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ORIGINAL PAPER

Comparison of solid phase microextraction and hollow fiber liquid phase microextraction for the determination of pesticides in aqueous samples by gas chromatography triple quadrupole tandem mass spectrometry

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Abstract This work compares two miniaturised sample preparation methods, solid phase microextraction (SPME) and hollow fiber liquid phase microextraction (HF-LPME), in combination with gas chromatography coupled to tandem mass spectrometry with a triple quadrupole analyzer for the determination of 77 pesticides in drinking water. In the case of SPME, extraction temperature and time were optimized by experimental design, although other parameters, as desorption time, pH, and ionic strength, were also evaluated. The extraction and desorption solvents [octanol/dihexyl ether (75:25, v/v) and cyclohexane, respectively], as well as the extraction and desorption time, ionic strength, and pH, were studied for the HF-LPME procedure. Under the optimal conditions, recoveries (70.2–113.5% for SPME and 70.0–119.5% for HF-LPME), intra-day precision (2.1–19.4% for SPME and 4.3–22.5% for HF-LPME), inter-day precision (5.2–21.5% for SPME and 8.4–27.3% for HF-LPME), and limits of detection, between 0.1 and 28.8 ng/L for SPME and 0.2 and 47.1 ng/L for HF-LPME and overall uncertainty (9.6–25.2% for SPME and 13.3–27.5% for HF-LPME) were established for both extraction procedures. Finally, the proposed methods were successfully applied to the analysis of 41 drinking water samples, and similar results were obtained with both extraction approaches.

Keywords SPME · HF-LPME · Pesticides · Gas chromatography–mass spectrometry · Triple quadrupole

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Introduction

Pesticides are essential in modern agricultural practices. However, due to their biocide activity, they represent a serious hazard to human health. For this reason, their detection in food samples is a very important element of consumer protection. Because the limits of pesticides in drinking water are getting stricter [1], more attention has to be given to the extraction procedure in order to determine low concentration levels.

Miniaturised extraction techniques, such as solid phase microextraction (SPME) and liquid phase microextraction (LPME), provide the extraction of a wide spectrum of pesticides at trace levels with good precision values, due to their simplicity and suitability. They have several advantages over the conventional techniques of sample treatment, liquid–liquid extraction or solid phase extraction, such as solvent-free or minimal consumption of harmful solvents, minimal amount of sample analyzed, and higher enrichment factor [2, 3].

SPME is a solvent-free extraction technique introduced by Pawliszyn and co-workers in the early 1990s [4, 5]. It is based on the partitioning of organic compounds between a

polymeric stationary phase that coats a fused silica fiber and the sample. Then, the absorbed compounds are desorbed into a gas or liquid mobile phase into a gas or liquid chromatograph injection port, respectively [4]. This technique is applied to the extraction of a wide variety of organic compounds, such as pesticides [6–12], polychlorinated biphenyls [7], polycyclic aromatic hydrocarbons [13], volatile organic compounds [14], or phenolic compounds [15] from water samples, by direct immersion (DI-SPME) or headspace (HS-SPME) modes. In addition, several articles have summarized the application of SPME in environmental, biomedical, food, or beverage samples [16–18].

The development of hollow fiber liquid phase microextraction (HF-LPME) was introduced by Pedersen-Bjergaard and Rasmussen in 1999 [19–21] as a variant of the first approaches developed in LPME [22, 23]. In HF-LPME, a polymeric membrane, usually made of polypropylene, serves as a support for the extracting solvent and an interface between the donor and acceptor phases, which enhanced stability of organic solvent. The analytes are extracted by diffusion from the sample into the organic solvent (two phase HF-LPME). HF-LPME has also been applied in biological, environmental, beverage, and food analysis [3, 24–28] in two- or three-phase system and static or dynamic mode. In general, a great number of environmental applications of the technique are focused on the extraction of pesticides from aqueous samples [6, 29–35].

The determination of pesticides in water samples is usually performed by gas chromatography (GC) or liquid chromatography (LC) coupled to mass spectrometry (MS) [36, 37]. The enhanced selectivity and sensitivity of tandem mass spectrometry (MS/MS), compared to single stage MS, has made it a frequent method of choice for structural and quantitative analysis of small molecules (molecular weight <1000, such as pesticides). The use of MS/MS based on a triple quadrupole analyzer (QqQ) makes possible a short pretreatment sample by reducing or even removing the clean-up stage due to its high selectivity and sensitivity [38] using the acquisition mode called selected reaction monitoring (SRM).

In this study, two miniaturised extraction methods based on SPME or HF-LPME have been developed and compared for the simultaneous determination of 77 pesticides in drinking water by GC coupled to MS/MS, using a QqQ analyzer. The proposed methods allow the analysis of a higher number of pesticides than other published methods using SPME [6–12] or HF-LPME [6, 29–35]. In addition, to our knowledge, this is the first approach in the simultaneous quantification–confirmation of more than 50 pesticides with a QqQ analyzer applied to SPME or HF-LPME. The developed methods have been validated and applied to the analysis of real samples taken from the Southeast of Andalusía (Spain).

Experimental

Chemicals, solvents, and standards

Certified pesticide standards were purchased from Dr. Ehrenstorfer GmbH (Ausgburg, Germany), Riedel de Haën (Seelze, Germany), and Fluka (Steinheim, Germany). The internal standard (IS), parathion ethyl d-10, was supplied by Dr. Ehrenstorfer GmbH. Sodium chloride (99.5%) was purchased from Panreac (Barcelona, Spain). Organic solvents, such as acetone (99.8%), 1-octanol (99.5%), dihexyl ether (97%), undecane (99%), ethyl acetate (99.8%), and cyclohexane (99.5%), were supplied from Fluka, while toluene (99.7%) was from J.T. Baker (Deventer, Holland). All of them were residue analysis grade. Extracts were dried using magnesium sulfate (98%), obtained from Riedel de Haën. Different buffers (pH 4, 6, and 8) were prepared using glacial acetic acid (99.7%) purchased from Panreac, sodium acetate anhydrous (99.0%), hydrochloric acid (37–38%), and sodium hydroxide (97.0%) supplied from J.T. Baker, and potassium dihydrogen phosphate (99.5%) and anhydrous di-sodium hydrogen phosphate purchased from Merck (Darmstadt, Germany).

Stock standard solutions of each individual compound (with concentrations between 200 and 500 mg/L) were prepared by weighing of the powder or the liquid and dissolving it in 100 mL of acetone and stored at −18 °C in a dark place. A multicompound working standard solution (2 mg/L concentration of each compound) was prepared by corresponding dilution of the stock solutions with acetone and stored under refrigeration at 4 °C. Finally, a working standard solution of parathion ethyl d-10 (4.5 mg/L) was prepared by appropriate dilution of the stock solution with acetone and stored under the aforementioned conditions.

Ultrapure water was obtained from a Milli-Q water system (Millipore, Bedford, MA, USA).

SPME procedure

StableFlex SPME fibers 65 μm poly(dimethylsiloxane)-divinylbencene (PDMS-DVB), extraction vials, septa, and magnetic cap were supplied by Supelco (Bellefonte, PA, USA). Fibers were conditioned in the bake-out station for 120 min at 250 °C according to supplier's instructions before use.

A Combi Pal auto-sampler (CTC Analytics AG, Zwingen, Switzerland) was used with an SPME module, an agitator, a heater module, and an SPME fiber conditioning station (bake-out station).

The SPME procedure was performed under the following conditions: 14 mL aliquots of the sample with 2 μL of standard solution of parathion ethyl d-10 (IS) were extracted by immersion of a StableFlex 65 μm PDMS-

DVB fiber during 60 min at 70 °C and sample agitation at 500 rpm. Ionic strength was adjusted by 15% adding NaCl (15%, *w*/*v*), and the pH was adjusted to 6. Afterwards, desorption of the pesticides was carried out at 250 °C for 5 min in the split–splitless injector and held for 4 min more with the split ratio at 100:1. Subsequently, the fiber was put into the bake-out station during 10 min at 250 °C in order to perform a clean-up. Blanks were run periodically to confirm the absence of carryover.

HF-LPME procedure

Q3/2 Accurel PP hydrophobic polypropylene hollow fiber tubing (200 μm wall thickness, 600 μm i.d., and 0.2 μm pore size) was obtained from Membrana GmbH (Wuppertal, Germany). Ten-microliter syringe plungers were provided by Hamilton (Bonaduz, Switzerland). The fibers were cleaned with acetone by sonication and then dried.

The HF-LPME procedure was based on the method developed by Bolaños et al. [26]. Briefly, a syringe plunger was inserted into the hollow fiber (pieces of 2 cm length), soaked with 1-octanol:dihexyl ether (75:25, *v*/*v*; 1 min), and then placed into a 15 mL screw top vial containing 14 mL of sample, adjusting the ionic strength with NaCl (15%, *w*/*v*) and the pH to 6. The vial was shaken in a rotary agitator (Reax 2 from Heidolph, Schwabach, Germany) for 90 min at 90 rpm. After the extraction step, the fiber was transferred on a 2 mL vial containing 1.5 mL of cyclohexane, and the extracted analytes were desorbed by means of agitation for 5 min in the rotary agitator at 30 rpm. Subsequently, the fiber was removed from the vial, and the extract was passed through $MgSO_4$ to remove any water that it might contain. Then, the extract was evaporated to dryness under a gentle stream of nitrogen. Finally, the residue was re-dissolved with 250 μL of cyclohexane and 5 μL of a solution of parathion ethyl d-10 (IS) for subsequent analysis by GC-QqQ-MS/MS.

