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Microwave-assisted extraction versus Soxhlet extraction in the analysis of 21 organochlorine pesticides in plants ☆

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Abstract

A method to determine 21 organochlorine pesticides in vegetation samples using microwave-assisted extraction (MAE) is described and compared with Soxhlet extraction. Samples were extracted with hexane–acetone (1:1, v/v) and the extracts were cleaned using solid-phase extraction with Florisil and alumine as adsorbents. Pesticides were eluted with hexane–ethyl acetate (80:20, v/v) and determined by gas chromatography and electron-capture detection. Recoveries obtained (75.5–132.7% for Soxhlet extraction and 81.5–108.4% for MAE) show that both methods are suitable for the determination of chlorinated pesticides in vegetation samples. The method using microwave energy was applied to grass samples from parks of A Coruña (N.W. Spain) and to vegetation from the contaminated industrial area of Torneiros (Pontevedra, N.W. Spain).

Keywords: Extraction methods; Microwave-assisted extraction; Plant materials; Environmental analysis; Pesticides; Organochlorine compounds

1. Introduction

Organochlorine pesticides (OCPs) are an important group of environmental contaminants. When pesticides are released into the environmental they may be broken down, or they may resist degradation and thus remain unchanged in the environment for long periods of time. They have been particularly effective in the control of pests and diseases but their resistance to degradation has resulted in their being universal contaminants in water, soil and also in foods. Their high toxicity has made their use very restrictive reaching the point of being forbidden in most countries [1,2]. OCPs are compounds with a very low solubility in water and because of their lipid character they can not be metabolised by organism accumulating in fats, often becoming more concentrated as they move up the food chain [2,3].

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These compounds are known of inducing or aggravating certain health problems in humans such as cancer, immune systems and the disruption of hormonal functions [3,4].

Once released into the environment, they are widely distributed in all compartments such as atmosphere, groundwater, soil, sediment, surface water and living tissues. The analysis of vegetation samples is of great interest since contaminant levels in vegetation samples can be used as indicators of environmental pollution as plants can suffer adhesion and absorption of compounds from soil and deposition and absorption of volatile compounds from atmosphere [5]. Furthermore, plants can be employed in bioremediation processes and so can be used for decontamination of polluted sites. On the other hand, animal and human diet is based in a wide variety of vegetables which begin the introduction of OCPs in living organisms.

A large number of multiresidue extraction methods has been developed in the last 20 years. The more frequently used methods employ solvent extraction and gas chromatography (GC) with selective and sensitive detection. The more widely used extraction techniques for pesticides in plants are Soxhlet [6], shake-flask [7], sonication [8,9], and more recently, supercritical fluid extraction (SFE) [10], accelerated solvent extraction (ASE) [11] and microwave-assisted extraction (MAE) [12]. MAE offers many advantages over conventional classical extraction techniques such as shortened extraction times and lower consumption of solvents; furthermore, stirring is possible in some microwave ovens and it makes the extraction conditions more homogeneous.

Although Soxhlet extraction is a traditional extraction technique which presents many disadvantages, it has been widely used for organochlorine pesticides and provides efficient extractions which allows its employment as reference method. The purpose of this work was to develop a method to determine 21 organochlorine pesticides in vegetation samples using microwave energy and compare the obtained results with Soxhlet extraction. Though comparisons between MAE and Soxhlet extraction for pesticides has been published in different matrixes, vegetation is one of the less studied using microwave energy.

2. Material and methods

2.1. Samples

Samples were collected from two different areas: (a) grass samples collected in parks in A Coruña city (N.W. Spain). Samples were lyophilised, ground and conserved in glass recipients out of light exposure before their analysis. (b) Wild samples collected in the industrial area of Torneiros (Pontevedra, N.W. Spain) involved in the production of lindane between 1947 and 1964. Samples were washed with distilled water in an ultrasonic bath and then dried in an air oven at ambient temperature.

