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Rapid analysis of persistent organic pollutants by solid phase microextraction in serum samples

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ABSTRACT

A simple and rapid headspace solid-phase microextraction (HS SPME) based method is presented for the determination of Persistent Organic Pollutants (POPs) in human serum by gas chromatography (GC) coupled to mass detector (MS) with electron impact ionization (EI). As an outcome of the assessment of several polymer phases; the one with the best result was the PDMS fiber (100 μm). A multivariate analysis of variance by permutations (PERMANOVA) was performed to establish the optimal extraction conditions as a function of temperature and time variables. The results were 1 mL serum + 200 μL H_2SO_4 9 M + 1 mL of deionized water at 600 rpm with a temperature of 80 °C for 50 min to expose the fiber. The limits of detection (LOD) for POPs pesticides fell within the 0.22–5.41 ng/mL interval, and within 0.07–1.79 ng/mL for PCBs; a linear method was used with correlation coefficients (r) higher than 0.99. Recovery percentages at low concentrations (15 ng/mL) were 67.8–120.2%, and at high concentrations (75 ng/mL) 80.2–119.2%. Evaluated precision as percentage Relative Standard Deviation (RSD%) of repeatability and reproducibility was within a range of 0.5–9% and 0.3–21%, respectively. This analytical method prevents some of the main problems for quantifying POPs in human serum, such as the elimination of the solvents, sample handling, integration of extraction steps, pre-concentration and introduction of samples; consequently, the time and cost of analyzing the sample can be significantly reduced. The method developed was applied to determine exposure to POPs in samples of children living in different polluted sites in Mexico. In children living in indigenous communities results show exposure to DDE (median 29.2 ng/mL range 17.4–52.2 ng/mL) and HCB (median 2.53 ng/mL range 2.50–2.64 ng/mL); whereas in the industrial scenario, exposure to HCB (median 2.81 ng/mL range 2.61–3.4 ng/mL) and PCBs (median Σ-PCBs 22.2 ng/ml range 8.2–74.6 ng/mL) and finally in petrochemical scenario was demonstrated exposure to HCB (median 2.81 ng/mL range 2.61–3.4 ng/mL) and PCBs (Σ-PCBs median 7.9 ng/mL range 5.4–114.5 ng/mL).

1. Introduction

Persistent Organic Pollutants (POPs) are organic chemicals, which are generated naturally or due to human activities. They have specific physical and chemical characteristics that allow them to remain chemically intact in the environment for long periods of time; they can be dispersed through different environmental matrices (soil, water, sediment, and air), stored in fatty tissues and biomagnified in the food chain; furthermore, they are toxic to humans and wildlife [1–4].

Current methodologies used for quantifying POPs in environmental and biological matrices result from a great deal of research in analytical chemistry. However, establishing an analytical laboratory and applying internationally acceptable techniques is a relatively expensive task. Moreover, the trend of using isotopically-labeled analytical standards and high-resolution mass spectrometry further increases analysis costs. This represents one of the main constraints that lead to a lack of biomonitoring programs in developing countries such as in Latin America. It is vital to emphasize that studies are needed in such countries due to an uncontrolled use of large amounts of POP compounds [5]. Furthermore, lowering costs would facilitate the continuity of biomonitoring schemes at these sites.

Liquid–liquid extraction (LLE) methods exist for POPs extraction in serum, plasma and whole blood [6,7]. LLE allows extraction

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of large amounts of the sample, but this method is labor-intensive and it is not possible to have high performance analysis. Solid-phase extraction (SPE) method is a better choice for serum extraction, also proven useful of POPs extraction in human serum samples at large scale monitoring project [8,9].

SPE has additional advantages over LLE method; SPE use of lower volume of solvent and the sample is simultaneous cleaning by using solid phase column. Unfortunately, SPE is not susceptible to particle-laden samples, such as serum (specifically for high molecular weight proteins). Both these methods involve various extraction steps, large volumes of solvents, the use of concentrated sulfuric acid with hexane to eliminate interferences mainly of lipid origin, among others [10].

Sample treatment is identified as the main problem in analytical processes, especially when it comes to trace analysis. New trends for developing analytical methods mainly focus on: (i) reproducibility, accuracy and lower detection limits, using surrogate standards or other analytical tools; (ii) optimization of the analytical procedure to reduce costs, time, waste and amount of sample required [11,12]. Our work met these two criteria.

Solid phase microextraction (SPME) appears to be a solvent-free extraction technique for preparing samples and in some cases, faster than conventional methods. Sample preparation extraction, cleaning and concentration steps are simplified into one step [13].

SPME consists in directly exposing a polymer coated fiber to the sample for a certain time. Once the fiber is exposed, analytes are immediately transported from the sample to the polymer. Extraction is considered complete when analyte concentration has reached equilibrium distribution between the sample matrix and fiber coating, which means that the analyte's concentration will remain constant beyond equilibrium time, regardless of an increase in extraction time.

There are several reports that use SPME to determine POPs in matrix such as water, soil, sediment and whole blood [14–17]. However, there are few studies that determine POPs in human serum using SPME [18–21]. In the literature review, there were no references on the technique potential for simultaneous extraction from human serum of POP pesticides and industrial products, and from compounds listed as future POPs such as atrazine.

The aim of this study was to develop and validate an analytical method to determine POPs in human serum using solid phase microextraction technique (SPME) coupled with gas chromatography–electron impact-mass spectrometry (GC-EI-MS), and demonstrate how effective this technique is for biomonitoring POPs in serum samples from children living in polluted sites.

2. Methods

2.1. Materials and reagents

For PCBs analysis a mixture of standard references (28, 52, 99, 101, 118, 138, 153 and 180) and individual (105, 128, 170, 183, 187, and 156) PCBs, with 95% purity at a concentration of 100 μg/mL in hexane, and use surrogate standard of PCB 141 (C13- isotopically labeled) at a concentration of 40 μg/mL in nonane. For POP pesticides (OCPs), a mixture (-α,-β,-γ,-δ-HCH, 4,4′ DDD; DDE; DDT, aldrin; heptachlor; heptachlor epoxide; -α,-β and sulfate endosulfan) and individual standards (HCB, atrazine) were used, with 99% purity at a concentration of 100 μg/mL in hexane-toluene and 1000 μg/mL for HCB. With a surrogate standard of α-HCH (C13) and DDE (C13) isotopically labeled at a concentration of 100 μg/mL in nonane; all standards purchased from Chemservice.

