# Accelerated Solvent Extraction Applications Summary

Fat Determination • Food Safety

Thermo SCIENTIFIC

# Contents

# The Accelerated Solvent Extraction (ASE) System

## Better extractions in less time with less solvent

Accelerated Solvent Extraction (ASE) eliminates many of the manual steps involved in preparing food samples for analysis, which helps ensure increased reproducibility and accelerates the process significantly. ASE is an automated extraction technique that uses elevated temperatures and pressures to achieve extractions in very short periods of time (a 10-g sample can be extracted in less than 15 minutes using less than 15 mL solvent).

Many of the organic solvents used in extractions boil at relatively low temperatures. This is a limitation to techniques such as Soxhlet or automated Soxhlet the highest temperatures at which extractions take place in these techniques will be the boiling point of the solvent.

If sufficient pressure is exerted on the solvent during the extractions, temperatures above the boiling point can be used. This means that all of the advantages of working at elevated temperature can be realized even with solvents with relatively low boiling points. Operating at elevated pressures also helps the extraction process to happen more quickly. Pumping solvent through a packed bed is easier at elevated pressures; pressurized solvent is forced into the pores of the sample matrix. Hence, the combination of elevated temperatures and pressures allows extractions to occur rapidly and completely.

When extractions are achieved at elevated temperatures, several factors contribute to improved speed, efficiency and reduced solvent use: 1) Solvent strength is higher 2) Diffusion rates are faster 3) Solvent viscosity is decreased 4) Solute-matrix interactions (dipole attractions, van der Waals forces, hydrogen bonding, etc.) are more easily disrupted allowing the analytes to be removed from the matrix. The net result means performing extractions at elevated temperatures uses less time and with less solvent.

In the food industry, time can be critical. Quality control checks need to be completed quickly and accurately. Whether a laboratory is analyzing the fat content of food for labeling or analyzing food for environmental contaminants, ASE is a powerful tool for preparing these samples in the fastest time possible while ensuring accurate results.

The Application Briefs compiled here show conditions for extracting fats and environmental contaminants from a variety of food and beverage samples. To view the complete Application Notes, visit our website at www.thermoscientific.com/dionex.

Thermo Scientific Dionex ASE 150 and ASE 350 Accelerated Solvent Extraction instruments.

# Determination of Unbound Fat in Various Food Matrices Using Accelerated Solvent Extraction (ASE)

## Introduction

Accelerated Solvent Extraction (ASE) is an extraction method that significantly streamlines sample preparation. A commonly used solvent is pumped 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 withlow solvent consumption. Up to 24 samples can be loaded and extracted sequentially without requiring operator intervention. Recently, the requirements for accurate labeling of fats in foods were revised by the U.S. Food and Drug Administration (U.S. FDA) and the U.S. Department of Agriculture (U.S. DA). This occurred as a result of the Nutrition Labeling and Education Act, which requires the labeling of total saturated and unsaturated fats contained in foods. Though these laws do not directly affect food sold outside of the U.S., there seems to be increased awareness worldwide of fat content in foods. In addition, food manufacturers require a method for the consistent determination of fat content for quality-control purposes.

## Accelerated solvent extraction of unsaturated fats in popular snack foods

### Equipment

Dionex ASE 200 Accelerated Solvent Extractor* equipped with either 11 or 22 mL stainless steel extraction cells

Analytical balance

Collection vials, 40 mL (P/N 048783) and 60 mL (P/N 048784)

Cellulose filters (P/N 049458)

### Solvents

Petroleum ether
Chloroform
Hexane
Isopropanol
Ethanol

All solvents are pesticide-grade or equivalent and are available from Fisher Scientific.

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

### Analysis

Gravemetric

### Extraction Conditions

| | |
|---|---|
| Oven Temperature: | 125 °C |
| Pressure: | 1500 psi |
| Oven Heatup Time: | 6 min |
| Static Time: | 5–25 min |
| Flush Volume: | 60% |
| Purge Time: | 60 s |
| Solvent: | Petroleum ether, chloroform, hexane, or hexane/isopropanol (3:2), chloroform/ethanol (1:1), depending on application |
| Static Cycles: | 1 to 3 |

### Results

Extraction of fat from snack crackers: comparison of results by soxhlet and ASE.

| Sample | Method | Avg % Fat (wt.%) | Std. Dev. | RSD (%) |
|---|---|---|---|---|
| Cracker 1 | Soxhlet* | 15.4 | N/A | N/A |
| Cracker 1 | ASE**, n = 3 | 14.6 | 0.09 | 0.65 |
| Cracker 2 | Soxhlet* | 28–30 | N/A | N/A |
| Cracker 2 | ASE**, n = 3 | 28.1 | 0.20 | 0.70 |

* After acid hydrolysis

** Conditions: 5 g sample, 125 °C, 1500 psi, 6 min heatup, 25 min static, 60% flush, 60 s purge, 1 static cycle, hexane/isopropanol (3:2).

# Determination of Total Fat in Powdered Infant Formula Using Accelerated Solvent Extraction (ASE)

## Introduction

Accurate determination of fat in certain foods is difficult due to the binding or entrapment of the fat by the matrix. Most methods used to determine fat in these difficult matrices include a pretreatment step to denature or destroy the physical structure of the matrix and allow greater accessibility to the fat.

Common fat determination methods used by the dairy industry are the Roese-Gottlieb and Modified Mojonnier methods (AOAC Intl. Methods 905.02 and 989.05, respectively). These methods specify the use of ammonium hydroxide to dissolve casein and liberate the fat. Here, Accelerated Solvent Extraction is compared to the Mojonnier method for extraction of fat from infant formula.

## Saturated and unsaturated fats

### Equipment

Dionex ASE 200 Accelerated Solvent Extractor* equipped with 11-mL stainless steel extraction cells

Cellulose filters (P/N 049458)

Analytical balance (0.001 g or better)

Mortar and pestle (Fisher Scientific or equivalent)

Solvent evaporator

Forced air oven

Ottawa Sand Standard (P/N S23-3)

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

### Reagent

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

### Solvents

Acetone

Hexane

Water

All solvents are pesticide-grade or equivalent and available from Fisher Scientific.

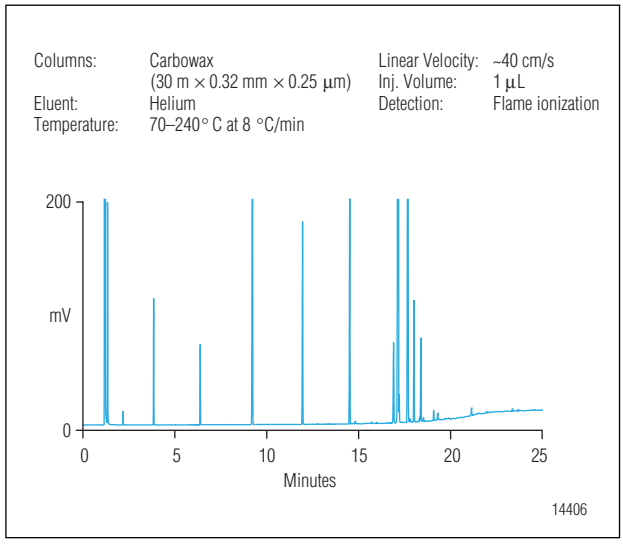
### Analysis

GC-FID

### Extraction Conditions

| | |
|---|---|
| Extraction Solvent: | Hexane acetone, 4:1 volume |
| Temperature: | 125 °C |
| Pressure: | 1500 psi |
| Heat Time: | 6 min |
| Static Time: | 5 min |
| Flush Volume: | 100% |
| Purge Time: | 60 s |
| Static Cycles: | 3 |
| Total Extraction Time: | 24 min per sample |

### Results




Chromatogram of FAMEs from ASE extraction of infant formula.

