DIONEX

Application Note 342

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Determination of PCBs in Large-Volume Fish Tissue Samples Using Accelerated Solvent Extraction (ASE)

INTRODUCTION

Extraction and analysis of PCBs from fish and other marine tissues continues to be a necessary step in the monitoring of the aquatic food supply. Due to the large number of samples requiring analysis, automated extraction systems have proven useful in this area. ASE technology has been shown to produce good recoveries of naturally occurring PCBs from fish tissue samples,[1,2,3] and is approved for use in U.S. EPA SW-846 Method 3545 for the extraction of PCBs, OCPs, BNAs, OPPs, herbicides, and dioxins and furans.[4] ASE was designed to replace time-consuming and solvent-intensive methods such as Soxhlet and sonication in the environmental area. ASE operates at temperatures higher than those possible in traditional techniques, thus increasing the efficiency of the extraction process.

The continued need for lower analyte detection limits in monitoring the bioaccumulation of priority organic pollutants (POPs) has resulted in the use of large sample sizes. Many automated extraction systems are limited in their ability to extract large samples; however; the ASE 300 extraction system was designed for these larger sample size applications. With sample cell sizes of 34, 66, and 100 mL, it can extract 30-g samples of raw fish tissue under both selective and nonselective conditions. Under nonselective conditions, the extracts produced are similar in composition those from traditional methods and require the usual cleanup steps prior to GC analysis. Using selective ASE conditions, extracts can be produced that are free of coextracted lipid material. These sample extracts can be analyzed without laborious and time-consuming cleanup steps.

EQUIPMENT

Dionex ASE 300 Accelerated Solvent Extractor* equipped with 100-mL cells

Dionex bottles (250 mL) for collection of extracts (P/N 056785)

Cellulose filters (P/N 056780)

Gas Chromatograph equipped with electron capture detector (ECD)

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

REAGENTS AND STANDARDS

Methylene chloride (Optima Grade, Fisher Scientific)

ASE Prep DE (diatomaceous earth) (P/N 062819)

Alumina (basic, Brockman activity I, Fisher Scientific)

PCB standards (ULTRA Scientific)

SAMPLE PREPARATION

Thirty grams of raw fish tissue (cod fillet, purchased locally) was weighed out and spiked with 50 µL of a PCB congener standard solution (ULTRA Scientific) in hexane, containing 50–250 µg/mL individual PCB congeners. This resulted in a final sample concentration of 80–400 ng/g. The samples were mixed with 20 g of ASE Prep DE in a mortar and pestle and then loaded into 100-mL cells containing 10 g of alumina and a cellulose filter.

EXTRACTION CONDITIONS

Extraction Solvent:	Methylene chloride
Temperature:	125 °C
Pressure:	1500 psi*
Heatup Time:	5 min
Static Time:	3 min
Flush Volume:	60%
Purge Time:	120 s
Static Cycles:	3
Total Extraction Time:	18 min per sample
Total Solvent Use:	120–140 mL per sample

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

POST EXTRACTION

Sample extracts were dried by sodium sulfate treatment, concentrated to 10 mL under nitrogen, and analyzed by GC/ECD. Sodium sulfate treatment can be performed in traditional cartridges or funnels, or simply added to the extraction bottle and shaken.

QUANTIFICATION

Analysis was performed using a gas chromatograph equipped with an ECD. A fused silica 30 m × 0.32 mm column was used. The injector was maintained at 280 °C and the detector at 300 °C. Temperature programming was performed from 100 to 300 °C (5 min) at 15 °C/min after a 1 min hold. Recoveries were determined by external standard calibration (three levels) with five replicate samples and duplicate GC injections.

RESULTS AND DISCUSSION

The fish tissue used in this study was cod fillet obtained from a local source. The sample had a fat content of 0.25% and a moisture level of 81%. The samples were premixed with 20 g of pelleted diatomaceous earth (ASE Prep DE) prior to cell loading. Extraction results are shown in Table 1. Average recovery for the nine PCB congeners was 96.9% with an average %RSD of 6.1 (n = 5). Selective extraction was performed by loading 10 g of alumina into the cell outlet. The alumina removes the coextracted lipid material from the extract as it passes from the cell. (The ratio of sample volume to alumina may have to be changed depending on the fat content of the tissue sample.)

Generally, the alumina will retain approximately 75 mg of lipid per gram of material under the conditions described in this application note. Figure 1 shows comparative chromatograms. Chromatogram A shows a PCB standard analysis and chromatogram B shows a tissue extract produced using the conditions outlined. The material present in the nonselective (no alumina treatment) ASE extract (chromatogram C) would have to be removed by a cleanup procedure such as sulfuric acid treatment or gel permeation chromatography (GPC) after traditional extraction methods.

CONCLUSION

The data presented in this application note indicate that ASE provides good recovery and precision for the extraction of PCBs from 30-g fish tissue samples. Using the selective method described, tissue extracts can be produced that can be immediately dried and concentrated, eliminating the traditional cleanup steps normally required in this analysis. Using ASE, extraction times can be reduced and the sample preparation process automated to make more efficient use of laboratory resources.

