

METHOD 3545A

PRESSURIZED FLUID EXTRACTION (PFE)

1.0 SCOPE AND APPLICATION

1.1 Method 3545 is a procedure for extracting water insoluble or slightly water soluble organic compounds from soils, clays, sediments, sludges, and waste solids. The method uses elevated temperature (100 - 180°C) and pressure (1500 - 2000 psi) to achieve analyte recoveries equivalent to those from Soxhlet extraction, using less solvent and taking significantly less time than the Soxhlet procedure. This procedure was developed and validated on a commercially-available, automated extraction system.

1.2 This method is applicable to the extraction of semivolatile organic compounds, organophosphorus pesticides, organochlorine pesticides, chlorinated herbicides, PCBs, and PCDDs/PCDFs, which may then be analyzed by a variety of chromatographic procedures.

1.3 This method has been validated for solid matrices containing 250 to 12,500 µg/kg of semivolatile organic compounds, 250 to 2500 µg/kg of organophosphorus pesticides, 5 to 250 µg/kg of organochlorine pesticides, 50 to 5000 µg/kg of chlorinated herbicides, 1 to 1400 µg/kg of PCBs, and 1 to 2500 ng/kg of PCDDs/PCDFs. The method may be applicable to samples containing these analytes at higher concentrations and may be employed after adequate performance has been demonstrated for the concentrations of interest (see Method 3500, Sec. 8.0).

1.4 This method is applicable to solid samples only, and is most effective on dry materials with small particle sizes. Therefore, waste samples must undergo phase separation, as described in Chapter Two, and only the solid phase material is to be extracted by this procedure. If possible, soil/sediment samples may be air-dried and ground to a fine powder prior to extraction. Alternatively, if the loss of analytes or during drying is a concern, soil/sediment samples may be mixed with anhydrous sodium sulfate or pelletized diatomaceous earth. (Drying and grinding samples containing PCDDs/PCDFs is *not* recommended, due to safety concerns). The total mass of material to be prepared depends on the specifications of the determinative method and the sensitivity required for the analysis, but 10 - 30 g of material are usually necessary and can be accommodated by this extraction procedure.

1.5 This method is restricted to use by or under the supervision of trained analysts. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 Samples are prepared for extraction either by air drying the sample, or by mixing the sample with anhydrous sodium sulfate or pelletized diatomaceous earth. The sample is then ground and loaded into the extraction cell. Drying and grinding samples containing PCDDs/PCDFs is *not* recommended, due to safety concerns. Grinding may also be a concern for other more volatile analytes. (See Sec. 7.1).

2.2 The extraction cell containing the sample is heated to the extraction temperature (see Sec. 7.8), pressurized with the appropriate solvent system, and extracted for 5 minutes (or as recommended by the instrument manufacturer). Multiple extractions are recommended for some

groups of analytes. The solvent systems used for this procedure vary with the analytes of interest and are described in Sec. 5.5.

2.3 The solvent is collected from the heated extraction vessel and allowed to cool.

2.4 The extract may be concentrated, if necessary, and, as needed, exchanged into a solvent compatible with the cleanup or determinative step being employed.

3.0 INTERFERENCES

3.1 Refer to Method 3500.

3.2 If necessary, Florisil and/or sulfur cleanup procedures may be employed. In such cases, proceed with Method 3620 and/or Method 3660.

3.3 Samples for PCDD/PCDF analysis should be subjected to the various cleanup procedures described in the determinative methods (8280 and 8290).

4.0 APPARATUS AND MATERIALS

4.1 Pressurized fluid extraction device

4.1.1 Dionex Accelerated Solvent Extractor or Supelco SFE-400 with appropriately-sized extraction cells. Currently, cells are available that will accommodate 10-g, 20-g and 30-g samples. Cells should be made of stainless steel or other material capable of withstanding the pressure requirements (2000+ psi) necessary for this procedure.

4.1.2 Other system designs may be employed, provided that adequate performance can be demonstrated for the analytes and matrices of interest.

4.2 Apparatus for determining percent dry weight

4.2.1 Oven - drying

4.2.2 Desiccator

4.2.3 Crucibles - porcelain or disposable aluminum

4.3 Apparatus for grinding - capable of reducing particle size to < 1 mm.

4.4 Analytical balance - capable of weighing to 0.01 g.

4.5 Vials for collection of extracts - 40-mL or 60-mL, pre-cleaned, open top screw-cap with PTFE-lined silicone septum (Dionex 049459, 049460, 049461, 049462 or equivalent).

