Sample preparation

EXTREVA ASE Accelerated Solvent Extractor accelerates time to insight for persistent organic pollutants (POPs) analyses in tuna

Department of Veterinary Medicine and Animal Science, University of Milan laboratory of inspection of food of animal origin

"The time required in the laboratory is reduced 50% by combining the extraction and the two cleanup steps (i.e., GPC and SPE) into one single accelerated solvent extraction step, thus doubling the number of samples that can be analyzed per day."

—Giacomo Mosconi, Research Fellow, Laboratory of Analysis of Foods of Animal Origin, Department of Veterinary and Animal Science, University of Milan

Introduction

At the top of the ocean food chain, tuna bioaccumulate pollutants as they age.[1] This a serious concern because tuna is a popular food. The risks are particularly high when tuna have fed in areas with high levels of toxic chemicals such as industrial locations. Research has determined that tuna near these areas can contain over 36 times higher levels of persistent organic pollutants (POPs) than farm-raised tuna and tuna not feeding near those locations.[2] Therefore, quantification of the various POP contaminants in tuna is necessary to ensure food safety. However, measurement of POPs in fish presents an analytical challenge due to the limitations of typical extraction techniques which are labor-intensive and require high solvent consumption. In addition, interfering compounds may be extracted along with the desired analytes, reducing quality of results.

"The determination of the presence as well as the quantification of various contaminants in foods of animal origin is pivotal to ensuring food safety for consumers. Our goal was to develop an appropriate analytical method for the extraction, purification and concentration of the sample using the new EXTREVA ASE instrument, which proved to be easy to use and very intuitive."

—Giacomo Mosconi

This is why the Laboratory of Analysis of Foods of Animal Origin of the University of Milan's Department of Veterinary and Animal Science applied their validated extraction, purification, and concentration method for the analysis of 33 POPs in fish that uses the Thermo Scientific™ EXTREVA ASE™ Accelerated Solvent Extractor prior to GC-MS/MS analysis. The method is the first to apply pressurized fluid extraction with inline cleanup using Supel™ QuE Z-Sep sorbent. The EXTREVA ASE system automates sample extraction, in-cell cleanup, and evaporation to prepare solid and semi-solid samples for GC, GC-MS, or LC-MS analyses. With the ability to extract four samples in parallel, the system increases laboratory throughput, while its patented gas-assisted extraction uses less solvent and produces less waste. Application of the method offers a faster path to addressing important food safety concerns.

POPs of interest

The POPs the laboratory targeted in the method included 6 polychlorinated biphenyls (PCBs), 16 organochlorine pesticides (OCPs), and 7 polybrominated diphenyl ethers (PBDEs) and 4 polycyclic aromatic hydrocarbons (PAHs). PCBs were manufactured in the United States from 1929 until banned in 1979. 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. Recently recognized as a major environmental pollutant, PBDEs have been used as flame retardants in electrical equipment, construction materials, coatings, textiles, and polyurethane foam. Several nations have banned PBDEs and introduced legislation that bans the sale of certain products containing PBDEs. At the recommendation of the European Food Safety Authority (EFSA), the European Commission has asked member states to monitor for the presence of PBDEs. PAHs are derived from both anthropogenic activities (i.e., incinerators, industrial processes, motor vehicles, and combustion of wood and fossil fuels) and natural sources (i.e., incomplete combustion of organic matter).

Extraction challenges

Techniques such as Soxhlet (U.S. EPA Method 3540), sonication (U.S. EPA Method 3550), and microwave extraction (U.S. EPA Method 3546) are typically used to extract POPs from food and environmental samples prior to their determination. These techniques are very labor intensive, use large amounts of solvent, and may allow extraction of interfering compounds along with the target analytes. Unwanted co-extractables can cause buildup of nonvolatile materials on the GC injection port and the analytical column, leading to poor results and increased instrument maintenance costs. Gel permeation chromatography (GPC) is often used as a post-extraction cleanup for fish and meat tissues prior to POPs analysis. However, the disadvantage of GPC is that it is difficult to remove all lipids, which must be removed in a second cleanup procedure. Additionally, for samples with a high lipid content, lipophilic pesticides may remain in the fatty layer even after the extraction.

"The novelty of this project was use of the EXTREVA ASE, which is the only instrument that allows extraction, inline purification, and concentration of a sample. Moreover, we introduced a freeze-drying step in order to get rid of the water in the samples. With accelerated solvent extraction, extractions can be completed in very short periods of time with minimal amounts of solvent compared to conventional sample extraction techniques."

