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Determination of Persistent Organic Pollutants in Fish Tissue by Accelerated Solvent Extraction and GC-MS/MS

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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. The capacity of the halogenated hydrocarbons 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. Due to this potential toxicity, the extraction and analysis of halogenated hydrocarbons from matrices such as fish tissue is required by the U.S. Environmental Protection Agency (EPA). Techniques, such as Soxhlet and sonication, are used for the extraction of halogenated hydrocarbons from environmental samples prior to their analytical determination. These techniques are, however, very labor intensive and suffer from high solvent consumption. Accelerated solvent extraction was developed to meet the new requirements of increased throughput and reduced solvent usage in sample preparation.

The work presented in the poster demonstrates workflow methods for halogenated hydrocarbon extraction and analysis using GC-MS/MS from fish tissue. An analytical method was developed and applied to evaluate POP residues in tuna samples from different Food and Agricultural Organization areas. The method reported here is applicable for the determination of 29 halogenated hydrocarbons (6 PCBs, 16 OCPs, and 7 PBDEs). The method proved to be simple and rapid, requiring small sample sizes and minimizing solvent consumption, due to use of accelerated solvent extraction with an in-line clean up step.

MATERIALS AND METHODS

Sample Collection and Preparation

A total of 79 Bluefin tuna (*Thunnus thynnus*) originating from different Food and Agriculture Organization (FAO) catch areas were selected for this study (**Table 1**). Representative samples from each fish were obtained by sampling tissue from three different anatomic zones (proximal, ventral, and caudal); each sample was then stored at -22 ° C until analysis.

Table 1. Number of Bluefin Tuna Samples from Each FAO Region.

Number of Samples	FAO Catch Area	Geographical Area
20	51	Indian Ocean, Western
20	71	Pacific Ocean, Western Central
20	34	Atlantic Ocean, Eastern Central
19	37	Mediterranean Sea

The extractions were carried out using a Thermo Scientific™ Dionex™ ASE™ 350 Accelerated Solvent Extractor (**Figure 1**) equipped with 34 mL stainless steel extraction cells. The extracts were collected in 60 mL vials treated with sodium sulfate and directly concentrated in a 2 mL autosampler glass vial with the Thermo Scientific™ Rocket™ Evaporator (**Figure 1).** A solution of PCBs congeners (PCB 28; PCB 52; PCB 101; PCB 138; PCB 153 and PCB 180), PCB 209 (internal standard for PCBs), solution of PBDEs (PBDE 28; PBDE 33; PBDE 47; PBDE 99; PBDE 100; PBDE 153 and PBDE 154) and FBDE, and an internal standard (IS) for flame retardants was used to spike the fish tissue. Working solutions were prepared by diluting the stock solution in hexane and then storing at -40 °C. The mixed compound calibration solution, in hexane, was prepared from the stock solutions and also used as a spiking solution (10 µg/mL).

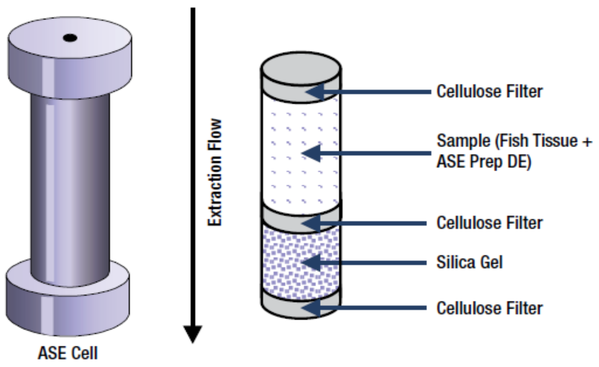
Figure 1. Dionex ASE 350 Accelerated Solvent Extractor and Rocket Evaporator.

Dionex ASE 350

Genevac Rocket Evaporator

The extraction cells were prepared by placing a cellulose filter in the bottom of a 34 mL extraction cell (**Figure 2**), followed by 10 g of activated silica gel and another cellulose filter. A representative portion of tuna (300 g) was obtained from each fish and minced. A 3 g sample was homogenized with an equal weight of Thermo Scientific™ Dionex™ ASE™ Prep DE (diatomaceous earth) and sodium sulfate, and transferred into the cell. A 1.0 mL isooctane solution containing the three internal standards was added to this mixture. The remaining empty volume was filled with Dionex ASE Prep DE. The accelerated solvent extractor was programmed according to the method conditions listed **Table 2**. The extracts were collected in 60 mL vials and treated with sodium sulfate to remove any possible humidity. After filtration, the organic phase was concentrated to dryness in the Rocket Evaporator system, dissolved in 200 µL of isooctane, and analyzed by GC-MS/MS.

