

## **SOLID-PHASE EXTRACTION OF PESTICIDES** **5.3**

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Samples collected for analysis of dissolved pesticides can be processed at the laboratory or onsite through a column containing pesticide-specific sorbents. Onsite solid-phase extraction (SPE) is useful, especially at remote sites, because pesticides isolated on the sorbent are less susceptible to degradation than when in water. Also, the SPE cartridges are less expensive to ship than water samples. However, onsite SPE is not required, and in some situations, laboratory SPE might be preferred.

All SPE methods require that the water sample be filtered (section 5.2.2.A) as soon as possible after collection. General equipment and supply needs for SPE for a broad-spectrum analysis of pesticides are listed in table 5-7 and general instructions are given in sections 5.3.1 and 5.3.2. More detailed information on SPE methods and procedures can be found in Sandstrom and others (1992), Sandstrom (1995), Zaugg and others (1995), Lindley and others (1996), and Werner and others (1996).

- ▶ Filter the environmental sample (section 5.2.2.A): this is necessary to prevent blockage of the SPE column by particulate material.
- ▶ Process the pesticide sample through an SPE column within 4 days of collection.
- ▶ Determine the reagents needed for the SPE method to be used (for example, conditioning solution, surrogate solution, and field-matrix spike solution).

**Table 5-7.** Checklist of general equipment and supplies required for broad-spectrum pesticide analysis by onsite solid-phase extraction

[SPE, solid-phase extraction; mm, millimeter;  $\mu$ L, microliter;  $\mu$ m, micrometer; mL, milliliter; NWQL, National Water Quality Laboratory]

✓	General equipment and supplies <sup>1</sup>	Description	Number required
	Aluminum foil	Heavy duty	1 box
	Blank water <sup>2</sup>	Pesticide grade (NWQL)	4 L
	Filter media	Glass microfiber, 147-mm diameter, 0.7- $\mu$ m pore diameter, precleaned <sup>3</sup>	1 per sample
	Detergent, nonphosphate laboratory	0.2-percent solution	4 L
	Glass bores	Disposable, for 100- $\mu$ L micropipet	ample supply
	Gloves, disposable	Powderless, latex or nitrile, assorted sizes	ample supply
	Graduated cylinder or beaker	50 mL, glass	2
	Luer™ connector <sup>2</sup> , Tefzel™ male	P-625	1 or more
	Metering pump, valveless, piston-type	FMI Model RHB OCKC	1
	Methanol	Pesticide grade (NWQL)	4 L
	Micropipet	Fixed volume (100 $\mu$ L)	1 or more
	Nut and union <sup>2</sup> , Tefzel™	P-623	1 or more
	Plastic beaker	1 L, for collecting extracted water	1 or more
	Plate-filter assembly	147-mm diameter, aluminum or stainless steel	1
	Portable balance	(Check method for weight requirements.)	1
	Sample bottles <sup>3</sup> and vials (40 mL) <sup>2</sup>	Amber glass, precleaned	1 per sample
	SPE column adapter <sup>2</sup>	(Check method requirements)	1 or more
	SPE columns, precleaned <sup>2</sup>	C-18: Analyticum™ C-18, 500 mg; Carbopak-B™, 500 mg; Other: as required	1 or more of each, as required
	SPE solutions <sup>2</sup>	(Check method requirements for conditioning, surrogate, and spike solutions)	as required by method
	Stopwatch	Standard	1
	Wash bottle, fluorocarbon polymer	250 mL, for methanol	1
	Wash bottle, fluorocarbon polymer	250 mL, for pesticide-grade water	1

<sup>1</sup>Filtration equipment and supplies are described in section 5.2.2.A, table 5-5, and figure 5-1.

<sup>2</sup>Supplies ordered by USGS personnel through E-mail to NWQL-DENSUPPL.

<sup>3</sup>Supplies ordered by the USGS personnel from Quality of Water Service Unit in Ocala, Fla. (QWSU).

