Customer application note | 001959

Sample preparation

# Determination of persistent organic pollutants in fish tissues by EXTREVA ASE system and GC-MS/MS


## Authors

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## Keywords

Pressurized fluid extraction, polychlorinated biphenyls, organochlorine pesticides, polybrominated diphenyl ethers, polycyclic aromatic hydrocarbons, fish tissue, in-line clean up, TSQ 8000 triple quadrupole GC-MS/MS, Xcalibur software, TraceFinder software, POPs


## Goal

To demonstrate a method for the determination of persistent organic pollutants (POPs) in fish tissues using a Thermo Scientific™ EXTREVA™ ASE™ Accelerated Solvent Extractor: a fully automated parallel extraction and evaporation system

## Introduction

Polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), and polybrominated diphenyl ethers (PBDEs) belong to a broad family of synthetic organic compounds known as halogenated hydrocarbons. These compounds, together with polycyclic aromatic hydrocarbons (PAHs), are part of the larger persistent organic pollutants (POPs) family. PCBs were manufactured in the United States from 1929 until their manufacture was banned in 1979. They have a range of toxicity and vary in consistency from thin, light-colored liquids to yellow or black waxy solids. Due to their non-flammability, chemical stability, high boiling point, and electrical insulating properties, PCBs were used in hundreds of industrial and commercial applications, such as coatings for electrical, heat transfer, and hydraulic equipment; plasticizers in paints, plastics, and rubber products; and in pigments, dyes, and carbonless copy paper. OCPs were widely used as insecticides throughout the 1950s and 1960s until their use was banned in western countries in the 1970s. PBDEs have recently emerged as a major environmental pollutant and have been used as flame retardants in consumer goods such as electrical equipment, construction materials, coatings, textiles, and polyurethane foam (furniture

padding). Several nations have recently banned PBDEs and introduced legislation that bans the sale of certain products containing PBDEs. Furthermore, based on the recommendation of the European Food Safety Authority (EFSA), the European Commission has asked member states to monitor the presence of PBDEs in food over the next two years. PAHs are derived from both anthropogenic activities (incinerators, industrial processes, motor vehicles, combustion of wood and fossil fuels, oil spills, etc.) and natural sources (incomplete combustion of organic matter and pyrolysis). Many studies have reported the mutagenic and carcinogenic effects of PAHs and chronic metabolite exposure as described by the EFSA. Indeed, the International Agency for Research on Cancer has classified several high molecular weight PAHs as recognized (Class 1), probable (Class 2A), or possible (Class 2B) human carcinogens.[1] The ability of halogenated hydrocarbons and PAHs to bioaccumulate in fatty tissues and biomagnify up the food chain, in combination with their resistance to degradation and their toxicity, make this class of chemicals a serious threat to environmental and human health.

Techniques such as Soxhlet (U.S. EPA Method 3540), sonication (U.S. EPA Method 3550), and microwave extraction (U.S. EPA Method 3546) are currently used for the extraction of POPs from food and environmental samples prior to their analytical determination. Those techniques are, however, very labor intensive, suffer from high solvent consumption, and interfering compounds may be extracted along with the desired analytes. These unwanted co-extractables can cause buildup of nonvolatile materials on the GC injection port and the analytical column, resulting in poor analytical results and high instrument maintenance costs. Gel permeation chromatography (GPC) is often used as a post extraction clean-up for fish and meat tissues prior to determination of POPs like PCBs, OCPs, PAHs, and PBDEs. However, the main disadvantage of the GPC systems is incomplete lipid removal. The remaining lipids must be removed in a second clean-up procedure, e.g., on an additional silica column or by a second GPC step. Additionally, challenges remain with high lipid content in which lipophilic pesticides may remain in the fatty layer even after the extraction.

Accelerated solvent extraction (ASE) was developed to meet the requirements for reducing solvent usage in the preparation of solid samples.[2] With ASE, extractions can be completed in very short periods of time with minimal amounts of solvent compared to conventional sample extraction techniques. The EXTREVA ASE system (Figure 1) is based on many proprietary technologies including gas-assisted solvent delivery[3] and parallel accelerated solvent extraction.[4] This fully automated system combines the extraction and evaporation capabilities in one instrument, and it can be conveniently used for extracting and concentrating/evaporating extracts from up to 16 solid and semi-solid samples.


**Figure 1. EXTREVA ASE Accelerated Solvent Extractor**

The method reported here is applicable for the determination of 29 halogenated hydrocarbons (6 PCBs, 16 OCPs, and 7 PBDEs) and four PAHs in fish tissues. The concentration ranges are 1–50 ng/g for all tested compounds. This also demonstrates an in-line clean up on an EXTREVA ASE system.