2.2. Reagents

A mix of organochlorine pesticides named ‘‘Appendix IX Organochlorine Pesticide Mix’’ containing: aldrin, α-hexachlorocyclohexane (α-HCH), β-hexachlorocyclohexane (β-HCH), δ-hexachlorocyclohexane (δ-HCH), dieldrin, α-endosulfan, β-endosulfan, endosulfan sulfate, endrin, endrin aldehyde, γ-hexachlorocyclohexane (γ-HCH), heptachlor, heptachlor epoxide (isomer B), methoxychlor; *p,p′*-DDD; *p,p′*-DDE and *p,p′*-DDT were 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, NY, USA). Acetone, *n*-hexane 95%, dichloromethane and methanol 205 gradient quality were super purity solvents from Romil (Cambridge, UK). Ethyl acetate (PAR) for instrumental analysis was from Panreac (Barcelona, Spain).

2.3. Apparatus

Soxhlet and thermostatic bath (Precis-Bat S-147-200) from Selecta were used. 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 six-position extraction rotor. Rotary evaporator Büchi R-3000 (Büchi, Switzerland) was used in the evaporation

step and Visiprep vacuum distribution manifold from Supelco was employed in the purification.

The GC equipment consisted of a Perkin-Elmer (Norwalk, CT, USA) Autosystem XL chromatograph equipped with a ^{63}Ni electron-capture detection (ECD) system, an autosampler, split-splitless injector, PPC (programmed pneumatic control) and a computer running Turbochrom 4 data processor. For separation a 5% diphenyl 95% dimethyl siloxane capillary column (30 m×0.25 mm×0.25 μm) TBR-5-Tracer (Tracer Analitica, Spain) was employed.

2.4. Sample preparation

2.4.1. Extraction

For the analysis 0.3 g of lyophilised grass sample were spiked with 1 ml of a standard solution with the organochlorine pesticides studied in a concentration of 0.1 mg/l, sonicated for 3 min and let stay 17 min before extraction procedure.

2.4.2. Soxhlet extraction

Sample was introduced into a glass fibre filter and extracted in a Soxhlet equipment with 80 ml of hexane–acetone (1:1, v/v) during 20 h. After cooling, the extract was concentrated to 1 ml using a rotary evaporator.

2.4.3. Microwave extraction

Sample was weighed into an extraction vessel and 15 ml of hexane–acetone (1:1, v/v) were added. After adding a magnetic stir bar the vessels were stopped and placed in the rotor. The vegetation samples were extracted 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 W. After cooling, the vessel contents were filtered through 0.6-μm glass fibre filter MN GF-6 (Macherey Nagel, Düren, Germany) and the filtrate was concentrated to 1 ml using a rotary evaporator.

2.4.4. Clean-up

In both cases the extraction process was followed by a clean-up step using solid-phase extraction with florisil and alumine as adsorbents.

Alumine was pretreated before its use. Soxhlet extraction of alumine was performed with dichoromethane–methanol (2:1, v/v) during 12 h and then another 12 h with dichloromethane–hexane (30:70, v/v). Once dried, alumine was activated at 350 °C for 12 h and further deactivated with Milli-Q water (10%). Then 2 g alumine was added to a Florisil Sep-Pak cartridge (5 g) (Waters, Milford, MA, USA) connected to a Visiprep vacuum distribution manifold. Previously to its use the cartridge was washed with the elution solvent and air dried during 30 min. Then the cartridges were loaded with the concentrated extract and pesticides were eluted with 35 ml of hexane–ethyl acetate (80:20). Finally the eluate was evaporated to a drop in rotary-evaporator and to dryness by a gentle nitrogen stream. Once dissolved in hexane, the solution was filtered through a syringe filter PTFE of 0.45 μm (Lida, Kenosha, WI, USA) and pesticides were determined by GC–ECD.

2.5. Chromatographic procedure

Helium (99.999%) was used as carrier gas flowing at 1.2 ml/min. The oven temperature was programmed from 60 °C (1 min) to 180 °C at a rate of 30 °C/min, 180 °C (3 min) to 300 °C at a rate of 3 °C/min. 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%). Quantification was performed using TCMX as internal standard. Under these chromatographic conditions α-chlordane and α-endosulfan coeluted so they were quantified together.

3. Results and discussion

3.1. Extraction

The solvent selected for the extraction was hexane–acetone (1:1, v/v) because this mixture is one of the more adequate for the extraction of pesticides due to good recoveries are obtained and also the time consumed in the evaporation step is much lower with this one than with other solvents [13].