4.6 Filter disk - 1.91 cm, Type D28 (Whatman 10289356, or equivalent).

4.7 Cell cap sealing disk (Dionex 49454, 49455, or equivalent).

5.0 REAGENTS

5.1 Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

5.2 Organic-free reagent water. All references to water in this method refer to organic-free reagent water, as defined in Chapter One.

5.3 Drying agents

5.3.1 Sodium sulfate (granular anhydrous), Na_2SO_4 .

5.3.2 Pelletized diatomaceous earth.

5.3.3 The drying agents should be purified by heating at 400°C for 4 hours in a shallow tray, or by extraction with methylene chloride. If extraction with methylene chloride is employed, then a reagent blank should be prepared to demonstrate that the drying agent is free of interferences.

5.3.4 Quartz sand. Although not strictly a drying agent, clean sand may be used to facilitate grinding of some sample matrices, to fill void volumes in the extraction cell, and to increase the flow of solvent through the sample. It may be prepared as described in Sec. 5.3.3.

5.4 Acids

5.4.1 Phosphoric acid solution (see Sec. 5.5.5). Prepare a 1:1 (v/v) solution of 85% phosphoric acid (H_3PO_4) in organic-free reagent water.

5.4.2 Trifluoroacetic acid solution (see Sec. 5.5.5). Prepare a 1% (v/v) solution of trifluoroacetic acid in acetonitrile.

5.4.3 Glacial acetic acid (see Sec. 5.5.6).

5.5 Extraction solvents

The extraction solvent to be employed depends on the analytes to be extracted, as described below. All solvents should be pesticide quality or equivalent. Solvents may be degassed prior to use.

5.5.1 Organochlorine pesticides may be extracted with acetone/hexane (1:1, v/v), $\text{CH}_3\text{COCH}_3/\text{C}_6\text{H}_{14}$ or acetone/methylene chloride (1:1, v/v), $\text{CH}_3\text{COCH}_3/\text{CH}_2\text{Cl}_2$.

5.5.2 Semivolatile organics may be extracted with acetone/methylene chloride (1:1, v/v), $\text{CH}_3\text{COCH}_3/\text{CH}_2\text{Cl}_2$ or acetone/hexane (1:1, v/v), $\text{CH}_3\text{COCH}_3/\text{C}_6\text{H}_{14}$.

5.5.3 PCBs may be extracted with acetone/hexane (1:1, v/v), $\text{CH}_3\text{COCH}_3/\text{C}_6\text{H}_{14}$ or acetone/methylene chloride (1:1, v/v), $\text{CH}_3\text{COCH}_3/\text{CH}_2\text{Cl}_2$ or hexane, C_6H_{14} .

5.5.4 Organophosphorus pesticides may be extracted with methylene chloride, CH_2Cl_2 or acetone/methylene chloride (1:1, v/v), $\text{CH}_3\text{COCH}_3/\text{CH}_2\text{Cl}_2$.

5.5.5 Chlorinated herbicides may be extracted with an acetone/methylene chloride/phosphoric acid solution (250:125:15, v/v/v), $\text{CH}_3\text{COCH}_3/\text{CH}_2\text{Cl}_2/\text{H}_3\text{PO}_4$, or an acetone/methylene chloride/trifluoroacetic acid solution (250:125:1, v/v/v), $\text{CH}_3\text{COCH}_3/\text{CH}_2\text{Cl}_2/\text{CF}_3\text{COOH}$. (If the second option is used, the trifluoroacetic acid solution should be prepared by mixing 1% trifluoroacetic acid in acetonitrile.) Make fresh solutions before each batch of extractions.

5.5.6 PCDDs/PCDFs may be extracted with toluene, $\text{C}_6\text{H}_5\text{CH}_3$. Fly ash samples to be extracted for PCDDs/PCDFs may be extracted with a toluene/acetic acid solution (5% v/v glacial acetic acid in toluene) in lieu of the HCl pretreatment described in Methods 8280 and 8290.

5.5.7 Other solvent systems may be employed, provided that the analyst can demonstrate adequate performance for the analytes of interest in the sample matrix (see Method 3500, Sec. 8.0).

CAUTION: For best results with very wet samples (e.g., $\geq 30\%$ moisture), reduce or eliminate the quantity of hydrophilic solvent used.

