Organochlorine Pesticide Residues in Strawberries from Integrated Pest Management and Organic Farming

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ABSTRACT: A rapid, specific, and sensitive method based on the Quick Easy Cheap Effective Rugged and Safe (QuEChERS) method and a cleanup using dispersive solid-phase extraction with $MgSO_4$, PSA, and C18 sorbents has been developed for the routine analysis of 14 pesticides in strawberries. The analyses were performed by three different analytical methodologies: gas chromatography (GC) with electron capture detection (ECD), mass spectrometry (MS), and tandem mass spectrometry (MS/MS). The recoveries for all the pesticides studied were from 46 to 128%, with relative standard deviation of <15% in the concentration range of 0.005−0.250 mg/kg. The limit of detection (LOD) for all compounds met maximum residue limits (MRL) accepted in Portugal for organochlorine pesticides (OCP). A survey study of strawberries produced in Portugal in the years 2009−2010 obtained from organic farming (OF) and integrated pest management (IPM) was developed. Lindane and β-endosulfan were detected above the MRL in OF and IPM. Other OCP (aldrin, o,p'-DDT and their metabolites, and methoxychlor) were found below the MRL. The OCP residues detected decreased from 2009 to 2010. The QuEChERS method was successfully applied to the analysis of strawberry samples.

KEYWORDS: organochlorine pesticides, gas chromatography, mass spectrometry, tandem mass spectrometry, electron capture detection, strawberries, organic farming

■ INTRODUCTION

Despite the usefulness of pesticides in agricultural practices, there are concerns about their excessive use and their presence and levels in the environment and foodstuffs. Integrated pest management (IPM) has been adopted to maximize crop yield while maintaining a quality environment with regard to pesticide contamination, solving ecological problems and contributing to the sustainability of agriculture.[1] These preventive practices were preceded by other actions. For instance, OCP have been banned since the 1970s because they are extremely persistent in the environment and due to their ability to accumulate in sediments, plants, and animals.[2] OCP have an extensive range of both acute and chronic health effects, including cancer, neurological damage, and birth defects. Many OCP are also suspected to act as endocrine disruptors.[3−5] Consequently, routine and comprehensive testing of OCP is given consideration in the scientific literature and enforcement actions.[3,6,7] Particularly, the control of OCP in fruits and vegetables is important for regulatory agencies to ensure that concentrations of toxic pesticides are below tolerance levels,[8] especially because these foods, in most cases, are eaten raw and often unwashed.

Traditional methods for trace analysis of OCP are characteristically time and solvent-consuming, and their sample throughput is too low.[9] Development of simple and reliable methods is a challenging task. The Quick Easy Cheap Effective Rugged and Safe (QuEChERS) method has recently attracted attention for pesticides analysis given its advantages of (i) high recovery for wide polarity and volatility ranges of pesticides; (ii) high sample throughput; (iii) the use of smaller amounts of organic solvent and the use of nonchlorinated solvents; (iv) the need for very little labware and increased safety for laboratory workers.[10] The main advantage of QuEChERS is that it is comprehensive, being

useful for the analysis of pesticides of varying polarities, by virtue of the fact that the sorbent used focuses on binding sample matrix compounds without interacting with the target analytes.[11]

The determination of OCP residues is generally performed by GC using specific detectors, such as ECD[12−14] and MS.[11,15,16] The use of MS detectors[17] is widely extended due to their selectivity and low detection limits.[18] Selected ion monitoring (SIM) mode allows reducing background noise but does not eliminate matrix interferences in all cases. Certain MS detectors can perform in tandem mass spectrometry (MS/MS), which provides higher selectivity and sensitivity.[19]

The aim of this study was to set up a multiresidue methodology for the determination of 14 OCP (α-, β-, γ-, and ζ-hexachlorocyclohexanes (HCHs), hexachlorobenzene (HCB), o,p'-DDT ([1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane]), and their degradation products 2,2-bis(p-chlorophenyl)-1,1-dichloroethylene (p,p'-DDE) and dichlorodiphenyldichloroethane (p,p'-DDD), aldrin, dieldrin, endrin, α- and β-endosulfan, and methoxychlor) in strawberries, by the QuEChERS method and GC. The developed approach was, first, the use of GC-ECD due to the high sensitivity of detector to chlorinated compounds for quantification method, followed by GC-MS operated in SIM mode and finally GC-MS/MS, the last ones as a confirmation method. Nowadays, this sequence is being used by some pesticide laboratories for multiresidue targeted screening of pesticides in food samples.[20,21] The methods were applied for the detection of OCP in

strawberries sampled from organic farming (OF) and IPM cultivated in central Portugal. Adequate sampling of strawberry collection was done in May of 2009 and 2010. To compare samples produced by different cultivation methods, a careful sampling was carried out taking into account (i) the maturity (75% of the strawberry should be red); (ii) the type of farming (at least 5 years in IPM and OF); (iii) the variety of strawberry; (iv) similar geographic location; and (v) type of soil, in order to perform a statistical correlation that may affect the studied factors.[22,23]

■ MATERIALS AND METHODS

The pesticide standards in this study were obtained from Chemservice (West Chester, PA), Dr. Ehrenstorfer GmbH (Augsburg, Germany),

Table 1. Quantitation and Identification Ions for the GC-MS and GC-MS/MS Analyses of Selected OCP

	SIM		MS/MS
OCP	identification ions (m/z)	quantification ion (m/z)	precursor ions (m/z)
(α, β, δ) HCH	109, 181, 219	181	219
HCB	142, 284, 286	284	142
lindane	181, 183, 219	181	183
aldrin	66, 263, 293	263	263
α-endosulfan	195, 207, 241	241	195
dieldrin	79, 263, 277	263	243
p,p'-DDE	176, 246, 318	246	318
endrin	81, 263, 281	263	245
β-endosulfan	195, 207, 241	195	195
p,p'-DDD	165, 235, 318	235	235
o,p'-DDT	165, 235, 352	235	235
methoxychlor	227, 237	227	227

