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Determination of organochlorine pesticides and polychlorinated biphenyls in human serum using headspace solid-phase microextraction and gas chromatography-electron capture detection

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Abstract

A simple procedure for the determination of organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) in human serum using headspace solid-phase microextraction (HS-SPME) was developed. The analysis was carried out by gas chromatography (GC) equipped with electron capture detector (ECD). A 2^{7-4} Plackett–Burman reduced factorial design for screening and a central composite design for optimizing the significant variables were applied. A 100 μm PDMS fiber, 3/5 headspace ratio (3 ml in 5 ml vial), 85 °C extraction temperature, 50 min extraction time, and 1 ml of acidic solution (pH 3) added to 1 ml of diluted serum (1:1) were chosen for the best response in HS extraction mode. The detection limits found were from 1 pg/ml (PCB 167) to 52 pg/ml (β-HCH), the relative standard deviation for the procedure varied from 3% (PCB 52) to 12% (PCB 189) and the accuracy was checked by using validated solid-phase extraction (SPE) procedure. The method that avoids the use of clean-up steps and the hazardous solvents enabled reliable determinations of the OCPs and the PCBs except β-HCH. The method was applied to the analysis of 33 human serum samples. The most abundant target compound was *p-p′*-DDE (range, 0.3–8.0 ng/ml; median value, 2.1 ng/ml). Among the PCBs the prevalent congeners were 138, 153 and 180.

Keywords: Organochlorine pesticides (OCPs); Polychlorinated biphenyls (PCBs); Headspace solid-phase microextraction; GC-ECD; Plackett–Burman design; Central composite design; Human serum

1. Introduction

Most organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) are persistent organic pollutants (POPs) in environment. Nine of the OCPs, as well as PCBs, were the subjects of the Stockholm convention on POPs. The proposed treaty called for urgent global actions to reduce and eliminate releases of these compounds [1]. However, these chemicals already occur in the environment and in food chains, and because of their resistance to degradation and high solubility in organic solvents and lipids these compounds bioaccumulate in human tissues and fluids, and pose a risk of causing adverse effects to human health. The PCBs have been shown to cause cancer in animals and other non-cancer effects including effects on the immune, reproductive, nervous and endocrine systems [2]. Studies in humans provide supportive evidence for potential carcinogenic and non-carcinogenic effects [2].

Measurements of the OCPs and the PCBs or their metabolites in body tissues and fluids (often called biological monitoring) have been done as useful approach for assessing the exposure risk in the epidemiological studies. Human serum is one of the biological materials that can be conveniently obtained and used for these types of studies [3–6].

Determination of PCBs and OCPs in serum is carried out using a liquid–liquid extraction (LLE) [7,8] or solid-phase extraction (SPE) by columns [9], C_{18} cartridges or disks [10,11]. Most of the reported procedures require posterior clean steps to remove interferences from the coextracted bulk fatty matrix material. Laborious operations such as conditioning, washing, elution, and solvent evaporation are included in the steps of

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sample preparation. Also, clotting, channeling and percolation are inconveniences of the SPE procedures. Those laborious and time-consuming clean-up steps lead to cleaner extracts and, consequently, to lower detection limits. However, the risk of analytical error increases because of the incorporation of more steps in the sample preparation. The cleaned extract is later analyzed using capillary gas chromatography (GC) with electron capture detector (ECD) [7–9,11], mass spectrometry (MS) detection [10,11], high-resolution mass spectrometry with isotope-dilution quantification (IDHRMS) [12,13] or isotope dilution time-of-flight mass spectrometry (IDTOFMS) [13].

Nowadays, solid phase microextraction (SPME) is considered an useful alternative to LLE or SPE. SPME does not require solvents and it can be carried out directly from the liquid phase or from headspace over the liquid samples (HS). Also, SPME involves fewer steps and less sample handling. The HS sampling is more advisable when the matrix could affect the determination of a target analyte. In addition, HS *versus* immersion extraction often shows an important reduction of extraction time [14]. The application of SPME technique, including advantages and disadvantages, to biological fluids has been reviewed [15,16]. These works agreed that an HS method should be applied whenever possible in body fluids analysis. Among other advantageous characteristics, the burden of the fiber with proteins is considerably decreased and the fiber lifetime greatly increased [15]. With respect to the target analytes of this study, an SPME technique has been reported for the determination of OCPs and PCBs in different environmental matrices including water and biological tissues [17–20]. However, only few works have been devoted to OCP determination in human serum using SPME or HS-SPME [21,22]. Nevertheless, in the revised literature there are no references about the potential of HS-SPME technique for simultaneous PCBs and OCPs determination in serum.

