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Selective Extraction of PCBs from Fish Tissue Using Accelerated Solvent Extraction (ASE)

INTRODUCTION

Accelerated Solvent Extraction (ASE) is a new extraction method that significantly streamlines sample preparation. A solvent is delivered into an extraction cell containing the sample, which is then brought to an elevated temperature and pressure. Minutes later, the extract is transferred from the heated cell to a standard collection vial for cleanup or analysis. The entire extraction process is fully automated and performed in minutes for fast and easy extraction with low solvent consumption.

The analysis of extracts containing PCB contaminants from fish tissue and fish homogenates can be hindered by the presence of coextracted fatty materials that interfere with the chromatographic analysis. It is standard procedure to perform some form of cleanup to remove the coextracted lipids from such samples prior to analysis. These clean-up procedures include size-exclusion chromatography (SEC), column chromatography, and acid treatment. These procedures add time to sample preparation and increase the potential for analyte losses. As an alternative, selective extraction procedures have been developed using ASE.

The data presented in this application note demonstrate that selective extractions can be performed using ASE with the proper choice of solvent and sorbent in the extraction cell. Results are given for the recovery of PCBs from contaminated fish tissue showing that extracts can be obtained using ASE that do not require further cleanup prior to analysis by gas chromatography.

EQUIPMENT

ASE 200 Accelerated Solvent Extractor
equipped with 11, 22, or 33 mL cells
Analytical balance
Dionex vials for collection of extracts
(40 mL, P/N 49465; 60 mL, P/N 49466)
Cellulose filter disks (P/N 49458)
Gas chromatograph (GC) with electron capture
detector (ECD)

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

SOLVENTS

Hexane (pesticide-grade or equivalent)

EXTRACTION CONDITIONS

Extraction Solvent: Hexane
Temperature: 100 °C
Pressure: 1500 psi*
Heat Time: 5 min
Static Time: 5 min
Flush Volume: 60%
Purge Time: 90 s
Static Cycles: 2
Total extraction time: 17 min per sample

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

SAMPLE INFORMATION

The sample chosen for this study was obtained from the National Research Council of Canada (NRC-CNRC). Characterized as a ground whole Carp reference material for organochlorine compounds (CARP-1), the sample contains certified concentrations of 14 PCB congeners and 9 dioxin compounds. The moisture content is approximately 85%, and the lipid content approximately 4%.

SAMPLE PREPARATION

Sample preparation was performed by mixing 3 g of the homogenate with 15 g of ASE Prep DE (diatomaceous earth) (P/N 062819) in a mortar and pestle. Given the high water content of the sample and the nonpolar nature of the extraction fluid, complete drying of the sample is essential. A 33 mL extraction cell was loaded by inserting a disposable cellulose filter into the cell outlet, followed by 5 g of alumina (acid, Brockman activity I, 60-325 mesh). After the addition of the alumina, a second disposable cellulose filter was inserted. The sample/ASE Prep DE mixture was then added to the cell on top of the alumina. It is important that the orientation of the cell be maintained when it is loaded onto the extraction system.

PROCEDURE

After extraction, the extracts were then measured and analyzed by GC/ECD (U.S. EPA Method 8081). No cleanup was performed on the extracts from the selectivity experiments prior to GC analysis. This method is a dual-column GC method with electron capture detection (ECD). Extract analysis was performed by Mountain States Analytical Laboratory in Salt Lake City, Utah. Results are reported on a wet weight basis.

RESULTS AND DISCUSSION

Two different batches of homogenized tissue were extracted in triplicate and analyzed. Tables 1 and 2 show the data from these extractions. The certified values for the tissue are included for reference.

For comparison, additional samples from sample batch 2 were extracted nonselectively under the same ASE conditions, except that methylene chloride/acetone (1/1, v:v) was used as the extraction fluid. Extracts were passed over 2 g of sodium sulfate; the solvent was exchanged to hexane; and then mixed with an equal volume (10 mL) of sulfuric acid for fat removal. The extracts cleaned in this manner were analyzed, as were the selective extractions. The results of these extractions are given in Table 3.

Figure 1 compares chromatograms obtained from the nonselective hexane ASE extraction of the fish tissue with selective ASE extraction of a portion of the same sample. As can be seen, the use of alumina in the outlet of the extraction cell prevents lipids and other coextractable materials from coming out in the extract which would complicate the quantification of the analytes of interest due to chromatographic interferences.

