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Determination of organochlorine pesticides in complex matrices by single-drop microextraction coupled to gas chromatography–mass spectrometry

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ABSTRACT

A rapid and simple single-drop microextraction method (SDME) has been used to preconcentrate eighteen organochlorine pesticides (OCPs) from water samples with a complex matrix. Exposing two microlitre toluene drop to an aqueous sample contaminated with OCPs proved an excellent preconcentration method prior to analysis by gas chromatography–mass spectrometry (GC–MS). A Plackett-Burman design was used for screening and a central composite design for optimizing the significant variables in order to evaluate several possibly influential and/or interacting factors. The studied variables were drop volume, aqueous sample volume, agitation speed, ionic strength and extraction time. The optimum experimental conditions of the proposed SDME method were: 2 μL toluene microdrop exposed for 37 min to 10 mL of the aqueous sample containing 0% w/v NaCl and stirred at 380 rpm.

The calculated calibration curves gave high-level linearity for all target analytes with correlation coefficients ranging between 0.9991 and 0.9999. The repeatability of the proposed method, expressed as relative standard deviation, varied between 5.9 and 9.9% ($n = 8$). The detection limits were in the range of 0.022–0.101 μg L^{-1} using GC–MS with selective ion monitoring. The LOD values obtained are able to detect these OCPs in aqueous matrices as required by EPA Method 625. Analysis of spiked effluent wastewater samples revealed that the matrix had no effect on extraction for eleven of the analytes but exerted notable effect for the other analytes.

1. Introduction

The use of pesticides in food production affords numerous benefits in terms of increasing production and quality; however, it is harmful to human health if applied inappropriately. Organochlorine pesticides (OCPs) are associated with many chronic diseases. Moreover, these compounds may behave as pseudohormones, disrupting the endocrine system in wildlife, humans, as well as aquatic biota. Many problems have been linked to these endocrine disrupters, such as neurological damage, Parkinson's disease, birth defects, respiratory illness, early sexual development, behavioural changes, breasts cancer, lowered sperm counts and immune system dysfunction [1,2].

Although the use of OCPs has been banned by the Stockholm Convention of Certain Persistent Organic Pollutants, previous widespread use of these pesticides caused significant environmental contamination. Therefore, monitoring trace levels of OCPs in food and waters is still imperative for health protection and environmental control because OCPs are of difficult degradation, easy accumulation and high toxicity [3].

Recently, researchers have paid more attention to developing analytical approaches to OCPs detection, including gas chromatography (GC-ECD) [4–6] and gas chromatography–mass spectrometry (GC–MS) [7,8]. Most OCPs determinations require a preparative processes, such as liquid–liquid extraction (LLE) [9], solid-phase extraction (SPE) [10,11], solid-phase microextraction (SPME) [12–14] and hollow fiber-liquid phase microextraction (HF-LPME) [15,16]. Liquid–liquid extraction (LLE) is one of the oldest pretreatment procedures and is commonly used because of its simplicity and low cost. However, conventional LLE requires relatively large volumes of organic solvents, is time-consuming, labor-intensive and hazardous to health and environment. SPE demands a large volume of organic solvents, analytes may be adsorbed and complex matrices can cause settling in cartridges. SPME, a robust, sensitive and accurate method, has achieved tremendous success and is widely used for testing foods and environmental pollutants; however, its application has been hindered by its relatively high price and fragile coating layer. Fibers tend to degrade with use and the partial loss of stationary phase leads to peaks that may coelute with the analytes, thus affecting precision. In addition, when SPME is used, carryover of the fiber between

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extractions may occur and is hard to eliminate even at high desorption temperatures.

Single-drop microextration (SDME), developed in 1996 by Jeannot and Cantwell [17], provides an alternative technique, which integrates sampling, extracting and concentrating into a single step. The advantages of simple processes using fewer organic solvents in SDME are attractive. Other advantages of SDME include low cost, since it requires only common laboratory equipment, as well as simplicity, speed and the potential for easy automation.

In recent years, SDME has become highly developed and is used for OCPs extraction [18–22]. de Jager and Andrews [18] reported a preliminary work using SDME for the extraction of 11 kinds of OCPs from river water. Qian and He [20] introduced a funnel-form single-drop microextraction to extract 11 organochlorine and two pyrethroid pesticides for the analysis of tea samples by gas chromatography-electron-capture detection (GC-ECD). Zhang et al. [22] investigated the extraction of 9 kinds of OCPs from vegetable samples by suspending a 1.00 μL mixed drop of *p*-xylene and acetone (8:2). Other applications of SDME for the extraction of OCPs from fish [21] and, natural and tap water [19] have also been developed.

