Simultaneous Extraction of PAHs and PCBs from Environmental Samples Using Accelerated Solvent Extraction

Brett Murphy, Selvan Lingam, Bruce Richter, and Richard Carlson;
Thermo Fisher Scientific, Salt Lake City, UT, USA

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Key Words
ASE, persistent organic pollutants (POPs), mussel tissue, soil, environmental analysis

Introduction

Accelerated solvent extraction is an established technique used for the extraction of solid and semisolid sample matrices using common solvents. Typical accelerated solvent extraction parameters include elevated temperatures and pressures, which enhance the kinetics of the extraction process. This, in turn, results in extraction efficiencies that are similar to or greater than those achieved with a Soxhlet extractor, but in a fraction of the time while consuming much less solvent. Accelerated solvent extraction has been approved for use in U.S. EPA Method 3545A for the extraction of polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), base/neutral/acid (BNA) compounds, organophosphorus pesticides (OPPs), herbicides, dioxins and is in compliance with U.S. EPA SW-846. Traditional extraction methods, such as Soxhlet and sonication, typically, take hours and produce large amounts of organic solvent waste. Accelerated solvent extraction reduces solvent consumption and simplifies and automates sample preparation. In addition, the accelerated solvent extraction platform brings unique flexibility to sample preparation, allowing some steps to be combined and automated.

One of the steps that can be combined is the introduction of an adsorbent into the extraction cell. Without the in-cell cleanup, Gel Permeation Chromatography (GPC) would need to be done, thus adding an hour or more to the analysis process. This will, in turn, necessitate cleaning the injection port more frequently.

Polyaromatic Hydrocarbons (PAHs) and Polychlorinated Biphenyls (PCBs) are considered toxic and carcinogenic. They are classified as persistent organic pollutants (POPs). Typically, PAHs and PCBs are extracted separately, using different solvent combinations. The purpose of this study is to report on the development of a single extraction method for PAHs and PCBs from mussel tissue and soil using accelerated solvent extraction. Our study consists of two parts:

1. Extraction of PAHs and PCBs from spiked mussel tissue at two different temperatures (see Table 1); and
2. Extraction of PAHs and PCBs from a soil as per standard reference materials (SRMs) provided by Resource Technology Corporation

Table 1. Accelerated solvent extraction conditions.

	Method 1	Method 2
System Pressure	10 MPa (1500 psi)	10 MPa (1500 psi)
Oven Temperature	125 °C	100 °C
Sample Size	5 g	5 g
Oven Heatup Time	6 min	5 min
Static Time	6 min	4 min
Static Cycles	4	5
Rinse Volume	40 mLs (60% of extraction cell volume)	40 mLs (60% of extraction cell volume)
Solvent	Dichloromethane	Dichloromethane
Nitrogen Purge	300 s	300 s
Extraction Time	30 min	25 min
Cell Size	66 mLs	66 mLs

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Equipment

- Thermo Scientific™ Dionex™ ASE™ 350 Accelerated Solvent Extractor equipped with 66 mL Stainless Steel Extraction Cell Kit, (P/N 074838)
- Filters, Glass Fiber, or 34, 66, or 100 mL Cell (P/N 056781)
- 250 mL Clear Collection Bottles for ASE 350/300/150/100 (P/N 056284)
- Analytical Balance (read to the nearest 0.001 g or better)
- Mortar and Pestle (Fisher Scientific or equivalent)
- Gas Chromatograph with Electron-Capture Detector (ECD)
- Gas Chromatograph/Mass Spectrometer
- Capillary Column 40 m × 0.18 mm i.d., d_f = 0.18 µm
- 5% Diphenyl Capillary Column 30 m × 0.25 mm i.d., d_f = 0.5 µm
- Nitrogen Evaporator (or equivalent)

Solvents and Standards

- Dichloromethane
- Hexane
- Fisherbrand™ Robotic Screw Top Autosampler; Amber with Write-on Patch (P/N 03-391-9)
- Diatomaceous Earth (DE) Dispersant for ASE™, 1 kg Bottle (P/N 062819)
- Alumina, Acidic, 60-325 Mesh, Fisher Chemical (P/N A948-500)
- Semi-Volatile Internal Standard Mix
- 8270 Calibration Mix
- 8270 Base/Neutrals Surrogate Mix
- Aroclor 1254 Mix
- 2, 4, 5, 6-Tetrachloro-m-xylene (surrogate)
- Decachlorobiphenyl (Internal Standard)

All solvents are optima-grade or equivalent and available from Fisher Scientific.

