Multiresidue Screening of Pesticides in Fruits Using an Automatic Solid-Phase Extraction System

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About 20 pesticides were determined in lyophilized fruits using a semiautomatic multiresidue method, based on solid-phase extraction (SPE) with a silica column. The lyophilization of the sample, besides the SPE procedure selected, provided clean extracts despite the complexity of the matrixes studied. In addition, the lyophilization process allows sample preservation for at least three months without changes in the concentrations of the pesticides. Determination and quantitation of organochlorine and pyrethroid residues was carried out using a gas chromatograph equipped with an electron capture detector (GC−ECD), and a mass spectrometric detector (GC−MS) was used for confirmation purposes. Organochlorine pesticides provided average recoveries (spiked at three concentration levels in eight different fruits) near 93 ± 4%, being lower (89 ± 8%) for pyrethroids as a consequence of their higher degradation and interaction with the sample matrix. On the other hand, the detection limits achieved for all pesticides (0.5−8 ng per g of lyophilized fruit) allow their determination at the MRLs established by the European Union, with good precision (∼5%). Finally, from the 100 different fruits screened, only 10 positive responses were obtained, which were further confirmed by GC−MS.

Keywords: *Organochlorine and pyrethroid pesticides; fruits; solid-phase extraction; gas chromatography with electron capture detection*

INTRODUCTION

Pesticides are necessary and essential in agricultural production. With their use, the risk of residues remaining on the food consumed is present. For this reason, governments and international organizations (EU) have published a list of pesticides and their tolerances or maximum residues limits (MRLs) (*1*). Fresh fruits must be screened for pesticide residues before marketing, but because of their short lifetime, a rapid, simple analytical process, as well as high accuracy in the identification and quantitation of the analytes detected, is required. Organochlorine and pyrethroid pesticides are a large group of fungicides and insecticides extensively used in recent decades against pests all over the world (*2*). Organochlorine pesticides (OCPs) are very persistent because of their high lipophilic properties and stability (*3*, *4*). Pyrethroids are less harmful for mammals; only the parent pyrethroids show toxicity as the existence of significant toxic metabolites has not yet been proven (*2*). In any case, the identification and quantitation of pyrethroid residues, along with OCPs, is necessary to monitor and regulate their usage on crops to protect consumers from unsafe levels.

Most determinations of OCPs and pyrethroids have been developed using chromatographic techniques, mainly gas chromatography (GC). Some of these pesticides have one or more halogen atoms in their chemical structure, so the electron capture detector (ECD) is used because of its high selectivity (*5*−*8*). For confirmation purposes, mass spectrometry (MS) is also employed (*5*, *6*).

Fruits are very complex matrixes, requiring a pretreatment step which usually includes extraction and cleanup processes, normally tedious and time-consuming, to provide clean extracts. Basically, a homogenized sample is extracted, once or several times, with a single solvent, typically acetonitrile (*9*, *10*), ethyl acetate (*6*, *11*, *12*), acetone (*5*, *7*), or *n*-hexane (*13*); or a solvent mixture, such as toluene−acetonitrile (*14*), acetone−dichloromethane−hexane (*15*), acetone−*n*-hexane (*16*), or acetone−petroleum ether (*17*). Normally, there are too many co-extractives and the extracts obtained must be cleaned up by liquid−liquid partition (LLP) and/or solid-phase extraction (SPE). If a polar solvent is used as the first extractant, a LLP is subsequently carried out with a nonpolar solvent (*7*). The SPE can be applied on RP-C_{18} (*9*), silica gel (*11*), Florisil (*7*, *16*), or alumina (*6*).

The majority of the methods developed to date are based on the direct extraction of the pesticides from fresh samples. However, lyophilization of fruits and vegetables leads to a higher stability of the samples without losses of analytes. In this regard, few contributions have been recently published (*8*, *18*), although only the latter implements the automatic extraction of pyrethroids from lyophilized agricultural samples. In this paper, the solid-phase extraction system proposed elsewhere (*18*) for pyrethroids determination was initially adopted, but we studied other solvents and sorbents in order to extend the use of the system to organochlorine pesticides. Fruit samples were lyophilized after collection in a raw state, then conserved at −20 °C to retain the concentrations of OCPs and pyretroids residues at constant levels for at least three months. The method is rapid and does not require either laborious sample manipulation nor use of a complicated SPE system. The

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10.1021/jf0011687 CCC: $20.00
Published on Web 02/10/2001