—Giacomo Mosconi

Workflow: determination of POPs in tuna using the EXTREVA ASE and GC-MS/MS

Validated method overview and validation

To carry out the tuna study, the laboratory applied their previously developed and validated method for multiresidue POP determination in fish.[3] Validation of the developed method was according to SANTE 11312/2021.[4] The EXTREVA ASE system performs sample extraction, purification, and concentration, saving time and solvent. The Thermo Scientific™ TRACE™ 1310 Gas Chromatography System with the Thermo Scientific™ TSQ™ 8000 Triple Quadrupole Mass Spectrometer is used for GC-MS/MS detection and quantitation of 6 PCBs, 16 OCPs, 7 PBDEs and 4 PAHs.

Detailed method performance and validation results are available in Thermo Scientific Customer Application Note CN001959.[3] Here, the method was determined to provide good linearity with coefficients of determination equal to or higher than 0.99 for all the compounds targeted, as well as good repeatability, confirming that it can be used to monitor compounds belonging to different chemical classes. Recoveries ranged from 93 to 100% for PCBs, from 93 to 104% for PBDEs, from 84 to 103% for OPCs, and from 99 to 109% for PAHs.

Sample prep innovations

Thirty tuna samples obtained from different Food and Agriculture Organization (FAO) Major Fishing Areas (FAO 37.2, 37.3, 57, 71 and, 77)[5] were collected for study. The FAO major fishing areas are shown in Figure 1. Sample (300 g) was minced and freeze-dried. As Giacomo Mosconi, Research Fellow, Laboratory of Analysis of Foods of Animal Origin, Department of Veterinary and Animal Science, University of Milan, explained, "freeze-drying removes up to 99% of the water content of the samples, therefore avoiding water co-extraction and the need for manual drying with sodium sulphate/moisture absorbing polymer before concentration and GC-MS analysis." An aliquot (0.70 g) corresponding to 3 g of wet tuna sample was homogenized in a beaker with 5 g of Thermo Scientific™ Dionex™ ASE™ Prep DE dispersant (diatomaceous earth). The dispersant prevents sample compaction during the compression phase of extraction, ensuring efficient solvent contact with the sample.

In addition to freeze-drying samples, another improvement was the replacement of the silica gel in the Thermo Scientific™ Dionex™ ASE™ stainless steel extraction cell with Supel QuE Z-Sep sorbent. Supel QuE Z-Sep is a Zirconium-based sorbent recommended for the analysis of hydrophobic analytes in fatty matrices. It increases the robustness of GC-MS and LC-MS methods by removing more fat and pigments than traditional C_{18} and PSA phase sorbents. The one-step accelerated solvent extraction method using Z-Sep as a fat retainer is rapid and cost-effective and minimizes waste generation compared to the classic methods. "To the best of our knowledge" noted Mosconi, "this is the first example of pressurized fluid extraction with inline cleanup using this type of sorbent."

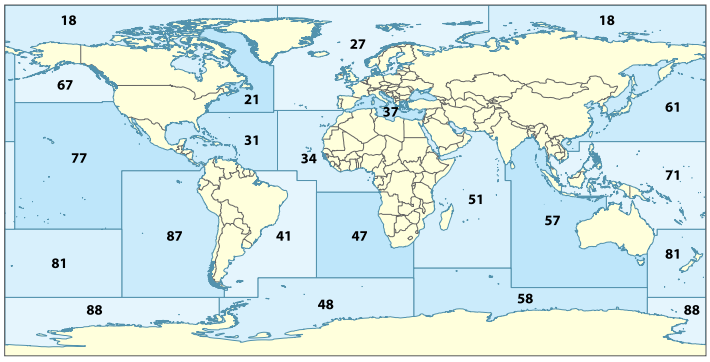


Figure 1. FAO major fishing areas.[6]

"The method proved to be simple and rapid, requiring small sample sizes and minimizing solvent consumption, due to use of accelerated solvent extraction combined with inline cleanup and concentration. Detection via MS/MS provided both quantitative information and confirmation of POP residues in the tuna samples, confirming that the one-step accelerated solvent extraction method is a valid faster alternative to classic methods because the analytical quality is comparable."

—Giacomo Mosconi

Extraction cell layout

Figure 2 shows the extraction cell layout. A cellulose filter was placed in the 22 mL extraction cell body and the end cap was hand-tightened. 500 mg of Supel QuE Z-Sep sorbent were added into the extraction cell, followed by another cellulose filter. Then the sample-dispersant mixture was poured into the extraction cell and spiked with 20 µL of hexane solution containing 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. Before extracting of samples in the EXTREVA ASE, it was rinsed with 10 mL of hexane. Hexane was also used during evaporation as a rinse solvent and additionally added during the concentration phase. Samples were concentrated to a final volume of 0.5 mL. The extraction time for four samples was 100 min. Following concentration, the samples were analyzed by GC-MS/MS in selected reaction monitoring (SRM) mode. Two MS/MS transitions were chosen per compound to meet the generally applied identification criteria outlined in SANTE 2021.[4]

