DIONEX

Application Note 359

Extraction of Contaminants, Pollutants, and Poisons from Animal Tissue Using Accelerated Solvent Extraction (ASE)

INTRODUCTION

Accelerated Solvent Extraction (ASE®) uses solvents at elevated temperatures and pressures to extract organic materials from solid and semisolid samples in a fraction of the time required by traditional extraction procedures. ASE is approved under EPA Method 3545A for extraction of organochlorine pesticides (OCPs), semivolatile compounds (BNAs), polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins and furans (PCDDs and PCDFs), and polynuclear aromatic hydrocarbons (PAHs) from samples such as soils and sediments.

Extensive documentation is available describing accelerated solvent extraction of environmental contaminants from soils and sediment samples.[1,2,3,4] ASE can also be used also to extract organic materials from matrices such as milk, foodstuffs, plant material, plasma, serum, and tissue. This Application Note details procedures for extracting the following contaminants from animal tissues:

- Dioxins/Furans
- Polybrominated Flame Retardants (PBDE)
- PCBs
- Pesticides
- PAHs
- Organotin

EQUIPMENT

Dionex ASE 200 Accelerated Solvent Extractor* with ASE Solvent Controller (P/N 059087)
11 mL stainless steel extraction cells (P/N 049560) or
22 mL stainless steel extraction cells (P/N 049561) or
33 mL stainless steel extraction cells (P/N 049562)
Cellulose filters (P/N 049458)
Collection vials 40 mL (P/N 048783) or
Collection vials 60 mL (P/N 048784)
Dionex SE 500 Solvent Evaporation system (P/N 063221)
Analytical balance (to read to nearest 0.0001 g or better)
Tissue homogenizer (Buchi B-400 or equivalent)
Freeze drier (for PCB extraction)
Centrifuge (for organotin extraction)
Mechanical shaker (for organotin extraction)

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

ANALYTES

Dioxins/Furans

Extraction of pesticides was performed using a modification of the method described by Raccanalli, et al.[1]

Chemicals and Reagents

Toluene (Pesticide-Free, Reagent Grade, Fisher Scientific)
Acidified silica (40% H_2SO_4) alumina and carbon
Native and $^{13}C_{12}$-labeled PCDD and PCDF standards (Cambridge Isotope Laboratories.)
ASE Prep DE (P/N 062819)

Samples

Fish tissue (Certified Reference Material (CRM) CARP-1, National Research Council, Halifax Nova Scotia, Canada)

Sample Preparation

Prepare the ampule of CRM fish tissue according to the manufacturer's directions. Weigh out the desired amount of tissue sample and mix with ASE Prep DE (1:1). Transfer the sample mixture to a 22 mL stainless steel extraction cell containing a cellulose filter. Spike the sample mixture with a series of 13 C_{12}-labeled 2,3,7,8 PCDD/F substituted isomers as internal standards.

ASE Conditions

Pressure: 1500 psi*
Temperature: 175 °C
Solvent: 100% Toluene
Static Time: 10 min
Static Cycles: 2
Flush: 60%
Purge: 60 sec

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

Extraction

Place the extraction cells containing the tissue samples into the ASE 200 carousel. Label 60 mL collection vials and place them on the vial carousel. Configure the ASE method as listed above, and press Start to begin extraction. Once the extraction is complete, remove the extracts and perform a solvent transfer by evaporating the toluene then dissolving with hexane.

Cleanup

Clean the extracts by using any automated offline cleanup system available from various vendors. Alternately, offline cleanup can be eliminated by using the proper in-cell cleanup procedure described in Dionex Technical Note 210. Analyze using high resolution gas chromatography/high resolution mass spectrometry.[1]

Results and Discussion

Table 1 shows comparative results between the ASE and Soxhlet methods for extraction of dioxins and furans from fish tissue. Total extraction time for ASE was 30 min per sample using approximately 50 mL of toluene, as compared to Soxhlet extraction, which required a total time of 36 h per batch and 300 mL of toluene per sample.