There are several experimental variables affecting the HS-SPME procedure such as type of fiber, stirring rate, temperature, extraction time, volume of the headspace, and salt addition. The study considering the variables one by one to get the best possible conditions for HS-SPME has been showed in several studies working with volatile compounds in biological fluids [23,24], PCBs in water [17] and OCPs in water [20] and in human serum [22]. However, this procedure requires a high number of runs and is also time consuming. A structured experiment design that could simultaneously take into account several variables, seems a more convenient approach searching for the optimal operational conditions in a reasonable number runs [25]. As an example in aqueous medium, a response surface methodology (Doehlert design) has been used for optimization of SPME extraction conditions to determine organochlorine pesticides [26]. The response surface methodology uses a multifactor space model where a set of experiments is carried out in a systematic way in order to predict the optimum and the interaction effects. Also, a design with the screening and optimization steps has been applied to find the best experimental conditions using HS-SPME for the determination of alkyl ethers as well as benzene, toluene, ethylbenzene, and xylene isomers (BTEX) in water [27]. Hence, the use of this type of structured methodology should be a short and useful approach to find the best experimental conditions for HS-SPME working with human serum.

This work mainly focused on obtaining a convenient method for the simultaneous determination of the OCPs and PCBs in serum using HS-SPME-GC-ECD. In the method developing the advantageous characteristics of the HS-SPME technique that avoid the use of clean-up steps and the hazardous solvents was considered. A Plackett–Burman reduced factorial design was planned in order to know the significant experimental variables, and a central composite design had run out trying to get the best experimental conditions for the HS-SPME extraction of analytes from serum. Finally, the procedure was applied to the analysis of 33 human serum samples.

2. Experimental

2.1. Reagents and materials

Two mixture standard solutions PCB "Key" (28, 52, 101, 118, 138, 153 and 180) and PCB "dioxin-like" (77, 81, 105, 114, 123, 126, 156, 157, 169 and 189) each at 10 ng/μl in isooctane, and PCB 46 and 143 solutions (internal standards) each at 100 ng/μl, were purchased from LGC Promochem (Middlesex, UK). Organochlorine pesticides (HCB, β-HCH, Heptachlor epoxide, *p*,*p*'-DDE and *p*,*p*′-DDT) were purchased from Dr. Ehrenstorfer (LGC Promochem). The criteria for the standard solution selection were based on reported abundance (PCB "Key" and OCPs) and toxicity (PCB "dioxin-like"). The PCB 118 included in PCB "Key" is one of the twelve "dioxin-like" congeners. These "dioxin-like" congeners display all the following characteristics: (a) there are "co-planar" with non-ortho chlorine substitution or only one mono-ortho of the 2, 2′, 6 or 6′ positions, (b) have a total of four or more chlorine substituents, (c) have both para positions (4 and 4′) chlorinated, and d) have two or more of the meta positions (3, 3′, 5, 5′) chlorinated. H_3PO_4 acid and Triton X-100 were from Fluka (Buchs, Switzerland). NaH_2PO_4 (99% purity) was supplied by Carlo Erba (Milan, Italy). Acetonitrile and *n*-hexane of HPLC grade, 37% HCl acid, sodium hydroxide and Na_2SO_4 (99% purity) were obtained from Panreac (Panreac Química S.A., Barcelona, Spain).

Two stock mixture solutions containing 2.5 mg/l of each PCB and 0.1 mg/l of OCPs respectively were prepared in cyclohexane. These solutions were stored at −20 °C. From these mixtures a 20 μg/l solution of each PCB and OCP was prepared in acetonitrile. Two standard solutions 0.1 M H_3PO_4 and 0.074 M NaH_2PO_4 were prepared and stored at 4 °C. From these solutions a 3 pH buffer was prepared.

SPME holders and fibers [100 μm thickness poly(dimethylsiloxane) (PDMS) and 65 μm poly(dimethylsiloxane)-divinylbenzene (PDMS-DVB)], 5 ml sample vials and PTFE-silicone septa were obtained from Supelco (Bellefonte, PA, USA). Before using, vials were heated at 300 °C for 10 h.

A thermo bath Lauda RE 104 (Lauda Dr. R. Wobser GmbH & Co. KG, Lauda-Königshofen, Germany) was used to maintain the temperature and a Heidolph MR 3003 (Heidolph Elektro GmbH & Co KG, Kelheim, Germany) was used to

stir the samples at 900 rpm. 10 mm PTFE coated stir bars were put in the 5 ml vials just before runs. The stir bars cleaning included washing with distilled water and thorough rinsing with *n*-hexane HPLC grade.

2.2. Serum samples

The pooled serum used for the development and the validation of the method was obtained from Laboratorio de Salud Pública de Guipúzcoa (San Sebastián, Spain). This pooled serum was composite from 35 individual serum samples and was named as Gipuzkoa-serum (GS). The method was applied to 33 individual samples obtained from volunteer donors following ethical approval. Blood was extracted by venipuncture and collected on Vacutainer blood tubes. The blood was centrifuged and serum was separated from the tube. Samples were stored at −20 °C until analysis.