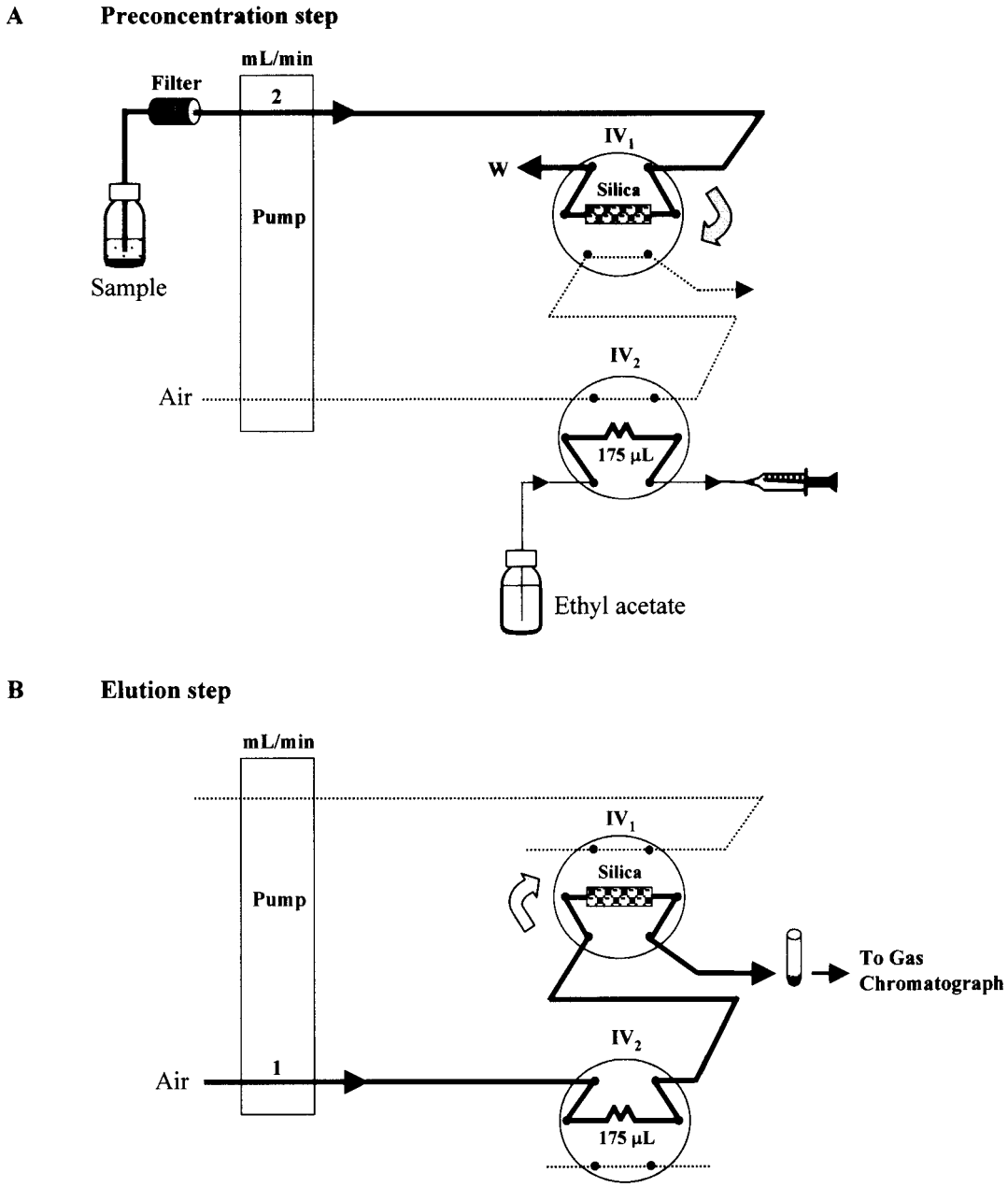

Figure 1. FI manifold for the on-line preconcentration of pesticides and their off-line determination by gas chromatography. IV, injection valve; W, waste.

sensitivity and selectivity of the method allows the screening of at least 19 pesticides at concentrations lower than their established MRLs.

MATERIALS AND METHODS

Apparatus. Analyses were performed on a Hewlett-Packard 5890 A gas chromatograph equipped with a ^{63}Ni electron-capture detector (ECD) and a fused-silica capillary column (30 m × 0.25 mm i.d.) coated with 5% phenylmethylpolysiloxane (film thickness 0.25 μm) (Supelco, Madrid, Spain), connected to a Hewlett-Packard 3392 A integrator. The injector and detector were operated at 225 °C and 325 °C, respectively. The chromatographic temperature program was 150 °C for 2 min, raised to 170 °C (8 °C/min) and held for 4 min; then raised to 255 °C (8 °C/min) and held for 15 min; and, finally, raised to 285 °C (5 °C/min) and held for 6 min. Nitrogen (6.0, Air Liquide, Seville, Spain) at a flow rate of 1 mL/min was used as carrier gas. To confirm the identity of eluted GC peaks, a Fisons 8000 GC instrument interfaced to a Fisons MD800 mass spectrometer and controlled by a computer running MASSLAB software (Thermo, Madrid, Spain) was also used; the chromatographic column and temperature program were both similar to those used with ECD. Ultrapure helium (6.0, Air Liquide), at a flow rate of 1 mL/min, was employed as carrier gas. The injection port and transfer line temperatures were maintained at 225 °C and 300 °C, respectively, throughout the experiments. The ion source temperature was 200 °C for the 70 eV electron impact mode. Mass spectra were recorded from m/z 70 to 500. Samples (1 μL) were injected in the split mode (1:25 ratio).

The flow system was constructed with a Gilson Minipuls-2 peristaltic pump (Villiers-le-Bel, France) furnished with Solvaflex pumping tubes, two rheodyne 5041 injection valves, PTFE tubing (0.5 mm i.d.), and commercially available connectors. The sorbent glass column (2 cm × 4 mm i.d.) was hand-packed with 50 mg of silica and sealed at both ends with small cotton beads to prevent material losses. The sorbent column was sequentially conditioned with 0.5 mL of acetonitrile and 1 mL of *n*-hexane prior to retention. This column can be reused for at least three months, working daily, including a washing step with 0.5 mL of 2-propanol before conditioning.

