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# Determination of polychlorinated biphenyls in biota samples using simultaneous pressurized liquid extraction and purification ☆

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**Abstract**

In order to reduce time and cost of analysis, a new pressurised liquid extraction method that automatically and rapidly achieves quantitative and selective (i.e., lipid-free) extraction of polychlorinated biphenyls (PCBs) in biota tissues was optimized. It consists of on-line clean-up by inclusion of sorbents in the extraction cell. The freeze–dried sample is dispersed with Florisil and loaded in the extraction cell containing an extra amount of Florisil. The extraction is performed under mild conditions using 55 ml of a dichloromethane–pentane (15:85) mixture, a temperature of 40 °C, a static extraction time of 10 min and two extraction cycles. The Florisil retains coextracted lipids from the matrix, and the extract, after pre-concentration, is clean enough for direct injection into GC–MS and GC–electron-capture detection (ECD). Quantitative recoveries (from 90 to 106%) are obtained for both native and spiked PCB congeners in samples with a high lipidic content (up to 42% dry mass, in spoonbill eggs). The reproducibility of replicate extractions was better than 11% relative standard deviation. Method detection limits were in the ranges of 0.001–0.004 and 0.002–0.07 ng $g^{-1}$ dry mass for GC–ECD and GC–MS–MS, respectively. The method was validated using the standard reference material SRM 2974 (a mussel tissue) from the US National Institute of Standards and Technology, compared to Soxhlet and matrix solid-phase dispersion extraction methods, and used to evaluate the contamination by PCBs in bivalves from South of Spain. © 2002 Elsevier Science B.V. All rights reserved.

*Keywords:* Pressurized liquid extraction; Extraction methods; Environmental analysis; Polychlorinate biphenyls

## 1. Introduction

Analysis of polychlorinated biphenyls (PCBs) in environmental matrices usually necessitates of an extraction step followed by gas chromatographic (GC) analysis. Extractions are traditionally performed by means of Soxhlet or sonication [1]. Unfortunately, these techniques demand long extraction times and large volumes of highly purified and hazardous organic solvents, generating dirty extracts that involve extensive clean-up steps before analysis. As a consequence there has been an increasing demand for new techniques, such as microwave-assisted extraction (MAE) [2], supercritical fluid extraction (SFE) [3] and pressurised liquid extraction (PLE) [4], that overcome some of the

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previous problems [5]. However, recoveries obtained with SFE are matrix dependent [6], and MAE requires long cool-down periods and filtration to separate the extract from the solid material once the extraction is finished [7].

PLE is gaining in popularity for the extraction of persistent organic pollutants (POPs) in environmental matrices [4]. This technique uses conventional liquid solvents at high temperatures (up to 200 °C) and pressures (up to 3000 p.s.i.; p.s.i.=6894.76 Pa) to extract compounds from solid samples quickly (less than 30 min) and with a small solvent volume (less than 100 ml). By pressuring the sample cell, it is possible to keep the solvent in a liquid phase at the high extraction temperatures used, and force solvent into the sample matrix. Temperature rise increases solubility, diffusion rate and mass transfer, lowering solvent viscosity and surface tension. Certain intermolecular bonds can only be broken at high temperatures and pressures, and this is probably the decisive advantage of PLE compared to traditional techniques [8,9].

PLE has been used to extract different POPs including PCBs [4,10]. Most applications on PCB extractions have been performed using soil [8,11] and sediments [4,12,13]. Biota have been paid relatively little attention and the number of papers presented is limited so far [9,14,15]. Moreover, one of the major drawbacks of those methods is the presence of a large amount of coextracted lipid. The lipids must be separated from PCBs, or chromatographic performance will seriously deteriorate. The extraction must be followed by a clean-up step to obtain extracts that may be analyzed by GC, but the time involved gives rise to high labour costs. As a consequence, there is a demand of methods that simultaneously extract and purify the sample, such as that called matrix solid-phase dispersion (MSPD). In MSPD, the sample is blended together with a suitable sorbent, and their mixture is used to fill a chromatographic column. Then, the analytes are selectively eluted with an appropriate solvent [16].

To our knowledge, two simultaneous extraction–purification methods for PCBs in biota samples using PLE have been reported so far [14,17]. Alumina was successfully used as a retainer sorbent in a Dionex Application Note [14]. Recently, Björklund et al. [17] concluded that the use of sulphuric acid impregnated silica gel inside the PLE cell was superior in some extent to the use of Florisil.

The objectives of this work are the development and validation of a new selective pressurized liquid extraction (SPLE) method for PCB analysis, and its comparison to a MSPD method and two conventional methods, such as a non-selective pressurized liquid extraction (NSPLE) and Soxhlet, follow by column clean-up. The possibilities of removing the lipids from biota samples were investigated by including several sorbents into the PLE cells, obtaining extracts that may be directly analyzed by GC–MS and GC–electron-capture detection (ECD). The influence of several experimental variables affecting the extraction efficiency of PCBs with PLE was also studied. Recoveries of PCBs with different chlorine atom numbers from biota samples were evaluated.

## 2. Experimental

### 2.1. Chemicals

PCBs standards, vinclozolin and ethyl parathion were purchased from Supelco (Bellefonte, PA, USA), AccuStandard (New Haven, CT, USA) and Riedel-de Haën (Seelze, Germany). Working solutions were prepared in hexane. Extraction solvents were HPLC grade (Romil, Barcelona, Spain).

Florisil, silica gel, alumina and anhydrous $Na_2SO_4$ (supplied by Merck, Darmstadt, Germany) were activated at 150 °C for 24 h prior to use. Other sorbents, such as 2,3-dihydroxypropoxypropyl (Diol) and cyanopropyl (CN) bonded silica, obtained from IST (Mid Glamorgan, UK), were used as received. Celatom was supplied by Aldrich (Milwaukee, WI, USA).