and Sigma-Aldrich Co. n-Hexane and acetonitrile were of HPLC grade from Merck. All solutions were prepared in n-hexane, and the internal standard (IS) solution of 4,4'-dichlorobenzophenone was 350 μg/L. Mixed fortification standards, each containing 14 OCP (α-, β-, γ- and ζ-HCHs, HCB, o,p'-DDT, p,p'-DDE, and p,p'-DDD, aldrin, dieldrin, endrin, α- and β-endosulfan, and methoxychlor) were prepared by diluting stock standard solutions. All working solutions were stored in glass vials at −20 °C prior utilization. The selected QuEChERS (EUMIV50CT-VP) and cleanup (CUMPS15C18CT) were obtained from UCT (Bristol, PA).
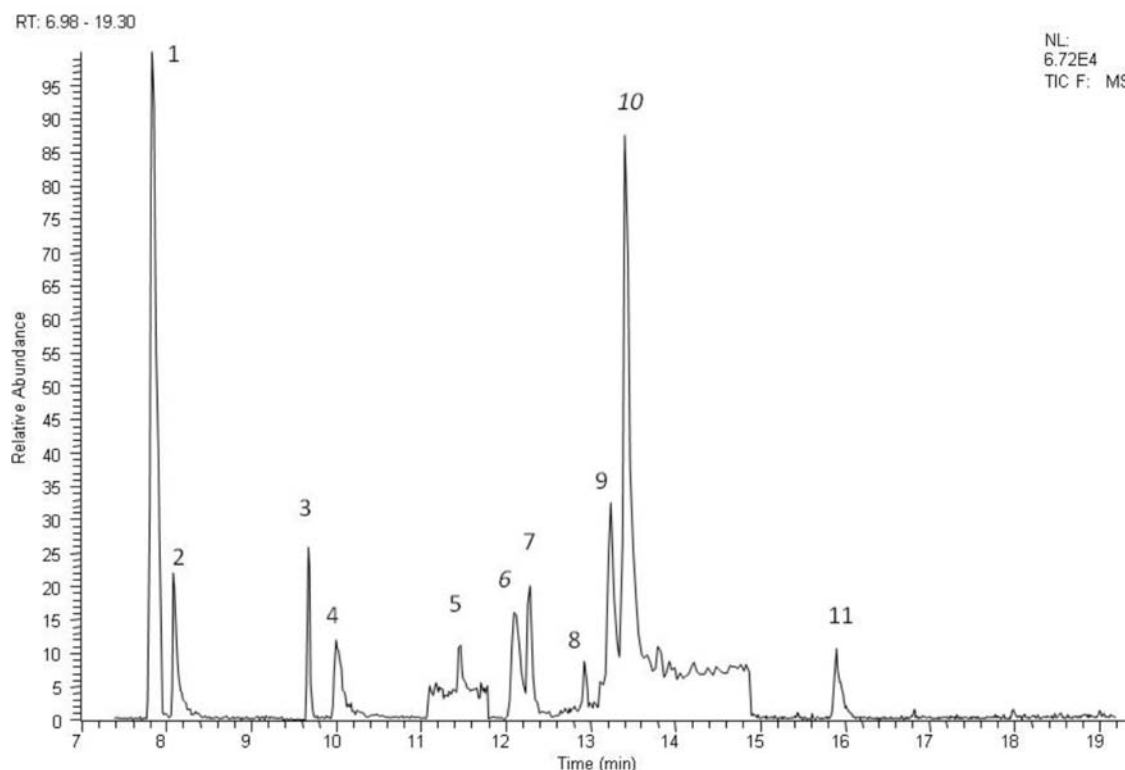
Sampling. The sampling was scheduled with producers during the two years of harvest and was performed by the authors. Strawberry samples were collected in two types of cultivation: OF (N 41° 12.040′; W 8° 39.943′) and IPM (N 38° 35.100′; W 8° 41.446′), both from Palmela in central Portugal. We collected different varieties of strawberries including Siba, Camarosa, Festival, and Albion. The sampling period was the first week of May in 2009 and 2010. The 2009 and 2010 fields of OF were close due to crop rotation.

Procedure. The extraction and cleanup steps were not optimized because the original method[24] provided adequate recoveries and precision. Samples were crushed and homogenized using a blender jar. Strawberry samples were frozen and stored at −20 °C until required for analysis. An amount (10 g) of strawberries was weighed into a 50 mL centrifuge tube, and 50 μL of IS solution was added. The strawberry sample was left during 30 min at room temperature to let the n-hexane evaporate before the addition of 10 mL of acetonitrile.

The resulting solution was shaken for 1 min, followed by the addition of 6 g of anhydrous magnesium sulfate, 1.5 g of sodium chloride, 1.5 g of trisodium citrate dehydrate, and 0.75 g of disodium hydrogencitrate sesquihydrate. The centrifuge tube was capped and shaken vigorously for 1 min to prevent salt agglomeration before centrifugation at 3000 rpm for 5 min. An aliquot of 1.5 mL was sampled from the upper layer into a 2 mL cleanup vial containing 150 mg of primary secondary amine (PSA), 150 mg of MgSO$_4$, and 50 mg of C18 and again vortexed for 1 min and then centrifuged for 5 min at 4000 rpm. From the upper layer of the prepared sample, an aliquot of 1.0 mL was transferred into a vial and put under a mild stream of nitrogen to dryness. Finally, 1 mL of hexane
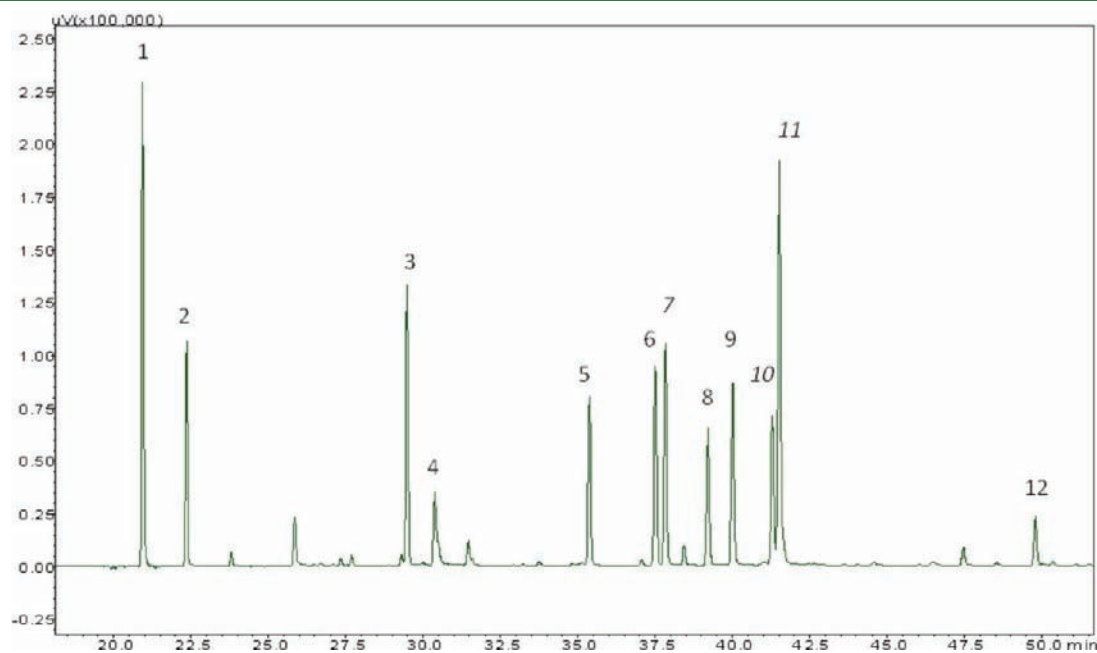
Figure 1. GC-ECD chromatogram of mixture OCP (70 μg/L). Peak identification in order of increasing retention time: 1, HCB; 2, lindane (γ-HCH); 3, aldrin; 4, IS; 5, α-endosulfan; 6, dieldrin; 7, p,p'-DDE; 8, endrin; 9, β-endosulfan; 10, p,p'-DDD; 11, o,p'-DDT; 12, methoxychlor.