For Soxhlet extraction three materials were assayed to contain sample during extraction: cellulose cartridge, cellulose filter and glass fibre filter. Previ-

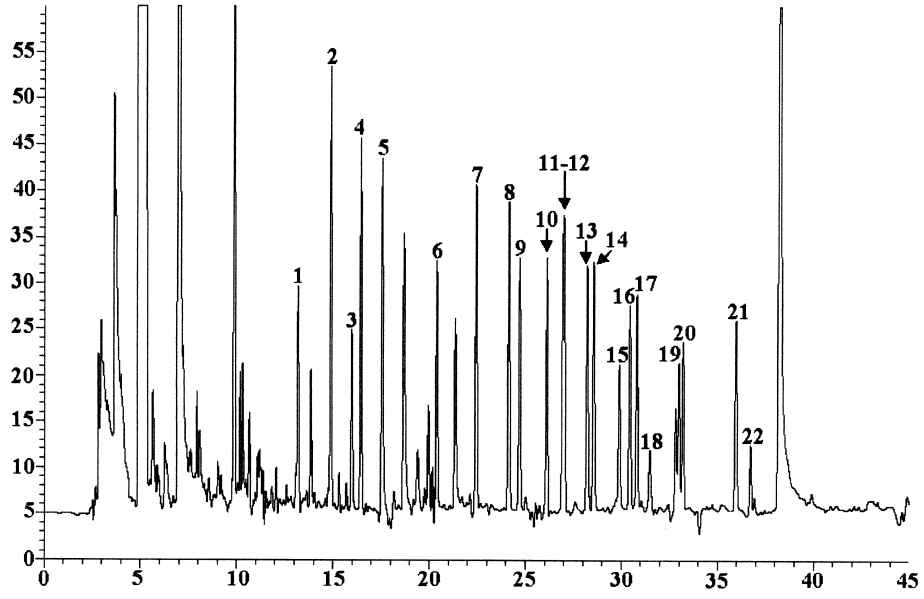
ously to its use, the three materials were washed in the Soxhlet extractor with hexane–acetone (1:1, v/v) during 8 h. The chromatographic injection of the three extracts showed that glass fibre filter gave cleanest blanks, so it was selected for containing sample during Soxhlet extraction.

The use of microwave energy to heat the solvent/sample can produce degradation or conversion of compounds by different ways such us exposure to the temperature and pressure inside the microwave extraction vessel, interaction with other analytes under these conditions and influence by the matrix. Earlier studies on the extractability of OCPs in soils under several microwave-assisted extraction conditions have showed that temperature has the most significant effect on the extraction of OCPs from soils followed by the matrix [14].

Extraction using microwave energy was carried out employing the cited microwave power program according to previous studies in our laboratory [15], with two ramps separated by a ventilation step to avoid an overheating of the system. Temperatures measured during extraction showed that 95–107 °C are achieved into vessels; these temperatures are adequate for the determination of OCPs which are stable under irradiation with microwave energy within 50–145 °C [14].

3.2. Clean up

As vegetation is a very complex matrix a clean up step is recommended to decrease the presence of interferents in the final extract and also to avoid the deterioration of the chromatographic column. Florisil was chosen as adsorbent because it has been often recommended in the residue analysis of fruit and vegetables and it has been used for the clean-up of plant extracts by many authors [5,8]; preliminary experiments were carried out using a SepPak cartridge containing 1 g of Florisil, but this amount of sorbent material was not enough for the cleaning of the extract so finally a SepPak cartridge containing 5 g of Florisil was employed. Alumine was employed as additional adsorbent because it is par-



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

Fig. 1. Chromatogram of fortified grass sample obtained using Soxhlet extraction.