# Rapid Determination of Fat in Meat Using Accelerated Solvent Extraction (ASE)

## Introduction

Soxhlet extraction is an accepted technique for extracting fat from meat samples. Though it is simple and robust, there are drawbacks to Soxhlet extraction, such as long drying and extraction times, lack of automation, and the amount of solvent used per sample. ASE is a technique that was developed to replace Soxhlet and other extraction techniques for many samples. The automation and rapid extraction time of ASE overcome the shortcomings of Soxhlet extraction.

## Fresh and processed meats

### Equipment

ASE 200 Accelerated Solvent Extractor,* with 11 or 22 mL stainless steel extraction cells

Cellulose Filters (P/N 049458)

Collection Vials, 40 mL (P/N 048783)

Ottawa Sand Standard (Fisher Scientific)

Analytical balance (to read to nearest 0.0001 g or better)

Mortar and pestle (Fisher Scientific or equivalent)

Solvent evaporator

Forced air oven

Microwave oven (800 W) with carousel

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

### Reagent

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

### Solvents

Petroleum ether or Hexane. All solvents are pesticide-grade or equivalent and available from Fisher Scientific.

### Analysis

Gravemetric

### Extraction Conditions

| | |
|---|---|
| Solvent: | Petroleum ether or hexane* extraction solvent |
| Temperature: | 125 °C |
| Pressure: | 1500 psi |
| Heatup Time: | 6 min |
| Static Time: | 1 or 2 min** |
| Flush Volume: | 60% |
| Purge Time: | 60 s |
| Cycles: | 2 |
| Total Time: | 12 min |
| Total Solvent: | 20 mL |

**Petroleum ether and hexane were found to be equivalent as extraction solvents for fat in meat.*

***When extracting more than 1 g of a high-fat sample, a 2 min static time may be beneficial.*

### Results

Percent fat in low-fat processed meat samples (ASE vs soxhlet).

| Sample | ASE Run #1 | ASE Run #2 | ASE Run #3 | ASE Average | Standard Deviation | Soxhlet |
|---|---|---|---|---|---|---|
| Beef | 2.82 | 2.90 | 2.83 | 2.85 | 0.046 | 2.81 |
| Chicken | 0.84 | 0.84 | 0.79 | 0.82 | 0.025 | 0.75 |
| Ham | 1.85 | 1.74 | 1.87 | 1.82 | 0.069 | 1.72 |
| Franks | 1.90 | 1.97 | 1.97 | 1.94 | 0.041 | 1.54 |
| Turkey | 1.04 | 1.00 | 1.04 | 1.02 | 0.026 | 0.94 |

# Determination of Fat in Dried Milk Products Using Accelerated Solvent Extraction (ASE)

## Introduction

Many extraction techniques for the determination of fat in food are labor-intensive or require long extraction times. The Roese-Gottlieb (RG) method requires alkaline pretreatment of the sample before a labor intensive liquid-liquid extraction. The Schmidt-Bondzynski-Ratzlaff (SBR) method calls for acid digestion before liquid-liquid extraction of the sample. Some Soxhlet methods require extraction times from 4 to 24 hours in duration.

## Low- and high-fat samples

### Equipment

Dionex ASE 200 Accelerated Solvent Extractor* equipped with 11-mL stainless steel extraction cells

Cellulose filters (P/N 049458)

Analytical balance (0.0001 g or better)

Solvent evaporator

Forced air oven

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

### Solvents

Hexane

Dichloromethane

Methanol

Petroleum ether (40-60 °C boiling range)

All solvents are pesticide-grade or equivalent and available from Fisher Scientific.

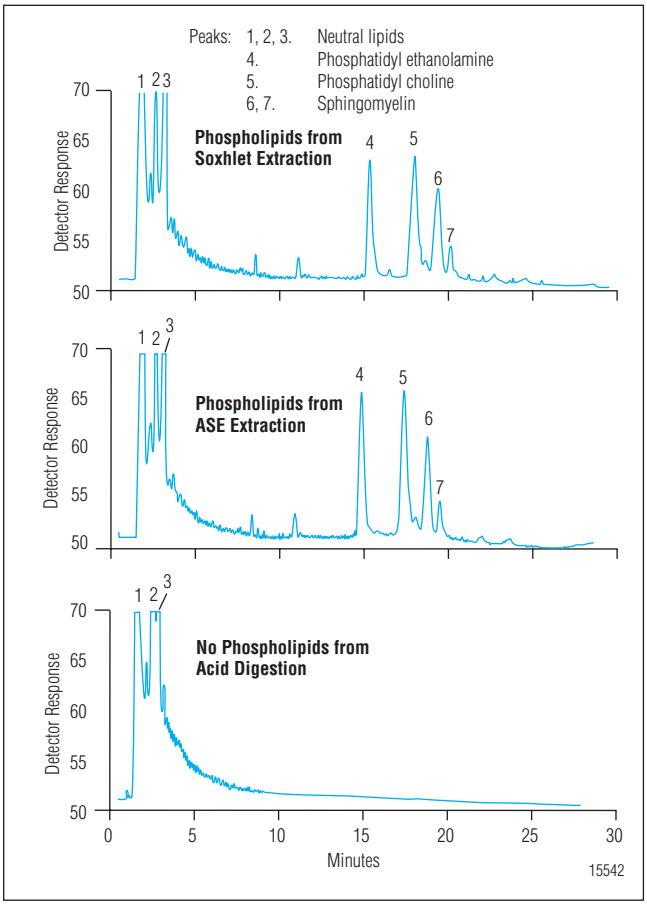
### Analysis

HPLC

### Extraction Conditions

| | |
|---|---|
| Temperature: | 80 °C |
| Pressure: | 1500 psi |
| Heatup Time: | 5 min |
| Static Time: | 1 min |
| Flush Volume: | 100% |
| Purge Time: | 40 s |
| Static Cycles: | 3 |
| Total Time: | 11 min |
| Total Solvent: | <30 mL |
| Solvent: | Mixtures of hexane, dichloromethane, and methanol in various volume ratios |

### Results




HPLC with ELSD traces of lipids extracted from whey protein concentrate.

