DIONEX

Application Note 337

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 determined 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)

Gas Chromatograph

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)

**ASE 150 and 350 can be used for equivalent results.*

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:	1500 psi*
Oven Temperature:	25 °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

**Pressure studies show that 1500 psi is the optimum extraction pressure for all ASE applications.*

Gas Chromatograph

Column:	RTX-5
Carrier Gas:	Helium (16 psi)
Detector:	Electron Capture Detector
Temperature:	300 °C
Temperature 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

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	0.40

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.

Table 3. PCBs in Low-Fat Fish Tissue: Comparison of Soxhlet and ASE Results

Method	Solvent	Avg. PCBs (µg/g)	Std. Dev. (µg/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 (µg/g)	Std. Dev. (µg/g)	RSD (%)
Soxhlet	Hexane/ Acetone (1:1)	0.19	NA	NA
ASE, n = 3	Hexane	0.21	0.01	4.8

CONCLUSION

The method described here 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 using a conventional oven in 1–2 h compared to 2.5 min by a microwave oven per every four samples.

SUPPLIERS

Fisher Scientific, 2000 Park Lane, Pittsburgh, PA 15275-1126 USA, Tel: (800) 766-7000, www.fishersci.com.

Restek Corporation, 110 Benner Cir., Bellefonte, PA 16823 USA, Tel: (814) 353-1300, www.restekcorp.com.

Chem Service, Inc., P.O. Box 599, West Chester, PA 19381-0599 USA, Tel: 610-692-3026, www.chemservice.com.

Organomation Associates, Inc., 266 River Road West, Berlin, MA 01503 USA, Tel: (978) 838-7300, www.organomation.com.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the contributions of Jeff McAnally, Ron Lilenthal, Ron Jardine, and C.C. Chou from the City of San Diego Metropolitan Waste Department. The authors also thank Celestine Geyston of the State of Illinois Environmental Protection Agency for performing the Soxhlet extraction and analysis of the fish tissue samples cited.

Dionex Corporation
1228 Titan Way
P.O. Box 3603
Sunnyvale, CA
94088-3603
(408) 737-0700

North America
U.S./Canada (847) 295-7500

South America
Brazil (55) 11 3731 5140

Europe
Austria (43) 1 616 51 25 Benelux (31) 20 683 9768; (32) 3 353 4294
Denmark (45) 36 36 90 90 France (33) 1 39 30 01 10 Germany (49) 6126 991 0
Ireland (353) 1 644 0064 Italy (39) 02 51 62 1267 Sweden (46) 8 473 3380
Switzerland (41) 62 205 9966 United Kingdom (44) 1276 691722

Asia Pacific
Australia (61) 2 9420 5233 China (852) 2428 3282 India (91) 22 2764 2735
Japan (81) 6 6885 1213 Korea (82) 2 2653 2580 Singapore (65) 6289 1190
Taiwan (886) 2 8751 6654

www.dionex.com

ISO 9001 REGISTERED

LPN 1118-05 PDF 6/11