since characteristics such as oil content may differ substantially between varieties. **Table 1** gives details of the olive samples included in the study.

Chemicals and Materials. All solvents were pesticide residue analysis grade. Analytical standards were kindly supplied by Bayer or were purchased and had a purity ranging from 94.9 to 99.8%. The target pesticides were selected according to their importance in oleiculture in Greece and covered a wide range of polarity (log $K_{o/w}$ 0.2−4.8). In order to satisfy the need for compliance monitoring, some compounds recently withdrawn from use in olive groves in the European Union (EU) were included. The compounds tested included 16 insecticides (azinphos-ethyl, chlorpyriphos, cypermethrin, deltamethrin, diazinon, dimethoate, endosulfan, fenthion, fenvalerate, malathion, methidathion, λ-cyhalothrin, parathion, parathion-methyl, permethrin, and phosalone) and three triazine herbicides (atrazine, prometryn, and simazine), residues of which may be found in olives through contamination from treated soil (*3*). The oxidative metabolites of fenthion, some of which are known to contribute significantly to the total residue of this insecticide (*10*), were also included. The use of fenthion is temporarily allowed in Greek olive groves as an essential use under EU legislation.

Sample Preparation, Extraction, and Cleanup. Olives were homogenized using a Waring blender. For large olives, the pits were removed by hand before homogenization. For small olives, the whole sample was cut roughly in the blender for approximately 10 s, the pits were removed using a pair of tweezers, and the flesh was returned to

* To whom correspondence should be addressed. Tel: +30 210 2827202. Fax: +30 210 2942129. E-mail: lizavram@otenet.gr.
† National Agricultural Research Foundation.
‡ National Technical University of Athens.

10.1021/jf062826b CCC: $37.00
Published on Web 01/03/2007

Table 1. Olive Samples

sample	description	scientific name[a]
R1	raw, small, black for oil production	*O. e.* var. *minor rotunda*
R2	raw, small, black for oil production	*O. e.* var. *microcarpa alba*
R3	raw, large, green for table olives	*O. e.* var. *rotunda*
P1	processed, medium, black table olives	*O. e.* var. *media oblonga*
P2	processed, medium, green table olives	*O. e.* var. *rotunda*
P3	processed, medium, green table olives	*O. e.* var. *rotunda*
P4	processed, medium, black table olives	*O. e.* var. *rotunda*
P5	processed, medium, black table olives	*O. e.* var. *pyriformis*
P6	processed, large, green table olives	*O. e.* var. *maxima*
P7	processed, large, black table olives	*O. e.* var. *rotunda*
P8	processed, large, black table olives	*O. e.* var. *ceraticarpa*

[a] *O. e.*, *Olea europaea*.

the blender for homogenization. The mass of the sample before removal of the pits was recorded, since the European Community maximum residue limit refers to the whole fruit after removal only of the stems (*11*). A 25 g sample of homogenized olive flesh was weighed into a 250 mL Teflon centrifuge container with a screw top lid, spiked with a mixture of pesticides, and treated as follows. The olives were extracted with 50, 60, 80, or 100 mL acetonitrile in the presence of 50 g of anhydrous sodium sulfate using an Ultra-Turrax T25 (IKA Labortechnik Staufen) at 9500 rpm, and the sample was centrifuged at 4000 rpm for 3 min. The liquid phase was either carefully decanted into a second vessel to which 5 g of anhydrous sodium sulfate had been added or was left over the precipitated solids and oil in the original vessel. The container was then closed tightly and stored in the freezer at −20 or −30 °C for 2, 4, or 24 h. On removal from the freezer, the organic phase was emptied immediately into a small beaker leaving the solids, which included the frozen oil, behind as far as possible. An aliquot of the cold extract equal to 20% of the original volume of acetonitrile was pipetted into a 100 mL round-bottomed flask and rotary evaporated to dryness. The residue was collected in 2 mL of acetone for determination of residues with NPD (removing traces of acetonitrile by rinsing twice with a small volume of acetone) or in 2 mL of acetonitrile for further cleanup and determination with electron capture detection (ECD). Any solid tarry residue was left behind in the flask.