### 2.2. Sample collection

Eggs of spoonbill (*Platalea leucordia*), and oysters (*Crassostrea gigas*) were collected from the Odiel Marshes Natural Park (Huelva, Spain). Clams (*Chamalea gallina*, *Scrobicularia plana*, *Gerastoderma edulis* and *Donax* sp.) and fish (*Solea senegalensis*) were collected from the south coast of Spain, and mussels (*Mytilus edulis*) from a local market in Cordoba (Spain). Soft tissues were freeze–

dried for 48 h (Virtis, New York, NY, USA), and then grinded and sieved to 100 μm particle size to enhance the analyte transport to particle surfaces and extractability [4].

Spiked samples were made mixing 2-g aliquots with a 20-ml standard solution containing 1.4 and 18 ng for low and high spike levels, respectively, of each PCB in hexane for 24 h (IUPAC numbers 1, 11, 29, 47, 121, 136, 185, 194, 206 and 209). Then the solvent was evaporated under a nitrogen stream. This PCB mixture was selected because of their distribution throughout the chromatogram and coverage of the chlorination range.

### *2.3. Non-selective PLE*

Pressurized solvent extraction was carried out using a fully automated ASE 200 system (Dionex, Camberley, UK). Biota samples of 2 g (dry mass) were weighed, and ground with pre-cleaned Celatom (1:2) in an agate mortar with a pestle until the mixture became homogeneous. The mixture was placed into the stainless-steel extraction cell (11 ml capacity) prepared for PLE by securing the bottom and inserting two cellulose filters (Dionex) to prevent clogging of the metal frit present at the cap. The dead volume was filled with Celatom and the cell was sealed with the top cell cap. After temperature equilibration (5 min), a static extraction was carried out at 1600 p.s.i. and 100 °C for 10 min using pentane. The flush volume amounted to 150% of the extraction cell volume. The extraction was cycled twice and the cell was finally purged with a nitrogen stream for 60 s. The volume of the resulting extract was about 30 ml. Extractions were made in triplicate.

### *2.4. Selective PLE*

The PLE conditions were optimized for the extraction of PCB congeners from spoonbill eggs as discussed below in Section 3. Florisil, silica gel, alumina, Diol and CN were tested as sorbents in the extraction cell. The final optimized method was as follows: a 22-ml extraction cell was loaded by inserting two cellulose filters into the cell outlet, followed by 6 g of Florisil. Samples of 2 g were weighed, and ground with Florisil (1:2) in an agate mortar with a pestle until the mixture became homogeneous. The mixture was loaded into the extraction cell on top of Florisil. The dead volume was filled with Celatom, and the cell was sealed with the top cell cap. The extraction cell was heated to 40 °C and filled with a dichloromethane (DCM)–pentane (15:85, v/v) mixture until the pressure reached 2000 p.s.i.. After an oven heat-up time of 5 min, under these conditions, two static extractions of 10 min at constant pressure and temperature were developed. After this static period, fresh solvent was introduced to flush the lines and cell, and the extract was collected in the vial. The flush volume amounted to 150% of the extraction cell. The remaining solvent in the extraction cell was forced out into the collection vial with pressurised nitrogen. The volume of the resulting extract was about 55 ml. Extractions were made in triplicate.

### *2.5. Soxhlet extraction*

Soxhlet extraction for comparison of the PLE results was accomplished in cellulose thimbles containing 2-g sample aliquots. Triplicate extractions were done using 150 ml of a mixture of pentane–acetone (2:1, v/v) for 13 h with an approximately 10 min reflux cycle time.

In order to test the efficiency of the Soxhlet extraction, a subsequent extraction was performed with increasing strength of extraction using 150 ml of a mixture of pentane–acetone (1:1, v/v) for 13 h.

### *2.6. Matrix solid-phase dispersion extraction*

A 4-g portion of Florisil and 2 g of sample were added to an agate mortar. The mixture was gently blended for 4 min with a pestle to yield a homogeneous-appearing material. The mixture was then placed in a glass column containing a frit and 6 g of Florisil packing at the bottom. The column was first eluted with 20 ml of a mixture of DCM–pentane (15:85) by gravitational flow (1 ml $min^{-1}$) and, after a static extraction time of 10 min, with 16 ml of extractant. The procedure was repeated once, and finally, when the flow had ceased, the remaining solvent was purged by applying a $N_2$ stream. Experiments were made in triplicate.

*2.7. Clean-up*

After extraction with NSPLE and Soxhlet methods, the extract was concentrated to 2 ml by vacuum rotatory evaporation with a temperature controlled bath (40 °C), and then transferred on top of a 6-g Florisil column pre-rinsed with 50 ml of 3% DCM in pentane. PCBs were eluted with 50 ml of this mixture at a flow-rate of 1.5 ml $min^{-1}$, and the majority of lipids and biogenic materials were retained longer. However, the clean up procedure was repeated once before acceptable low levels of contaminants were reached in the extract.

The eluates from clean up (for NSPLE and Soxhlet methods) and the extracts from SPLE and MSPD methods were concentrated to 2 ml by rotatory evaporation, and then to dryness under a nitrogen stream. The residue was dissolved in 100 μl of hexane containing either 100 ng $ml^{-1}$ of vinclozolin or 10 000 ng $ml^{-1}$ of ethyl parathion as internal standards for GC–ECD and GC–MS analysis, respectively.

*2.8. Chromatographic analysis by GC–MS*

Extracts were analyzed on a Varian Model 3800 gas chromatograph directly connected to a Saturn 2000 ion-trap mass spectrometry detector (Varian, Sunnyvale, CA, USA). The gas chromatograph was equipped with a 1.5 m×0.32 mm deactivated fused-silica guard column connected to a VA5-MS fused-silica capillary column (30 m×0.25 mm I.D., film thickness 0.25 μm) from Varian. The carrier gas was helium at a flow-rate of 1.3 ml $min^{-1}$. A sample volume of 1.0 μl was injected into a split–splitless injector, operated in the splitless mode (splitless time: 60 s) at a temperature of 280 °C. The temperature of the GC–MS transfer line was 280 °C. The oven temperature program started at 80 °C for 1 min. Subsequently, the temperature was increased to 250 °C at 5 °C $min^{-1}$, held at 250 °C for 1 min and finally ramped to 280 °C at 20 °C $min^{-1}$ and held for 4.5 min.