2.3. Instrumentation

A 6890N gas chromatograph equipped with split-splitless injector and an ECD detector (Agilent Technologies, Wilmington, DW, USA) was used in all the measurements. The injection port fitted with a 0.75 mm i.d. injection liner (Supelco) was operated in the splitless mode, with the split–splitless purge valve opened at 4 min after injection. The injection port temperature was 270 °C and helium served as a carrier gas at a pressure of 20 psi with a flow-rate of 5.0 ml/min. Chromatographic separation was accomplished with a DB-XLB column (30 m × 0.32 mm × 0.50 μm). The DB-XLB column has a stationary liquid phase equivalent in polarity to a 12% (phenylmethyl)-polysiloxane [8]. The temperature program used was: 60 °C for 1 min, 20 °C/min to 180 °C, 1.5 °C/min to 240 °C, 30 °C/min to 300 °C and held for 8 min. The detector temperature was set at 300 °C with an N_2 make-up flow of 20 ml/min. A PC interfaced to the GC using Chemstation software (Agilent Technologies) was used for data acquisition and processing.

The Plackett–Burman and the central composite matrix designs were performed and evaluated using the STATISTICA software package (StatSoft, Tulsa, USA).

2.4. Analytical procedure

One milliliter of diluted serum (0.5 ml serum:0.5 ml distilled water) was put into 5 ml vial. One milliliter of the 3 pH buffer was added to the diluted serum. Sampling extraction was performed by HS mode exposing the PDMS fiber over the stirred samples. For all the runs a stirring rate of 900 rpm was used. The extraction was done for 50 min at 85 °C. After sampling the fiber was withdrawn into the needle of the holder and was immediately placed in the GC injector. The desorption conditions were 270 °C and 10 min. No carryover was observed after this desorption time.

In order to quantify the OCPs and PCBs compounds in the serum samples the standard addition procedure was used. Five mixed standards with closer concentrations to levels of the analytes present in the sample were used for quantification. In these runs 4, 7, 10, 15 or 20 μl of the standard mixture (20 μg/l PCBs and OCPs in acetonitrile) was added to the 2 ml previously introduced in the vial. The extraction and desorption conditions were the same as cited in the sample without the mixed standards.

3. Results and discussion

In order to develop an adequate method using HS-SPME for PCBs and OCPs determination in serum, it is necessary to consider and optimize several parameters that affect the extraction procedure. A design with two steps (screening and optimization) was used for searching the best experimental conditions. In order to evaluate the results we considered the PCB 46 and PCB 143 usually used as internal standards (ISTDS) [11]. In all the runs, 2.5 ng of each ISTD was added per ml of the GS serum. Also, 1 ml of the acidic or basic solution was put in the vials. The latter solution either contained or did not contain the additives (salt or surfactant) as described in the screening design.

3.1. Screening design

Screening is the first step in the efficient assessment of the factors involved in the studied analytical system. If a large number of factors are involved reduced factorial designs, such as Plackett-Burman design, are applied. These designs are useful as with few experiments it is possible to detect the most significant factors or variables. The main limitation concerned with the reduced factorial designs is that the main effects are confounded with interaction terms. In the Plackett–Burman design is assumed that the interactions can be completely ignored and only the main effects are calculated. However, it can be used trying to look for large main effects, which can be later thoroughly studied using other type of designs.

On the basis of the literature and the experience of the laboratory [14–24,26–28] seven variables were selected to define the experimental field (one qualitative or categorical, and the other six quantitative or continuos). These were the variables considered: type of fiber, extraction temperature, extraction time, headspace volume, pH, concentration of anhydrous sodium sulfate, and concentration of Triton X-100. The latter three variables are referred to the solution added to the human serum with the goal of modifying the protein properties (deproteinization). In order to contrast the coating phases the PDMS and PDMS-DVB fibers were chosen. The extraction time was from 20 to 40 min, and the extraction temperature was from 40 to 70 °C. The headspace volume varied from 3/5 (3 ml in 5 ml vial) to 4/5 (8 ml in 10 ml vial). The pH ranged from 2 to 10. These pHs were achieved with HCl acid or NaOH solutions. The concentration of anhydride Na_2SO_4 was 0 and 35% (350 mg/ml). The Triton concentration was 0 and 20% (200 mg/ml). The variables, the codes and the low and high levels considered are shown in Table 1.

In Plackett–Burman designs the number of experiments is a multiple of four (4, 8, 12, etc., experiments) and exceeds the number of factors by one. In this study, a 2^{7-4} Plackett–Burman design was applied to evaluate the main effects of the seven fac-

Table 1
Experimental variables, levels, 2^{7-4} Plackett–Burman design matrix, and results (in peak area units) for PCBs 46 and 143 (ISTDs) determination in human pooled serum using HS-SPME

Variable	Coded	Level	
		Low	High
Fiber type	Fiber	PDMS/DVB	PDMS
Extraction temperature (°C)	Temp	40	70
Extraction time (min)	Time	20	40
Headspace volume ratio	HS	3/5	4/5
pH value	pH	2	10
Na_2SO_4–Salt concentration (%)	Salt	0	35
Triton-surfactant concentration (%)	Surf	0	20

Run	Fiber	Temp	Time	HS	pH	Salt	Surf	PCB 46	PCB 143
1	PDMS	40	20	4/5	2	35	20	276	342
2	PDMS-DVB	70	40	4/5	2	35	0	4238	1937
3	PDMS	70	40	3/5	10	35	20	154	142
4	PDMS	40	40	4/5	10	0	0	270	388
5	PDMS-DVB	40	20	3/5	10	35	0	1208	2214
6	PDMS-DVB	70	20	4/5	10	0	20	195	182
7	PDMS	70	20	3/5	2	0	0	2994	6578
8	PDMS-DVB	40	40	3/5	2	0	20	63	229

tors. In total the design matrix had eight runs. The design matrix and the response for the studied PCBs (peak area in arbitrary units) are also given in Table 1.

