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# Simple solid-phase extraction method for determination of polychlorinated biphenyls and selected organochlorine pesticides in human serum

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**Abstract**

A simple off-line solid-phase extraction (SPE) method for isolation of polychlorinated biphenyls (PCBs) and selected organochlorine pesticides (OCPs) from human serum has been developed. The procedure includes denaturation of serum proteins by a mixture of water–1-propanol, application of the sample by aspiration twice repeatedly through the SPE column and elution with a mixture of *n*-hexane–dichlormethane. After final clean-up the compounds of interest were analysed by gas chromatography with micro-electron capture detection (GC–μECD). The recoveries achieved for PCB congeners using spiked porcine serum samples were 99–120% and for OCPs 88–115%. Relative standard deviations (RSD) ranged from 3 to 7%. The method was applied to real human serum samples and the recoveries of analytes in the serum were proportionally recalculated considering the recovery of the internal standard PCB-174. PCB-103 served as a syringe standard to correct volume of samples analysed. The aim of this study was to develop an effective off-line SPE procedure by optimization of existing SPE methods to supply laborious, solvent- and time-consuming liquid–liquid extraction (LLE) in routine analytical process.


*Keywords:* Polychlorinated biphenyls; Organochlorine pesticides; Solid-phase extraction; Human serum

## 1. Introduction

Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are persistent organic pollutants (POPs) present in environmental, food and biological materials along with polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans. In general, they are characterised by low solubility in water and on the contrary high solubility in organic solvents and lipids. Their lipophilicity and resistance to degradation leads to bioaccumulation of these compounds in human tissues and fluids. PCBs and selected OCPs are considered as risk factors because of their estrogenic and anti estrogenic properties and potential to act as direct or indirect carcinogens [1,2]. Their health effects such as chloracne, hyperpigmentation, endocrine disruption, impairment of immune responses, hepatotoxicity, reproductive effects have been intensively studied recently [3,4]. For that reason it seems to be very important to monitor organochlorine pollutants in human population. Human blood serum appears to be a suitable material for this purpose. Off-line solid-phase extraction (SPE) was chosen to prepare samples for determination of the PCBs and OCPs like a method superior to liquid–liquid extraction (LLE).

## 2. Experimental

### 2.1. Samples

The method was applied to the real sample of human serum (Institute of Preventive and Clinical Medicine, Bratislava, 1992). Recovery experiments were performed with porcine serum (slaughterhouse Modra, 2001) spiked with standard solution.

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The blood sample collected was allowed to clot for 60 min at room temperature. The serum was isolated by centrifugation for 10 min at 3000 × *g*. The isolated blood serum was transferred by Pasteur pipette into a Teflon screw-caped vial and kept frozen at −18 °C until next processing.

### 2.2. Chemicals

*n*-Heptane of spectroscopy grade quality, *n*-hexane, dichloromethane (DCM) and methanol for organic trace analysis, water, 1-propanol, isooctane of chromatography grade quality, sulphuric acid (95–97%) for analysis, silica gel 60 (0.063–0.200 mm) for column chromatography, anhydrous sodium sulphate of residual analysis grade quality, 2-propanol and formic acid (98–100%) for analysis were used. All these chemicals were from Merck (Darmstadt, Germany). Florisil (60–100 mesh) of chromatographv grade quality was from Fluka (Buchs, Switzerland).

### 2.3. Standards and analytes

Based on reported abundance and toxicity, a working stock solution was prepared from individual PCB standards (Ehrenstorfer, Accustandard): 28, 52, 77, 101, 103, 105, 114, 118, 123, 126, 138, 153, 156, 157, 167, 169, 170, 174, 180, 189 (IUPAC nos.) and selected OCPs (IIOC Warsaw, Supelco): HCB, α-,β-,γ-HCH, *p,p′*-DDT, *p,p′*-DDE. This standard solution was used for preparation of calibration standard solutions on five concentration levels (Table 1). The third level calibration solution was used as a spiking solution for recovery check experiments with porcine serum. PCB-174 served as a recovery standard and PCB-103 as a syringe standard to correct volume of samples analysed.