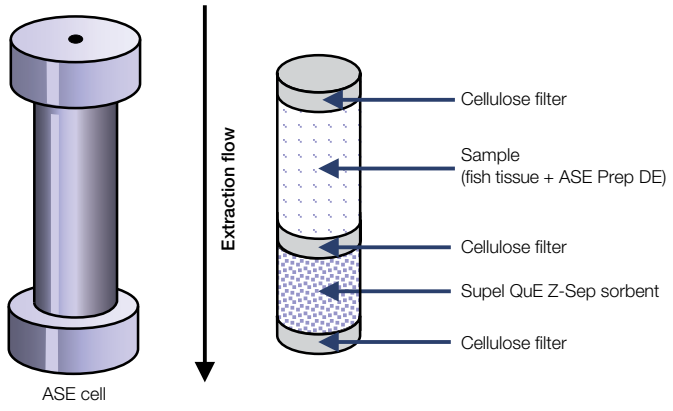


Figure 2. Extraction cell schematic.

Research study results

POP levels in tuna

Analysis of real tuna samples produced results of substantial interest to the food safety community. The laboratory detected PCBs in all tuna samples with concentrations ranging between 1.43 and 59.79 ng/g. Among the PBDEs, PBDE 28 was detected in 73.3% of the samples. PBDE 47, 100, and 153 were detected in over 30% of the samples. PBDE 33, 99 and 154 were detected in less than 25% of the samples at concentrations ranging from 1.00 to 8.48 ng/g. Despite being banned for agricultural uses in the early 70s, DDT, along with its reductive dechlorination products DDD and DDE, was detected in 80% of the tuna samples. Detected in every sample, hexachlorobenzene was the most frequently found OCP. The other OCPs were detected in 7 to 50% of the samples at concentrations ranging from 1.42 to 44.50 ng/g. The only PAH detected, Benzo(*b*)fluoranthene, was found in only two samples at a concentration below the LOQ.

Fishery comparison

Comparisons of tuna sampled from the various FAO fishing areas revealed notable differences. For example, there were statistically significant differences in the concentration of PCB 180 between FAO 37.2 where the tuna was more contaminated, and FAO 77, FAO 57, and FAO 71 ($p < 0.01$, $p < 0.01$ and $p < 0.001$ respectively). A visual comparison of the levels of various selected POPs detected in tuna from different FAOs sampled is shown in Figure 3. Taken together, the results from the laboratory's method indicated that tuna from FAO 37.2 is significantly more contaminated than the other areas sampled.

"Based on the results, future work will certainly have to be directed towards continuous monitoring of the presence of these contaminants in various food chains. In addition, the development of new analytical methods for different classes of analytes, such as PFASs, will be essential."