Full scan electron impact ionization data were acquired under the following conditions: solvent delay 11 min, 70 eV electron impact energy, emission current 30 μA, scan rate 0.8 scans $s^{-1}$, manifold and trap temperatures 50 and 200 °C, respectively. The automatic gain control (AGC) was switched on with a target fixed at 2000 counts. To obtain better signal-noise rates, the overall run time was splitted into three segments which were assigned to PCBs with different grades of chlorination scanning the following ranges ($m/z$): 175–340 in the second segment (15–29 min) and 300–600 in the third segment (29–42 min).

In order to lower the detection limits, the option MS–MS was used. To program the isolation of precursor ions for every compound along the chromatographic run, the overall run time was split into 23 segments. Each segment was assigned to a fragment ion chosen as the base peak observed in the EI mass spectrum for each compound. Precursor ions were isolated using a 3 amu isolation window and subjected to collision-induced dissociation (CID) for 20 ms. Automated method development toolkit software was used to optimize the CID parameters (low-mass cutoff and CID voltage) to obtain maximum sensitivity. The excitation storage level was selected as the minimum value that allowed the dissociation of the precursor ion. The MS–MS parameters are shown in Table 1. In most cases, the precursor ions dissociates to a single, intense product ion, which is ideal for trace analysis since all the ion current belonging to the precursor ion will be transferred to the ion current of the single product ion thus improving sensitivity. High CID energies were required due to the stable nature of the selected precursor ions.

The quantitation of the individual PCB congeners was performed by internal standard multi-point calibration using five standard solutions covering the ranges 25–1000 and 2–300 ng $g^{-1}$ for GC–MS and GC–MS–MS, respectively. The compounds are quantified using the ratio of the analyte and internal standard response (peak area). The internal standard (ethyl parathion) was added to the sample extract just prior to the instrumental analysis. Calibration curves were obtained for all compounds by regression analysis of peak areas versus injected concentrations. In all cases, regression coefficients were higher than 0.995. Instrumental (absolute) detection limits were in the ranges of 10 and 0.2 pg for GC–MS and GC–MS–MS, respectively. Each calibration standard and sample extract were injected three times. Every

Table 1
MS–MS parameters for PCB analysis

| PCB congener | Precursor ion | Product ions | CID parameters | |
|---|---|---|---|---|
| | | | Store level ($m/z$) | Amplitude (V) |
| PCB 1 | 188 | 151–153 | 70 | 71.4 |
| PCB 11 | 222 | 152–153 | 80 | 85.0 |
| PCB 29 | 258 | 186–188 | 90 | 94.0 |
| PCB 47 | 292 | 220–223 | 95 | 88.0 |
| PCB 121 | 326 | 244–256 | 95 | 88.0 |
| PCB 87, 99, 101 | 326 | 254–256 | 100 | 90.0 |
| PCB 136,153, 128, 138, 156 | 360 | 288–291 | 100 | 87.0 |
| PCB 105, 118 | 326 | 250–336 | 95 | 88.0 |
| PCB 170, 180, 183, 185 | 394 | 322–325 | 100 | 80.0 |
| PCB 194 | 430 | 355–440 | 189 | 0.85 |
| PCB 206 | 464 | 427–429 | 204 | 1.30 |
| PCB 209 | 498 | 428+291 | 219 | 0.75 |

four sample extracts, one of the calibration solutions was injected to check any drift.

### *2.9. Chromatographic analysis by GC–ECD*

Extracts were analyzed on a Hewlett-Packard Model 6890 gas chromatograph (Palo Alto, USA), equipped with a fused-silica capillary column (SFE 74, 50 m×0.23 mm I.D., film thickness 0.25 μm) from Análisis Vínicos (Madrid, Spain). The carrier gas was helium at a flow-rate of 1.33 ml min$^{-1}$. A sample volume of 3.0 μl was injected into a split–splitless injector, operated in the splitless mode (splitless time: 45 s) at a temperature of 280 °C. The temperature of the detector was 300 °C. The oven temperature program started at 40 °C, ramped to 150 °C at 20 °C min$^{-1}$, then to 300 °C at 3 °C min$^{-1}$, and finally held at 300 °C for 6 min.

Six standard solutions covering the range of 2–600 ng g$^{-1}$, and vinclozolin as internal standard were used for quantitation. Regression coefficients higher than 0.995 were obtained. Instrumental detection limits were in the range of 0.2–0.4 pg, except for PCB 1 (10 pg) and PCB 11 (2.3 pg).

### *2.10. Validation of the SPLE method*

In order to estimate the accuracy and precision of the analytical method, the reference material SRM 2974 from NIST (US National Institute of Standards and Technology, Gaithersburg, MS, USA), a mussel tissue, was extracted in triplicate and analyzed by GC–ECD and GC–MS–MS.

Method detection limits (MDLs) of the SPLE method were evaluated from standard deviation ($\sigma$) of seven replicates of the mussel sample from Cordoba market, spiked with PCB congeners at levels of 0.145 and 0.134 ng g$^{-1}$ for GC–ECD and GC–MS–MS, respectively. MDL was calculated as:

$$\mathrm{MDL} = t_{(6\ \mathrm{df},\ 1-\alpha=0.99)}\sigma$$

where $t$ is the student's $t$ distribution for 6 degrees of freedom at the 99% confidence level (3.143).

### *2.11. Determination of the lipid content*

In order to determine the amount of lipids in biota samples, 2 g of homogenized sample were Soxhlet extracted with a pentane–acetone (7:10, v/v) mixture for 13 h [18]. The solvent was removed by rotatory evaporation and the lipid content was gravimetrically determined. In addition, the lipid content in every extract obtained from NSPLE, SPLE and MSPD was evaluated by the same gravimetric procedure. Experiments for determining PCB and lipid concentrations were performed separately. All results are expressed in dry mass basis.