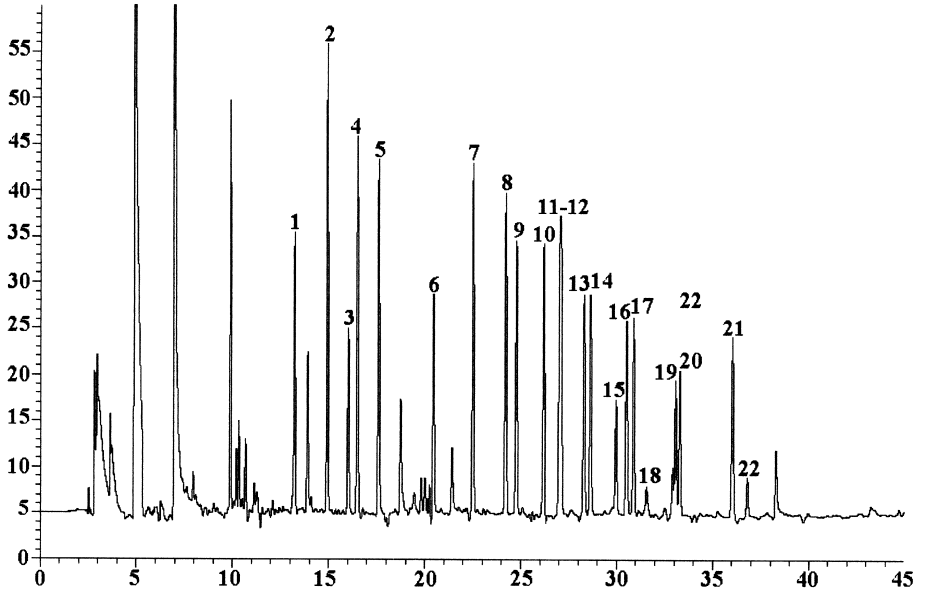


Fig. 2. Chromatogram of fortified grass sample obtained using MAE.

Table 1
Recovery studies for OCPs by Soxhlet procedure and by MAE (fortification level 0.33 μg/g lyophilised grass sample)

Pesticide	Soxhlet extraction		MAE	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
α-HCH	95	6.1	94	6.1
β-HCH	93	5.1	101	3.4
γ-HCH	99	4.1	98	1.3
δ-HCH	116	6.4	108	3.6
Heptachlor	109	1.8	94	3.7
Aldrin	86	3.6	92	2.2
Isodrin	88	5.0	92	5.9
Heptachlor epoxide	82	4.6	92	6.2
γ-Chlordane	76	8.4	90	3.9
α-Chlordane+α-endosulfan	86	3.0	89	6.4
p,p′-DDE	82	4.2	91	5.5
Dieldrin	99	5.9	101	7.2
Endrin	121	4.2	101	6.4
β-Endosulfan	98	5.7	96	6.8
p,p′-DDD	132	5.2	105	7.2
Endrin aldehyde	51	15.5	50	8.4
p,p′-DDT	113	5.1	103	6.0
Endosulfan sulfate	121	9.0	96	4.8
Endrin ketone	115.6	4.0	82	1.8
Methoxychlor	106.0	14.7	99	13.4

ticularly indicated for retention of waxes and other lipid compounds [16]. The order of these adsorbents (alumina at the top and Florisil at the bottom) is important; alumina retains the more polar interfering materials and Florisil the less polar materials, thus large amounts of interfering material are trapped at the top of the cartridge rather than the bottom, so the risk of co-extracting impurities is low. In fact, the use of a single step clean-up on the two adsorbents diminished the presence of peaks due to the matrix in comparison when only Florisil was used as adsorbent.

3.3. Recovery studies

Figs. 1 and 2 show the chromatograms of grass sample extracted using Soxhlet extraction and MAE, respectively. It can be seen that MAE resulted in cleaner chromatograms.

Recovery studies were taken account with grass samples because grass is widely distributed and so it is easy to obtain. Analytical recoveries were evaluated by spiking three samples (0.33 μg/g) and then analysed in duplicate. The recovery values as well as the RSD expressed as % for both methods are shown in Table 1. The recoveries obtained (75.5–132.4% for Soxhlet extraction and 81.5–108.4% for MAE) show that both methods are suitable for the determination of chlorinated pesticides in vegetation samples except for endrin aldehyde. However, as Soxhlet extraction is more laborious and requires higher solvent consumption and longer extraction time, MAE is the most recommended of both techniques.

The low recovery of endrin aldehyde in both cases is due to this pesticide is strongly retained by alumina deactivated with 10% water because when only Florisil is used as adsorbent the recovery obtained for this pesticide is satisfactory (91.7%). In order to know the role of the rate of deactivation of alumina on the recovery of endrin aldehyde other rates of deactivation (5 and 20%) were assayed. Our results indicated that the rate of deactivation of alumina does not affect the recovery of the rest of pesticides but the recovery of endrin aldehyde is significantly decreased when alumina deactivated with 5% water is used (18.6%) and significantly improved when alumina deactivated with 20% water is employed (88.7%).

