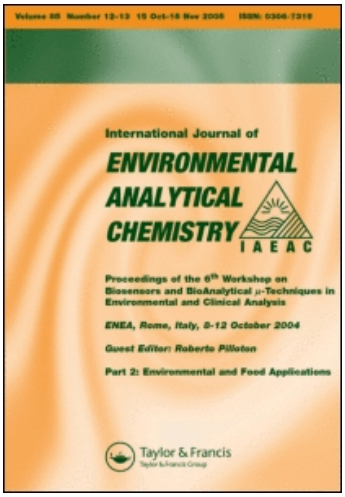

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Determination of organochlorine pesticides in horticultural samples by microwave assisted extraction followed by GC-ECD

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A method to determine 21 organochlorine pesticides in horticultural samples (lettuce, pepper, tomato, spinach and potato) based on microwave assisted extraction (MAE) followed by solid-phase extraction (SPE) clean-up is described. After extraction with hexane : acetone (50 : 50), a carbon cartridge was employed for the clean-up step. Pesticides were eluted with 5 mL of hexane : ethyl acetate (80 : 20) and determined by gas chromatography and electron capture detection (GC-ECD). Results were confirmed by GC-MS analysis. Analytical recoveries obtained were ca. 100% for most of the studied pesticides with the proposed method in each analysed matrix. The method was applied to analyse 35 commercial samples from A Coruña (NW Spain); only two samples contained pesticide residues, but none of them exceeded the MRLs established by EU legislation.

Keywords: Organochlorine pesticides; Horticultural samples; Microwave assisted extraction; Solid-phase extraction; GC-ECD; GC-MS

1. Introduction

Use of pesticides is necessary in the production and conservation of food sources but results in the presence of pesticide residues in agricultural foods. Organochlorine pesticides (OCPs) are an important group of contaminants in the environment due to their low biodegradability and high persistence. Moreover, these chemicals are very toxic, and known to induce cancer and endocrine disruption in several organisms, so they pose a significant risk to natural ecosystems and human health [1].

Public concern over pesticide residues in food has increased during the last 20 years to the point where it has become a significant food safety issue. The detection and

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identification of these compounds in food for human consumption is of growing concern for producers, consumers and governments due to the potential risks of these compounds [2]. Therefore, pesticide monitoring programs are established by governments for the protection of consumers and to control the quality of foods [3,4].

Analytical methods for pesticide residues are mainly used to control foods for human consumption, particularly fruits and vegetables which usually receive direct application of pesticides [5]. Despite their banning several years ago, the analysis of organochlorine pesticide residues are of special interest as their high chemical stability results in their persistence and bioaccumulation in the environment and animal tissues [6]. The European Union and the governments of its Member States have established maximum recommended limits (MRLs) for organochlorine pesticide residues in a variety of agricultural foods [7, 8]. These limits can be as low as $10\,\mu g\,kg^{-1}$ depending on the particular pesticide and sample type which make necessary high sensitive methods of analysis.

The determination of OCPs in vegetable samples usually comprises three steps: extraction, clean-up and chromatographic analysis [9]. Due to the low detection levels required by European Union regulations and the complex nature of the vegetable matrixes, efficient sample preparation and trace level detection are very important aspects [10]. This kind of analysis is also difficult because of the large concentration difference between the vegetable matrix components and the pesticides.

The aim of this work is the determination of 21 organochlorine pesticides in different horticultural samples purchased at several local markets in A Coruña, Spain using microwave assisted extraction (MAE) followed by a clean-up step with solid phase extraction (SPE). Vegetable samples were extracted. Finally organochlorine pesticides were determined by gas-chromatography with electron capture detection (GC-ECD) and confirmed by gas-chromatography with mass spectrometry detection (GC-MS).

2. Experimental

2.1. *Samples*

Five horticultural vegetables (lettuce, pepper, tomato, spinach and potato) were collected from seven local markets in A Coruña city, NW Spain, during February 2004. In order to select the sampling point's location a map of the city was divided in seven regular sectors and a market addressed in each sector was chosen.

