Microwave-assisted extraction through an aqueous medium and simultaneous cleanup by partition on hexane for determining pesticides in agricultural soils by gas chromatography: A critical study

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Abstract

A simple microwave-assisted extraction and partitioning method (MAEP) using water–acetonitrile and *n*-hexane for desorption and simultaneous partitioning, respectively, together with gas chromatography (GC) was studied to determine representative pesticides (trifluralin, metolachlor, chlorpyriphos and triadimefon) with a broad range of physico-chemical properties in agricultural soils. Three points were considered crucial in this study: instrumental and sample-associated factors affecting extraction of the target compounds were studied through experimental design; the spiking procedure at trace levels was carried out to reproduce the solute-soil sorption taking place in the environment as closely as possible; and results were analyzed taking into account the adsorption behaviour of the compounds on different kinds of soils. The complete analytical procedure proposed consisted of the MAEP of pesticides from 1.0 g of soil with 1 mL of 1:1 water/acetonitrile mixture, and 5 mL of hexane for trapping. The microwave heating program applied was 2 min at 250 W and 10 min at 900 W, and 130 °C maximum temperature. After extraction, the hexane layer was evaporated to dryness; the residue was re-dissolved and directly analyzed by gas chromatography electron capture detection (GC-ECD). Clean chromatograms were obtained without any additional cleanup step. Besides the four pesticides used to optimise MAEP, the method was applied to determine an additional group of pesticides (triallate, acetochlor, alachlor, endosulphan I and II, endrin, methoxychlor and tetradifon) in different soils. Most of the compounds studied were recovered in good yields with relative standard deviations (R.S.D.s) below 9% and detection limits ranged from 0.004 to 0.036 $\mu g\,g^{-1}$. The described method is efficient and fast to determine hydrophobic pesticides at $ng\,g^{-1}$ level in soil with different clay-to-organic matter ratios.

Keywords: Microwave-assisted extraction; Water–hexane partition; Pesticides; Soils

1. Introduction

The ubiquitous presence of pesticides as environmental contaminants has created concern about their fate and transport in natural waters, sediments and soils. Soils function as chemical and biological filters that diminish the environmental impact of pesticides introduced into the biosphere by design or accident. Thus, monitoring of these compounds in soils with intensive agriculture is clearly necessary. However, because of the lipophilic properties of some compounds and the complexity of

this kind of matrix, analytical procedures to determine pesticides in soils are usually tedious and time-consuming, especially at the extraction stage.

For solid samples, Soxhlet extraction (SE) followed by cleanup of the extracts by solid phase extraction is a common analytical method and has reached official status in many countries (AOAC) [1]. This extraction technique requires refluxing large volumes of solvent through the sample for several hours. Newer extraction techniques developed for environmental analysis of organic contaminants are mainly instrumental and include supercritical fluid extraction (SFE), sub-critical water extraction (SBWE), pressurised liquid extraction (PLE) and microwave-assisted extraction (MAE) [2–4]. SFE and SBWE are selective and not solvent-consuming techniques; however,

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for SFE, a relatively complex and expensive system is needed. On the other hand, PLE and MAE require a small amount of solvent, short extraction time and, pressurised MAE, permitting the simultaneous extraction of several samples. Nevertheless, because of its low selectivity, the main drawback of this extraction technique is the need of a cleanup procedure to determine pesticides in the final extracts. The use of non-polar solvents reduces the co-extraction effect, allowing the determination of a large number of pesticides by gas chromatography coupled with different detection systems. However, according to the principles of microwave energy transfer, the solvent must possess a dipole; thus, non-polar solvents would not be the most suitable for microwave extraction. To overcome this disadvantage in MAE, water could be used as the extracting solvent, since at high-pressure and temperature its dielectric constant, viscosity, and surface tension become reduced, these facts making it effective to extract organic compounds having a broad spectrum of polarities from solid samples. On comparing water, methanol, acetone–hexane and dichloromethane, water has turned out to be as efficient as organic solvents to extract triazines from soil samples in MAE at 600 W [5]. Water capability to extract polar and medium polar compounds under pressure and high temperature has been applied in PLE on aged spiked soils through hot phosphate buffered water [6]. Sub-critical water extraction in a hot-water percolation setup has been proposed for the extraction of six selected pesticides from forest soils; recoveries ranged from 44% to 95% depending on the compound solubility in water [7]. Although water is a good alternative in MAE and PLE, the necessary cleanup procedure to eliminate co-extracted polar interferences from soils, the non-compatibility of extract with GC techniques, and the low extraction efficiency in the case of less hydrophilic compounds are the principal limitations.