—Giacomo Mosconi

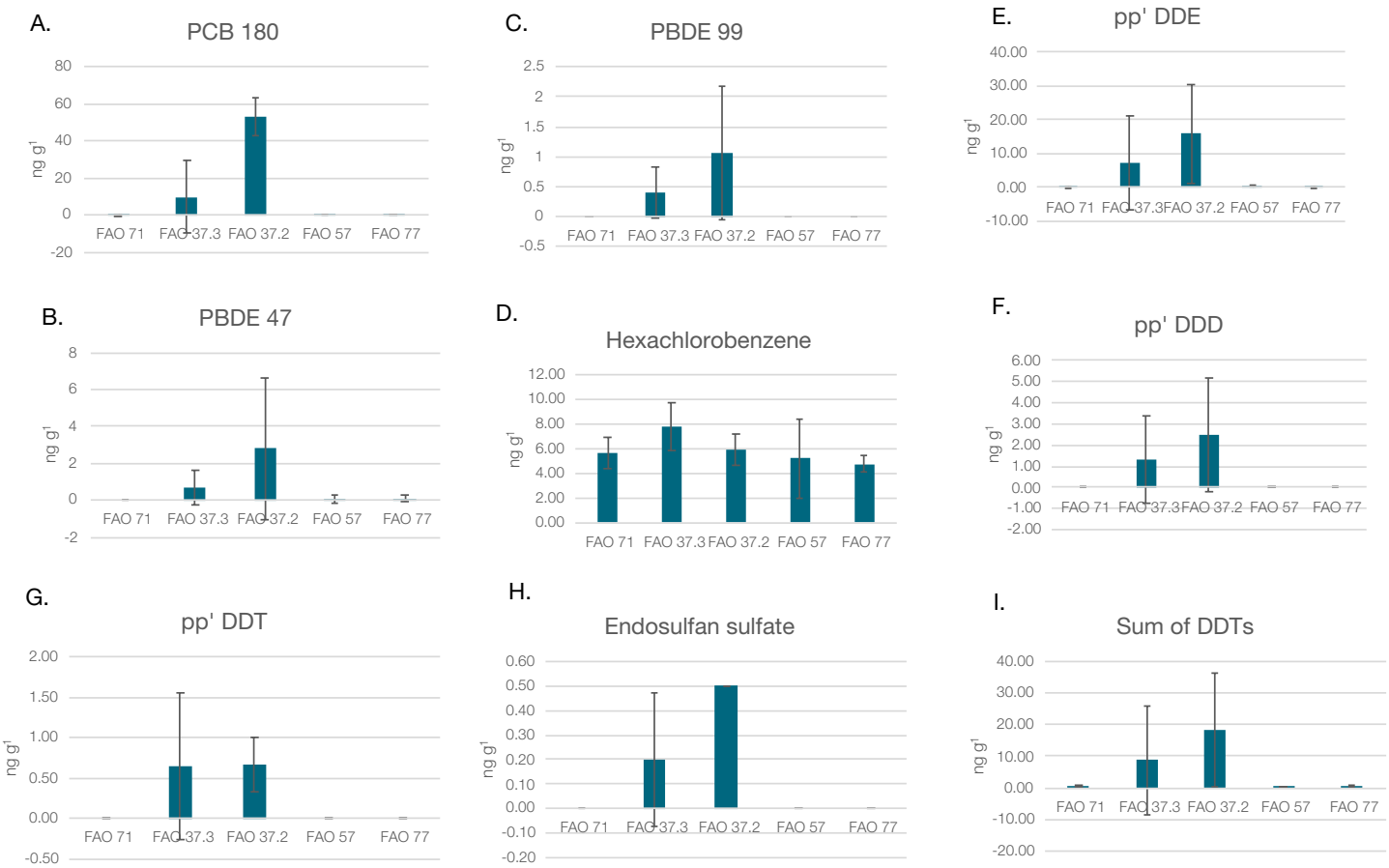


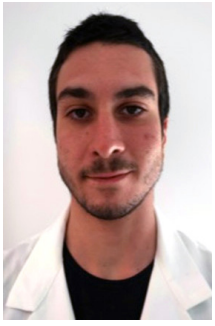
Figure 3. Selected POPs found in each fishery studied. A.) PCB 180, B.) PBDE 47, C.) PBDE 99, D.) Hexachlorobenzene, E.) *pp*'-DDE, F.) *pp*'-DDD, G.) *pp*'-DDT, H.) Endosulfan sulfate, I.) Sum of DDTs

Conclusion

The analytical method for the determination of POP residues in tuna was simple and rapid, required small sample sizes, and minimized solvent consumption compared to previous methods. Detection using GC-MS/MS provided quantitation and confirmation of target POP residues in tuna, demonstrating that the one-step accelerated solvent extraction method using the EXTREVA ASE is a valid faster alternative to classic extraction methods. Using the workflow, the laboratory detected POPs in real tuna samples at concentrations ranging from 1.0 and 59.79 ng/g. Comparisons of tuna sampled from the various FAO fishing areas revealed significant differences and indicated that tuna from FAO 37.2 is significantly more contaminated than the other areas studied.

The laboratory introduced two key improvements to the sample preparation and extraction method: freeze-drying the samples, and replacement of the silica gel in the ASE cell with Supel QuE Z-Sep sorbent for the inline cleanup. Freeze-drying removes up to 99% of the water in fish tissue samples, therefore avoiding water co-extraction and the need for manual drying prior to concentration. The Supel QuE Z-Sep also increases the robustness of GC-MS and LC-MS method by removing more fat and pigments than traditional C_{18} and PSA phase sorbents.

About Giacomo Mosconi



Giacomo Mosconi Is a Ph.D. Research Fellow at the Laboratory for the Analysis of food of Animal Origin in the Department of Veterinary Medicine, University of Milan. His experimental thesis is quantitative analysis of the change in protein profiles due to the action of HNE in intestinal epithelium cells using a proteomic approach. Mosconi has a Master's Degree in Medicinal Chemistry, from the University of Milan. His interest is in analytical chemistry applied to food matrices, in particular, chromatography and mass spectrometry.

About the Department of Veterinary Medicine and Animal Sciences, University of Milan

The Department of Veterinary Medicine and Animal Sciences organizes, guarantees and promotes research studies in collaboration with public institutions/bodies and private, national and international companies. Its research group operates in the fields of inspection and hygiene of food of animal origin including shelf-life studies; food labeling, compliance checks during preparation and administration of meals; analysis of food production processes; food recovery feasibility studies; and methods to support inspection and certification, risk analysis, and traceability for effective control of food quality along the entire supply chain. Research is also focused on the investigation of molecules of interest for inspection purposes and the quality of foods of animal origin in compliance with current European regulations and related national transpositions (National Residue Plan).

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