Table 1. PCDDs/PCDFs in Fish Tissue Samples (ng/kg or ppt) Using ASE[1]

Compound	Soxhlet	ASE	Certified
2,3,7,8-TCDD	7.6	7.6	6.6
1,2,3,4,8-PCDD	4.3	4.3	4.4
1,2,3,4,7,8-HCDD	1.4	1.4	1.9
2,3,4,7,8-TCDF	13.4	12.6	11.9
1,2,3,7,8-PCDF	5.4	5.1	5.0
1,2,3,4,7,8-HCDF	12.5	12.2	12.2
OCDD	12.4	6.4	6.3
Total TEQ	21.4	21.1	21.0

PBDEs

Extraction of PBDEs was performed using a modification of the method described by Oros, et al.[2]

Chemicals and Reagents

Acetone (HPLC grade, Fisher Scientific)

Dichloromethane (DCM) (HPLC grade, Fisher Scientific)

ASE Prep DE (P/N 062819)

Bio Beads®

Samples

Freshwater mussel, oysters, and clam samples were collected from estuary sites near San Francisco, California.

Sample Preparation

Rinse the bivalve samples with reagent grade water to remove any extraneous material. Shuck each sample into a homogenizing container and homogenize. Weigh 1–5 g of tissue homogenate and dry in a 70 °C oven for 48 hours to determine moisture content. Weigh 10 g of the dried tissue homogenate and mix with 7 g ASE Prep DE until the mixture is homogenous and free flowing. Transfer the sample mixture to a 33 mL stainless steel extraction cell containing a cellulose filter.

ASE Conditions

Pressure:	1500 psi
Temperature:	100 °C
Solvent:	Acetone:DCM (1:1)
Static Time:	5 min
Static Cycles:	1
Flush:	60%
Purge:	60 sec

Extraction

Place the extraction cells with samples onto the ASE 200 carousel. Label 60 mL vials and place them on the vial carousel. Configure the ASE method listed above and save as "Method 1." Create a Schedule to extract each cell twice using Method 1. (Using this method, the ASE will extract the same cell twice, but will deliver each extract to separate vials.) When both extractions are complete, combine the extracts from the two vials and evaporate to approximately 0.5 mL, then dilute to 10 mL with DCM. Remove a 2-mL aliquot for lipid determination if needed. Clean the remaining extract using gel permeation chromatography (70 g Bio-Beads in 100% DCM) and fractionate using Florisil® or using in-cell cleanup as described in Dionex Technical Note 210. Perform a solvent exchange for a final volume of sample in 2 mL isooctane. Analyze by gas chromatography.[2]

Results and Discussion

The data from ASE extraction of mussel, clam, and oyster tissue collected at various sites from the San Francisco area over a two year period shows a range of 9–106 ppb PBDE dry weight. For quality assurance, cleaned sample extracts and blanks were spiked with surrogate recovery standards prior to extraction with ASE. Certified Reference Materials (CRM) for PBDE were not available at the time of testing and so were not used. Replicates of the spiked matrix and field samples were analyzed and determined to be within the accepted RSD of <20%.

PCBs

Extraction of PCBs was performed using a modification of the method described by Gomez-Ariza, et al.[3]

Chemicals and Reagents

Dichloromethane (DCM) (HPLC grade, Fisher Scientific)

Pentane (HPLC grade, Fisher Scientific)

Florisil (U.S. Silica Company)

Ottawa sand (Fisher Scientific)

Samples

Eggs of the Common Spoonbill (Platalea leucorodia) and freshwater oysters (species unidentified) were collected from Marshes National Park, Huelva, Spain.

Assorted clams and fish were collected from multiple areas around the south coast of Spain. Assorted mussels (species unidentified) were purchased from a local market in southern Spain.

