

Standard Operation Procedure (SOP) For the Extraction and Cleanup of PCBs from Air Samples Collected With Polyurethane Foam (PUF), Amberlite® XAD-2 Resin, and Quartz Fiber Filters Using Dionex Accelerated Extraction Systems

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1.0 Scope and Application

This method covers the extraction and cleanup in preparation for the analysis of PCBs collected in polyurethane foam (PUF), Amberlite XAD-2 Resin (XAD), and quartz filter air samples. Briefly, samples are spiked with surrogate standard (SS), extracted using either a Dionex ASE 300 or ASE 350 Accelerated Extraction System, purified of water (if necessary) with sodium sulfate, taken through an acidified silica cleanup process, and spiked with internal standard (IS) before being run on an Agilent 7000C or 7000D Triple Quadrupole gas chromatography tandem mass spectrometry (GC/MS/MS) instrument for analysis. For an experienced technician, 12 samples can be comfortably taken through to completion in 8 hours.

2.0 Apparatuses and Materials

2.1 Apparatuses

- Dionex ASE 350 or ASE 300 Accelerated Solvent Extraction System
- Biotage TurboVap II Concentration Evaporator Workstation
- Agilent 7000C or 7000D Triple Quadrupole GC/MS System
- 9" Pasteur Pipette Holder (custom)
- Thermolyne 30400 Furnace

2.2 Glassware, Materials, and Supplies

- Fisher Scientific 9" Disposable Pasteur pipettes, borosilicate & non-sterile, Cat. No. 13-678-20D
- 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
- Dionex™ ASE™ 100 mL Stainless Steel Extraction Cells, Cat. No. 068091
- Biotage 200 mL Evaporation Tube w/ 1 mL endpoint, Cat. No. C128506
- Biotage 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

3.0 Chemicals and Solvents

3.1 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 |
| • Sulfuric Acid certified ACS plus, A300-212 | Fisher Scientific |
| • Sodium Sulfate, 99% pure, anhydrous, 7757-82-6 | Acros Organics |
| • Amberlite XAD-2 Resin | Supelco |
| • 1/2" x 5 1/2" (unwashed) poly-urethane vapor Collection substrates, TE-1014 | Tisch Environmental |
| • High Purity Quartz Microfibre Filters 8 x 10 ins | Whatman |

3.2 Preparation of Acidified Silica

Acidified silica is used as the primary sample cleaning material in this procedure, and should be made in the lab regularly. Combust silica overnight at 450 °C and allow it to cool. Weigh and pour a portion of the silica into a clear glass container that can be securely closed. Add enough concentrated sulfuric acid to the same container such that a ratio by weight of 2:1, silica:acid, is achieved. Securely close the container, and thoroughly mix the silica and acid mixture by vigorously shaking it until no lumps can be seen.

4.0 Quality Assurance and Quality Control

4.1 Sample Handling Before Extraction

Air samples, whether PUF, XAD, or quartz filters, need to be stored in a freezer that is no warmer than -21°C (-6°F) prior to extraction.

4.2 Method blanks

Each sample batch must contain one method blank of each type of sampling media being extracted. The method blanks are taken through the same handling procedure as the samples, and are typically run as the last sample in the batch. Method blanks are included as a means of control to account for unwanted and unknown sources of contamination, such as glassware interferences, impurities in solvents and standards, laboratory air, and

instrument carryovers. Method blanks from individual projects are later used in determining project and media specific limits of quantification.

4.3 Cleaning of Laboratory Materials

All reusable glassware is rinsed with methylene chloride immediately after use and subsequently washed with warm soapy water and allowed to air dry. Prior to use, all glassware associated with the ASE and Turbovap instruments is immediately thoroughly “triple rinsed” with methanol, acetone, and finally hexane. Combusting the glassware associated with these instruments causes warping, which interferes with sensor operation, and brittleness, which makes the glassware very fragile. The stainless steel ASE cells are also triple rinsed, as combusting at high heat causes them to corrode rapidly, diminishing their ability to form a tight seal under pressure.