# Extraction of Fat from Chocolate Using Accelerated Solvent Extraction (ASE)

## Introduction

Accelerated Solvent Extraction (ASE) is a new way to speed up gravimetric fat determination of chocolate products and greatly reduce the amount of solvent used. The ASE system uses a combination of elevated temperature and pressure to increase the extraction kinetics, thus decreasing time and solvent consumption. Current methods for determining the fat content in chocolate are labor-intensive and require large amounts of solvent and time. For example, the Mojonnier ether extraction method takes 2–3 h and over 110 mL of solvent and requires the laboratory technician to be present for most of the extraction. Using ASE, extraction time is reduced to 18 min and solvent use to 20 mL. ASE has been shown to produce comparable if not better results than the current methods. Furthermore, the ASE process is fully automated, making it possible to extract up to 24 samples unattended.

## Fats in solid and powdered chocolate samples

### Equipment

Dionex ASE 200 Accelerated Solvent Extractor* with 11-mL stainless steel extraction cells

Cellulose Filters (P/N 049458)

Collection Vials, 40 mL (P/N 048783)

Analytical balance (to read to the nearest 0.0001 g or better)

Mortar and pestle (Fisher Scientific or equivalent)

Solvent evaporator

Forced air oven

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

### Reagent and Solvent

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

Petroleum ether (pesticide grade or equivalent; Fisher Scientific)

### Analysis

Gravemetric

### Extraction Conditions

| | |
|---|---|
| Solvent: | Petroleum ether 100% |
| Temperature: | 125 °C |
| Pressure: | 1500 psi |
| Heatup Time: | 6 min |
| Static Time: | 3 min |
| Flush Volume: | 60% |
| Purge Time: | 60 s |
| Cycles: | 3 |
| Total Time: | 18 min |
| Total Solvent: | 20 mL |

### Results

Baking chocolate (top) and milk chocolate (bottom) % fat* recovery: ASE vs mojonnier method (n = 3).

| | ASE | Mojonnier |
|---|---|---|
| Average | 52.80 | 51.69 |
| SD | 0.35 | 0.26 |
| RSD | 0.67 | 0.50 |

*%fat = (residue/sample wt.) × 100

| | ASE | Mojonnier |
|---|---|---|
| Average | 31.80 | 32.34 |
| SD | 0.32 | 0.33 |
| RSD | 1.02 | 1.02 |

*%fat = (residue/sample wt.) × 100

# Extraction of Fat from Dairy Products (Cheese, Butter, and Liquid Milks) Using Accelerated Solvent Extraction (ASE)

## Introduction

The current methods for determining fat in dairy products, though acceptable, have several drawbacks. Many dairy-based products require a pretreatment prior to extraction. This denatures the casein, allowing greater exposure of the fat to the solvents. For example, a 1-g sample of cheese must be pretreated with ammonium hydroxide followed by hydrochloric acid and boiled for several minutes. The standard fat extraction methods, including the pretreatment, are very time consuming. Large amounts of solvents are required to remove the fat from each sample matrix, which can be costly. For example, manual extraction of pretreated cheese usually requires 2–3 h and more than 110 mL of solvent per sample. Therefore, the standard fat extraction methods are not time- or costefficient and can expose laboratory technicians to potentially dangerous solvents.

## Extraction of dairy sample matrices made simple!

### Equipment

ASE 200 Accelerated Solvent Extractor* equipped with 11- and 33-mL stainless steel extraction cells

Cellulose Filters (P/N 049458)

Collection Vials, 40 mL (P/N 048783) and 60 mL (P/N 048784)

Analytical balance (to read to nearest 0.0001 g or better)

Mortar and pestle (Fisher Scientific or equivalent)

Solvent evaporator

Heated block

Forced air oven

Ottawa Sand Standard (P/N S23-3)

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

### Reagent

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

### Solvents

Petroleum ether

Acetone I

Isopropanol

Hexane

All solvents are pesticide-grade or equivalent and are available from Fisher Scientific.

### Analysis

Gravemetric

### Extraction Conditions

| *Cheese* | | *Butter* | |
|---|---|---|---|
| Solvent: | Hexane: isopropanol (3:2) | Solvent: | Petroleum ether: acetone (3:2) |
| Temperature: | 110 °C | Temperature: | 100 °C |
| Pressure: | 1500 psi | Pressure: | 1500 psi |
| Cell Heatup Time: | 6 min | Heatup Time: | 5 min |
| Static Time: | 2 min | Static Time: | 2 min |
| Flush Volume: | 100% | Flush Volume: | 60% |
| Purge Time: | 60 s | Purge Time: | 60 s |
| Cycles: | 3 | Cycles: | 1 |
| Total Time: | 10 min | Total Time: | 8 min |
| Total Solvent: | <30 mL | Total Solvent: | <30 mL |

*Milk and Cream*

| | |
|---|---|
| Sample: | Cream (40%) |
| Solvent: | Petroleum ether:acetone:isopropanol (3:2:1) |
| Sample: | Whole milk (4–6%) |
| Solvent: | Petroleum ether:isopropanol (2:1) |
| Sample: | Homogenized/UHT milk (3%) |
| Solvent: | Petroleum ether:isopropanol (3:2) |
| Sample: | Skim milk (0.1%) |
| Solvent: | Petroleum ether:isopropanol (3:2) |
| Temperature: | 120 °C |
| Pressure: | 1500 psi |
| Cell Heatup Time: | 6 min |
| Static Time: | 1 min |
| Flush Volume: | 100% |
| Purge Time: | 60 s |
| Cycles: | 3 |
| Total Time: | 10 min |
| Total Solvent: | <30 mL |

### Results

Milk and cream, % fat recovery: ASE vs. RG method.

| Sample | ASE Mean ± SD (n) | RG Method |
|---|---|---|
| Cream | 40.62 ± 0.06 (3) | 40.58 |
| Whole Milk | 4.42 ± 0.02 (4) | 4.50 |
| Homogenized Milk | 3.39 ± 0.03 (6) | 3.39 |
| Skim Milk | 0.053 ± 0.010 (7) | 0.053 |

# Extraction of Total Fat from Food Samples After Acid Hydrolysis Using Accelerated Solvent Extraction (ASE) with GC-MS Analysis

## Introduction

Sample preparation—specifically, solvent extraction—is an important step in the analytical process. For many years, analysts have used an array of solvent extraction techniques including Soxhlet, shaking, sonication, and blending.

ASE technology provides a flow-thru solvent extraction system that increases productivity while decreasing cost and providing a platform for automation. Complex matrices such as food typically require acid hydrolysis or pretreatment prior to solvent extraction.

Pretreatment or hydrolysis of these matrices is often necessary to facilitate complete extraction of lipids from the sample. Time-consuming and labor-intensive liquid extraction techniques such as Soxhlet, automated Soxhlet, and Mojonnier extraction are typically used to extract fatty acids after acid hydrolysis.

## Turnkey extraction and analysis of snack and processed foods

### Equipment

Dionex ASE 150 or 350 with pH-hardened pathway (P/N 066401 or 066230)

Dionium extraction cells (100 mL) (P/N 068103)

Glass fiber filters (P/N 056781)

Collection bottles (250 mL) (P/N 056284)

Collection vials (40 mL) (P/N 048783)

GC-MS

Carbowax capillary GC column

Pressure tubes (ACE Glass Inc.)