Table 1. Analytical Figures of Merit of the Proposed Method for Determination of 19 Pesticides

compound	detn. limit (ng/mL)	linear range (ng/mL)	r^2	RSD (%)	m/z[a]
dicloran	0.1	0.2−20	0.998	4.5	**124**, 176, *206*
lindane	2.0	5−200	0.999	5.3	111, **181**, 219
vinclozolin	0.1	0.2−20	0.998	3.0	**187**, 212, *285*
dichlofuanid	0.05	0.2−20	0.992	4.0	**123**, 224, *332*
captan	2.0	8−200	0.998	6.5	**79**, 264, *299*
procymidone	0.1	0.2−20	0.998	5.2	67, **96**, *283*
α-endosulfan	0.1	0.2−20	0.996	5.4	**195**, 241, 339
β-endosulfan	0.05	0.2−20	0.998	3.8	**195**, 241, 339
endosulfan sulfate	0.5	1−200	0.996	4.7	**272**, 387, *420*
bifenthrin	0.1	1−200	0.998	6.0	152, 165, **181**
fenpropathrin	0.1	1−200	0.998	5.0	**97**, 181, *349*
λ-cyhalothrin	0.1	1−200	0.996	3.5	141, **181**, 209
permethrin	0.8	2−200	0.996	5.8	163, **183**, *390*
cyfluthrin	0.4	1−200	0.994	5.0	**77**, 163, 227
β-cyfluthrin	0.2	1−200	0.998	4.7	**77**, 163, 227
cypermethrin	0.4	1−200	0.996	3.6	77, 181, **209**
α-cypermethrin	0.1	1−200	0.998	3.3	77, 181, **209**
fenvalerate	0.2	1−200	0.996	6.1	**169**, 181, *419*
deltamethrin	0.2	1−200	0.994	5.8	**181**, 209, 253

[a] m/z values used for confirmation: in italics, M^+ ions; and in bold face, base peaks.

A glass column (3 cm × 5 mm i.d., packed with cotton wool) was used to filtrate the *n*-hexane phase.

A Hetossic laboratory freeze-dryer, type CD-53-1 (Birkerod, Denmark) was also employed.

Chemicals and Standard Solutions. All chemicals and sorbents were of analytical grade or better. The following pesticides were studied: dicloran, lindane, vinclozolin, dichlofuanid, captan, procymidone, α- and β-endosulfan (3:1), endosulfan sulfate, bifenthrin, λ-cyhalothrin, deltamethrin, fenpropathrin, fenvalerate (cis and trans isomers), permethrin (cis and trans isomers), cyfluthrin isomers, β-cyfluthrin isomers, cypermethrin isomers, α-cypermethrin, and piperonyl butoxide internal standard (IS) were obtained from Riedel-de-Haën (Seelze, Germany). Cyfluthrin and cypermethrin have four isomers: 1(RS)cis,α(RS) = isomer I; 1(RS)trans,α(RS) = isomer III; 1(RS)cis,α(SR) = isomer II; and 1(RS)trans,α(SR) = isomer IV; α-cypermethrin is the isomer III of cypermethrin and β-cyfluthrin is the mixture of isomers II and IV of cyfluthrin at a ratio of 1:2. The RP-C_{18} HPLC sorbent was supplied by Sigma (Madrid, Spain) and the silica sorbent was obtained from Varian (Zug, Switzerland). Solvents (ethyl acetate, *n*-hexane, 2-propanol, and acetonitrile) were purchased from Merck (Darmstadt, Germany).

Stock standard solutions of each pesticide were prepared in *n*-hexane at concentrations of 5 mg/mL, and stored in glass stoppered bottles in the dark at 4 °C. Working standard solutions were obtained by appropriate dilution with *n*-hexane.

Fruit Materials and Sample Preparation. The trial was carried out with fruits purchased at local markets in Córdoba. Because legally established limits of pesticides residues have been set for raw materials, samples were analyzed unwashed, in a raw state (*19*). Sampling was done according to the legally established protocol of the EU (*20*). Thus, a raw global sample consisting of ∼5 kg of each fruit was reduced by quartering it to ∼500 g (laboratory sample). For melon, the raw sample (∼5 units) was cut into slices of ∼100 g and then reduced by quartering to ∼500 g. Laboratory samples were pulped in a high-speed blender and fractions of ∼50 g were lyophilized by freeze-drying at 6 Pa for 8 h, after which they were conserved in glass containers, at −20 °C in the dark, until analysis. Under these conditions, the concentration of the pesticides assayed remained constant for at least three months.

The lyophilized sample was prepared by the following method. An accurately weighed amount of 0.1−1 g (n = 5), containing between 2 ng and 2 μg of pesticides, was placed into a 50-mL amber glass bottle with 10 mL of *n*-hexane and 0.1 mL of 200 μg/mL piperonyl butoxide (IS). After the bottle was stoppered, the mixture was mechanically shaken for 10 min and allowed to settle. Then, 5 mL of the *n*-hexane phase, containing the analytes and the IS, was continuously aspirated and filtered into the SPE system of Figure 1.

Analytical Method. The SPE system used for the screening and determination of organochlorine and pyrethroid pesticides in fruits is depicted in Figure 1. In the preconcentration step, 5 mL of a standard solution or the treated sample containing 0.2−200 ng/mL of pesticides (for lyophilized samples, between 2 ng/g and 2 μg/g) plus 2 μg/mL of IS in *n*-hexane, was continuously aspirated at 2 mL/min through the cotton column, which filters the fine particles occasionally present in the organic phase, to avoid clogging the system, and then passed through the sorbent column (50 mg of silica), located in the loop of the injection valve 1 (IV_1), with the analytes being retained and the sample matrix being wasted (position in bold lines). Simultaneously, the loop of the second valve (IV_2) was filled with the eluent (ethyl acetate) by means of a syringe. Prior to the elution, by switching IV_1, residual organic solution inside the column and the connectors was flushed by passing an air stream through the carrier line of the IV_2 at 1 mL/min for 4 min. In the elution step, by switching IV_2, 175 μL of the eluent was injected into an air stream and passed through the sorbent column (in the direction opposite to that of the sample) to elute the pesticides (position in bold lines). The eluate was collected in a glass vial containing anhydrous sodium sulfate, and a 1-μL aliquot was injected into the gas chromatograph. After each determination, the sorbent column was cleaned with 0.5 mL of 2-propanol, to remove residual compounds from the matrix, and then conditioned with 0.5 mL of acetonitrile and 1 mL of *n*-hexane.