## Experimental

### Sample collection

A total of 30 Mediterranean shad *(Alosa agone)* were selected for this study. All the samples were purchased at a fish market in Milan, Italy. Each sample was stored at –22°C until analysis.

### Equipment and consumables

- EXTREVA ASE accelerated solvent extractor (P/N 22184-60101)
- Thermo Scientific™ TRACE™ 1310 Gas Chromatography System
- Thermo Scientific™ TSQ™ 8000 Triple Quadrupole Mass Spectrometer
- Thermo Scientific™ Dionex™ Cellulose Filter (P/N 056780)
- Concentration flask assembly 100 mL (P/N 22184-62235)
- 1.5 mL short thread vial DN9, 32 × 11.6 mm, clear, label (VWR International P/N 548-8012)
- 9 mm PP cap red hole red rubber/PTFE (VWR International P/N 548-3297)
- Fused-silica GC capillary column (RXI-XLB 30 m × 0.25 mm × 0.25 µm Restek™ P/N 13723)
- Topaz liner, 2 mm baffled PTV, 2.75 × 120 mm for Thermo Scientific GCs (Restek P/N 23438)

### Solvents and chemicals

- Hexane (Sigma-Aldrich® P/N 139386)
- Acetone (Sigma-Aldrich P/N 650501)
- PCB calibration mix (AccuStandard® P/N AE-00059-H-2X)
- PBDE calibration mix (AccuStandard P/N BDE-CAE-1)
- OCP calibration mix (Restek P/N 32094)
- PAH calibration mix (Restek P/N 32469)
- Thermo Scientific™ Dionex™ ASE™ Prep Diatomaceous Earth (DE) Dispersant, 1 kg (P/N 062819)
- Supel™ QuE Z-Sep sorbent (Sigma-Aldrich P/N 55418-U)
- Internal standard 1: PCB 209 (AccuStandard P/N C-209S-TP)
- Internal standard 2: FBDE (AccuStandard P/N FBDE-5002S-0.5X)

### Extraction, concentration, and measurement

The PCBs, PBDEs, OCPs, and PAHs standards were diluted with hexane to produce a 10 µg/mL stock solution. Working solutions were prepared by diluting the stock solution in hexane and then storing at –40°C. Calibration standards with concentrations of 0.1 and 1.0 µg/mL were prepared by diluting the stock solution. The internal standard solution of PCB209 and FBDE had a concentration of 1 µg/mL. Thirty microliters were added to each calibration standard.

A cellulose filter was placed on top of a 22 mL extraction cell body and the end cap was hand tightened. Five hundred milligrams of Supel QuE Z-Sep sorbent were added into the extraction cell, followed by another cellulose filter. A representative portion of Mediterranean shad (300 g) was minced and freeze-dried. An aliquot (0.75 g) corresponding to 3 g of wet sample was homogenized in a beaker with 5 g of diatomaceous earth (Dionex ASE Prep DE). The resulting mixture was poured carefully into the extraction cell and spiked with 20 µL of hexane solution containing the two internal standards. Any empty volume was filled with diatomaceous earth while lightly tapping the extraction cell. After placing another cellulose filter on top of the cell body, the second end cap was hand tightened. The extraction cell schematic is depicted in Figure 2. The Dionex ASE Prep DE dispersant plays a key role in preventing sample compaction during the compression phase and in ensuring efficient solvent contact with the sample.

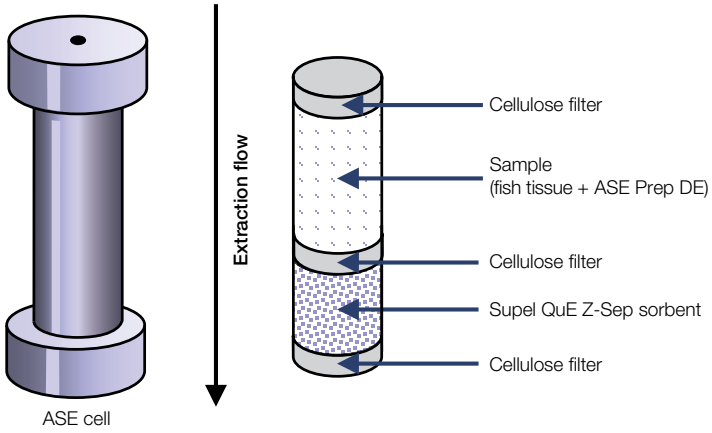


**Figure 2. Extraction cell schematic**

The instrument was programmed according to the conditions reported in Table 1. Before proceeding to the extraction of the samples, the system was rinsed with 10 mL of hexane. Hexane was also used during evaporation as a rinse solvent and 1.6 mL were added during the concentration phase. After concentration to a final volume of 0.5 mL, the samples were analyzed by GC-MS. The GC-MS conditions are summarized in Table 2.