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Figure 2. GC-MS/SIM chromatogram of mixture OCP (150 μg/L). Peak identification in order of increasing retention time: 1, HCB; 2, lindane (γ-HCH); 3, aldrin; 4, IS; 5, α-endosulfan; 6, dieldrin; 7, p,p'-DDE; 8, endrin; 9, β-endosulfan; 10, o,p'-DDT; 11, methoxychlor.

Table 2. Calibration, LOD, and S/N for the Studied OCP in Spiked Strawberries by GC-ECD, GC-MS/SIM, and GC-MS/MS

| | GC-MS/SIM | | | | | GC-MS/MS | | | | | GC-ECD | | | |
| | equation[a] | | | | | equation[a] | | | | | equation[a] | | | |
OCP	m	b	correl coeff	LOD (μg/kg)	S/N[b]	m	b	correl coeff	LOD (μg/kg)	S/N[b]	m	b	correl coeff	LOD (μg/kg)
α-HCH[c]	7.78×10^4	-6.18×10^2	0.9984	7.34	2.92	5.78×10^4	-2.12×10^2	0.9989	7.02	31.30	4.24×10^8	4.92×10^5	0.9963	4.49
β-HCH[c]	8.11×10^4	3.52×10^1	0.9987	6.92	2.38	6.78×10^4	4.56×10^1	0.9990	6.52	28.53	3.85×10^8	5.29×10^5	0.9980	3.84
δ-HCH[c]	7.47×10^4	6.45×10^2	0.9991	7.56	2.87	5.34×10^4	5.88×10^2	0.9991	7.11	26.02	2.96×10^8	3.60×10^5	0.9974	4.10
HCB[c]	1.18×10^6	-5.13×10^3	0.9982	9.20	9.15	6.66×10^5	5.16×10^3	0.9990	7.65	36.40	6.36×10^8	-1.46×10^6	0.9971	7.44
lindane[c]	2.92×10^5	5.36×10^2	0.9982	9.10	2.60	1.91×10^5	4.28×10^3	0.9991	7.03	29.27	2.71×10^8	-6.36×10^3	0.9974	7.08
aldrin[c]	2.04×10^5	2.08×10^2	0.9988	8.20	20.09	2.03×10^5	-8.12×10^2	0.9994	5.99	28.65	3.68×10^8	7.76×10^5	0.9987	5.05
α-endosulfan[d]	8.69×10^4	1.43×10^3	0.9989	9.90	1.94	1.11×10^5	1.13×10^3	0.9981	8.76	300.66	1.74×10^8	1.17×10^6	0.9962	8.58
dieldrin[c]	1.92×10^5	9.06×10^2	0.9982	9.60	2.02	1.82×10^5	-7.39×10^1	0.9990	8.02	108.31	2.45×10^8	1.13×10^6	0.9984	4.48
p,p'-DDE[d]	3.17×10^5	-1.13×10^3	0.9990	9.50	1.20	2.04×10^5	-6.55×10^2	0.9995	7.50	56.21	2.65×10^8	6.29×10^5	0.9989	5.61
endrin[c]	7.33×10^4	9.33×10^2	0.9987	8.70	2.85	7.84×10^4	3.67×10^2	0.9990	7.80	162.90	2.52×10^8	1.66×10^6	0.9973	7.14
β-endosulfan[d]	4.07×10^5	5.53×10^2	0.9985	8.60	15.61	3.33×10^5	-1.37×10^3	0.9988	7.64	148.41	1.87×10^8	8.52×10^5	0.9984	5.61
p,p'-DDD[d]	4.77×10^2	-2.26×10^4	0.9978	8.91	4.41	5.01×10^2	-3.18×10^4	0.9985	6.12	180.88	1.89×10^8	5.54×10^4	0.9994	3.43
o,p'-DDT[d]	1.51×10^6	-9.12×10^3	0.9976	9.90	3.04	4.96×10^5	-3.05×10^3	0.9991	6.02	192.95	3.17×10^8	-6.66×10^5	0.9980	6.18
methoxychlor[c]	1.94×10^5	-6.86×10^2	0.9983	9.32	5.35	1.54×10^5	-3.62×10^2	0.9988	7.86	48.64	2.40×10^7	-4.96×10^4	0.9961	8.68

[a] m, slope; b, intercept. [b] S/N, concentration 10 μg/L. [c] MRL for each pesticide in Europe in strawberries = 10 μg/kg. [d] MRL for each pesticide in Europe in strawberries = 50 μg/kg.

was added to dissolve the residue, and then 2 μL of this solution was injected onto the gas chromatograph.

Method Validation. The standard working solutions were used for spiking strawberries for recovery assays and for the matrix-matched calibration standards in strawberry blank used for quantification of the OCP. The developed method was completely validated in a strawberry matrix. A validation procedure was carried out to guarantee the feasibility of the method.

The performance of the method was evaluated considering the following validation parameters.

Linearity. The calibration curves for all of the compounds in matrix were obtained by plotting the peak area against the concentration of the corresponding calibration standards at seven calibration levels ranging between 0.005 and 0.250 mg/kg.