Based on reference standards, independent solutions of 1000 ng/mL were prepared for PCBs, OCPs and three surrogate standards in hexane. Solutions remained stored at −70 °C.

A 9 M of H_2SO_4 (JT Baker) solution in Milli-Q deionized water (18.3 MΩ, Millipore) was used. Supelco supplied SPME fibers (PDMS, 100 μm; Carboxen (CAR)/PDMS 75 μm; Carboxen (CAR)/PDMS 85 μm; CarboWax (CW)/DVB 65 μm), the manual holder and the 10 mL amber vials.

Samples and calibration curves were analyzed in a gas chromatograph (GC) 6890 (Agilent) equipped with a split/splitless injector coupled with a mass spectrometry detector (MS) 5975 (Agilent) with electron impact ionization (EI). The injection port was operated in splitless mode with a 0.75 mm liner without glass wool. Injection port temperature fixed at 230 °C; helium used as carrier gas at a pressure of 36 psi with a constant flow of 1 mL/min. The chromatographic separation was through a HP 5 ms (60 m × 0.25 mm × 0.25 μm) column (Agilent). Setting of the oven was as follows: 90 °C (2 min), 180 °C (30 °C/min), 200 °C (1 °C/min), 265 °C (2 °C/min), 310 °C (30 °C/min) with a run time of 59 min.

MS conditions were established for the source's temperature and its quadruple at 230 and 150 °C. The tune parameters were: emission: 34.6; energy: 69.9: repeller: 26.6 and EMVolts: 1341. SCAN mode (50–500 m/z) was used to identify compounds and identification and quantification ions were selected for SIM mode (Table 1). Results were obtained and processed using Chemstation software (Agilent).

2.2. Analytical procedure

Blank samples, non-spiked serum samples, were analyzed to check for any contamination throughout the analytical procedure. No background interference appeared to be introduced in this method.

After verifying the blank matrix, one milliliter of serum was fortified with PCBs and OCPs with independent concentrations from the quantification limit up to 100 ng/mL and 20 ng/mL of surrogate standards; it stored at 4 °C for 2 days to stabilize the compounds and evaporate hexane. The presence of an organic solvent in an aqueous sample may decrease the distribution constant; therefore, the amounts of organic solvents should be kept at a minimum. Tipically, for optimum extraction efficiencies, organic solvent amount should not exceed 1% given that above this threshold the properties of water and distribution constant values change substantially [22]

2.3. Establishing optimal conditions

Two processes can be clearly distinguished in the SPME technique: adsorption (retention of analytes in fiber) and desorption (GC injection port). Developing a procedure in order to determine analytes by SPME requires the optimization of a number of variables related to the stages mentioned earlier [23]: fiber coating, the extraction mode, the stirring method, sample amount, pH, ionic strength, sample temperature, extraction time and desorption conditions.

The three most important conditions of POPs adsorption process are: (i) fiber type, (ii) temperature and (iii) performance time. Assessment performed on two levels of concentration (low and high) in four types of fiber coatings, for nonpolar, semi-polar and polar compounds (PDMS, 75 μm > CAR/PDMS 75 μm > CAR/PDMS 85 μm > CW/DVB 65 μm, respectively); the activation of each fiber went under the conditions indicated by the manufacturer. To evaluate temperature conditions (80, 100, 120 °C) and the extraction time (30, 40, 50 and 60 min), each one was performed three times.

Optimal conditions were established. One mL of serum, 1 mL of deionized water, 200 μl of H_2SO_4 9 M and a magnetic bar were added to a 10 mL amber vial. The vial was sealed, preheated at 80 °C and magnetically stirred at 600 rpm for 10 min. Expose fiber

Table 1
Parameters for HS-SPME-GC–MS analytical method validation in fortified serum.

Compound	Ion	rt	LOD	LOC	*r*	*m*	Low	High
*α-HCH C_{13}	187	12.65	–	–	–	–	–	–
*DDE C_{13}	258	30.54	–	–	–	–	–	–
*PCB 141C_{13}	372	36.97	–	–	–	–	–	–
α-HCH	183, 181	12.65	0.24	2.38	0.99	0.437 ± 0.001	103	100
HCB	284, 286	13.04	0.79	2.02	0.99	1 ± 0.001	104	103
Atrazine	200, 215	13.43	5.41	10.09	0.99	0.40 ± 0.001	78.4	82.7
β-HCH	183, 181	13.97	2.67	6.01	0.99	0.42 ± 0.001	67.8	92.9
γ-HCH	183, 181	14.19	0.91	3.67	0.99	1.86 ± 0.086	103.8	100.8
δ-HCH	183, 181	15.56	1.2	3.4	0.99	5.17 ± 0.095	101.5	90.8
PCB 28	256, 258	17.41	1.43	3.07	0.99	5.47 ± 0.691	118.9	119.9
Heptaclor	272, 256	18.43	0.55	3.33	0.99	1.89 ± 0.046	101.5	92.6
PCB 52	292, 290	19.85	1.26	2.7	0.99	3.38 ± 0.224	120.2	113
Aldrin	263, 261	21	0.77	3.78	0.99	0.49 ± 0.001	85.8	95.7
Heptaclor epox	353, 355	24.48	0.69	1.46	0.99	0.43 ± 0.001	111.6	113.2
PCB 101	326, 324	27.64	0.95	2.07	0.98	2.79 ± 0.058	117	110.3
α-Endosulfan	241, 239	27.85	1.95	2.22	0.99	4.47 ± 0.195	75.4	77.8
PCB 99	326, 324	28.15	1.31	2.85	0.99	2.96 ± 0.059	115.9	103.1
DDE	246, 248	30.54	0.22	1.57	0.99	3.27 ± 0.261	100.9	107.3
β-Endosulfan	241, 239	33.17	0.33	2.04	0.99	2.63 ± 0.028	83.5	89.8
PCB 118	326, 324	33.88	1.32	2.88	0.99	1.54 ± 0.011	104.5	98.4
DDD	235, 237	34.53	0.55	1.97	0.99	0.81 ± 0.015	115.1	101.4
PCB 153	360, 362	35.82	1.46	2.41	0.99	1.67 ± 0.017	106.1	98
PCB 105	326, 324	36.14	1.35	2.91	0.99	2.14 ± 0.018	102.3	98
Endosulfan sulfate	272, 282	37.4	1.09	2.03	0.99	2.18 ± 0.110	77.3	80.2
DDT	235, 237	38.02	1.65	3.55	0.99	6.58 ± 0.350	108.8	97.4
PCB 138	360, 362	38.39	1.17	2.53	0.98	1.25 ± 0.025	110.6	100
PCB 187	394, 396	39.95	0.83	1.79	0.99	0.99 ± 0.016	113.3	99.8
PCB 183	394, 362	40.41	1.79	2.89	0.99	0.97 ± 0.016	103	99.7
PCB 128	360, 362	40.88	0.07	1.57	0.99	1.00 ± 0.017	113	100.2
PCB 156	360, 362	43.08	1.68	2.6	0.99	0.65 ± 0.007	110.9	100.8
PCB 180	394, 396	44.67	1.74	2.73	0.98	0.66 ± 0.008	111.3	100.9
PCB 170	394, 396	47.21	1.07	2.34	0.99	0.51 ± 0.005	112.4	102.4

