Standard operation procedure (SOP) for the extraction and cleanup of PCBs from water samples, Amberlite® XAD-2 Resin

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1.0 Scope and Application

This method covers the extraction and cleanup for the analysis of PCBs in Amberlite XAD-2 resin utilized in water sampling. The samples are cleaned up by applying sulfuric acid, different sulfuric acid impregnated silica gel columns, and sodium sulfate columns. The instrumental analyses are performed by gas chromatography (GC) with tandem mass spectrometry (MS/MS) and are described elsewhere.

2.0 Interferences and QA/QC

2.1 Method blanks

Interferences from glassware, solvent and chemicals are monitored by running method blanks. The method blank is spiked with surrogate standards and run through the entire extraction process and clean up steps in parallel with the samples. In every series of 6-11 water samples, one method blank should also be run. The method blank is prepared by saturating approximately 35-70 grams of XAD-2 resin with deionized water in an ASE300 cell. The saturated resin is then spiked with the surrogate standards and processed with the samples.

2.2 Control of GC Instruments

Pure hexane is run as the first sample for all instruments to check for instrumental interferences. Hexane should also be run between calibration standards and samples for control of memory effects. Also the last sample should be pure hexane to check the instrument.

A calibration standard with concentrations close to the limit of detection should be included to check the response of the instrument.

2.3 Cleaning of glassware

All glassware is rinsed with hot water as soon as possible after use. The glassware is left in soapy water no longer than 24 hrs and rinsed with tap water until soap bubbles are gone followed by 2 rinses with DI water. All glassware is heated in a furnace at 450 °C overnight. After cooling, the glassware is capped with aluminum foil and stored in a clean environment to be protected from dust and other contaminants. Plastic caps are washed in the same method as the glassware, followed by a triple-rinsing of methanol, hexane, and acetone. Caps are also stored in a clean environment protected from dust and other contaminants.

2.4 Surrogate Recovery

Before starting the extraction procedure all samples are spiked with surrogate standards (SS). At the same time, one empty GC vial is also spiked with the

same surrogate standard for each class of compounds. After the water samples have gone through the extraction and clean up steps the samples are evaporated to an appropriate volume. Then the samples AND the GC vial that contains the SS are spiked with the internal standard. These GC vials represent 100% of what has been added to the samples and will be used for the calculation of the recovery of the SS in the samples.

3.0 Apparatus

3.1 Apparatus

- Dionex ASE 300 Accelerated Solvent Extraction System
- Biotage TurboVap II Concentration Evaporator Workstation
- Mettler Toledo AG245 Analytical Balance
- Agilent 7000C Triple Quadrupole GC/MS System
- Silica Column Holder (custom)
- Beckman J2-21 Floor Model Centrifuge
- Barnstead International LABQUAKE Shaker/Rotator with double-deck trays and bar w/ 32 clips, Cat. No. C415110 or C415220
- Thermolyne 30400 Furnace

3.2 Glassware and Supplies

- Fisher Scientific 9" Disposable Pasteur pipettes, borosilicate & non-sterile, Cat. No. 13-678-20D
- 16x125 mm Pyrex test tubes w/ Teflon-lined screw caps
- Glass funnel (custom)
- Fisher Scientific Amber Large ID Crimp GC sample vials (2mL capacity), Fisher Cat. No. 03-391-6
- Teflon aluminum crimp caps with Teflon septa, SUN-Sri 200 100 TFE/RUB 11MM seal
- Glass wool, Pyrex borosilicate, Fisher Cat. No. 11-388 from Corning, Inc. X3950
- Fisher Scientific polytetrafluoroethylene (PTFE) – Silicone Septa, Item No. 288-7222
- Restek Peek Washer, Cat. No. 25394
- Fisher Scientific 500 mL Pyrex Separatory Funnel, No. 6402
- Biotage 200 mL Evaporation Tube w/ 1 mL endpoint, Cat. No. C128506
- Blotage 50 mL Evaporation Tube w/ 1 mL endpoint, Cat. No. C128511
- Dionex Corp. 30mm Quartz Filters for ASE, P/N 056781
- Kimberly-Clark Purple Nitrile Powder-Free Exam Gloves
- Reynolds FOODSERVICE FOIL, 620 Heavy Duty Aluminum Foil
- Hamilton 1710 Gastight Syringe, 100 µL, Cemented, 22s G, 2"

4.0 Chemicals and Solvents

4.1 Raw Materials

- Silica gel, Flash Chromatography Grade, 70-230 Mesh, S826-1 — Fisher Scientific
- Hexanes, Pesticide Grade, H300-4 — Fisher Scientific
- Methylene Chloride, Pesticide Grade, D142-4 — Fisher Scientific
- Acetone, Pesticide Grade, A40-4 — Fisher Scientific
- Methanol, Pesticide Grade, A450-4 — Fisher Scientific
- Water optima, W7-4 — Fisher Scientific
- Sulfuric Acid certified ACS plus, A300-212 — Fisher Scientific
- Sodium Sulfate, 99% pure, anhydrous, 7757-82-6 — Acros Organics
- Amberlite XAD-2 Resin — Supelco

4.2 Extraction Solvent

- 1:1 Hexane:Acetone (v/v)

4.3 Preparation of Acidified Silica Gel Columns

Combust the silica gel overnight at 450 °C. When it has cooled, transfer the gel to a screw-capped glass bottle. Weigh a portion of the gel in a new screw-capped glass bottle and add by weight concentrated H_2SO_4 to a relationship of 2:1 (silica gel:acid). Shake the bottle thoroughly until no lumps are seen.