5.6 High-purity gases such as nitrogen, carbon dioxide, or helium are used to purge and/or pressurize the extraction cell. Follow the instrument manufacturer's recommendation for the choice of gases.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

See the introductory material to this chapter, Organic Analysis, Sec. 4.1, and Method 3500.

7.0 PROCEDURE

7.1 Sample preparation

7.1.1 Sediment/soil samples - Decant and discard any water layer on a sediment sample. Mix the sample thoroughly, especially composited samples. Discard any foreign objects such as sticks, leaves, and rocks. Air dry the sample at room temperature for 48 hours in a glass tray or on hexane-rinsed aluminum foil. Alternatively, mix the sample with an equal volume of anhydrous sodium sulfate or pelletized diatomaceous earth until a free-flowing powder is obtained.

NOTE: Dry, finely-ground soil/sediment allows the best extraction efficiency for nonvolatile, nonpolar organics, e.g., 4,4'-DDT, PCBs, etc. Air-drying may not be appropriate for the analysis of the more volatile organochlorine pesticides (e.g., the BHCs) or the more volatile of the semivolatile organics because of losses during the drying process. *Drying of samples for PCDDs/PCDFs is not generally recommended, due to safety concerns with samples containing these analytes.* The use of sodium sulfate as a drying agent can lead to clogging of the frits in the cell with recrystallized sodium sulfate. (See "Caution" following Sec. 5.5.6.)

7.1.2 Waste samples - Multiphase waste samples must be prepared by the phase separation method in Chapter Two before extraction. *This extraction procedure is for solids only.*

7.1.3 Dry sediment/soil and dry waste samples amenable to grinding - Grind or otherwise reduce the particle size of the waste so that it either passes through a 1-mm sieve or can be extruded through a 1-mm hole. Disassemble grinder between samples, according to manufacturer's instructions, and decontaminate with soap and water, followed by acetone and hexane rinses. *Grinding of samples for PCDDs/PCDFs is not generally recommended, due to safety concerns with samples containing these analytes.*

NOTE: The note in Sec. 7.1.1 also applies to the grinding process.

7.1.4 Gummy, fibrous, or oily materials not amenable to grinding should be cut, shredded, or otherwise reduced in size to allow mixing and maximum exposure of the sample surfaces for the extraction. The analyst may add anhydrous sodium sulfate, pelletized diatomaceous earth, sand, or other clean, dry reagents to the sample to make it more amenable to grinding.

7.1.5 Solid samples for PCDD/PCDF analysis are generally carefully mixed with clean sand and a drying agent such as diatomaceous earth or sodium sulfate, breaking up lumps with a spatula or other suitable tool.

7.1.6 Fly ash samples may be pretreated with an HCl solution prior to extraction (See Sec. 7 of Method 8280 or 8290). Alternatively, they may be extracted with the toluene/acetic acid solution described in Sec. 5.5.6.

7.2 Determination of percent dry weight - When sample results are to be calculated on a dry weight basis, a second portion of sample should be weighed at the same time as the portion used for analytical determination.

WARNING: The drying oven should be contained in a hood or vented. Significant laboratory contamination may result from drying a heavily contaminated sample.

7.2.1 Immediately after weighing the sample for extraction, weigh 5 - 10 g of the sample into a tared crucible. Dry this aliquot overnight at 105°C. Allow to cool in a desiccator before weighing. Calculate the % dry weight as follows:

$$\% \text{ dry weight} = \frac{\text{g of dry sample}}{\text{g of sample}} \times 100$$

7.3 Grind a sufficient weight of the dried sample from Sec. 7.1 to yield the sample weight needed for the determinative method (usually 10 - 30 g). Grind the sample until it passes through a 10 mesh sieve. *Grinding of samples for PCDDs/PCDFs is not generally recommended, due to safety concerns with samples containing these analytes.*

7.4 Transfer the ground sample to an extraction cell of the appropriate size for the aliquot. Generally, an 11-mL cell will hold about 10 g of material, a 22-mL cell will hold about 20 g of material, and a 33-mL cell will hold about 30 g of material. The weight of a specific sample that a cell will contain depends on the bulk density of the sample and the amount of drying agent that

must be added to the sample in order to make it suitable for extraction. Analysts should ensure that the sample aliquot extracted is large enough to provide the necessary sensitivity and choose the extraction cell size accordingly. Use disposable cellulose or glass fiber filters in the cell outlets. Clean sand may be used to fill any void volume in the extraction cells.