### Solvents and Reagents

Chloroform

Pyrogallol

Alcohol; reagent-grade

Hexane

Ethyl ethern

ASE Prep DE P/N 062819)

ASE Prep CR P/N 080024)

8.3 M HCL

Toluene

12% BF3 in MeOH

$Na_2SO_4$

All solvents are pesticide-grade or equivalent and are available from Fisher Scientific.

### Analysis

GC-MS

### Extraction Conditions

| | |
|---|---|
| Pressure: | 1500 psi* |
| Temperature: | 100 °C |
| Solvent: | Hexane |
| Static Time: | 5 minutes |
| Static Cycles: | 3 |
| Flush Volume: | 70% |
| Purge Time: | 120 sec |

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

### Results

Extraction results using Mojonnier techniques and ASE (n = 3).

| Mayonnaise | Average | RSD | %RSD |
|---|---|---|---|
| Mojonnier | 75.1 | 0.89 | 1.18 |
| ASE | 74.2 | 0.43 | 0.575 |
| **Corn Chips** | | | |
| Mojonnier | 30.41 | 0.37 | 1.21 |
| ASE | 29.85 | 0.33 | 1.10 |
| **Parmesan Cheese** | | | |
| Mojonnier | 26.41 | 0.284 | 1.08 |
| ASE | 26.27 | 0.220 | 0.839 |
| **Baked Shortbread** | | | |
| Mojonnier | 13.95 | 0.033 | 0.238 |
| ASE | 14.07 | 0.451 | 3.20 |
| **Bologna** | | | |
| Mojonnier | 25.58 | 0.275 | 0.968 |
| ASE | 28.60 | 0.375 | 1.31 |

# 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 data presented in this application brief 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.

## Environmental contaminants in fish

### Equipment

Dionex ASE 200 Accelerated Solvent Extractor* equipped with 11-, 22-, or 33-mL stainless steel extraction cells

Analytical balance

Collection vials, 40 mL (P/N 048783) and 60 mL (P/N 048784)

Cellulose filter (P/N 049458)

Gas chromatograph (GC) with electron capture detector (ECD)

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

### Solvents

Hexane

All solvents are pesticide-grade or equivalent and are available from Fisher Scientific.

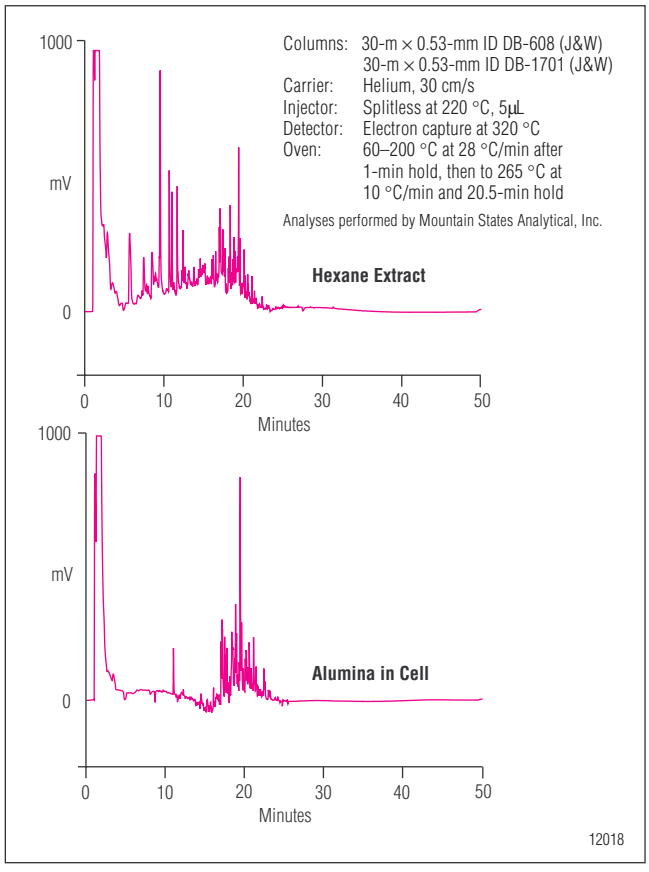
### Analysis

GC-ECD

### 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 |

### Results




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).

# Accelerated Solvent Extraction (ASE) of Pesticide Residues in Food Products

## Introduction

Residue analysis in crops and food products is routinely performed in regulatory and industrial laboratories around the world. Many of the traditional procedures used to perform these extractions are time-consuming and solvent-intensive. Accelerated Solvent Extraction (ASE) is an extraction technique that speeds the extraction process and reduces the total amount of solvent used. The system uses conventional liquid solvents at elevated temperatures and pressures, which results in increased extraction kinetics. Extraction of samples ranging from 1 to 30 g typically requires 12–17 min and 15–50 mL of solvent.

## Organochlorine residues in bananas and potatoes

### Equipment

Dionex ASE 200 Accelerated Solvent Extractor* equipped with 11, 22, or 33 mL stainless steel extraction cells

Dionex vials for collection of extracts (40 mL, P/N 049465; 60 mL, P/N 049466)

Cellulose filter (P/N 049458)

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

### Reagents

Acetonitrile, Optima grade (Fisher Scientific)

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

### Solvents

Acetone

Acetonitrile

Hexane

Sodium sulfate, anhydrous (added after extraction)

All solvents are pesticide-grade or equivalent and are available from Fisher Scientific.

### Analysis

GC-ECD

### Extraction Conditions

| | |
|---|---|
| Temperature: | 100 °C |
| Pressure: | 1500 psi |
| Heatup Time: | 5 min |
| Static Time: | 5 min |
| Flush Volume: | 60% |
| Purge Time: | 100 s |
| Static Cycles: | 1–2 |
| Total Extraction Time: | 14–18 min per sample |
| Total Solvent Used: | 15–45 mL per sample |

### Results

Recovery of organochlorine pesticides spiked onto raw banana at the 100 ppm level.*

| Compound | Av. Recovery (%) | SD (µg/kg) | RSD (%) |
|---|---|---|---|
| α-BHC | 100.3 | 2.3 | 2.3 |
| α-BHC | 102.2 | 2.3 | 2.3 |
| α-BHC | 98.9 | 3.2 | 3.2 |
| Heptachlor | 89.2 | 7.6 | 8.5 |
| Aldrin | 89.4 | 2.2 | 2.5 |
| Heptachlor Epoxide | 93.5 | 2.1 | 2.2 |
| Dieldrin | 93.7 | 1.6 | 1.7 |
| 4,4'-DDE | 92.1 | 1.8 | 1.9 |
| 2,4'-DDD | 95.4 | 2.5 | 2.6 |
| Endrin | 94.4 | 2.7 | 3.0 |
| 4,4'-DDD | 88.0 | 2.7 | 3.0 |
| 4,4'-DDT | 89.6 | 5.8 | 6.4 |

*N = 3

# 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.

## Fats and contaminants in fish

### Equipment

ASE 200 Accelerated Solvent Extractor* equipped with 33 mL stainless steel extraction cells

ASE Solvent Controller (optional)

Gas Chromatograph

Methylsiloxane column

Microwave oven (800 W) with carousel

Analytical balance

Graduated Conc. Vial (P/N 055442)

Analytical Evaporator

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.*

### Solvents

Hexane

PCB Aroclor Standard

Sulfuric Acid, ACS grade or equivalent

### Reagent

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

### Analysis

GC-ECD

### Extraction Conditions

*Accelerated Solvent Extractor*

| | |
|---|---|
| Solvent: | Hexane |
| System Pressure: | 1500 psi* |
| Oven Temperature: | 25 °C |
| Sample Size: | 10 g |
| 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: | Methylsiloxane |
| 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 |

### Results

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 |

# 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,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. 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.