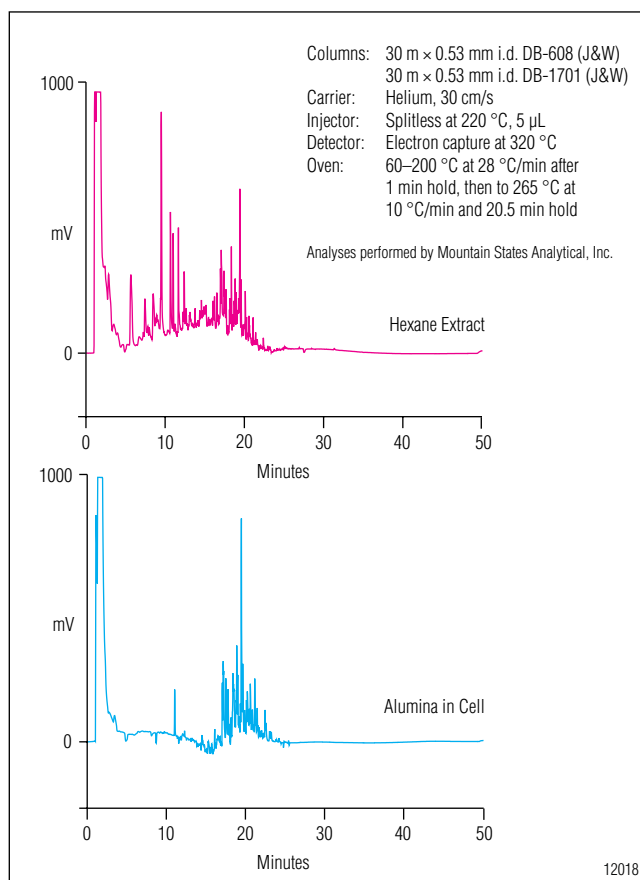


Figure 1. Chromatograms obtained from the nonselective ASE extraction of the fish tissue (top) and from the selective ASE extraction of a portion of the same sample (bottom).

As can be seen in Tables 1 and 2, the selective extraction using ASE gives acceptable results, and the need for additional cleanup such as sulfuric acid treatment or size exclusion chromatography is eliminated. Only one value obtained by ASE with selective extraction was below the 95% confidence interval and two values were above (Tables 1 and 2).

Table 1. Batch 1: Recovery of PCBs from Fish Tissue using Selective ASE
(Concentration Expressed as µg/kg)

Congener	Cert.* Value	Extract 1	Extract 2	Extract 3	Average	Standard Deviation	RSD (%)
52	124 ± 32	100	107	99	102	4.4	4.3
101/90	124 ± 37	101	103	100	101	1.5	1.5
105	54 ± 24	124	128	125	126**	2.1	1.7
118	132 ± 60	107	109	107	108	1.2	1.1
138/163/164	102 ± 23	48	48	48	48**	0.0	N/A
153	83 ± 39	48	48	48	48	0.0	N/A
170/190	22 ± 8	30	31	31	31	0.58	1.9
180	46 ± 14	65	62	64	64**	1.5	2.4
187/182	36 ± 16	30	30	30	30	0.0	N/A

* 95% confidence limits are given

** Values fall outside the 95% confidence limits

Table 2. Batch 2: Recovery of PCBs from Fish Tissue using Selective ASE
(Concentration Expressed as µg/kg)

Congener	Cert.* Value	Extract 1	Extract 2	Extract 3	Average	Standard Deviation	RSD (%)
52	124 ± 32	99	104	97	100	3.6	3.6
101/90	124 ± 37	93	100	93	95.3	4.0	4.2
105	54 ± 24	119	127	121	122**	4.2	3.4
118	132 ± 60	97	105	108	103	5.7	4.8
138/163/164	102 ± 23	41	44	40	42**	2.1	5.0
153	83 ± 39	41	44	40	42**	2.1	5.0
170/190	22 ± 8	28	31	28	29	1.7	3.4
180	46 ± 14	54	57	54	55	1.7	3.1
187/182	36 ± 16	35	38	35	36	1.7	4.7

* 95% confidence limits are given

** Values fall outside the 95% confidence limits

In contrast, when using the conventional cleanup procedure with sulfuric acid, three values were low, and one was high (Table 3). In addition, the precision was superior for the samples that were extracted using the selective extraction procedure (Tables 1 and 2).

The amount of sample that can be selectively extracted is 1–4 g due to the necessity for sample drying and the volume of alumina in the extraction cell. If larger samples are required, up to 10 g (depending on the moisture content) can be nonselectively extracted. These samples should be prepared as described (smaller amounts of ASE Prep DE may be used), and extracted according to the conditions listed using hexane or methylene chloride/acetone (1:1) as the extraction fluid. In these cases, the fat will be coextracted, and standard extract cleanup steps and solvent exchanges will have to be employed. If the tissue is freeze dried or air dried, larger sample sizes may be used. Dried samples may be extracted without any pretreatment; however, mixing the sample with ASE Prep DE or sand may allow better penetration of the sample matrix. For selective extraction of dried tissues, add 2 g of alumina for every gram of sample (samples with higher fat content may require more alumina).

Table 3. Batch 2: Recovery of PCBs from Fish Tissue using *Nonselective ASE*
(Concentration Expressed as µg/kg)

Congener	Cert.* Value	Extract 1	Extract 2	Extract 3	Average	Standard Deviation	RSD (%)
52	124 ± 32	99	101	100	100	1.0	1.0
101/90	124 ± 37	145	138	134	139	5.6	4.0
105	54 ± 24	114	119	118	117**	2.6	2.2
118	132 ± 60	69	94	92	85	14	17
138/163/164	102 ± 23	54	37	37	43**	9.8	23
153	83 ± 39	54	37	37	43**	9.8	23
170/190	22 ± 8	42	ND	ND	14**	24	171
180	46 ± 14	64	58	57	60	3.8	6.4
187/182	36 ± 16	ND	39	47	29	25.1	87

* 95% confidence limits are given

** Values fall outside the 95% confidence limits

CONCLUSION

The method outlined in this application note demonstrates that selective extractions can be performed using ASE with the proper choice of solvent and sorbent in the extraction cell. In this case, the technique was used on fish tissue extracts containing PCB contaminants in which the selective extraction using ASE gives acceptable results, and the need for additional cleanup, such as sulfuric acid treatment or size-exclusion chromatography, is eliminated. Using this method, it is possible to decrease both the time for sample preparation and the potential for analyte losses.

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