A 1–2 kg sample of each vegetable was chopped and homogenized. An aliquot of about 100 g was weighted on an Erlenmeyer flask and freeze-dried. Then samples were ground in a mill and stored at room temperature in glass bottles in darkness until their analysis.

2.2. *Reagents*

2.2.1. Pesticide standards. A mix of organochlorine pesticides named "Appendix IX Organochlorine Pesticide Mix" containing: Aldrin; α-HCH; β-HCH; δ-HCH; Dieldrin; α-Endosulfan; β-Endosulfan; Endosulfan Sulfate; Endrin; Endrin Aldehyde; γ-HCH; Heptachlor; Heptachlor epoxide (isomer B); Methoxychlor; p,p'-DDD; p,p'-DDE and p,p'-DDT was obtained from Supelco (Bellefonte, PA, USA).

Individual standards of Endrin ketone, α-Chlordane, γ-Chlordane and 2,4,5,6-Tetrachloro-*m*-xylene (TCMX) were also obtained from Supelco. Isodrin was purchased from ChemService (West Chester, USA).

2.2.2. Solvents. Acetone and *n*-Hexane 95% were Super Purity Solvents from Romil (Cambridge, UK). Ethyl acetate (PAR) for instrumental analysis was from Panreac (Barcelona, Spain).

2.2.3. Sorbents. ENVI^{TM}-Carb of 100 m^2g^{-1} Packing 12 mL (1 g) was from Supelco (Bellefonte, PA, USA).

2.3. *Materials and apparatus*

Microwave extraction was carried out using a laboratory microwave oven (Anton Paar Multiwave, Graz, Austria) equipped with a built-in magnetic stirrer, a fiber-optic temperature sensor, a pressure sensor and a basic 6-position extraction rotor.

A rotary evaporator Büchi R-3000, (Büchi Labortechnic AG, Flawil, Switzerland) was used in the evaporation step. A Visiprep® vacuum distribution manifold from Supelco (Bellefonte, PA, USA) was employed in the purification step. An ultrasonic bath Branson 3200 (Energieweg, The Netherlands) was used.

The GC equipment consisted of a Perkin-Elmer (Norwalk, CT, USA) Autosystem XL chromatograph equipped with a ^{63}Ni electron capture detector (ECD), an auto-sampler, split–splitless injector, programmed pneumatic control and a computer running Turbochrom 4 data processor. For separation a J&W (Folsom, CA, USA) DB-35MS capillary column (30 m × 0.32 mm × 0.25 μm) was employed.

The GC-MS equipment consisted of a Trace 2000 GC coupled to a Thermo Finnigan (Austin, Texas, USA) Polaris-Q ion trap mass spectrometer detector (MS). The gas chromatograph is equipped with a PTV (programmed temperature vaporisation) injector and a J&W DB-XLB (60 m × 0.25 mm × 0.25 μm) capillary column.