## Large-volume capacity yields increased sample throughput

### Equipment

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

Collection vials, 250 mL (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.*

### Analysis

GC-ECD

### 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 Inc.)

### 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 |

### Results

Recovery of spiked PCB congeners from 30-g fish tissue samples using selective ASE extraction conditions.

| Cogener | 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 |

# Determination of Pesticides in Large-Volume Food Samples Using Accelerated Solvent Extraction (ASE)

## Introduction

Pesticide residue analysis in crops and food products is performed in regulatory and industrial laboratories around the world. Many of the traditional procedures used to perform the extractions for these analyses are time consuming and solvent intensive. Accelerated Solvent Extraction (ASE) is an extraction technique that speeds the extraction process and reduces the total amount of solvent used. The system uses conventional solvents at elevated temperatures and pressures, which results in improved extraction kinetics. The extraction of samples ranging from 1 to 30 g typically requires 12–17 min and 15–50 mL of solvent.

Extraction of samples up to 30 g have been reported using the Dionex ASE 200 extractor with an upper limit sample cell size of 33 mL. However, for many pesticide residue analyses, this volume is insufficient. Food samples such as fruit and vegetables have very high water contents and must be mixed with desiccants such as sodium sulfate to achieve quantitative pesticide recovery. In this case, the actual weight of the sample extracted will be much less than 30 g. The Dionex ASE 300 has the capability toextract samples with volumes as large as 100 mL. This capability allows the direct extraction of food and vegetable samples with weights in the 30 to 50-g range.

## High-volume capacity for ultralow detection levels of pesticide residues

### Equipment

Dionex ASE 300 Accelerated Solvent Extractor* with 34-, 66-, or 100 mL stainless steel extraction cells

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

Cellulose filters (P/N 056780)

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

### Analysis

GC

### Solvents and Reagents

Acetone, Optima grade (Fisher Scientific)

Methylene chloride, Optima grade (Fisher Scientific)

Ethyl acetate, Optima grade (Fisher Scientific)

Hexane, Optima grade (Fisher Scientific)

Cyclohexane, Optima grade (Fisher Scientific)

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

Sodium sulfate, anhydrous (Fisher Scientific)

### Extraction Conditions

Temperature: 100 °C

Pressure: 1500 psi

Solvent: Ethyl acetate/cyclohexane or $MeCl_2$/acetone (1:1, v/v)

Heatup Time: 5 min

Static Time: 5 min

Flush Vol.: 60%

Purge Time: 180 s

Static Cycles: 1–2

Total Extraction Time: 14–20 min per sample

Total Solvent: 135–145 mL per sample

### Results

Percent recovery of organophosphorus pesticides from apple puree fortified at 50 ppb.

| Apples | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | Mean (%) | SD | RSD (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dichlorvos/Naled | 76 | 80 | 93 | 82 | 80 | 97 | 102 | 95 | 97 | 90 | 67 | 92 | 87 | 10 | 12 |
| Mevinphos | 91 | 96 | 105 | 93 | 90 | 108 | 115 | 111 | 110 | 104 | 71 | 106 | 100 | 12 | 12 |
| TEPP | 117 | 141 | 124 | 120 | 96 | 126 | 144 | 137 | 150 | 107 | 79 | 115 | 121 | 20 | 16 |
| Demeton-O | 64 | 78 | 47 | 44 | 64 | 67 | 83 | 71 | 75 | 51 | 64 | 77 | 65 | 12 | 19 |
| Ethoprophos (Ethoprop) | 84 | 86 | 105 | 91 | 87 | 106 | 110 | 106 | 103 | 91 | 70 | 97 | 95 | 11 | 12 |
| Sulfotep | 94 | 100 | 101 | 95 | 88 | 102 | 105 | 101 | 108 | 87 | 72 | 90 | 95 | 10 | 10 |
| Phorate | 80 | 84 | 85 | 77 | 83 | 88 | 100 | 93 | 95 | 83 | 71 | 89 | 86 | 8 | 9 |
| Demeton-S | 60 | 68 | 45 | 46 | 72 | 55 | 65 | 55 | 72 | 41 | 63 | 73 | 59 | 11 | 18 |
| Dimethoate | 128 | 125 | 146 | 133 | 106 | 141 | 148 | 140 | 149 | 115 | 81 | 121 | 128 | 19 | 15 |
| Diazinon | 87 | 92 | 101 | 86 | 86 | 99 | 107 | 101 | 104 | 91 | 73 | 93 | 93 | 9 | 10 |
| Disulfoton | 59 | 73 | 46 | 44 | 66 | 59 | 78 | 63 | 75 | 52 | 66 | 80 | 63 | 11 | 18 |
| Parathion-methyl | 91 | 95 | 104 | 89 | 88 | 103 | 108 | 104 | 101 | 94 | 70 | 95 | 95 | 10 | 10 |
| Fenchlorphos | 82 | 89 | 101 | 86 | 85 | 100 | 103 | 99 | 96 | 91 | 71 | 93 | 91 | 9 | 10 |
| Malathion | 87 | 96 | 106 | 97 | 84 | 106 | 116 | 104 | 105 | 82 | 62 | 89 | 94 | 14 | 15 |
| Fenthion | 82 | 89 | 87 | 79 | 83 | 91 | 98 | 93 | 94 | 82 | 71 | 90 | 86 | 7 | 8 |
| Chlorpyrifos | 89 | 97 | 94 | 82 | 84 | 101 | 99 | 100 | 95 | 89 | 70 | 87 | 91 | 9 | 10 |
| Parathion-ethyl | 100 | 104 | 105 | 99 | 87 | 104 | 109 | 106 | 118 | 92 | 75 | 91 | 99 | 11 | 11 |
| Trichloronat | 80 | 91 | 98 | 83 | 82 | 99 | 98 | 95 | 96 | 90 | 68 | 90 | 89 | 9 | 10 |
| Tetrachlorvinphos | 87 | 90 | 100 | 87 | 85 | 95 | 100 | 97 | 98 | 91 | 71 | 94 | 91 | 8 | 9 |
| Prothiofos | 76 | 87 | 93 | 78 | 78 | 93 | 93 | 91 | 97 | 85 | 64 | 90 | 85 | 9 | 11 |
| Merphos | 78 | 79 | 91 | 76 | 74 | 88 | 89 | 88 | 96 | 82 | 63 | 83 | 82 | 9 | 10 |
| Fensulfothion | 91 | 92 | 113 | 93 | 90 | 106 | 110 | 106 | 105 | 100 | 71 | 95 | 98 | 11 | 11 |
| Sulprofos | 70 | 85 | 80 | 70 | 76 | 86 | 92 | 82 | 88 | 76 | 64 | 85 | 80 | 8 | 10 |
| EPN | 96 | 103 | 100 | 97 | 88 | 105 | 111 | 103 | 111 | 89 | 70 | 93 | 97 | 11 | 11 |
| Azinphos-methyl | 95 | 99 | 106 | 87 | 84 | 105 | 111 | 104 | 111 | 99 | 75 | 104 | 98 | 11 | 11 |
| Coumaphos | 95 | 98 | 102 | 92 | 89 | 102 | 110 | 102 | 106 | 101 | 80 | 96 | 98 | 8 | 8 |