## 3. Results and discussion

### 3.1. Optimization of SPLE method

A sample of egg was subjected to PLE with the aim of finding conditions under which this extraction technique would achieve both an extraction efficiency comparable with the Soxhlet extraction of the PCB congeners, and an extract ready for injection into the GC–MS. Since eggs contain a high amount of lipid, this matrix seemed to be one of the most difficult ones from which to obtain satisfactory recoveries and purification. The appropriate conditions for eggs should be sufficient for bivalve and fish samples with lower lipid contents.

To optimize the SPLE method, an amount of 2 g of sample spiked with 9 ng $g^{-1}$ of the PCBs (IUPAC numbers 1, 11, 29, 47, 121, 136, 185, 194, 206 and 209) was dispersed with 4 g of either an inert matrix (Celatom) or a polar sorbent (Florisil). The extraction cell was charged with 6 g of either Celatom or Florisil, respectively, and pentane or DCM–pentane mixtures (5, 10, 15 and 50% DCM) were tested as extracting solvents using a temperature of 175 °C, a pressure of 2000 p.s.i., 15 min of static extraction time, a flush volume of 150% of the cell volume, and three cycles. Pentane was selected instead of hexane owing to its lower boiling point and the shorter time required to prepare the extracts. Results for both native and spiked PCBs are presented in Table 2, and may be compared to those obtained from Soxhlet (Table 6). Quantitative recoveries were obtained using Celatom, but a high amount of lipids was also extracted, and a clean-up step was necessary before chromatographic analysis. When Florisil was added to the extraction cell, at least a 15% of DCM was

Table 2
PCB concentration found in spoonbill eggs by PLE using solvents with different polarities, and filling the extraction cell (22 ml volume) with 6 g Florisil or Celatom (2 g of sample dispersed with 4 g of Florisil or Celatom, 175 °C, 2000 p.s.i., 3×15 min static extraction, 150% flush). Experiments were made in triplicate

| PCB | Concentration±SD (ng $g^{-1}$, dry mass) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Pentane | | DCM–Pentane (5:95) | | DCM–Pentane (10:90) | | DCM–Pentane (15:85) | | DCM–Pentane (50:50) | |
| | Florisil | Celatom | Florisil | Celatom | Florisil | Celatom | Florisil | Celatom | Florisil | Celatom |
| 1 | 5.8±0.6 | 9.1±0.6 | 6.7±0.7 | 8.7±0.5 | 7.6±0.4 | 9.2±0.3 | 8.5±0.4 | 8.6±0.4 | 8.7±0.7 | 9.2±0.6 |
| 11 | 5.6±0.4 | 8.4±0.5 | 6.3±0.6 | 8.1±0.6 | 6.6±0.4 | 9.5±0.8 | 8.5±0.9 | 9.8±0.7 | 8.8±0.4 | 8.7±0.5 |
| 29 | 6.1±0.6 | 9.1±0.7 | 6.8±0.6 | 9.3±0.4 | 8.2±0.5 | 8.7±0.4 | 9.2±0.5 | 8.1±0.5 | 9.1±0.3 | 9.4±0.6 |
| 47 | 5.1±0.2 | 8.3±0.7 | 6.3±0.6 | 8.9±0.6 | 6.8±0.7 | 9.4±0.7 | 9.5±0.6 | 9.5±0.4 | 8.2±0.8 | 8.4±0.7 |
| 87 | 10.7±0.7 | 14.7±0.8 | 11.7±0.7 | 14.5±0.7 | 11.8±1.0 | 15.1±0.7 | 15.8±1.4 | 14.0±0.8 | 15.5±1.7 | 14.3±1.3 |
| 99 | 19.6±2.0 | 27.6±2.4 | 19.9±1.7 | 35.7±3.2 | 23.5±2.1 | 28.4±2.9 | 27.4±2.0 | 26.4±1.9 | 28.6±1.9 | 28.1±1.2 |
| 101 | 2.7±0.3 | 3.8±0.4 | 3.0±0.3 | 3.6±0.3 | 3.0±0.2 | 3.3±0.3 | 3.6±0.2 | 3.5±0.4 | 4.1±0.2 | 3.7±0.2 |
| 105 | 12.0±0.8 | 17.2±1.5 | 13.2±1.2 | 16.7±0.4 | 14.4±0.6 | 18.1±1.0 | 17.6±1.5 | 17.0±0.8 | 17.2±1.3 | 16.8±1.7 |
| 118 | 40.0±2.9 | 57.1±3.8 | 44.0±3.8 | 55.2±3.6 | 46.3±4.0 | 61.0±4.2 | 54.5±3.3 | 57.4±4.8 | 55.7±4.2 | 60.2±4.3 |
| 121 | 6.3±0.4 | 9.4±1.0 | 6.9±0.5 | 9.7±0.7 | 7.5±0.4 | 8.5±0.9 | 9.2±0.7 | 9.1±0.6 | 9.0±0.6 | 9.4±0.6 |
| 128 | 13.0±1.2 | 18.9±1.7 | 12.6±1.1 | 20.1±2.1 | 15.3±1.2 | 17.9±1.7 | 18.2±1.8 | 18.0±1.2 | 18.4±1.1 | 20.3±1.9 |
| 136 | 6.3±0.5 | 9.3±0.7 | 6.9±0.6 | 9.7±0.5 | 7.8±0.5 | 8.6±0.9 | 8.8±0.5 | 8.9±0.4 | 8.9±0.5 | 9.1±0.4 |
| 138 | 142±10 | 209±16 | 163±12 | 205±9 | 169±10 | 215±13 | 209±14 | 199±18 | 203±14 | 193±14 |
| 153 | 427±35 | 610±49 | 478±30 | 610±50 | 482±28 | 643±47 | 638±44 | 647±31 | 610±39 | 657±51 |
| 156 | 10.2±0.6 | 14.0±0.9 | 7.7±0.5 | 12.9±0.7 | 11.3±0.5 | 15.8±0.5 | 14.7±1.1 | 15.2±1.3 | 14.0±1.0 | 13.6±0.9 |
| 170 | 73.6±4.3 | 100±6 | 76.8±4 | 91.4±5.7 | 84.7±5.3 | 103±6 | 100±6 | 93.1±4.3 | 99.7±4.5 | 101±4 |
| 180 | 211±21 | 351±25 | 278±24 | 334±26 | 288±14 | 363±32 | 381±28 | 350±18 | 339±17 | 351±20 |
| 183 | 35.5±3.6 | 52.2±4.1 | 39.6±1.9 | 51.2±5.2 | 41.8±2.8 | 56.7±4.0 | 51.6±2.4 | 55.0±2.3 | 53.5±3.1 | 54.6±4 |
| 185 | 5.2±0.4 | 8.5±0.9 | 6.6±0.3 | 8.7±0.4 | 7.3±0.6 | 9.5±0.4 | 7.9±0.8 | 8.6±0.5 | 8.4±0.4 | 8.8±0.4 |
| 194 | 27.1±2.4 | 38.1±3.7 | 30.1±3.1 | 34.6±2.9 | 31.2±2.8 | 41.2±3.1 | 37.6±3.6 | 40.5±2.1 | 37.2±3.0 | 37.3±3.8 |
| 206 | 6.3±0.6 | 8.8±0.7 | 6.4±0.4 | 9.1±0.5 | 7.3±0.5 | 8.4±0.3 | 8.5±0.4 | 9.4±0.4 | 8.5±0.3 | 9.1±0.6 |
| 209 | 6.6±0.7 | 9.4±0.8 | 7.5±0.4 | 8.7±0.3 | 8.0±0.4 | 8.7±0.4 | 9.0±0.3 | 9.1±0.6 | 9.6±0.4 | 9.4±0.7 |
| Lipids % | 14.3±1.1 | 27.0±1.4 | 19.8±1.2 | 35.8±2.3 | 25.9±2.0 | 38.3±2.2 | 27.7±1.1 | 40.5±2.2 | 36.5±2.2 | 41.7±2.1 |