RESULTS AND DISCUSSION

Solid-Phase Extraction System. Recently, our working group has developed a flow system for the determination of organochlorine pesticides (OCPs) in fresh fruits (*21*) and another method for screening of pyrethroids in lyophilized fruits (*18*). Petroleum ether/*n*-hexane and RP-C_{18}/silica were found to be the most efficient extraction solvents and sorbents for OCPs/pyrethroids, respectively. In both cases, the eluent was ethyl acetate. In this work, the development of a multiresidue method for both types of pesticides using the same SPE system was afforded. Therefore, taking into account that lyophilized samples gave better results for pyrethroids than fresh samples (*18*) through a simplified extraction procedure which provided cleaner chromatograms as well as higher recoveries, the stability of the OCPs during the lyophilization process was initially studied using melon as the model fruit. For this purpose, 100 g of fresh melon, free from OCPs, was chopped and spiked with 20 ng/g of each of the OCPs studied, and then divided into two fractions. One of them was extracted following a standard method (*7*) which used sequential extraction with acetone and *n*-hexane. The *n*-hexane extract was further evaporated, redissolved, and extracted again; finally, Florisil cartridges were used for clean up purposes, and the extract was evaporated and redissolved in *n*-hexane prior to its injection into the GC−ECD instrument. The other fraction of 50 g of sample was lyophilized at 6 Pa for 8 h and then directly extracted with *n*-hexane following the procedure described above for the fresh fraction. This operation was repeated four times. On the basis of the results obtained, it was concluded that no losses of OCPs occurred during the lyophilization process. In addition, chromatograms for lyophilized melon were cleaner than those for fresh melon. Therefore, all samples were lyophilized for further experiments.

The study of the extractant and sorbent was carried out using a flow system similar to that depicted in Figure 1. For this purpose, two working standard

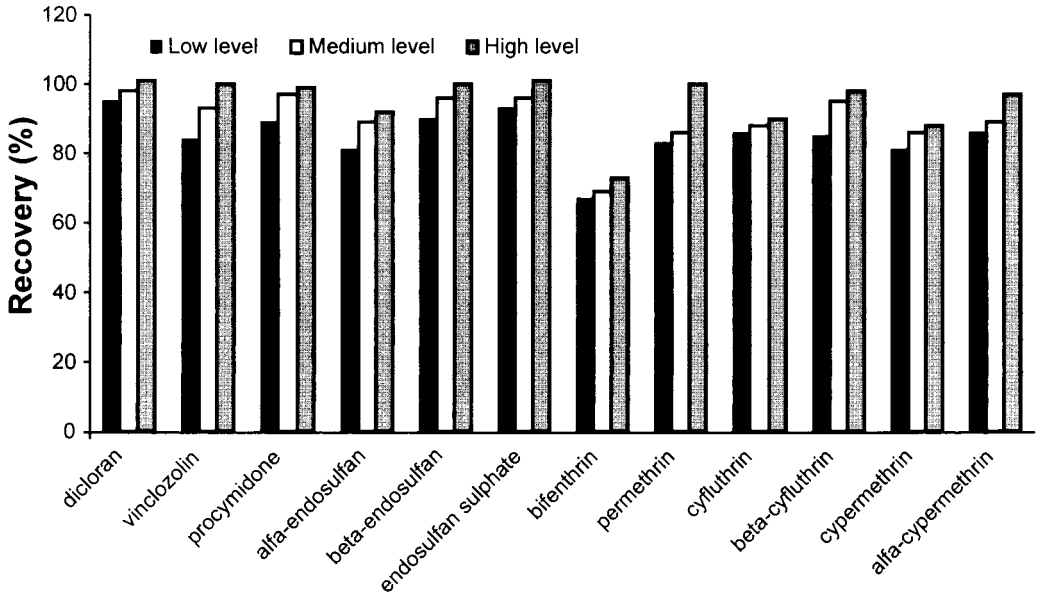

Figure 2. Recovery of pesticides added to a peach sample at three concentrations. Low, medium, and high levels correspond to pesticide concentration spiked at levels of 6−30, 12−60, and 24−100 ng per g of fresh fruit. For more details, see text.

solutions containing 10 ng/mL of each OCP and 20 ng/mL of each pyrethroid, plus 2 μg/mL of the IS were prepared in *n*-hexane and petroleum ether. Two columns packed with 60 mg of RP-C_{18} and silica were separately inserted into the flow system, with an eluent of 250 μL of ethyl acetate in all instances. A volume of 5 mL of each working standard solution was aspirated through the flow system first with the RP-C_{18} column and then with the silica column. On the assumption that ethyl acetate provided quantitative elution, the following conclusions can be addressed: silica was a much better sorbent than RP-C_{18} because all pesticides in both organic solvents were only retained onto RP-C_{18} sorbent up to 20% while pyrethroids were quantitatively retained (100%) onto the silica column, and for OCPs the retention efficiency varied from 40% (e.g., lindane, dicloran) to 90% (e.g., captan, endosulfan sulfate). On the other hand, *n*-hexane and petroleum ether behave equally as solvents for standards preparation. To select the most efficient extractant for real samples, two fractions of 0.5 g of lyophilized melon were spiked with 150 ng and 300 ng of each OCP and pyrethroid, respectively, extracted with 15 mL of *n*-hexane or petroleum ether and then passed through the flow system with the silica column; *n*-hexane showed slightly better extraction properties than petroleum ether because it provided cleaner chromatograms. So, *n*-hexane as extractant and silica as sorbent were finally selected.