# Rapid Determination of Organochlorine Pesticides in Animal Feed Using Accelerated Solvent Extraction (ASE)

## Introduction

Animal feed contaminated with organochlorine pesticides (OCPs) has begun to attract worldwide attention. When ingested, the OCPs from animal feed tend to accumulate in certain animal products, especially those rich in fat, such as meat, milk, and butter. Because these types of animal products are widely consumed by humans, methods are needed that quickly extract and determine OCPs in the feeds of animals used to produce products for human consumption. Traditional methods used to extract OCPs from animal feed require large amounts of organic solvents and take from one to several hours per extraction. Also, many of the traditional methods are very labor intensive and require constant analyst attention.

## Environmental contaminants in feedstocks

### Equipment

Dionex ASE 200 Accelerated Extractor* with Solvent Controller

11-mL stainless steel extraction cells (P/N 055422)

Dionex cellulose filters (P/N 049458)

Collection vials, 40 mL (P/N 048783)

Analytical balance (accurate to the nearest 0.0001 g or better)

Laboratory grinder

Ottawa Sand Standard (P/N S23-3)

Dichloromethane silica gel, 0.063–0.200 mm, water content 2.62% (Merck, Darmstadt, Germany)

S-X3 Bio-Beads® (Bio Rad Laboratories)

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

### Analysis

GC

### Reagents

For reagents, use either:

Bulk Isolute® Sorbent (International Sorbent Technology Ltd., UK)

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

### Solvent

Petroleum ether (All solvents are pesticide-grade or equivalent and available from Fisher Scientific.)

### Extraction Conditions

| | |
|---|---|
| Solvent: | Petroleum ether 100% |
| Temperature: | 125 °C |
| Pressure: | 1500 psi |
| Heatup Time: | 6 min |
| Static Time: | 3 min |
| Flush Volume: | 60% |
| Purge Time: | 60 s |
| Cycles: | 3 |
| Total Time: | 18 min |
| Total Solvent: | 20 mL |

### Results

Concentration values (ng $g^{-1}$) and RSD (%) for the extraction of CRM BCR 115.

| Compounds | Certified Value | | ASE (n = 3) | |
|---|---|---|---|---|
| | C (ng $g^{-1}$) | RSD (%) | C (ng $g^{-1}$) | RSD (%) |
| α-HCH | * | * | 21.5 ± 0.5 | 2.5 |
| HCB | 19.4 ± 1.4 | 7.2 | 20.6 ± 0.4 | 1.8 |
| β-HCH | 23 ± 3 | 13.0 | 26.0 ± 2.3 | 8.7 |
| γ-HCH | 21.8 ± 2 | 9.2 | 27.1 ± 1.4 | 5.3 |
| Heptachlor | 19 ± 1.5 | 7.9 | 20.0 ± 0.5 | 2.7 |
| Aldrin | * | * | 56.0 ± 3.1 | 5.5 |
| p,p′–DDE | 47 ± 4 | 8.5 | 54.6 ± 2.6 | 4.7 |
| Dieldrin | 18 ± 3 | 16.7 | 22.0 ± 0.6 | 2.6 |
| Endrin | 46 ± 6 | 13.0 | 52.1 ± 1.9 | 3.6 |
| p,p′ –DDD | * | * | 91.8 ± 2.6 | 2.8 |
| o,p′ -DDT | 46 ± 5 | 10.9 | 49.8 ± 0.5 | 1.1 |
| p,p′ -DDT | * | * | 59.4 ± 1.8 | 3.1 |

* Present but not certified.

# Extraction of Zearalenone from Wheat and Corn by Accelerated Solvent Extraction (ASE)

## Introduction

Zearalenone (ZON) is a mycotoxin produced by the Fusarium fungus. ZON can be found in a wide variety of plants and soils, and can have negative health effects on animal husbandry and humans. Traditional methods for extracting ZON from soils or animal feed include wrist shaking or blending. These methods normally take 30–60 min per sample with constant lab technician attendance. Because of the time-consuming nature of these traditional extraction techniques, many sample prep labs experience large bottlenecks that hinder the flow of samples to the analytical lab.

## Mycotoxins in grains

### Equipment

Dionex ASE 200 Accelerated Solvent Extractor* equipped with 22 mL stainless steel extraction cells (P/N 048764)

Cellulose Filters (P/N 049458)

Collection Vials, 60 mL (P/N 048784)

Analytical Balance (to read to nearest 0.0001 g or better)

Ottawa Sand Standard (P/N S23-3)

Laboratory grinder or blender (Fisher Scientific)

Tyler Sieve 0.5 mm (Fisher Scientific)

PTFE Syringe Filter 0.45 µm (Fisher Scientific)

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

### Analysis

LC-MS

### Reagents

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

### Solvents

Methanol Acetonitrile (All solvents are pesticide-grade or equivalent and available from Fisher Scientific.)

### Extraction Conditions

| | |
|---|---|
| Solvent: | 50% methanol, 50% acetonitrile |
| Temperature: | 80 °C |
| Pressure: | 1500 psi |
| Heatup Time: | 5 min |
| Static Time: | 5 min |
| Static Cycles: | 2 |
| Flush Volume: | 75% |
| Purge Time: | 100 s |
| Total ExtractionTime: | 15 min |
| Volume of Solvent Use: | 25–35 mL |

### Results

Results of extraction of ZON from wheat and corn using ASE.

| Sample | Target Value (ng/g) | Average Recovery (ng/g) n=3 | Percent Recovery | Percent RSD |
|---|---|---|---|---|
| Wheat | 112 | 132 | 118 | 5.2 |
| Corn | 285 | 305 | 107 | 2.2 |

# Rapid Determination of Persistent Organic Pollutants (POPs) Using Accelerated Solvent Extraction (ASE)

## Introduction

The United Nations Environmental Program (UNEP) has been implemented in an effort to combat the release of selected persistent organic pollutants (POPs). POPs are found in environmental samples such as soils, sludges, solid and semi-solid waste, and sediments. POPs are also found in biological samples such as human breast milk, and fish tissue. UNEP is interested in eliminating POPs from the environment because these compounds are considered toxic, carcinogenic, and mutagenic, and degrade slowly in the environment, posing a threat to the global environment. The following compounds are listed by UNEP to be POPS:

- Pesticides: Aldrin, Chlordane, DDT, Dieldrin, Endrin, Heptachlor, Mirex, and Toxaphene
- Industrial chemicals: Hexachlorobenzene, and PCB (polychlorinated biphenyl)
- Chemical by-products (Dioxins): Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD and PCDF)

## Persistent organic pollutants in environmental samples

### Equipment

Dionex ASE 200 Accelerated Extractor* with Solvent Controller

Use either:

22 mL Stainless Steel Extraction Cells (P/N 048764)

11 mL Stainless Steel Extraction Cells (P/N 048765)

33 mL Stainless Steel Extraction Cells (P/N 048766)

Cellulose Filters (P/N 049458)

Collection Vials 60 mL (P/N 048784) or 40 mL (P/N 048783)

Analytical Balance (to read to nearest 0.0001 g or better)

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

### Solvents

Hexane

Dichloromethane

Acetone

Toluene

(All solvents are pesticide-grade or equivalent and available from Fisher Scientific.)