GC-QqQ-MS/MS analysis

Chromatographic analysis was performed with a GC system Varian 3800 (Varian Instruments, Sunnyvale, CA, USA). Injections were carried out with a Combi Pal autosampler (CTC Analytics, Zwingen, Switzerland) into an SPI/1079 split/splitless programmed temperature injector. A fused-silica untreated capillary column (2 m× 0.25 mm i.d.) supplied by Supelco was used as guard column connected to a Varian FactorFour capillary column VF-5 ms analytical column (30 m×0.25 mm i.d.×0.25 μm film thickness). Helium (99.9999%) at a constant flow rate of 1 mL/min was used as carrier gas.

For SPME, the initial injector temperature was set at 250 °C (held for 9 min) and then increased to 300 °C at 200 °C/min (held for 10 min). The injector split ratio was initially set at 30:1. The splitless mode was switched on at 0.01 until 5 min. At 5 min, the split ratio was 100:1; it reduced to 50:1 at 9 min and to 30:1 at 13 min. The column oven temperature, initially 70 °C, was held for 5 min. Then, the temperature was increased at a rate of 35 °C/min up to 180 °C and at a rate of 10 °C/min up to 300 °C (held for 7 min). The total running time was 27.14 min.

For HF-LPME, aliquots of 10 μL of the final extract were injected into the chromatographic system at 10 μL/s using a 100-μL syringe. The glass liner was equipped with a Carbofrit plug (Resteck, Bellefonte, PA, USA). The large volume injection technique was used, with a split/splitless programmed temperature vaporization injection. The injector temperature program started at 70 °C (held for 0.5 min) and then was increased with a rate of 100 °C/min until 310 °C (held for 10 min). The injector split ratio was initially set at 30:1. The splitless mode was switched on at 0.50 min until 3.50 min. At 3.50 min, the split ratio was 100:1, and at 12 min, it was 30:1. The column oven temperature was similar to the SPME method described previously. Cryogenic cooling with carbon dioxide (CO_2, 99.9%) was applied when the injector temperature was 170 °C, in order to reach the initial injector temperature of 70 °C as soon as possible before the next injection.

The GC was interfaced to a Varian 1200 L triple quadrupole mass spectrometer operated in electronic ionization mode (EI, 70 eV). The computer controlling the system held an EI MS/MS library created specifically for the target analytes under our experimental conditions. The mass spectrometer was calibrated weekly with perfluorotributylamine. Varian Workstation software was used for instrument control and data analysis. Argon (99.999%) was used as collision gas at a pressure in the range 1.90–2.10 mTorr.

The QqQ mass spectrometer operated in the SRM acquisition mode. For endrin the selected ion monitoring (SIM) mode was applied because lower signals were obtained in SRM mode. The temperatures of the transfer line, ionization source, and manifold were set at 300, 280, and 40 °C, respectively. The scan time was set at 0.221 s (segment 1), 0.216 s (segment 2 and 6), 0.261 s (segment 3), 0.308 s (segment 4), and 0.288 s (segment 5), respectively. The electron multiplier voltage was set at 1,750 V (+200 V offset above the value obtained in the auto-tuning process). The analysis was performed with a filament-multiplier delay of 7.5 min for both SPME and HF-LPME in order to prevent instrument damage.

Sample collection

Natural spring water, as well as tap and commercial mineral waters from the Southeast area of Andalusia (Spain), were collected between February and April of 2009. All water

samples were used without previous treatment or filtration. The samples were stored in dark place under refrigeration at 4 °C until the analysis.

Experimental design

A central composite design (CCD) was applied in the optimization of the extraction method to analyze the simultaneous effect of the extraction time and temperature parameters in SPME procedure. Experimental design analysis was performed using the Statgraphics Statistical Computer Package Statgraphics Plus 5.1.

Results and discussion

Optimization of SPME procedure

All optimization experiments, both in SPME and HF-LPME, were carried out using an uncontaminated commercial mineral water spiked at 200 ng/L of each target pesticide.

The parameters to be optimized for SPME were extraction time and temperature, desorption time, ionic strength, and pH. The agitation speed was fixed at 500 rpm, as high agitation improves the extraction of the analytes [9]. The desorption temperature was kept to 250 °C, 20 °C below the highest recommended temperature by the manufacturer to avoid thermal degradation of the fiber [9, 39]. PDMS-DVB fiber of intermediate polarity was chosen because, according to previous works [9–12], this is the most versatile fiber for the extraction of pesticides with a wide range of polarity.

Extraction temperature and time in SPME procedure

In order to optimize the suitable extraction conditions of the selected pesticides, a response surface methodology approach was used [40]. A CCD was used to optimize extraction time and temperature. Taking into account that the extraction conditions are usually a compromise, since the best conditions for each analyte can be quite different, overall desirability function D was selected as analytical response. This is based on the transformation of each estimated response, relative peak area, into a dimensionless desirability (d_i) scale, which ranges between 0 (undesired response) and 1 (fully desired response). Then, the overall desirability function D is calculated combining the individual desirability values (d_i), according to the expression $(\prod d_i)^{1/m}$, where m indicates the number of pesticides under study.

A circumscribed CCD (CCCD) was applied, in which the axial points were located at $\alpha = \sqrt{2} = 1.414$ [41]. The experimental variables and levels of the two CCCD matrices (CCCD-1 and CCCD-2) are given in Table 1. In both cases, replicates at the central point were included. Therefore, the overall matrix of CCCD-1 and CCCD-2 involved ten experiments.

First, extraction time was studied from 12 to 68 min and temperature from 34 °C to 70 °C (CCCD-1), and Fig. 1a shows the response surface. This figure indicates that a maximum value for D was not achieved, observing that it increases at higher temperature and extraction time. Taking into account these results, a second CCCD design (CCCD-2) was carried out, selecting higher temperatures and extraction times (Table 1). It can be observed that the best response was obtained at 85 °C and 122 min (Fig. 1b). However, it must be indicated that the most volatile pesticides, such as alachlor, aldrin, α- and β-lindane, α-, β-, ether-, and lactone-endosulfan, etoprophos, heptachlor, hexachlorbenzene, and procymidone were better extracted at 70 °C, whereas the pyrethroids showed better signals at 80 °C, although acceptable values were also obtained at 70 °C. Considering these results, 70 °C was selected as optimum temperature. Furthermore, this effect was not significant (p=0.20), and only minor variations on D values were observed when temperature was changed from the optimum value (85 °C) to the selected one (70 °C).

In relation to the extraction time, and despite that the optimum value is very long (122 min), this is pesticide dependent. Compounds, such as feranimol, chlorfenvinphos, or esfenvalerate, presented a similar behaviour in the range 38–122 min (Fig. 2a), while for other compounds (ethion, dieldrin, endrin, or permethrin), an increase in the signal (Fig. 2b) was obtained with the time (122 min). Finally, an extraction time of 60 min was selected as a compromise, taking into account that this time allows the detection of pesticides at the levels required in drinking water, and sample throughput can be increased. In addition, this effect was not significant (p=0.47), except for some

Table 1 Values of the experimental variables of the circumscribed central composite design 1 (CCCD-1) and 2 CCCD-2 (values in parenthesis)

Point	Temperature (°C)	Time (min)
(0,0)	55 (75)	40 (80)
(−α, 0)	34 (65)	40 (80)
(−1, −1)	40 (68)	20 (50)
(−1, 1)	40 (68)	60 (110)
(0, −α)	55 (75)	12 (38)
(0, α)	55 (75)	68 (122)
(1, −1)	70 (82)	20 (50)
(−1, 1)	40 (68)	60 (110)
(α, 0)	70 (85)	40 (80)

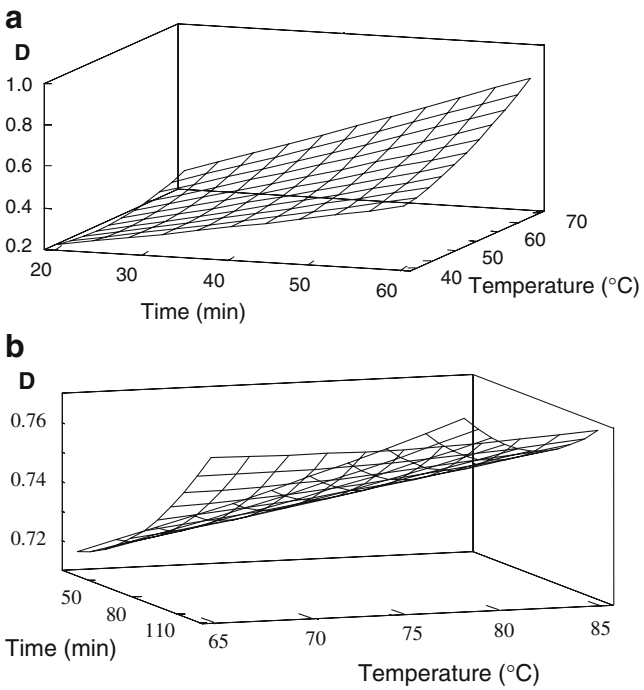

Fig. 1 Response surface for the desirability function: **a** experimental design 1 and **b** experimental design 2