The data obtained were evaluated by an ANOVA test (not included) and the main effects visualized using Pareto chart (Fig. 1). In the chart, the bar lengths are proportional to the absolute value of the estimated main effects. The sign of the effect showed that the response would or would not be improved on passing a given factor from the lowest to the highest level. The Triton concentration was the most important variable with a negative effect for the highest concentration. Therefore, the surfactant was eliminated for the next optimization step. As the pH factor showed a negative effect with basic values a pH of 3 was chosen. Both analytes showed higher responses at the highest time and temperature. These factors were considered in the next optimization step. There were three factors with opposite results. For PCB 46, PDMS/DVB fiber, high salt concentration and high HS volume ratio were the favorable conditions. For PCB 143, PDMS fiber, no salt and low HS volume ratio were the best experimental conditions. The PDMS fiber was also chosen for working with water for PCBs [17], fish tissues for OCPs [19] and human fluids for OCPs determination [21]. Since, the method had to be useful for other PCBs with molecular weight higher than the PCB 143, we chose the conditions that should enhance the extraction of less volatile compounds (represented by PCB 143). Briefly, the salt and surfactant addition was eliminated. Three variables PDMS fiber, 3/5 HS volume ratio, and 3 pH were fixed. And the other two factors, the time and the extraction temperature, were considered for the optimization step.

3.2. Optimization design

The second step was done to optimize the chosen significant variables using a central composite design (CCD). The two variables and their low, central and high levels are as follows: extraction temperature (temperature, 35–60–85 °C) and extraction time (time, 15–30–45 min). Those values are included in Table 2.

CCD consisted of the points of factorial design (2^N) increased with ($2N+1$) star points N being the number of variables. In this

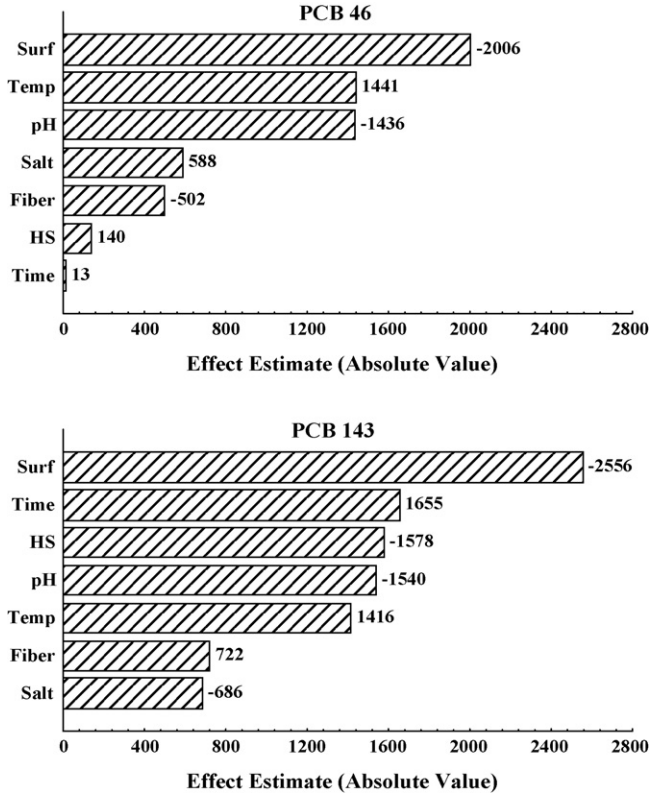

Fig. 1. Pareto charts of the main effects obtained from 2^{7-4} Plackett-Burman design for PCB 46 and 143 determination in human pooled serum using HS-SPME.

Table 2
Experimental variables, levels, central composite design (CCD) matrix, and results (in peak area units) for PCB 46 and 143 (ISTDs) determination in human pooled serum using HS-SPME

Variable	Coded	Level		
		Low	Centre	High
Extraction temperature (°C)	Temp	35	60	85
Extraction time (min)	Time	15	30	45

Run	Block	Temp	Time	PCB 46	PCB 143
1	1	35	15	889	453
2	1	35	45	2686	2459
3	1	85	15	5332	2725
4	1	85	45	5549	3743
5 (C)	1	60	30	1464	1119
6 (C)	1	60	30	1794	814
7 (C)	1	60	30	1444	835
8	2	35	30	544	462
9	2	85	30	4618	3063
10	2	60	15	1767	826
11	2	60	45	3066	1014
12 (C)	2	60	30	1793	848
13 (C)	2	60	30	2475	1338
14 (C)	2	60	30	1744	805

(C), central point.

work, 2^2 increased with $(2 \times 2 + 1)$ star points. The star points were face centered ($\alpha \pm 1$). There were two blocks, one on each day of the experiment. The runs at the center of the experimental field were performed twice more. Therefore, in total the matrix of CCD design consisted of 14 experimental runs randomly carried out. The average values of the two data (in arbitrary units of peak area) are shown in Table 2.