The following experiments were carried out by aspirating into the SPE system a volume of 5 mL of a working standard solution containing 10 ng/mL of each OCP, 20 ng/mL of each pyrethroid, and 2 μg/mL of IS. The amount of silica material was optimized into the range 25−100 mg, with the optimum being 50 mg. The influence of the flow rate was studied for the sample and the eluent (using an air stream as carrier), over the range 0.1−5.0 mL/min. Pyrethroids were more affected than OCPs for both variables; sample and eluent flow rates of 2 and 1 mL/min, respectively, were selected. The volume of the eluent (ethyl acetate) was examined by changing the loop of IV_2 (Figure 1), the optimum volume being 175 μL. A second elution with the same eluent volume showed the absence of carry over.

As has been stated above, pyrethroids were quantitatively retained onto silica, whereas retention for OCPs was not complete. So, after the SPE system was optimized, the sorbent capacity of the silica column for OCPs was evaluated. For this purpose, a standard solution containing 10 ng/mL of each OCP and 2 μg/mL of IS in *n*-hexane was prepared; an aliquot of 5 mL was passed through the SPE system and collected at the end of the column (this eluate fraction corresponds to unretained OCPs). The standard solution (5 mL) (100% adsorption) and the collected eluate fraction were evaporated near dryness and reconstructed with 200 μL of *n*-hexane (for preconcentration purposes). A 1-μL aliquot of each extract was injected into the GC−ECD. The chromatograms obtained (the process was repeated four times) were compared. The highest retention (80−90%) was obtained for procymidone, β-endosulfan, dichlofuanid, captan, and endosulfan sulfate, and the lowest retention (40−50%) corresponded to lindane, α-endosulfan, and dicloran (vinclozolin excepted ca. 65%). Finally, piperonyl butoxide was selected as an internal standard among other compounds as it was quantitatively retained on the silica column and eluted with ethyl acetate; it was added directly to the standards or samples at a concentration of 2 μg/mL, thus acting as a procedural internal standard.

The breakthrough volume, defined as the sample volume above which the analyte starts to elute from the column bottom (it depends on the strength with which the analytes are retained by each sorbent, on the amount of sorbent, and on the packing efficiency of the sorbent bed), was evaluated in the optimized SPE system in order to know the maximum sample volume that can be used and, hence, the maximum preconcentration factor that can be achieved. For this purpose, different volumes of *n*-hexane containing always the same amount of analytes (50 ng of each OCP, 100 ng of each pyrethroid, and 10 μg of IS) were aspirated into the flow system. The results showed that 20 mL was the breakthrough volume, with the pesticides with minor affinity by the sorbent (viz. lindane, α-endosulfan, and dicloran) the most affected at higher volumes. Based on these results, and taking into account the high

Table 2. Average Recoveries[a] of Pesticides Added at Three Variable Concentrations to Fruit Samples

compound	apple	peach	pear	strawberry	kiwi	melon	cherry	orange
dicloran	97 (4)	98 (3)	92 (4)	97 (1)		98 (1)	99 (4)	95 (2)
lindane	96 (7)				89 (5)	100 (3)	94 (6)	95 (7)
vinclozolin	95 (9)	92 (8)	94 (9)	93 (7)	92 (6)	93 (9)	94 (7)	91 (6)
dichlofuanid	97 (2)		95 (5)	91 (6)		95 (7)	95 (5)	94 (6)
captan	85 (6)			84 (6)		86 (7)		88 (5)
procymidone	95 (2)	95 (5)	96 (2)	97 (1)		97 (3)		94 (4)
α-endosulfan	84 (7)	87 (6)	83 (8)		84 (8)	88 (7)	85 (7)	
β-endosulfan	92 (8)	95 (5)	93 (5)		93 (7)	94 (7)	91 (5)	
endosulfan sulfate	95 (1)	97 (4)	96 (2)		96 (3)	93 (1)	95 (3)	
bifenthrin	66 (2)	70 (3)	76 (4)	68 (1)		70 (2)		69 (1)
fenpropathrin	91 (4)		92 (1)	93 (9)	92 (8)			
λ-cyhalothrin			93 (8)	98 (7)		95 (7)	97 (6)	98 (8)
permethrin	88 (8)	90 (7)						91 (5)
cyfluthrin		88 (2)					90 (2)	
β-cyfluthrin		93 (7)					92 (6)	
cypermethrin	97 (2)	85 (4)	97 (1)	98 (2)	93 (5)	97 (2)		
α-cypermethrin		91 (6)	92 (3)	95 (8)	95 (3)			
fenvalerate					92 (4)		90 (5)	
deltamethrin						84 (6)	80 (4)	82 (5)

[a] Standard deviations ($n = 9$) are given in parentheses. For experimental details, see text.

sensitivity of the method, the volume of extractant required to achieve the MRLs was established as 5 mL, with it being possible to increase it up to 20 mL if required.