### 2.4. Extraction

Serum sample (5 g) was placed into a 24 ml vial and spiked with extraction standard solution. The fortified serum sample was allowed to equilibrate for 24 h in a refrigerator. After equilibration to the room temperature, the equivalent amount of water–1-propanol (85:15, v/v) mixture was added to the sample and it was sonicated for 5 min. SPE column (1 g/6 ml Alltech Extract-Clean High Capacity $C_{18}$ endcapped, Alltech Associates Inc., Lokeren, Belgium) was conditioned with 5 ml of methanol. Methanol was removed with 5 ml of water–1-propanol (85:15, v/v). Afterwards, the treated sample was applied aspirating twice repeatedly through the SPE column. The matrix residues were washed out with 5 ml of water–1-propanol (85:15, v/v) and the SPE column was dried for 1 h by aspiration of ambient air. The analytes were eluted from the dry SPE column with 5 ml of *n*-hexane–DCM (1:1, v/v) into a vial. The extract was concentrated by gentle stream of nitrogen nearly to dryness. The residues were diluted in 1 ml of *n*-hexane.

### 2.5. Clean-up

Final purification of the human serum extract was performed on florisil-silica gel column treated with sulphuric acid [5]. Glass column (i.d. = 7 mm) was sealed with silanized glass wool and gradually filled with: 0.5 g of activated florisil, 1 g of sulphuric acid on silica gel (0.475 ml of sulphuric acid/1 g of activated silica gel), 0.5 g of activated florisil and 1 g of anhydrous sodium sulphate. The column was washed with 8 ml of *n*-hexane. Residues diluted in 1 ml of *n*-hexane were applied on the column and the vial was rinsed three times with 0.5 ml of n-hexane. Then analytes were eluted with 10 ml of 10% DCM–*n*-hexane. Isooctane (10 μl) was added as a keeper to prevent the loss of more volatile compounds. The eluate was concentrated by vacuum rotary evaporator, transferred into a vial and carefully concentrated with nitrogen. The residues were diluted using syringe standard and analysed by GC–μECD.

### 2.6. Chromatographic conditions

The extract was injected in splitless mode (2 μl, purge time 1.5 min) onto a Gas Chromatograph 6890N (Agilent Technologies, USA) equipped with a 60 m × 0.25 mm i.d. × 0.25 μm film thickness DB-5 (J&W Scientific, USA) capillary column, a μECD and Agilent Chemstation software. Helium was used as a carrier gas (column head pressure 200 kPa) and nitrogen as a make-up gas (60 ml $min^{-1}$).

Temperatures of the injector and the μECD were 280 and 320 °C, respectively.

The following temperature programme was used: from initial temperature 110 °C (1.5 min) to 200 °C (0.2 min) at a rate of 30 °C $min^{-1}$ and then 2.5 °C $min^{-1}$ to final temperature 305 °C (5 min).

## 3. Results and discussion

### 3.1. Denaturation of serum proteins

SPE $C_{18}$ column mentioned above was chosen as a suitable one for extraction of target compounds. Experiments with acid denaturation method (formic acid) and organic solvents (water–1-propanol, water–2-propanol) were performed. Some authors prefer the use of formic acid for denaturation of serum or plasma proteins. Formic acid is added to the plasma to ensure the analytes are fully released from the sample matrices. The reduced pH due to formic acid inhibits protein binding and increase the extraction efficiency of the analytes by the $C_{18}$ sorbent [6]. Another laboratory published a method based on 2-propanol–formic acid for precipitation of blood. To maintain a low resistance of the SPE column during sample loading a dilution of the sample after ultrasonication by water–2-propanol was performed [7].