2.4. *Sample preparation*

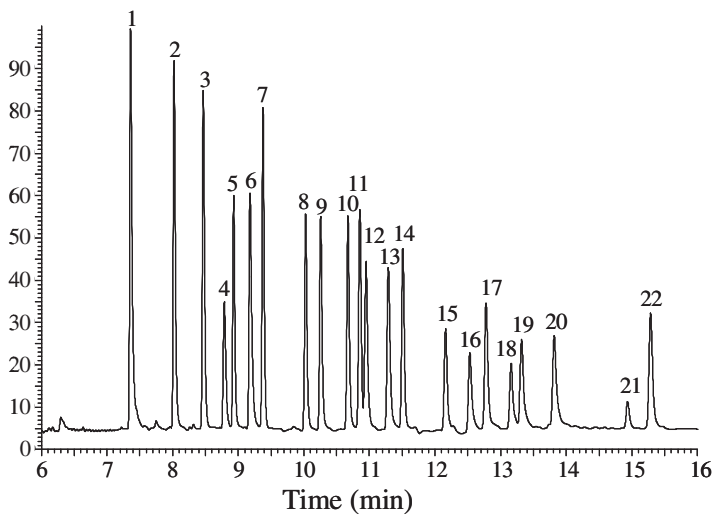
Horticultural samples (0.3 g) were weighed into an extraction vessel and extracted with 15 mL of hexane : acetone (50 : 50) with stirring using the following microwave program: 1 min ramp from 100 to 800 W, a 4 min hold at 800 W, 0 W for 2 min, 1 min ramp from 100 to 800 W, a 4 min hold at 800. After cooling, the vessel's content was filtered through 0.6 μm glass fibre filter MN GF-6 (Macherey Nagel, Düren, Germany) and the filtrate was concentrated to ca. 1 mL using a rotary evaporator.

SPE cartridges were connected to a Visiprep® vacuum distribution manifold. Previously to their use cartridges were washed with elution solvent and dried with nitrogen during 30 min. Then cartridges were loaded with concentrated extract and pesticides were eluted with 10 mL of hexane : ethyl acetate (80 : 20). Finally eluates were evaporated to a drop in rotary-evaporator and got to dryness by a gentle nitrogen stream. Once dissolved in 1 mL hexane, the solution was filtered through a syringe filter PTFE of 0.45 μm (Lida, Manufacturing Corp, Kenosha, WI) and pesticides were determined by gas chromatography and electron capture detection (GC-ECD).

2.5. *Chromatographic procedure*

2.5.1. GC-ECD conditions. Helium (99.999%) was used as carrier gas flowing at $1\,mL\,min^{-1}$. The oven temperature was programmed from 60°C (1 min) to 220°C at a rate of $25°C\,min^{-1}$, 220°C to 300°C at a rate of $6°C\,min^{-1}$. The temperature of the injector operating in splitless mode (volume injected 1 μL) was held at 300°C and electron capture detector temperature was 350°C. The detector auxiliary gas was nitrogen (99.999%) with a flow of $30\,mL\,min^{-1}$. Quantification was performed using TCMX as internal standard. Figure 1 shows a chromatogram of a standard solution containing $0.1\,mg\,L^{-1}$ of each pesticide injected under these chromatographic conditions.

2.5.2. GC-MS conditions. Transfer line temperature 290°C; ion source temperature 240°C and multiplier voltage 1500 V. A PTV (programmed temperature vaporization) injector operating in solvent-split mode was employed. The volume injected was 8 μL, split flow $20\,mL\,min^{-1}$, splitless time: 2.50 min, injection time: 0.50 min, injection flow: $20\,mL\,min^{-1}$. Injector temperature programme: 80°C increased at $3.3°C\,s^{-1}$ to 300°C (held for 15 min). The oven temperature program was: 80°C (1 min) to 180°C at $30°C\,min^{-1}$, 180°C (3 min) to 300°C (13 min) at $3°C\,min^{-1}$. Carrier gas flow: $1\,mL\,min^{-1}$ constant flow (He). Ion trap mass detection was operated in full scan mode from 50 to 450 amu.



Pesticides					
1 TCMX (2,4,5,6-tetrachloro-*m*-xylene)			11 α-Chlordane	15 Endrin	19 Endrin aldehyde
2 α-HCH	5 Heptachlor	8 Isodrin	12 α-Endosulfan	16 *p,p'*-DDD	20 Endosulfan sulfate
3 γ-HCH	6 δ-HCH	9 Heptachlorepoxide	13 *p,p'*-DDE	17 β-Endosulfan	21 Metoxichlor
4 β-HCH	7 Aldrin	10 γ-Chlordane	14 Dieldrin	18 *p,p'*-DDT	22 Endrin ketone

Figure 1. GC-ECD chromatogram of a standard solution with $0.1\,mg\,L^{-1}$of OCPs.