**Table 1. Extraction and evaporation conditions for the EXTREVA ASE system**

| Extraction | |
|---|---|
| Cell type | Stainless steel |
| Cell size | 22 mL |
| Oven temperature | 80°C |
| Purge time | 45 s |
| Nitrogen flow (gas assisted extraction) | 10 mL/min per channel |
| Cell fill volume | 70% |
| Solvent flow rate | 0.5 mL/min |
| Extraction solvent | Hexane-acetone 4:1 |
| Pre-run rinse | 10 mL, Hexane |
| Extraction volume | 26 mL (estimated) (22 mL cell, flow rate 0.5 mL/min) |
| Extraction time (four samples) | 20–25 min (22 mL cell, flow rate 0.5 mL/min) |
| **Concentration** | |
| Mode | Fixed volume |
| Collection flask | 2 mL vial assembly |
| Final volume | 0.5 mL |
| Rinse solvent | Hexane, 1.6 mL |
| Evaporation temperature | 40°C |
| Nitrogen flow rate | 100 mL/min per channel |
| Vacuum | 6 psi (~315 torr/~420 mbar) |
| Evaporation time (four samples) | 75–80 min |

**Table 2. GC-MS conditions**

| GC and injector conditions | |
|---|---|
| **Split/splitless injector** | |
| Injector temperature | 250°C |
| Liner | Topaz, 2 mm baffled PTV, 2.75 × 120 mm |
| Injected volume | 1 µL |
| Splitless time | 0.5 min |
| Split flow | 10 mL/min |
| Surge pressure | 5 kPa |
| **GC program and column** | |
| Column | RXI-XLB 30 m × 0.25 mm × 0.25 µm |
| Carrier gas | Helium, 99.999% purity |
| Flow rate | 1.0 mL/min, constant |
| Initial temperature | 80°C (3 min) |
| | 10°C/min to 170°C |
| | 3°C/min to 240°C |
| | 10°C/min to 310°C |
| Final temperature | 310°C (5 min) |
| **Mass spectrometer parameters** | |
| Source temperature | 250°C |
| Ionization | EI |
| Electron energy | 70 eV |
| Emission current | 50 µA |
| Q2 gas pressure (Argon) | 1.5 mTorr |
| Collision energy | 10 to 30 eV |
| Q1 peak width FWHM | 0.7 Da |
| Q3 peak width FWHM | 0.7 Da |

The monitored selected reaction monitoring (SRM) transitions for PCBs, PBDEs, PAHs, and OCs are given in Tables 3–6.

**Table 3. SRM transitions for PCBs**

| PCB # | Retention time (min) | Nominal mass | Exact mass | Precursor ion (*m/z*) | Product ion (*m/z*) | Collision energy (eV) |
|---|---|---|---|---|---|---|
| 28 | 21.00 | 258 | 255.9613 | 256 | **186*** | 20 |
| | | | | 258 | 186 | 25 |
| 52 | 22.41 | 292 | 294.9194 | 292 | **222*** | 25 |
| | | | | 292 | 257 | 10 |
| 101 | 27.09 | 326 | 325.8804 | 324 | **254*** | 25 |
| | | | | 326 | 256 | 25 |
| | | | | 328 | 256 | 25 |
| 138 | 31.93 | 361 | 359.8415 | 360 | **290*** | 25 |
| | | | | 360 | 325 | 10 |
| 153 | 33.49 | 361 | 359.8415 | 360 | **290*** | 20 |
| | | | | 360 | 325 | 30 |
| 180 | 37.07 | 395 | 393.8025 | 394 | **324*** | 25 |
| | | | | 394 | 359 | 10 |
| | | | | 396 | 324 | 25 |