Chemometrics have been employed to optimize analytical methods, whose advantages include the need for fewer resources (i.e., time, reagents and experimental work). Chemometrics offer a sound theoretical basis to optimize chemical systems and processes. Moreover, chemometric tools, which employ mathematical models, can assess the statistical significance of the effects of the independent variables under study as well as evaluating the effects of their interaction. Multivariate optimization employs designs in which the levels of all variables can be changed simultaneously. Some applications make use of the microextraction and chemometric aspects with SDME simultaneously [23–25]. To our knowledge, no studies have been reported using multivariate analysis to optimize the SDME of organochlorine pesticides.

In this work, SDME was used to extract eighteen OCPs, some of which have not been considered previously, from complex aqueous matrices, and then, determinations were carried out on GC–MS. SDME extraction parameters (i.e., drop volume, aqueous sample volume, agitation speed, ionic strength and extraction time) were optimized by the experimental design. The procedure was then applied to the determination of organochlorine pesticides in water samples.

2. Experimental

2.1. Chemicals and "real-world" water samples

Eighteen organochlorine pesticides were used in the present study, namely: alpha-hexachlorocyclohexane (HCH), beta-HCH, gamma-HCH, delta-HCH, Heptachlor, Aldrin, Heptachlor epoxide, alpha-Endosulfan, *p*,*p*′-DDE, Dieldrin, Endrin, beta-Endosulfan, *p*,*p*′-DDD, Endrin aldehyde, Endosulfan sulfate, *p*,*p*′-DDT, Endrin ketone and Methoxychlor, all obtained from Dr. Ehrenstorfer (Agsburg, Germany). Methanol, toluene, isooctane and hexane were pesticide-grade and were obtained from Sigma–Aldrich (St. Louis, MO, USA). De-ionized water was prepared in a water purification system (Gradient A10) supplied by Millipore (Billerica, MA, USA). Sodium chloride from Merck (Darmstadt, Germany) was used to adjust the ionic strength of the aqueous samples. Stock standard solution of 10 mg L^{-1} of target compounds was prepared in methanol. Working solutions were prepared by dilution of standard stock solution. All solutions were stored in the dark at 4 °C.

The recovery studies were carried out using effluent wastewater (Torrevieja, Spain) from a municipal and industrial wastewater treatment plant (WWTP). Samples were stored in the dark at 4 °C and were analyzed after being filtered through common lab filter paper. Initial analysis confirmed that they were free of all target analytes.

2.2. Single-drop microextraction (SDME)

For SDME, 10 mL of the sample solution was placed in a 15 mL crimp top glass vial containing a stirring bar and fitted with a Mininert Valve from Supelco (Bellefonte, PA, USA). In order to eliminate volatilization losses, all aqueous samples were freshly prepared before each SDME experiment.

A 10 μL SGE syringe (Melbourne, Australia) with a bevel-needle tip (length: 5.0 cm, i.d.: 0.047 cm, bevel 26°) typically containing 2 μL of toluene was clamped above the vial containing the water sample. The microsyringe was then lowered and its needle passed through the Mininert valve until the tip of the needle was immersed in the sample. The plunger was depressed and the 2-μL drop of the extractant phase was exposed to the sample with magnetic stirring at 380 rpm. The analytes were then allowed to partition between the aqueous sample and toluene droplet at room temperature for 37 min (unless otherwise stated in the text). After extraction, the microdrop was retracted into the microsyringe and then injected into the GC–MS system for analysis.

2.3. GC–MS determination

All analyses were carried-out on a Varian 3800-Saturn 2000 Gas Chromatography/Mass Spectrometer system (Walnut Creek, CA, USA) equipped with a VF-5-Ms Factor-four Varian column (30 m × 0.25 mm, 0.25 μm). The mass spectrometer employed was an ion trap (20 μA) with 0.82 s of scan time. The injector was maintained at 250 °C and operated in the splitless mode with the split closed for 0.75 min. Helium (>99.999% pure) was used as the carrier gas at a flow rate of 1.0 mL min^{-1}. The column oven was initially set at 70 °C for 2 min, programmed to 160 °C at a 15 °C min^{-1} rate for 5 min, and finally to 240 °C at 3.5 °C min^{-1} rate, where it was held for 5 min. The interface temperature was set at 200 °C and the detector voltage at 4 V. A 10-min solvent cut time was allowed for all analyses. The base peak ion and two other significant ions of each analyte were chosen as the quantifying ions. The base peak ions (*m*/*z*) for the target analytes were: alpha, beta, gamma and delta-HCH: 183, Heptachlor: 272, Aldrin: 263, Heptachlor epoxide: 289, alpha-Endosulfan: 241, *p*,*p*′-DDE: 318, Dieldrin: 79, Endrin: 281, beta-Endosulfan: 195, *p*,*p*′-DDD: 235, Endrin aldehyde: 345, Endosulfan sulfate: 272, *p*,*p*′-DDT: 235, Endrin ketone: 317 and Methoxychlor: 227. Prior to quantification, the identification of all target compounds was based on their mass spectra and GC retention times. Fig. 1 shows a typical chromatogram of a real spiked aqueous sample containing 10 μg L^{-1} of all the target analytes.