Sample Preparation

Freeze dry samples and grind to a particle size of 100 µm. (The authors chose to freeze dry the tissue samples prior to extraction and mix the samples with ASE Prep DE at a 1:1 ratio.) Prepare the 22 mL extraction cell by inserting two cellulose filters into the outlet followed by 6 g of Florisil.* Weigh 2 g of freeze-dried sample and mix with Florisil (1:2) using a mortar and pestle, until sample is homogenous and free-flowing. Load the sample mixture into the extraction cell on top of the Florisil (Figure 1). Fill the remainder of the cell with Ottawa sand.

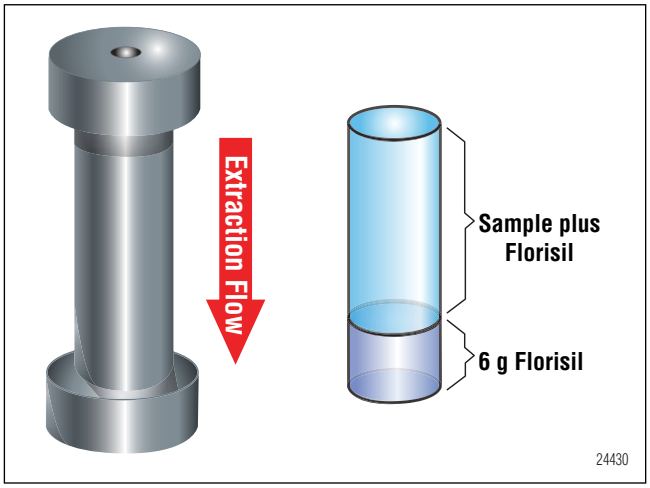

Figure 1. Schematic of in-cell cleanup.

*The addition of the Florisil to the extraction cell or in-cell cleanup as described in Dionex Technical Note 210 may eliminate the need for a post-extraction cleanup step.

ASE Conditions

Pressure:	2000 psi
Temperature:	40 °C
Solvent:	DCM: Pentane (15:85, v/v)
Static Time:	10 min
Static Cycles:	2
Flush:	150%
Purge:	60 sec

Extraction

Place the extraction cells containing the tissue samples onto the ASE 200 carousel. Label 60 mL vials and place them on the vial carousel. Enter the ASE method listed above and begin the extraction. Once complete, remove the extracts and concentrate to dryness using the SE 500. Dissolve the residue in 100 µL of hexane containing an internal standard. Analyze by gas chromatography with electron capture detection.[3]

Results and Discussion

Table 2 shows recovery data of ASE with in-cell cleanup compared to Soxhlet extraction of PCB from spoonbill eggs. The Soxhlet extraction required a post-extraction cleanup step using Florisil due to high concentrations of lipids present in the extracts. Total extraction time for ASE was 25 min using approximately 40 mL of solvent per sample. Extraction time using Soxhlet was 13 hours per batch with 150 mL of solvent used per sample.

Table 2. PCB Concentration Found in Spoonbill Eggs Using Using ASE and Soxhlet

PCB	Recovery ASE vs Soxhlet
1	96.47
11	106.67
29	98.91
47	97.78
87	98.72
99	100.36
101	97.44
105	97.48
118	97.67
121	92.47
128	102.78
136	110.59
138	106.15
153	97.12
156	106.02
170	113.36
180	97.44
183	97.02
185	87.36
194	92.02
206	106.59
209	103.53
Avg.	100.18

Pesticides

Extraction of pesticides was performed using a modification of the method described by Curren, et al.[4]

Chemicals and Reagents

Ethanol (HPLC grade, Fisher Scientific)

Methanol (HPLC grade, Fisher Scientific)

Amberlite XAD-7 HP resin (Supelco)

Atrazine standard (Chem Service Inc.)

Water (HPLC grade, Fisher Scientific)

Samples

Beef kidney (purchased at a local retail outlet in Salt Lake City, UT)

Sample Preparation

Homogenize kidney samples using a standard tissue homogenizer or blender. Freeze each sample homogenate until ready for analysis.

Prepare the XAD-7 resin prior to preparing the kidney samples for extraction using the following steps:

- Weigh an appropriate amount of dry XAD-7 resin into a clean beaker and cover with methanol for 15 min.
- Decant methanol and soak resin in water for 5 min.
- Rinse resin with water at least three times or until all methanol has been removed.