7.5 Add the surrogates (or labeled internal standards for PCDDs/PCDFs) listed in the determinative method to each sample. Add the matrix spike/matrix spike duplicate compounds listed in the determinative method to the two additional aliquots of the sample selected for spiking.

7.6 Place the extraction cell into the instrument or autosampler tray, as described by the instrument manufacturer.

7.7 Place a precleaned collection vessel in the instrument for each sample, as described by the instrument manufacturer. The total volume of the collected extract will depend on the specific instrumentation and the extraction procedure recommended by the manufacturer and may range from 0.5 to 1.4 times the volume of the extraction cell. Ensure that the collection vessel is sufficiently large to hold the extract.

7.8 Recommended extraction conditions

7.8.1 Semivolatiles, organophosphorus pesticides, organochlorine pesticides, herbicides, and PCBs

Oven temperature:	100°C
Pressure:	1500 - 2000 psi
Static time:	5 min (after 5 min pre-heat equilibration)
Flush volume:	60% of the cell volume
Nitrogen purge:	60 sec at 150 psi (purge time may be extended for larger cells)
Static Cycles:	1

7.8.2 PCDDs/PCDFs

Oven temperature:	150 - 175°C
Pressure:	1500 - 2000 psi
Static time:	5-10 min (after 5 min pre-heat equilibration)
Flush volume:	60 - 75% of the cell volume
Nitrogen purge:	60 sec at 150 psi (purge time may be extended for larger cells)
Static Cycles:	2 or 3

7.8.3 Optimize the conditions, as needed, according to the manufacturer's instructions. In general, the pressure is not a critical parameter, as the purpose of pressurizing the extraction cell is to prevent the solvent from boiling at the extraction temperature and to ensure that the solvent remains in intimate contact with the sample. Any pressure in the range of 1500 - 2000 psi should suffice.

7.8.4 Once established, the same pressure should be used for all samples extracted for the same analysis type.

7.9 Begin the extraction according to the manufacturer's instructions. For PCDD/PCDF extraction, 2 to 3 static extractions are recommended.

7.10 Collect each extract in a clean vial (see Sec. 7.7). Allow the extracts to cool after the extractions are complete.

7.11 The extract is now ready for concentration, cleanup, or analysis, depending on the extent of interferants and the determinative method to be employed. Refer to Method 3600 for guidance on selecting appropriate cleanup methods. Excess water present in extracts may be removed by filtering the extract through a bed of anhydrous sodium sulfate. Certain cleanup and/or determinative methods may require a solvent exchange prior to cleanup and/or sample analysis.

7.12 If the phosphoric acid solution in Sec. 5.5.5 is used for the extraction of chlorinated herbicides, then the extractor should be rinsed by pumping acetone through all the lines of the system. The use of other solvents for these analytes may not require this rinse step.

8.0 QUALITY CONTROL

8.1 Refer to Chapter One and Method 8000 for guidance on quality control procedures. Refer to Method 3500 for specific guidance on extraction and sample preparation procedures.

8.2 Before processing any samples, the analyst should demonstrate that all parts of the equipment in contact with the sample and reagents are interference-free. This is accomplished through the analysis of a solid matrix method blank (e.g., clean sand). Each time samples are extracted, and when there is a change in reagents, a method blank needs to be extracted and analyzed for the compounds of interest. The method blank should be carried through all stages of the sample preparation and measurement.

8.3 Standard quality assurance practices should be used with this method. Field duplicates should be collected to validate the precision of the sampling procedures. A matrix spike/matrix spike duplicate, or matrix spike and duplicate sample analysis, and a laboratory control sample should be prepared and analyzed with each batch of samples prepared by this procedure, unless the determinative method provides other guidance.

8.4 When listed in the appropriate determinative method, surrogate standards should be added to all samples prior to extraction. For PCDDs/PCDFs, the labeled internal standards listed in the determinative methods should be added to all samples prior to extraction.

9.0 METHOD PERFORMANCE

9.1 Chlorinated pesticides and semivolatile organics

Single-laboratory accuracy data were obtained for chlorinated pesticides and semivolatile organics at three different spiking concentrations in three different soil types. Spiking concentrations ranged from 5 to 250 µg/kg for the chlorinated pesticides and from 250 to 12500 µg/kg for the semivolatiles. Spiked samples were extracted both by the Dionex Accelerated Solvent Extraction system and by a Perstorp Environmental Soxtec™ (automated Soxhlet). Extracts were analyzed either by Method 8270 or Method 8081. Method blanks, spikes and spike duplicates were included for the low concentration spikes; matrix spikes were included for all other concentrations. The data are reported in detail in Reference 1, and represent seven replicate extractions and analyses for each sample. Data summary tables are included in Methods 8270 and 8081.