necessary to obtain equivalent recoveries to the Soxhlet extraction ($t$-test, $P>0.051$). Significant lower recoveries were obtained with lower percentages of DCM (analysis of variance, ANOVA, $P<0.021$). A high percentage of lipids was associated with the surface of Florisil, although they were not totally removed from the extract.

In order to obtain cleaner extracts, several extraction temperatures (30, 40, 60, 125 and 175 °C) were tested. Results for the PCB congeners 118, 138 and 153 are presented in Fig. 1 as examples, together with the percentage of lipid extracted. Similar results were obtained for the other PCBs. No significant differences were found for PCB recoveries using temperatures between 40 and 175 °C (ANOVA, $P>0.20$). However, lower values were observed at 30 °C for several PCB congeners (IUPAC numbers 1, 29, 87, 153, 105, 206 and 209) (post-hoc comparison test, $P<0.009$). The lipid content also decreased using lower temperatures, and the extract obtained at 40 °C (0.83% of lipid) was sufficiently clean for the GC–MS analysis. This temperature was selected for further experiments. A low temperature (40 °C) helping to obtain cleaner extracts has also been described for PCB extraction from mosses using PLE and hexane as solvent [9].

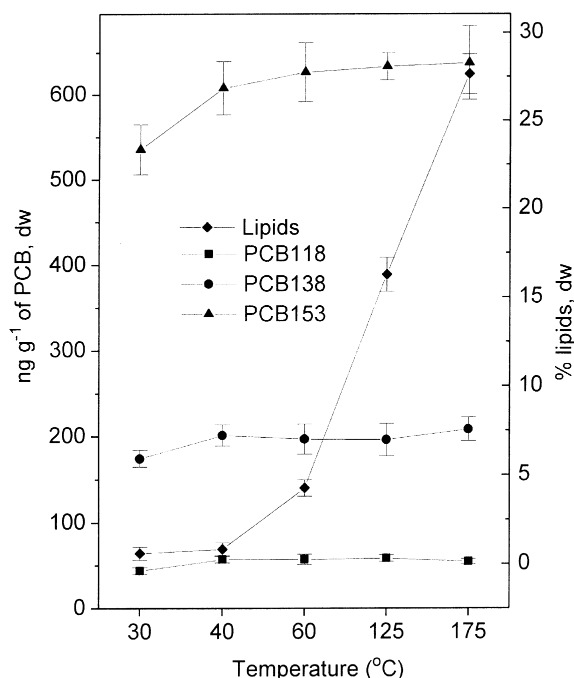


Fig. 1. Influence of the temperature on the extraction of selected PCBs and lipids from egg samples using PLE (2 g of sample dispersed with 4 g of Florisil on top of 6 g of Florisil in a 22 ml cell, 15% of dichloromethane in pentane, 2000 p.s.i., 3×15 min static extraction, 150% of flush volume). Error bars represent standard deviation (experiments were made in triplicate). dw = Dry mass.

The effect of the pressure on the recovery was studied in the range between 500 and 2500 p.s.i., and did not have any significant influence on the extraction (ANOVA, $P>0.24$).

The duration of extraction was tested in two steps. Firstly, the time of static extraction period was decreased, and secondly, the sample was subjected to one or two extraction cycles. Results of these experiments are presented in Table 3. At least, a static extraction time of 10 min and two cycles were needed to obtain quantitative recoveries ($t$-test, $P>0.05$), and were selected for further experiments.

The consumption of solvent depend on both the number of extraction cycles and the portion of fresh solvent (measured as a percentage of the cell volume) used to rinse the cell after the static extraction step. The influence of this latter variable (flush percentage) on the PCB recoveries was studied, and results showed that the extraction yields improved by factors of 1.23 and 1.38 as flush percentage increased from 50 to 100 and 150%, respectively. The maximum value of flush percentage technically available was selected, which resulted in a total solvent volume of 55 ml to quantitatively extract the PCBs.

Under these optimised conditions, several sorbents were tested, and results are presented in Table 4. Quantitative recoveries were observed with all the sorbents, but the cleanest extracts (with the lowest percentages of lipid) were obtained using Florisil. For the other sorbents, a clean-up step after extraction was needed for chromatographic analysis.