3. Results and discussion

MAE was carried out according to a method previously developed in our laboratory [11]. MAE was chosen as the extraction method as this technique offers advantages: it is amenable to automation, requires short extraction times, and reduces organic solvent consumption and costs of analysis [12]. However, lipid compounds as well as other molecules present in the samples are coextracted with the analysed pesticides so a clean-up step is recommended to diminish the presence of interferents in the final extract.

For purification step SPE with carbon (1 g) commercial cartridges was employed according to a previous study which compared four different sorbents (Florisil®, tandem Florisil® + alumina, silica gel and carbon) for the clean up in tree leaves [13]. This study showed that the efficiency of clean up was as follows: silica < florisil® < florisil® + alumina ≅ carbon; moreover carbon was the only one that gave colourless eluates. Furthermore the full scan GC-MS chromatograms corresponding to leaf extracts purified with the sorbents considered showed that carbon is the most efficient sorbent to remove other matrix compounds, which though present are not detected by the selective electron capture detector, avoiding deterioration of the chromatographic column.

Regarding linearity, linear calibration curves for all pesticides over six calibration levels, from 0.005 to 0.100 mg L^{-1} were constructed using TCMX as internal standard. The calibration curves were linear over the whole concentration tested for all the OCPs with correlation coefficients (R^2) ranging between 0.9926 for methoxychlor and 0.9968 for *p*,*p*′-DDE.

Limit of detection (LOD) and limit of quantitation (LOQ) were calculated at 3 and 10 times the standard deviation above the blank signal respectively. Table 1 shows these values in ng g^{-1} of freeze dried samples calculated with blank pepper extracts. Limits of detection ranged from 0.7 to 8.5 ng g^{-1}, and LOQ ranged from 6.2 to 44.1 ng g^{-1} for *p*,*p*′-DDE and methoxychlor respectively. These values show that this method is very useful in the control of pesticide residues in several vegetables at concentrations established by the EU in the Maximum Residue Levels (MRLs).

Differences in the plant material (such as water, fat and pigment contents) and the texture of the samples influence extraction and clean-up efficiencies [14]. Because a certified reference material was not available, a study of recoveries for each pesticide was carried out to assess the extraction efficiency of the proposed method. For that, four samples of each matrix were spiked with 1 mL of a standard solution with the organochlorine pesticides studied in a concentration of 0.05 mg L^{-1}, sonicated for 3 min and let stand for 17 min before extraction procedure. Figure 2 shows the GC-ECD chromatogram for a pepper sample unfortified and fortified with the target OCPs.

Average recovery data and relative standard deviations (RSDs) ($n = 4$) obtained are shown in table 2. In most cases analytical recoveries were in the range between 80 and 120%, and the RSD was lower than 15% in all cases except for endrin aldehyde in spinach. The recoveries obtained are comparable to those provided by existing methods for the determination of these compounds in horticultural samples [5, 15, 16]. As can be seen, analytical recovery is below 70% for endrin aldehyde in lettuce (53%) and especially for spinach (14%), which can be attributed to an interaction of this compound with these leafy vegetables. These results show that the sample matrix

Table 1. LOD and LOQ in $ng\,g^{-1}$ of freeze dried sample.

Pesticides	LOD	LOQ
α-HCH	2.3	6.4
γ-HCH	4.0	8.6
β-HCH	8.5	17.5
Heptachlor	4.8	12.6
δ-HCH	6.3	11.1
Aldrin	3.6	7.9
Isodrin	6.1	11.4
Heptachlor epoxide	6.5	11.7
γ-Chlordane	6.6	11.6
α-Chlordane	1.4	6.9
α-Endosulfan	5.9	11.3
p,p'-DDE	0.7	6.3
Dieldrin	4.0	9.4
Endrin	6.5	15.8
p,p'-DDD	5.3	13.9
β-Endosulfan	4.9	12.1
p,p'-DDT	2.4	15.4
Endrin aldehyde	4.9	13.1
Endosulfan sulfate	5.4	13.5
Methoxychlor	7.8	44.1
Endrin ketone	4.3	10.6