The columns are prepared by stuffing a small amount of glass wool in the bottom of a 9" Pasteur pipette and then using a small glass funnel to add 0.1 g of pure activated silica gel. Then 1 g acidified silica gel is added on the top.

5.0 Standards

5.1 Surrogate Standards (SS)

5.1.1 PCBs

3,5-Dichlorobiphenyl	(^{13}C PCB 14)
Deuterated 2,3,5,6-Tetrachlorobiphenyl	(^{13}C PCB D65)
2,3,4,4',5,6-Hexachlorobiphenyl	(^{13}C PCB 166)

5.2 Internal Standards (IS)

5.2.1 PCBs

Deuterated 2,4,6-Trichlorobiphenyl	(^{13}C PCB D30)
2,2',3,4,4',5,6,6'-Octachlorobiphenyl	(^{13}C PCB 204)

5.3 Calibration Standards

5.3.1 PCBs

PCB calibration mixture contains all 209 PCB congeners, the SS, and the IS.

6.0 Sample handling

The water samples should be kept in a freezer at approximately -20 °C until extraction. When it is time for extraction, let the water sample warm up to room temperature.

7.0 Blank Sample Preparation

1. Collect all necessary materials (Figure 1 provides an example)

Figure 1: Blank Sample Preparation Materials

2. Pack quartz wool into one end of an ASE cell and wrap foil to cover the end.
3. Pour approximately 35 grams of cleaned Amberlite XAD-2 Resin into the ASE cell.
4. Saturate the sampling media with deionized water (100-200 mL).
5. Remove quartz wool with tweezers.
6. Place two quartz filters into one end of the ASE cell and tighten extraction cap to that end.
7. Place the cell upright and cover top with aluminum foil. Method blank is now ready to be spiked with the surrogate standard (SS). (PCB-14, PCB-D65, PCB-166)
8. Repeat steps 2-7 for each method blank then proceed to SS spiking.

8.0 Surrogate Standard Spike

1. Remove SS from freezer and place in fume hood to allow SS to reach approximately room temperature.
2. While waiting for SS to warm up, clean a 100 µL gas-tight syringe with dichloromethane (DCM) and hexane. Use alternating rinse technique, hexane then DCM. Complete three rinses of each and finish with three hexane rinses.
3. Create reference sample. Place 0.5-0.75 mL of hexane into an Amber Large ID Crimp GC sample vial.
4. Once SS is approximately room temperature, remove aluminum foil from a sample and spike with a desired amount of SS (typically 25-30 ng).
5. After spike, place two quartz filters into the open end of the ASE cell and tighten on extraction cap.
6. Repeat step 3 and 4 for each sample. Remember to spike reference with the same amount as a sample.
7. Now that all samples are spiked and capped, place ASE cells and sample collection bottles on ASE 300 (Figure 2).

Figure 2: Dionex ASE 300 Accelerated Solvent Extraction System

9.0 Sample Extraction

1. Rinse the ASE 300.
2. Load extraction method and start extraction process. If ASE errors occur, consult manual for handling the situation.

ASE 300 Method Conditions	
Heat	5 min
Static Time	5 min
Flush Volume %	55%
Purge Time	60 sec
Pressure	1500 psi
Temperature	75°C
Cycles	1

3. Once samples have been extracted, empty out ASE cells into appropriate container for disposal (double layered foil is adequate).

10.0 Liquid-Liquid Extraction

1. Pour sample into clean separatory funnel (Figure 3).
2. Transfer the aqueous layer back into the same ASE collection bottle and transfer the organic layer into a 200 mL evaporation tube. Cover sample with aluminum foil.
3. Add 30 mL of hexane to the sample collection bottle containing the aqueous layer.
4. Pour mixture into separation funnel, cap the funnel, and swirl the mixture for two minutes. Let the mixture sit for 2-4 minutes so that, if an emulsion occurred, it will separate.
5. Remove the water layer back into the same ASE collection bottle and transfer the organic layer into a 200 mL evaporation tube. Cover sample with aluminum foil.
6. Repeat steps 3-5 (three times)
7. Complete the same process for each sample. Remember to triple rinse the separatory funnel in between samples to prevent cross contamination.
8. Concentrate samples to 2 mL utilizing the TurboVap II Concentration Evaporator Workstation. After concentration, a water layer (typically 0.25-1 mL) will be present (Figure 3). Remove the water through the use of sodium sulfate columns or sulfuric acid cleanup. The choice between which is utilized is based upon the chemicals being analyzed. PCBs only – either method, Other POPs – sodium sulfate columns.