LOD: limit of detection; LOQ: limit of quantification; *r*: coefficient of correlation; *m*: sensibility expressed as a linear slope; recovery percentage at low concentration: 15 ng/mL and high concentration: 75 ng/mL. (*) surrogate standard.

into vial a depth of 2.5 cm for 50 min and then retracted. The device was inserted into the GC's injection port for 5 min at a temperature of 230 °C. This desorption condition is sufficient to ensure that the fiber is ready for another extraction.

2.4. Statistical analysis

A multivariate analysis was performed to infer the difference between treatment patterns; The choice for the aforementioned was PRIMERv6 software with a PERMANOVA package [24,25]. The multivariate analysis of variance based on two-way distance permutations (PERMANOVA) was performed to assess significant differences in the abundance of POPs response as a function of the following factors: (1) Time (30, 40, 50, 60 min) and (2) Temperature (80, 100, 120 °C), with three replicates for each treatment at a concentration of 100 ng/mL.

This analysis on Euclidean distance matrices calculated from data normalization, followed by 9999 random permutations (Monte Carlo analysis) to obtain test probability values (p). Significant values ($p < 0.05$) were researched with pairwise comparisons, which also use 9999 random permutations to obtain p values. Non-metric multidimensional scaling graphs (nMDS) were constructed in order to visualize the patterns in the multivariate data cloud and evaluate its consistency with results provided by PERMANOVA.

2.5. Analytical technique validation

The internal quality control and validation of the method were performed based on the Guide for the Validation of Analytical Methods for the Determination of Organic Compounds at Trace Levels AOAC/FAO/IAEA/IUPAC [26], by evaluating the following parameters: limit of detection (LOD) and limit of quantification (LOQ), linearity (r), sensitivity, percentage of recovery and precision (repeatability and reproducibility).

LOD and LOQ were calculated using results obtained from the triple calibration curve of each compound determined in a concentration range of 2.5 to 15 ng/mL. The linearity expressed by the correlation coefficient (r) and the sensitivity determined by the slope of the working range curve (LOQ-100 ng/mL) resulted from the average of eleven curves worked for five days. The percentage of recovery of the method for each analyte derived from evaluating 10 different fortified points on the calibration curve of a low (15 ng/mL) and high (75 ng/mL) concentration (Table 1).

The method's precision is measured based on repeatability and reproducibility, evaluating different concentration in triplicate on the same day, and in duplicate five different days (Table 3).

2.6. Biomonitoring of POPs in children

The method was applied to 96 serum samples from children living in polluted sites; 19 children in Rincon de San Jose (RSJ) in Mexquitic de Carmona S.L.P., which houses the first hazardous waste landfill in Mexico [5]; 42 children in Coatzacoalcos (COA), Veracruz, identified as a malarious area and the country's main petrochemical zone [27]; and 35 children in Santa Maria Picula (SMP) S.L.P., an indigenous community located in a malarious area [28].

Inclusion criteria for children's participation in the study were: (i) informed, voluntary and signed consent by the parents of each child for taking the sample; (ii) a minimum residence period of

two years; (iii) aged between 4 and 9 years old. Research methodology initiated post approval by the bioethics committee of the Faculty of Medicine of the Autonomous University of San Luis Potosi.

Extraction of the blood samples was by venipuncture of the antecubital vein with vacuum blood collection tubes (Vacutainer tubes) and without anticoagulant. The samples were then centrifuged at 3500 rpm for 15 min; serum was separated and stored at −20 °C until it was analyzed.

3. Results and discussion

3.1. Method optimization

3.1.1. Extraction mode and type fiber

There are several parameters affecting the sensitivity of the SPME procedure: fiber coating, extraction mode, distribution constant (Kfs) between the fiber and the sample, and variables such as temperature, stirring, salt addition and extraction time [22]. In this work parameters, such as fiber type, extraction time, extraction temperature, and addition salt underwent examination for POPs extraction.

Headspace extraction mode (HS-SPME) was selected for POPs extraction: these analytes are semi-volatile, and hence transported through its gas phase before being adsorbed by the polymer; this difference prevents fiber damage from having direct contact with interferences of high molecular weight and other non-volatile substances in the serum samples [23]. In addition, this extraction mode also allows pH modification without damaging the fiber. Also, the addition of acid promotes the hydrolysis of lipids, which are one of the major interferences in POPs analysis [10]. Additionally, HS extraction is more suitable for the extraction of analytes of low-to-medium polarity [22].

SPME extraction efficiency is dependent on the fiber coating/sample matrix distribution constant (Kd) [29]. Four types of polymers chosen for evaluation were: PDMS 75 μm, CAR/PDMS 75 μm, CAR/PDMS 85 μm, and CW/DVB 65 μm. Fig. 1 shows the comparison of the chromatograms obtained from POPs extraction for each of the polymers, assessing the extraction capacity at a low and a high concentration (25 and 75 ng/mL, respectively). The PDMS polymer (Fig. 1D) shows the best recovery of all compounds; this is evident by means of higher responses it generated and because all the compounds were extracted. The partition equilibrium of this polymer phase has been related to the octanol–water constant (log Kow), where an analyte with high log Kow values allows for higher recoveries, as in the case of POPs [23,30].

3.1.2. Ionic strength

Addition of salt (sodium chloride) to the sample has frequently been used in HS-SPME to improve recoveries [31]. Therefore, different increasing salt concentrations were used in the extraction procedure (0, 7.5 and 15% addition in 100 ng/mL of compound); in our conditions, the tested salt amounts did not influence the method sensitivity.

3.1.3. PERMANOVA analysis

A study of the temperature (80, 100, 120 °C) and time (30, 40, 50 and 60 min) extraction was performed using PDMS fiber. PERMANOVA analysis results indicate an effect of the factors in the following order of importance: Time > Temperature > Time × Temperature (Table 2). This data pattern is evident in the nMDS graph that shows a clear grouping for both factors (time and temperature, Fig. 2).