strongly affects the behaviour of these OCPs in the extraction and clean-up procedures used. Similar results have been reported by Obana *et al.* [17] who have obtained low recoveries for some pesticides in spinach, even though these pesticides are sufficiently recovered in other vegetables. Recovery of metoxychlor was over 130% in potato which can be attributed to the presence of this pesticide in the blank sample, although the direct analysis of this compound in the sample gave values under the detection limit and for this reason, this attempt could be not confirmed. According to Adou *et al.* [18] organochlorine pesticide recoveries higher than 100% might be caused by the sample matrix, which acts as a shield for the analyte molecules against loss in hot injectors ensuring a more complete transfer from injector to column compared to results obtained with sample free standard solutions.

The applicability of the method to the routine testing was assayed by the analysis of 35 samples (five horticultural species in the seven sampling points of A Coruña city). Only two samples analysed, tomato and pepper supplied from the market in point 4, contained residues of pesticides. Results were confirmed by GC-MS by its Full Scan mode and also by Selected Ion Monitoring (SIM) because Full Scan often does not provide enough sensitivity but SIM, which improves sensitivity, reduces considerably the qualitative information. Since SCAN mode is not sensitive enough at very low levels, extracts were concentrated 10 times prior to injection. In tomato three pesticides were found: α-Endosulfan ($18.2\,\mu g\,kg^{-1}$), β-Endosulfan ($28.2\,\mu g\,kg^{-1}$) and Endosulfan Sulfate ($19.7\,\mu g\,kg^{-1}$); in pepper only α-Endosulfan was found ($28.2\,\mu g\,kg^{-1}$). For positive GC–MS confirmation both retention time (rt) and m/z were used (α-Endosulfan, rt 17.15 min, m/z 195; β-Endosulfan rt 20.20 min, m/z 195; Endosulfan Sulfate, rt 22.47 min, m/z 229). Maximum recommended limit (MRLs) established by EU for the sum of these three organochlorine pesticides is $1\,mg\,kg^{-1}$, therefore these samples are within the limits established by European and Spanish legislations.