*Quantifier ion

**Table 4. SRM transitions for PBDEs**

| PBDE # | Retention time (min) | Nominal mass | Exact mass | Precursor ion (*m/z*) | Product ion (*m/z*) | Collision energy (eV) |
|---|---|---|---|---|---|---|
| 28 | 30.68 | 407 | 405.8027 | 246 | **139*** | 10 |
| | | | | 248 | 139 | 10 |
| | | | | 408 | 248 | 10 |
| 33 | 31.07 | 407 | 405.8027 | 246 | **139*** | 30 |
| | | | | 248 | 139 | 30 |
| | | | | 406 | 246 | 10 |
| 47 | 37.41 | 486 | 485.7111 | 326 | **217*** | 30 |
| | | | | 328 | 219 | 30 |
| | | | | 484 | 326 | 30 |
| 99 | 40.21 | 565 | 563.6216 | 410 | **297*** | 30 |
| | | | | 406 | 294 | 30 |
| | | | | 564 | 404 | 20 |
| 100 | 40.93 | 565 | 563.6216 | 410 | **297*** | 30 |
| | | | | 406 | 297 | 30 |
| | | | | 564 | 404 | 10 |
| 153 | 42.45 | 644 | 643.5301 | 484 | **377*** | 25 |
| | | | | 642 | 482 | 10 |
| 154 | 43.41 | 644 | 643.5301 | 484 | **324*** | 30 |
| | | | | 486 | 326 | 30 |
| | | | | 644 | 484 | 20 |

*Quantifier ion

Table 5. SRM transitions for OCPs

| Compound name | Retention time (min) | Nominal mass | Exact mass | Precursor ion (*m/z*) | Product ion (*m/z*) | Collision energy (eV) |
|---|---|---|---|---|---|---|
| *α*-HCH | 16.92 | 297 | 295.8772 | 181 | **145*** | 10 |
| | | | | 181 | 146 | 10 |
| | | | | 219 | 183 | 10 |
| Hexachlorobenzene | 17.20 | 285 | 283.8102 | 284 | **249*** | 20 |
| | | | | 286 | 214 | 30 |
| | | | | 286 | 251 | 20 |
| β-HCH | 18.36 | 297 | 295.8772 | 181 | **145*** | 10 |
| | | | | 183 | 148 | 10 |
| | | | | 219 | 183 | 10 |
| Lindane (γ-HCH) | 20.01 | 297 | 295.8772 | 181 | **145*** | 10 |
| | | | | 183 | 145 | 10 |
| | | | | 219 | 183 | 10 |
| Heptachlor | 21.12 | 373 | 371.8181 | 272 | **237*** | 10 |
| | | | | 274 | 237 | 10 |
| | | | | 274 | 239 | 10 |
| Aldrin | 22.52 | 365 | 363.8728 | 261 | **191*** | 30 |
| | | | | 263 | 193 | 30 |
| | | | | 265 | 193 | 30 |
| Heptachlor epoxide | 25.17 | 389 | 387.8130 | 353 | **263*** | 10 |
| | | | | 353 | 282 | 10 |
| | | | | 355 | 265 | 10 |
| *trans*-Chlordane | 27.03 | 410 | 409.7919 | 373 | 264 | 20 |
| | | | | 373 | **266*** | 20 |
| | | | | 375 | 266 | 20 |
| Endosulfan I | 27.26 | 407 | 405.8139 | 373 | **266*** | 20 |
| | | | | 375 | 266 | 20 |
| | | | | 377 | 268 | 20 |
| pp'-DDE | 28.74 | 318 | 317.9351 | 246 | **176*** | 30 |
| | | | | 248 | 176 | 30 |
| | | | | 328 | 248 | 20 |
| Endrin | 29.95 | 381 | 379.8677 | 245 | 173 | 30 |
| | | | | 263 | **193*** | 30 |
| | | | | 281 | 245 | 10 |
| Endosulfan II | 31.73 | 407 | 405.8139 | 195 | **159*** | 10 |
| | | | | 241 | 206 | 10 |
| pp'-DDD | 31.76 | 320 | 319.9507 | 235 | **165*** | 20 |
| | | | | 237 | 165 | 20 |
| op'-DDT | 30.87 | 354 | 353.9117 | 235 | **165*** | 20 |
| | | | | 237 | 165 | 20 |
| pp'-DDT | 33.47 | 354 | 353.9117 | 235 | **165*** | 20 |
| | | | | 237 | 165 | 20 |
| Endosulfan sulfate | 34.14 | 423 | 421.8088 | 272 | **237*** | 10 |
| | | | | 274 | 237 | 10 |
| | | | | 274 | 239 | 10 |

*Quantifier ion

Table 6. SRM transitions for PAHs

| Compound name | Retention time (min) | Nominal mass | Exact mass | Precursor ion (*m/z*) | Product ion (*m/z*) | Collision energy (eV) |
|---|---|---|---|---|---|---|
| Chrysene | 36.74 | 228 | 228.0939 | 226 | **224*** | 30 |
| | | | | 228 | 202 | 20 |
| | | | | 228 | 226 | 20 |
| Benz(*a*)anthracene | 36.96 | 228 | 228.0939 | 226 | 223 | 30 |
| | | | | 226 | **224*** | 30 |
| | | | | 228 | 202 | 20 |
| Benzo(*b*) fluoranthene | 41.33 | 252 | 252.0939 | 250 | 224 | 30 |
| | | | | 250 | 248 | 30 |
| | | | | 252 | **250*** | 30 |
| Benzo(*a*)pyrene | 42.29 | 252 | 252.0939 | 252 | 226 | 30 |
| | | | | 252 | **250*** | 30 |
| | | | | 252 | 227 | 20 |