In a Dionex Application Note [14], alumina was successfully used as a retainer sorbent in PLE cells for PCBs extractions. However, we obtained better results with Florisil. The higher lipid content of eggs using in this study compared to that of fish used in the Dionex Application Note may account for these results. Otherwise, Björklund et al. [17] concluded that the use of sulphuric acid impregnated silica gel

Table 3
PCB concentration found in spoonbill eggs by PLE using different static extraction times and cycles (22 ml cell, 2 g of sample dispersed with 4 g of Florisil on top of 6 g of Florisil, 15% dichloromethane in pentane, 40 °C, 2000 p.s.i., 150% flush). Experiments were made in triplicate

| PCB | Concentration±SD (ng $g^{-1}$, dry mass) | | | | |
|---|---|---|---|---|---|
| | 3 cycles/5 min | 3 cycles/10 min | 3 cycles/15 min | 2 cycles/10 min | 1 cycle/10 min |
| 1 | 6.8±0.5 | 8.4±0.3 | 8.7±0.5 | 8.2±0.7 | 6.9±0.4 |
| 11 | 7.9±0.6 | 9.4±0.8 | 9.5±0.6 | 9.6±0.4 | 7.5±0.4 |
| 29 | 7.4±0.6 | 9.7±0.4 | 9.2±0.6 | 9.1±0.8 | 6.9±0.4 |
| 47 | 6.3±0.7 | 8.6±0.6 | 8.8±0.5 | 8.8±0.5 | 6.2±0.5 |
| 87 | 13.6±1.0 | 16.0±1.4 | 15.2±1.7 | 15.4±1.4 | 12.2±1.0 |
| 99 | 21.5±1.9 | 28.6±2.5 | 27.2±2.7 | 27.6±2.4 | 24.7±2.1 |
| 101 | 3.0±0.3 | 3.6±0.2 | 3.6±0.2 | 3.8±0.4 | 3.0±0.1 |
| 105 | 13.7±0.5 | 16.3±1.3 | 16.5±1.5 | 15.5±1.3 | 11.8±0.6 |
| 118 | 40.6±3.1 | 57.1±3.9 | 57.0±3.7 | 54.4±3.9 | 45.8±4.1 |
| 121 | 7.2±0.5 | 8.5±0.9 | 8.7±0.9 | 8.6±0.5 | 6.8±0.4 |
| 128 | 14.4±0.6 | 19.0±2.0 | 18.9±2.0 | 18.5±1.9 | 15.5±1.4 |
| 136 | 6.9±0.5 | 9.4±0.8 | 9.7±0.9 | 9.4±1.0 | 7.0±0.6 |
| 138 | 170±6 | 206±17 | 201±12 | 207±16 | 157±12 |
| 153 | 486±33 | 593±42 | 608±31 | 607±45 | 496±26 |
| 156 | 11.3±0.8 | 14.6±1.0 | 13.9±1.0 | 14.1±0.9 | 10.8±0.9 |
| 170 | 78.3±2.0 | 90.9±3.4 | 95.0±3.8 | 94.2±5.7 | 72.2±4.4 |
| 180 | 294±21 | 384±18 | 371±29 | 380±18 | 299±19 |
| 183 | 42.8±2.3 | 52.8±5.1 | 52.8±5.3 | 48.8±1.3 | 38.7±3.7 |
| 185 | 6.3±0.5 | 7.6±0.3 | 7.9±0.3 | 7.6±0.4 | 6.5±0.5 |
| 194 | 27.6±2.9 | 36.4±2.5 | 37.8±1.8 | 34.6±2.8 | 29.2±3.1 |
| 206 | 7.4±0.8 | 9.4±0.8 | 9.2±0.8 | 9.7±0.4 | 7.7±0.7 |
| 209 | 7.1±0.4 | 9.1±0.7 | 9.1±0.3 | 8.8±0.2 | 6.7±0.3 |
| Lipids % | 0.8±0.2 | 0.6±0.3 | 0.8±0.4 | 0.6±0.3 | 0.9±0.5 |

inside the PLE cell was superior in some extent to the use of Florisil. However they used an extraction temperature of 100 °C, at which we have also observed that the lipidic content is not totally retained in this sorbent.

### *3.2. Application of SPLE method to several biota matrices*

Biota samples with a range of lipid content (10.2, 8.8, 3.8 and 2.9% for oyster, mussel, fish and clam, respectively) were spiked at two levels (0.7 and 9 ng $g^{-1}$ dry mass) with PCB congeners ranging from mono- to decachlorobiphenyls (IUPAC numbers 1, 11, 29, 47, 121, 136, 185, 194, 206 and 209). Recoveries were satisfactory (higher than 85%) irrespective of the PCB congener, level of spike and matrix investigated.

### *3.3. Validation of the SPLE method*

The results obtained for the SRM 2974 using the SPLE method and both GC–MS–MS and GC–ECD, together with the certified values, are presented in Table 5. All values fell within the 95% confidence interval established during the certification process, except for PCB 156 using GC–ECD. The RSDs were mostly below 11%. The high value found for PCB 156 using GC–ECD (10.3±0.5 ng $g^{-1}$) compared to the certified one (7.4±1 ng $g^{-1}$) may be attributable to an interference in the chromatographic analysis as far as no discrepancy was found using GC–MS–MS (7.5±0.4 ng $g^{-1}$).