Sensitivity. The limit of detection (LOD) and the limit of quantification (LOQ) were determined by considering the slope of the calibration line and the residual standard deviation of the regression line.[25]

Fortification. Strawberries with no pesticides detected previously were used for the fortification experiments. Ten grams of homogenized
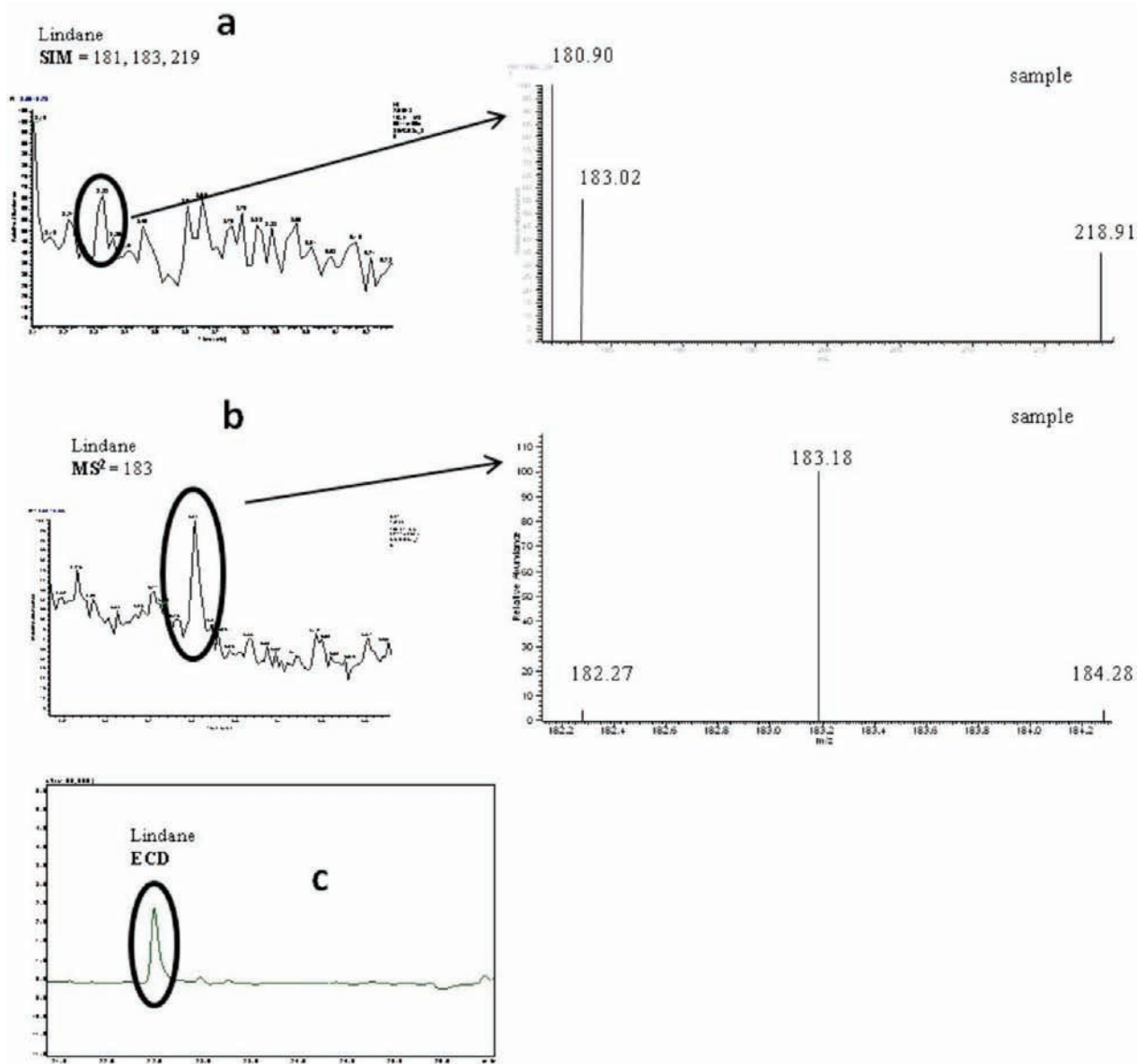
Figure 3. GC-MS/SIM (a), GC-MS/MS (b), and GC-ECD (c) chromatograms of OF strawberry sample contaminated with lindane (13 μg/kg) with spectra comparison of the sample by GC-MS and GC-MS/MS.

sample was spiked prior to the determination procedure by the addition of a mixture of standard pesticides solution to give 0.030, 0.090, and 0.180 mg/kg of each compound. The fortification tests were done in triplicate. Spiking samples were left to stand for 3 h to allow pesticide absorption onto the sample and evaporate the solvent. They were then prepared according to the determination procedure described above. Quantification was performed by using the peak area of the analyte and calculating the concentration by preparing a calibration curve.

Gas Chromatography—Electron Capture Detector (GC-ECD). OCP were analyzed using a Shimadzu GC-2010 with an ECD apparatus, equipped with a capillary column of 30 m, TRB-5MS (0.25 mm i.d., 0.25 μm film thickness, Teknokroma). The oven temperature was programmed starting at 65 °C and held for 2 min, followed by increases of 8 °C/min to 160 °C, then 2 °C/min to 235 °C, and then 15 °C/min to 250 °C. The injection port was at 250 °C in splitless mode, and the detection was carried out at 300 °C. Helium (Linde Sogás) was used as carrier gas at a constant flow rate of 1.3 mL/min, whereas nitrogen (Linde Sogás, purity ≥ 99.999%) was employed as makeup gas

at a flow of 30 mL/min. The system was operated by GCsolution Shimadzu software.

Gas Chromatography—Mass Spectrometry (GC-MS). In addition, the real samples were analyzed using a Thermo Trace-Ultra gas chromatograph coupled to an ion trap mass detector Thermo Polaris, operated in the electron impact ionization (EI) at 70 eV. The ion source temperature was 250 °C and the MS transfer temperature, 250 °C. The system was operated by Xcalibur v 1.3 software. Confirmation of residues was carried out by GC-MS/SIM and MS/MS using a Supelco column fitted with an SLB-5MS (30 m × 0.25 mm, 0.25 μm film thickness) column operating in the splitless mode; helium was used as carrier gas at a constant flow rate of 1.3 mL/min. The injector was maintained at 240 °C. The oven temperature was programmed starting at 40 °C and held for 2 min, followed by increases of 30 °C/min to 220 °C, held for 5 min, then 10 °C/min to 270 °C, and held for 1 min. For the identification of pesticides, the retention time, and three ions, the NIST and Wiley pesticide libraries were used. Selected ions (m/z) used for confirmation are summarized in Table 1. The MS/MS conditions