We found out that the denaturation of serum proteins by formic acid caused difficulties with serum throughput. We

Table 1
Levels of PCBs and OCPs in five calibration standard solutions

| Compound | Level 1 (ng ml$^{-1}$) | Level 2 (ng ml$^{-1}$) | Level 3 (ng ml$^{-1}$) | Level 4 (ng ml$^{-1}$) | Level 5 (ng ml$^{-1}$) |
|---|---|---|---|---|---|
| PCB-28 | 1 | 4 | 20 | 80 | 200 |
| PCB-52 | 1 | 4 | 20 | 80 | 200 |
| PCB-101 | 1 | 4 | 20 | 80 | 200 |
| PCB-138 | 1 | 4 | 20 | 80 | 200 |
| PCB-153 | 1 | 4 | 20 | 80 | 200 |
| PCB-180 | 1 | 4 | 20 | 80 | 200 |
| PCB-105 | 0.5 | 2 | 10 | 40 | 100 |
| PCB-114 | 0.5 | 2 | 10 | 40 | 100 |
| PCB-118 | 0.5 | 2 | 10 | 40 | 100 |
| PCB-123 | 0.5 | 2 | 10 | 40 | 100 |
| PCB-156 | 0.5 | 2 | 10 | 40 | 100 |
| PCB-157 | 0.5 | 2 | 10 | 40 | 100 |
| PCB-167 | 0.5 | 2 | 10 | 40 | 100 |
| PCB-170 | 0.5 | 2 | 10 | 40 | 100 |
| PCB-189 | 0.5 | 2 | 10 | 40 | 100 |
| PCB-103 | 5 | 20 | 100 | 400 | 1000 |
| PCB-174 | 5 | 20 | 100 | 400 | 1000 |
| HCB | 4 | 16 | 80 | 320 | 800 |
| α-HCH | 0.5 | 2 | 10 | 40 | 100 |
| β-HCH | 0.5 | 2 | 10 | 40 | 100 |
| γ-HCH | 1 | 4 | 20 | 80 | 200 |
| *p,p′*-DDE | 8 | 32 | 160 | 640 | 1600 |
| *p,p′*-DDT | 1 | 4 | 20 | 80 | 200 |

observed various flow of identical serum samples through the SPE column. To avoid coarse precipitation resulting in loss of analytes due to binding to serum proteins by occlusion in the precipitate [8], we preferred another approach.

Therefore, we finally compared water–2-propanol and water–1-propanol mixtures (both 85:15, v/v) for denaturation of serum proteins [9]. The compounds of interest were eluted in this procedure with *n*-hexane. The use of water–2-propanol (85:15, v/v) resulted in lower recoveries especially for PCBs having a high degree of chlorination (33–69% PCBs, 36–62% OCPs). The recoveries achieved for PCB congeners using water–1-propanol (85:15, v/v) ranged between 65 and 98% and for pesticides 63 and 78% (Table 2).

### 3.2. SPE conditions

Optimization of the sample application, washing, and elution steps was performed. Although the capacity of the SPE column was declared as adequate, a single aspiration seems to be insufficient especially for highly chlorinated PCBs (67–80%). We observed that repeated aspiration and replacement of n-hexane as eluting solvent by *n*-hexane–DCM (1:1) mixture gave better recoveries (Table 2). All recovery experiments were performed with tested clean porcine serum spiked with a standard solution consisted of 15 PCBs and 6 OCPs (Fig. 1). The concentration levels of analytes in the spiking solution were chosen on the basis of their concentrations in real human serum samples. Although the first recovery experiments were performed by porcine serum fortified with the second level calibration solution, the third level of calibration standard was determined as the appropriate solution for porcine serum spiking.

### 3.3. Method application to real human serum

The method was applied to real human serum samples. The recoveries of analytes in the serum were proportionally recalculated considering the recovery of the internal standard PCB-174. PCB-103 served as a syringe standard to correct volume of samples analysed. Analytical batch consisted of 10