The MDLs evaluated for SPLE method ranged from 0.002 to 0.07 ng $g^{-1}$ for GC–MS–MS, and from 0.001 to 0.004 ng $g^{-1}$ for GC–ECD (except for PCBs 1 and 11 with GC–ECD, which are 30 and ten times higher, respectively). The repeatability of the

Table 4
PCB concentration found in spoonbill eggs by PLE using different sorbents (22 ml cell, 2 g of sample dispersed with 4 g of sorbent on top of 6 g of sorbent, 15% dichloromethane in pentane, 40 °C, 2000 p.s.i., 2×10 static extraction, 150% flush). Experiments were made in triplicate

| PCB | Concentration±SD (ng $g^{-g}$, dry mass) | | | | |
|---|---|---|---|---|---|
| | Florisil | Alumina | Silica gel | Cianopropyl- | Dihydroxipropoxypropyl- |
| 1 | 8.2±0.7 | 9.2±0.4 | 9.7±0.6 | 9.1±0.3 | 8.6±0.7 |
| 11 | 9.6±0.4 | 8.8±0.5 | 8.6±0.9 | 9.3±0.4 | 9.0±0.4 |
| 29 | 9.1±0.8 | 8.6±0.8 | 9.1±0.3 | 8.6±0.4 | 9.0±0.5 |
| 47 | 8.8±0.5 | 9.0±0.6 | 9.0±0.4 | 8.7±0.5 | 9.3±0.5 |
| 87 | 15.4±1.4 | 13.0±1.0 | 15.4±0.8 | 12.2±1.0 | 12.4±1.2 |
| 99 | 27.6±2.4 | 24.1±2.1 | 26.3±2.8 | 25.1±1.4 | 28.6±2.1 |
| 101 | 3.8±0.4 | 3.5±0.2 | 3.8±0.3 | 3.7±0.3 | 3.3±0.3 |
| 105 | 15.5±1.3 | 11.3±0.5 | 14.4±0.4 | 15.9±1.6 | 12.2±0.9 |
| 118 | 54.4±3.9 | 58.9±3.4 | 50.2±3.6 | 57.6±4.7 | 52.5±4.1 |
| 121 | 8.6±0.5 | 9.0±0.5 | 9.2±0.6 | 9.3±0.5 | 8.9±0.6 |
| 128 | 18.5±1.9 | 14.4±1.0 | 17.0±1.4 | 15.6±0.9 | 17.6±1.3 |
| 136 | 9.4±1.0 | 10.0±1.0 | 9.1±0.4 | 8.7±0.4 | 8.9±0.4 |
| 138 | 207±16 | 220±14 | 226±22 | 215±13 | 211±11 |
| 153 | 607±45 | 582±29 | 577±22 | 566±24 | 595±25 |
| 156 | 14.1±0.9 | 16.0±0.8 | 17.0±1.4 | 15.6±0.7 | 13.7±0.5 |
| 170 | 94.2±5.7 | 97.2±4.1 | 95.0±1.4 | 99.7±5.7 | 93.2±3.0 |
| 180 | 380±18 | 390±21 | 366±15 | 360±15 | 281±17.2 |
| 183 | 48.8±1.3 | 43.0±1.9 | 46.0±3.4 | 44.5±3.2 | 49.0±2.4 |
| 185 | 7.6±0.4 | 7.9±0.5 | 8.4±0.8 | 8.0±0.6 | 8.6±0.5 |
| 194 | 34.6±2.8 | 32.1±3.0 | 36.0±0.9 | 31.3±2.0 | 36.2±3.7 |
| 206 | 9.7±0.4 | 8.6±0.4 | 9.0±0.9 | 9.0±0.4 | 9.7±0.7 |
| 209 | 8.8±0.2 | 8.4±0.7 | 9.0±0.4 | 8.7±0.7 | 8.9±0.4 |
| Lipids % | 0.6±0.3 | 3.6±0.3 | 5.2±0.3 | 15.1±0.7 | 11.2±1.1 |

Table 5
PCB concentrations found in the reference mussel tissue SRM 2974 (NIST, Gaithersburg, MS, USA) using SPLE. Average of three separate samples

| PCB | Concentration±SD (ng $g^{-1}$, dry mass) | | |
|---|---|---|---|
| | GC–ECD | GC–MS–MS | Certified value |
| 47 | 24.5±2.0 | 27.7±2.6 | – |
| 99 | 72.7±2.7 | 70.0±3.6 | 70.9±4.5 |
| 101 | 122±5 | 125±4 | 128±10 |
| 105 | 53.1±2.4 | 53.0±4.8 | 53.0±3.8 |
| 118 | 130±6 | 132±6 | 130.8±5.3 |
| 121 | 13.7±1.3 | 14.9±1.7 | – |
| 128 | 21.0±2.6 | 23.1±2.8 | 22.0±3.5 |
| 136 | 8.2±0.4 | 8.0±0.3 | – |
| 138 | 133±9 | 134±5 | 134±10 |
| 153 | 142±7 | 141±10 | 145.2±8.8 |
| 156 | 10.3±0.5 | 7.5±0.4 | 7.4±1.0 |
| 170 | 5.7±0.6 | 5.2±0.3 | 5.5±1.1 |
| 180 | 16.8±1.6 | 17.2±1.1 | 17.1±3.8 |
| 183 | 15.6±1.1 | 16.0±1.0 | 16.0±2.4 |
| 194 | 3.6±0.1 | 3.7±0.2 | – |

extraction step was estimated analyzing five aliquots of egg sample each day, and during 5 days. RSD values within and between days were below 10.6 and 11.5%, respectively.

### *3.4. Comparison of NSPLE, SPLE, MSPD and Soxhlet methods and determination of PCBs in bivalves and fish from the South of Spain*

The SPLE, NSPLE, MSPD and Soxhlet extraction methods were compared using samples of egg of spoonbill, bivalves (*C. gallina*, *S. plana*, *C. gigas*, *G. edulis*, *Donax* spp.) and fish (*S. senegalensis*) collected from ten locations along the south coast of Spain. Results obtained for eggs are reported in Tables 4 and 6. Two values are presented for Soxhlet extraction. The second corresponds to the re-extraction of the sample using a more polar solvent mixture (pentane–acetone, 1:1), and resulted in very