2.4. Data handling and processing

According to previous works, the response of the instrument used in the screening study was based on each area of the individual peaks eluted during GC–MS analysis (see Section 3.1.1 for explanation) [26]. By contrast, the optimization study was based on the sum of all the areas of the individual peaks [23,27–29], in order to obtain one unique set of optimum conditions for the extraction of all the organochlorine pesticides.

Experimental design matrices were constructed and results were evaluated using the Statgraphics Statistical Computer Package "Statgraphics Plus 5.1".

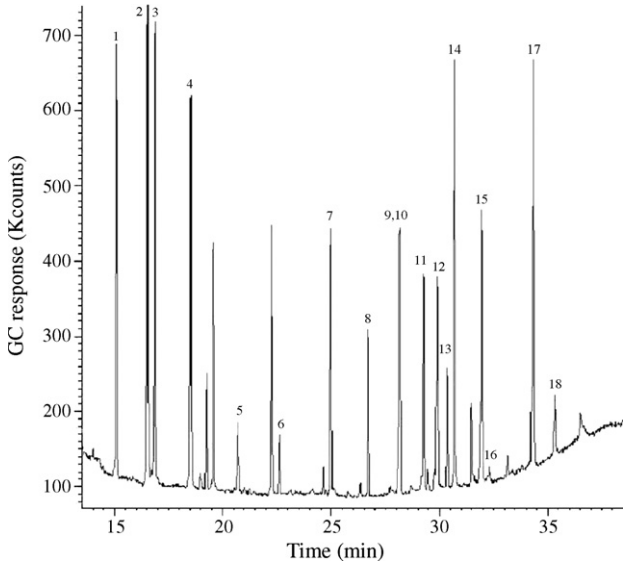

Fig. 1. Typical chromatogram of organochlorine pesticides studied. (1) α-HCH; (2) β-HCH; (3) γ-HCH; (4) δ-HCH; (5) Heptachlor; (6) Aldrin; (7) Heptachlor epoxide; (8) α-Endosulfan; (9) *p,p′*-DDE; (10) Dieldrin; (11) Endrin; (12) β-Endosulfan; (13) *p,p′*-DDD; (14) Endrin aldehyde; (15) Endosulfan sulfate; (16) *p,p′*-DDT; (17) Endrin ketone; (18) Methoxychlor.

3. Results and discussion

3.1. Study of experimental variables involved in SDME

The first step in the optimization procedure was to select an appropriate extraction solvent. Toluene, *n*-hexane and isooctane were tested as potential acceptor phases. Solvent selectivity was evaluated after exposing 2 μL of organic solvent drop immersed in a10 mL de-ionized water sample, spiked at 100 μg L^{-1} with all target analytes and stirred at 300 rpm for 10 min. Enrichment factors were calculated for each solvent and are shown in Table 1. Of the three solvents tested toluene gave the best results, as can be seen in Table 1; contrarily, hexane presented drop-stability problems. Therefore, toluene was chosen for the next optimization procedure for the extractant phase.

Different variables can affect the extraction yield in the SDME procedure and in most cases they are correlated. Therefore, a multivariate approach is recommended for their optimization. However, some of them might not have a significant effect and they can, thus, be obviated. In this respect, a screening study, prior to optimization, is helpful in order to assess the significant variables involved in the analytical system under study.

In this case, based on the literature and previous experience of our group [23,24,26], the influence of five variables, namely drop volume, sample volume, ionic strength, agitation speed and extraction time, were studied in order to maximize the extraction yield of the SDME procedure.