To overcome these limitations we propose MAE of low water-soluble pesticides (trifluralin, chlorpyriphos, triadimefon and metolachlor) from agricultural soils using an aqueous phase containing an organic modifier (acetonitrile) for desorption and *n*-hexane for simultaneous partitioning and cleanup (MAEP method). Factors affecting extraction of the target compounds from soils were studied and optimised by using experimental designs. Moreover, in order to reproduce the solute-soil adsorption occurring in the environment as closely as possible, special attention was paid to the spiking procedure used in the recovery studies. Thus, soils samples were contaminated through a batch equilibrium method with aqueous solution to ensure a whole pesticide sorption process. Fortified soil samples with different physico-chemical properties were used to verify the applicability of the method to different types of soils. Ageing effects in the sorption process were also considered of particular concern. The developed MAEP and GC-ECD method was applied to determine a wide range of pesticides in agricultural soils and compared with an ultrasound-based extraction method using ethyl acetate as extracting solvent. The MAEP method has the advantages resulting from the use of a low volume of organic solvent – an unnecessary cleanup step – and good efficiency to extract pesticides actually adsorbed on different soils at residual levels.

2. Experimental

2.1. Chemicals and reagents

The pesticides used had purity $\geq 99\%$ (Pestanal®, Riedel de Häen and ChemService). All the solvents used were residue analysis grade (Fisher). Water was purified with a NANOPure system (Barnstead Thermolyne). Pentachloronitrobenzene (PCNB Aldrich) was used as internal standard for GC determinations. Stock solutions were prepared in acetone at $1\,g\,L^{-1}$, except for PCNB, which was prepared in hexane. Working standard solutions for GC analysis were diluted with hexane and with water for spiking purposes.

2.2. Equipment and experimental GC conditions

For MAEP, a Milestone MLS 1200 MEGA high-pressure microwave oven extraction system was used, equipped with pressure and temperature sensors and an exhaust module EM-45/A.

GC-ECD. A Hewlett Packard 5890 Series II gas chromatograph equipped with split/splitless injector, an ECD and HP 3395 integrator were employed. An HP-5 capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness) was used. Helium and nitrogen were selected as carrier and auxiliary gas, respectively. Halogenated pesticides were separated and determined under the following conditions: injector temperature, 250 °C; detector temperature, 300 °C; column temperature program, 170 °C, held for 1 min, increased at 2.5° min^{-1} up to 210 °C, and increased at 15° min^{-1} up to 260 °C, held for 5 min. A 1 μL volume of the extract was injected in the split mode in ECD (split ratio 1:30). The carrier gas flow in the column was 1.3 mL min^{-1}. Under these conditions, the mixture of 12 pesticides (those used for MAEP optimisation and a group of eight additional halogenated pesticides) and the internal standard was well resolved in 25 min using the oven temperature program.

2.3. Soil samples for sorption

Recovery and optimisation studies were carried out using sieved samples (2 mm mesh) of six soils with diverse physico-chemical properties, collected (0–20 cm depth) in different agricultural zones in Chile: three non-allophanic soils, two from the Aconcagua Valley (V Region of Chile), Pocuro (PCR) and Quillota (QTA) and one from an apple orchard in the Cachapoal Valley (VI Region of Chile), Rancagua (RGA) and three allophanic soils from the Araucanía Region (IX Region of Chile), Vilcún (VLC), Temuco (TEM) and Galvarino (GLV). Some of the most relevant physical and chemical properties of these soils are given in Table 1.

2.4. Preparation of spiked soil samples

In the preliminary studies performed to evaluate time and temperature effects and the presence of acetonitrile in the aqueous phase on MAEP efficiency, freshly spiked QTA soil was used owing to its medium content of organic matter. For this

Table 1
Physico-chemical characteristic of soils

Soil	OM (%)	pH	% Clay	% Silt	% Sand	% Clay/% OM	Texture
TEM	12.0	6.3	22.6	58.6	18.8	1.9	Silty clay loam
VLC	13.4	6.3	32.7	33.0	34.3	2.4	Clay loam
QTA	3.1	7.4	15.8	54.1	30.1	5.1	Silt loam
RGA	1.9	6.9	15.7	44.9	39.4	8.3	Loam
PCR	1.8	7.2	17.5	50,7	31.8	9.7	Silt loam
GLV	4.0	5.6	41.7	43.9	14.3	10.4	Silty clay

Compound	Solubility ($mg\,L^{-1}$)	$1/n$	K_f ($cm^3\,g^{-1}$)
Trifluralin	0.75	0.69	52.2
Chlorpyriphos	1.2	0.87	48.8
Triadimefon	260	0.64	7.45
Metolachlor	530	0.72	4.26

Water solubility and Freundlich parameters for the adsorption of compounds on QTA soil.

purpose, 1 g of soil was weighed in a tetrafluoromethaxil (TFM) microwave extraction vessel with addition of 1 mL of an aqueous spiking solution containing trifluralin, chlorpyriphos and triadimefon ($2\,\mu g\,mL^{-1}$) and metolachlor ($8\,\mu g\,mL^{-1}$). Samples were equilibrated by shaking for 2 h and standing overnight at 4 °C before microwave extraction.