Further cleanup for analysis using GC-ECD was achieved by passing the acetonitrile extract, or the acetone extract after replacement of the solvent with acetonitrile, through a 500 mg SepPak alumina-N column cartridge using the method developed in our laboratory for olive oil extracts (*12*). The cartridge was fitted with a 10 mL reservoir via a Teflon adapter and prewashed with 3 mL of acetonitrile. A 0.6 mL aliquot of extract, corresponding to 1.5 g of olives, was loaded onto the column when the solvent had reached the bottom of the reservoir, and gentle pressure was applied until all solvent had been expelled from the cartridge. Elution of the pesticides was achieved by adding 3 mL of acetonitrile to the reservoir and applying gentle pressure to achieve a flow of approximately 1 drop per second until all of the solvent had passed through the cartridge. The 3 mL of eluate was collected in a 4 mL glass brown bottle with Teflon-lined, screw top lid and stored at −20 °C until GC determination.

During method development, the olive residue in the extract before cleanup was determined by weighing the flask following the evaporation step and reweighing it after collection of the soluble residue and evaporation of any remaining solvent. The mass of the remaining residue was also determined in a similar way. The mass of residue following cleanup on an alumina-N cartridge was determined by evaporating the solvent from a known volume of extract.

GC Analysis. A Varian CP (Walnut Creek, PA) 3800 gas chromatograph with a Varian CP 8200 autosampler, equipped with NPD and ECD (both at 300 °C) and operated in the splitless mode (240 °C, 1 min, and 1 μL injection), was used for the analysis of most of the samples. All capillary columns used had dimensions 30 m × 0.25 mm i.d. × 0.25 μm film thickness, and operating conditions were as follows. With NPD, an Rtx-1701 column (14% cyanopropylphenyl) was used with temperature program 75 °C, hold 1 min; 170 °C, 15 °C/min; 200 °C, 1.5 °C/min; 280 °C, 4 °C/min. With ECD, an Optima 5 MS column (5% phenyl) was used with temperature program 120 °C, hold 1 min; 200 °C, 15 °C/min; 240 °C, 2.5 °C/min; and 280 °C, 5 °C/min, hold 11 min. In addition, a few samples were analyzed using a Hewlett-Packard 6890 gas chromatograph with NPD (325 °C) or a Hewlett-Packard 5890 Series II gas chromatograph with ECD, both with injection temperatures of 220 °C.

Quantification was carried out using standards in the matrix extract, since the GC response for pesticides in olive oil extracts has been shown to be matrix-dependent (*3*). With the Varian instrument, internal standards (ethion for NPD and kresoxim-methyl and cyfluthrin for ECD) were used to correct for small variations in injection volume.

Table 2. Mass of Coextracted Material (mg/g ± SD) in Extracts of 25 g of Olive Flesh with 80 and 60 mL of Acetonitrile, Following 24 h at −20 °C

sample	80 mL of CAN	60 mL of ACN
R1	12.5 ± 1.0 (n = 3)	9.8 ± 1.0 (n = 3)
R2	14.5 ± 1.2 (n = 3)	11.6 ± 1.5 (n = 22)
P3	10 ± 0.7 (n = 3)	8.6 (n = 2)
P4	17.6 ± 2.5 (n = 3)	13.7 (n = 2)
P6	7.5 ± 0.9 (n = 6)	6.7 (n = 2)