Figure 2. Extraction Cell Schematic.



Parameter	Setting
Solvent	n-Hexane/Acetone (4:1, v/v)
Temperature	80 °C
Pressure	1500 psi
Static Cycles	3
Static Cycle Time	10 min
Rinse Volume	90%
Purge Time	90 s
Extraction Time per Sample	~ 40 min
Solvent Used per Sample	~ 40 mL

Analytical Methods

The samples were analyzed using a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph equipped split/splitless injector, a fused-silica capillary column (Rt-5MS Crossbond-5% diphenyl 95% dimethylpolysiloxane, 35 m × 0.25 mm × 0.25 µm,) and a Thermo Scientific™ TSQ™ 8000 Triple Quadrupole GC-MS/MS (**Figure 3**). The method conditions for the gas chromatograph and mass spectrometer are listed in **Tables 3 and 4**.

Figure 3. TSQ 8000 Triple Quadrupole GC-MS/MS.

Table 3. GC and Injector Conditions.

Parameter	Setting
Injector Type	Split/Splitless
Injector Temperature	250 °C
Liner	2 x 2.75 x 120 mm
Injected Volume	1 µL
Splitless Time	0.5 min
Splitflow	10 mL/min
GC Column	Rt-5MS (35m x 0.25 mm x 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 195 °C 2 °C/min to 240 °C 3 °C/min to 280 °C 10 °C/min to 310 °C
Final Temperature	310 °C (5 min)

Table 4. Mass Spectrometer Parameters.

Parameter	Setting
Source Temperature	250 °C
Ionization	EI
Electron Energy	70 eV
Emission Current	50 µA
Q2 Gas Pressure	1.5 mTorr
Collision Energy	10 to 30 eV
Q1 Peak Width FWHM	0.7 Da
Q3 Peak Width FWHM	0.7 Da

RESULTS

The proposed method was optimized for the multiresidue analysis of 29 persistent organic pollutants (POPs). Total ion current chromatograms (GC-MS/MS) of tuna spiked with POPs and a naturally contaminated fish sample are shown in **Figures 4 and 5**. The optimization of the MS/MS method consisted of:

(1) Acquisition of respective MS spectra in full-scan mode (m/z 100–1000 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 2015 (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. 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, makes possible 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 whole GC-MS system. All results obtained confirm the efficacy of the present method for the determination of multiresidue pollutants in fish tissue.

Figure 4. Total ion current (GC-MS/MS) chromatogram of tuna spiked sample

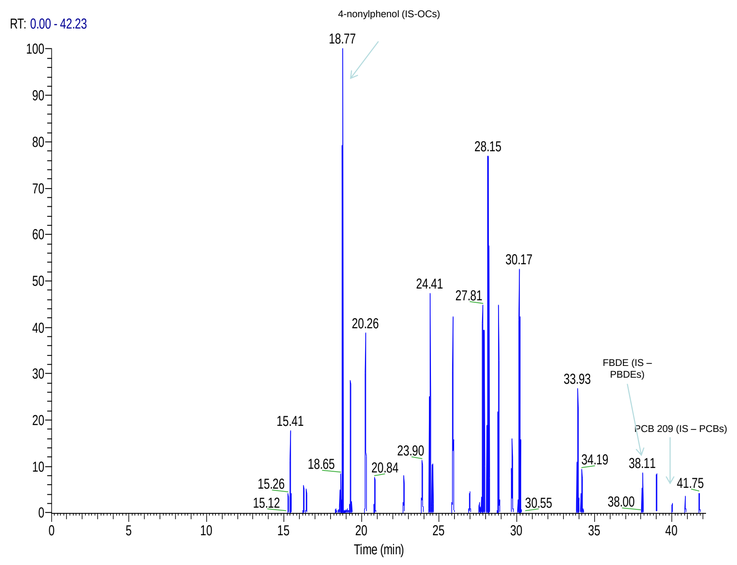
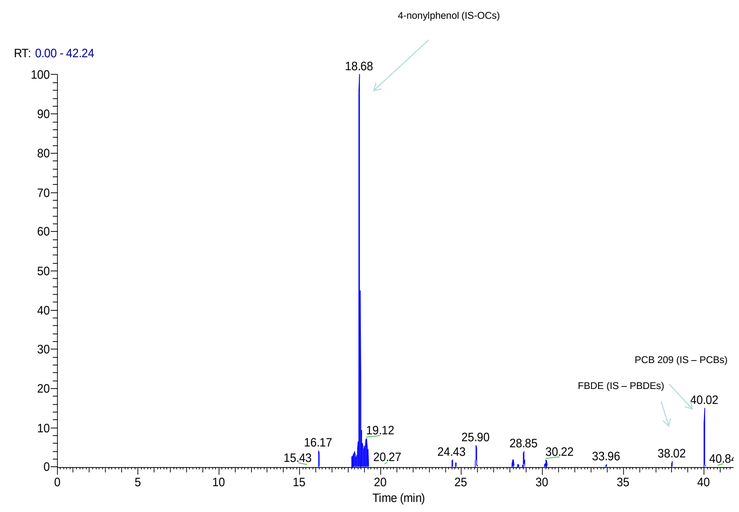