*Quantifier ion

## Results and discussion

Compared to the application note using the Thermo Scientific™ Dionex™ ASE 350 Accelerated Solvent Extractor on tuna samples from the same research group, two key improvements were introduced: the freeze drying of the samples and the replacement of the silica gel with Supel QuE Z-Sep sorbent for the in-line clean-up. Freeze drying allows the removal of up to 99% of the water content of the fish tissue samples, therefore avoiding water co-extraction and the need of manual drying with sodium sulphate/moisture absorbing polymer (MAP, P/N 083475) prior to concentration and GC-MS analysis. Supel QuE Z-Sep is a Zirconium-based sorbent recommended for the analysis of hydrophobic analytes in fatty matrices. It increases the robustness of LC-MS and GC-MS methods by effectively removing more fat and pigments than traditional C18 and PSA phase sorbents. To the best of our knowledge, this is the first example of pressurized fluid extraction with in-line clean-up by using this type of sorbent.

The described method was optimized for the multi-residue analysis of 33 POPs. The optimization of the MS/MS method consisted of:

1. Acquisition of respective MS spectra in full-scan mode (*m/z* 100–1,000 mass range)
2. Selection of precursor ions
3. Product ion scans at different collision energies (10, 20, and 30 eV)
4. Final tuning of the collision energy in selected reaction monitoring mode.

For each compound, two MS/MS transitions were chosen to fulfill the generally applied identification criteria: according to SANTE 2021 (guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed), one precursor ion with two product ions, or two precursor ions with one product ion should be available for unbiased identification of the target analyte. An overview of the quantitative and confirmation MS/MS transitions and the collision energies selected for each compound in EI mode is given in Tables 3 to 6. In general, MS/MS allows for minimal matrix component interferences, and at the same time, due to the possibility of selecting suitable precursor and product ions, enables identification and quantification of the above-mentioned contaminants even at (ultra) trace concentrations. Notwithstanding that a highly selective triple quadrupole mass spectrometer is used, because GC-MS instruments are generally rather intolerant of non-volatile matrix impurities, the choice of an appropriate sample preparation strategy is also important to avoid poor ionization, background noise, and contamination of the GC-MS system. All results obtained confirm the efficacy of the present method for the determination of multiresidue pollutants in fish tissues. The method showed a good linearity with coefficients of determination equal to or higher than 0.99 for all the compounds investigated, as well as good repeatability, confirming the present method as useful to monitor compounds belonging to different chemical classes (Table 7). Recovery rates and relative standard deviation (RSD) were calculated on six replicates at final concentration of 10 ng/g. Calibration standards were spiked on the real matrix sample. The recoveries ranged from 93 to 100% for PCBs, from 93 to 104% for PBDEs, from 84 to 103% for OCs, and from 99 to 109% for PAHs. The RSDs ranged from 4 to 19%. The one-step accelerated solvent extraction method using Z-Sep as fat retainer is both rapid and cost-effective and minimizes waste generation compared to the classic methods. The time required in the laboratory is reduced 50% by combining the extraction and the two clean-up steps (i.e., GPC and SPE) in one single accelerated solvent extraction step, thus doubling the number of samples that can be analyzed per day.

Table 7. Recoveries rates, RSD, LOD, LOQ, and coefficient of determination ($r^2$)