3.1.1. Screening design

Screening is the first step in the efficient assessment of the variables involved in the analytical system under study. If a large number of variables are involved, reduced factorial designs are employed. One particular type of those designs is the Plackett-Burman design [30], which assumes that the interactions can be completely ignored and so the main effects are calculated with a reduced number of experiments. A saturated Plackett-Burman matrix was employed because many parameters were to be tested. A matrix with 11 variables (five real variables and six dummy variables) was used. The effects of dummy variables are used to estimate the experimental error used in the statistical interpretation [31,32].

For each variable, shown in Table 2, two levels were considered. The matrix of the Plackett-Burman design comprised 12 experiments. The experiments were carried out at random in order to nullify the effect of extraneous or nuisance variables, by using aqueous solutions of 100 μg L^{-1} and evaluating the GC peak area for each experiment and for each analyte. The screening study was done with each area because there were many variables and many analytes. Therefore, it is possible that confusion could arise when the sum of the area is used, because of the different effects exerted by the variables on the different analytes. The aim of this preliminary study was to choose the significant variables in common for all the analytes rather than the optimum conditions, therefore, the use of individual peak area is the best option, and the significant variables for all the analytes should be compared.

An ANOVA test was used to evaluate the data and statistically significant effects were determined using a *t*-test with 95% probability [31,32] and visualized by using main effects Pareto charts (Fig. 2). The Pareto charts, shown in Fig. 2, belong to alpha, beta, delta-HCH and Endrin aldehyde. The charts for the rest of the analytes are not shown as they are similar.

According to the results, agitation speed was the most significant variable having a positive effect for almost all target analytes. The next most significant variables were ionic strength followed by extraction time, exerting a negative and a positive effect, respectively.

Pareto charts also reveals that drop volume appeared as a non-significant effect showing a negative effect for all the analytes except for the Endrin aldehyde. In general, an increase in the signal is expected on increasing droplet volume; however, larger organic solvent drops sometimes require extended equilibration times because mass transfer inside the drop is by diffusion alone [33]. However, in this study 2 μL of organic solvent drop was cho-

Table 1
Enrichment factor of various organic solvents[a].

Analyte	Enrichment (-fold)		
	Toluene	*n*-Hexane	Isooctane
Alpha-HCH	68	29	35
Beta-HCH	61	24	30
Gamma-HCH	107	44	54
Delta-HCH	110	32	39
Heptachlor	25	11	12
Aldrin	5	3	4
Heptachlor epoxide	23	11	14
Alpha-Endosulfan	28	14	17
p,p′-DDE	3	2	2
Dieldrin	20	10	13
Endrin	23	8	13
Beta-Endosulfan	51	19	27
p,p′-DDD	13	6	7
Endrin aldehyde	50	11	13
Endosulfan sulfate	93	28	34
p,p′-DDT	12	5	8
Endrin ketone	100	30	43
Methoxychlor	232	61	97

[a] Water samples spiked at a concentration of 100 μg L^{-1} of each compound.

Table 2
Experimental variables and levels of the Plackett-Burman design.

Variables	Level	
	Low (−1)	High (+1)
Drop volume (μL)	1	2
Sample volume (mL)	8	10
Ionic strength (NaCl concentration; %, w/v)	0	3
Agitation speed (rpm)	0	300
Extraction time (min)	10	30