Figure 5. Total ion current (GC-MS/MS) chromatogram of raw fish sample



The method showed 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 5**). The recoveries ranged from 108–119% for PCBs, from 91–102% for PBDEs, and from 75–96% for OCPs. The CVs ranged from 4–14%. The one-step accelerated solvent extraction method using silica 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 5. Recoveries (%, RSD), LOD, LOQ, and coefficient of determination (r^2).

Contaminants	LOD (ng/g)	LOQ (ng/g)	Recovery % (RSD)	Coefficient of Determination (r2)
Polychlorinated Biphenyls (PCBs)				
PCB 28	0.08	0.24	102 (7)	0.9994
PCB 52	0.07	0.21	103 (7)	0.9999
PCB 101	0.04	0.12	97 (4)	0.9999
PCB 138	0.05	0.15	105 (4)	0.9999
PCB 153	0.02	0.06	102 (4)	0.9999
PCB 180	0.06	0.18	98 (9)	0.9999
Polybrominated Diphenyl Ethers (PBDEs)				
PBDE 28	0.01	0.03	100 (9)	0.9991
PBDE 33	0.02	0.06	98 (9)	0.9999
PBDE 47	0.02	0.06	97 (8)	0.9996
PBDE 99	0.03	0.09	102 (7)	0.9998
PBDE 100	0.01	0.03	103 (7)	0.9998
PBDE 153	0.03	0.09	97 (10)	0.9992
PBDE 154	0.02	0.06	100 (12)	0.9999
Organochlorine Pesticides (OCPs)				
α-HCH	0.99	2.97	78 (10)	0.9959
Hexachloro-benzene	1.26	3.78	80 (12)	0.9945
β-HCH	1.17	3.51	85 (12)	0.9995
Lindane	0.79	2.39	96 (10)	0.9985
Heptachlor	0.95	2.84	93 (12)	0.9996
Aldrin	0.85	2.55	75 (14)	0.9991
Heptachlor epoxide	0.91	2.73	77 (14)	0.9994
trans-Chlordane	1.48	4.44	92 (10)	0.9993
Endosulfan I	1.13	3.38	80 (13)	0.9992
pp‘-DDE	0.85	2.55	97 (12)	0.9994
Endrin	0.99	2.98	88 (11)	0.9998
Endosulfan II	1.14	3.42	90 (10)	0.9993
pp‘-DDD	0.91	2.74	87 (14)	0.9986

CONCLUSIONS

An analytical method was developed and applied to evaluate POP residues in tuna samples from different FAO areas. The method proved to be simple and rapid, requiring small sample sizes and minimizing solvent consumption, due to use of accelerated solvent extraction with an in-line clean up step. Detection via MS/MS provides both quantitative information and confirmation of POP residues in tuna, confirming that the one-step accelerated solvent extraction method is a valid faster alternative to classic extraction methods because the analytical quality is comparable.

REFERENCES

1. Thermo Fisher Scientific Customer Application Note (CAN) 122: Determination of Persistent Organic Pollutants in Fish Tissues by Accelerated Solvent Extraction and GC-MS/MS.
2. Chiesa, L.M.; Labella, G.F.; Panseri, S.; Pavlovic, R.; Arioli, F. Distribution of persistent organic pollutants (POPS) in wild Bluefine tuna (*Thunnus thynnus*) from different FAO capture zones. *Chemosphere*. **2016,** 153, 162-9**.**

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TRADEMARKS/LICENSING

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