Sample Information

New Zealand green-lipped mussels were purchased from a local grocery and stored in a refrigerator at 4 °C until extraction.

Contaminated soil used in this study was a certified reference material (CRM911-50G) for Aroclor 1254-Loam, purchased from Resource Technology Corporation (Laramie, Wyoming, USA).

Mussel Tissue Preparation and Extraction

Clean each extraction cell with soap and water, prior to use. Attach the bottom cell cap and then add a glass fiber filter, followed by twenty grams of acidic alumina and another filter. Prior to use, bake the alumina overnight at 350 °C. Weigh out a 5-gram portion of mussel tissue. Place it into a mortar containing ten grams of diatomaceous earth. Spike the tissue samples with the appropriate amount of PAH spike and Base/Neutrals Surrogate solutions. Each compound will have a final concentration of 5.0 µg/g. Then spike the tissue samples with PCB surrogate (30 ng/g) and an Aroclor standard (2 mg/g). Homogenize the spiked tissue samples with the DE Dispersant for ASE and add to the prepared ASE extraction cell.

Load the extraction cells onto the Dionex ASE 350 Accelerated Solvent Extractor system and extract using the methods specified in Table 1. Concentrate the resulting extracts to 5 mL using nitrogen flow and heat (40 °C). Remove a 1 mL portion of the Methylene Chloride extract for PAH analysis. Evaporate the remaining extract almost to dryness. Add 10 mL of hexane and again evaporate until almost to dryness. Add a second 10 mL portion of hexane and evaporate to 5 mL. Prior to the PCB analysis, remove 500 µL of the resulting extract and add 500 µL of hexane. The appropriate amount of internal standard is then added. Load the extracts onto the GC Autosampler for analysis.

Soil Preparation and Extraction

Weigh out 5 g of the aforementioned CRM. Spike with the appropriate aliquots of PAH (5 µg/g) and Base/Neutrals Surrogate (5 µg/g) solutions. Then spike with the PCB surrogate solution (30 ng/g). After the sample is spiked, mix with DE Dispersant for ASE and transfer to the 66 mL stainless steel cells, which have been prepared as previously mentioned with acidic alumina. (See Mussel Tissue Preparation and Extraction section). These samples were extracted by Method 2.

Concentrate the extract to approximately 5 mL and remove the 1 mL PAH portion. The exchange to hexane was performed as previously mentioned. An aliquot of the 5 mL hexane extract is further diluted by a factor of five. Transfer a 1 mL portion of the diluted sample to a GC vial and add Decachlorobiphenyl.

Post Extraction Cleanup

For soil and tissue extracts, GPC is a very good method for removing sulfur and lipids, but the initial setup can be very expensive. The processing time per sample is between 30 to 70 minutes. For these reasons, alumina was added to the extraction cells. No further cleanup was required for the accelerated solvent extracts.

Analysis of Extracts

GC-MS and GC-ECD were used to separate and identify PAHs and PCBs, respectively.

GC-MS Conditions	
Column:	5% Diphenyl Capillary Column 30 m × 0.25 mm i.d., d_f = 0.5 µm
Injection Port Temperature:	280 °C
Injection Mode:	Splitless
Column Flow Rate:	1.4 (mL/min) constant flow
Oven Temp.:	50 °C (hold for 1 min) to 320 °C at 6 °C/min (hold for 10 mins)

GC-ECD Conditions	
Column:	Capillary Column 40 m × 0.18 mm i.d., d_f = 0.18 µm
Injection Port Temperature:	250 °C
Injection Mode:	Splitless
Purge Time:	1.00 min
Makeup Gas:	Nitrogen
Column Flow Rate:	1.5 (mL/min) constant flow
Oven Temp.:	100 °C (hold 1 for min) to 200 °C at 30 °C/min to 320 °C at 2 °C/min (hold 2 min)

Results and Discussion

As mentioned previously, the purpose of this work was to develop a single extraction method for both PAHs and PCBs. As shown in Tables 2 and 3, the percent recoveries for all compounds with both accelerated solvent extraction methods are within acceptable EPA recovery limits. While the results from accelerated solvent extraction Method 1 were very

Table 2. Data for mussel and soil samples extracted by Method 1.