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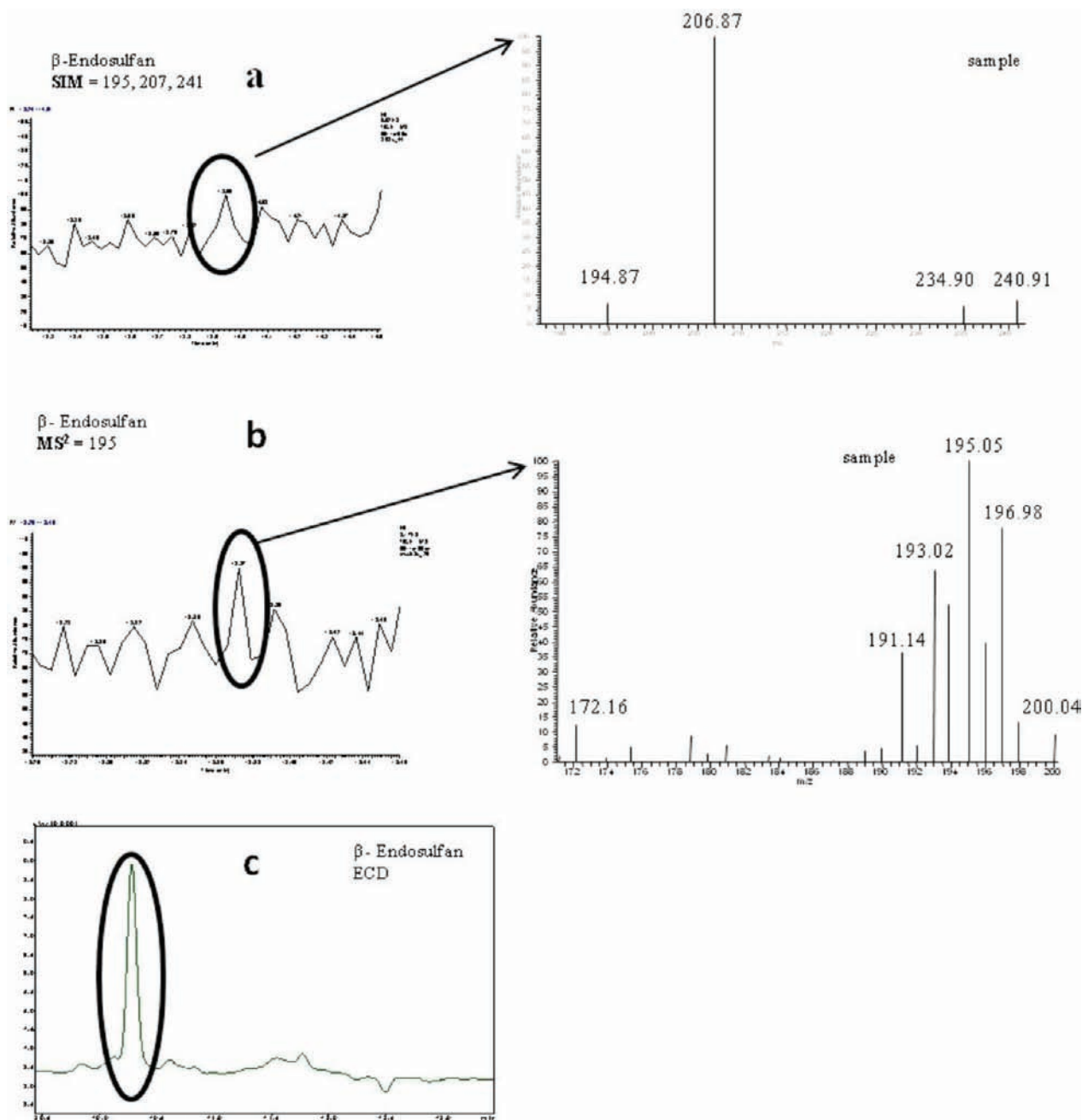
Figure 4. GC-MS/SIM (a), GC-MS/MS (b), and GC-ECD (c) chromatograms of OF strawberry sample contaminated with β-endosulfan (7 μg/kg) with spectra comparison of the sample by GC-MS and GC-MS/MS.

were fixed for each compound, trying to select as precursor ion the one with the highest m/z ratio and abundance (Table 1).

■ RESULTS AND DISCUSSION

Sample Extraction and Cleanup. Matrix effects can generate serious problems in pesticide residue analysis using GC coupled with MS due a possible over- or underestimation of analyte concentration when compared with the same analyte in organic solvent. Acetonitrile was preselected for sample preparation in this study as it is a common solvent for the extraction of OCP from fruit and vegetable samples and was also used in the QuEChERS procedure. However, a preliminary assay showed

that acetonitrile resulted in extracts that were heavily pigmented, containing large amounts of matrix coextractants. At this point, acetonitrile extracts are unsuitable for further analysis due to the high levels of endogenous interferences coextracted with the pesticides during solvent extraction. The sample matrix coextractants may have a deleterious effect on the capillary columns, may interfere with the detection of pesticides at trace levels, and/ or may result in a sample matrix-induced enhancement effect, dispersive solid phase extraction is necessary for further cleanup prior to chromatographic analysis. The cleanup procedure in this work employs 150 mg of PSA, which is higher compared with the amount commonly used,[10,26,27] and was shown to be effective for the removal of a variety of matrix interferents such as sugars,

Table 3. Percent Recoveries ± RSD Based on Three Replicate Experiments for the Analysis of OCP by GC-ECD in Strawberries Produced by OF and IPM

| | recoveries[a] (%) | | | | | |
| | OF | | | IPM | | |
name	0.030 mg/kg	0.090 mg/kg	0.180 mg/kg	0.030 mg/kg	0.090 mg/kg	0.180 mg/kg
α-HCH	107 ± 8	101 ± 10	98 ± 7	105 ± 5	100 ± 7	97 ± 9
β-HCH	103 ± 10	100 ± 9	99 ± 8	103 ± 7	99 ± 8	99 ± 10
δ-HCH	109 ± 7	99 ± 10	100 ± 10	106 ± 8	101 ± 9	100 ± 7
HCB	120 ± 3	85 ± 1	91 ± 1	112 ± 5	88 ± 6	92 ± 5
lindane	121 ± 1	97 ± 8	110 ± 12	119 ± 10	99 ± 7	107 ± 10
aldrin	101 ± 5	80 ± 2	110 ± 3	99 ± 3	85 ± 10	107 ± 5
α-endosulfan	116 ± 6	97 ± 1	91 ± 2	118 ± 11	100 ± 2	90 ± 10
dieldrin	111 ± 1	111 ± 10	123 ± 7	113 ± 10	108 ± 11	117 ± 9
p,p'-DDE	51 ± 13	46 ± 11	54 ± 12	59 ± 13	49 ± 9	57 ± 11
endrin	105 ± 3	128 ± 4	124 ± 8	100 ± 8	121 ± 7	118 ± 8
β-endosulfan	122 ± 7	111 ± 8	108 ± 12	115 ± 1	107 ± 10	105 ± 10
p,p'-DDD	100 ± 4	112 ± 12	128 ± 5	105 ± 3	115 ± 10	121 ± 10
o,p'-DDT	111 ± 10	90 ± 10	118 ± 7	104 ± 7	97 ± 8	117 ± 11
methoxychlor	94 ± 12	89 ± 12	121 ± 11	99 ± 9	92 ± 9	114 ± 8

[a] Mean percent recovery ± RSD of OCP in strawberries at 0.030, 0.090, and 0.180 mg/kg fortification levels ($n = 3$).

fatty acids, organic acids, and anthocyanin pigments, yielding a clear supernatant.