Fig. 2 represents nMDS related to the Euclidean distance matrix of the normalized responses for the 30 analyzed compounds. Each point resulted from a triplicate of each compound. An increase in response occurs when there is a shift towards the right of the nMDS diagram and the difference between treatments is visualized in the separation in both the aggregate and individual variables. Thus, the best treatment, the one with the greatest overall abundance across all compounds would be 50 min and 80 °C. This was proven by comparing abundances between pairs of factors and the nMDS diagram, finding the following patterns: (1) time 50 > 60 ($t=3.947$, $p=0.0001$) > 40 ($t=7.827$, $p=0.0001$) > 30 ($t=15.282$, $p=0.0001$), and (2) temperature 80 > 100 ($t=3.60$, $p=0.0006$) > 120 ($t=2.720$, $p=0.005$).

Thus, the previous information, and comparisons between pairs of interacting factors (Table 2) proves that treatment with the highest abundances detected for all POPs was 50 °C/80 min (time/temperature). These conditions are consistent with other works for OCPs (90 °C/30 min) [17,18] and PCBs in serum (85 °C/50 min) [20] using PDMS fiber (100 μm) in HS mode.

Different responses for OCPs and PCBs showed to be influenced by time and temperature and directly related to the physicochemical properties of the analytes (volatility, distribution constant and structure of the compounds).

Fig. 3 depicts some of the analytes' profiles, through a response surface graph, as a function of the optimization variables (time and temperature). Fig. 3(A) describes the response obtained by γ-HCH; this response was similar to that of the other HCH isomers (α,-β,-δ) where maximum responses are achieved at lower temperatures and times compared to other compounds (about 30 min and 80 °C). Fig. 3(B) shows HCB response; in this graph temperature variation levels do not exert a significant effect on the response, as it does not present a significant change between 80 and 120 °C. This was the same for all other compounds (Fig. 3A–I). However, the time variable was the most notorious factor, showing an increase that was directly proportional to the response, except HCH isomers.

The extraction of analytes by SPME is based on distribution processes; the equilibrium time is reached when the maximum amount of analyte has been extracted by the fiber. Said equilibrium times are altered mainly by the analytes' physicochemical characteristics. High molecular weights of the PCBs and some OCPs are expected to have longer equilibrium times due to low diffusion between the gas phase and the fiber. Most compounds, except for the HCH family, have long equilibrium times where even compounds such as PCBs (Fig. 3H, I) and DDE (Fig. 3G) did not reach equilibrium in the proposed time (50 min). This behavior has been reported for soil, where some OCPs such as DDE and endosulfan reach equilibrium times of 220 and 120 min, respectively [32].

The temperature's effect is directly related to the distribution coefficient between the SPME fiber and the analytes, causing fluctuations in adsorption equilibrium. Elevated temperatures may reduce OCPs and PCBs partition coefficient in serum, and significantly increase the diffusion of analytes during the gas phase [22]. However, sensitivity decreased among HCH isomers when temperature exceeded 80 °C (Fig. 3A). These compounds have relatively high vapor pressures ($1.25\text{-}23.3\times10^{-3}$–$3.33\times10^{-3}$ Pa); with increasing temperature, the equilibrium shifts towards the gas phase. Additionally, these analytes reached equilibrium in less time (30 min) because their higher volatility allows a greater range of diffusion between the sample and fiber. However, the conditions proposed in the methodology are sufficient to ensure a detectable concentration of all analytes (Fig. 4).

After establishing the extraction conditions, the method obtained validation in a fortified sample by evaluating linearity, sensitivity, percentage of recovery, repeatability and reproducibility (Tables 1, 3). The resolution of chromatographic peaks was appropriate for evaluating and quantifying the analytes of interest (Fig. 4).