After checking the adequacy of the model, the next step was to find the conditions of the factors or independent variables (extraction temperature and extraction time) that maximize the response of the dependent variable (PCB 46 and 143 peak areas). For CCD a second-degree polynomial model was used including main effects for the two factors, their interaction and their quadratic components. For both the PCBs with this second order model ANOVA shows a lack of fit not significant, and a good values for R^2 (0.9732 for PCB 46 and 0.9166 for PCB 143) and adjusted R^2 (0.9502 for PCB 46 and 0.8452 for PCB 143). R^2 is the proportion of variance accounted for by the respective model, in the measurements of the dependent variable. The adjusted R^2 applies to the R^2 value an adjustment for the number of terms in the respective model. Those data were considered as indication of the adequacy of the model. The regression coefficients obtained using this second-degree polynomial model were used in computing predicted values for the dependent variables at different combinations of the independent variables levels.

The most common way to summarize the results of a CCD experiment is in the form of a response surface. This can be done in 3D plots selecting two factors (i.e. temperature and time). In this study, two 3D plots belonging to each of the PCBs (46 and 143) can be obtained. Instead of this independent response surfaces we chose to get the global desirability surface. First, the

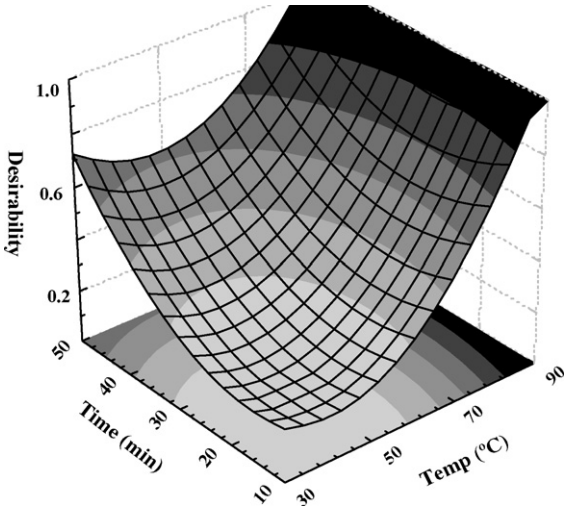

Fig. 2. Desirability response surface from the CCD design considering time and temperature extraction for PCBs 46 and 143 determination in human pooled serum using HS-SPME.

desirability function for each dependent variable was fixed by assigning desirability values of 0.0 (for undesirable, lowest result in this work), 0.5 (medium), and 1.0 (for very desirable, highest result in this work). After doing the specifications, prediction profiles and desirability graphs for each dependent variable were obtained. Also, the global desirability surface response developed by the model in a 3D plot (Fig. 2) was obtained. This graph is useful for interpreting graphically the effect on overall response desirability of each pair of independent variables. In this study of extraction time and temperature, as can be seen, in the experimental domain the best global response was reached at higher extraction temperature (85 °C). Also, we can observe that an increase of extraction time could slightly enhance the response at higher temperatures. Temperatures higher than 85 °C were not considered because of the difficulty to get with a water bath and trying to avoid the boiling of the sample that could reach and damage the fiber positioned very closed to the surface sample. With respect to the time, getting the equilibrium for several of the target analytes could take a long extraction time (even hours) that could diminish the usefulness of the procedure. The 50 min time could be enough to assure a detectable amount of the OCPs and PCBs. Furthermore, this time was similar to the time of the chromatographic run that allowed the simultaneity between the extraction and the chromatographic steps.

A temperature of 90 °C and 30 min of extraction time were the conditions chosen for the determination of the OCPs in serum working with PDMS in headspace mode [22]. However, high molecular weight compounds such as PCBs are expected to have longer equilibration times because of their low diffusion. Furthermore, a decrease in the partition coefficient with increase the chlorine number is observed. These facts were shown in a study of extraction efficiencies of SPME (immersion mode) for the determination of pesticides and PCBs in water [28]. In this work, the proposed time for extraction was 150 min working at room temperature.