A second spiking procedure was carried out to obtain dry spiked soil used to evaluate the effect of sample-associated factors (soil properties, ageing time, and mass) on the extraction efficiency and to optimise the factors associated with the MAEP method. To this end, three soils were selected (PCR, QTA, VLC) and spiked according to the following procedure: 20 g were equilibrated with 10 mL of an aqueous standard solution at a proper concentration to obtain $0.2\,\mu g\,g^{-1}$ for trifluralin, chlorpyriphos and triadimefon and $0.8\,\mu g\,g^{-1}$ for metolachlor. The mixture was shaken for 2 h and subsequently dried at 30 °C overnight, homogenised by grinding with a mortar and pestle and stored at −20 °C until microwave extraction (2, 16 and 30 days after this procedure). The residual moisture content was also determined by drying a soil spiked sample at 105 °C overnight. In order to optimise factors associated with the MAEP method, a similar spiking procedure was carried out using QTA soil spiked at $0.1\,\mu g\,g^{-1}$ for the target compounds ($0.4\,\mu g\,g^{-1}$ for metolachlor). Finally, all soils extracted under optimal MAEP conditions were also spiked with this procedure.

2.5. Experimental designs

Two full factorial designs with four central points were developed to evaluate the effect of instrumental and sample-associated factors. An additional central composite design, comprising a 2^3 full factorial design, six added axial points (faced centered), and four central points, was applied to optimise the MAEP-associated factors through the evaluation of response surfaces. Factors and corresponding boundary levels associated to the sample and those optimised through central composite design are listed in Tables 2 and 3, respectively. Statistical software (Statgraphics Plus v 5.1 for Windows, Rockville, MD) was used to build the experimental design and to analyse the data from the experimental values.

2.6. Microwave-assisted extraction and partitioning method (MAEP)

Aliquots of spiked soil were weighed and transferred to a TFM microwave extraction vessel. Subsequently, the extraction solution (water–acetonitrile) was added in 1:1 sample-to-solvent ratio and the sample was manually shaken for homogenisation. Then, 5 mL of hexane was added over the soaked soil for partitioning, TFM vessels were covered with pressure-resistant holders, and preheated for 2 min at 250 W and then at 900 W, using the microwave oven system (which allows the simultaneous heating of six vessels). An optic-fiber probe inside the monitoring cell was used to control temperature. After microwave irradiation, vessels were water-cooled, opened, and the hexane was carefully transferred with a Pasteur pipette to 10 mL conic-bottom tubes, rinsing the inner wall with a small amount of hexane. The

Table 2
Design matrix and recovery values of pesticides for the factorial design to study the effect of sample-associated factors on MAEP efficiency

Factor	Level		
	−1	0	+1
A: type of soil	PCR	QTA	VLC
B: mass of soil (g)[a]	1	2	3
C: ageing time (days)	2	16	30

Run	A	B	C	% Recovery			
				Trifluralin	Metolachlor	Chlorpyriphos	Triadimefon
1	−	−	−	62	103	62	98
2	+	−	−	66	92	95	82
3	−	+	−	73	72	21	55
4	+	+	−	78	86	51	54
5	−	−	+	65	97	52	85
6	+	−	+	62	91	92	76
7	−	+	+	51	70	23	46
8	+	+	+	62	70	53	40
9	0	0	0	50	74	64	59
10	0	0	0	54	83	64	56
11	0	0	0	56	81	68	63
12	0	0	0	58	80	69	61

[a] Samples extracted with 1:1 soil–water/CH_3CN 50% (v/v).

Table 3
Design matrix, recovery values and predicted composite desirability (PCD) evaluated at each point in the central composite design to optimize MAEP

Factor	Level		
	−1	0	+1
A: CH$_3$CN (%, v/v)	25	50	75
B: extraction time (min)	2	5	8
C: extraction temperature (°C)	70	100	130

Run	A	B	C	% Recovery				
				Trifluralin	Metolachlor	Chlorpyriphos	Triadimefon	PCD
1	−	−	−	53	80	66	101	0.5961
2	+	−	−	57	57	73	47	0.1957
3	−	+	−	59	78	71	104	0.7343
4	+	+	−	53	63	76	52	0.3544
5	−	−	+	49	71	60	101	0.2637
6	+	−	+	55	62	74	54	0.3620
7	−	+	+	65	77	75	102	0.8066
8	+	+	+	64	53	75	45	0.0
9	−	0	0	29	90	68	80	0.1564
10	+	0	0	42	72	68	47	0.2229
11	0	−	0	49	89	78	87	0.7408
12	0	+	0	45	87	68	77	0.5296
13	0	0	−	59	91	75	88	0.8063
14	0	0	+	60	97	76	106	0.9316
15	0	0	0	61	92	77	100	0.9013
16	0	0	0	58	91	72	86	0.7446
17	0	0	0	57	95	75	91	0.8181
18	0	0	0	57	91	74	93	0.7982

extract was evaporated to dryness under nitrogen and the residue re-dissolved with 1 mL of hexane. Internal standards (20 μL of PCNB at 2.5 mg L^{-1}) were added and the extracts analyzed by GC-ECD under the experimental conditions described above. In Fig. 1, the main steps of the procedure are schematically depicted. Depending on each particular experiment, the mass of soil, acetonitrile content in the extraction solution, temperature, and time of extraction at 900 W were varied according to the experimental designs.