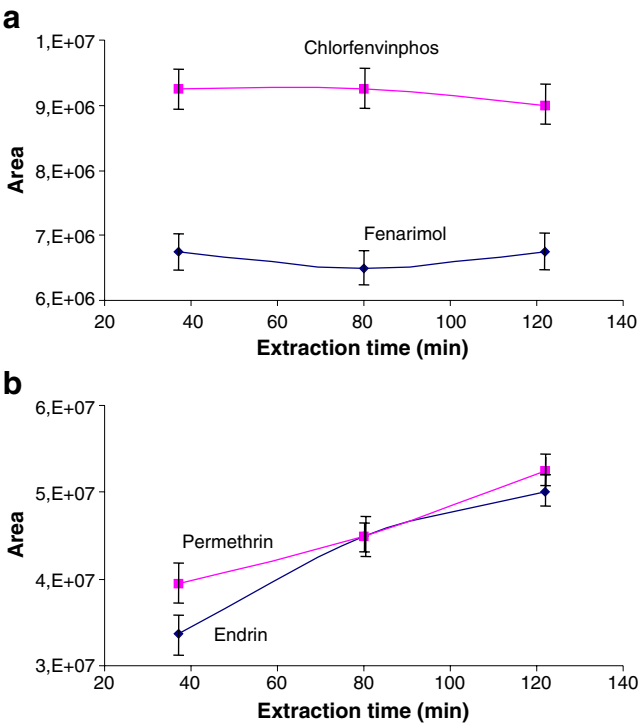

Fig. 2 **a**, **b** Effect of extraction time on the SPME peak area. Conditions: sample volume, 14 mL; sample agitation, 500 rpm; extraction temperature, 75 °C; desorption temperature, 250 °C; and without pH modification neither salt addition. *Error bars* indicated the standard deviation (n=3)

pyrethroids, such as tetramethrin, dieldrin, and permethrin, with $p<0.05$.

Desorption time in SPME procedure

In order to determine the optimum time conditions for a complete desorption of the extracted analytes, different desorption times were tested on the injection port (2, 3, 4, and 5 min) at 250 °C. Figure 3 shows the behavior of representative pesticides taking into account different chemical families (organochlorine, organophosphorus, or pyrethroids) and retention times. Although some pesticides showed a better result at 3 min, as chlorpyriphos methyl and endosulfan ether, or at 4 min for piperonyl butoxide, most of them were better desorbed after 5 min. Consequently, a desorption time of 5 min was selected for subsequent studies. Afterwards, the split valve was switched on at a split ratio of 100:1 during 4 min remaining the fiber into the injector, followed with 10 min on the bake-out station at 250 °C to remove the carry over effect. Using the selected conditions, a vial with doubly distilled water was analyzed after each sample injection, and no carryover effect was observed.

Optimization of HF-LPME procedure

For HF-LPME, the following parameters were optimized: extraction and desorption solvents, extraction and desorption time, ionic strength, and pH. The fiber length and the agitation speed were based on previous study carried out in our research group [26].

Extraction and desorption solvents in HF-LPME procedure

For HF-LPME, it is necessary to choose a suitable organic solvent capable to extract the analytes, immiscible with the samples and compatible with the hollow fiber that is made of polypropylene with hydrophobic properties. Owing to the fact that samples are highly stirred during the extraction, the solvent should be held without leaking from the fiber and has low vapor pressure to reduce losses (stable at room temperature). Therefore, 1-octanol, undecane, toluene, and dihexyl-ether were tested. The worst results were obtained with the undecane and toluene solvents (data not shown). Both 1-octanol and dihexyl-ether were equally effective for most of the pesticides, although dichloran, difenoconazole, ethoprophos, methidathion, myclobutanil, nuarimol, or triadimefon were better extracted in dihexyl-ether, while achrinathrin, azoxystrobin, bifenthrin, famphur, fenpropathrin, lamda-cyhalothrin, or malathion were better extracted in 1-octanol.

On a second experiment, different ratios of 1-octanol and dihexyl-ether were evaluated (Fig. 4). Pesticides, as

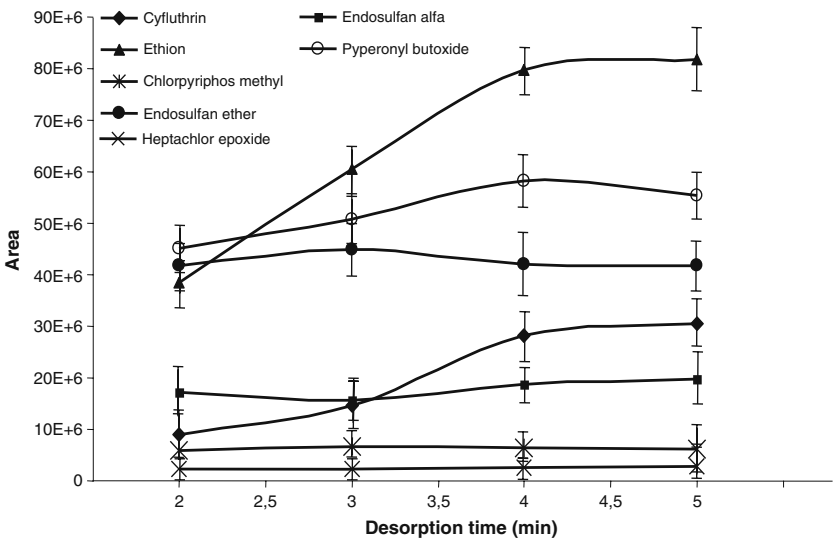

Fig. 3 Desorption time profiles of SPME. Conditions: sample volume, 14 mL; sample agitation, 500 rpm; extraction time, 60 min; extraction temperature, 70 °C; desorption temperature, 250 °C; and without pH modification neither salt addition. *Error bars* indicated the standard deviation (n=3)

acrinathrin, triadimefon, endosulfan (α, β, ether, lactone, and sulphate), difenoconazole, fenitrothion, flucythrinate, or pyridaben showed better responses when a mixture of 1-octanol/dihexyl-ether (75:25 *v*/*v*) was used. Deltamethrin, etrimfos, metoxichlor, or pyrazophos were better extracted when a mixture 1-octanol/dihexyl-ether (50:50 *v*/*v*) was applied, and a mixture of 1-octanol/dihexyl-ether (25:75 *v*/*v*) was more suitable for the extraction of lindane (α, β, γ and δ), sulfotep, pendimethalin, *p*-*p*′-DDD+*o*,*p*′-DDT, and *p*,*p*′-DDT. Bearing in mind that most of the compounds were extracted when a mixture 1-octanol/dihexylether (75:25 *v*/*v*) was used, it has been applied for further experiments.

The desorption solvent used to re-extract the analytes from the fiber should be compatible with GC/MS, and cyclohexane and ethyl-acetate were tested. The obtained results (data not shown) indicated that cyclohexane was better than ethyl-acetate, and consequently, it was selected.

Extraction and desorption time in HF-LPME procedure

The extraction time was studied in the range between 30 and 240 min under stirring at 90 rpm and room temperature [25, 34]. The effect of the extraction time on the peak areas of the analytes can be seen in Fig. 5. The signals rise with the increase in the extraction time for most of the pesticides, as acrinathrin, α-lindane, bifenthrin, endosulfan-β, endrin, heptachlor, mirex, oxifluorfen, *p*,*p*-DDE and parathion methyl. However, for some compounds such as famphur and tetramethrin (see Fig. 5), signal

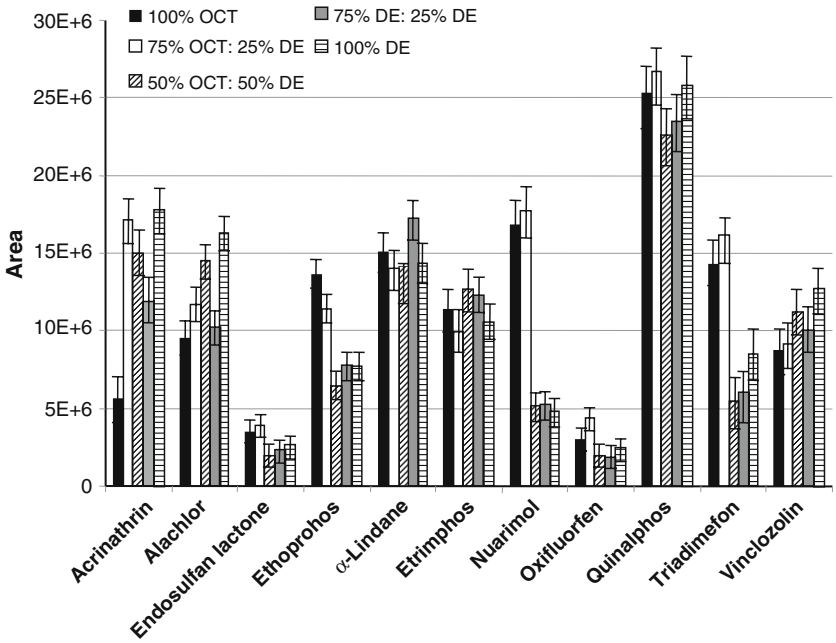

Fig. 4 Effect of the extraction solvents in HF-LPME. Conditions: sample volume, 14 mL; sample agitation, 90 rpm; extraction time, 120 min; desorption time, 5 min; without pH modification neither salt addition; and stripping solvent, cyclohexane. *Error bars* indicated the standard deviation (n=3)