Table 3
Linear ranges, correlation coefficients, detection limits and repeatability of the HS-SPME-GC-ECD method for the OCPs and PCBs determination

Compound	PCB-chlorine substitution	Retention time (min)	Linear range (pg/ml)	Correlation coefficient (r^2)	LOD[a] (pg/ml)	RSD[b] (%)
HCB		12.88	51.8–207	0.9996	13.4	6
β-HCH		16.04	51.8–207	0.9990	51.7	5
Heptachlor epoxide		23.14	51.8–207	0.9996	28.0	4
p,p′-DDE		28.60	104–414	0.9999	21.0	5
p,p′-DDT		36.94	51.8–207	0.9964	5.4	9
PCB 28	2,4,4′	17.40	51.8–207	0.9974	12.7	5
PCB 52	2,2′,5,5′	19.07	51.8–207	0.9990	7.3	3
PCB 101	2,2′,4,5,5′	26.20	51.8–207	0.9982	5.2	4
PCB 81	3,4,4′,5	29.55	51.8–207	0.9962	3.9	5
PCB 77	3,3′,4,4′	30.59	51.8–207	0.9930	2.0	5
PCB 123	2,3′,4,4′,5	32.13	51.8–207	0.9972	5.3	6
PCB 118	2,3′,4,4′,5	32.53	51.8–207	0.9964	4.8	6
PCB 114	2,3,4,4′,5	33.48	51.8–207	0.9976	6.7	7
PCB 153	2,2′,4,4′,5,5′	34.10	51.8–207	0.9958	3.9	8
PCB 105	2,3,3′,4,4′	35.13	51.8–207	0.9958	4.5	7
PCB 138	2,2′,3,4,4′,5′	36.94	51.8–207	0.9964	5.4	9
PCB 126	3,3′,4,4′,5	39.67	51.8–207	0.9795	2.3	8
PCB 167	2,3′,4,4′,5,5′	40.99	51.8–207	0.9902	1.0	10
PCB 156	2,3,3′,4,4′,5	43.19	51.8–207	0.9884	1.6	10
PCB 157	2,3,3′,4,4′,5′	43.64	51.8–207	0.9878	2.1	10
PCB 180	2,2′,3,4,4′,5,5′	44.08	51.8–207	0.9938	31.0	9
PCB 169	3,3′,4,4′,5,5′	47.99	51.8–207	0.9848	3.8	11
PCB 189	2,3,3′,4,4′,5,5′	49.10	51.8–207	0.9970	2.4	12

[a] LOD, limit of detection.
[b] RSD, relative standard deviation ($n = 7$) (each compound 70 pg/ml, except *p,p′*-DDE 140 pg/ml).

Taking into account the previous results, the working extraction conditions to obtain the best response were PDMS fiber, 3/5 headspace ratio (3 ml in 5 ml vial), 85 °C extraction temperature, 50 min extraction time, and 1 ml of acidic solution (3 pH) added to 1 ml of the diluted serum.

3.3. Method evaluation

After establishing the extraction conditions, the HS-SPME-GC-ECD method was firstly checked with respect to linearity, limits of detection, and precision. For this evaluation the mixture of five OCPs (HCB, β-HCH, Heptachlor epoxide, *p,p′*-DDE, and *p,p′*-DDT) and 18 PCBs (28, 52, 77, 81, 101, 105, 114, 118, 123, 126, 138, 153, 156, 157, 167, 169, 180, 189) was used. Five different concentrations of the standard mixture (See range in Table 3) were added to 2 ml of 3 pH buffer solution. The volume of 2 ml was chosen in order to maintain the headspace volume ratio (3/5). The GS pooled serum was not used as a "blank" since the sample contained several target OCPs and PCBs.

In Table 3 the retention time of each compound is shown. The peak resolution was adequate for the target analytes identification and quantification with the exception of *p,p′*-DDT and PCB 138 that co-eluted. This co-elution behavior has been observed working with DB-XLB columns and could be corrected using alternative RTX-5MS column [8].

Calibration curves (peak area *versus* concentration) were linear in the concentration range tested. For the 5 OCPs, and 14 of the PCBs, the correlation coefficients (r^2) were higher than 0.99 (Table 3). The LOD was considered as the lowest concentration of the analyte that in the whole process could differentiate from the background levels. Some works used LOD calculated by a signal-to-noise ratio of 3 [7,9,21]. Other works obtained it from the calibration plot [27]. The latter was the option in this study. The LOD of each compound was calculated considering the intercept of the calibration plot (as blank signal, y_B) with a standard deviation estimated by $S_{y/x}$ (in place of the standard deviation of the blank, S_B) [29]. The detection limits ranging from 1.0 to 51.7 pg/ml are shown in Table 3. The comparison with other works using SPME and GC-ECD that determined these analytes in aqueous matrix showed LODs similar than others found using PDMS commercial fiber [17] and higher than the levels found (few pg/ml magnitude order) working with a more selective fibers only for OCPs determination [18,20].

The precision of HS-SMPE-GC-ECD was obtained using the analysis of seven independent samples with 70 pg/ml of each compound (*p,p′*-DDE, 140 pg/ml). The repeatability, measured as a relative standard deviation (RSD), was from 3% to 12%. These values were similar to those found using HS mode for OCPs determination in water [18,20] and direct SPME for OCPs determination in serum samples [21].