RESULTS AND DISCUSSION

Optimization of Extraction Parameters. Efficient homogenization of the sample with the Ultraturrax during extraction was achieved by delaying the addition of anhydrous sodium sulfate until after the olive flesh had been cut more finely by blending with the solvent for 1 min. Initial tests showed that variations in the extraction parameters described above (extraction volume, decanting the liquid phase immediately following extraction, freezer temperature, and time in the freezer) generally had little effect on the mean recovery of the pesticides determined with NPD and that, in this respect, the method was very robust. However, the amounts of coextracted material, and consequently, the appearance of interferences in the chromatogram and the rate of deterioration of the chromatographic column, were significantly affected by variations in several of the parameters. In addition, the volume of extraction solvent used affected the recoveries of pesticides determined with ECD following cleanup of a 0.6 mL aliquot of extract on a SepPak alumina-N cartridge. From the results for some organophosphorus compounds determined with both NPD and ECD, it was concluded that this reduction resulted from the effect of coextractants on the cleanup process.

Tables 2−4 show the effect on the mass of coextracted material of (i) the extraction solvent volume, (ii) the time allowed in the freezer for precipitation of the solids and oil, (iii) decanting the liquid phase from the solids before low-temperature precipitation, and (iv) the size of the aliquot of extraction solvent taken. Increasing the volume of acetonitrile from 50 to 100 mL increased the mass of material coextracted. The increase with a volume of 80 mL as compared to 60 mL was approximately 20%, as shown by the data in **Table 2**. The final method used 60 mL of extraction solvent, since 50 mL was not always adequate to allow removal of the required aliquot without also taking unwanted frozen material. In addition, extraction with 60 mL of solvent gave satisfactory recoveries (70−110%) for ECD compounds following cleanup with solid-phase extraction (SPE), whereas 80 mL of solvent gave recoveries 10−20% lower for many compounds. However, given the relatively low recoveries for many of the pyrethroid pesticides, further work is needed to better understand the cleanup procedure and to optimize its parameters, e.g., the amount of matrix loaded onto the column.

Table 3. Mass of Coextracted Material (mg/g) for Different Times in the Freezer of Extracts of 25 g of Olive Flesh with 80 mL of Acetonitrile

aample	2 h	4 h	24 h
R1	17.7 ± 8.3 (n = 3)	6.0 ± 8.3 (n = 3)	12.4 ± 0.8 (n = 4)
P6		8.0 (n = 2)	7.0 ± 1.1 (n = 3)
P7		13.7 (n = 2)	14.8 ± 2.2 (n = 3)
P8		13.6 (n = 2)	13.5 ± 0.7 (n = 3)

The minimum time for which the sample needed to be left in the freezer for fat precipitation and satisfactory removal of water was found to be 4 h. The mass of coextracted residue after 2 h was more variable than that for 4 or 24 h (**Table 3**), and although it was not always greater, more interfering peaks appeared in the chromatograms. Recoveries for some compounds determined with ECD after cleanup with SPE were also reduced. No difference was observed between freezer temperatures of −20 and −30 °C. The effect of decanting the liquid phase from the solids after centrifugation and before freezing is shown in **Table 4**. A paired *t*-test applied to these data showed a significant increase in the mass of coextracted material at the 95% confidence level when the liquid phase was decanted (p = 0.042). Extraction of a second aliquot of the organic phase (25 g of sample, 80 mL of acetonitrile, and 24 h in freezer) gave no significant difference (paired *t*-test, p = 0.242) in the amount of coextracted material as compared to the first aliquot (13.8 ± 3.9 and 13.2 ± 3.6 mg/kg, respectively; n = 6), showing that the size of the aliquot could be doubled with no negative effects. This depended, however, on the possibility of removing sufficient extract without also taking any solid frozen material. Occasionally, small clumps of dispersed frozen material were present in an extract and this appeared to lead to reduced recoveries as well as to more coextracted material in the final extract.

Following the rotary evaporation of an aliquot of extract to dryness, not all of the residual solid in the flask was soluble in acetone or acetonitrile. Tests on the material remaining after collection of the sample showed that it contained no detectable pesticide residues. The insoluble solids from raw olives (sample R2) were greater for acetonitrile (35% of total residue, n = 13) than for acetone (9% of total residue, n = 13). However, the greater solubility of the residue in acetone did not lead to noticeable differences in the chromatograms or to changes in the efficiency of the SPE cleanup procedure.