### 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)

### Analysis

GC-ECD

### Extraction Conditions

***Pesticides and PCBs (8081/8082)***

| | |
|---|---|
| Solvent: | Hexane/acetone (1:1) (v/v) |
| Temperature: | 100 °C |
| Pressure: | 1500 psi* |
| Static Time: | 5 min |
| Static Cycles: | 1–2 |
| Flush Vol.: | 60% |
| Purge Time: | 60–120s |

***Hexachlorobenzene (8270)***

| | |
|---|---|
| Solvent: | Dichloromethane/ acetone (1:1), (v/v) |
| Temperature: | 100 °C |
| Pressure: | 1500 psi |
| Static Time: | 5 min |
| Static Cycles: | 1–2 |
| Flush Vol.: | 60% |
| Purge Time: | 60–120 s |

***Dioxins (PCDD and PCDF) (8290)***

| | |
|---|---|
| Solvent: | Toluene (100%) or toluene/acetic acid (5%, v/v) if HCl pretreatment currently used |
| Temperature: | 175–200 °C |
| Pressure: | 1500 psi |
| Static Time: | 5–15 min |
| Static Cycles: | 2–3 |
| Flush Volume: | 60–70% |
| Purge Time: | 60–120 s |

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

### Results

PCB recovery from oyster tissue[a]

| PCB Congener | Average Recovery, n = 6 (as % of Soxhlet) | RSD (%) |
|---|---|---|
| PCB 28 | 90.0 | 7.8 |
| PCB 52 | 86.9 | 4.0 |
| PCB 101 | 83.3 | 1.5 |
| PCB 153 | 84.5 | 3.5 |
| PCB 138 | 76.9 | 3.0 |
| PCB 180 | 87.0 | 4.3 |

[a] Analyte concentration range: 50–150 µg/kg per component

# Rapid Extraction and Determination of Arsenicals in Fish Tissue and Plant Material Using Accelerated Solvent Extraction (ASE)

## Introduction

The toxicity of arsenic is species dependent. Inorganic arsenic species such as arsenite (As[III]) and arsenate (As[V]) have been classified as carcinogens. Methylated forms such as monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) have recently been labeled as cancer promoters. Arsenobetaine (AsB), arsenocholine (AsC), and arseno sugars have been found to be relatively nontoxic. Two major pathways for toxic arsenic exposure include drinking water and diet. Seafood (including fish and seaweed) accounts for the majority of ingested arsenic, most of which is nontoxic, however, fruits and vegetables grown in contaminated soils and sediments contribute another significant source. Due to the variable levels of toxicity associated with arsenic species in foods, total arsenic determination is not sufficient to assess potential harmful contamination. Determination of individual arsenic species is necessary. This has increased the need to improve separation and detection methods for organometallic speciation.

## Arsenic species extracted from a variety food sample matrices

### Equipment

Dionex ASE 200 Extractor* equipped with 11-mL stainless steel extraction cells

Cellulose Glass-fiber Filters (P/N 049458 or 047017)

Collection Vials (40 or 60 mL) (P/N 048783 or 048784)

Analytical Balance (to read to the nearest 0.0001 g or better)

Solvent Evaporator

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

### Reagents

Methanol (HPLC grade)
HPLC water
Ottawa Sand Standard (P/N S23-3)

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

### Solvents

Hexane

Dichloromethane

Acetone

Toluene

(All solvents are pesticide-grade or equivalent and available from Fisher Scientific.)

### Analysis

LC-ICP-MS

### Extraction conditions

*Fish Tissue*

| | |
|---|---|
| Solvent: | Methanol 100% |
| Temperature: | 100 °C |
| Pressure: | 1500 psi Cell |
| Heatup Time: | 5 min |
| Static Time: | 2 min |
| Flush Volume: | 60% |
| Purge Time: | 60 s |
| Cycles: | 5 |
| Total Time: | 7 min |
| Total Solvent: | <30 mL |

*Carrots*

| | |
|---|---|
| Solvent: | Water |
| Temperature: | 100 °C |
| Pressure: | 1500 psi |
| Cell Heat-up Time: | 5 min |
| Static Time: | 1 min |
| Flush Volume: | 100% |
| Purge Time: | 90 s |
| Cycles: | 3 |
| Total Time: | 18 min |
| Total Solvent: | <30 m |

*Ribbon Kelp*

| | |
|---|---|
| Solvent: | 30/70 (w/w) Methanol/ $H_2O$ |
| Temperature: | Ambient |
| Pressure: | 1500 psi |
| Heatup Time: | N/A |
| Static Time: | 1 min |
| Flush Volume: | 90% |
| Purge Time: | 120 s |
| Cycles: | 3 |
| Total Time: | 7 min |
| Total Solvent: | <30 mL |

### Results

Results of ASE extraction of fish tissue CRMs (n=6). Data obtained for AsB in two certified reference materials and a candidate reference material* extracted with ASE.

| | Measured Value | Certified Value |
|---|---|---|
| DORM-2 (dogfish muscle) | 16.3 ± 0.9 (±1s) | 16.4 ± 1.1 (±95% C.I.) |
| BCR 627 (tuna fish) | 3.69 ± 0.21 (±1s) | 3.90 ± 0.22 (±95% C.I.) |
| BCR 710** (oyster tissue) | 31.8 ± 1.1 (±1s) | 32.7 ± 5.1 (±1s) |

* Expressed as mg/kg As, unless otherwise stated.
** Concentration as species. The data shown for this material is based on the consensus mean of the final certification round after the removal of statistical outliers.

# Determination of Perchlorate in Vegetation Samples Using Accelerated Solvent Extraction (ASE) and Ion Chromatography

## Introduction

Perchlorate ($ClO_4^-$) is an environmental contaminant that has been found in drinking, ground, and surface waters in several states within the United States. Most of the contaminated sites have been traceable to sources near military installations or manufacturing sites where perchlorate salts are used to manufacture rocket propellant, munitions, or fireworks. The solubility, mobility, and persistence of perchlorate have resulted in the contamination of drinking water, soil, and vegetation in several areas.