Allow the kidney sample to thaw slightly and weigh approximately 0.5 g sample and 1 g ASE Prep DE in a plastic weighing dish. Spike the sample with a 1 µL aliquot of atrazine (or other pesticide) standard prepared with methanol (2 µg/g). Allow 30 min for the standard to permeate the meat sample. Add an additional 1 g ASE Prep DE to the sample. Place the contents of the plastic weigh dish into a mortar and add 1–2 g prepared XAD-7 HP resin. Grind the entire mixture using a mortar and pestle until the sample is homogenous and free-flowing. Transfer the sample mixture to an 11 mL extraction cell containing a cellulose filter.

ASE Conditions

Pressure: 735 psi
Temperature: 100 °C
Solvent: Ethanol/Water (30:70, v/v)
Static Time: 5 min
Static Cycles: 3
Flush: 50%
Purge: 60 sec

Extraction

Place the extraction cells containing the kidney sample mixture onto the ASE 200 carousel. Label 40 mL collection vials and place them onto the vial carousel. Enter the ASE method listed above and begin the extraction. When complete, use Solid Phase Microextraction (SPME) to sample the extracts directly by placing a 1.5 mL aliquot in a sampling vial. SPME adsorption time is 30 minutes. Analyze the SPME fibers by gas chromatography.

Results and Discussion

Table 3 summarizes the recovery data for kidney samples spiked with different concentrations of atrazine. The data shows the described extraction method produced excellent recoveries of pesticide from animal tissue.

Table 3. Percent Recovery for Atrazine in Beef Kidney

	% Recovery[a] (%RSD)		
Atrazine in Beef Kidney (µg/g)	**Sample 1**	**Sample 2**	**Sample 3**
2	104 (14)	103 (8)	104 (7)
0.2	115 (19)	127 (3)	90 (3)

[a]n = 3

PAHs

Extraction of PAHs was performed using a modification of the method described by Yusa, et al[5]

Chemicals and Reagents

Dichloromethane (DCM) (HPLC grade, Fisher Scientific)

Acetone (HPLC grade, Fisher Scientific)

Sodium sulfate (Anhydrous, Fisher Scientific)

Samples

Mussels (SRM 2977, NIST)

Sample Preparation

Homogenize tissue. Store sample at -18° C until ready to analyze. Thaw tissue, weigh out approximately 4 g of sample and grind with ASE Prep DE (1:1) using a mortar and pestle until the sample is homogenous and free flowing. Add this sample mixture to a 22 mL cell containing a cellulose filter.

ASE Conditions

Pressure: 1500 psi

Temperature: 125 °C

Solvent: DCM: Acetone (50:50, v/v)

Static Time: 5 min

Static Cycles: 1

Flush: 60%

Purge: 60 sec

Extraction

Place the extraction cells containing the sample onto the ASE 200 carousel. Place labeled 60 mL vials onto the vial carousel and begin the extraction using the ASE parameter listed above. Once complete, add 1–2 g sodium sulfate to the extraction vials and shake. Evaporate the solvent to approximately 0.5 mL using the SE 400 or SE 500. Dilute to 5 mL with DCM. Clean the extracts using gel permeation chromatography (GPC).[5]* Analyze by HPLC-Fl to remove interferances from lipids.

*It may be possible to eliminate post-extraction GPC cleanup by using an adsorbent such as Florisil in the bottom of the extraction cell with sample added to the top.

Results and Discussion

Table 4 shows ASE data compared to MSE (methanol solvent extraction) for extraction of PAHs from mussel tissue. Total extraction time for ASE was 10 min per sample, with approximately 25 mL solvent used. ASE completed the extraction 24 times faster than the MSE method and used 1/12 the amount of solvent.