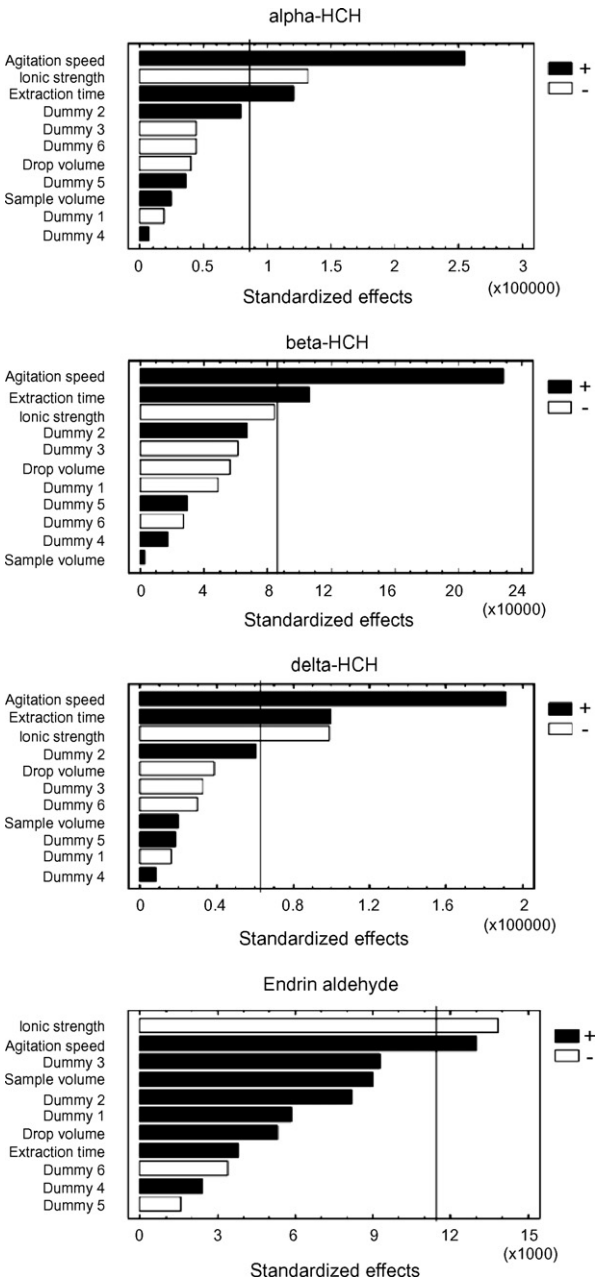

Fig. 2. Pareto charts of the main effects obtained from a Plackett-Burman design for alpha-HCH, beta-HCH, delta-HCH and Endrin aldehyde.

sen to keep at least 1 μL of the droplet needed for the injection after extraction, due to problems of dissolution and instability.

Sample volume appeared to have a positive non-significant effect upon extraction. This positive effect agrees with the fact that increasing the aqueous sample volume also led to an increase in the total amount of analytes present in the solution, given that all samples were spiked at the same concentration level. Consequently, a greater amount of target pollutants was transferred to the droplet.

Overall, the results of this first screening study revealed that two variables could be fixed, i.e., sample volume at 10 mL and drop volume at 2 μL, for the following optimization.

3.1.2. Optimization design

The second study was concerned with optimizing the values of the significant variables in order to obtain the best response (in our case the sum of all peak areas). Different experimental designs can be found in the literature, many of which are based on the so-called response surface designs. Box-Wilson or central composite design (CCD) is one of the most commonly used response surface designs, and is constructed by several superimposed designs. It consists of a factorial design (2^k) augmented with ($2k$) star points, where k is the number of variables to be optimized, and with a central point, which can be run n times [30]. A circumscribed central composite design (CCCD) was employed, where the star points were located at $\pm\alpha$ from the centre of the experimental domain, which was located at 0. In order to establish the rotatability of the experimental design, n was set at 2 and $\alpha = \sqrt[4]{2^k} = 1.682$ [30]. Therefore, the overall matrix of CCCD design involved 16 experiments.

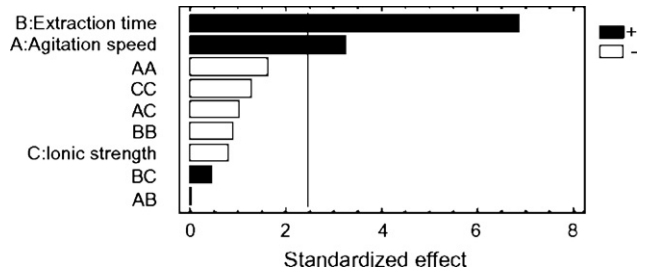

Fig. 3. Pareto chart of the three main factors obtained from the circumscribed central composite design.

In this study, the three variables considered were: extraction time, agitation speed and sodium chloride concentration (ionic strength). The low (−1), central (0), and high (+1) levels of these variables, as well as the location of their star points, are also given in Table 3.

The data obtained were evaluated by ANOVA test, and the effects were visualized by using a Pareto chart (Fig. 3). As can be seen, extraction time and agitation speed proved significant showing a positive effect, whilst ionic strength showed a non-significant negative effect upon extraction.

The effects exerted by pairs of variables were considered separately as it is not possible to plot the instrumental response as a function of all the variables controlling the extraction process simultaneously. Accordingly, the plots shown in Fig. 4 are useful to interpret the variation of the instrumental response as a function of each pair of independent variables graphically. Accordingly, Fig. 4a shows the response surface obtained by plotting extraction time vs. ionic strength with the agitation speed fixed at 400 rpm, Fig. 4b shows the response surface developed for extraction time and agitation speed, whilst keeping a sodium chloride concentration of 3% (w/v) and finally, Fig. 4c shows the response surface obtained as a function of agitation speed and ionic strength, for an extraction time of 20 min.