Table 2
Comparison of PCB and OCP recoveries in spiked porcine serum eluted with *n*-hexane and *n*-hexane:DCM (1:1) from the SPE column

| Compound | *n*-Hexane ($n = 5$) | | *n*-Hexane:DCM (1:1) ($n = 10$) | |
|---|---|---|---|---|
| | *R* (%) | RSD (%) | *R* (%) | RSD (%) |
| α-HCH | 63 | 12 | 99 | 3 |
| HCB | 67 | 11 | 88 | 4 |
| β-HCH | 77 | 10 | 115 | 4 |
| γ-HCH | 72 | 12 | 102 | 5 |
| PCB-28 | 83 | 3 | 109 | 6 |
| PCB-52 | 82 | 8 | 113 | 5 |
| PCB-101 | 83 | 12 | 120 | 3 |
| *p,p′*-DDE | 73 | 13 | 94 | 4 |
| PCB-123 | 86 | 12 | 119 | 5 |
| PCB-118 | 85 | 12 | 114 | 5 |
| PCB-114 | 77 | 13 | 105 | 4 |
| PCB-153 | 82 | 13 | 112 | 4 |
| PCB-105 | 84 | 12 | 112 | 5 |
| *p,p′*-DDT | 78 | 12 | 120 | 7 |
| PCB-138 | 82 | 12 | 112 | 5 |
| PCB-167 | 77 | 14 | 109 | 5 |
| PCB-156 | 75 | 13 | 108 | 5 |
| PCB-157 | 76 | 13 | 109 | 5 |
| PCB-180 | 69 | 13 | 104 | 5 |
| PCB-170 | 73 | 14 | 107 | 5 |
| PCB-189 | 65 | 15 | 99 | 6 |

*R*: recovery; RSD: relative standard deviation.

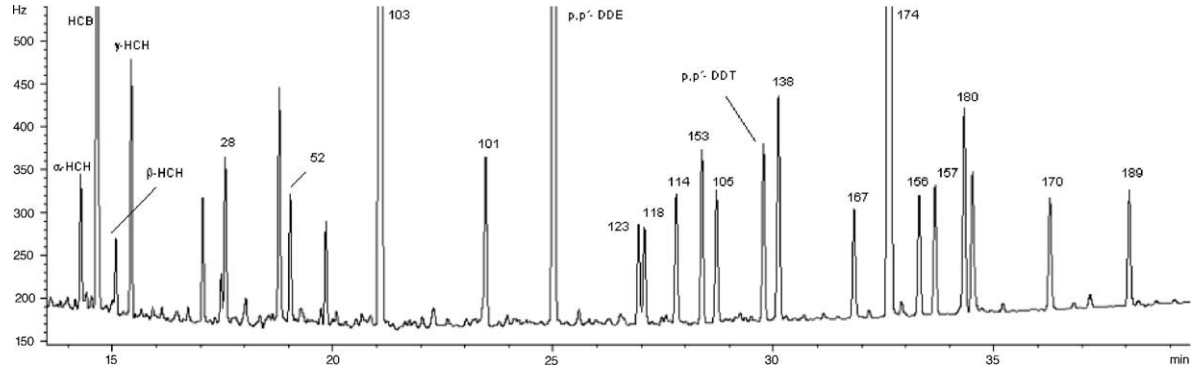


Fig. 1. GC–μECD analysis of spiked porcine serum.

serum specimens, one solvent blank and one recovery sample (spiked porcine serum). Relative standard deviations (RSD) ranged from 2.3 to 8.3% for compounds with significantly higher concentration than limit of detection (LOD) and from 12.6 to 26.4% for γ-HCH, PCB-52 and PCB-101 because of their very low concentration and potential interferences in real human serum (Table 3, Fig. 2).

### 3.4. Lipid determination

As the POPs are compounds characterised by high solubility in lipids, in most cases the results are expressed on the lipid basis, and the knowledge of the whole lipid content of the sample is needed. It is not possible to determine total lipids with SPE method. If it is required to evaluate results on lipid basis, another method such as LLE or enzymatic "summation" method [10] is necessary to use. We decided to express the results on serum basis during the method evaluation.

### 3.5. Quality control

The linearity of μECD was studied by preparing of calibration curves from five calibration standard solutions on five concentration levels (Table 1). Ratio of the PCB-174 concentration in recovery sample (spiked porcine serum) and PCB-174 in human serum samples was used for proportional recalculation of recoveries of other analytes. PCB-103 was chosen as an appropriate syringe standard as described above.