3. Results and discussion

3.1. Preliminary studies on adsorption of compounds in soil

In order to determine the adsorption behaviour of pesticides in the soil matrix, the adsorption isotherms of compounds were determined in a soil with medium organic content (QTA) and data were fitted to the empirical Freundlich model [8]. Results are summarised in Table 1. Adsorption
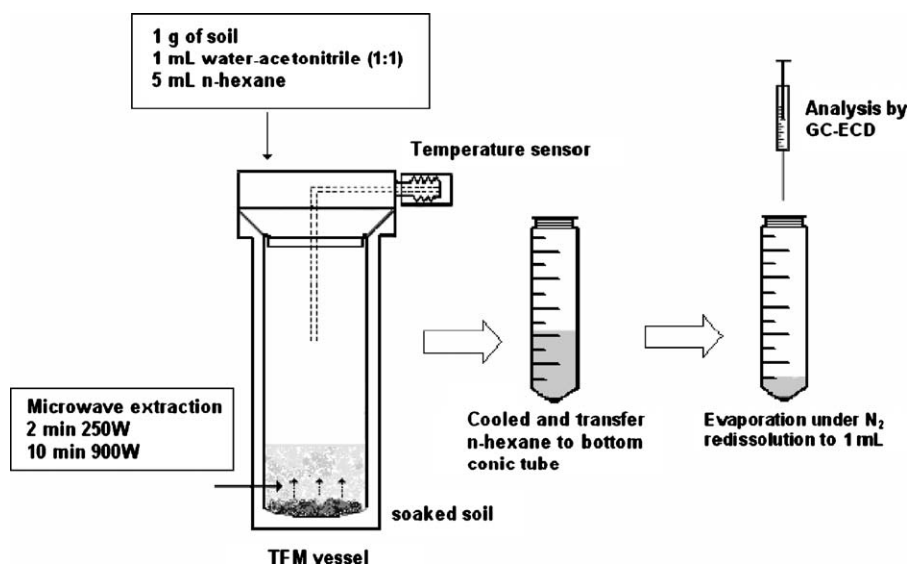

Fig. 1. Schematic representation of the MAEP procedure.

coefficients observed in QTA soil decreased in the order trifluralin > chlorpyriphos > triadimefon > metolachlor and correlated with their hydrophobicity, reflecting sorption on soil organic matter. The non-linearity of isotherms observed in all cases ($1/n$ value ranging from 0.87 for chlorpyriphos to 0.64 for triadimefon) shows, in addition to partition in organic matter from aqueous solution, that adsorption on this soil is probably due to adsorption at heterogeneous sites in organic matter and clay. This behaviour has already been observed for different pesticides [9,10] and attributed to adsorption on carbonaceous material [11] or water mineral interfaces on smectite clays [12–14].

3.2. Spiking procedure

Recovery studies to validate extraction techniques are often carried out with spiked soils obtained by adding a low volume of spiking solution, frequently in methanol or acetone. Samples are allowed to air dry with occasional stirring [15–18], assuming that contaminants are uniformly distributed in the sample. However, in spite of the "equilibrium time", analytes will persist partially as a deposit on the adsorbent surface, without undergoing a whole adsorption process, the extent being controlled by the soil adsorption capacity. The validation of multiresidual extraction methods by using this fortification approach could overestimate their efficiency and underestimate contamination in a field sample. Since sorption of non-ionic organic compounds is principally due to a partitioning process between the aqueous soil solution and organic matter [19,20] and adsorption on clay surfaces [21], as in the case of the compounds under study, samples were contaminated through a "batch" equilibrium method by the addition of an aqueous solution of pesticides to soils using a low water-to-soil ratio, followed by a dry processing. Consequently, the efficiency of MAEP was evaluated to extract the residues actually sorbed.

3.3. Screening analysis of MAEP-associated factors

Three factors were defined to evaluate their contribution to pesticides extraction efficiency from soil: % (v/v) of CH_3CN as organic modifier (0, 25 and 50%, v/v), time of extraction (5, 10 and 15 min) and temperature of extraction (60, 90 and 120 °C). The ANOVA test ($p < 0.05$) showed that organic modifier and temperature had a significant positive effect on chlorpyriphos extraction. The percentage of CH_3CN also had a positive effect on trifluralin extraction, whereas for triadimefon and metolachlor it was negative. The organic modifier added to water improved the extraction efficiency of poorly soluble compounds such as chlorpyriphos and trifluralin. However, at the same time, the medium became less efficient to extract more water-soluble compounds such as triadimefon and metolachlor. Non-quantitative trapping by *n*-hexane was discarded, since recoveries higher than 98% were observed in preliminary studies on the partitioning of compounds between water and acetonitrile and *n*-hexane assisted by microwaves. According to these facts, the amount of acetonitrile added to the aqueous phase, temperature and time of extraction require optimisation

to achieve the maximum efficiency for all compounds under a unique optimal condition.