As can be seen, extraction time shows a positive effect upon extraction (Fig. 4a and b). Indeed, increasing extraction time augments the total amount of analyte extracted, reaching a maximum at 37 min (+1.682). As expected, agitation speed also shows a positive effect (Fig. 4b and c), reaching a maximum at 380 rpm (+0.956), and becoming stable afterwards. Increasing the speed of sample agitation is expected to enhance the extraction rate of the analyte, since mass transference is increased; however, higher values cause drop instability. On the other hand, ionic strength exerts a negative effect (Fig. 4a and c), reaching a maximum at 2.6% (w/v) (−0.35). Apart from the salting-out effect, the presence of salt was assumed to cause a secondary effect and change the physical properties of the extraction film, thus reducing the diffusion rates of the analytes toward the drop [34]. Since this variable had a non-significant effect and the presence of salt also caused drop instability, the following extractions were done without NaCl.

Table 3
Experimental variables and levels of the circumscribed central composite design (CCCD).

Variables	Level			Star points ($\alpha = 1.682$)	
	Low (−1)	Central (0)	High (+1)	$-\alpha$	$+\alpha$
Agitation speed (rpm)	300	400	500	230	570
Extraction time (min)	10	20	30	3	37
Ionic strength (NaCl concentration; %, w/v)	2	3	4	1	5

According to this optimization study, the GC sum peak area for target analytes is expected to be maximized for sodium chloride concentration, reaching a value of 0% (w/v), extraction time reaching a value of 37 min and agitation speed reaching a value of 380 rpm. Overall, summarizing the results of both screening and optimization studies yield the following optimum experimental conditions: sodium chloride concentration, 0%; extraction time, 37 min; agitation speed, 380 rpm; drop volume, 2 μL; and sample volume, 10 mL.

3.2. Analytical figures of merit

The optimum SDME conditions were used to test the applicability of the proposed method for quantitative determination of target analytes. A calibration study was performed by spiking pure water with analytes over the concentration range of 0.5–16 $\mu g\,L^{-1}$. The calculated calibration curves gave a high level of linearity for all target analytes with correlation coefficients (r) ranging between 0.9991 and 0.9999 as shown in Table 4. The repeatability of the proposed method, expressed as relative standard deviation (R.S.D.), was evaluated by extracting eight consecutive aqueous samples (spiked at 10 $\mu g\,L^{-1}$ with each target analyte) and was found to vary between 5.9 and 9.9% (Table 4). The limits of detection (LODs) for all target analytes were determined according to the signal-to-noise-ratio (S/N) of three and the limits of quantification (LOQs) as ten times the above-mentioned ratio. As shown in Table 4, the LOD and LOQ values were found to be in the low $\mu g\,L^{-1}$ level, ranging from 0.022 to 0.101 $\mu g\,L^{-1}$ and between 0.074 and 0.337 $\mu g\,L^{-1}$, respectively. As we can see in Table 4, limits of detection values obtained in this work are much lower than the LOD values obtained with the EPA method 625 [35]. Furthermore, in comparison with previously reported works on the same topic, analyzing clean waters [18,19], we obtained higher or similar LOD values for more OCPs. Moreover, the LODs obtained by the method proposed here are similar to, or lower than, those obtained in other previous works on organochlorine pesticide determination using other microextraction methods (solvent cooling assisted dynamic hollow-fiber-supported headspace liquid-phase microextraction) [36].