## SOLID-PHASE EXTRACTION BY 5.3.1 C-18 COLUMN

The C-18 SPE column is used for samples that will be analyzed by capillary column gas chromatography/mass spectrophotometry with selected ion monitoring using NWQL schedule 2010 for a broad spectrum of pesticides.<sup>8</sup> Detailed descriptions of the method and laboratory and field extraction procedures are found in Zaugg and others (1995). For C-18 SPE processing, obtain a precleaned Analytichem™ SPE column (500 mg) and the other supplies and equipment described in the spike kit available from the NWQL (table 5-7).

***Quality-control samples are required as an integral part of the sampling program.***

- ▶ Process an initial field blank and then after about every 10 to 20 samples.
  - Use pesticide-grade blank water (PBW, obtained from the laboratory).
  - Process the blank in the same manner as you process the environmental water sample.
- ▶ Process a field matrix spike about every 20 samples. When processing a field matrix spike:
  - Collect duplicate samples.
  - Use a 100- $\mu$ L micropipet to add the spike solution (mixture) to one of the duplicate samples. The concentration of spike solution can vary, depending on availability and the needs of the study (1 ng/ $\mu$ L concentration is commonly used at this time). Follow the instructions provided with the spike kit.
  - Add the surrogate to every spiked sample and an associated unspiked sample.
  - Record lot number and concentration of spike mixture on the NWQL Schedule 2010 Reporting Form (worksheet) (fig. 5-2).

<sup>8</sup>C-18 solid-phase extraction method is used for isolation and concentration of 41 pesticides and pesticide metabolites with concentrations of 4 mg/L or less in natural water samples (atrazine, alachlor, cyanazine, and metolachlor have upper concentration limits of 20 mg/L) (Zaugg and others, 1995).

<b>Schedule 2010 Field Extraction Checklist and Reporting Form</b> <b>U.S. Geological Survey/National Water Quality Laboratory</b> <b>Solid-Phase Extraction and GC/MS Analysis Filtered Water</b>	
Station ID or Unique Number: _____ Station Name _____ Date: _____ Time: _____ Collector: _____ Telephone Number of Collector: _____ Comments: _____	
<b>NWQL INFORMATION</b>	
<input type="checkbox"/> SPE Column	Brand or Type: _____ Lot #: _____ Dry weight (wt.): _____ grams (g)
<b>ON-SITE INFORMATION</b>	
<input type="checkbox"/> Filter Sample (0.7- $\mu$ m glass fiber filter) Prior to filtration record bottle tare wt.: _____ g	
<input type="checkbox"/> SPE column Conditioning Methanol (2 mL): _____ milliliters (mL) Pesticide-grade water (2 mL): _____ mL	
<b>(DO NOT LET COLUMN GO DRY ONCE CONDITIONING STARTED)</b>	
<input type="checkbox"/> Sample      Sample + bottle wt: _____ g - Bottle tare wt: _____ g = Sample wt: _____ g Add methanol conditioner (1% of sample wt.): _____ mL Sample + bottle + methanol: _____ g	
<input type="checkbox"/> Surrogate      Solution ID: _____ Volume added (100 $\mu$ L): _____ $\mu$ L	
<input type="checkbox"/> QA Samples – Spike Mixture Solution ID: _____ Volume added (100 $\mu$ L): _____ $\mu$ L	
<input type="checkbox"/> Sample through column _____ g Sample + plastic beaker _____ g Plastic beaker _____ g	
<input type="checkbox"/> Flow Rate (= Sample wt. extracted/Time) _____ g Start time _____ hr:min Finish time _____ hr:min	
<input type="checkbox"/> Remove excess water. Write station ID,date,time, on column. Store in 40-mL vial @ 4°C.	
<b>NWQL INFORMATION</b>	
Lab ID: _____ Set#: _____ Date Received _____ <input type="checkbox"/> Dry Column with N <sub>2</sub> or CO <sub>2</sub> : _____ Date: _____ Pressure: _____ lb/in <sup>2</sup> Time: _____ min Dry SPE column wt.: _____ g	
<input type="checkbox"/> SPE Elution _____ Date: _____ Add 1.8 mL elution solvent _____ mL	
<input type="checkbox"/> Internal Standard (PAH-dn mixture in toluene keeper) Solution ID: _____ Volume added (100mL): _____ mL	
<input type="checkbox"/> Evaporate solvent - nitrogen Pressure: _____ lb/in <sup>2</sup> Time: _____ min	
Analysis: Instrument ID: _____ Date: _____ Comments: _____	