3.4. Screening analysis of sample-associated factors

We evaluated whether the extraction efficiency of MAEP was affected by three defined sample-associated factors (type of soil, ageing time and mass of soil). Organic matter content in soil was the parameter selected to define the complexity of soil and then the code level in the experimental design. The soil mass-to-extractant ratio was 1:1 and the process was carried out for 2 min at 250 W and 5 min at 800 W (120 °C maximum temperature), with 50%, v/v of acetonitrile in the aqueous phase. Levels for each factor, design matrix, and recoveries obtained for each experiment for the four pesticides are presented in Table 2. In Fig. 2, the standardized Pareto chart shows the influence that every sample-associated factor had on the recovery of the compounds under study as well as the possible cross effects. Organic matter content in soil had a significant positive effect on the extraction of clorpyriphos and, although not significantly, it also increased trifluralin recovery through MAEP. Earlier studies on soil uptake of non-ionic organic compounds from water have demonstrated the contribution of soil organic matter to this retention by partitioning as a sorption mechanism [21,22]. The fraction of pesticides dissolved in soil organic matter was assumed to be easily extractable by MAEP through a re-partitioning process between this component and the water–acetonitrile solution, while the fraction bound to clays is more difficult to extract. Authors have proposed that planar aromatic structures with electron withdrawing substituents, such as $-NO_2$, favour pesticide adsorption on clay surface by the formation of an electron donor–acceptor complex between polarized aromatic ring and the siloxane oxygens of smectite on the soil surface [14]. So, trifluralin and chlorpyriphos might be more effectively sorbed by clays of soil than triadimefon and metolachlor owing to their aromatic structures with electron withdrawing substituents ($-NO_2$, $-CF_3$, $-Cl$). Some authors [23,24] have effectively reported a strong adsorption of trifluralin on soils with a clay content higher than 15% and a low organic matter content. Although the organic matter content was selected initially as a representative parameter of soil complexity, the clay-to-organic matter ratios seem to be more adequate for this purpose (Table 1).

Analyte residence time was a significant negative factor on the extractability of chlorpyriphos, triadimefon and metolachlor. Decreasing recoveries resulting from ageing on matrices is a well-known fact. This is explained according to whether the analytes are incorporated by adsorption in short contact periods, where H-bonding and Van der Waals forces are involved, or by sequestration in long contact periods, which involves sorption at remote microsites within the soil matrix [25]. The negative effect of ageing time could be also caused by degradation of compounds. However, spiked soils were stored dry, in the dark at -20 °C, reducing the probable degradation process.

Soil mass had a significant negative effect on the extractability of trifluralin and triadimefon, and only a small negative influence on the extractability of chlorpyriphos and metolachlor. This effect is related to the fraction of microwave energy effectively
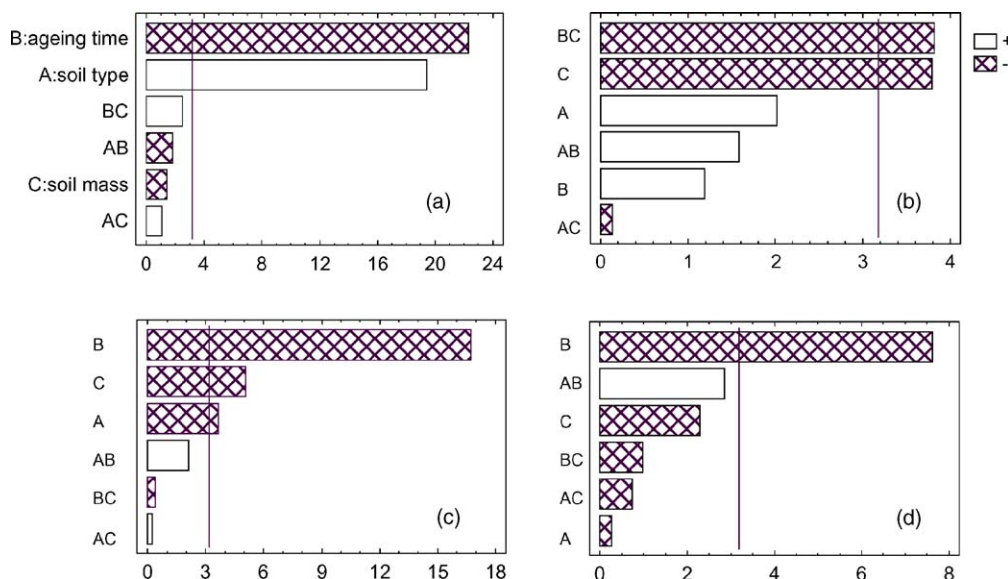
Fig. 2. Pareto charts for the standardized effects of sample-associated factors on MAEP efficiency (a) chlorpyriphos, (b) trifluralin, (c) triadimefon, and (d) metolachlor. Vertical lines indicate both statistically significant effects and cross effects (95% confidence level).

absorbed by the soaked soil, which diminishes by heat dissipation and low penetration effects produced by increasing sample size [26].