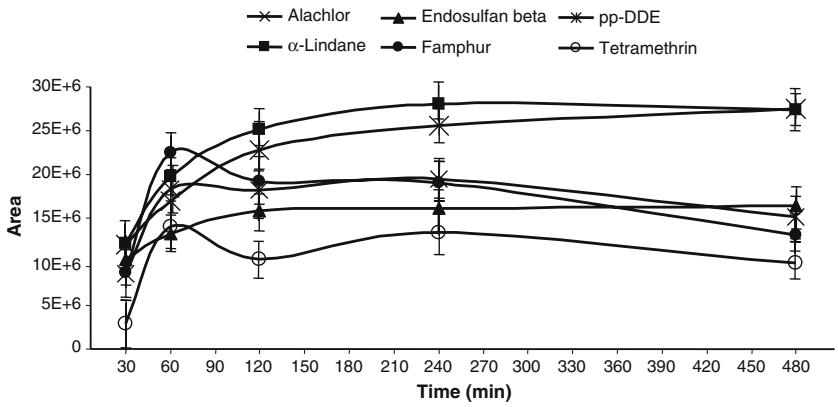

Fig. 5 Extraction time profiles of HF-LPME. Conditions: sample volume, 14 mL; sample agitation, 90 rpm; desorption time, 5 min; room temperature, without pH modification neither salt addition; and stripping solvent, cyclohexane. *Error bars* indicated the standard deviation (n=3)

slightly decreased for extraction time longer than 60 min, and this can be due to pesticides were extracted back to the sample. After 120 min of extraction, it was observed that the extraction time profile kept almost constant, meaning that the equilibrium between the phases was reached after 120 min. Considering that 90-min extraction provides optimal recoveries as well as sample throughput is increased, it was selected for further experiments.

The desorption time was studied at 5, 10, 15, and 30 min. As can be seen in Fig. 6, acrinathrin, alachlor, heptenophos, famfur, λ-cyhalothrin, and parathion-ethyl reached the maximum at 15 min and then decreased, while other compounds as α-lindane, chlorpyriphos-ethyl, endosulfan ether, heptachlor, or procymidone reached the maximum peak area at 5 min, and they decreased when desorption time was longer. As a consequence, 5 min was chosen as desorption time in order to reduce analysis time.

Optimization of common parameters: pH and ionic strength

When acidic or basic analytes must be analyzed, pH is an important factor for both techniques, SPME and HF-LPME, changing the proportion of non-ionized pesticides that could be extracted [3, 42, 43]. The influence of pH was evaluated, studying the pH from 4 to 8. The results indicate that when both techniques (SPME and HF-LPME) were applied, non-significant differences were obtained at values studied. However, in order to standardize the extraction conditions, pH 6 was selected.

It is well known that ionic strength can decrease or increase the extraction of pesticides using either SPME [9, 11, 12] or HF-LPME [6, 26, 31, 34]. The effect of the salt addition was studied by adding NaCl to water samples at different concentrations ranging from 0% to 30% (*w*/*v*) (three replicates). As can be observed on Fig. 7, depending on the chemical properties of the target analytes, an increase in the ionic strength of the aqueous solution may have various effects upon extraction. It enhances the extraction of most polar compounds, such as alachlor and azoxystrobin. Ionic strength did not affect the extraction of some compounds, such as diflufenican or β-lindane, and the extraction of non-polar compounds, such as fenpropathrin, permethrin, or pentachlorobenzene, decreased when ionic strength increased. For most of them, better extractions were obtained when the percentage of NaCl ranged from 10% to 20% (*w*/*v*). Therefore, 15% NaCl (*w*/*v*) was chosen as a compromise value in the extraction process of both techniques.

Validation

The proposed methodology was validated in terms of identification and confirmation of the analytes, linearity, trueness (expressed as recovery), precision (intra- and inter-

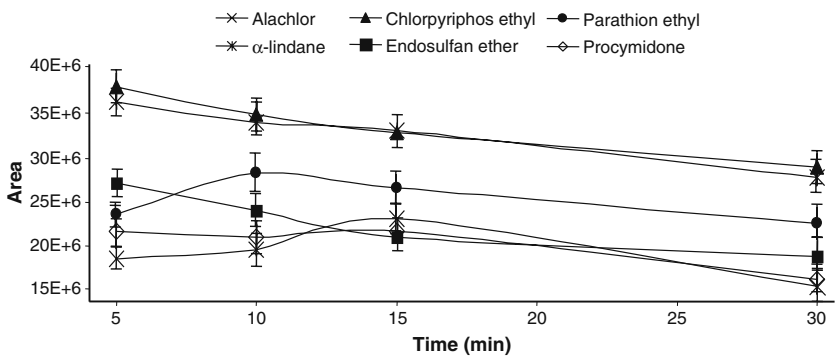

Fig. 6 Desorption time profiles of HF-LPME. Conditions: sample volume, 14 mL; sample agitation, 90 rpm; extraction time, 90 min; room temperature, without pH modification neither salt addition; and stripping solvent, cyclohexane. *Error bars* indicated the standard deviation (n=3)

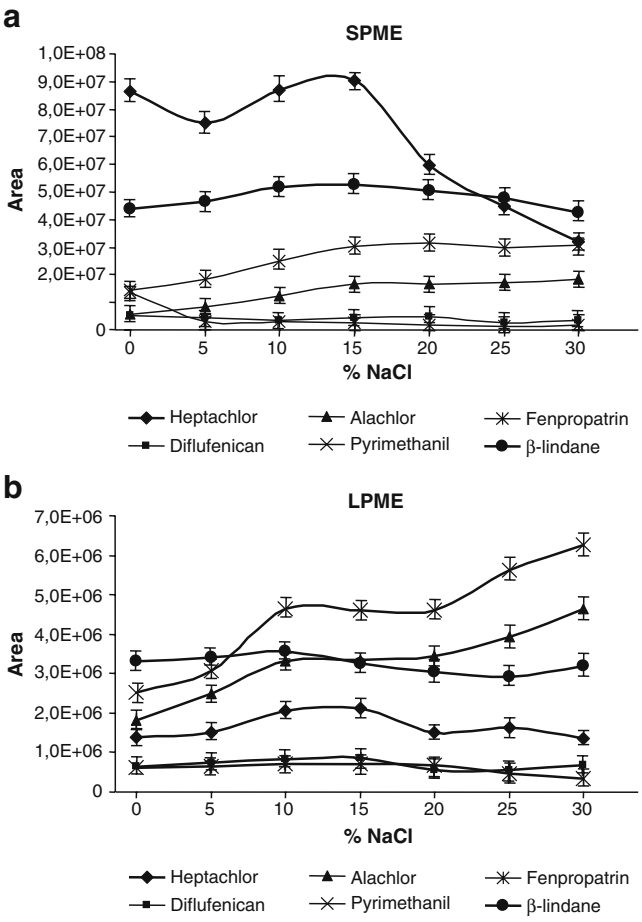

Fig. 7 Effect of the ionic strength on the peak area on the extraction of pesticides: **a** SPME and **b** LPME. SPME conditions: sample volume, 14 mL; sample agitation, 500 rpm; extraction time, 60 min; extraction temperature, 70 °C; desorption temperature, 250 °C; and pH 6. HF-LPME conditions: sample volume, 14 mL; sample agitation, 90 rpm; extraction time, 90 min; room temperature; pH 6; and stripping solvent, cyclohexane. *Error bars* indicated the standard deviation (n=3)

day precision), detection limit (LOD) and quantification limit (LOQ) using aliquots of an uncontaminated commercial mineral water sample. In addition, an estimation of the uncertainty of analytical results was carried in accordance with the recommendations of EURACHEM/CITAC guide [44] using in-house validation date [45].

Identification and confirmation of the pesticides

Identification of pesticides was based on the retention time windows, defined as the retention time average±three standard deviations of the retention time when ten blank water samples spiked at 100 ng/L were analyzed (Tables 2 and 3). The identity was then confirmed by acquisition of two or more MS/MS transitions or three SIM transitions for endrin, bearing in mind European guidelines [46]. The selectivity of the method was evaluated by running control blank samples. The absence of any signal at the same retention time as target pesticides indicated that there were no matrix interferences that may give a false positive signal.

Matrix effect and linearity

It is well known that matrix components can enhance or inhibit the target analyte signal in relation to the signal in pure solvent. In this study, the matrix effect was evaluated by comparison of the slopes of the calibration curves in pure solvent (for HF-LPME) or distillated water (for SPME) and in a mineral water sample. In both methods, the slopes were very similar, which verify the absence of matrix effect. Consequently, calibration curves were measured in pure solvent (for HF-LPME) or distillated water (for SPME).

The linear range was tested using nine calibration levels over a range between 0.6 and 600 ng/L for aldrin, dieldrin, heptachlor, and heptachlor epoxide, and from 1 to 1000 ng/L for the rest of the pesticides (injections in triplicate). Linear range and determination coefficients (R^2) are presented in Tables 2 and 3, and two examples for each developed method are shown in Figs. 8 and 9. The calibration graphs showed a good linearity (R^2>0.98) for all compounds in SPME using two calibration ranges, except for pentachlorobenzene, heptachlor, and phosmet. For most of them, determination coefficients were higher than 0.99. For HF-LPME, the calibration graphs were also good (R^2>0.96), although most of them use one calibration range. Residuals of the calibration curves, both in SPME as in HF-LPME, were always below 20%. To conclude, SPME presented a better linear range and determination coefficients than HF-LPME.