Certified reference material, Mackerel oil (CRM no. 350. Community bureau of reference, Brussels, Belgium) was

Table 3
Levels of PCBs and OCPs with appropriate standard deviations (SD), relative standard deviations (RSD), recoveries (R) and limits of detection (LOD) concerning 10 identical human serum samples treated by the developed solid-phase extraction procedure

| Compound | $n = 10$ | | | | |
|---|---|---|---|---|---|
| | $C_A$[a] (ng ml$^{-1}_{serum}$) | SD (ng ml$^{-1}_{serum}$) | RSD (%) | $R$[b] (%) | LOD (ng ml$^{-1}_{serum}$) |
| α-HCH | <0.0018 | | | 86 | 0.0018 |
| HCB | 7.33 | 0.227 | 3.1 | 76 | 0.0015 |
| β-HCH | 0.540 | 0.018 | 3.3 | 99 | 0.0050 |
| γ-HCH | 0.091 | 0.024 | 26.1 | 88 | 0.0019 |
| PCB-28 | 0.085 | 0.007 | 8.3 | 94 | 0.0026 |
| PCB-52 | 0.029 | 0.004 | 12.6 | 97 | 0.0051 |
| PCB-101 | 0.017 | 0.004 | 26.4 | 103 | 0.0026 |
| *p,p′*-DDE | 11.8 | 0.340 | 2.9 | 81 | 0.0022 |
| PCB-123 | <0.0025 | | | 103 | 0.0025 |
| PCB-118 | 0.178 | 0.006 | 3.3 | 98 | 0.0027 |
| PCB-114 | 0.025 | 0.001 | 5.5 | 90 | 0.0019 |
| PCB-153 | 1.81 | 0.046 | 2.5 | 97 | 0.0037 |
| PCB-105 | 0.023 | 0.002 | 7.7 | 97 | 0.0028 |
| *p,p′*-DDT | 0.285 | 0.012 | 4.3 | 104 | 0.0016 |
| PCB-138 | 1.06 | 0.029 | 2.7 | 97 | 0.0019 |
| PCB-167 | 0.070 | 0.004 | 5.9 | 94 | 0.0014 |
| PCB-156 | 0.244 | 0.006 | 2.5 | 93 | 0.0013 |
| PCB-157 | 0.023 | 0.001 | 3.2 | 94 | 0.0012 |
| PCB-180 | 1.69 | 0.051 | 3.0 | 90 | 0.0013 |
| PCB-170 | 0.714 | 0.018 | 2.5 | 92 | 0.0019 |
| PCB-189 | 0.025 | 0.001 | 4.5 | 85 | 0.0019 |

[a] Average concentration.
[b] Recovery of analytes proportionally recalculated considering the recovery of the internal standard PCB-174.