9.2 Organophosphorus pesticides and chlorinated herbicides

Single-laboratory accuracy data were obtained for organophosphorus pesticides (OPPs) and chlorinated herbicides at two different spiking concentrations in three different soil types. Spiking concentrations ranged from 250 to 2500 µg/kg for the OPPs and from 50 to 5000 µg/kg for the chlorinated herbicides. Chlorinated herbicides were spiked with a mixture of the free acid and the ester (1:1). Spiked samples were extracted both by the Dionex Accelerated Solvent Extractor and by Soxhlet for the OPPs. Extracts were analyzed by Method 8141. Spiked chlorinated herbicides were extracted by the Dionex Accelerated Solvent Extractor and by the shaking method described in Method 8151. Extracts were analyzed by Method 8151. Method blanks, spikes and spike duplicates were included for the low concentration spikes; matrix spikes were included for all other concentrations. The data are reported in detail in Reference 2, and represent seven replicate extractions and analyses for each sample. Data summary tables are included in Methods 8141 and 8151.

9.3 PCBs

Single-laboratory accuracy data were obtained for PCBs from a soil sample with PCB content certified by NIST (Standard Reference Material, SRM 1939, River Sediment). A PCB-contaminated soil was purchased from a commercial source. Spiking or certified concentrations ranged from 1 to 1400 µg/kg. Samples were extracted by the Dionex Accelerated Solvent Extractor and by Soxtec™ (Perstorp Environmental). Extracts were analyzed using Method 8082. Method blanks, spikes and spike duplicates were included. The data are reported in Reference 2, and represent seven replicate extractions and analyses for each sample. Data summary tables are included in Method 8082.

9.4 PCDDs/PCDFs

Single-laboratory data were obtained for PCDDs/PCDFs from ground chimney brick, urban dust, fly ash, a relatively highly contaminated soil sample (EC-2, National Water Research Institute, Burlington, Ontario, Canada), a low-level sediment sample (HS-2, National Research Council Institute of Marine Biosciences, Halifax, Nova Scotia, Canada) and various field-contaminated soils and sediments. Concentrations of PCDDs/PCDFs ranged from low ng/kg to mid µg/kg levels. Samples were extracted by the Dionex Accelerated Solvent Extractor and by traditional Soxhlet techniques. Extracts were analyzed by a high resolution mass spectrometric method employing isotope dilution quantitation. The data are reported in Reference 3. Data summary tables are included in Method 8290.

10.0 REFERENCES

1. B. Richter, Ezzell, J., and Felix, D., "Single Laboratory Method Validation Report. Extraction of TCL/PPL (Target Compound List/Priority Pollutant List) BNAs and Pesticides using Accelerated Solvent Extraction (ASE) with Analytical Validation by GC/MS and GC/ECD"; Document 116064.A, Dionex Corporation, June 16, 1994.
2. B. Richter, Ezzell, J., and Felix, D., "Single Laboratory Method Validation Report. Extraction of TCL/PPL (Target Compound List/Priority Pollutant List) OPPs, Chlorinated Herbicides and PCBs using Accelerated Solvent Extraction (ASE)". Document 101124, Dionex Corporation, December 2, 1994).

3. B. E. Richter *et al.*, "Extraction of Polychlorinated Dibenzo-*p*-Dioxins and Polychlorinated Dibenzofurans from Environmental Samples Using Accelerated Solvent Extraction (ASE)." *Chemosphere*, 34(5-7), pp. 975-987, 1997.

11.0 SAFETY

The use of organic solvents, elevated temperatures, and high pressures in Method 3545 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.

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

11.2 During the gas purge step, some solvent vapors may exit through a vent port in the instrument. Follow the manufacturer's directions regarding connecting this port to a fume hood or other means to prevent release of solvent vapors to the laboratory atmosphere.

11.3 The instrument may contain flammable vapor sensors and should be operated with all covers in place and doors closed to ensure proper operation of the sensors. If so equipped, follow the manufacturer's directions regarding replacement of extraction cell seals when frequent vapor leaks are detected.

METHOD 3545A

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