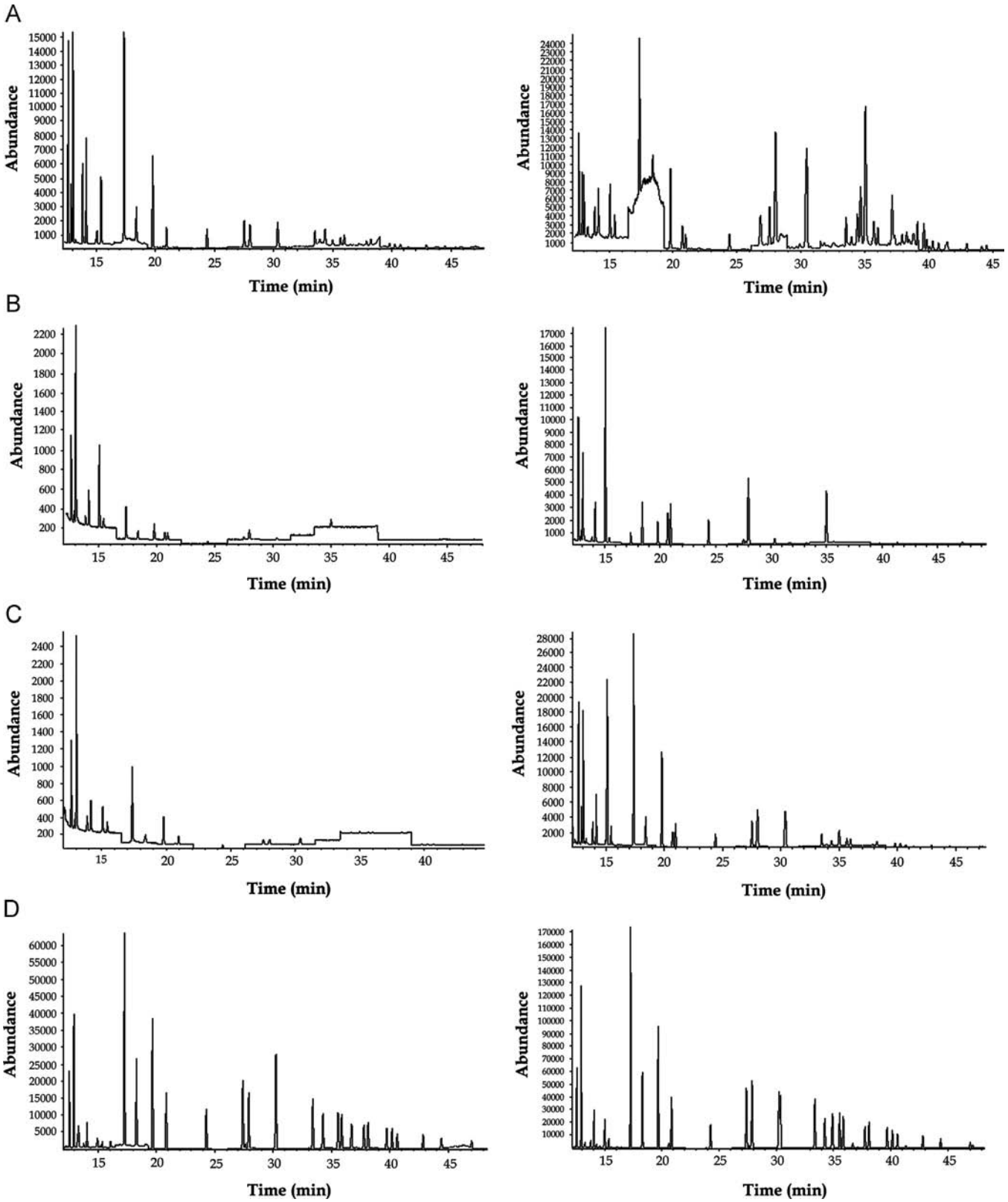

Fig. 1. Chromatograms obtained by GC–MS with different SPME fibers at high and low concentration. (A) CW-DVB, (B) CAR/PDMS 75 μm, (C) Carboxen/PDMS 85 μm, (D) PDMS 100 μm. Low concentration: 25 ng/mL (1) high concentration 75 ng/mL (2).

3.2. Validation of the method

3.2.1. Linearity and limit of detection and quantification

The calibration curve was linear over the working range (LOQ-100 ng/mL), and correlation coefficients (r) were higher than 0.99. LOD and LOQ were determined by a linear curve at low concentrations, adding a confidence limit of 95% [33,34]. Values were obtained through the slope method, which consists of measuring variation in the lower zones of the curve (where there is more uncertainty) to verify that response values obtained are different from a blank

response. This is calculated from the intercept (YB) value plus the deviation that estimates random errors in the direction of the intersection (Sy/x), YB+3 Sy/x for LOD and YB+10 Sy/x for LOQ. The 95% confidence limit of the slope variation in the interpolation zone is added to the value obtained so that the final value contemplates the greatest uncertainty in the curve's lowest area, ensuring analyte presence and quantification with acceptable precision. The compounds' LOD interval was 0.07–5.41 ng/mL; these results are similar to those reported by Beltrán for OCPs [18] (0.1–6 ng/mL) using GC–MS-EI. In another study, LOD intervals were 1.0–51.7 pg/mL [20], difference between these values are due to the type of detector used. For OCPs and PCBs, the Electron Capture Detector (ECD) has higher sensitivity; however, the mass detector presents greater selectivity as it only quantifies the ions in the compounds (SIM mode) (Table 1). Also, this ensures that only the analyte is quantified, hence eliminating most interference from the sample.

3.2.2. Recovery

The feasibility of the SPME method developed was evaluated by analyzing serum spiked at different concentrations levels. The recovery of the OCPs and PCBs investigated at the 15 ng/mL level were (the lowest point of recovery) 67.8% for β-HCH and 120.2% for PCB 52, with the highest concentration 75 ng/mL, the lowest point was 80.2% for endosulfan sulfate and 119.9 for PCB 128; all compounds fell within the acceptable range indicated by the validation guide used (70–120% for concentrations between 10 ng/mL and 100 ng/mL). That is results were similar than reported by Kim et al. [21] and better than other values published using similar PDMS SPME fiber [18], except to atrazine, which no previous report can be found before this work.

3.2.3. Repeatability and reproducibility

Precision was measured as repeatability and reproducibility, and each point in the calibration curve was evaluated as relative standard deviation (RSD%) of the area ratios; all compounds are below the acceptable RSD% [35], which shows that the method was reliable in different working days.

3.3. Biomonitoring of OCPs and PCBs in Mexican children

Having demonstrated the method's effectiveness, a multiresidue analysis (HS-SPME-GC–MS) was applied to serum samples from children living in polluted sites, the medians, percentile 25 (P25) and 75 (P75) are presented in Table 4. Fig. 5 shows examples of chromatograms obtained from the analytical procedure in the children's serum. In the indigenous scenario of Santa Maria Picula, the following POPs were detected in descending order of concentration: DDE > β-endosulfan > HCB > γ-HCH in 82.8, 30, 51.4 and 37.1% of the sampled children, respectively. Indoor use of wood

Table 2
Details of the two-factor PERMANOVA test for abundances of Persistent Organic Pollutants (POPs) on biological samples in response to the time and temperature factors. Conditions: (1) time (30, 40, 50, 60 min) and (2) temperature (80, 100, 120 °C). Pair-wise test of factors is also shown.

Source	df	MS	Pseduo-F	p (perm)
Main test				
Time (Ti)	3	844.74	168.230	0.001
Temperature (Te)	2	62.502	18.671	0.001
Ti × Te	6	67.589	6.730	0.001
Residual	24	40.171		
Total	35	1015.0		
Source			**t-stat**	**p (perm)**
Pairwise test				
Within level: time '50'				
80 vs 100			2.670	**0.0141**
80 vs 120			4.101	**0.0023**
100 vs 120			2.383	**0.0214**
Within level: temperature '80'				
30 vs 40			8.830	**0.0002**
30 vs 50			12.462	**0.0001**
30 vs 60			9.999	**0.0001**
40 vs 50			6.959	**0.0009**
40 vs 60			5.073	**0.0013**
50 vs 60			2.644	**0.0152**

Bold values indicate significant differences at $p < 0.05$. df, degrees of freedom; SS, sum of squares; MS, mean squares; perm, permutations.

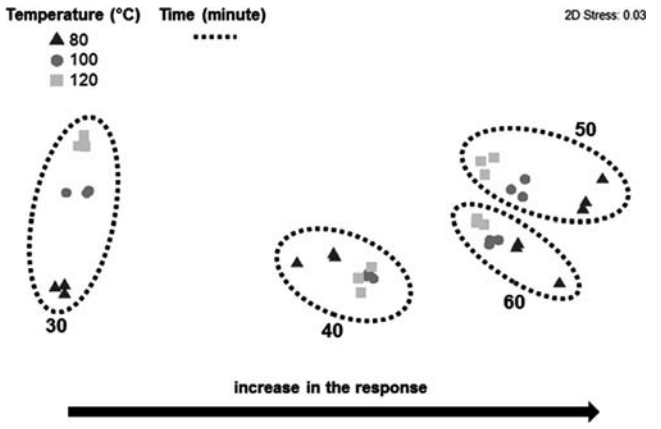

Fig. 2. Multidimensional scaling plots (nMDS) of the Euclidean distance matrix based on normalized abundance data for the 30 analyzed compounds. Each point represents a replicate. Patterns for the time factor are shown in circles.