Results showed that differences may be found on pesticide recoveries when different amounts of soil with different physicochemical characteristics are analyzed, thus experimental conditions of MAEP must be optimised to determine the pesticides under study in different soil samples.

3.5. Optimisation of MAEP method

A central composite design was applied based on a two-level full factorial design, supported by the low number of factors to be optimised, in order to obtain the response surface for recovery percentage of pesticides from soil, considering the significant factors previously determined in the screening experiment: % (v/v) of CH_3CN in the extraction solution, extraction time and temperature. QTA soil stored for a 10-day period before extraction was used for MAEP optimisation due to its medium value in the clay-to-organic matter ratio.

ANOVA of the results indicated that % (v/v) of CH_3CN, temperature of extraction and/or its quadratic terms appeared to have a significant effect on the extraction efficiency of all compounds ($p < 0.05$). With these terms, 2nd order polynomial models were fitted for each compound and the corresponding response surfaces were obtained (these models are well fitted for all individual compounds, $R^2 > 90\%$). As the responses were different for each compound, a simultaneous optimisation was considered for all compounds. This multiple response optimisation proceeds through the maximization of a desirability function. The desirability is 0.0 for the lowest recovery values obtained in the design for each compound; it increases as recovery increases, and is 1.0 for the highest recovery obtained. Then a composite desirability that combines individual desirability into a single measure is maximised. Table 3 shows 18

experiments with the corresponding recoveries for each pesticide and the composite desirability function evaluated at each point in the design. Fig. 3 shows response surfaces and a residual plot of the composite desirability function estimated from the central composite design and multiple response optimisation. A random pattern was observed for residual plot, which is an indication that the models describe adequately the observed data (composite desirability). The content of acetonitrile in the extraction solution and temperature had a significant influence on composite desirability, whereas time of extraction appears with a minimal effect. Maximal desirability is reached with a medium content of acetonitrile, and at the highest temperature in the range under study. Then, 50% CH_3CN and 130 °C (900 W) were established as the experimental values that simultaneously gave the highest extraction efficiency of MAEP. Although time was a non-significant factor on extraction efficiency, 10 min was chosen as extraction time to maintain a stable instrumental temperature condition.

3.6. Analytical applications

In order to evaluate the extraction efficiency of the proposed method, besides PCR, QTA and VLC soils, other three soils (TEM, RGA and GLV soils) spiked according to procedure 2 and stored for 10 days were extracted under the selected conditions. Results are summarised in Table 4. Except for trifluralin, recoveries in the different soils were higher than 80% with R.S.D. below 9%. Trifluralin recovery was lower, but still in the range or higher than the values previously reported for hot phosphate buffer extraction and SFE methods from silt loam soils [6,27]. In addition to pesticides used to optimize the method, a wide range of halogenated pesticides (water solubility ranged 0.1–250 mg L^{-1}) was determined in QTA, PCR and VLC soils at 0.1–0.4 µg g^{-1}. As can be seen in Table 4, recoveries of these compounds varied from 72 to 101%. As a result of the MAEP
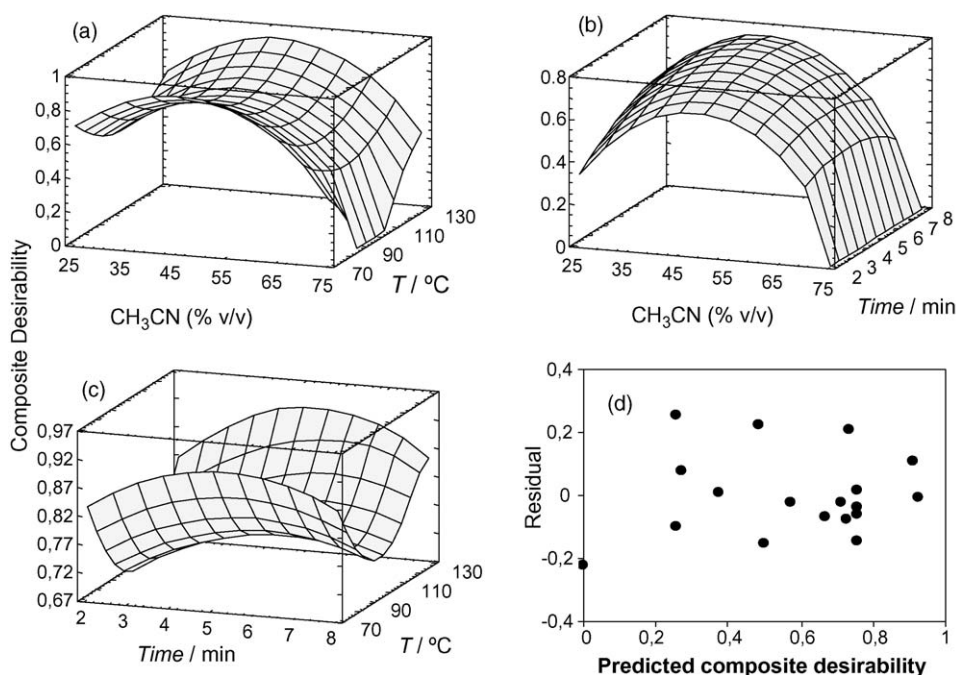
Fig. 3. Response surfaces (a–c) and residual plot (d) for the composite desirability function estimated from the central composite design and multiple response optimisation. Response surfaces at: (a) 5 min; (b) 100 °C; (c) 50% CH$_3$CN.