PAH Recoveries – Mussel (N = 6)			
Compound	**% Recovery**	**SD**	**% RSD**
Nitrobenzene-d5**	83.3	0.54	13.05
2-Fluorobiphenyl**	95.1	0.43	9.13
p-Terphenyl-d4**	91.4	0.27	5.92
Naphthalene	89.1	0.28	6.33
Acenaphthylene	101.2	0.30	5.91
Acenaphthene	98.3	0.28	5.65
Fluorene	107.5	0.46	8.65
Phenanthrene	104.6	0.30	5.70
Anthracene	100.1	0.29	5.77
Fluoranthene	97.1	0.30	6.24
Pyrene	88.9	0.24	5.31
Benzo(a)anthracene	85.4	0.21	4.85
Chrysene	95.5	0.27	5.66
Benzo(b)fluoranthene	91.7	0.31	6.72
Benzo(k)fluoranthene	88.3	0.20	4.43
Benzo(a)pyrene	89.9	0.28	6.29
Benzo(ghi)perylene	94.1	0.31	6.60
Dibenzo(a,h)anthracene	92.3	0.28	6.06
Indeno(1,2,3-cd) pyrene	91.1	0.31	6.72
PCB Recoveries – Mussel (N = 6)			
Compound	**% Recovery**	**SD**	**% RSD**
2,4,5,6-tetrachloro-m-xylene**	93.1	0.48	5.21
Aroclor 1254	95.9	0.06	3.26

PAH Recoveries – Soil (N = 6)			
Compound	**% Recovery**	**SD**	**% RSD**
Nitrobenzene-d5**	94.6	0.81	17.20
2-Fluorobiphenyl**	101.2	0.25	4.87
p-Terphenyl-d4**	102.1	0.10	1.94
Naphthalene	79.0	0.47	6.29
Acenaphthylene	76.3	0.21	5.44
Acenaphthene	102.9	0.33	6.40
Fluorene	80.3	0.21	5.31
Phenanthrene	114.8	0.37	6.39
Anthracene	91.4	0.51	11.19
Fluoranthene	103.6	0.12	2.23
Pyrene	97.4	0.14	2.90
Benzo(a)anthracene	99.0	0.17	3.35
Chrysene	91.2	0.09	1.90
Benzo(b)fluoranthene	96.3	0.14	2.82
Benzo(k)fluoranthene	92.8	0.13	2.70
Benzo(a)pyrene	83.0	0.23	5.52
Benzo(ghi)perylene	82.4	0.13	3.22
Dibenzo(a,h)anthracene	78.9	0.15	3.68
Indeno(1,2,3-cd) pyrene	84.6	0.11	2.65
PCB Recoveries – Soil (N = 6)			
Compound	**% Recovery**	**SD**	**% RSD**
2,4,5,6-tetrachloro-m-xylene**	86.7	1.2	4.72
Aroclor 1254	101.6	0.19	3.15

**Surrogate Spike

Table 3. Data for mussel samples extracted by Method 2.

PAH Recoveries - Mussel (N = 6)			
Compound	**% Recovery**	**SD**	**% RSD**
Nitrobenzene-d5**	84.8	0.11	12.46
2-Fluorobiphenyl**	112.3	0.06	5.12
p-Terphenyl-d4**	105.8	0.10	9.09
Naphthalene	72.5	0.08	10.85
Acenaphthylene	82.3	0.09	10.50
Acenaphthene	81.2	0.07	9.20
Fluorene	79.5	0.06	7.41
Phenanthrene	95.3	0.06	6.49
Anthracene	85.2	0.07	8.01
Fluoranthene	90.8	0.08	8.43
Pyrene	86.2	0.07	7.82
Benzo(a)anthracene	84.7	0.09	10.48
Chrysene	114.0	0.11	9.99
Benzo(b)fluoranthene	89.2	0.07	7.97
Benzo(k)fluoranthene	84.7	0.05	5.33
Benzo(a)pyrene	77.7	0.08	10.39
Benzo(ghi)perylene	87.5	0.14	16.46
Dibenzo(a,h)anthracene	77.7	0.08	10.85
Indeno(1,2,3-cd) pyrene	83.5	0.07	7.97
PCB Recoveries – Mussel (N = 6)			
Compound	**% Recovery**	**SD**	**% RSD**
2,4,5,6-tetrachloro-m-xylene**	94.67	3.75	3.96
Aroclor 1254	85.68	1.87	2.18

**Surrogate Spike

good, matrix interferences due to coextractable compounds were evident in the chromatograms (see Figure 1). These interferences necessitate frequent injection port cleanings. When the conditions of accelerated solvent extraction Method 2 were employed, the amount of co-extractables in the extracts was negligible (see Figure 2). However, as shown in Table 3, the recoveries for some of the higher molecular weight compounds (Benzo(a)pyrene, Benzo(ghi)perylene, Dibenzo(a,h)anthracene, Indeno(1,2,3-cd) pyrene and Aroclor 1254) were reduced, compared to the higher extraction temperature of Method 1.