**Figure 5-2.** Worksheet for C-18 solid-phase extraction of pesticides.

***Prepare to process samples onsite using the C-18 SPE column:***

1. Cover a bench or table with a sheet of aluminum foil to make a clean work surface. Put on appropriate disposable, powderless gloves.
2. Collect and split samples using the appropriate procedures (NFM 4; NFM 5.1; Sandstrom and others, 1995). Filter the samples as instructed in section 5.2.2. Wear gloves (usually latex or nitrile) during sample collection and processing.
3. Set up the necessary equipment and supplies and assemble them on the clean work surface. Remove the aluminum foil wrapping from the precleaned equipment.
4. Record the sampling site information, the lot number and dry weight of the C-18 SPE column, and the surrogate solution identification number on the Schedule 2010 worksheet (fig. 5-2).
5. Change gloves.
6. Tare the weight of a clean amber glass 1-L sample bottle and a 1-L plastic beaker to the nearest gram using an analytical balance and record the weights on the Schedule 2010 worksheet.

***Extract the sample:***

Use the appropriate surrogate solution mixture supplied by the NWQL for the C-18 SPE method with each environmental sample.

**SAMPLE EXTRACTION SHOULD BE COMPLETED ONSITE, IF POSSIBLE. If onsite extraction is not possible, extract the sample within 4 calendar days of collecting the sample.**

1. Condition the SPE column:
  - a. Pipet 2 mL of pesticide-grade methanol into the C-18 SPE column and allow it to flow through the column by gravity. Collect the methanol rinse in a proper container for disposal.
  - b. Remove any excess methanol by rinsing approximately 2 mL of PBW, by gravity, through the column. The rinse water/methanol mixture must be disposed of according to local, State, or Federal regulations.

c. **Do not allow the SPE column to go dry once the conditioning has started.**

- If the column goes dry, repeat the conditioning process.
  - To keep the column from drying out once the conditioning has started, maintain water in the C-18 SPE column by replacing water that drained through the column. Alternatively, attach an on/off valve-to-column outlet to prevent complete draining before the sample is extracted.
2. Following the filtration instructions for general organic compounds (5.2.2.A or Sandstrom, 1995), pass about 1 L of sample through the a glass microfiber filter into the tared bottle, leaving about 2 cm of headspace.
  3. Weigh the filled bottle and record the weight on the worksheet (fig. 5-2).
  4. Add about 10 mL of methanol to the filtered sample using the bottle-top dispenser or a volumetric pipet. Weigh and record the sample-plus-methanol weight on the worksheet.
  5. Add the surrogate solution contained in the 2-mL amber screw-cap vial to the filtered sample as follows (refer to Spike Kit Instruction Manual for detailed information and instructions on use of a micropipet):
    - a. Withdraw the surrogate solution from the 2-mL amber screw-cap vial using a clean 100- $\mu$ L micropipet and a clean glass bore.
    - b. Insert the tip of the glass bore into the sample bottle below the surface of the sample, and depress the plunger to deliver the surrogate to the sample. (Tip the bottle on its side, if necessary, to reach below the surface of the sample with the glass bore.)
    - c. Keeping the plunger depressed, swirl pipetor in water several times and then withdraw the micropipet from the bottle. Release the plunger, then remove the used glass bore from the micropipet and discard properly.
    - d. Rinse the fluorocarbon polymer tip of the micropipet with methanol.
    - e. Add the field-matrix spike as dictated by the study's quality-assurance plan, as required.
    - f. Cap and swirl the sample to mix the sample + surrogate. (For spiked samples, mix sample + surrogate + spike solution.)
    - g. If a duplicate will be submitted for analysis, repeat steps 5a–f on the duplicate sample.