Table 3
Repeatability and reproducibility of the HS-SPME-GC–MS analytical method in fortified serum.

Compounds	**Repeatability (%RDS)**					Reproducibility (%RDS)				
	5	10	25	50	100	5	10	25	50	100
Isomers HCH	3–10	1–7	1–4	6–9	1–4	3–14	3–9	1–15	1–5	2–3
HCB	2	0.8	1	9	4	5	6	15	7	1
Atrazine	–	6.2	4.8	8	4	–	4	1	4	3
Heptaclor's	1.6–2.4	1–1.6	1.6–5.3	7–8	2	4–16	3–7	8–12	5-6	2–16
Aldrin	4.11	2.1	4	8	0.5	10	8	8	2	2
Endosulfan's	0.5–11	2	6–7	2–4	1-2	6	11	13	11.7	4
DDTs	1–5	1–6	6–7	1–4	1.9–2	10–14	12	9	2–15	0.3–1
PCBs	3–6	0.5–1	0.7–3	5–7	0.1–4	6–12	3–11	2–17	3–18	0.3–16
%RDS acceptable [35]	**17.7**	**16**	**13.9**	**12.5**	**10.1**	**26.6**	**21**	**18.5**	**16.7**	**15**

Concentration: ng/mL; Isomers α-, β-, γ-, δ-HCH; HCB: Hexachlorobencene; Heptaclor's: heptachlor, heptachlor epoxide; Endosulfa's endosulfan sulfate; DDTs: DDD, DDE, DDT; PCBs: 28, 52, 99, 101, 105, 118, 128, 138, 153, 156, 170, 183, 180 and 187.

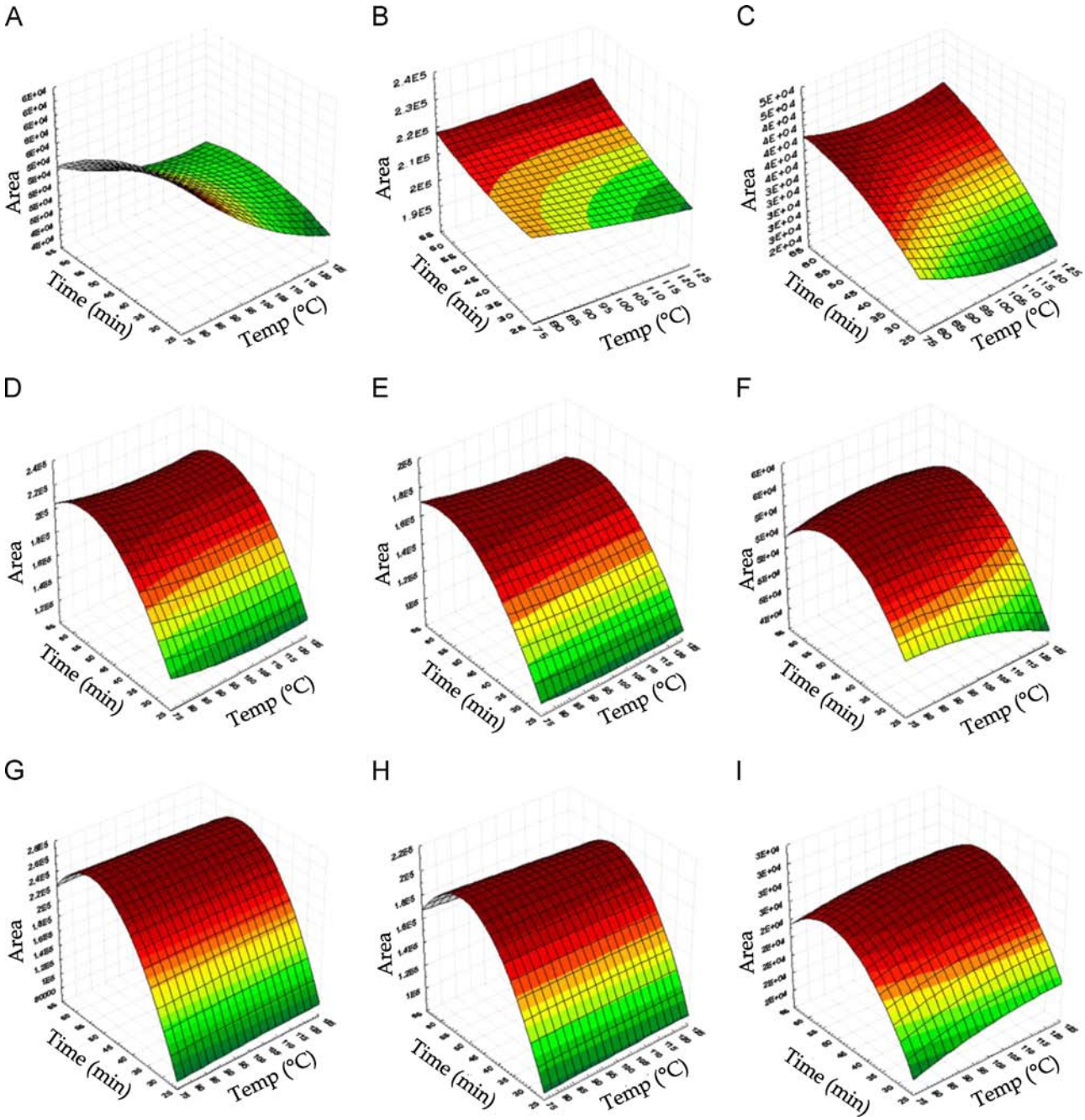

Fig. 3. Estimated size optimization design based on temperature and time. Results were obtained at three temperature conditions (80, 100 and 120 °C) and four different times (30, 40, 50 and 60 min) at a concentration of 100 ng/mL. Each figure represents the optimization of distinctive compounds in each group of analytes. (A) HCH isomers: α,-β,-γ,-δ-HCH, (B) HCB, (C) Atrazine, (D) heptachlor, heptachlor epoxide, (E) Aldrin, (F) endosulfan, sulfate and endosulfan-α,-β, (G) DDE, DDT, DDD, (H) PCB 99, 28, 52, 101.118, (I) 180, 138, 153, 105, 128, 170, 183, 187, 156.