Method Validation: Calibration, Linearity, S/N, and LOD. Standard mixtures of OCP in *n*-hexane were initially used to determine their characteristic parameters such as retention time (GC-ECD), mass spectra, and acquisition time segments for both GC-MS/SIM and GC-MS/MS. All analyses were performed by GC-ECD, GC-MS/SIM, and MS/MS mode. Pesticides were identified according to their retention times and selected and precursor ions. First, the strawberry extracts were analyzed by GC-ECD. The GC-ECD demonstrated a high performance for the detection and quantification of OCP (Figure 1) compared with GC-MS/SIM (Figure 2). In SIM, the identification of pesticide peaks was confirmed by matching retention times of standards and by the presence of major ions. In MS analysis, the MS/MS conditions were fixed for each compound, trying to select as precursor ion the one with the highest m/z ratio (greater selectivity) and abundance (greater sensitivity). Analysis of the targeted pesticides for quantification and identification purposes from the strawberry extracts indicates the desired specificity by selecting the precursor-to-product ion transitions used in GC-MS/MS over the nonspecific qualifier and target ions used in GC-MS/SIM. Although a cleanup procedure was used, many of the pesticides were still very difficult to detect and identify in low concentrations by GC-MS/SIM. Matrix interferences can contribute to the pesticide target and qualifier ion abundances that affect the qualifier-to-target percentage ratios used for identification in GC-MS/SIM. GC-MS/SIM can lead to false negatives due to the difficulty of distinguishing the peak from the baseline noise. However, demand for the precursor ion in MS/MS allows confirmation due to better resolution of the peak. In the analysis by GC-MS/SIM, noise baseline is high, which does not allow proper identification of the pesticide found. On the other hand, the specificity of GC-MS/MS provided transition ions that are only specific to the pesticide even in the presence of the strawberry matrix.

Table 4. Pesticides Residues in Portuguese Strawberries from OF and IPM in Several Varieties (Siba, Camarosa) and Their Concentrations in 2009
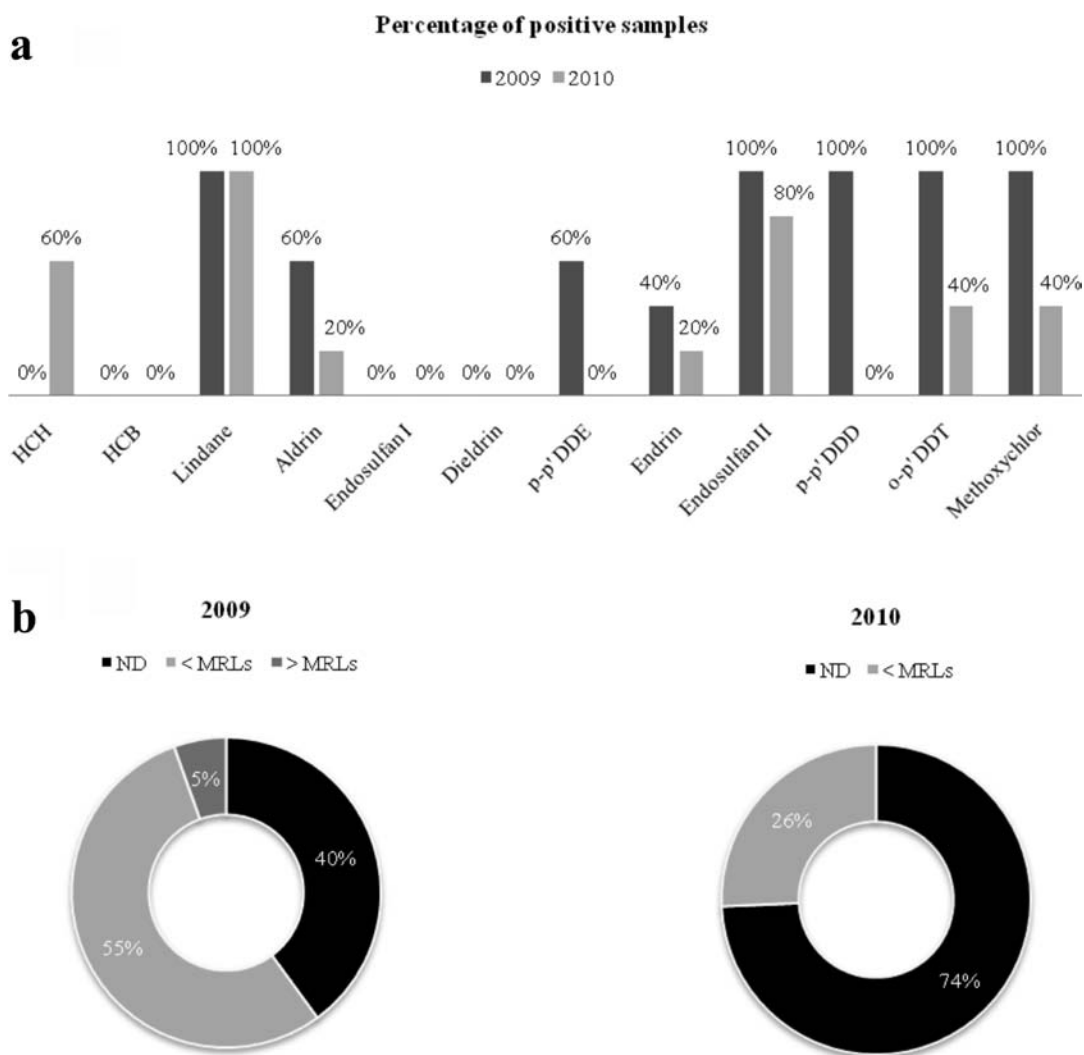
| | OF (μg/kg) | | IPM (μg/kg) |
	Siba	Camarosa	Camarosa
lindane	13.00	27.30	14.60
β-endosulfan	7.02	<LOD[a]	<LOD

[a] <LOD, below the LOD.