6. Extract the sample through the SPE column using a metering pump fitted with 3.18 mm (1/8 in.) fluorocarbon polymer tubing with appropriate connectors (Sandstrom, 1995; NFM 2).
  - a. Insert clean tubing from the inlet side of the pump into the sample bottle.
  - b. Turn on the pump, flush air from the tubing (be careful to minimize any sample discharge from the end of the tubing), and then attach the outlet side of the tubing to the small end of the SPE column.
  - c. Invert the SPE column to drain any remaining conditioning water left in the SPE column reservoir.
  - d. Begin extraction by pumping the sample through the column at a rate of 20 to 25 mL/min and collect the extracted water into the tared 1-L plastic beaker.
7. After the sample has been pumped through the SPE column, turn the pump off and disconnect the column.
8. Remove excess sample from the SPE column using a syringe with 10 to 20 mL of air to push excess sample into a plastic beaker.
9. Weigh the beaker containing the volume of sample extracted through the SPE column. Subtract the tare weight of the beaker from the weight of the beaker plus the extracted sample and record this weight on the worksheet.
10. Write the sample identification number and the sampling date and time on the side of the SPE column. Place the SPE column into a 40-mL glass or plastic shipping ampoule and wrap it in aluminum foil.
11. Finish filling out the worksheet (fig. 5-2). Wrap the completed worksheet around the shipping ampoule and secure it with a rubber band or tape. Place in a sealable plastic bag.
12. Chill the SPE column immediately and maintain between 4°C and 25°C during storage and shipping.
13. Keep a copy of the worksheet for the field folder.

14. Field clean all equipment, including the pump and tubing, immediately after use and before going to the next site (NFM 3).
  - a. Rinse thoroughly with about 50 mL of a 0.2-percent solution of a phosphate-free laboratory detergent, followed by about 50 mL of tap water (or DIW) to remove the detergent.
  - b. Final rinse with about 30 to 50 mL of methanol. Collect used methanol into an appropriate container for disposal.
15. After cleaning, wrap all the equipment apertures with aluminum foil.

**Ship the SPE column to the laboratory immediately. Elution from the SPE column must be completed within 7 days of extraction.**

### 5.3.2 SOLID-PHASE EXTRACTION BY CARBOPAK-B™ COLUMN

The Carbopak-B™ method currently (November 1998) is used for NWQL schedule 2051, which is for analysis of a broad spectrum of field-extracted pesticides.<sup>9</sup> Detailed descriptions of the method and the laboratory and field-extraction procedures can be found in Werner and others (1996). General equipment needs for solid-phase extraction are listed in table 5-7. For Carbopak-B™ SPE processing, obtain the SPE column, Carbopak-B™, 500 mg, precleaned; surrogate mixture and field-matrix spike-solution mixture for Carbopak-B™ SPE and PBW; ascorbic acid solution, 10 g/L; and reagent-grade sodium chloride (NaCl) 10 g/sample.

<sup>9</sup>The Carbopak-B™ method is graphitized carbon-based solid-phase extraction used with a high-performance liquid chromatographic analytical method for determining 41 pesticides and pesticide metabolites that are not readily amenable to gas chromatography or other high-temperature analytical techniques (Werner and others, 1996).

***Quality-control samples are required as an integral part of the sampling program.***

- ▶ Process a field blank with the first sample. Process additional field blanks about every 10 to 20 samples:
  - Use pesticide-grade blank water (PBW).
  - Process the blank in the same manner as the environmental water sample.
- ▶ Process field-matrix spikes about every 20 samples. When processing a field-matrix spike:
  - Use a 100- $\mu$ L micropipet to add the field-matrix-spike solution to two of the triplicate samples. Follow the instructions provided in the spike kit.
  - Add the surrogate to every matrix-spiked sample and associated unspiked sample.
  - Record lot number and concentration of spike-solution mixture on the NWQL Schedule 2051 worksheet (fig. 5-3).