Table 4. Extraction of PAH from SRM 2977: Comparison of ASE and MSE

		ASE	MSE	ASE	MSE
	Certified Concentration[a,b]	Mean[a]	Mean[a]	% Recovery	% Recovery
Compound					
Anthracene	8 ± 4	7.6	7.5	95.0	93.75
Fluoranthene	35.1 ± 3.8	33.3	35	94.87	99.72
Pyrene	78.9 ± 3.5	76.4	72	96.83	91.25
Benzo (a) anthracene	20.34 ± 0.78	19.5	20	95.87	98.33
Chrysene	49 ± 2	47	46	95.92	93.88
Benzo (e) pyrene	13.1 ± 1.1	13	11	99.24	83.97
Benzo (b) fluoranthene	11.01 ± 0.28	10.8	10	98.09	90.83
Benzo (k) fluoranthene	4 ± 1	3.9	3.8	97.50	95.0
Benzo (g,h,i) perylene	9.53 ± 0.43	9.1	8	95.49	83.95
Indeno (1,2,3-e,d) pyrene	4.84 ± 0.81	4.6	4.4	95.04	90.91

(n = 5). [a]Concentrations in µg/Kg dry weight:

[b]The dispersion is expressed as expanded uncertainty.

Organotin

Chemicals and Reagents

Extraction of organotin was performed using a modification of the method described by Wasik, et al.[6]

Acetic acid (HPLC grade, Fisher Scientific)
Sodium acetate (Analytical grade, Sigma-Aldrich)
Tropolone (Analytical grade, Sigma-Aldrich)
Methanol (HPLC grade, Fisher Scientific)
Hexane (HPLC grade, Fisher Scientific)
Sodium Sulfate (Analytical grade, Fisher Scientific)
Aluminum Oxide (3% water, Sigma-Aldrich)
Tetrabutyltin (TetraBT) (Internal Standard, Sigma-Aldrich)
Sodium tetraethylborate ($NaBEt_4$, Derivatization agent Sigma-Aldrich)

Prepare the stock solution of the internal standards (TetraBT) by dissolving in methanol to the desired concentration, and storing in the dark at 4 ºC. Prepare an acetic acid/sodium acetate buffer solution to pH 5.

Samples

Certified Reference Material NIES No. 11 (freeze-dried fish tissue sample, certified for total tin and TBT (tributyltin chloride)

Sample Preparation

Weigh approximately 1 g of sample into a beaker and mix with 9 g Ottawa sand. Add the sample mixture to an 11 mL cell containing a cellulose filter.

ASE Conditions

Pressure:	800 psi
Temperature:	125 °C
Solvent:	1 mol acetic acid + 1 mol sodium acetate + 0.3 g tropolone per 1 mL methanol/ water mixture (90% v/v methanol)
Static Time:	3 min
Static Cycles:	4
Flush:	20%
Purge:	100 sec

Extraction

Place the extraction cells containing the sample mixture onto the ASE 200 carousel, and place labeled 40 mL vials onto the vial carousel. Enter the ASE method listed above and begin the extraction. Once extraction is complete, transfer approximately 10 mL of the extract into a glass, round-bottomed centrifuge vial using a volumetric pipette. Add 10 mL of acetic acid/sodium acetate buffer solution (pH=5), 2 mL hexane, and 4 µL TetraBT standard solution (internal standard). Secure the centrifuge cap tube and shake the sample mechanically for 10 min at 520 cpm. Next, centrifuge the sample at 4400 rpm for 3 min. Using a pipette, decant approximately 1.5 mL of the top hexane layer and clean by passing through a column made from a Pasteur pipette closed with a piece of silanized glass wool and packed with 1 g Al_2O_3 (3% water) and a 1 mL layer of anhydrous Na_2SO_4 on the top. Elute the organotin compounds with 10 mL hexane and evaporate to 1 mL using the SE 500. Decant a 2 µL aliquot for analysis by gas chromatography.

RESULTS AND DISCUSSION

The ASE recoveries show the sum of TBT and DBT to be very close to the TBT certified value (96%) whereas the sum of MPhT, DphT, and TPhT is only 76% of the TPhT reference value. This may indicate that a significant amount of TPhT was degraded to inorganic tin and was thereby not detectable using this method.