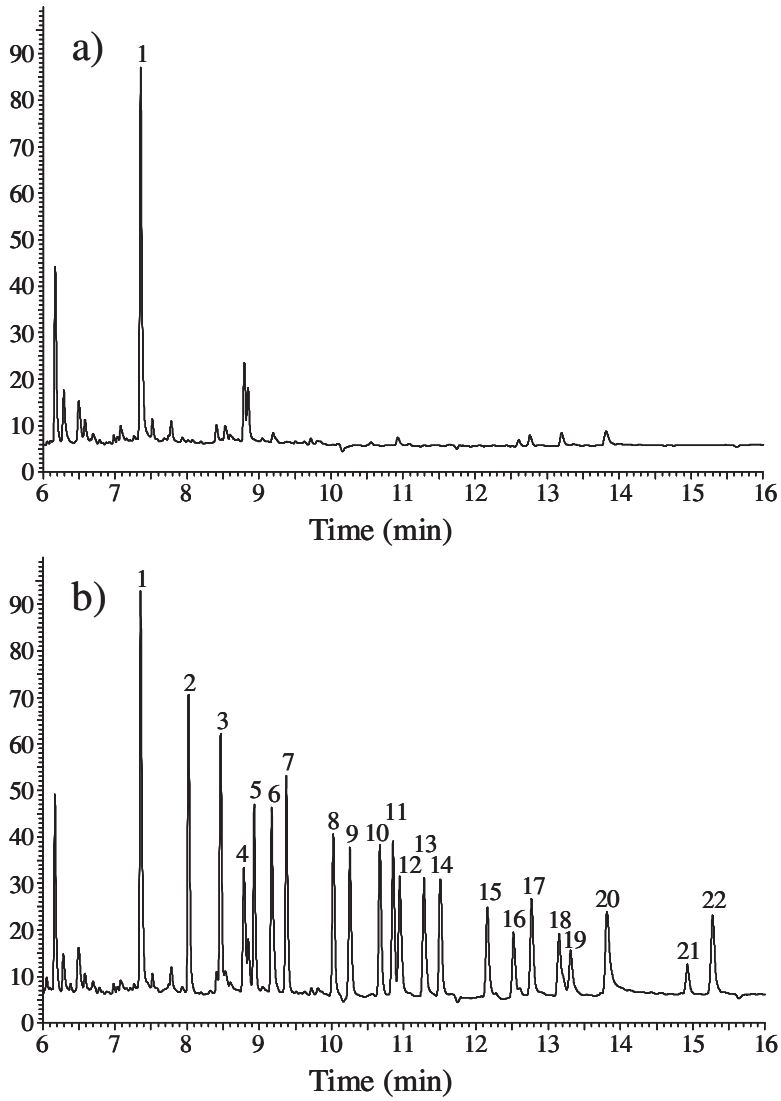


Figure 2. (a) Pepper extract chromatogram. (b) Spiked pepper extract chromatogram. Spiked level: $0.17\,\mu g\,g^{-1}$. Peak numbers as in figure 1.

4. Conclusions

A rapid and useful method for the analysis of 21 organochlorine pesticides from horticultural samples has been outlined. This method is based on MAE, and SPE clean-up of the extracts prior to GC-ECD identification and quantification. Confirmation with GC-MS was taken into account.

The method was validated for different matrices. This method has shown suitable precision, accuracy and sensitivity for monitoring of pesticide residues in vegetables.

Table 2. Analytical recoveries (%) and RSD (%) of OCPs added to horticultural samples* ($n=4$).

	Lettuce		Pepper		Tomato		Spinach		Potato	
Pesticides	%R	RSD	%R	RSD	%R	RSD	%R	RSD	%R	RSD
α-HCH	89	8	97	7	99	3	87	3	92	2
γ-HCH	82	9	100	6	92	3	81	4	93	5
β-HCH	95	12	118	11	104	13	108	15	112	9
Heptachlor	90	7	106	7	106	4	109	12	110	2
δ-HCH	88	8	97	6	93	3	89	2	86	3
Aldrin	85	5	94	4	94	3	74	8	91	3
Isodrin	92	2	108	5	102	3	86	3	94	4
Heptachlor epoxide	90	4	106	4	103	4	86	5	96	4
γ-Chlordane	94	5	104	5	108	5	91	2	101	5
α-Chlordane	93	5	102	5	106	4	84	2	95	5
α-Endosulfan	91	5	94	5	108	5	89	3	97	4
p,p'-DDE	91	10	110	5	110	5	99	7	110	3
Dieldrin	104	7	106	7	112	6	101	4	113	5
Endrin	111	4	120	4	115	7	120	7	120	4
p,p'-DDD	89	10	107	5	99	4	114	6	117	3
β-Endosulfan	88	6	99	5	104	3	89	4	99	4
p,p'-DDT	129	7	107	6	132	4	98	10	99	6
Endrin aldehyde	53	13	84	13	95	5	14	20	86	9
Endosulfan sulfate	88	5	116	3	104	5	80	9	87	8
Methoxychlor	116	9	124	8	117	10	130	3	143	6
Endrin ketone	100	8	113	4	112	6	107	7	114	4

*Spiked level: 0.17 $\mu g\,g^{-1}$ in freeze dried samples.

The applicability of the method to routine analysis was tested in real samples with good results. Only two samples contained residues of pesticides but none of them exceeded the MRLs established by legislation of Spain and EU.

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