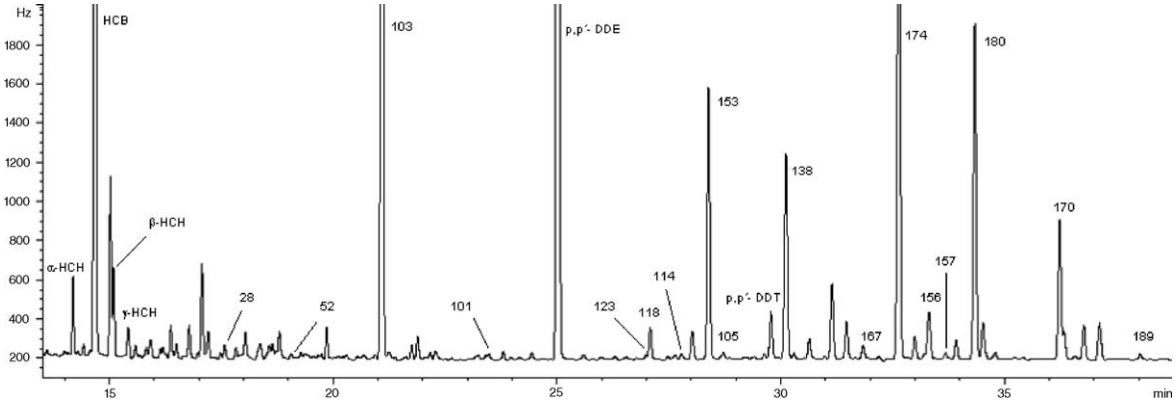


Fig. 2. GC–μECD analysis of human serum spiked with internal standard PCB-174 and syringe standard PCB-103.

Table 4
Comparison of analysed certified reference material of mackerel oil (CRM no. 350) with certified values

| Compound | ($ng\,g^{-1}$) | | | | | | | D[c] (%) |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | $C_A$[a] | $C_C$[b] | |
| PCB-28 | 16.5 | 18.1 | 17.8 | 15.6 | 17.0 | 17.0 | 22.5 | −24.4 |
| PCB-52 | 59.4 | 67.3 | 58.0 | 67.5 | 59.0 | 62.2 | 62.0 | 0.4 |
| PCB-101 | 151.1 | 177.0 | 152.3 | 186.9 | 157.0 | 164.9 | 164.0 | 0.5 |
| PCB-118 | 112.8 | 132.2 | 120.6 | 125.2 | 127.1 | 123.6 | 142.0 | −13.0 |
| PCB-153 | 277.4 | 317.7 | 286.3 | 320.8 | 311.1 | 302.7 | 317.0 | −4.5 |
| PCB-138 + 163 | 239.1 | 273.9 | 242.6 | 272.7 | 269.1 | 259.5 | 274.0[d] | −5.3 |
| PCB-180 | 64.0 | 70.8 | 64.1 | 73.3 | 70.7 | 68.6 | 72.0 | −4.8 |

[a] Average concentration of five analysis.
[b] Certified concentration.
[c] Difference between mean value measured by our method and the certified value declared by producer in percentage.
[d] Value declared by producer as not certified.

used to test the clean-up procedure on the florisil-silica gel column with sulphuric acid. The average PCB and OCP concentrations of five measurements show good agreement with certified values (Table 4).

The analysis of two blanks was performed: solvent blank from SPE and solvent blank from clean-up on the florisil-silica gel column with sulphuric acid. After extraction, the SPE column was dried by aspiration of ambient air. The SPE blank and the clean-up solvent blank show no contamination or interference with PCBs or OCPs from aspirated air or solvents as well.

Limits of detection (LOD) were evaluated from the ratio of noise/peak height. The peak of analyte should be at least three times higher than the noise. Limits of quantification (LOQ) represent concentration values in the sample, which correspond to the lowest concentration of calibration solutions (level 1 in Table 1). The values of LOQ were 0.01–0.02 for PCBs and $0.01 - 0.16\,ng\,ml^{-1}_{serum}$ for OCPs.

The values of standard deviation of the determination of PCBs given in Table 3 demonstrate that results with sufficient repeatability and accuracy can be obtained using this method.

In the case of method application for routine analysis a solvent blank sample (from the whole SPE and clean-up process as well) and a recovery sample (fortified porcine serum) were analysed with the batch of 10 human serum samples. The fortified porcine serum functioned as a recovery sample for the recovery evaluation as mentioned above. The μECD response and the calibration curve were checked by injection of the daily standard solution of the same concentration as the third calibration level. The relative deviation of concentrations obtained could not differ more than 15% of the calibration standard concentrations. The clean-up procedure was verified repeatedly every 3 months analysing certified reference material CRM no. 350 as described above.

## 4. Conclusions

SPE in comparison with LLE appears to be more effective, solvent and time saving, mainly for laboratories providing large number of analysis with rapid reporting time. Since the method showed sufficient reliability, accuracy, and repeatability it was applied to real human serum samples taken within the fifth FP project “Evaluating human health risk from low-dose and long-term PCB exposure (PCBRISK)”.

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