

## **Pressurized Solvent Extraction of Organochlorines from Fish Liver**

### **Introduction**

Organochlorines include such compounds as chlordane, toxaphene, dieldrin, polychlorinated biphenyls, DDT, hexachlorobenzene, and hexachlorocyclohexanes. Contamination of waterways with organochlorine compounds can result in toxic levels of those compounds in fish. The analytic method used to test for organochlorine compounds in fish typically requires fish tissue to be dried through processes like lyophilization before organochlorines can be extracted by the labor intensive Soxhlet technique.

Pressurized solvent extraction is a new technique that reduces solvent consumption and sample preparation time. Solvent is pumped into an extraction vessel containing the sample and is heated and pressurized. The pressurized solvent at high temperature accelerates the extraction process by increasing the solubility of the analyte in the solvent and also increasing the kinetic rate of desorption of the analyte from the sample matrix.

The *fast* PSE is an automated system which processes six samples simultaneously. The parallel processing technology of the *fast* PSE dramatically increases sample throughput compared to Soxhlet and pressurized solvent extraction systems that employ serial processing. In addition to rapid extraction times, significant reduction in solvent consumption is achieved.

This application describes the rapid extraction of organochlorines from fish liver by pressurized solvent extraction (PSE) through a method that bypasses the time-consuming step of conventional sample drying procedures. Pressurized solvent extraction is approved for use as EPA Method 3545A and can be used to replace Soxhlet and sonication techniques with significant time savings and solvent reduction.

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#626





## Equipment

- ✓ Applied Separations' *fast* PSE Pressurized Solvent Extractor
- ✓ 33 mL Extraction Vessels-Cat.#10627
  - Note: the *fast* PSE can run 6 samples simultaneously
- ✓ Solvent Concentration System
- ✓ Analytical balance
- ✓ Mortars (90mm o.d. Coors #60316) and pestles (Coors #60317)
- ✓ Gas chromatograph

## Solvents and Materials

- ✓ Ethyl acetate
- ✓ cyclohexane
- ✓ Na<sub>2</sub>SO<sub>4</sub>
- ✓ Collection Vials (60mL for extract collection)- Cat. #10650
- ✓ Cellulose Filter Disks- Cat. #10711
- ✓ S/S Vessel Frits- Cat. #10710
- ✓ Ottawa Sand- Cat. #10584

## Summary of Method

|   |  |
|---|--|
|  <p><b>1. Prepare Sample</b></p> |  <p><b>2. Load Sample</b></p>      |
|  <p><b>3. Run Sample</b></p>     |  <p><b>4. Collect Extract</b></p> |

**Applied  
Separations**

930 Hamilton Street  
Allentown, PA 18101  
610-770-0900  
610-740-5520 (FAX)

[www.appliedseparations.com](http://www.appliedseparations.com)

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## Procedure

### *Prepare Sample*

Blend 5 g of fish tissue with 20 g of Na<sub>2</sub>SO<sub>4</sub> (1+4, w/w). Pour the weighed material carefully into the mortar and grind with the pestle until uniform. (Scrape all particles from pestle into the mortar and set the pestle aside.) Ensure that the fish product sample is homogeneous before proceeding with this method. Failure to prepare a totally uniform sample may result in inconsistent results.

### *Load Sample*

Prepare the extraction vessels for analysis by placing a cellulose filter disk in the bottom opening followed by a 10µm s/s frit, and secure them in place with a retaining nut. Place adaptor and funnel in top opening. Pour the sample mixture through a funnel into the extraction vessel. Once sample is in vessel, add clean Ottawa sand to within 1 cm of the top of the vessel's interior flange as directed by User's Manual.

Place the extraction vessel into the instrument as described in the *fast* PSE operator's manual. Ensure that the pump is primed and that the extraction solvent is ethyl acetate-cyclohexane. Place a pre-cleaned collection vial in the instrument for each sample, and program the instrument using the following parameters:

### *Extraction Conditions*

*Program the following extraction parameters on the fast PSE*

Program A Mode – 33 mL vessels

|                   |   |
|-------------------|---|
| Solvent:          | Ethyl acetate-cyclohexane (1+1,v/v)     |
| Temperature:      | 125 ° C                                 |
| Pressure:         | 120 Bar                                 |
| Static:           | 10 minutes                              |
| Solvent Module:   | 1*                                      |
| Cycles:           | 2                                       |
| Pause:            | N=0                                     |
| Flushing Program: | Solvent/gas/repeat flush: 1 min/2 min/0 |

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**\*Note:** If automatic solvent selection module is used, enter the appropriate position number (i.e. 2, 3, or 4).

Optimize the conditions as needed. In general, the pressure is not a critical parameter, as the purpose of pressurizing the extraction vessel is to prevent the solvent from boiling at the extraction temperature and to ensure that the solvent remains in intimate contact with the samples. Any pressure in the range of 100 BAR should suffice. Once established, the same parameters should be used for all samples extracted for the same analysis type.

### ***Collect Extract***

Collect each extract in a clean 60mL vial. Allow the extract to cool after the extraction is complete.

### **Analysis**

After the extraction procedure is complete, pass the extracts through 10g of Na<sub>2</sub>SO<sub>4</sub> to remove any water and then adjust volume to 50mLs. Filter extracts through a .45 micron membrane filter and analyze by Gel Permeation Chromatography.

### **References**

US EPA Method 3545A – Pressurized Fluid Extraction  
Weichbrodt et al. *Journal of AOAC International*. Vol. 83, No.6, 2000.

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#626

## Safety

The use of organic solvents, elevated temperatures, and high pressures present potential safety concerns in the laboratory. Common sense laboratory practices can be employed to minimize these concerns. However, the following sections describe additional steps that should be taken.

Extraction vessels in the *fast* PSE oven are hot enough to burn unprotected skin. Allow the vessels to cool before removing them from the oven, or use appropriate protective equipment (e.g. insulated gloves or tongs) as recommended by the manufacturer.

During the gas purge step, some solvent vapors may exit through a vent port in the instrument. Connect this port to a fume hood or other means to prevent release of solvent vapors to the laboratory atmosphere. This precaution also applies to the removal of post extraction solvent from the collected extract.