For accuracy, the HS-SPME method was tested against a validate procedure followed by the laboratory using GS serum in both the procedures. Briefly, the contrast procedure consisted of an SPE C_{18} extraction on 96-well plate, followed by a clean-up done on sulfuric acid/silica gel column. The final hexane-dichloromethane eluate was concentrated under a nitrogen stream, reconstituted in cyclohexane, and transferred to a vial prior to a GC-ECD analysis. In order to confirm the identity of the suspected OCPs and PCBs present in the GS pooled serum a GC–MS instrument was used. Results showed a good agreement between the procedures for HCB, *p,p′*-DDE, and PCBs 118, 153 and 138. The found values expressed as percentages

Table 4
Range and median values (ng/ml) of OCPs and PCBs in human serum samples obtained in this study ($n = 33$) Comparison with data obtained in samples from different countries

Compound	This study range (ng/ml)	This study median (ng/ml)	USA [7][a] (ng/ml)	Spain [10][b] (ng/ml)	Romania [11][c] (ng/ml)	Belgium [11][d] (ng/ml)	Italy [6][e] (ng/ml)
HCB	0.08–7.24	0.73	0.63–14.0	0.3–2.2	0.20–0.69	0.15–1.13	
β-HCH	nd–1.42	0.48	1.65–8.68	nd–1.5			
Heptachlor epoxide	nd–0.12	0.01	nd–0.43				
p,p′-DDE	0.29–8.01	2.12	7.24–124	1.0–10.0	4.49–74.65	2.25–9.22	
p,p′-DDT	0.05–1.06	0.31	0.29–1.62	nd–<0.9			
PCB 105	nd–0.10	0.02	nd–0.08		nd–0.33	nd–0.11	na
PCB 118	0.03–0.33	0.09	0.22–0.62		0.02–1.12	0.14–0.49	nd–1.58
PCB 138	0.07–1.01	0.26	0.76–2.59	nd–0.4	0.11–1.10	0.60–1.46	nd–5.39
PCB 153	0.19–4.31	1.14	0.94–4.14	>0.15–0.60	0.26–2.12	1.13–3.16	nd–9.26
PCB 156	nd–0.25	<0.01	nd–0.36		nd–0.15	0.12–0.38	nd–0.84
PCB 180[f]	0.19–1.61	0.78	0.78–3.33	>0.08–0.60	0.28–2.01	0.92–2.75	nd–13.81

nd: not detected; na: not available.
[a] Ten archived samples.
[b] Ten samples (exposed and non exposed agricultural workers).
[c] Five individual samples.
[d] Four individual and 1 pooled samples.
[e] Three-hundred eleven subjects.
[f] ($n = 25$).

calculated from the results of validate procedure were from 86% to 105%. The value for β-HCH was less than 40%. Hence, the β-HCH that was obtained later in the application of the HS-SPME procedure to the serum samples has to be taken with precaution.

3.4. Application to serum samples

The HS-SPME-GC-ECD procedure obtained in the present study was applied to 33 human serum samples. The standard addition method was followed trying to avoid the matrix influence on the extraction efficiency from the fiber. A 4, 7, 10, 15 or 20 μl of standard mixture (20 μg/l) was added to the diluted serum sample, with supposed concentrations from 0.08 to 0.4 μg/l (or ng/ml serum sample). The standard addition procedure has been used for the quantification of volatile compounds in water [27] and the OCPs in fish tissue [19]. Fig. 3 shows a chromatogram obtained using HS-SPME applied (a) in human serum alone and (b) after the addition of OCPs and PCBs mixed standards.

The range and the median values (in ng/ml) of the detected OCPs and PCBs are given in Table 4. Also, results obtained in other countries are included in Table 4. In this study, HCB, *p,p′*-DDE, *p,p′*-DDT, and PCBs, 118, 138, 153, and 180 were detected in all the samples analyzed. The highest concentration was for *p,p′*-DDE (range, 0.3–8.0 ng/ml; median, 2.1 ng/ml) which is the main metabolite of *p,p′*-DDT that enters into human body mainly through food chain. Among the PCBs the highest concentration was for PCB 153 (range 0.2–4.3 ng/ml; median, 1.1 ng/ml). PCBs 138 and 180 were detected at lower concentrations. These results agree with other data obtained in human serum from different population [10,11].

The 33 samples were also analyzed following the SPE procedure in the laboratory. With these data and those obtained with HS-SPME we wanted to validate the procedure by calculating the correlation between the two methods for each of following analytes: HCB, *p,p′*-DDE, PCBs 118, 138, and 153. The Pearson correlation analysis showed that the two data sets were highly and significantly correlated. The correlation coefficient *r* was from 0.8875 (PCB 138) to 0.9953 (HCB). The slopes of