On the basis of the tests described above, the optimized method was as follows. A 25 g analytical sample was weighed out into a 250 mL Teflon centrifuge container with a screw top lid, and 60 mL of acetonitrile was added. The mixture was homogenized using an Ultraturrax for 1 min at 9500 rpm, 50 g of anhydrous sodium sulfate was added, and homogenization was continued for a further 2 min. The container was closed, the sample was centrifuged at 4000 rpm for 3 min, and the vessel was transferred to the freezer, taking care not to disturb the precipitated solids, where it was left overnight or for a minimum of 4 h. On removal from the freezer, part of the organic phase was emptied immediately into a small beaker leaving the solids, which included the frozen oil, behind as far as possible. A 12 mL (9.43 g) aliquot of the cold extract, measured by mass, was transferred using a Pasteur pipet into a 100 mL round-bottomed flask and rotary evaporated to dryness. The residue was collected in 2 mL of acetone after removing traces of acetonitrile by rinsing twice with a small volume of acetone for determination of pesticides using GC-NPD or in 2 mL of acetonitrile for further cleanup followed by determination using GC-ECD. Alternatively, cleanup for determination with GC-ECD was carried out on a 0.6 mL aliquot of the extract dissolved in acetone after replacement of the solvent with acetonitrile.

Table 4. Effect on Mass of Coextracted Material (mg/g) of Decanting the Liquid Phase before Freezing (24 h at –20 °C)[a]

sample	decanted	in contact with solids
R1	12.5 ± 1.0[b]	11.0 ± 0.3[b]
R2	15.0	13.2
R3	18.0	14.4
P1	24.7	22.1
P2	15.4	15.0 ± 1.4[b]
P3	10.7	9.7
P4	15.8	18.6
P5	10.9	11.0
P6	7.7	5.7
P7	15.2	14.1
P8	13.6	13.2

[a] Twenty-five grams of olive flesh was extracted with 80 mL of acetonitrile. [b] Mean value ± standard deviation; n = 3.

Cleanup Efficiency. As described above, the cleanup efficiency was assessed by determination of the mass of material coextracted from the olives and taken up in the extract. For the optimized method, this was evaluated for the three varieties of raw olives and eight varieties of processed olives tested and found to be 10.9 ± 1.4 mg/g (n = 26, collected in acetone) and 10.4 ± 0.4 mg/g (n = 36, collected in acetone), respectively. This represented approximately 1% of the sample mass. The range of values for raw olives was 9.9−13.0 mg/g (varieties R1 and R3, respectively) and for processed olives was from 7.0 to 22.1 mg/g (varieties P6 and P1, respectively). Approximately 90% of this was removed by cleanup on the alumina-N cartridge. The coextracted material both before and after SPE cleanup was quantitatively the same as that found with the low-temperature precipitation method developed for olive oil, with a similar range of variation between different olive varieties.

The sample cleanup achieved with this simple method was sufficient for the chromatographic system to maintain its separation efficiency for a large number (>100) of sample injections. **Figures 1** and **2** show NPD and ECD chromatograms, respectively, of a typical raw olive sample and of a mixture of the pesticides at 0.5 and 0.025 μg/mL, respectively, matrix-matched with the same olive extract. Gas chromatograms were largely free of interfering peaks.

Method Validation. The specificity of the analysis for the pesticides included was confirmed by testing the method with the different varieties of raw and processed olives given in **Table 1**. Interferences in the chromatograms were noted for endosulfan sulfate with ECD and phosalone with NPD, but interference peaks from the different blank samples were always less than 30% of the compound peak at the limit of quantification (LOQ) of 0.01 mg/kg. In addition, negative peaks sometimes reduced the precision of the quantitative determination of α-endosulfan and cypermethrin at low concentrations. In contrast, the liquid−liquid partitioning method (*8*) previously used in our laboratory gave two large interference peaks in the NPD chromatogram that prevented determination of several of the pesticides tested.