method, a colourless organic solvent phase was recovered and no interference was observed in the determination of pesticides by GC-ECD. Clean chromatograms were obtained without any additional cleanup step (Fig. 4b).

Malathion was also determined by MAEP and GC-FPD, low recoveries in the three soils under study were obtained (15–17%). Similar to trifluralin, strong adsorption of malathion on soil clays probably occurs rather than sorption to the organic matter, which make their extraction by MAEP difficult. In this sense, MAEP method could underestimate the content in soil of pesticides strongly bound to clay.

Both microwave-assisted and ultrasound-based extraction methods have been widely used as instrumental fluid-phase partitioning methods to extract different organic compounds from soils [28], including pesticides [15,16,29,30]. Therefore, the yield of MAEP to extract the target pesticides was compared with that provided by an ultrasonic method recently proposed [16], using ethyl acetate as solvent at ambient temperature (5 g of soil in columns extracted twice with 4 mL of solvent for 15 min, volume extract adjusted to 10 mL). Although both methods produced clean chromatograms (Fig. 4b and c), MAEP was similar or more efficient than the ultrasonic approach to extract the twelve compounds from soils (Table 4).

Among the soils analyzed by MAEP and GC-ECD, only RGA soil showed the presence of naturally weathered pesticide. In this soil, chlorpyriphos was detected (<0.03 µg g^{-1}) and its presence was confirmed by HPLC-DAD through UV spectra matching with a standard solution. Result reflects the use of this pesti-

Table 4
Limit of detection, recoveries of pesticides (%, $n=3$) and standard deviations (S.D.) for different soils spiked at 0.1 µg g^{-1} level (triallate 0.2 µg g^{-1}; acetochlor, alachlor and metolachlor, 0.4 µg g^{-1}) obtained with the optimised MAEP and the ultrasonic-based extraction method

	LOD (µg g^{-1})	% Recovery in soil					
		TEM	VLC	QTA	RGA	PCR	GLV
Trifluralin	0.006	69 ± 5	80 ± 3 (74 ± 3)	68 ± 3 (42 ± 2)	70 ± 1	67 ± 2 (48 ± 2)	52 ± 3
Triallate	0.018	–	82 ± 4 (79 ± 4)	80 ± 4 (52 ± 3)	–	79 ± 6 (57 ± 3)	–
Acetochlor	0.036	–	100 ± 3 (84 ± 5)	89 ± 3 (54 ± 3)	–	90 ± 7 (70 ± 4)	–
Alachlor	0.030	–	101 ± 6 (79 ± 4)	91 ± 3 (51 ± 2)	–	91 ± 5 (65 ± 4)	–
Metolachlor	0.036	90 ± 6	104 ± 3 (83 ± 4)	99 ± 3 (57 ± 3)	101 ± 5	90 ± 8 (73 ± 5)	85 ± 7
Chlorpyriphos	0.010	80 ± 6	100 ± 3 (90 ± 5)	89 ± 6 (38 ± 2)	80 ± 3	100 ± 7 (28 ± 1)	80 ± 7
Triadimefon	0.018	78 ± 7	92 ± 5 (114 ± 6)	93 ± 3 (87 ± 8)	85 ± 7	98 ± 5 (98 ± 6)	89 ± 8
Endosulphan I	0.008	–	78 ± 6 (70 ± 4)	78 ± 3 (44 ± 2)	–	83 ± 6 (49 ± 2)	–
Endrin	0.009	–	78 ± 5 (77 ± 5)	79 ± 4 (47 ± 3)	–	89 ± 8 (52 ± 3)	–
Endosulphan II	0.008	–	72 ± 7 (71 ± 3)	76 ± 4 (45 ± 3)	–	89 ± 6 (52 ± 3)	–
Methoxychlor	0.010	–	73 ± 5 (64 ± 3)	72 ± 6 (97 ± 5)	–	87 ± 7 (64 ± 4)	–
Tetradifon	0.004	–	76 ± 5 (75 ± 4)	80 ± 1 (36 ± 2)	–	97 ± 9 (41 ± 3)	–

