Extraction of Organochlorine Pesticides from Oyster Tissue Using Accelerated Solvent Extraction

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Key Words
Persistent organic pollutants, moisture absorbing polymer, wet samples, accelerated solvent extraction, sample preparation

Introduction

Organochlorine pesticides (OCPs) are a class of chemicals that were used to control insect pests since the 1940s. The use of OCPs was banned in the later part of the last century due to their longevity, a trait that made them effective for long term pest control, but also increased concerns of potential health outcomes such as cancer in humans and ecosystem disruption. Pesticides are regulated in the U.S. by the Environmental Protection Agency (EPA) under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Some states also regulate pesticides under FIFRA, in a more restrictive manner than the EPA. In the European Union, water intended for human consumption must meet a maximum level of 0.1 µg/L for each pesticide and a maximum of 0.5 µg/L for total pesticides, except for aldrin, dieldrin, heptachlor, and heptachlor epoxide, which are each limited to maximum levels of 0.03 µg/L. Maximum contaminant levels have been established for OCPs by the United States Environmental Protection Agency ranging from 0.2 µg/L for Lindane to 2 µg/L for Endrin.

Many OCPs are endocrine disrupting chemicals, meaning they have subtle toxic effects on the body's hormonal systems. Endocrine disrupting chemicals often mimic the body's natural hormones, disrupting normal functions contributing to adverse health effects. OCPs are persistent organic pollutants (POPs), a class of chemicals that are ubiquitous environmental contaminants because they break down very slowly in the environment and accumulate in lipid rich tissue such as body fat. According to the Centers for Disease Control and Prevention (CDC), most people have OCPs present in their bodies. Exposure to low concentrations of organochlorine chemicals over a long period may eventually lead to a substantial body burden of toxic chemicals. Organochlorine compounds have long been recognized as the most deleterious contaminants to biota in the world's marine and estuarine waters. Various biomonitoring strategies have therefore been developed to monitor and evaluate the adverse impact of these compounds on the marine ecosystems. Analyses of OCPs are becoming increasingly important,

and often with the need to isolate and analyze trace levels of compounds from a variety of matrices such as soil, sediment, animal tissue, fruits, and vegetables. Sample pretreatment constitutes an important step prior to analysis. The purpose of the sample pretreatment step is to selectively isolate the analytes of interest from matrix components and present a sample suited for routine analysis by an established analytical techniques such as gas chromatography or high-pressure liquid chromatography. Accelerated solvent extraction is an established technique for extracting analytes of interest from a solid, semisolid or an adsorbed liquid sample using an organic solvent at an elevated temperature and pressure. The elevated pressure elevates the boiling temperature of the solvent thereby allowing faster extractions to be conducted at relatively high temperatures. Thus the extraction process is significantly faster than traditional methods such as Soxhlet extraction.

This Application Brief discusses the use of Thermo Scientific™ Dionex™ ASE Prep MAP, a proprietary polymer designed to remove moisture and increase extraction efficiencies from wet samples including soils, tissues and food products. This polymer is useful for in-cell extraction of trace level organics from a variety of moisture containing samples with no additional pre or post extraction steps. The Dionex ASE Prep MAP polymer has a high-capacity for water removal and does not suffer from some of the limitations of clumping or precipitation observed in some of the traditional drying methods.

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Equipment

- Thermo Scientific™ Dionex™ ASE™ 350 Accelerated Solvent Extractor system, equipped with 34 mL Stainless Steel Extraction Cell Kit, (P/N 060071)
- Filters, Glass Fiber Cell (P/N 056781)
- 250 mL Clear Collection Bottles (P/N 056284)
- Analytical Balance (read to the nearest 0.001 g or better)
- Mortar and Pestle (Fisher Scientific or equivalent)
- Gas Chromatograph (GC) with Electron-Capture Detector (ECD)

Consumables, Regents and Standards

- Dionex ASE Prep Map, Moisture Absorbing Polymer (P/N 083475)
- Thermo Scientific Dionex ASE Prep DE (diatomaceous earth) Dispersant, 1 kg Bottle (P/N 062819)
- Sodium Sulfate
- Acetone
- Hexane
- Heptachlor
- Lindane
- Aldrin
- Dieldrin
- Endrin
- Dichlorodiphenyltrichloroethane (DDT)

All solvents are optima-grade or equivalent and are available at Fisher Scientific.