Calibrations with six levels were linear over the range of concentrations used to quantify samples (0.005−0.25 μg/mL). Analyte stability in both standard solutions and extracts was tested over an 8 week period (n = 5), and no degradation was observed. Recoveries were measured for five replicate samples of raw olives at each of three spiking levels (1.0, 0.05, and 0.01

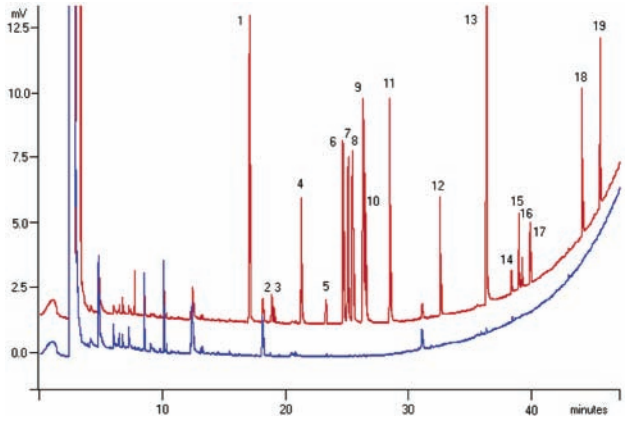

Figure 1. NPD chromatograms on the Varian 3800 GC of an olive extract and a 0.5 μg/mL mixture of pesticides, matrix-matched with the same olive extract. Key: 1, diazinon; 2, atrazine; 3, simazine; 4, dimethoate; 5, prometryn; 6, chlorpyriphos; 7, parathion-methyl; 8, fenoxon; 9, fenthion; 10, malathion; 11, parathion; 12, methidathion; 13, ethion (internal standard); 14, fenoxon sulfoxide; 15, fenthion sulfoxide; 16, fenoxon sulfone; 17, fenthion sulfone; 18, phosalone; and 19, azinphos-ethyl.

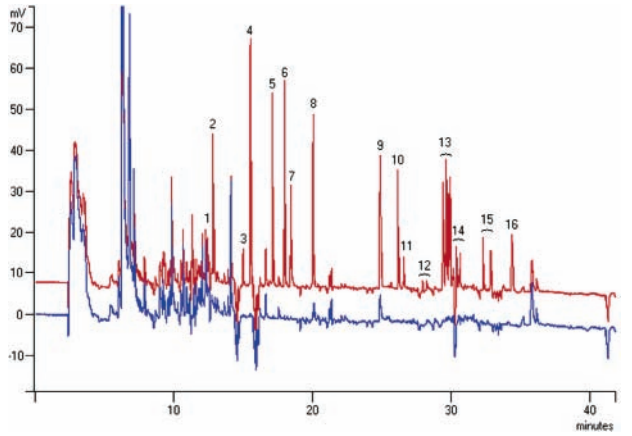

Figure 2. ECD chromatograms (Varian 3800 GC, Optima 5 MS column) of an olive extract and a 0.025 μg/mL mixture of pesticides, matrix-matched with the same olive extract. Key: 1, malathion; 2, parathion and chlorpyriphos; 3, methidathion; 4, α-endosulfan; 5, kresoxim-methyl (internal standard); 6, β-endosulfan; 7, fenthion sulfone; 8, endosulfan sulfate; 9, phosalone; 10, λ-cyhalothrin; 11, azinphos-ethyl; 12, permethrin; 13, cyfluthrin (internal standard); 14, cypermethrin; 15, fenvalerate; and 16, deltamethrin.

mg/kg) for NPD and at two spiking levels (1.0 and 0.01 mg/kg) for ECD for most pesticides. For a few pesticides, atrazine, simazine, prometryn, and permethrin, the LOQ, which was taken to be the lowest level tested at which recovery and relative standard deviation (RSD) values were satisfactory, was 0.05 instead of 0.01 mg/kg, and in these cases, the lowest spiking level was 0.05 mg/kg. The results of recovery tests are given in **Table 5** for NPD and in **Table 6** for ECD. Overall recoveries were between 84 and 99% for NPD and 71 and 84% for endosulfan and five pyrethroid insecticides determined with ECD. RSD values were nearly all below 10%.

