

Extraction of Lipids and Polychlorinated Biphenyls from Fish Tissue in a Single Run Using Accelerated Solvent Extraction (ASE[®])

INTRODUCTION

The presence of polychlorinated biphenyls (PCBs) in fish and other marine organisms is of immediate environmental and regulatory concern. To determine the concentrations of PCBs indicative of contaminant exposure, and lipids, which also characterize physiological conditions of fish, the analytes must first be extracted. Traditionally, this has been done using Soxhlet methods that are both time consuming and use large volumes of solvent. This application note summarizes the use of ASE to quickly and efficiently extract lipids and PCBs in a single 20-min extraction using only 40 mL of solvent.

ASE is an automated extraction technique that uses traditional liquid solvents at elevated pressure and temperature. The extraction is performed at temperatures higher than the atmospheric pressure boiling point of the solvent. High temperature substantially increases solvation kinetics and the high pressure ensures that the solvent remains a liquid.

While developing the ASE method, it was found that water typically found in fish tissue (>70%) can act as a polar solvent and produce coextractables which complicate extract cleanup prior to analysis. It was discovered, however, that drying samples before ASE extraction produced extracts free of troublesome coextractables. Samples can be dried in a conventional oven or, even faster, in a microwave oven after mixing and dispersing with ASE Prep DE (diatomaceous earth). This application note provides full details of the extraction parameters and a comparison of ASE and Soxhlet extraction results.

EQUIPMENT

ASE 200 Accelerated Solvent Extractor equipped with 33-mL extraction cells
ASE Solvent Controller (optional)
AutoASE[™] Software (optional)
Hewlett Packard Gas Chromatograph 5890 with an electron capture detector (now Agilent Technologies)
Restek RTX-5 column, 30-m × 0.32-mm i.d., film thickness 0.25 μm
Microwave oven (800 W) with carousel
Analytical balance
Dionex Graduated 40-mL vials for extract collection (P/N 055442)
Organomation N-EVAP[®] Analytical Evaporator (Model 112) or equivalent
Screw-on Stainless Steel Funnel (P/N 049288)
Cellulose Filter Insertion Tool (P/N 049495)
Cellulose Filters (P/N 049458)

REAGENTS AND STANDARDS

Hexane, Optima grade (Fisher Scientific)
ASE Prep DE (P/N 062819)
PCB Aroclor[™] standard (Chem Service)
Sulfuric Acid, ACS grade or equivalent (Fisher Scientific)

CONDITIONS

Accelerated Solvent Extractor

Solvent:	Hexane
System Pressure:	10 MPa (1500 psi)
Oven Temperature:	125 °C
Sample Size:	10 g
Oven Heatup Time:	6 min
Static Time:	5 min
Static Cycles:	2
Flush Volume:	60% of extraction cell volume
Nitrogen Purge:	1 MPa (150 psi) for 60 s
Total Extract Volume:	40 mL
Total Extraction Time:	20 min

Gas Chromatograph

Column:	RTX-5
Carrier Gas:	Helium (16 psi)
Detector:	Electron Capture Detector
Temperature:	300 °C
Program:	100 °C (hold for 2 min), 100–160 °C at 15 °C/min, followed by 160–270 °C at 5 °C/min

Injector

Temperature:	225 °C
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SAMPLE PREPARATION

The following procedure provides efficient extraction of lipids and PCBs from fish tissue. Fish have varying fat content depending on the species and other factors. Therefore, samples might require different drying times.

Cell Preparation

Insert a cellulose filter, using the filter insertion tool, into a 33-mL extraction cell.

Whole Fish and Fillet Preparation

Mix 10 g of fish tissue (whole fish or fillet) with 5 g of ASE Prep DE in a 250-mL beaker or equivalent. Transfer this mixture into a plastic weighing dish. Place the weighing dish in a microwave oven for 2.5 min. The time required may depend on the moisture content and lipid content of the particular species of fish. Typically, four samples can be dried at the same time.

Transfer the dried sample to the extraction cell. This transfer is completed using a stainless steel spatula and a screw-on stainless steel funnel.

ANALYSIS AND RESULTS

Gravimetric Lipid Determination

The extract is concentrated to 10 mL in a 40 mL graduated concentration vial using an N-EVAP evaporator. An aluminum weighing dish is tared on an analytical balance. One milliliter of the concentrated extract is placed into this aluminum weighing dish and then dried in an oven at 100 °C ± 5 °C for approximately 5 min. The dish is allowed to cool and then reweighed. The remainder of the concentrated extract may be used for PCB analysis (see below). A comparison of ASE and Soxhlet extraction of lipids is shown in Tables 1 and 2.

Table 1. Lipids in Low-Fat Fish Tissue: Comparison of Soxhlet and ASE Results

Method	Solvent	Avg. Lipids (%)	Std. Dev. (%)	RSD (%)
Soxhlet	Hexane/ Acetone (1:1)	2.20	NA	NA
ASE, n = 3	Hexane	2.40	0.01	0.4

Table 2. Lipids in High-Fat Fish Tissue: Comparison of Soxhlet and ASE Results

Method	Solvent	Avg. Lipids (%)	Std. Dev. (%)	RSD (%)
Soxhlet	Hexane/ Acetone (1:1)	15.00	NA	NA
ASE, n = 3	Hexane	16.01	0.58	3.62

PCB Determination

The extract is concentrated to 10 mL in a 40 mL graduated concentration vial using an N-EVAP evaporator. A 4-mL aliquot of this concentrate is placed into a 12-mL glass vial and shaken with 2 mL of concentrated sulfuric acid to destroy the lipids. This treated extract is then analyzed by gas chromatography using an electron capture detector. External standards are used to quantify the PCBs. A comparison of ASE and Soxhlet extraction results for PCBs is shown in Tables 3 and 4.

CONCLUSION

The method described in this application note demonstrates that a single ASE extraction for PCBs and lipid content can be achieved by first drying the sample and then extracting with hexane. Drying can be accomplished by a conventional oven in 1–2 h compared to 2.5 min by a microwave oven per every four samples.

SUPPLIERS

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Organomation Associates, Inc., 266 River Road West,
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**Table 3. PCBs in Low-Fat Fish Tissue:
Comparison of Soxhlet and ASE Results**

Method	Solvent	Avg. PCBs ($\mu\text{g/g}$)		RSD (%)
Soxhlet	Hexane/ Acetone (1:1)	1.7	NA	NA
ASE, n = 3	Hexane	1.8	0.01	0.56

**Table 4. PCBs in High-Fat Fish Tissue:
Comparison of Soxhlet and ASE Results**

Method	Solvent	Avg. PCBs ($\mu\text{g/g}$)	Std. Dev. ($\mu\text{g/L}$)	RSD (%)
Soxhlet	Hexane/ Acetone (1:1)	0.19	NA	NA
ASE, n = 3	Hexane	0.21	0.01	4.8

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N-EVAP is a registered trademark of Organomation Associates, Inc.
Arclor is a trademark of Monsanto Chemical Company.



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