Detection and quantification limits

LOD and LOQ were calculated as the lowest analyte concentration that yields a signal/noise (S/N) ratio of 3 and 10, respectively. The values obtained (see Tables 2 and 3) were satisfactory and allowed the determination of these pesticides at trace levels. As can be seen, the LODs values varied between 0.1 ng/L (hexachlorobenzene, pentachlorobenzene, tetraconozole, endosulfan sulphate, and difenoconazole) and 28.8 ng/L (phosmet) in SPME, and between 0.2 ng/L (sulfotep) and 47.1 ng/L (acrinathrin) in HF-LPME, which were similar [12] or lower than other reported results in SPME [6, 7, 9, 11], or better than other published data in HF-LPME [29–35]. SPME showed better sensitivity than HF-LPME for all pesticides, except for sulfotep and clodinafop-propargyl. This can be explained, taking into account that the whole extract is injected in SPME, while only 10 μL (a fraction of 1/25 of the extract) was analyzed in HF-LPME.

Table 2 Retention time windows (RTWs), linear ranges, determination coefficients (R^2), and lower limits for SPME procedure

Pesticide	RTWs (min)	1st linear range (ng/L)	R^2	2nd linear range (ng/L)	R^2	LODs (ng/L)	LOQs (ng/L)
Pentachlorobenzene	10.10–10.19	1–100	0.9912	—	—	0.1	0.3
Ethoprophos	10.75–10.85	5–100	0.9980	100–500	0.9984	1.7	2.9
Sulfotep	10.93–11.04	5–100	0.9961	100–500	0.9962	1.4	3.7
α-Lindane	11.37–11.48	1–100	0.9985	100–1,000	0.9897	0.3	0.7
Hexachlorobenzene	11.45–11.56	1–100	0.9890	100–500	0.9852	0.1	0.4
Dichloran	11.54–11.72	10–100	0.9910	100–500	0.9892	3.2	7.0
β-Lindane	11.76–11.87	10–100	0.9996	100–1,000	0.9976	0.2	0.6
γ-lindane	11.87–11.99	10–100	0.9991	100–1,000	0.9983	0.3	0.7
Pyrimethanil	12.01–12.11	10–100	0.9997	100–1,000	0.9981	0.4	0.9
Etrimfos	12.08–12.19	5–50	0.9982	50–500	0.9971	1.0	2.6
δ-Lindane	12.31–12.42	5–50	0.9979	50–500	0.9949	1.8	4.2
Endosulfan ether	12.61–12.71	10–100	0.9996	100–1,000	0.9992	0.2	0.6
Chlorpyriphos-methyl	12.69–12.78	5–100	0.9998	100–500	0.9993	1.4	3.7
Vinclozolin	12.72–12.82	5–100	0.9991	100–500	0.9982	0.5	1.7
Alachlor	12.78–12.88	50–200	0.9964	200–1,000	0.9986	0.7	1.4
Parathion methyl	12.80–12.91	5–50	0.9928	50–500	0.9919	0.2	0.5
Heptachlor	13.01–13.13	3–100	0.9991	–	–	0.7	1.3
Pyrimiphos methyl	13.06–13.16	5–50	0.9976	50–200	0.9974	1.7	4.4
Terbutryn	13.14–13.26	5–50	0.9984	50–500	0.9967	1.6	3.7
Fenitrothion	13.18–13.29	5–50	0.9992	50–500	0.9954	0.6	2.1
Malathion	13.25–13.35	10–100	0.9910	100–500	0.9873	2.5	6.0
Chlorpyriphos	13.42–13.54	10–100	0.9955	100–1,000	0.9935	0.2	0.5
Parathion ethyl d-10 (IS)	13.50–13.61	–	–	–	–	–	–
Parathion ethyl	13.56–13.68	1–100	0.9982	100–1,000	0.9946	0.3	0.8
Tetraconazole	13.57–13.69	5–50	0.9996	50–500	0.9972	0.1	0.5
Triadimefon	13.62–13.72	5–50	0.9967	50–500	0.9949	0.7	2.8
Aldrin	13.64–13.75	30–100	0.9987	100–1,000	0.9941	1.1	2.8
Isodrin	13.97–14.04	50–200	0.9982	200–500	0.9962	15.8	38.4
Chlorfenvinphos	13.94–14.08	5–100	0.9921	100–1,000	0.9987	1.9	4.3
Pendimethalin	14.01–14.12	1–50	0.9972	50–500	0.9958	0.2	0.6
Endosulfan lactone	14.27–14.38	1–100	0.9995	100–1,000	0.9992	0.3	0.7
Quinalphos	14.30–14.41	5–100	0.9943	100–1,000	0.9926	1.6	3.4
Heptachlor epoxide	14.35–14.47	5–100	0.9964	100–500	0.9941	1.8	4.2
Procymidone	14.37–14.49	1–50	0.9973	50–1,000	0.9945	0.4	0.9
Endosulfan alfa	14.95–15.06	1–100	0.9984	100–1,000	0.9993	0.4	0.8
Hexaconazole	15.05–15.23	20–100	0.9993	100–500	0.9972	5.4	11.1
Fludioxonil	15.13–15.27	1–100	0.9974	100–500	0.9925	0.3	0.7
Oxyfluorfen	15.21–15.31	1–100	0.9996	100–500	0.9972	0.3	0.9
p-p′-DDE	15.23–15.34	1–100	0.9923	100–1,000	0.9900	0.3	0.6
Kresoxym methyl	15.24–15.38	5–50	0.9993	50–500	0.9985	2.1	4.6
Dieldrin	15.42–15.55	3–50	0.9993	50–500	0.9962	0.6	1.5
Cyproconazole	15.66–15.80	5–100	0.9910	100–1,000	0.9899	1.8	3.8
Endrin	15.82–15.93	10–100	0.9982	100–500	0.9997	3.4	7.5
Ethion	15.88–16.00	10–100	0.9987	100–1,000	0.9965	2.8	7.8
p-p′-DDD + o.p′-DDT	16.01–16.13	1–100	0.9973	100–1,000	0.9952	0.3	0.8
Endosulfan beta	16.01–16.16	1–100	0.9998	100–1,000	0.9991	0.3	0.8
Famphur	16.34–16.48	5–100	0.9954	100–500	0.9967	1.2	3.7
Benalaxyl	16.37–16.48	10–100	0.9992	100–1,000	0.9981	5.3	8.9
Carbophenothion	16.43–16.55	30–100	0.9981	100–500	0.9943	4.7	19.1

Table 2 (continued)

Pesticide	RTWs (min)	1st linear range (ng/L)	R^2	2nd linear range (ng/L)	R^2	LODs (ng/L)	LOQs (ng/L)
Propiconazole	16.49–16.61	5–100	0.9998	100–1,000	0.9996	1.7	3.8
Clodinafop-propargyl	16.54–16.64	30–100	0.9920	100–500	0.9883	8.9	21.3
p-p′-DDT	16.67–16.78	1–50	0.9981	50–1,000	0.9969	0.2	0.7
Endosulfan sulphate	16.68–16.79	1–100	0.9987	100–1,000	0.9962	0.1	0.5
Diflufenican	16.82–17.01	5–100	0.9987	100–1,000	0.9972	1.6	4.4
Nuarimol	16.90–17.01	5–100	0.9962	100–500	0.9941	1.2	3.3
Tebuconazole	16.90–17.12	5–100	0.9995	100–500	0.9991	1.7	4.4
Piperonyl butoxide	16.91–17.03	1–100	0.9956	100–1,000	0.9910	0.2	0.5
Bifenthrin	17.35–17.47	5–100	0.9992	100–500	0.9986	1.7	4.0
Tetramethrin	17.46–17.57	5–100	0.9982	100–500	0.9969	1.5	3.6
Bromopropylate	17.53–17.64	5–100	0.9993	100–1,000	0.9990	1.9	4.2
Phosmet	17.53–17.67	100–500	0.9992	–	–	28.8	65.2
Fenpropathrin	17.57–17.70	5–100	0.9981	100–1,000	0.9932	1.5	3.6
Methoxychlor	17.61–17.72	5–100	0.9927	100–500	0.9919	1.6	3.9
Tetradifon	18.07–18.19	1–100	0.9995	100–1,000	0.9991	0.3	0.8
λ-Cyhalotrin	18.31–18.42	30–200	0.9991	200–1,000	0.9983	6.0	15.6
Acrinathrin	18.34–18.53	30–100	0.9942	100–500	0.9921	11.6	26.3
Pyrazophos	18.57–18.67	5–100	0.9999	100–500	0.9998	2.1	4.4
Mirex	18.69–18.80	1–100	0.9987	100–500	0.9954	0.2	0.4
Fenarimol	18.72–18.84	1–100	0.9993	100–1000	0.9991	0.2	0.5
Permethrin	19.09–19.33	30–100	0.9934	100–1,000	0.9920	11.4	27.2
Pyridaben	19.37–19.49	10–100	0.9965	100–1,000	0.9912	4.6	9.1
Cyfluthrin	19.65–20.05	50–200	0.9970	200–1,000	0.9954	12.5	28.2
Cypermethrin	19.95–20.23	30–100	0.9987	100–1,000	0.9965	7.4	18.1
Flucytrinate	20.14–20.26	5–100	0.9976	100–1,000	0.9936	1.4	3.5
Difenoconazole	21.72–21.83	1–100	0.9981	100–500	0.9991	0.1	0.5
Deltamethrin	21.92–22.07	50–200	0.9861	200–1,000	0.9914	11.3	27.6
Azoxyzstrobin	22.19–22.30	10–100	0.9995	100–500	0.9984	3.1	7.3

Trueness

Trueness was evaluated in terms of recovery using a spiked in-house reference material at two concentration levels, 30 and 600 ng/L for aldrin, dieldrin, heptachlor, and heptachlor epoxide, taking into account the maximum level established by Spanish authorities for these compounds [47], and 50 and 100 ng/L for the rest of pesticides (n=5) in both microextraction techniques, except for endosulfan beta, famphur, carbophenothion, acrinathrin, flucytrinate, and deltamethrin at 100 and 150 ng/L by HF-LPME.