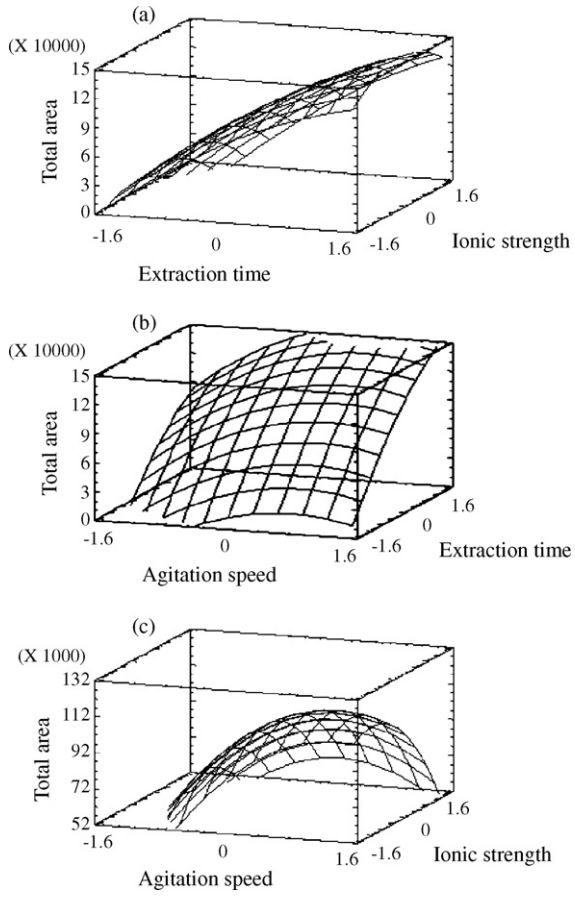

Fig. 4. Response surfaces for total chromatographic peak area using the circumscribed central composite design obtained by plotting: (a) extraction time vs. ionic strength (agitation speed: 400 rpm); (b) agitation speed vs. extraction time (NaCl: 3%, w/v); and (c) agitation speed vs. ionic strength (extraction time: 20 min).

3.3. "Real-world" water analysis

Different effluent wastewater samples from urban and industrial wastewater treatment plants were extracted using the SDME method developed here, and the extracts were analyzed by GC–MS. The preliminary results showed that they were free of organochlorine pesticide contamination. In order to investigate the effects of sample matrix upon the SDME procedure three replicate analyses were carried out of the effluent wastewater samples, spiked at 10 $\mu g\,L^{-1}$ with each target contaminant, filtrated with common lab filter paper and analyzed under optimized experimental conditions. Relative recoveries were determined as the ratio of the concentrations found in real and de-ionized water samples, spiked at the same contamination level. The results for each set of experiments are summarized in Table 5. As shown in the table, eleven out of the eighteen organochlorine pesticides did not exert matrix effects, considering 70% as the acceptable lower limit and 120% as the upper limit. However, the other seven organochlorine pesticides, mainly Heptachlor, alpha-Endosulfan, *p*,*p*′-DDE, beta-Endosulfan, *p*,*p*′-DDD, *p*,*p*′-DDT, and Methoxychlor, exerted a notable matrix effect. The decrease in relative recoveries observed could be attributed to three different phenomena [37]: (i) the possible competitive adsorption of target analytes by suspended particulate matter; (ii) adsorption by the containers and; (iii) losses during sample prefiltration. This phenomena could also be related with the structure of the analyte, where Endosulfans (alpha and beta) and Heptachlor have a similar structure, and on the other hand *p*,*p*′-DDE, *p*,*p*′-DDT, *p*,*p*′-DDD and Metoxychlor are also similar in structure. This effect can be observed in Table 5. The mean recovery values could be used to quantify these analytes in real water samples.

Table 4
Main method parameters for the extraction of organochlorine pesticides from water samples using the optimized SDME method.

Analyte	Slope ± S.D.	Intercept ± S.D.	Correlation coefficient (r)[a]	R.S.D. (%)[b]	LOD ($\mu g\,L^{-1}$)[c]	LOQ ($\mu g\,L^{-1}$)[d]	LOD EPA 625 ($\mu g\,L^{-1}$)[e]
Alpha-HCH	8600 ± 300	−4000 ± 2000	0.9993	6.7	0.087	0.290	NA[f]
Beta-HCH	3800 ± 100	4000 ± 1000	0.9993	6.5	0.093	0.311	4.2
Gamma-HCH	8000 ± 100	−3000 ± 1000	0.9998	6.5	0.045	0.149	NA
Delta-HCH	8200 ± 200	−900 ± 1000	0.9996	8.2	0.066	0.221	3.1
Heptachlor	800 ± 10	300 ± 100	0.9998	7.6	0.049	0.163	1.9
Aldrin	510 ± 10	260 ± 70	0.9997	9.9	0.053	0.177	1.9
Heptachlor epoxide	1290 ± 20	−500 ± 200	0.9997	6.8	0.054	0.178	2.2
Alpha-Endosulfan	1070 ± 20	0 ± 200	0.9996	7.6	0.064	0.213	NA
p,p′-DDE	2040 ± 20	800 ± 100	0.9999	9.0	0.025	0.083	5.6
Dieldrin	2840 ± 20	0 ± 200	0.9999	6.3	0.022	0.074	2.5
Endrin	1450 ± 30	0 ± 300	0.9996	9.8	0.068	0.227	NA
Beta-Endosulfan	1180 ± 30	400 ± 200	0.9996	7.9	0.071	0.237	NA
p,p′-DDD	4700 ± 30	6200 ± 300	0.9999	6.9	0.022	0.074	2.8
Endrin aldehyde	2260 ± 50	−700 ± 400	0.9999	5.9	0.069	0.230	NA
Endosulfan sulfate	1860 ± 50	300 ± 400	0.9993	9.3	0.087	0.290	5.6
p,p′-DDT	740 ± 20	900 ± 200	0.9991	7.8	0.101	0.337	4.7
Endrin ketone	4050 ± 90	−2400 ± 700	0.9996	9.1	0.060	0.200	NA
Methoxychlor	4390 ± 80	−200 ± 600	0.9999	7.4	0.050	0.167	NA