The 20% weathered alumina gives better results for endrin aldehyde without degrading method performance for the rest of pesticides. However, the 20% weathered alumina did not clean up efficiently the extracts which is necessary to avoid the deterioration of the chromatographic system. In fact, the presence of peaks due to the matrix was increased using the 20% weathered alumina which resulted in dirtier chromatograms. However in specific analysis in which endrin aldehyde is an analyte of special interest a 20% weathered alumina can be used.

3.4. Samples

The method using microwave energy was applied to analyse grass samples from two parks of A Coruña city. These samples did not contain pesticides residues at detectable concentrations.

This method was also applied to samples collected in an industrial area where high concentrations of pesticides could be found, so a second level of fortification was carried out. The second spiked level

Table 2
Recovery values for OCPs obtained by MAE (fortification level 5 μg/g lyophilised grass sample)

Pesticide	MAE	
	Recovery (%)	RSD (%)
α-HCH	103	2.5
β-HCH	93	4.7
γ-HCH	103	2.4
δ-HCH	102	2.0
Heptachlor	98	1.1
Aldrin	93	2.8
Isodrin	98	2.0
Heptachlor epoxide	96	4.0
γ-Chlordane	98	1.3
α-Chlordane+α-endosulfan	97	1.1
p,p′-DDE	104	1.4
Dieldrin	96	1.0
Endrin	100	5.4
β-Endosulfan	92	0.7
p,p′-DDD	104	2.6
Endrin aldehyde	56	7.1
p,p′-DDT	94	5.4
Endosulfan sulfate	99	4.0
Endrin ketone	86	3.2
Methoxychlor	108	13.6

Table 3
HCH content (mg/kg dry sample) of vegetation samples from Torneiros (NW Spain)

Sample	α-HCH	β-HCH	γ-HCH	δ-HCH
Rubus ulmifolius (leaf)	8.27	7.62	0.56	0.51
Paranthropophytia (leaf)	7.21	12.7	0.64	0.46
Rubus ulmifolius (root)	1.37	2.20	0.06	0.07
Paranthropophytia (root)	2.44	7.97	0.14	0.15

assayed was 5 μg/g lyophilised grass sample. The recoveries obtained, evaluated by spiking three samples and then analysed in duplicate, were satisfactory ranging from 85.7 to 107.7% as it is shown in Table 2, except for endrin aldehyde as it occurs with the first spiked level studied.

Roots and leaves of wild plants (*Rubus ulmifolius* and *Paranthropophytia*) were analysed. The clean up procedure of the concentrated extracts was adequate independently of the type of plant and part of the plant analysed. All samples contained high levels of HCHs isomers ranging from 4.87 to 21.0 mg/kg dry sample expressed as total content of HCH; none of the other pesticides studied were found in these samples. The distribution of each HCH isomer in each species and part of plant is shown in Table 3. Regardless of the species and part of the plant the results obtained show that the predominant isomers were β-HCH and α-HCH in all samples whereas δ-HCH and γ-HCH were present at lower levels. The proportion of each isomer in technical lindane (67–70% of α, 5–6% of β, 13% of γ and 6% of δ) can explain the high levels of α and the low levels of γ and δ, while that the high values of β can be attributable, as well as its physicochemical properties, to this isomer is the only one that can not be degraded aerobically [17]. Regarding to the part of the plant, level of contamination is higher in leaves than in roots. This can be explained due to atmospheric deposition and because of the green parts of higher plants are covered by a hydrophobic epicuticular wax that sorb hydrophobic compounds from the surrounding air [18].

4. Conclusions

MAE has been shown as a powerful technique for the extraction of organochlorine pesticides in vegetation samples with clear advantages versus Soxhlet extraction such us shorter extraction time and lower solvent consumption. Furthermore, the recoveries obtained with the described procedure are comparable to or better than those provided for the determination of OCPs in vegetation samples.

The combination of Florisil and alumina deactivated with 10% water for the clean-up step resulted in cleaner chromatograms than when only Florisil was used as adsorbent, but it has the disadvantage that endrin aldehyde is not quantitatively eluted from alumina. On the other hand, the rate of deactivation of alumina does not affect the recovery of the rest of pesticides but it plays an important role on the recovery of endrin aldehyde and also on the clean-up of the vegetation extracts.

The extraction and clean up procedures developed are satisfactory for different plants and plant materials, and they can be also applied to a wide range of concentrations of organochlorine pesticides.

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