REFERENCES

1. Dionex Corporation. *Selective Extraction of PCBs from Fish Tissue.* Application Note 327; Sunnyvale, CA.
2. Schantz, M.; Nichols, J.; and Wise, S. *Evaluation of Pressurized Fluid Extraction for the Extraction of Environmental Matrix Reference Materials*. Anal. Chem. **1997** 69 4210–4219.
3. Ezzell, J.; Richter B.; and Francis, E. *Selective Extraction of PCBs from Fish Tissue Using Accelerated Solvent Extraction*. Amer. Environ. Lab. December **1996**, 12–13.
4. Test Methods for Evaluating Solid Waste, Method 3545. U.S. EPA SW-846, Update III. Fed. Regist. Vol. 62, 114: 32451. U.S. GPO: Washington, DC, June 13, 1997.

SUPPLIERS

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

ULTRA Scientific, 250 Smith St., North Kingstown, RI 02852 USA, Tel: 800-338-1754, www.ultrasci.com.

Table 1. Recovery of Spiked PCB Congeners from 30-g Fish Tissue Samples Using Selective ASE Extraction Conditions

Congener	BZ #	Spike (µg)	% Recovery	% RSD
2-Chlorobiphenyl	1	2.5	99.8	3.0
2,3-Dichlorobiphenyl	5	2.5	103.8	8.8
2,4,5-Trichlorobiphenyl	29	2.5	107.1	3.1
2,2',4,6-Tetrachlorobiphenyl	50	5	98.4	2.4
2,2',3,4,5'-Pentachlorobiphenyl	87	5	92.3	7.9
2,2',4,4',5,6'-Hexachlorobiphenyl	154	5	89.0	5.9
2,2',3,4',5,6,6'-Heptachlorobiphenyl	186	7.5	91.1	8.5
2,2',3,3',4,5',6,6-Octachlorobiphenyl	200	7.5	96.0	6.5
Decachlorobiphenyl	209	12.5	94.2	8.7

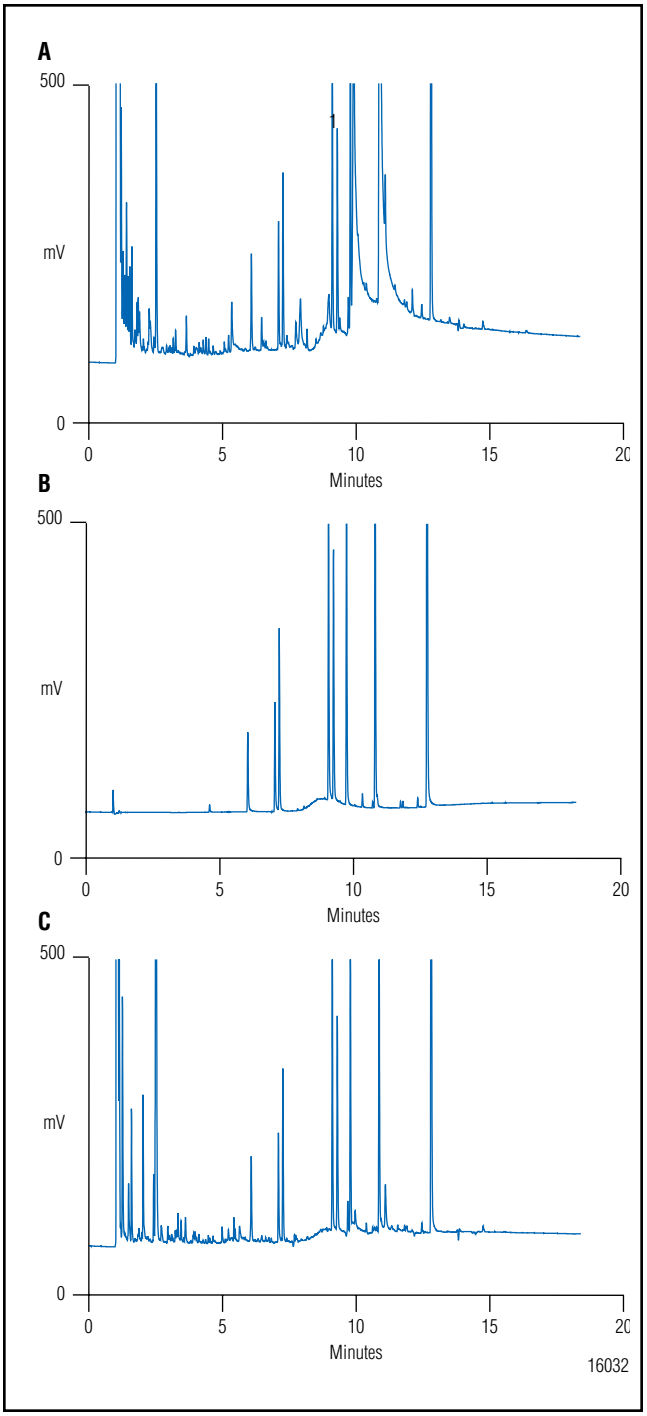

Figure 1. GC/ECD analysis of (A) PCB congener standards, (B) ASE fish tissue extract, and (C) ASE fish tissue extract produced nonselectively.