[a] Linear range: 0.5–16 $\mu g\,L^{-1}$ (number of calibration points = 4).
[b] Relative standard deviation (R.S.D.); mean value for eight replicate analyzes; spiking level 10 $\mu g\,L^{-1}$.
[c] Limits of detection (LODs) were calculated for a three signal-to-noise-ratio (S/N = 3).
[d] Limits of quantification (LOQs) were calculated for a ten signal-to-noise-ratio (S/N = 10).
[e] Data taken from Ref. [35]; EPA 625 (GC–MS).
[f] Not available.

Table 5
Relative and mean recoveries of the eighteen organochlorine pesticides in effluent wastewater samples.

Analyte	Relative recoveries (%) and R.S.D. values (%) in parentheses[a]							Mean recoveries ± S.D.
	Urban WWTP-1	Wastewater raft	Urban WWTP-2	Sanitary sewage	Urban and industrial WWTP	Urban WWTP-3	Urban WWTP-4	
Alpha-HCH	101(3)	94(5)	98(11)	117(9)	98(8)	106(2)	110(5)	103 ± 8
Beta-HCH	103(4)	104(9)	87(7)	113(4)	97(7)	98(5)	101(7)	100 ± 8
Gamma-HCH	95(1)	101(7)	92(11)	118(10)	99(8)	105(3)	104(4)	102 ± 8
Delta-HCH	101(8)	90(6)	101(6)	115(7)	98(10)	99(8)	105(5)	101 ± 8
Heptachlor	66(8)	63(1)	55(13)	45(10)	51(5)	57(5)	47(8)	55 ± 8
Aldrin	75(6)	90(10)	107(11)	97(13)	96(5)	100(8)	79(2)	90 ± 10
Heptachlor epoxide	74(1)	90(7)	91(7)	74(5)	87(7)	77(7)	78(4)	82 ± 8
Alpha-Endosulfan	46(8)	59(6)	43(10)	41(6)	48(9)	49(8)	46(11)	47 ± 6
p,p′-DDE	34(15)	48(11)	49(10)	44(8)	43(8)	39(3)	43(4)	43 ± 5
Dieldrin	94(5)	71(6)	79(12)	73(9)	80(9)	73(5)	73(11)	78 ± 8
Endrin	83(7)	75(6)	84(11)	74(10)	68(10)	79(6)	80(9)	78 ± 6
Beta-Endosulfan	46(9)	56(9)	59(9)	53(2)	51(9)	45(9)	54(10)	52 ± 5
p,p′-DDD	43(9)	54(7)	55(8)	47(2)	34(3)	51(6)	45(6)	47 ± 7
Endrin aldehyde	92(10)	76(9)	92(9)	93(6)	86(4)	84(1)	75(9)	85 ± 8
Endosulfan sulfate	79(3)	81(7)	88(4)	96(10)	84(10)	91(2)	94(6)	88 ± 7
p,p′-DDT	34(6)	71(11)	46(4)	52(2)	42(5)	45(7)	43(5)	50 ± 10
Endrin ketone	84(5)	72(11)	69(4)	87(11)	70(10)	76(5)	83(10)	77 ± 7
Methoxychlor	57(3)	64(7)	64(2)	58(9)	58(8)	61(3)	66(7)	61 ± 4

[a] Three replicate analyzes at a 10 $\mu g\,L^{-1}$ spiking level.

4. Conclusions

A sensitive analytical method comprising SDME coupled with GC–MS has been developed to quantify trace levels of eighteen organochlorine pesticides in complex water samples. The SDME method has been optimized by experimental design, which significantly reduces the resources used (time, reagents and experimental work). Sample preparation time, as well as consumption of toxic organic solvents, were minimized without reducing method sensitivity. This easy-to-handle and cost-effective method represents an attractive alternative to both traditional and more recently proposed sample preparation methods, as it affords better results.

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