CONCLUSIONS

The recovery data presented in this Application Note show ASE to be an excellent alternative to traditional methods for extraction of various environmental contaminants from animal tissues. ASE gives comparable or better results while providing a faster, easier way to prepare tissue samples for analysis.

LIST OF MANUFACTURERS

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

Sigma-Aldrich Chemical Company, 3050 Spruce St., St. Louis, MO 63103 USA, Tel: (800) 325-3010, Supelco Inc, Supelco Park Bellefonte, PA 16823 USA www.sigmaaldrich.com

National Institute of Standards & Technology (NIST), 100 Bureau Drive, Gaithersburg, MD 20899 USA, (301) 975-2000, www.nist.gov.

Fluid Management Systems, Inc. 56-58 Felton Street, Waltham, MA 02453 (781) 891-6522 www.fms-inc.com

Cambridge Isotope Laboratories 50 Frontage Road Andover, MA 01810-5413 (978) 749-8000 www.isotope.com

Chem Service, Inc PO Box 599 West Chester, PA 19381-0599, Phone: (610) 692-3026 www.chemservice.com

REFERENCES

1. Dionex Corporation *Methods Optimization in Accelerated Solvent Extraction.* Technical Note 208 (LPN 0982-02, Sept. **2004**), Sunnyvale CA
2. Dionex Corporation *Accelerated Solvent Extraction of Hydrocarbon Contaminanats (BTEX, Diesel, and TPH) in Soils.* Application Note 324 (LPN 0731-03, Sept. **2004**), Sunnyvale CA
3. Dionex Corporation *Extraction of Total Petroleum Hydrocarbon Contaminants (Diesel and Waste Oil) in Soils by Accelerated Solvent Extraction (ASE).* Application Note 338 (LPN 1167-02, Sept. **2004**), Sunnyvale CA
4. Dionex Corporation *Extraction of Total Petroleum Hydrocarbon Contaminants (Diesel and Waste Oil) in Soils by Accelerated Solvent Extraction (ASE).* Application Note 338 (LPN 1167-02, Sept. **2004**), Sunnyvale CA
5. Raccanelli, S., Bonamin, V., Favotto, M., Di Marco, V., Tirler, W. Comparative PCDD/Fs Analysis in Different Matrices (Fish, Harbour Sediment, Industrial Sludge, MSWI Ash) Extracted by Pressurized Fluid Extraction and by Traditional Soxhlet. Organohalogen Compounds. **1999**, 40: 239-242.
6. Oros, D., Hoover, D., Rodigari, F., Crane, D., Sericano, J. Levels and Distribution of Polybrominated Diphenyl Ethers in Water, Surface Sediments, and Bivalves from the San Francisco Estuary. *Env. Sci. Technol*. **2005**, 39: 33-41.
7. Gomez-Ariza, J.L., Bujalance, M., Giraldez, I., Velasco, A., Morales, E. Determination of Polychlorinated Biphenyls in Biota Samples Using Simultaneous Pressurized Liquid Extraction and Purification. *Journal of Chromatography A* **2002**, 946: 209-219.
8. Curren, M., King, J. Ethanol-Modified Subcritical Water Extraction Combined with Solid-Phase Microextraction for Determination Atrazine in Beef Kidney. J. *Agric. Food Chem*. **2001**, 49: 2175-2180.
5. Yusa, V., Pardo O., Marti, P., Pastor, A. Application of Accelerated Solvent Extraction Followed by Gel Permeation Chromatography and High-Performance Liquid Chromatography for the Determination of Polycyclic Aromatic Hydrocarbons in Mussel Tissue. *Food Additives and Contaminants* **2005**, 22(5): 482-489.
6. Wasik, A., Ciesielski, T. Determination of Organotin Compounds in Biological Samples Using Accelerated Solvent Extraction, Sodium Tetraethylborate Ethylation, and Multicapillary Gas Chromatography-Flame Photometric Detection. *Anal. BioChem.* **2004**, 378: 1357-1363.