Recoveries in SPME ranged from 70.2% (phosmet) to 113.5% (tetramethrin), while in HF-LPME, they ranged from 70.0% (difenoconazole) to 119.5% (vinclozolin) (see Tables 4 and 5). As can be seen from Table 5, a lower number of pesticides were correctly recovered in HF-LPME (56 compounds) than in SPME (77 compounds). Therefore, information about recovery and precision of these compounds by HF-LPME is not included.

Precision

Precision was expressed as relative standard deviation (RSD), and it was evaluated as intra- and inter-day precision (Tables 4 and 5). First, five real samples at the same concentration levels evaluated during trueness study were subjected to both microextraction systems during the same day to evaluate the intra-day precision. Second, other five samples at the same concentration levels were, equally, subjected to both microextraction systems in three different days to evaluate the inter-day precision. In general, both microextraction techniques showed adequate RSD values for intra-day precision in the range 2.1% (malathion) to 19.4% (hexaconazole) and 4.3% (parathion ethyl) to 22.5% (δ-lindane) for the SPME and HF-LPME, respectively. In relation to inter-day precision, RSD values ranging from 5.2% (endrin) to 23.9% (mirex) for SPME, and 8.4% (pyrazophos) to 27.3% (difenoconazole) for HF-LPME were obtained. However, most of the target compounds

Table 3 Linear ranges, determination coefficients (R^2) and lower limits for HF-LPME procedure

Pesticide	1st linear range (ng/L)	R^2	2nd linear range (ng/L)	R^2	LODs (ng/L)	LOQs (ng/L)
Pentachlorobenzene	10–500	0.9948	–	–	1.5	3.2
Ethoprophos	50–200	0.9874	–	–	13.8	36.2
Sulfotep	1–500	0.9932	–	–	0.2	0.7
α-Lindane	30–500	0.9917	–	–	4.1	18.7
Hexachlorobenzene	10–500	0.9978	–	–	1.5	4.8
β-Lindane	30–500	0.9930	–	–	7.7	27.3
γ-Lindane	50–500	0.9994	–	–	5.9	18.9
Pyrimethanil	30–200	0.9932	–	–	17.1	43.1
Etrimfos	50–500	0.9907	–	–	6.6	22.9
δ-Lindane	100–1000	0.9943	–	–	17.3	44.0
Endosulfan ether	30–500	0.9741	–	–	6.2	24.8
Chlorpyriphos-methyl	30–500	0.9983	–	–	12.4	27.1
Vinclozoline	30–500	0.9944	–	–	10.8	24.2
Alachlor	50–200	0.9940	200–1,000	0.9983	6.3	9.3
Heptachlor	30–500	0.9898	–	–	2.9	7.5
Fenitrothion	50–200	0.9978	200–1,000	0.9979	17.2	45.5
Malathion	30–500	0.9898	–	–	10.9	27.7
Chlorpyriphos	50–500	0.9810	–	–	7.9	15.1
Parathion ethyl d-10	–	–	–	–	–	–
Parathion ethyl	50–500	0.9853	–	–	8.6	21.6
Triadimefon	50–500	0.9810	–	–	11.9	31.5
Aldrin	30–100	0.9872	100–500	0.9862	7.3	12.1
Isodrin	50–500	0.9924	–	–	17.8	42.8
Chlorfenvinphos	50–200	0.9975	200–1,000	0.9854	6.1	14.7
Pendimethalin	30–500	0.9766	–	–	4.4	17.5
Endosulfan lactone	50–500	0.9906	–	–	15.8	39.3
Quinalphos	50–500	0.9836	–	–	12.5	46.2
Procymidone	10–500	0.9857	–	–	1.3	4.5
Endosulfan alfa	30–500	0.9849	–	–	5.5	18.4
p-p′-DDE	30–500	0.9813	–	–	5.8	14.2
Kresoxym methyl	50–200	0.9878	200–500	0.9812	15.6	42.3
Dieldrin	30–600	0.9851	–	–	11.7	28.3
Ethion	50–500	0.9935	–	–	7.5	27.3
p-p′-DDD + o.p′-DDT	10–500	0.9962	–	–	2.0	7.3
Endosulfan beta	100–500	0.9698	–	–	35.9	90.5
Famphur	100–500	0.9794	–	–	42.4	82.6
Carbophenothion	100–1000	0.9751	–	–	28.5	69.2
Clodinafop-propargyl	30–500	0.9805	–	–	7.1	21.1
p-p′-DDT	10–500	0.9931	–	–	1.6	6.3
Endosulfan sulphate	50–200	0.9801	200–1,000	0.9823	16.3	41.4
Diflufenican	100–1000	0.9951	–	–	15.1	41.8
Piperonyl butoxide	50–500	0.9988	–	–	17.0	41.4
Bifenthrin	30–500	0.9978	–	–	11.2	23.0
Methoxychlor	30–500	0.9860	–	–	6.4	22.0
Phosmet	50–500	0.9910	–	–	8.3	21.8
Tetradifon	50–200	0.9846	200–1,000	0.9991	1.5	3.8
λ-Cyhalotrin	50–500	0.9954	–	–	17.2	41.2
Acrinathrin	100–1000	0.9948	–	–	47.1	89.5
Pyrazophos	50–500	0.9893	–	–	18.4	44.2

Table 3 (continued)

Pesticide	1st linear range (ng/L)	R^2	2nd linear range (ng/L)	R^2	LODs (ng/L)	LOQs (ng/L)
Mirex	30–500	0.9953	–	–	4.4	10.8
Fenarimol	50–1000	0.9952	–	–	11.5	30.2
Permethrin	50–500	0.9897	–	–	15.7	40.4
Pyridaben	30–500	0.9879	–	–	7.5	25.4
Flucytrinate	10–1000	0.9888	–	–	33.5	85.1
Difenoconazole	50–200	0.9941	200–500	0.9821	16.6	43.0
Deltamethrin	50–200	0.9798	200–500	0.9871	30.5	72.4

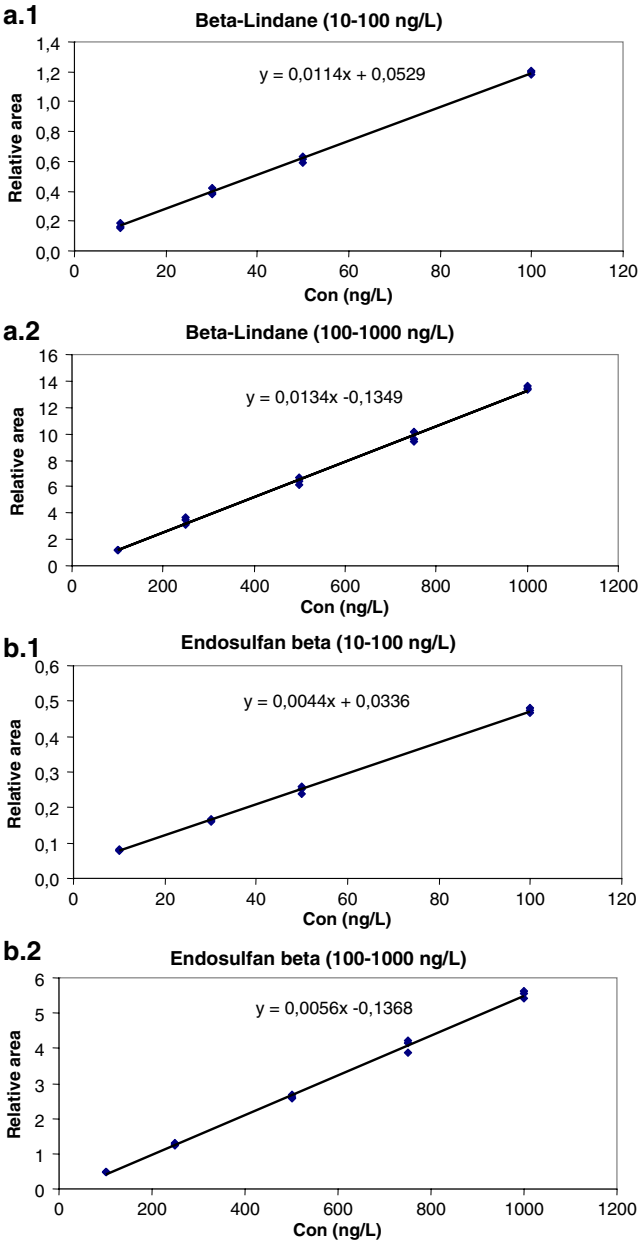

Fig. 8 Calibration curves for some pesticides after the application of the SPME method: β-lindane, *a.1* first calibration range and *a.2* second calibration range; endosulphan beta, *b.1* first calibration range and *b.2* second calibration

showed better intra- and inter-day precision values in SPME than in HF-LPME. This can be explained by considering that SPME is a fully automated method, whereas for HF-LPME procedure, the operator skill has a great influence. Therefore, SPME shows a better precision than HF-LPME. In addition, it can be observed that the highest precision values, i.e. 23.9 and 27.3% for SPME and HF-LPME respectively, were obtained at the low concentration level studied (50 ng/L). These inter-day precision values are acceptable taking into account the low spiking level.