Considering the previously referred to matrix effects, external calibrations were performed with pesticide-fortified samples. These samples were previously confirmed for the absence of OCP. Table 2 summarizes the matrix-matched calibration results, along with LOD values and signal-to-noise ratios (S/N) for the pesticides studied. Calibration curves were constructed for each compound using seven different concentration levels between 0.005 and 0.250 mg/kg and finding the minimum LOD expected for the method. An internal standard was employed at the final sample preparation stage to help control the significant losses of analytes during extraction. The calibration plots exhibit good linearity for pesticides ranging from 0.005 to 0.250 mg/kg. Average coefficients of determination were >0.996. Because the LOD is matrix dependent, it is recommended that matrix-matched calibration be performed for quantitative analysis for unknown samples in complex matrices such as, in this case, strawberries. Matrix interferences may be noted for some qualifier ions, especially those of low masses, at low concentrations. Despite these restrictions, the results indicate that the LOD are sufficient for strawberry safety purposes.

Calibration data were generated from three replicate samples of seven points at linearity range and LOD was determined as in eq 1, where m is the slope of the calibration line and $S_{x/y}$ the residual standard deviation of a regression line.[25]

$$\text{LOD} = \frac{3.3 S_{x/y}}{m} \qquad (1)$$

Percentage of positive samples

a

■ 2009 ■ 2010

b

2009

■ ND ■ < MRLs ■ > MRLs

2010

■ ND ■ < MRLs

Figure 5. Percentage of 12 pesticide residues in Portuguese strawberries produced in 2009 and 2010 above and below the MRL (a); percentage of all OCP studied in Portuguese strawberry samples that were below the MRL, above the MRL, and not detected in two years of study (b).

For all of the compounds assayed, the LOD are below their MRL, as shown in Table 2. The lowest LOD for all pesticides and the chromatogram with better resolution and sensibility were obtained through ECD.

Enhancement of S/N Ratio by GC-MS/MS. The specificity of MS/MS results in an improvement of the S/N ratio, allowing for improved sensitivity over MS/SIM and resulting in a higher number of pesticides detected and identified at low levels. In Table 2, S/N ratios are calculated for both GC-MS (SIM and MS/MS) analyses for all OCP through the Xcalibur v 1.3 software, in a strawberry sample contaminated at 10 μg/kg. The S/N ratios obtained by GC-SIM/MS mode are considerably lower than those obtained by GC-MS/MS. These results are especially important for pesticides with poor detectability and with the same quantification ions as the interferences of matrix by GC-SIM/MS. For example, lindane and β-endosulfan show similar quantification in the same strawberry sample (Figures 3 and 4). When the chromatograms obtained from ECD are compared, the peak that identifies the pesticide found offers the best resolution. The baseline GC-ECD has less noise, being thus considered a good technique for detecting OCP. In the case of GC-MS/MS analysis, the chromatogram for most analytes

showed higher peak resolution and low background noise compared to GC-MS/SIM (Figures 3b and 4b). This could possibly be due to the higher selectivity in the analysis by MS/MS compared to SIM. The confirmation performed in SIM and MS/MS results in similar mass spectra of sample and standard. Both GC-MS/SIM and GC-MS/MS were able to detect, quantify, and identify all of the compounds assayed, but the biggest advantage of GC-MS/MS over GC-MS/SIM is its instrument specificity and sensitivity.

Recovery Studies. The recovery study was based on the SANCO guideline[28] and European directive 96/46/EC.[29] Satisfactory recoveries (from 70 to 120%) were obtained with 43% of recovery data ranging from 90 to 105%, 6% of the data ranging from 70 to 89%, and 33% of the data ranging from 105 to 120%, with relative standard deviations (RSDs) of <13%. These results were obtained from two strawberry samples spiked ($n = 3$) at 0.030, 0.090, and 0.180 mg/kg. Table 3 lists the mean recoveries ($n = 3$) and RSD for 14 OCP fortified at 3 levels of OF and IPM measured by GC-ECD. For each pesticide, the absolute recovery value was obtained from the ratio between the peak area corresponding to the spiked strawberries and the peak area of the standard solution corresponding to the same pesticide
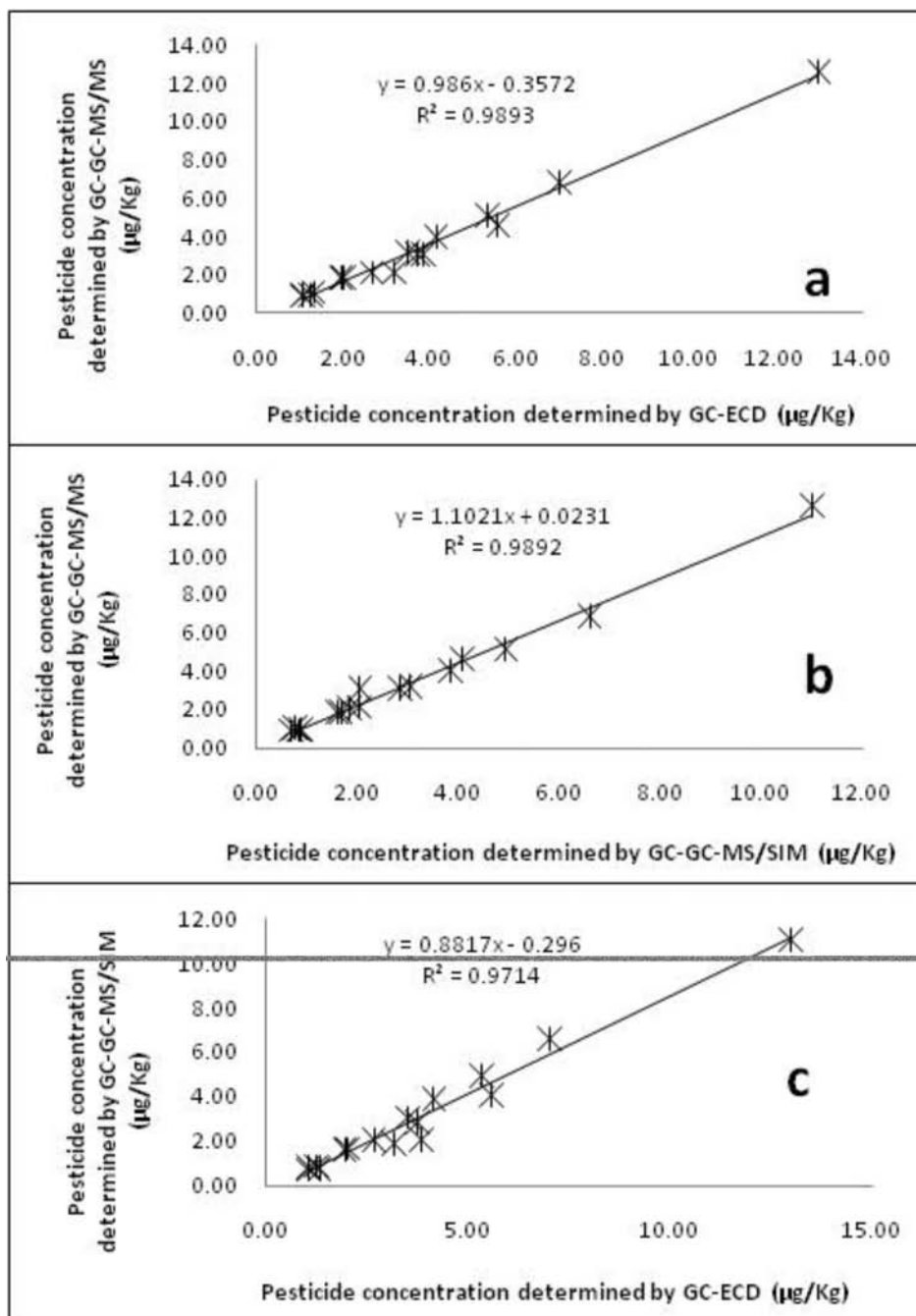
Figure 6. Correlation between (a) GC-ECD and GC-MS/MS, (b) GC-ECD and GC-MS/SIM, and (c) GC-MS/SIM and GC-MS/MS methods for determination of OCP in strawberries.