and residual DDT sprayed for malaria control has been shown to be an important source of these chemicals in indigenous communities such as Santa Maria Picula [28]. In Mexquitic, the main contribution of pollutants according to concentration were PCB 101 > 99 > HCB > 105 > 118 > 138 > 187 in 36.8, 47.3, 100, 52.6, 52.6, 42.1, 26.3% of children sampled; the other PCBs were found in less than 20%. In the case of HCB and PCBs, the source may be the hazardous waste landfill and an asphalt plant near the community. In the case of Coatzacoalcos, the quantified pollutants, in descending order, were PCB 99 > δ-HCH > 101 > β-endosulfan > endosulfan sulfate > HCB > DDE > 105 > 118, in 42.8, 40.4, 11.9, 95.2, 95.2, 95.2, 11.9, 95.2 and 95% of children sampled. A petrochemical area nearby and polluted food are thought to be the main source of PCBs and HCB; agricultural areas the study site could be the major source of insecticides [27].

Exposure to POPs varies significantly in all sites (Table 4) [36–38]. Although all studies were performed with children born in these sites, there are different patterns of exposure; a fact that is explained by their different types of culture and environment [39]. In the case of the HCB compound, children living in industrial settings exhibit greater concentrations and exposure prevalence; compared to children living in countries such as Spain, Romania and Germany, the children's serum concentration in Coatzacoalcos and Mexquitic are 2.4, 4.6 and 16-0 and 2.1, 4.0 and 14.0 times higher, respectively. Exposure to PCBs was only found in these scenarios; although there is no significant difference with respect to levels found; Mexquitic

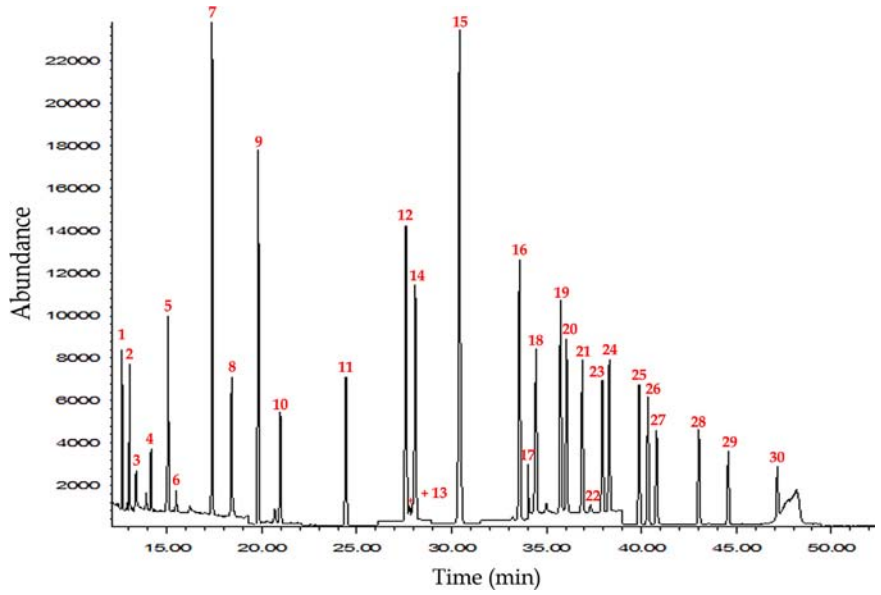

Fig. 4. Chromatogram HS-SPME-GC–MS (SIM mode) fortified serum to 5 ng/mL. Compounds: (1) α-HCH, (1*) α-HCH C13, (2) HCB, (3) atrazine, (4) β-HCH, (5) γ-HCH, (6) δ-HCH, (7) PCB 28, (8) heptachlor, (9) PCB 52, (10) Aldrin, (11) heptachlor epoxide, (12) PCB 101, (13) α-endosulfan; (14) PCB 99, (15) DDE, (15*) DDE C13, (16) PCB 118, (17) β-endosulfan; (18) DDD; (19) PCB 153, (20) PCB 105, (21) PCB 141C 13*, (22) endosulfan sulfate, (23) DDT, (24) PCB 138, (25) PCB 187, (26) PCB 183, (27) PCB 128, (28) PCB 156, (29) PCB 180 and (30) PCB 170.

Table 4
Exposure assessment of OCPs and PCBs in children's serum in this study compared with data from different countries.

Compound	SMP, S.LP. ($n=35$)	Mex, S.L.P. ($n=19$)	COA, Ver. ($n=42$)	USA [37]	Spain [47]	Rumania [46]	Germany [38]
HCB	2.53 (2.5–2.64)	2.81 (2.61–3.4)	3.21 (3.17–3.29)	0.63–14.0	1.32 (0.52–2.61)	0.20–0.69	0.2
γ-HCH	1.83 (1.53–2.18)	–	–	–	–	–	–
δ-HCH	–	–	35.6 (19.9–77.5)	–	–	–	–
DDD			0.2 (0.2-0.2)	–	–	–	–
DDE	29.2 (17.4–52.2)	–	3.1 (2.0–7.2)	7.24–124	2.53 (1.25–5.11)	4.49–74.65	1.4
β-Endosulfan	4.0 (2.8–6.1)	–	5.4 (4.6–7.5)	–	–	–	–
Endosulfan sulfate			4.95 (4.40–9.42)				
PCB 101	–	28.6 (8.3–56.6)	8.8 (4.4–54.9)	–	–	–	0.1
PCB 99		11.4 (5.6–33.8)	89.0 (58.3–105.3)				
PCB 118	–	2.5 (2.1–3.1)	2.5 (2.3–13.1)	0.22–0.62	0.17 (0.11–0.30)	0.14–0.49	–
PCB 153	–	1.20 (0.7–2.43)	3.1 (3.0–3.2)	0.94–4.14	0.71 (0.45–1.01)	1.13–3.16	0.4
PCB 105	–	2.8 (2.5–3.3)		nd–0.08	0.55 (0.32–0.77)	nd–0.11	-
PCB 138	–	1.2 (1.2-1.2)	–	0.76–2.59		0.60–1.46	0.3
PCB 187	–	0.8 (0.8–2.0)	–	–	–	–	–
PCB 183	–	1.44 (0.8–2.47)	–	–	–	–	–
PCB 128	–	0.78 (0.03–1.49)	–	–	–	–	–
PCB 156	–	1.30 (0.8–3.66)	–	nd–0.36		0.12–0.38	–
PCB 180	–	1.30 (0.8–2.55)	–	0.78–3.33	0.54 (0.37–0.77)	0.92–2.75	0.3
PCB 170	–	1.10 (0.5–3.71)	–	–	–	–	–

The values represent median, P25 and P75, the units are expressed in ng/mL; SMP: Santa Maria Picula, S.L.P.; SJR:Mex: Mexquitic, S.L.P. and COA: Coatzacoalcos, Ver.

children showed a higher quantity of PCB congeners, even some of the dioxin type (105, 118, 156, 170 and 180) [40].