Uncertainty

The "bottom–up" approach was used in order to estimate each individual uncertainty for every single step of the measurement process and obtain the combined standard uncertainty from the sum of each contribution. Global

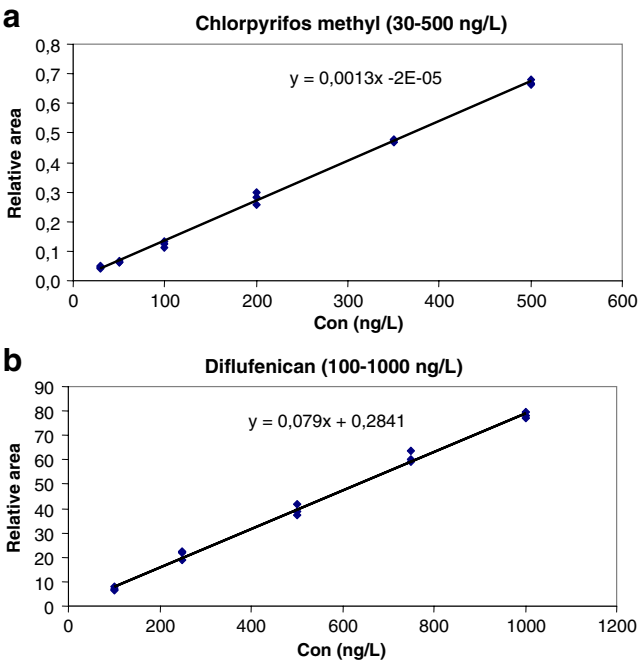

Fig. 9 Calibration curves for some pesticides after the application of the HF-LPME method: **a** chlorpyriphos-methyl and **b** diflufenican

Table 4 Recovery and precision values for SPME procedure

Pesticide	50 ng/L			100 ng/L			Expanded uncertainty (%)[b]
	Recovery (%)	Intraday precision (%)	Interday precision (%)	Recovery (%)	Intraday precision (%)	Interday precision (%)	
Pentachlorobenzene	85.0	11.7	18.9	90.3	9.7	14.0	20.2
Ethoprophos	70.3	6.0	13.9	84.0	5.1	9.9	15.7
Sulfotep	76.0	11.5	16.4	76.0	9.2	9.8	16.3
α-Lindane	71.0	3.6	9.6	76.0	7.0	15.7	21.5
Hexachlorobenzene	92.0	13.7	17.9	106.0	11.1	17.6	22.8
Dichloran	95.5	6.0	9.7	101.8	3.2	7.4	13.6
β-Lindane	78.5	3.8	12.4	73.5	4.8	18.0	24.6
γ-Lindane	78.0	5.1	11.3	77.8	4.9	9.2	14.3
Pyrimethanil	88.5	7.7	14.4	82.0	5.9	7.9	12.4
Etrimfos	86.7	7.3	16.4	96.0	3.8	11.2	16.7
δ-Lindane	77.5	10.0	11.7	74.8	3.7	6.4	11.9
Endosulfan ether	95.5	7.9	12.1	87.5	7.3	9.4	14.0
Chlorpyriphos-methyl	88.0	12.0	16.7	82.8	7.1	8.5	14.8
Vinclozoline	84.0	11.3	14.1	74.5	7.9	9.0	15.3
Alachlor	79.5	11.5	16.1	73.0	6.9	10.5	16.0
Parathion methyl	94.0	4.7	6.3	89.8	6.4	5.6	9.6
Heptachlor[a]	84.0	12.7	19.4	101.3	10.1	16.8	21.5
Pyrimiphos methyl	91.5	12.0	12.4	89.8	4.0	8.1	15.8
Terbutryn	87.0	6.6	8.2	94.3	3.4	6.0	13.6
Fenitrothion	86.0	8.1	13.1	83.0	4.2	8.1	14.9
Malathion	93.0	4.5	10.6	98.3	2.1	9.2	16.0
Chlorpyriphos	72.0	17.2	19.9	71.7	12.2	16.5	23.1
Parathion ethyl	80.5	9.4	10.5	86.0	5.5	7.7	13.5
Tetraconazole	82.5	11.1	11.4	73.7	4.4	6.2	12.8
Triadimefon	76.0	5.7	11.6	77.5	7.5	12.2	18.0
Aldrin[a]	92.7	17.1	20.1	101.0	16.4	18.1	23.6
Isodrin	77.4	13.8	17.7	78.3	15.4	19.9	24.6
Chlorfenvinphos	81.5	4.6	7.6	79.0	4.6	6.3	12.9
Pendimethalin	76.7	18.9	21.5	90.3	11.8	13.8	19.2
Endosulfan lactone	88.7	11.1	15.3	83.3	10.2	14.2	19.9
Quinalphos	82.5	10.4	11.3	88.8	6.8	7.2	14.1
Heptachlor epoxide[a]	77.5	12.8	18.6	73.0	10.0	14.3	20.4
Procymidone	84.0	8.0	11.1	74.0	5.3	9.3	15.7
Endosulfan alfa	94.7	8.7	9.9	90.8	6.4	8.9	16.2
Hexaconazole	75.5	19.4	21.7	77.8	14.9	18.4	23.5
Fludioxonil	71.0	4.9	9.6	75.5	6.7	11.1	17.2
Oxyfluorfen	106.0	8.0	15.0	93.3	12.4	11.9	18.1
p-*p*′-DDE	89.3	18.1	19.0	79.7	17.2	19.5	25.0
Kresoxym methyl	81.3	8.6	9.7	78.0	2.8	7.3	11.3
Dieldrin[a]	77.3	8.5	11.5	76.7	6.0	9.5	14.6
Cyproconazole	86.0	6.3	9.2	89.5	7.4	16.8	22.7
Endrin	84.0	7.0	10.1	76.8	3.6	5.2	9.8
Ethion	85.5	7.6	10.2	83.8	4.2	6.4	12.3
p-*p*′-DDD+*o*,*p*′-DDT	92.7	15.2	20.6	95.0	14.0	16.8	21.6
Endosulfan beta	95.3	4.4	8.7	91.7	5.8	10.7	16.9
Famphur	77.3	17.6	19.2	90.0	11.8	16.4	22.5

Table 4 (continued)

Pesticide	50ng/L			100ng/L			Expanded uncertainty (%)[b]
	Recovery (%)	Intraday precision (%)	Interday precision (%)	Recovery (%)	Intraday precision (%)	Interday precision (%)	
Benalaxyl	98.0	7.1	13.4	82.5	9.2	17.8	23.1
Carbophenothion	79.5	5.9	11.0	98.3	9.5	15.0	21.9
Propiconazole	77.3	10.5	11.5	77.5	6.5	7.9	14.5
Clodinafop-propargyl	82.5	9.2	12.4	77.0	10.1	13.1	18.1
p-p'-DDT	87.3	16.2	13.1	82.8	16.5	9.5	13.9
Endosulfan sulphate	85.5	8.8	12.7	77.0	6.5	10.8	16.2
Diflufenican	82.7	7.6	12.9	88.5	5.5	8.8	15.8
Nuarimol	71.0	8.8	12.6	70.7	4.1	6.1	13.5
Tebuconazole	106.0	4.1	6.4	94.7	2.2	6.0	12.9
Piperonyl butoxide	81.5	12.7	16.2	86.8	8.8	9.0	17.2
Bifenthrin	72.0	12.4	17.3	70.5	6.6	18.1	24.1
Tetramethrin	113.5	8.4	12.3	107.7	10.6	14.7	20.0
Bromopropylate	90.5	7.9	16.5	72.0	6.5	12.7	18.4
Phosmet	70.2	14.0	18.5	71.7	12.4	17.6	19.1
Fenpropathrin	80.7	12.2	13.4	74.3	5.6	8.3	14.2
Methoxychlor	82.0	6.5	9.9	70.3	4.3	6.9	13.9
Tetradifon	93.5	10.1	13.5	76.8	7.9	9.7	17.1
λ-Cyhalotrin	77.3	9.1	11.0	89.7	10.3	14.4	21.4
Acrinathrin	78.4	13.0	17.0	103.5	8.7	11.1	17.3
Pyrazophos	96.0	10.8	13.3	83.0	9.4	9.6	16.2
Mirex	83.2	18.6	23.9	77.5	17.0	20.1	25.2
Fenarimol	76.0	7.7	12.0	74.5	9.4	13.9	18.9
Permethrin	104.7	6.7	10.1	100.5	8.6	10.5	15.3
Pyridaben	93.0	8.0	12.6	77.3	5.6	9.3	14.6
Cyfluthrin	111.3	11.2	18.3	94.0	10.2	15.2	22.1
Cypermethrin	112.0	9.9	14.1	93.5	7.4	11.1	17.8
Flucytrinate	79.5	13.0	18.8	86.3	8.8	12.1	18.3
Difenoconazole	84.7	5.9	10.0	81.5	3.6	8.7	15.6
Deltamethrin	81.3	12.6	16.6	85.7	7.0	10.2	17.2
Azoxyzstrobin	72.7	6.9	11.8	92.8	11.0	15.4	21.4

[a] 30 ng/L and 60 ng/L respectively for recovery and precision studies

[b] 100 ng/L

uncertainty was determined for all of the pesticides at the level of 100 ng/L by multiplying the previous result by a coverage factor of $K=2$ (confidence level of 95%). Uncertainty values in SPME ranged from 9.6% (parathion methyl) to 25.2% (mirex), while in HF-LPME, they ranged from 13.3% (pyrazophos) to 27.5% (famphur) (see Tables 4 and 5).