standards injected directly onto the analytical column. Unsatisfactory recoveries (>120%) were obtained for the remainder of the pesticides due to the phenomenon commonly known as the "matrix-induced chromatographic response enhancement effect", contracted to matrix-induced response enhancement. Some factors may affect sample matrix enhancement such as the nature of the pesticide, the nature of the matrix, the pesticide-to-matrix ratio, and the GC system.[17,30] The performances of recoveries were similar for most pesticides at all three fortification concentrations when analyzed by GC-ECD.

Determination in Samples. The strawberry samples from local farmers in central Portugal were sampled and analyzed following the sample preparation method described above. The residues were found in all strawberry samples produced by OF and IPM. Pesticide levels encountered in the analyzed samples are shown in Table 4. Lindane was detected in all samples analyzed in 2009 and 2010 at concentrations ranging from 0.06 to 27.30 μg/kg (Table 4). In 2009, β-endosulfan and methoxychlor were detected in all samples. In only one of these samples (Siba OF 2009) was β-endosulfan detected above the LOD. o,p'-DDT and their metabolites were also identified in lower concentrations in all samples below the LOD. Endrin was detected in two samples and aldrin in three samples at concentrations ranging from 0.13 to 4.94 μg/kg and from 0.65 to 1.38 μg/kg,

respectively. HCB, dieldrin, and α-endosulfan were not detected in any samples. The residues obtained for 2009 are higher than for 2010. At least one of the OCP was detected in 100% of the analyzed samples (Figure 5).

The OCP concentration in strawberry samples was higher than the MRL in 5% of samples in 2009, whereas none of them were detected in 2010. About 55% of the samples were positive (<MRL) in 2009 versus 26% in 2010 (Figure 5).

Because all of these pesticides have not been used over the past 30 years, the data reported indicate that OCP persist in the environment. This study also shows that the proposed method to determine residues of OCP is rapid, simple, and sensitive and uses smaller amounts of organic solvents, reducing the risk for workers and the environment. The results showed that the amounts of pesticide residues detected were similar in samples produced by IPM and OF. These results also showed that strawberries were able to accumulate OCP from soils or as a result of atmospheric deposition of those pesticides. The OF and IPM fields have the same type of soil, and they are close enough to have the same type of environmental exposure.

The fields where we collected the OF and IPM strawberries are located in a large area of cultivation. In both cases, the fields are surrounded by other fields that may or not be cultivated. The OF and IPM fields are located near a small village, away from the city.

Correlation Studies. The variables from different groups were compared with the Student t test. The significance of the differences was tested at the level of $p < 0.05$. Figure 6 shows plots of correlation between OCP concentrations detected in strawberry samples. Correlation coefficients were 0.9893 and 0.9892 for all pesticides in a and b, respectively, which indicates a good agreement in results from GC-ECD versus GC-MS/MS and GC-MS/SIM versus GC-MS/MS. A correlation coefficient with lower value was obtained for GC-ECD versus GC-MS/SIM with $R^2 = 0.9714$ (Figure 6c). The slopes of correlation plots are slightly lower than 1 in a and b and slightly higher (1.1021) in c, indicating that the OCP concentrations detected by GC-ECD were higher than OCP concentrations obtained by GC-MS/SIM and that the pesticide concentrations obtained by GC-MS/MS were also higher than those obtained by GC-MS/SIM, respectively.

Moreover, statistical analysis (paired t test) shows that there is no significant difference between GC-ECD and GC-MS/MS (in most results $p > 0.05$), and in the case of GC-ECD versus GC-MS/SIM methods a statistical difference has been observed ($p < 0.05$). The concentrations of most OCP did not differ between the GC-ECD and GC-MS/MS results, whereas the OCP levels were significantly higher in the GC-ECD results than in the GC-MS/SIM.

In conclusion, these pesticides tend to be very persistent, and this method using QuEChERS sample preparation and GC-ECD, GC-MS/SIM, or MS/MS analysis showed a high sensitivity and confirmatory capacity necessary for the determination of pesticide residues at the levels required in EU MRL for strawberries. The proposed method not only allowed the simultaneous determination and confirmation of a large number of OCP with good recoveries and low detection limits but was also useful in routine analysis due to its speed and ease of performance. This work has demonstrated that OCP quantification by GC-ECD can be a powerful tool for a complex matrix. The results showed that GC-ECD can be used for screening these OCP. However, GC-MS/MS requires only one injection, rather than multiple injections for multiple instruments for the screening

identification and quantification of pesticides in the sample. The applicability of the method was demonstrated by analysis of real samples (strawberries produced by different agricultural methods), resulting in good quality control data and thus making possible reliable determination of the targeted pesticides.

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