Sample Preparation and Experimental Conditions

Sample Preparation Using Sodium Sulfate as the Drying Agent

The Oyster samples were prepared by blending or chopping to produce a unifrom homogenate. 2.5 g of the spiked oyster sample was treated with 9 g of sodium sulfate as the drying agent prior to in-cell extraction in the Dionex ASE 350 system. The extraction was pursued at 100 °C using hexane:acetone (1:1) as solvents. The extracts were analyzed by GC-ECD.

Sample Preparation Using Dionex ASE Prep MAP as the Drying Agent

A 5 g portion of the homogenate was accurately weighed and mixed with 1.7 g of Dionex ASE Prep DE and 1.7 g of Dionex ASE Prep MAP. Carefully transfer the samples to the extraction cells, ensuring that the sample is completely removed from the container. Load the extraction cells and collection vials into the Dionex ASE 350 system and perform the extraction according to the conditions listed. In the case of spiked samples the spikes were added to the sample prior to extraction.

Accelerated Solvent Extraction Conditions	
Oven Temperature:	100 °C
Pressure:	1500 psi
Static Time:	5 min
Static Cycles:	3
Rinse Volume:	60%
Solvent:	Hexane/Acetone (1:1, v/v)
Total Extraction Time:	22–25 min
Pure Time:	120 sec

Results and Discussion

Sample preparation is challenging for a wet animal tissue sample such as an oyster sample. The presence of water in such a sample can result in poor recoveries of the analyte of interest. A drying step is therefore needed before the extraction. Mixtures of six OCPs at concentrations of 500 ng/g each were spiked on to the wet oyster samples. The spiked oyster samples were mixed with Dionex ASE Prep MAP and Dionex ASE Prep DE (1:1) or mixed with sodium sulfate as the drying agent prior to in-cell extraction in the Dionex ASE system. The extraction was pursued at 100 °C using hexane: acetone (1:1) as solvents. The extracts were analyzed by GC-ECD. The results in Table 1 show recoveries ranging from 91% for Lindane to 114% for DDT when the extractions are done using Dionex ASE Prep MAP and Dionex ASE Prep DE. The recoveries for extractions done with sodium sulfate are considerably lower ranging from 69% for DDT to 81% for Lindane. The data shows that Dionex ASE Prep DE and Dionex ASE Prep MAP were an effective drying agent for wet oyster samples with excellent recoveries for the six OCPs. In contrast the sodium sulfate treated sample showed poorer recoveries.

Table 1. In-cell moisture removal of oyster sample using Dionex ASE Prep MAP and Dionex ASE Prep DE, in comparison to sodium sulfate.

Compound	% Recovery Oyster dried with Dionex ASE Prep MAP and Dionex ASE Prep DE* (n = 3)	% Recovery Oyster dried with sodium sulfate** (n = 3)
Lindane	91	81
Heptachlor	93	64
Aldrin	94	66
Dieldrin	105	75
Endrin	106	70
DDT	114	69
Total	101	71

* Data is courtesy of Dr. Todd Anderson from the Department of Toxicology, Texas Tech University, Lubbock

** In-cell drying with sodium sulfate is not recommended using accelerated solvent extraction

Conclusion

This Application Brief describes a simple and reliable method to extract OCPs from oyster tissue. This method also demonstrates the use of Dionex ASE Prep DE and Dionex ASE Prep MAP for in-cell extractions without any pre and post extraction steps to remove moisture and increase extraction efficiencies in wet samples. The method is ideal for routine extractions of OCPs from wet samples.

References

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