Analysis of real samples

The proposed methods were applied to the analysis of 41 samples of water: ten samples of natural springs (F1-10), 25 of tap water (T1-25), and six of commercial mineral water (B1-6).

In order to assure the quality of the results when the proposed methods were applied, an internal quality control was carried out in every batch of samples. This quality control implies the analysis of the following: a reagent blank sample in order to eliminate false positives by contamination in the extraction process; a blank sample spiked at the concentration of the second calibration level of each pesticide in order to control the extraction efficiency; and a calibration curve prepared daily in pure solvent (HF-LPME) or distilled water (SPME).

Table 5 Recovery and precision values for HF-LPME procedure

Pesticide	50 ng/L			100 ng/L			Expanded uncertainty (%)[c]
	Recovery (%)	Intraday precision (%)	Interday precision (%)	Recovery (%)	Intraday precision (%)	Interday precision (%)	
Pentachlorobenzene	82.8	12.4	14.7	83.3	17.1	18.5	24.9
Ethoprophos	85.2	8.9	16.7	104.6	16.3	16.1	23.4
Sulfotep	72.4	17.6	19.9	75.3	14.1	19.6	24.3
α-Lindane	89.2	16.2	12.9	86.6	13.6	16.0	21.8
Hexachlorobenzene	88.4	14.2	16.1	87.8	12.9	17.6	23.1
β-Lindane	81.2	18.5	18.1	100.8	8.3	11.9	18.4
γ-Lindane	92.0	20.3	18.9	90.0	19.4	19.8	24.6
Pyrimethanil	106.5	12.5	16.8	103.3	14.0	18.4	24.0
Etrimfos	100.2	14.9	20.5	90.5	18.7	17.5	23.8
δ-Lindane	72.1	22.5	23.8	81.0	21.5	20.1	26.1
Endosulfan ether	106.5	19.7	15.5	83.4	12.6	16.3	23.4
Chlorpyriphos-methyl	115.5	13.7	16.4	80.2	11.1	20.0	25.7
Vinclozoline	119.5	21.5	22.4	81.5	10.8	19.6	25.2
Alachlor	96.5	13.5	19.4	74.8	12.2	12.1	19.6
Heptachlor[a]	100.0	14.7	15.6	100.0	16.1	18.8	24.5
Fenitrothion	92.8	12.5	19.5	71.0	10.5	18.5	25.2
Malathion	100.4	15.3	15.5	107.8	14.7	19.4	25.9
Chlorpyriphos	106.0	20.3	22.0	116.5	11.7	18.6	24.7
Parathion ethyl	98.4	8.6	16.8	105	4.3	11.9	18.9
Triadimefon	84.8	14.4	16.2	85.5	17.0	20.8	26.0
Aldrin[a]	80.8	13.6	18.8	98.0	14.2	17.2	23.4
Isodrin	110.0	13.0	13.8	91.2	13.1	17.2	24.0
Chlorfenvinphos	71.0	18.1	21.5	81.0	15.1	15.5	21.6
Pendimethalin	80.0	22.2	21.2	102.8	8.5	20.0	24.7
Endosulfan lactone	86.8	17.7	19.0	104.3	13.9	18.7	25.1
Quinalphos	111.5	13.0	10.1	99.8	13.4	18.3	24.9
Procymidone	94.0	10.1	12.8	102.8	6.6	18.7	24.7
Endosulfan alfa	96.7	17.5	19.5	82.7	19.7	18.2	25.2
p-p′-DDE	106.0	14.4	18.9	106.2	17.4	19.9	25.9
Kresoxym methyl	95.0	17.6	18.1	96.8	8.0	16.5	23.8
Dieldrin[a]	116.7	18.0	20.6	115.0	13.7	19.7	26.1
Ethion	108.4	12.7	17.2	105.4	5.0	16.0	24.6
p-p′-DDD+*o,p′*-DDT	92.0	13.2	12.4	100.2	12.7	18.8	25.2
Endosulfan beta[b]	79.7	16.7	19.8	105.3	14.2	17.7	23.6
Famphur[b]	76.5	12.4	17.9	82.2	10.9	20.2	27.4
Carbophenothion[b]	113.2	8.8	15.8	106.4	6.2	10.9	17.3
Clodinafop-propargyl	94.5	14.5	13.9	100.6	13.2	19.3	25.1
p-p′-DDT	101.5	15.5	18.9	100.4	13.6	19.6	25.9
Endosulfan sulphate	106.0	16.5	18.5	88.0	20.3	19.8	26.3
Diflufenican	83.2	20.3	22.5	91.6	19.2	15.8	22.0
Piperonyl butoxide	108.4	18.1	13.7	99.6	7.2	12.3	18.9
Bifenthrin	101.2	10.2	10.1	103.0	13.4	15.8	21.5
Methoxychlor	113.0	10.4	15.1	117.6	7.3	17.3	22.8
Phosmet	94.8	16.7	19.1	81.3	12.8	17.8	23.2
Tetradifon	111.5	18.7	19.6	96.7	12.9	13.1	19.6
λ-Cyhalotrin	102.8	10.9	9.0	90.0	6.7	19.6	23.1

Table 5 (continued)

Pesticide	50 ng/L			100 ng/L			Expanded uncertainty (%)[c]
	Recovery (%)	Intraday precision (%)	Interday precision (%)	Recovery (%)	Intraday precision (%)	Interday precision (%)	
Acrinathrin[b]	108.0	7.4	14.3	96.5	7.4	12.3	18.5
Pyrazophos	111.6	17.4	19.1	112.4	10.0	8.4	13.3
Mirex	86.5	16.7	18.2	96.8	7.8	15.2	21.0
Fenarimol	72.6	19.5	22.4	78.5	17.5	20.7	26.7
Permethrin	85.9	16.5	19.4	114.6	15.1	18.4	24.2
Pyridaben	99.0	10.2	10.6	108.2	9.8	18.8	25.9
Flucytrinate[b]	86.5	14.8	16.9	93.8	12.2	19.8	26.4
Difenoconazole	70.0	13.4	27.3	73.0	16.6	14.9	19.8
Deltamethrin[b]	103.2	21.0	23.8	119.3	20.2	20.6	26.7

[a] 30 and 60 ng/L

[b] 100 and 150 ng/L for recovery and precision studies

[c] 100 ng/L

No pesticides were detected in the analyzed waters above legislated limits [1]. Most of them were not found above the LODs, and a small number of the analyzed samples presented pesticides at concentrations over the first calibration level (Table 6). As it can be seen, similar results were obtained when both methods were applied, except for pirimethanil and fenitrothion, which were detected in two samples when SPME procedure was used, but they were not detected when HF-LPME procedure was applied because the concentrations were lower than the estimated LOQ.

Conclusions

Two microextraction techniques, SPME and HF-LPME, were proposed for the simultaneous extraction of 77 pesticides in drinking water using GC-QqQ-MS/MS. SPME is simple, easy to handle, highly automatable and does not require much equipment, and in general, it provides better linearity, precision, and lower limits (LOD and LOQ) than HF-LPME. However, SPME has several disadvantages, such as the price of the fibers, the lifetime of fibers, and the presence of carryover. On the other hand, HF-LPME is also simple to use and operate, is inexpensive, and removes the carryover effect since the membranes are used only once. However, worse precision was obtained, probably because extraction procedure is not automated.

The total time of analysis in SPME and HF-LPME for ten samples was approximately 810 min; however, HF-LPME method requires more manual operations than SPME. On the other hand, the number of pesticides that can be analyzed by SPME with adequate performance characteristics is higher (77 compounds) than by HF-LPME (56 pesticides). Overall, the SPME-GC-MS method offers the best compromise in quality, speed, and reliability.

Table 6 Concentration of pesticides detected in the analyzed samples

Pesticide	Pesticide concentration (ng/L)									
	F7		T12		T2		F3		F10	
	HF-LPME	SPME	HF-LPME	SPME	HF-LPME	SPME	HF-LPME	SPME	HF-LPME	SPME
Piperonyl butoxide	51	45								
Pyrimethanil			ND[a]	36						
Fenitrothion					ND	41				
Malathion							35	32		
Chlorpyriphos									43	50

[a] Not detected

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