Features of the Proposed Automated Method. All pesticides studied exhibited good gas chromatographic properties. Analytical curves for standards of pesticides were obtained by using a sample volume of 5 mL of *n*-hexane containing variable concentrations (0.2−200 ng/mL) and the SPE system depicted in Figure 1. In the case of mixtures of isomers (permethrin (2 isomers), fenvalerate (2 isomers), *β*-cyfluthrin (2 isomers), cyfluthrin (4 isomers) and cypermethrin (4 isomers)), the global analytical signal was obtained by summing the peak areas of all isomers. The curves were constructed by plotting the analyte-to-IS peak area ratio against the analyte concentration. The detection limit, linear range, correlation coefficient (r^2), precision (as RSD), and *m*/*z* values for GC−MS confirmation are shown in Table 1. The detection limit was defined as the minimum concentration providing a chromatographic signal 3 times higher than background noise. The lowest detection limits were for dichlofuanid and *β*-endosulfan (0.05 ng/mL), and for the other pesticides it ranged from 0.1 to 0.8 ng/mL (lindane and captan excepted, which had limits of 2.0 ng/mL). For 1 g of lyophilized sample, the detection limits ranged from 0.5 to 8 ng. The sensitivity of the method (as slope of the calibration graph) was adequate to determine all pesticides at concentrations lower than the MRLs established by the European Union. Finally, the precision of the method, expressed as relative standard deviation, was checked on 10 samples containing 9 ng/mL of each pyrethroid, and lindane, captan, and α-endosulfan, 3.0 ng/mL of each OCP, and 2 *μ*g/mL of IS, and was acceptable for all analytes, ranging from 3.0 to 6.5%.

Recovery Test. To validate the proposed method, taking into account that there is no appropriate reference material containing both types of pesticides in fruit matrixes, a recovery test was carried out. Prior to this study, it was necessary to optimize the volume of *n*-hexane (extractant) and the extraction time. For this purpose, various samples of 1 g of lyophilized melon were fortified with variable amounts of pesticides, and then extracted, by mechanical shaking, with 10, 15, 20, 25, and 30 mL of *n*-hexane (at least 3 mL of solvent was required to soak the dry material) for different times (from 5 to 20 min). The short time elapsed between spiking of the lyophilized fruit with the pesticides and its analysis minimized analyte−matrix interactions, so the recoveries of pesticides were quantitative. The experiments were carried out by aspirating 5 mL of each extract into the SPE system, always containing the same amount of analytes (12 ng of dicloran, vinclozolin, dichlofuanid, procymidone, *β*-endosulfan, and endosulfan sulfate; 36 ng of α-endosulfan; 60 ng of lindane, captan, and each pyrethroid, and 10 *μ*g of IS). Aliquots of 1 *μ*L were injected into the GC−ECD instrument. From this experiment, it can be concluded that the recoveries were more marked by the extraction time than by the volume of extractant. Complete extraction was accomplished above 10 min and a volume of 10 mL of extractant was selected, taking into account the additional preconcentration provided by the lower volume.

Recoveries of analytes were studied in eight uncontaminated fruits (viz. apple, peach, pear, strawberry, kiwi, melon, cherry, and orange). For each sample, the spiked pesticides were selected according to their use and occurrence/appearance (*19*, *22*, *23*). About 10 g of each fresh fruit was pulped and then fortified at three levels of concentration: 6, 12, and 24 ng/g of dicloran, vinclozolin, dichlofuanid, procymidone, *β*-endosulfan, and endosulfan sulfate; 18, 36 ,and 72 ng/g of α-endosulfan; and 30, 60, and 100 ng/g of lindane, captan, and pyrethroids from standard solutions in acetone (1 mL of acetone containing from 60 ng to 1 *μ*g of pesticides). After the addition, the mixture was slightly shaken and left to stand for air-drying (∼2 h), and then lyophilized. Each sample was spiked three times at each of the levels indicated above ($n = 3$). The pesticides were assumed to be uniformly distributed in the 10 g of fresh fruit, so any analyte−matrix interactions were assumed to have occurred over the weathering period (∼2 h). The levels of residues of pesticides were quantified by comparison with standard solutions in *n*-hexane, which were passed through the SPE unit under identical conditions. Figure 2 shows the variation of the recoveries of pesticides with increased spiked amounts in fruits (exemplified for peach). In all fruits assayed, the initial contaminant concentration was found to influence the desorption rate. Thus, at the higher pesticides concentrations, recoveries increased, being near 100% for OCPs