Processed olives generally gave cleaner chromatograms than raw olives. However, recoveries were unchanged, as illustrated by the results of tests for duplicate samples of five different varieties (P1−P5) spiked at 0.05 mg/kg and shown in **Table 5** (determined with NPD) and for three different varieties (P3, P4, and P6) spiked at 0.05 mg/kg and shown in **Table 6** (determination with ECD).

Table 5. Mean Recoveries ± RSD with NPD for Fortified Samples of Raw and Processed Olives Following Extraction of 25 g of Olive Flesh with 60 mL of Acetonitrile and 24 h at –20 °C

	raw (n = 5)				processed[a]
pesticide	1 mg/kg	0.05 mg/kg	0.01 mg/kg	overall	0.05 mg/kg
diazinon	92 ± 2	84 ± 3	98 ± 5	91 ± 7	85 ± 6
atrazine	94 ± 1	91 ± 8		92 ± 6	90 ± 7
simazine	89 ± 4	92 ± 6		91 ± 5	92 ± 5
dimethoate	100 ± 3	89 ± 3	97 ± 12	96 ± 8	95 ± 5
prometryn	93 ± 3	99 ± 11		96 ± 8	86 ± 5
chlorpyriphos	88 ± 2	80 ± 4	83 ± 5	84 ± 5	82 ± 6
parathion-methyl	98 ± 4	86 ± 3	96 ± 7	93 ± 8	89 ± 4
fenoxon	98 ± 4	89 ± 2	93 ± 7	93 ± 6	93 ± 5
fenthion	100 ± 3	84 ± 2	88 ± 8	91 ± 9	89 ± 5
malathion	97 ± 1	85 ± 3	98 ± 7	93 ± 8	92 ± 5
parathion	102 ± 6	86 ± 2	97 ± 1	95 ± 8	88 ± 4
methidathion	102 ± 3	93 ± 7	97 ± 2	97 ± 6	92 ± 4
fenoxon sulfoxide	95 ± 5	95 ± 12	102 ± 5	97 ± 8	94 ± 10
fenoxon sulfone	96 ± 10	97 ± 7	93 ± 14	95 ± 10	95 ± 7
fenthion sulfoxide	105 ± 7	79 ± 9	90 ± 12	91 ± 15	91 ± 11
fenthion sulfone	98 ± 2	96 ± 14	97 ± 6	97 ± 8	93 ± 7
phosalone	101 ± 2	94 ± 2	102 ± 5	99 ± 5	89 ± 13
azinphos-ethyl	104 ± 6	94 ± 6	94 ± 7	97 ± 8	90 ± 6

[a] Two replicates for each of five different samples.

Table 6. Mean Recoveries ± RSD with ECD for Fortified Samples of Raw and Processed Olives Following Extraction of 25 g of Olive Flesh with 60 mL of Acetonitrile, 24 h at –20 °C, and Cleanup on a SepPak Alumina-N Column

	raw (n = 5)			processed (n = 3)
pesticide	1 mg/kg	0.01 mg/kg	overall	0.05 mg/kg
malathion	75 ± 8	76 ± 5	76 ± 6	80 ± 23
α-endosulfan	70 ± 8	74 ± 4	72 ± 7	67 ± 10
methidathion	80 ± 7	89 ± 5	85 ± 8	92 ± 6
β-endosulfan	78 ± 7	89 ± 5	84 ± 9	77 ± 10
endosulfan sulfate	81 ± 8	86 ± 4	84 ± 6	82 ± 7
fenthion sulfone	77 ± 8	84 ± 4	81 ± 7	87 ± 5
λ-cyhalothrin	81 ± 10	78 ± 4	80 ± 7	87 ± 7
phosalone	76 ± 9	81 ± 9	78 ± 9	82 ± 2
permethrin	70 ± 5	71 ± 5[a]	71 ± 5	72 ± 6
azinphos-ethyl	77 ± 9	76 ± 7	77 ±9	86 ±2
cypermethrin	75 ± 9	75 ± 10	75 ± 9	86 ± 6
fenvalerate	73 ± 10	81 ± 7	76 ± 9	78 ± 3
deltamethrin	72 ± 9	81 ± 5	76 ± 9	83 ± 15