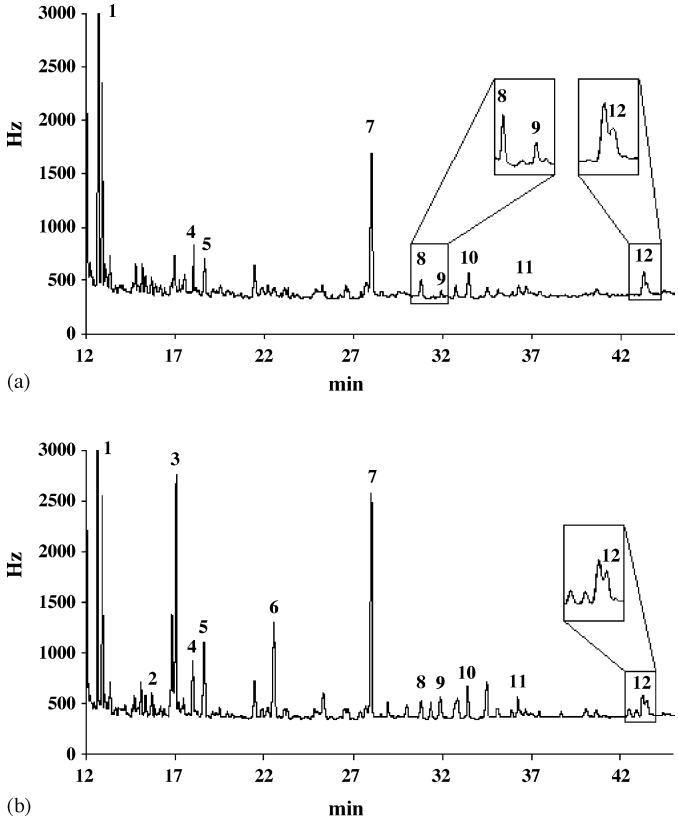

Fig. 3. Chromatograms obtained by HS-SPME GC-ECD method of (a) human serum sample and (b) serum sample with addition of PCBs and OCPs (0.3 ng/ml; *p,p′*-DDE 0.6 ng/ml). Peak assignment: (1) HCB, (2) β-HCH, (3) PCB 28, (4) PCB 46 (ISTD), (5) PCB 52, (6) heptachlor epoxide, (7) *p,p′*-DDE, (8) PCB 143 (ISTD), (9) PCB 118, (10) PCB 153, (11) PCB 138, (12) PCB 180.

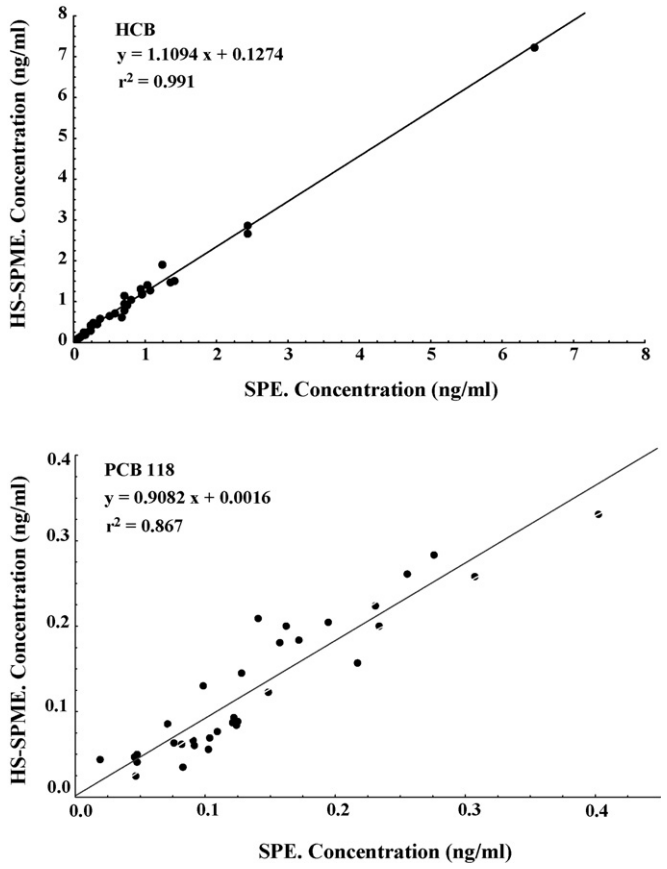

Fig. 4. Concentrations of HCB and PCB 118 (ng/ml) from the analysis of 33 human serum samples obtained using HS-SPME and SPE procedures.

the linear regression analysis were from 0.773 (PCB 138) to 1.384 (PCB 153). For example, the data for HCB and PCB 118 determination using both extraction procedures are represented in Fig. 4. These results indicated a good agreement among the methods for the studied analytes.

4. Conclusions

An HS-SPME method for the simultaneous determination of the OCPs and the PCBs in human serum was developed. The usefulness of experimental design taken in the optimization of extraction conditions for HS-SPME method was also shown. A PDMS fiber for the extraction and GC-ECD for the determination were the only equipments required. There were some remarkable advantages of the method compared with other techniques, as the clean-up steps and the use of hazardous solvents were avoided. A time of 50 min was enough for the extraction of the studied analytes. In these conditions the relevant OCPs and PCBs were reliably detected and quantified. The addition standard procedure was advisable for quantification when a complex matrix such as serum is analyzed. However, this manual addition standard procedure is time consuming. Hence, improvements by using automatic equipment or the quantification by the use of isotopic standard (GC–MS) could reduce the time drawback of this addition standard procedure. Considering the results, the proposed method using HS-SPME and quantification by standard addition can be useful for simultaneous detection and evaluation of OCPs and PCBs with a limited number of serum samples.

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