Another benefit derived from this work, is the use of alumina in the extraction cell to retain lipids. This bypasses the post extraction cleanup step. Using nonselective conditions, the extracts produced are similar to those from traditional methods and require the usual cleanup steps prior to GC analysis. The analysis of extracts containing PCB or PAH contaminants from soil or tissue can be hindered by the presence of coextractables. It is standard procedure to perform some type of cleanup to remove interferences and facilitate the analysis. These procedures may include a florisil, silica gel, or alumina cleanup step.

Using selective accelerated solvent extraction conditions coupled with in-cell cleanup (alumina), extracts can be produced that are free of matrix interferences. These sample extracts can be analyzed without time-consuming cleanup steps.

Suppliers

Fisher Scientific International, Pittsburgh, PA USA

Sigma-Aldrich Chemicals, St. Louis, MO USA

Millipore Corporation, Billerica, MA USA

Resource Technology Corporation, Laramie, WY USA

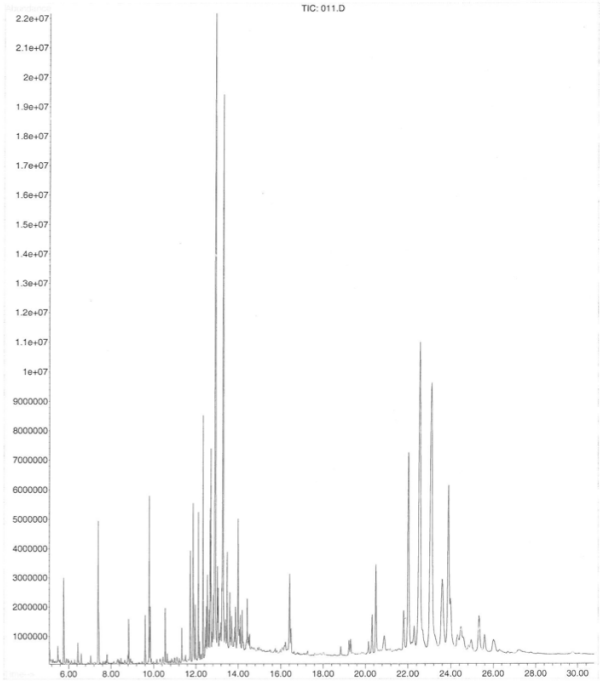

Figure 1. GC/MS analysis of mussel sample extracted by Method 1.

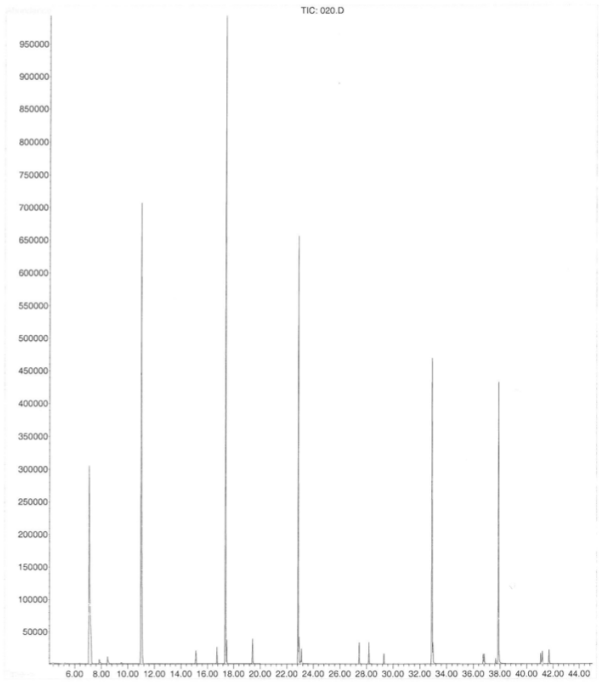

Figure 2. GC/MS analysis of mussel sample extracted by Method 2.

Australia +61 3 9757 4486
Austria +43 1 333 50 34 0
Belgium +32 53 73 42 41
Brazil +55 11 3731 5140
China +852 2428 3282
Denmark +45 70 23 62 60
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Germany +49 6126 991 0
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Japan +81 6 6885 1213
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UK/Ireland +44 1276 691722
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