## Environmental contaminants in a variety of vegetable matrices

### Equipment

Dionex ASE 200 or ASE 300* system

Collection vials, 60 mL (P/N 048784)

Collection bottles, 250 mL (P/N 056284)

Glass fiber filters (P/N 047017 for ASE 200, P/N 056781 for ASE 300)

OnGuard II Sample Pretreatment Cartridges

- Ag (P/N 057089)
- Ba (P/N 057093)
- H (P/N 057085)
- RP (P/N 057083)

ASE Prep DE (P/N 062819)

Analytical balance with 0.1 mg resolution

Dionex ICS-2500 chromatography system consisting of:

- GP50 Gradient Pump with vacuum degas option
- EG50 Eluent Generator with EluGen EGC II
- NaOH cartridge (P/N 058908)
- AS40 Autosampler
- LC30 Chromatography Oven
- CD25 Conductivity Detector with conductivity cell

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

### Analysis

IC

### Reagents and Standards

Deionized water (DI $H_2O$), Type I reagent grade, 18 Ω-cm resistance or better

Sodium perchlorate, 98% ACS reagent grade or better (Fisher Scientific)

ACS reagent grade sodium salts (Mallinckrodt, Fisher)

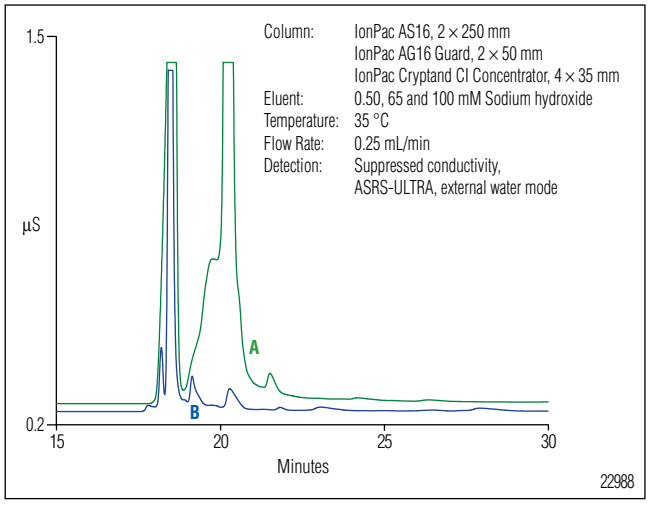
Sodium Hydroxide (NaOH) 50% w/w (Fisher Scientific)

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

### Extraction Conditions

| | |
|---|---|
| Extraction Solvent: | Water |
| Pressure: | 1,500 psi |
| Temperature: | 80 °C |
| Equilibration Time: | 5 min |
| Extraction Time: | 5 min (static) |
| Solvent Flush: | 30% (of cell volume) |
| Nitrogen Purge: | 1, 20 s (after extraction) |
| Extraction Cycles: | 3 |
| Cell Sizes: | 33 mL and 100 mL |

### Results




Alfalfa extracts obtained using (A) no in-line cleanup and (B) OnGuard resins combined with basic alumina in the ASE extraction cell.

# Extraction and Cleanup of Acrylamide in Complex Matrices Using Accelerated Solvent Extraction(ASE) Followed by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

## Introduction

Acrylamide is formed during the cooking process of certain plant-based foods which are rich in carbohydrates and low in protein. Specifically, it forms when asparagines reacts with sugars such as glucose at high temperatures. Acrylamide was detected in fried foods by the Swedish National Food Authority in 2002. Since then, many food laboratories have successfully performed determinations for this compound on a variety of different food matrices. Acrylamide is a known carcinogen in animals.

## Contaminants in coffee and chocolate

### Equipment

Dionex ASE 200* equipped with 33-mL stainless steel extraction cells

Cellulose filters (P/N 049458)

Collection vials, 60 mL (P/N 048784)

Dionex SE 500 Solvent Evaporator (P/N 063221, 120 v) (P/N 063218, 240 v)

Standard laboratory tissue homogenizer

Standard laboratory centrifuge (rated to 10,000 rpm or greater)

Centrifuge tubes (40–50 mL)

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

### Analysis

LC-MS/MS

### Chemicals and Reagents

Acrylamide, purity 99% (Fisher Scientific)

*d3*-Acrylamide (2,3,3-*d3*-2-propenamide) (Cambridge, Isotope Laboratories USA)

Florisil, 60–100 mesh (Fisher Scientific)

Potassium hexacyanoferrate (II) trihydrate (Carrez I) (Fisher Scientific)

Zinc sulfate heptahydrate (Carrez II) (Fisher Scientific)

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

Termamyl® 120 L (Type L thermostable amyloglucosidase enzyme) (Novozymes, Denmark)

Ethyl acetate (Fisher Scientific, HPLC Grade)

Dichloromethane (Fisher Scientific, HPLC Grade)

Methanol (Fisher Scientific, HPLC Grade)

### Extraction Conditions

| | |
|---|---|
| Solvent: | Ethyl acetate (100%) |
| Temperature: | Ambient |
| Pressure: | 1500 psi |
| Static Time: | 3 min |
| Static Cycles: | 3 |
| Flush Volume: | 100% |
| Purge Time: | 60 s |

### Results

Comparison of manual extraction versus ASE for quantification of acrylamide spiked samples in soluble chocolate powder (n = 6).

| | Manual Extraction | | ASE Extraction | |
|---|---|---|---|---|
| Spiking Levels | Recovery % | %RSD | Recovery % | %RSD |
| 12.7 µg/kg | 103.7 | 17.2 | 94.6 | 4.3 |
| 304.7 µg/kg | 108.0 | 6.3 | 102.2 | 7.0 |
| 2504 µg/kg | 104.3 | 5.3 | 101.5 | 2.4 |

ASE of roast ground coffee, soluble coffee, coffee surrogate, and cocoa.

| Materials | Acrylamide Level (µg/kg) | | | |
|---|---|---|---|---|
| | | Spiked at 150 µg/kg | | |
| | Incurred[a] | Expected[b] | Measured[a] | CV% |
| R&G coffee | 136 | 286 | 298 | 3.1 |
| Soluble coffee powder | 299 | 449 | 435 | 2.9 |
| Coffee surrogate | 632 | 782 | 782 | 1.0 |
| Cocoa powder | 192 | 342 | 343 | 1.1 |

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

## Introduction

ASE also be used also to extract organic materials from matrices such as milk, foodstuffs, plant material, plasma, serum, and tissue. This application brief details procedures for extracting the following contaminants from animal tissues:

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

## Environmental contaminants in fish and egg samples

### Equipment

Dionex ASE 200 Accelerated Solvent Extractor* with ASE Solvent Controller

Choose either 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 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*

### Analysis

GC

GC-MS

GC-ECD

HPLC

### Extraction Conditions

| | |
|---|---|
| Pressure: | 1500 psi |
| Temperature: | 175 °C |
| Solvent: | 100% Toluene |
| Static Time: | 10 min |
| Static Cycles: | 2 |
| Flush Volume: | 60% |
| Purge Time: | 60 sec |
| Static Time: | 1 or 2 min** |
| Flush Volume: | 60% |
| Purge Time: | 60 sec |
| Cycles: | 2 |
| Total Time: | 12 min |
| Total Solvent: | 20 mL |

* *Petroleum ether and hexane were found to be equivalent as extraction solvents for fat in meat.*

***When extracting more than 1 g of a high-fat sample, a 2 min static time may be beneficial.*

### Results

PCDDs/PCDFs in fish tissue samples (ng/kg or ppt)using ASE.

| 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 |

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