| Compound | LOD ng/g | LOQ ng/g | Recovery (%) | RSD (%) | Coefficient of determination ($r^2$) |
|---|---|---|---|---|---|
| **Polychlorobiphenyls (PCBs)** | | | | | |
| PCB 28 | 0.5 | 1 | 93 | 13 | 0.9981 |
| PCB 52 | 0.5 | 1 | 90 | 13 | 0.9993 |
| PCB 101 | 0.5 | 1 | 93 | 19 | 0.9937 |
| PCB 138 | 0.5 | 1 | 93 | 13 | 0.9991 |
| PCB 153 | 0.5 | 1 | 100 | 19 | 0.9979 |
| PCB 180 | 0.5 | 1 | 96 | 14 | 0.9994 |
| **Polybrominated Diphenyl Ethers (PBDEs)** | | | | | |
| PBDE 28 | 0.5 | 1 | 102 | 9 | 0.9965 |
| PBDE 33 | 0.5 | 1 | 103 | 11 | 0.9938 |
| PBDE 47 | 0.5 | 1 | 104 | 8 | 0.9972 |
| PBDE 99 | 0.5 | 1 | 96 | 12 | 0.9910 |
| PBDE 100 | 0.5 | 1 | 93 | 5 | 0.9958 |
| PBDE 153 | 0.5 | 1 | 104 | 17 | 0.9946 |
| PBDE 154 | 0.5 | 1 | 93 | 12 | 0.9989 |
| **Organochlorines (OCs)** | | | | | |
| *α*-BHC | 0.5 | 1 | 97 | 9 | 0.9975 |
| Hexachlorobenzene | 0.5 | 1 | 104 | 12 | 0.9959 |
| β-BHC | 0.5 | 1 | 95 | 11 | 0.9981 |
| Lindane (γ-BHC) | 0.5 | 1 | 90 | 14 | 0.9970 |
| Heptachlor | 0.5 | 1 | 98 | 15 | 0.9939 |
| Aldrin | 0.5 | 1 | 95 | 14 | 0.9949 |
| Heptachlor epoxide | 0.5 | 1 | 90 | 19 | 0.9939 |
| *trans*-Chlordane | 0.5 | 1 | 91 | 18 | 0.9903 |
| Endosulfan I | 0.5 | 1 | 91 | 19 | 0.9915 |
| pp'-DDE | 0.5 | 1 | 101 | 19 | 0.9980 |
| Endrin | 0.5 | 1 | 100 | 19 | 0.9983 |
| Endosulfan II | 0.5 | 1 | 88 | 7 | 0.9981 |
| pp'-DDD | 0.5 | 1 | 84 | 11 | 0.9992 |
| op'-DDT | 0.5 | 1 | 97 | 9 | 0.9957 |
| Endosulfan sulfate | 0.5 | 1 | 90 | 14 | 0.9984 |
| pp'-DDT | 0.5 | 1 | 94 | 16 | 0.9901 |
| **Polycyclic Aromatic Hydrocarbons (PAHs)** | | | | | |
| Chrysene | 0.5 | 1 | 99 | 8 | 0.9986 |
| Benz(*a*)anthracene | 0.5 | 1 | 101 | 7 | 0.9979 |
| Benzo(b)fluoranthene | 0.5 | 1 | 109 | 18 | 0.9927 |
| Benzo(*a*)pyrene | 0.5 | 1 | 107 | 18 | 0.9924 |

Representative calibration curves for PCB 138, PBDE 47, pp'-DDD and chrysene are reported in Figure 3.