Table 6
PCB concentration found in spoonbill eggs using different extraction methods (SPLE: 22 ml cell, 2 g of sample dispersed with 4 g of Florisil on top of 6 g of Florisil, 15% dichloromethane in pentane, 40 °C, 2000 p.s.i., 2×10 min static extraction, 150% flush; NSPLE: 11 ml cell, 2 g of sample dispersed with 4 g of Celatom, pentane, 100 °C, 1600 p.s.i., 2×10 min static extraction, 150% flush; Soxhlet-1: 2 g of sample, pentane–acetone (2:1), 13 h; MSPD: 2 g of sample dispersed with 4 g of Florisil on top of 6 g of Florisil, 15% dichloromethane in pentane, 2×10 min static extraction, 55 min, 1 ml $min^{-1}$). Experiments made in triplicate

| PCB | Concentration±SD (ng $g^{-1}$, dry mass) | | | | |
|---|---|---|---|---|---|
| | SPLE | NSPLE | Soxhlet-1 | Soxhlet-2[a] | MSPD |
| 1 | 8.2±0.7 | 8.8±0.5 | 8.5±0.5 | <DL[b] | 8.9±0.4 |
| 11 | 9.6±0.4 | 9.0±0.5 | 9.0±0.5 | <DL | 99.0±0.3 |
| 29 | 9.1±0.8 | 8.5±0.7 | 9.2±0.7 | <DL | 8.5±0.6 |
| 47 | 8.8±0.5 | 8.4±0.4 | 9.0±0.7 | <DL | 9.0±0.4 |
| 87 | 15.4±1.4 | 14.4±1.3 | 15.6±1.1 | <DL | 16.0±0.9 |
| 99 | 27.6±2.4 | 26.6±2.5 | 27.5±1.9 | 1.3±0.8 | 28.0±1.7 |
| 101 | 3.8±0.4 | 3.5±0.2 | 3.9±0.4 | <DL | 3.7±0.3 |
| 105 | 15.5±1.3 | 14.3±1.7 | 15.9±1.0 | <DL | 14.9±0.8 |
| 118 | 54.4±3.9 | 50.3±4.6 | 55.7±3.4 | 2.0±0.4 | 55.1±3.2 |
| 121 | 8.6±0.5 | 9.0±0.3 | 9.3±0.5 | <DL | 9.3±0.5 |
| 128 | 18.5±1.9 | 17.9±1.6 | 18.0±0.9 | <DL | 18.3±0.5 |
| 136 | 9.4±1.0 | 8.7±0.5 | 8.5±0.4 | <DL | 9.4±0.9 |
| 138 | 207±16 | 240±16 | 195±17 | 4.7±0.7 | 224±19 |
| 153 | 607±45 | 586±47 | 625±32 | 9.2±1.4 | 569±20 |
| 156 | 14.1±0.9 | 14.9±1.2 | 13.3±0.7 | <DL | 15.0±0.7 |
| 170 | 94.2±5.7 | 95.3±3.8 | 83.1±5.4 | 1.2±0.4 | 96.2±3.0 |
| 180 | 380±18 | 358±16 | 390±17 | 10.0±2.1 | 367±24 |
| 183 | 48.8±1.3 | 46.0±3.8 | 50.3±3.6 | 1.2±0.7 | 49.2±2.4 |
| 185 | 7.6±0.4 | 8.2±0.7 | 8.7±0.5 | <DL | 9.0±0.5 |
| 194 | 34.6±2.8 | 33.5±3.9 | 37.6±3.8 | <DL | 35.6±3.4 |
| 206 | 9.7±0.4 | 8.6±0.5 | 9.1±0.7 | <DL | 9.0±0.5 |
| 209 | 8.8±0.2 | 9.2±0.6 | 8.5±0.6 | <DL | 9.3±0.5 |
| Lipids % | 0.6±0.3 | 37.2±2.4 | 40.1±1.3 | – | 0.7±0.4 |

[a] Extraction of the residue obtained with Soxhlet-1, using 1:1 (v/v) pentane–acetone as extractant.
[b] Below detection limit.

low PCB yields. It is evident that the first extraction was virtually exhaustive, and counted for at least 96% of the extracted compounds.

Seven PCB congeners (IUPAC numbers 105, 118, 138, 153, 170, 180 and 194) were found in bivalves and fish, at concentrations ranging from below detection limit to 15 ng $g^{-1}$, dry mass. Therefore, these samples represented low PCB concentrations matrices. When comparing the four extraction methods, average recoveries did not have any significant difference irrespective of the PCB congener and matrix investigated (ANOVA, $P>0.29$). Precision achieved by the four methods was comparable (RSD<12%), with SPLE being slightly superior (RSD<10%).

The extracts obtained with both SPLE and MSPD methods were colourless and sufficiently clean (<0.1% of lipid content) for direct injection into the GC–MS and GC–ECD. However, the extracts from NSPLE and Soxhlet were yellow (with a lipid content ranging from 3 to 40%). Therefore, the presence of co-extracted matrix organics required a post-extraction clean-up step with Florisil before GC analysis.

In addition to extraction recoveries it is interesting to compare the relative merits of each extraction technique. The relative consumptions of organic solvent are: Soxhlet (350 ml)>NSPLE (230 ml)> SPLE=MSPD (55 ml), while for spent time the order is Soxhlet (17 h)>NSPLE (5 h)>MSPD (2 h)>SPLE (1 h). Based on the data presented in this study both SPLE and MSPD methods present a higher number of advantages, SPLE being less time consuming and more automatized than MSPD. How-

ever, the capital cost of the PLE techniques is much higher than that for MSPD and Soxhlet.

## 4. Conclusions

A simple, rapid and accurate method for the determination of PCBs in biota samples has been developed using a selective PLE and GC–MS or GC–ECD. The experimental setup mimicked a PLE setup followed immediately by an in situ and concurrent column chromatographic clean-up of the PLE extract. Results demonstrated that this SPLE approach compares satisfactorily with traditional extraction methods for PCB analysis, offering a single-step extraction and clean-up of biota samples with a high lipidic content with the proper choice of solvent, temperature and sorbent in the extraction cell. The obtained extract is clean enough for direct injection to GC–MS and GC–ECD. A total volume of 55 ml is required for the analysis. Similar advantages are obtained using a MSPD method, with a lower capital cost.

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