Application Note 322

Selective Extraction of PCBs from Fish Tissue Using Accelerated Solvent Extraction

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Introduction

Accelerated solvent extraction is a technique 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 polychlorinated biphenyl (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 cleanup 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 the accelerated solvent extraction technique.

The data presented here demonstrate that selective extractions can be performed using accelerated solvent extraction 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 do not require further cleanup prior to analysis by gas chromatography buffer, an ion-pairing agent, and acids such as ethylenediaminetetraacetic acid (EDTA)—all essential for good peak shape but detrimental to mass spectrometry—when using accelerated solvent extraction.

Equipment

- Thermo Scientific™ Dionex™ 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)

**Dionex ASE 150 and 350 systems an be used for improved or 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 optimumu extraction pressure for all accelerated solvent extraction applications.

Sample Information

The sample chosen for this study was obtained from the National Research Council of Canada. Characterized as 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%.

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Sample Preparation

Mix 3 g of the homogenate with 15 g of Dionex ASE Prep DE (diatomaceous earth) Dispersant (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. Load a 33 mL extraction cell 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, insert a second disposable cellulose filter. Add the sample/Dionex ASE Prep DE mixture 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, measure and analyze the extracts by GC/ECD per U.S. EPA Method 8081. No cleanup on the extracts from the selectivity experiments is necessary prior to GC analysis. This method is a dual-column GC method with 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 Discussions

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 accelerated solvent extraction technique 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 accelerated solvent extraction technique of the fish tissue with the selective accelerated solvent extraction technique 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.

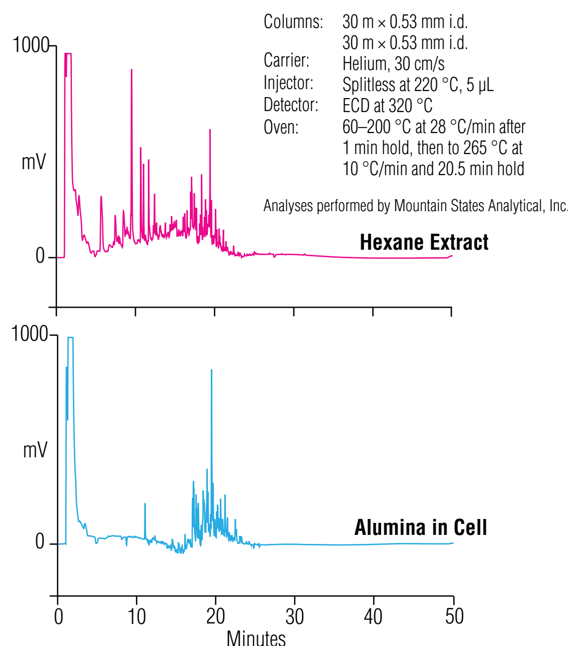

Figure 1. Chromatograms obtained from the nonselective accelerated solvent extraction technique of the fish tissue (top) and the selective accelerated solvent extraction technique of a portion of the same sample (bottom).

As can be seen in Tables 1 and 2, the selective extraction using the accelerated solvent extraction technique 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 the accelerated solvent extraction technique with selective extraction was below the 95% confidence interval and two values were above (Tables 1 and 2).

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).

Table 1. Batch 1: Recovery of PCBs from fish tissue using the selelctive accelerated solvent extraction technique (concentration expressed as µg/kg).

Congener	Certified 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	28	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 the selelctive accelerated solvent extraction technique (concentration expressed as µg/kg).

Congener	Certified 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

Table 3. Batch 2: Recovery of PCBs from fish tissue using the nonselelctive accelerated solvent extraction technique (concentration expressed as µg/kg).

Congener	Certified 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	92	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	N/D	14**	24	171
180	46 ± 14	64	57	57	60	3.8	6.4
187/182	36 ± 16	ND	47	47	29	25.1	87

* 95% confidence limits are given
** Values fall outside the 95% confidence limits

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 Dionex ASE Prep DE Dispersant 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 Dionex ASE Prep DE Dispersant 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).

Conclusion

The method outlined here demonstrates that selective extractions can be performed using accelerated solvent extraction 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, providing acceptable results and eliminating the need for additional cleanup, such as sulfuric acid treatment or size-exclusion chromatography. Using this method, it is possible to decrease both the time for sample preparation and the potential for analyte losses.

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