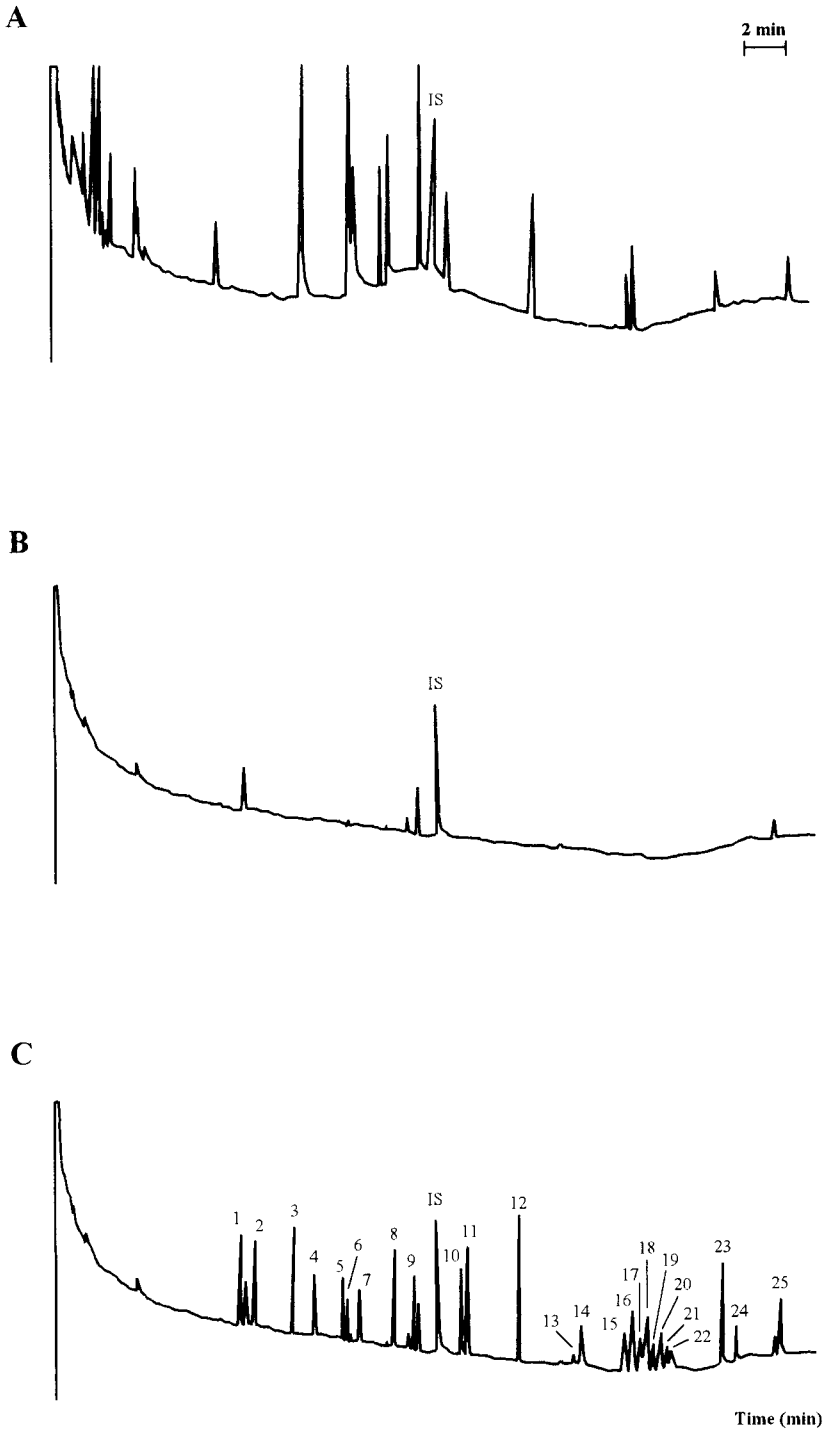

Figure 3. Gas chromatogram for 10 g of unfortified fresh (A) and lyophilized (B) melon sample and the lyophilized sample fortified (C) with all pesticides assayed. Peaks: dicloran (1), lindane (2), vinclozolin (3), dichlofuanid (4), captan (5), procymidone (6), α-endosulfan (7), *β*-endosulfan (8), endosulfan sulfate (9), bifenthrin (10), fenpropathrin (11), *λ*-cyhalothrin (12), permethrin cis (13), permethrin trans (14), cyfluthrin I (15), cyfluthrin III (16), cyfluthrin II (17), cyfluthrin IV (18), cypermethrin I (19), cypermethrin III (20), cypermethrin II (21), cypermethrin IV (22), fenvalerate cis (23), fenvalerate trans (24), deltamethrin (25), and internal standard (IS).

but lower for pyrethroids, above all for bifenthrin, probably due to their higher interaction with the sample matrix.

Table 2 gives the average recovery values obtained for each pesticide at the three concentrations spiked from three replicates ($n = 9$) for the fruits assayed. In all instances, the lowest recoveries were obtained for bifenthrin (average 70 ± 3%) followed by deltamethrin, α-endosulfan, and captan (average, ~85%) as the likely result of a higher interaction with the sample matrix. For the other pesticides assayed, no significant differences were observed, with average recovery values of 90% (viz. cifluthrin, permethrin, and fenvalerate) and 95% (viz. cypermethrin, dichlofuanid, lindane, endosulfan sulfate, procymidone, *λ*-cyhalothrin, and dichloran). Melon and pear are the fruits for which the recoveries are closest to the average values for each pesticide, and thus can be used as model fruits. On the other hand, there are no significant differences between the average recoveries for all pesticides in each fruit, ranging from

Table 3. Pesticides Found in the 100 Checked Fruit Samples (SD, $n = 5$)

sample	water (%)	pesticide found	concentration[a] (ng/g)	MRL[a,b] (ng/g)
apple	85	procymidone	400 (35)	—[c]
peach	80	vinclozolin	23 (4)	2000
pear	80	procymidone	136 (9)	1000
strawberry	90	procymidone	109 (8)	5000
kiwi	85	vinclozolin	2600 (365)	10000
melon	85	deltamethrin	28 (7)	500
cherry	70	λ-cyhalothrin	38 (4)	100
strawberry	90	λ-cyhalothrin	14 (4)	200
kiwi	85	fenvalerate	34 (7)	500
orange	80	captan	530 (25)	3000

[a] Amount per g of fresh sample. [b] Established by the European Union. [c] Value not established by the European Union.