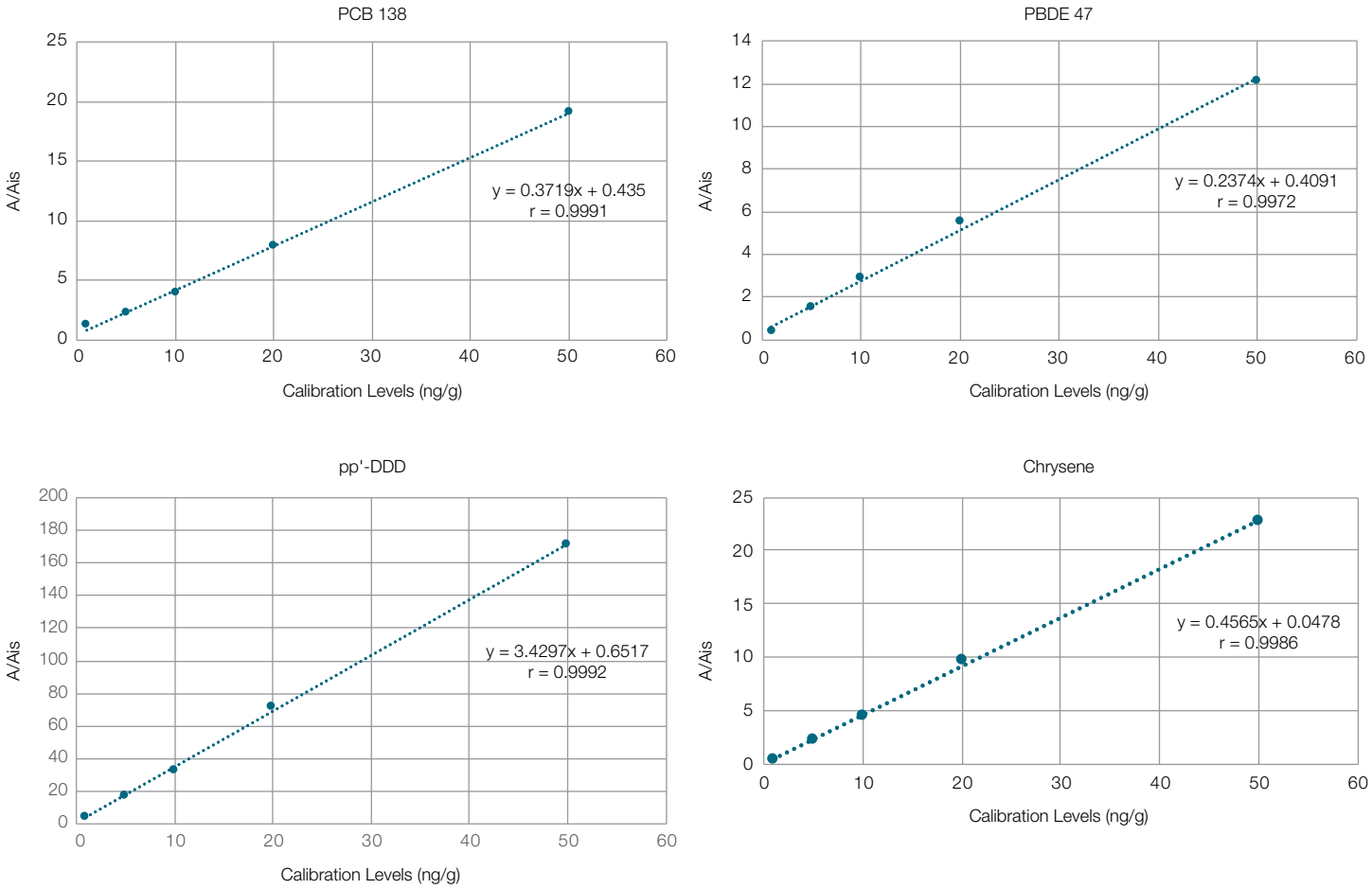


**Figure 3. Calibration curves for PCB 138, PBDE 47, pp'-DDD, and chrysene (1–50 ng/g)**

PCBs were detected in all Mediterranean shad samples (Figure 4) and the concentrations ranged between 1.09 and 11.8 ng/g. Among PBDEs, PBDE 47 was detected in 100% of the samples. PBDE 99, 28, and 100 were detected in over 70% of the samples. PBDE 153, 33, and 154 were detected in less than 40% of the samples. The concentrations ranged from 1.05 to 5.72 ng/g (Figure 5). Even though the use of DDT was banned for agricultural uses in the early 70s, it was still detected in 70% of the samples. Moreover, the very persistent reductive dechlorination products of DDT, DDD, and DDE, were detected in all analyzed samples. Hexachlorobenzene and Endosulfan I were the two other more frequently detected OCPs, present in 87% and 70% of samples respectively. The remaining OCPs were detected in between 7 and 57% of the samples. The concentrations ranged from 1.03 to 14.81 ng/g (Figure 6). The most frequently detected PAH was benz(*a*)anthracene (83% of the cases), followed by chrysene (63%), benzo(b)fluoranthene, and benzo(*a*)pyrene (13% and 7% respectively). Fortunately, none of the PAHs was present at a concentration over the LOD.

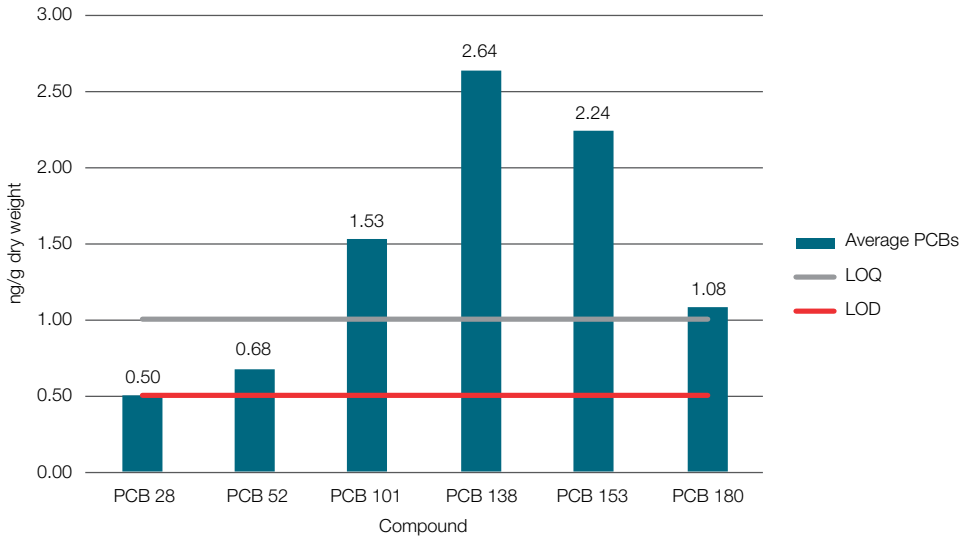


Figure 4. Average PCBs concentration in samples of Mediterranean shad (n = 30)