High concentrations of DDE, a residual presence of DDT, which was used extensively as an insecticide in campaigns against malaria [28], were found in indigenous scenarios. Compared to countries such as Spain and Germany, the indigenous scenarios in Mexico show concentrations that are 11.5 and 20.8 times higher, respectively. β-endosulfan and γ-HCH are used in agriculture and against ectoparasites, respectively. It is possible to assume that the source of HCB is garbage burning, especially plastic materials [41].

Finally, our work shows that in developing countries, such as Mexico, toxic substance management remains inadequate, risking both humans and the ecosystem. Children are potentially at a higher risk than adults [42]. In common risk scenarios in Mexico [43–45], children are one of the most susceptible populations, and the issue of health effects from exposure to mixtures of pollutants is a public health issue in those sites. Therefore, in future studies it is important to understand potential interactions of compounds with biomarkers of effect and to establish a biomonitoring program as a continuous assessment of concentration in these sites, as well as establish intervention measures needed to reduce exposure and its effects.

4. Conclusions

In the present study, HS-SPME-GC–MS method was used in order to analyze POPs levels in human serum. Applying a PERMANOVA

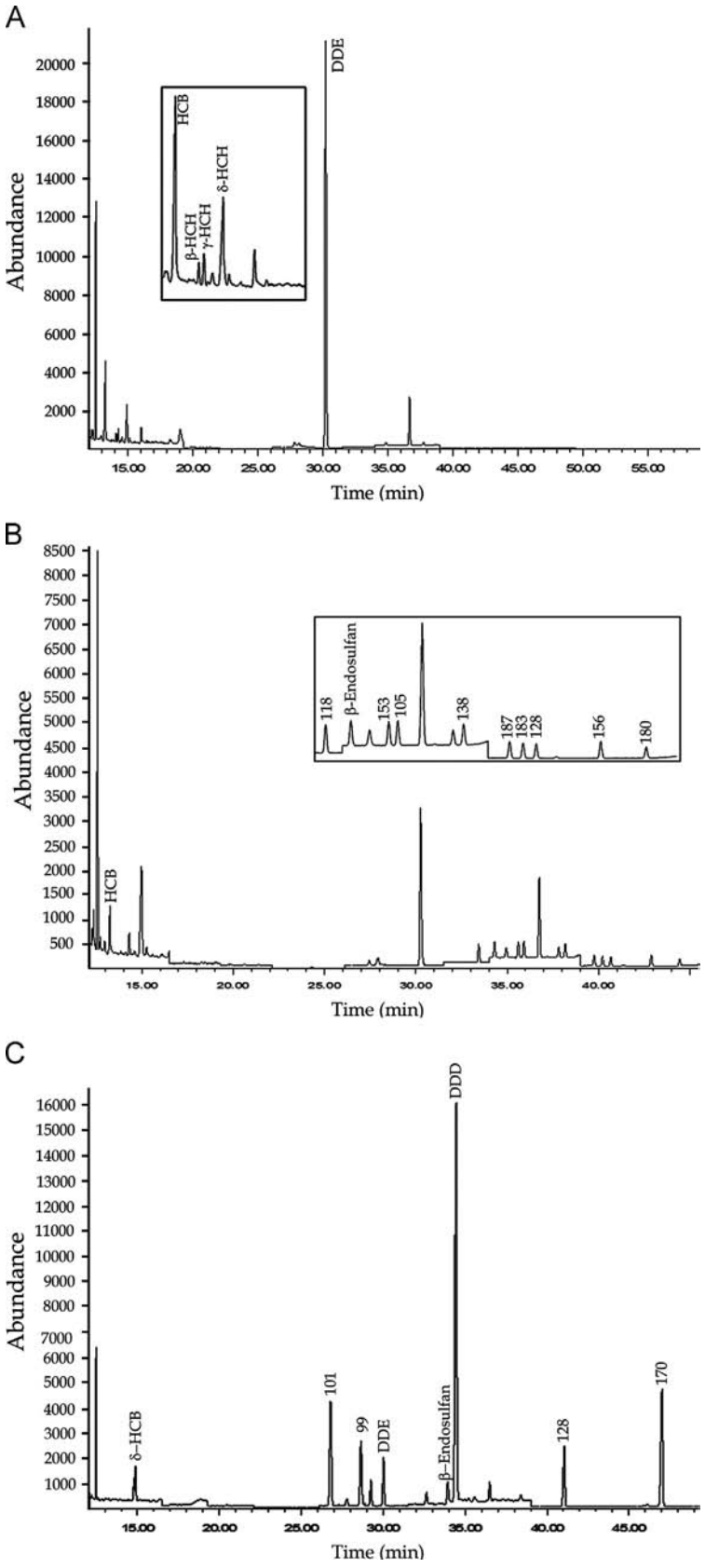

Fig. 5. Chromatograms (SIM mode) of serum samples from children exposed to POP polluted sites in Mexico. (A) Santa Maria Picula, S.L.P. (B) Mexquitic de Carmona, S.L.P. y (C) Coatzacoalcos Veracruz.

and nMDS analysis to the main experimental parameters affecting the HS-SPME step resulted in optimization which allowed us to obtain maximum information with a minimum number of assays

This solvent-free method gave respectable precision, linearity, recovery and detection limits. Moreover, high recovery and RSD% values obtained for the target compounds, support its feasibility for the fast POPs analysis.

The method developed is simple, fast (around 65 min) and involve a minimum organic solvent. Therefore, showed obvious advantages compared to other techniques related to solvent reduction or elimination, sample handling, a reduced matrix effect, integration of extraction phases, pre-concentration and introduction of the sample to the chromatographic system. All these factors reduce analysis costs.

On the other hand, in agreement to environmental and human exposure values to POPs [36,42–44], the detection and quantification limits found in this work are acceptable for biomonitoring. The simplicity and sensitivity of this technique allows it to be applied in biomonitoring programs in order to assess the presence of POPs mixtures in children and the general population, particularly at polluted sites, many of which exist in developing countries due to an extensive use of these compounds.

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