92% (viz. peach, kiwi, cherry, and orange) to 94% for strawberry (bifenthrin excepted in these average results).

The efficiency of pesticides extraction from lyophilized fruits and the sorbent cleanup system, together with the high selectivity of the proposed method, are revealed in the chromatograms of the different fruits assayed, where only a few peaks from the matrixes are present. By way of example, Figure 3 shows the chromatograms for unfortified fresh and lyophilized melon samples (blanks) and a lyophilized melon sample fortified with all pesticides assayed at the following concentrations: 12 ng/g of dicloran, vinclozolin, dichlofuanid, procymidone, β-endosulfan, and endosulfan sulfate; 36 ng/g of α-endosulfan; and 40 ng/g of lindane, captan, and pyrethroids. As was mentioned before, the chromatogram for the lyophilized melon sample was cleaner than that for the fresh sample. Indeed, the fresh melon fortified with all pesticides provided complex chromatograms, hindering the identification of some of the analytes.

Analysis of Fruits. Different kinds of fruits, including those listed above, were purchased at various local markets and about 100 samples were analyzed in quintuplicate ($n = 5$). Sampling was done as described in the Fruit Materials and Sample Preparation sections, and laboratory samples were lyophilized as soon as received. Initially, 0.1 g of each lyophilized fruit was accurately weighed and analyzed by the proposed method; when negative results were obtained, the sample amount was increased to 1 g. In all instances, quantitation was done by ECD and positive findings were confirmed by MS, using 2 g of lyophilized sample with 20 mL of *n*-hexane and passing 15 mL of extract through the SPE system, as the sensitivity was ∼10 times lower than with ECD. Only 10 samples were found to contain chlorinated or pyrethroid residues at detectable levels. The results thus obtained, for 0.1 g of lyophilized sample, are listed in Table 3. As can be seen, the concentrations found were lower than the EU maximum residue limits (see Table 3) for all compounds examined. Figure 4A shows the chromatogram for a cherry sample containing only λ-cyhalothrin obtained with electron capture detection. Figure 4B illustrates the identification of this pyrethroid by comparing the mass spectrum for the corresponding peak with that in the library. Spectral comparison resulted in coincidence of ca. 85%.

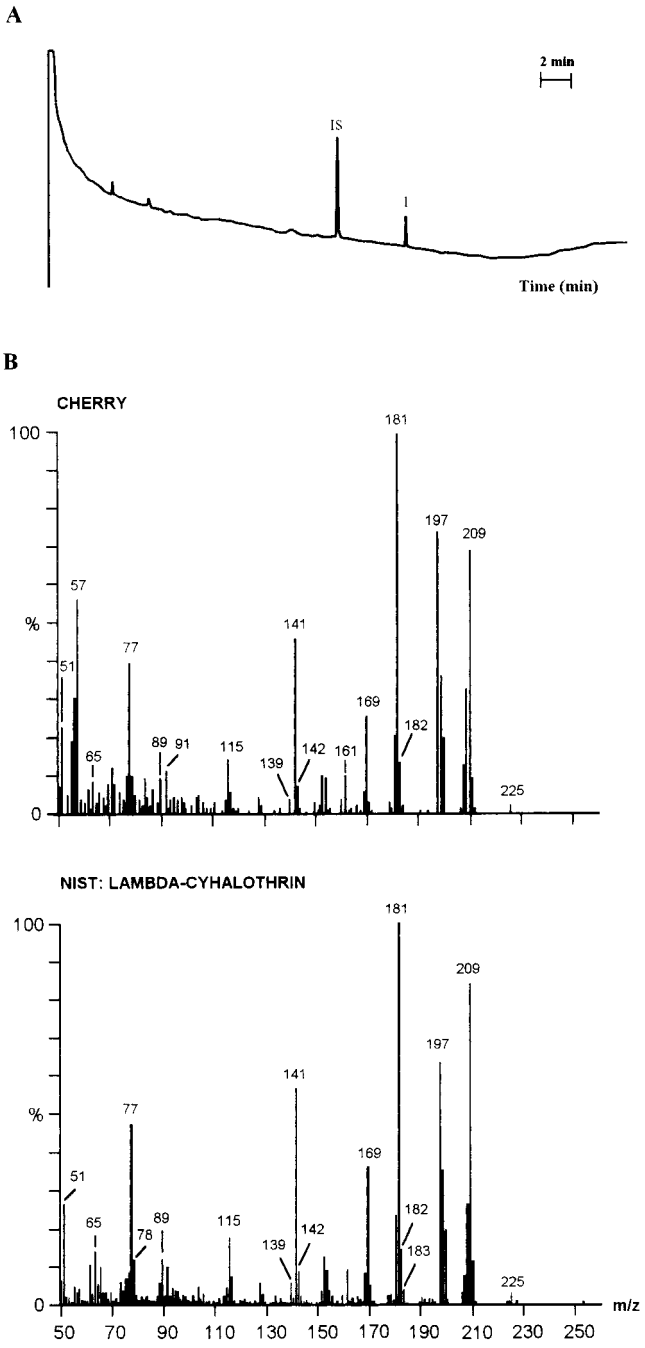

Figure 4. Gas chromatogram for λ-cyhalothrin (1) found in a cherry sample (A); IS, internal standard. Full scan EI mass spectra from the pyrethroid in the fruit sample and from pesticide library (B).

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Received for review September 22, 2000. Revised manuscript received December 7, 2000. Accepted December 17, 2000. This work was supported by grant PB-95-0977 from Spain's DGICyT.

JF0011687