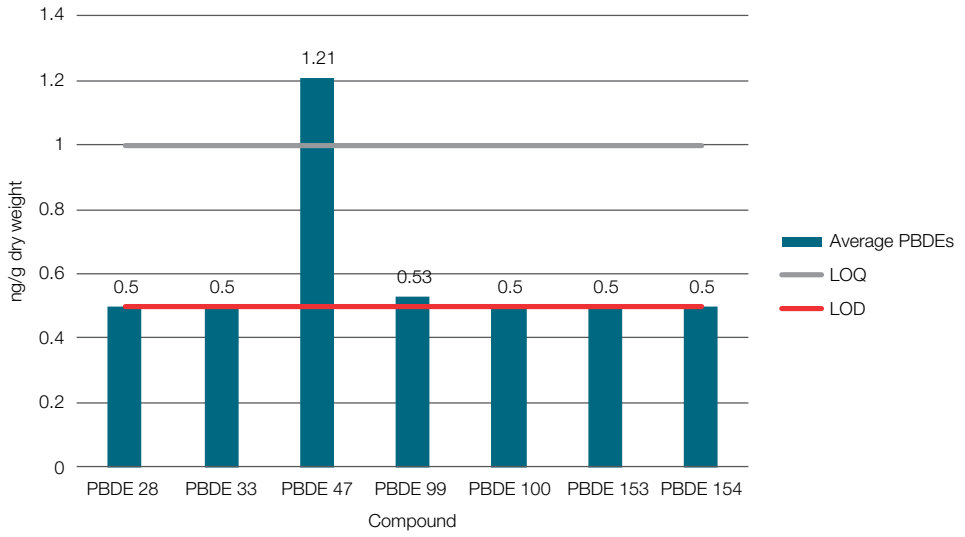


Figure 5. Average PBDEs concentration in samples of Mediterranean shad (n = 30)

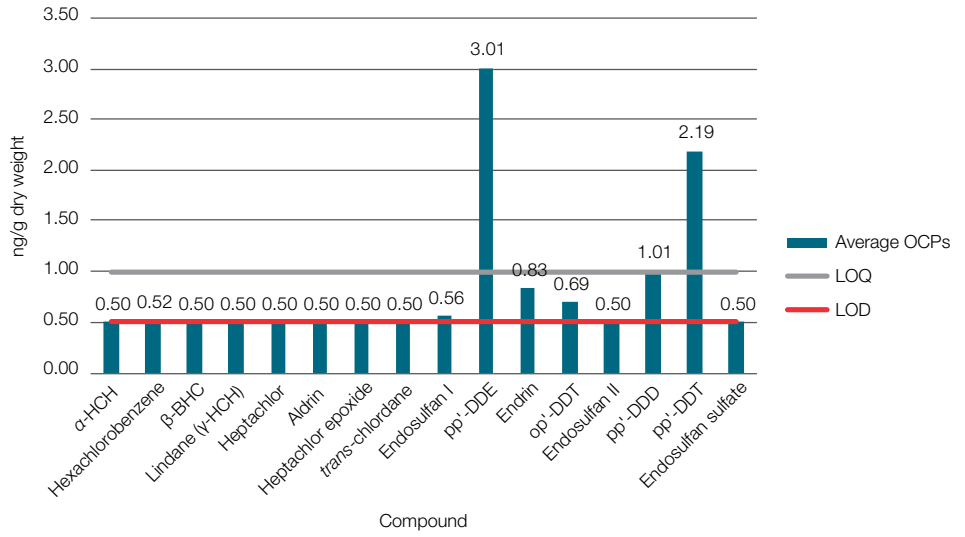


Figure 6. Average OCPs concentration in samples of Mediterranean shad (n = 30)

## Summary

An analytical method was developed and applied to evaluate POP residues in fish samples (Mediterranean shad). The method proved to be simple and rapid (about 1.6 times faster analyte extraction compared to the ASE 350 system), requiring small sample sizes and minimizing solvent consumption (about 1.6 times less compared to ASE 350 system), due to use of gas assisted accelerated solvent extraction combined with an in-cell clean up and concentration step. Detection via MS/MS provides both quantitative information and confirmation of POP residues in fish samples, confirming that the one-step accelerated solvent extraction method is a valid faster alternative to classic extraction methods because the analytical quality is comparable.

## Acknowledgments

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