[a] Spiked at 0.05 and not at 0.01 mg/kg.

Accurate determination of the oxon metabolites of fenthion was not possible on all of the chromatographic systems tested because of inadequate peak resolution and variable matrix effects, as has been noted previously for olive oil (*3*). However, their small contribution to the total fenthion concentration (*10*) means that it is not essential to analyze for these metabolites.

The application of low-temperature fat precipitation has been shown by this work to provide an effective method for the cleanup of olive extracts for analysis using GC with NPD and ECD detectors. The small amount of oil and other coextractives remaining in the final extract allowed the chromatographic system to maintain its separation efficiency for a large number of injections and gave very few interfering peaks. The method, which has been tested on pesticides with a wide range of polarity (log $K_{o/w}$ 0.2−4.8) and which requires only standard laboratory equipment, provides a simple, low-cost, and robust analytical method for olives. Further work needs to be carried out to

optimize the cleanup for determination with ECD, to ascertain that new pesticides introduced recently in olive groves are covered by the method and to verify that residues in the final extract may also be determined using MSD.

LITERATURE CITED

(1) Walters, S. M. Clean-up techniques for pesticides in fatty foods. *Anal. Chim. Acta* **1990**, *236*, 77−82.

(2) Armishaw, P.; Millar, R. G. Comparison of gel permeation chromatography, sweep codistillation and florisil column adsorption chromatography as cleanup techniques for the determination of organochlorine pesticide residues in animal fats. *J. AOAC Int.* **1993**, *76*, 1317−1322.

(3) 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.

(4) Morchio, G.; De Andreis, R.; Verga, G. R. Investigation of opganophosphorus residues in vegetable oils with special reference to olive oil. *Riv. Ital. Sostanze Grasse* **1992**, *69*, 147−157.

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

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

(7) Sanchez, R.; Vazquez, A.; Andini, J. C.; Villen, J. Automated multi-residue analysis of pesticides in olive oil by on-line reversed-phase liquid chromatography-gas chromatography using the through oven transfer adsorption-desorption interface. *J. Chromatogr. A* **2004**, *1029*, 167−172.

(8) Lentza-Rizos, Ch. Monitoring pesticide residues in olive products: Organophosphorus insecticides in olives and oil. *J. AOAC Int.* **1994**, *77*, 1096−1100.

(9) FAO. *Pesticide Residues in Food 2003, Evaluations, Part I—Residues*; FAO plant production and protection paper 177; FAO: Rome, Italy, 2004; pp 374−375.

(10) Lentza-Rizos, Ch.; Avramides, E. J. Organophosphorus insecticide residues in Virgin Greek olive oil, 1988−1990. *Pestic. Sci.* **1991**, *30*, 161−171.

(11) European Council Directive 90/642/EEC on the fixing of maximum levels for pesticide residues in and on cereals and foodstuffs of animal origin and certain products of plat origin, including fruit and vegetables. *Off. J. Eur. Commun.* **1990**, L350/71.

(12) Lentza-Rizos, Ch.; Avramides, E. J.; Visi, E. Determination of residues of electron captive insecticides in virgin olive oil. *J. Chromatogr. A* **2001**, *921*, 297−304.

Received for review October 2, 2006. Revised manuscript received November 16, 2006. Accepted November 28, 2006.

JF062826B