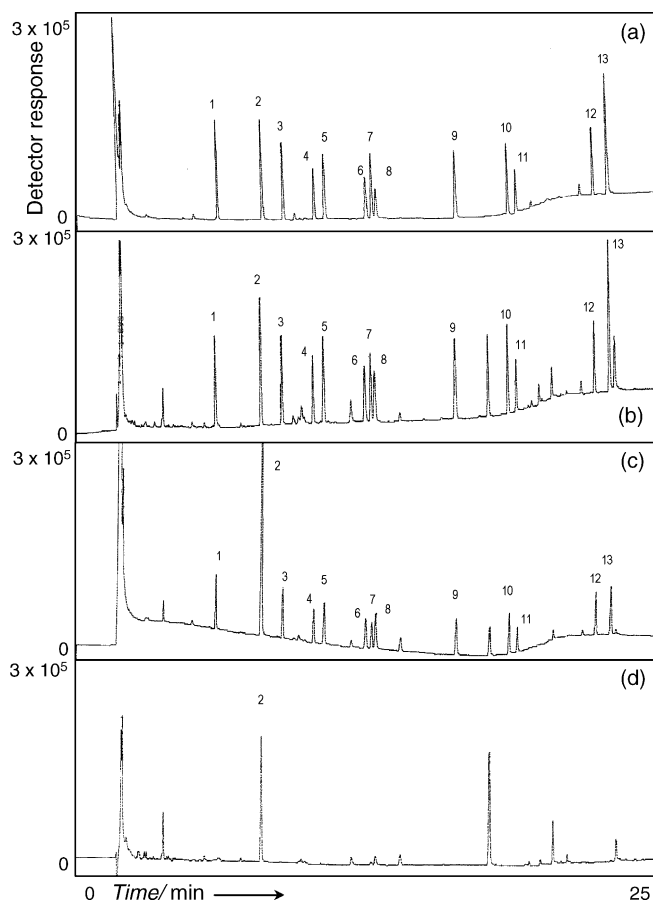Recoveries for ultrasonic method shown in parentheses.

Fig. 4. GC-ECD chromatograms of a pesticide mixture at 0.1 μg mL⁻¹ (triallate 0.2 μg mL⁻¹; acetochlor, alachlor and metolachlor 0.4 μg mL⁻¹) (a); of MAEP extract from QTA soil spiked at 0.1 μg g⁻¹ (triallate 0.2 μg g⁻¹; acetochlor, alachlor and metolachlor 0.4 μg g⁻¹) (b); of a ultrasound-based extraction (2 μL injected) (c); of unfortified QTA soil sample (d). Pesticides: (1) trifluralin, (2) PCNB (internal standard, 0.05 μg mL⁻¹), (3) triallate, (4) acetochlor, (5) alachlor, (6) metolachlor, (7) chlorpyriphos, (8) triadimefon, (9) endosulphan I, (10) endrin, (11) endosulphan II, (12) methoxychlor and (13) tetradifon.

cide in the phytosanitary control of an apple orchard where the soil was collected. According to Pesticides Market Report of 2003 [31], chlorpyriphos is present in high volumes through insecticides sales in VI Region. Once MAEP was fully applied, detection limits for each analyte were calculated (three times the standard error of the signal obtained for six QTA soil extracts, the soil previously contaminated at 0.1–0.4 μg g⁻¹ level). The values ranged from 0.004 to 0.036 μg g⁻¹ (Table 4) and are adequate for the analysis of extracts from 1 g of soil containing less than 0.06 μg g⁻¹ of all compounds (0.1 μg g⁻¹ for acetochlor, alachlor and metolachlor) extracted and dissolved in 1 mL of *n*-hexane.

4. Conclusions

A simple, selective, and sensitive method has been developed to determine hydrophobic pesticides at sub μg g⁻¹ level in different kinds of soils based on microwave-assisted extraction through aqueous media and simultaneous partition on hexane-GC-ECD. Partitioning on hexane operates as an "in situ"

cleanup. The method was successfully applied to the extraction of residues of triallate, acetochlor, alachlor, metolachlor, chlorpyriphos, triadimefon, endosulphan I and II, endrin, methoxychlor and tetradifon from soils with different clay-to-organic matter ratios, which was considered an appropriate index to establish the matrix complexity. The lower recoveries obtained for trifluralin were properly explained by their adsorption behaviour on a model soil, and also by the matrix complexity of very different kinds of soils. In this sense MAEP method could underestimate the content in soil of pesticides strongly bound to clay. The multivariate approach used in this study through experimental design taking into account instrumental and sample-associated factors and understanding the environmental behaviour of the organic compounds led us to a critical assessment of the proposed method.

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