Figure 3: (Left) Sample Present in Separatory Funnel (Right) Sample after TurboVap Treatment

11.0 Water Removal

11.1 Sodium Sulfate Columns

1. Place a small amount (1-2 cm) of heat-treated quartz wool into a 9" Pasteur pipette. Lightly pack the wool to the bottom of the pipette.
2. Pour 2-2.5 grams of heat-treated sodium sulfate into the pipette. Prepare one column for each sample.
3. Place sodium sulfate columns on column holder.
4. Prepare one 50 mL evaporation tube and a pipette for each sample. Fill an Erlenmeyer flask with 50-150 mL of hexane (dependent on batch size).
5. Prime the columns with hexane. Ensure that the columns NEVER dry out.
6. Pipette off the top, organic layer of a sample and transfer it to the corresponding column with an evaporation tube under it.
7. Add 1-2 mL of hexane to remaining aqueous layer and swirl for one minute.
8. Transfer the organic layer into the column.
9. Repeat steps 7 and 8.
10. Once all sample and sample rinses have been run through the column, rinse hexane through the column until 12 mL of solution is present in the evaporation tube.
11. Repeat steps 5-10 for each sample. Multiple columns can be performed at the same time just as long as the samples aren't cross contaminated or the columns do not run dry.
12. Concentrate samples to 0.75-1 mL utilizing the TurboVap II Concentration Evaporator Workstation.

11.2 Sulfuric Acid Cleanup

1. Transfer sample to 16x125 mm Pyrex test tube with Teflon-lined screw cap
2. Add 1 mL of concentrated sulfuric acid to the sample.
3. Screw on cap and use paper towel to ensure cap does not leak.
4. Repeat steps 1-3 for each sample.
5. Once all samples are prepared, tumble for two minutes on the Barnstead International LABQUAKE Shaker/Rotator (Figure 4).
6. Centrifuge samples in the Beckman J2-21 Floor Model Centrifuge on the following settings (Figure 4).

   Rotor: 10 Speed: 3000 rpm Temperature: 20°C
   Time: 5 minutes

7. After centrifuging, transfer organic layer to 50 mL evaporation tube. Cover the sample with aluminum foil.
8. Add 3 mL of hexane to remaining mixture in test tube. Check to ensure cap does not leak
9. Complete hexane extraction twice for each sample. Repeat steps 5-7.
10. Concentrate each sample to 0.75-1 mL utilizing the TurboVap II Concentration Evaporator Workstation.

Figure 4: Sulfuric Acid Cleanup

12.0 Column Cleanup

1. Place a small amount (1-2 cm) of heat-treated quartz wool into a 9" Pasteur pipette. Lightly pack the wool to the bottom of the pipette.
2. Pour 0.1 gram of heat-treated silica gel to the pipette.
3. Pour 1 gram of acidified silica gel on top of the heat-treated silica layer. Prepare one pipette for each sample.
4. Place silica gel columns on the column holder.
5. Prepare one 50 mL evaporation tube and a pipette for each sample. Fill an Erlenmeyer flask with 50-150 mL of hexane (dependent on batch size).
6. Prime the columns with hexane. Ensure that the columns NEVER dry out.
7. Transfer the sample to the corresponding column with an evaporation tube under it.
8. Rinse the sample container with 1 mL of hexane.
9. Transfer the hexane to the column.
10. Repeat steps 8 and 9
11. Once all sample and sample rinses have been run through the column, rinse hexane through the column until 12 mL of solution is present in the evaporation tube.
12. Repeat steps 5-11 for each sample. Multiple columns can be performed at the same time just as long as the samples aren't cross contaminated or the columns do not run dry (Figure 5).
13. Concentrate samples to 0.75-1 mL utilizing the TurboVap II Concentration Evaporator Workstation. After concentration, samples will be ready to be spiked with the internal standard (IS) (PCB-D30, PCB-204)

Figure 5: Silica Gel Column Cleanup

13.0 Internal Standard Spike

1. Remove IS from freezer and place in fume hood to allow IS to reach approximately room temperature.
2. While waiting for IS to warm up, clean a 100 µL gas-tight syringe with dichloromethane (DCM) and hexane. Use Use alternating rinse technique, hexane then DCM. Complete three rinses of each and finish with three hexane rinses.
3. Transfer all samples to corresponding Amber Large ID Crimp GC sample vials. Each vial should be labeled with lab technician's initials, current date, and sample/batch identification number.
4. Once IS is approximately room temperature, spike each sample and the reference with the desired amount of IS (typically 25-30 ng).
5. Crimp all vials. Proceed to sample analysis.

14.0 Sample Analysis

1. Place all GC sample vials on sample tray of Agilent 7000C Triple Quadrupole GC/MS System (Figure 6)
2. Write sequence for the batch.
3. Start sequence.
4. Once the calibration standard has been injected and begun analysis, recap the GC vial.
5. Wait for run to finish.
6. Recap all GC vials.
7. Analyze peak information.

Figure 6: Agilent 7890B GC System Equipped with 7693 Series Automatic Liquid Sampler Coupled to a 7000C Triple Quadrupole MS System.