***Before beginning field work, prepare an ascorbic acid solution in the office laboratory:***

Each Carbopak-B™ requires 15 mL of ascorbic acid solution. Check that you have the volume needed before leaving for the field site(s).

The ascorbic acid solution must remain capped and chilled unless in use. The shelf life of the solution is 28 days—discard if shelf life has been exceeded or if the solution has been left uncapped or unchilled.

1. Place a tared, 1-L amber glass pesticide bottle (cleaned at the NWQL) on an analytical balance and fill to 500 g with PBW (pesticide-grade organic-free water purchased from NWQL DENSUPPL).
2. Empty a 5-g vial of ascorbic acid into the 500 g of PBW to obtain a 10-g/L ascorbic acid solution. Cap immediately and shake to dissolve.
3. Label the bottle with the date and preparer's name, contents of the solution, and the concentration of ascorbic acid.
4. Refrigerate the solution immediately and keep chilled until ready for field use. Transport to the field on ice in a foam sleeve.

**Schedule 2051 Field Extraction  
Checklist and Reporting Sheet  
Solid-Phase Extraction, HPLC  
Analysis, Filtered Water**

Station ID or Unique Number: \_\_\_\_\_  
 Station Name: \_\_\_\_\_  
 Date: \_\_\_\_\_  
 Time: \_\_\_\_\_  
 Collector: \_\_\_\_\_

- |  |                                   |               |
|--|-----------------------------------|---------------|
| <input type="checkbox"/> Filter Sample   | 0.7- $\mu$ m glass fiber filter   |               |
| <input type="checkbox"/> SPE Cartridge Conditioning                              | Ascorbic acid solution<br>[15 mL] | _____ mL      |
| <input type="checkbox"/> Sample  | Sample + bottle weight            | _____ g       |
|  | - bottle tare weight              | _____ g       |
|  | = sample weight                   | _____ g       |
| <input type="checkbox"/> Surrogate   | Solution lot number               | _____         |
|  | Volume added                      | _____ $\mu$ L |
| - QA Samples Spike Mixture   | Solution lot number               | _____         |
|  | Volume added                      | _____ $\mu$ L |
| <input type="checkbox"/> Sample through Cartridge                                | Sample + plastic beaker           | _____ g       |
|  | - plastic beaker                  | _____ g       |
|  | = volume of sample extracted      | _____ mL      |
| <input type="checkbox"/> Flow Rate   | Start time                        | _____ hr:min  |
|  | Stop time                         | _____ hr:min  |
| <input type="checkbox"/> Write Station ID Number and Sampling Date on Cartridge  |                                   |               |
| <input type="checkbox"/> Remove Excess Water                                     |                                   |               |
| <input type="checkbox"/> Replace Cartridge in Shipping Container and Store @ 4°C |                                   |               |
| <input type="checkbox"/> Comments:   |                                   |               |

**Figure 5-3.** Worksheet for Carboxpak-B™ solid-phase extraction of pesticides.

***Prepare to process samples onsite using the Carbopak-B™ column:***

1. Put on disposable, powderless gloves during sample collection and processing. Cover a bench or table with a sheet of aluminum foil to make a clean work surface.
2. Collect and split samples using the procedures described in NFM 4 and NFM 5.1 (refer also Sandstrom and others, 1995; Werner and others, 1996).
3. Set up the equipment and assemble supplies on the clean work surface. Remove the aluminum foil wrapping from equipment.
4. Begin to fill out the NWQL Schedule 2051 worksheet (fig. 5-3), recording the type, lot number, and dry weight of the Carbopak-B™ SPE column.
5. Put on a new pair of gloves.
6. Tare the weight of a clean amber glass, 1-L sample bottle and a 1-L plastic beaker to the nearest gram using an analytical balance. Record the weight on the worksheet provided with each column.
7. Following the filtering instructions for general organic compounds (section 5.2.2.A or Sandstrom, 1995), filter about 1 L of sample through a glass microfiber filter into the tared bottle, leaving about 2 cm of headspace.
8. Weigh the filled bottle and record the weight on the worksheet (fig. 5-3).
9. Calculate and record the sample weight.

***Extract the sample:***

When extracting the sample, be sure to use the appropriate surrogate solution mixture supplied by the NWQL for the Carbopak-B™ SPE method. Add surrogate solution to all samples including field blanks, replicates, and field-matrix spikes.

**Sample extraction must take place within 4 days of sample collection.**

1. Withdraw surrogate mixture using a clean 100- $\mu$ L micropipet and glass bore (detailed instructions on the use of a micropipet are included in the NWQL spike kit).
2. Insert the tip of the glass bore below the surface of the sample in the sample bottle and depress the plunger to deliver the surrogate mixture. (Tip the bottle, if necessary, to reach below the surface of the sample with the micropipet tip.) Keeping the plunger depressed, swirl sample with the pipetor several times and then withdraw the micropipet. Release plunger, then remove and discard the used glass bore.
3. Leave approximately 2 cm of headspace for the addition of NaCl.
4. Rinse the tip of the micropipet with methanol.
5. Add 10 g of NaCl to each sample. Cap and swirl the sample.
6. Process field-matrix spikes, if dictated by the study's quality-assurance plan. To process spikes, set aside three subsamples and spike two of the three subsamples with spike-solution mixture obtained from the NWQL spike kit. Follow the instructions provided with the kit.
7. Fill a clean glass graduated cylinder or beaker with 15 mL of ascorbic acid solution.
8. Using a metering pump fitted with 1/8-in. fluorocarbon polymer tubing and appropriate connectors:
  - a. Turn on the pump.
    - Adjust the pump flow rate to deliver about 20 to 25 mL/min (1 drop per second).
    - Test the flow rate by pumping the cleaning solution into a graduated cylinder or beaker and timing with a stopwatch.
  - b. Attach the outlet end of the pump tubing to the SPE-column adapter.
  - c. Remove the SPE column from the shipping container and attach to the adapter. (The open end of the SPE column should fit tightly over the adapter; make sure the column is sealed completely against the lip of the adapter to create a leak-proof seal.)
  - d. Place the inlet end of the pump tubing into 15 mL of ascorbic acid and pump the ascorbic acid solution through the column at a rate of 20 to 25 mL/min.
9. After all ascorbic acid solution has been pumped through the column, continue to pump air through the column for 1 minute. The conditioned column is now ready for sample extraction.  
**Extract sample onto the column within 8 hours of conditioning with ascorbic acid.**

10. Insert the inlet end of the pump's fluorocarbon polymer tubing into the sample bottle to begin sample extraction.
11. Pump sample through the Carboxpak-B™ SPE column at a rate of 20 to 25 mL/min and collect extracted water in tared 1-L plastic beaker.
12. After the sample has been pumped through the column, turn off the pump and disconnect the SPE column.
13. Remove excess sample from the SPE column by using a syringe with 10 to 20 mL of air to push the excess sample into the tared, 1-L plastic beaker.
14. Weigh the beaker with the volume of sample processed through the SPE column (subtract tare weight of beaker from weight of beaker plus sample) and record the weight of the sample processed through the column on the worksheet (fig. 5-3).
15. Write the station identification number and the sampling date and time on the side of the SPE column and place the SPE column in a shipping container (40-mL glass or plastic ampoule). Complete the worksheet, wrap it around the shipping ampoule, and secure it with a rubber band or tape. Place SPE-column sample in a sealable bag. Keep a copy of the worksheet for the field folder.
16. Chill the SPE-column immediately and maintain at 4°C during storage and shipping.

**Ship the SPE column to the laboratory immediately. Elution from the SPE column must be completed within 7 calendar days of extraction.**

17. Field clean all equipment including the pump and tubing immediately after use (NFM 3) and before going to the next site. Rinse thoroughly with about 50 mL of a 0.2-percent solution of phosphate-free laboratory detergent, followed by about 50 mL of tap water or DIW to remove the detergent. Final rinse with 30 to 50 mL of methanol. Collect methanol rinse into an appropriate container. After cleaning, wrap all equipment apertures with aluminum foil.