Disposable items, such as Pasteur pipette test tubes, and reusable glassware that does come into direct contact with samples, can be combusted overnight at 450 °C before use. Reusable glassware still must be rinsed with methylene chloride and washed with warm soapy water after use and before combustion.

4.4 Control of GC/MS/MS Instruments

Clean hexane is run at the beginning of each sample batch analysis to assess baseline quality and any instrumental interferences. Clean hexane is also run after the calibration standard to control for carryover effects, as well as at the end of the sample batch to ensure that the instrument is free of any contamination. The inlet septa and liner are also regularly replaced, as their degradation over time is responsible for the majority of instrument interferences.

4.5 Surrogate Recovery

Extraction efficiency and sample losses are monitored with the use of surrogate standards. A predetermined amount of surrogate standard is added to each sample before extraction as well as to a reference GC vial containing only pure hexane. During the analysis, the amount of surrogate present in each sample is compared to the amount of surrogate present in the reference, which represents the amount of surrogate added to each sample before losses.

5.0 Standards

5.1 Surrogate Standards (SS)

Surrogate standard is added to samples before extraction, and is used to monitor extraction efficiency and sample losses. In our lab we have two different sets of surrogate standards we use depending on the project that the samples belong to. One set is a mixture of 10 different ¹³C labeled PCBs, with one PCB from each homolog group. The other is a set of 3 PCBs, one of which is deuterated.

5.1.1 ¹³C Labeled PCB Surrogate Standards

4-monochlorobiphenyl	(¹³ C PCB 3)
4,4'-dichlorobiphenyl	(¹³ C PCB 15)
2,4,4'-trichlorobiphenyl	(¹³ C PCB 28)
2,2',5,5'-tetrachlorobiphenyl	(¹³ C PCB 52)
2,3',4,4',5-pentachlorobiphenyl	(¹³ C PCB 118)
2,2',4,4',5,5'-hexachlorobiphenyl	(¹³ C PCB 153)
2,2',3,4,4',5,5'-heptachlorobiphenyl	(¹³ C PCB 180)
2,2',3,3',4,4',5,5'-octachlorobiphenyl	(¹³ C PCB 194)
2,2',3,3',4,5,5',6,6'-nonachlorobiphenyl	(¹³ C PCB 208)
Decachlorobiphenyl	(¹³ C PCB 209)

5.1.2 Non-¹³C Labeled PCB Surrogate Standards

3,5-Dichlorobiphenyl	(PCB 14)
Deuterated 2,3,5,6-Tetrachlorobiphenyl	(PCB D65)
2,3,4,4',5,6-Hexachlorobiphenyl	(PCB 166)

5.2 Internal Standards (IS)

Internal Standard is added immediately before analysis on the GC/MS/MS, and it is used to obtain the congener specific mass of all 209 PCBs. In our lab we use the following two congeners as internal standards:

Deuterated 2,4,6-Trichlorobiphenyl	(PCB D30)
2,2',3,4,4',5,6,6'-Octachlorobiphenyl	(PCB 204)

5.3 Calibration Standard

The PCB calibration mixture contains known concentrations of all 209 PCB congeners, the SS, and the IS.

6.0 Sample Prep in ASE Cells, Surrogate Standard Spike, and Reference Creation

6.1 Surrogate Prep

1. Before beginning any work, make sure you have a clean work station set up in the fume hood. Place a clean piece of heavy duty aluminum foil down to cover the entire area that you intend to work in.
2. Remove the surrogate standard from the freezer and allow it to warm up to room temperature inside of the fume hood.
3. Clean a gas tight syringe (usually either 25 or 100 μ L) by fully rinsing it 5 times with dichloromethane followed by 5 rinses with hexane.

6.2 Sample Prep

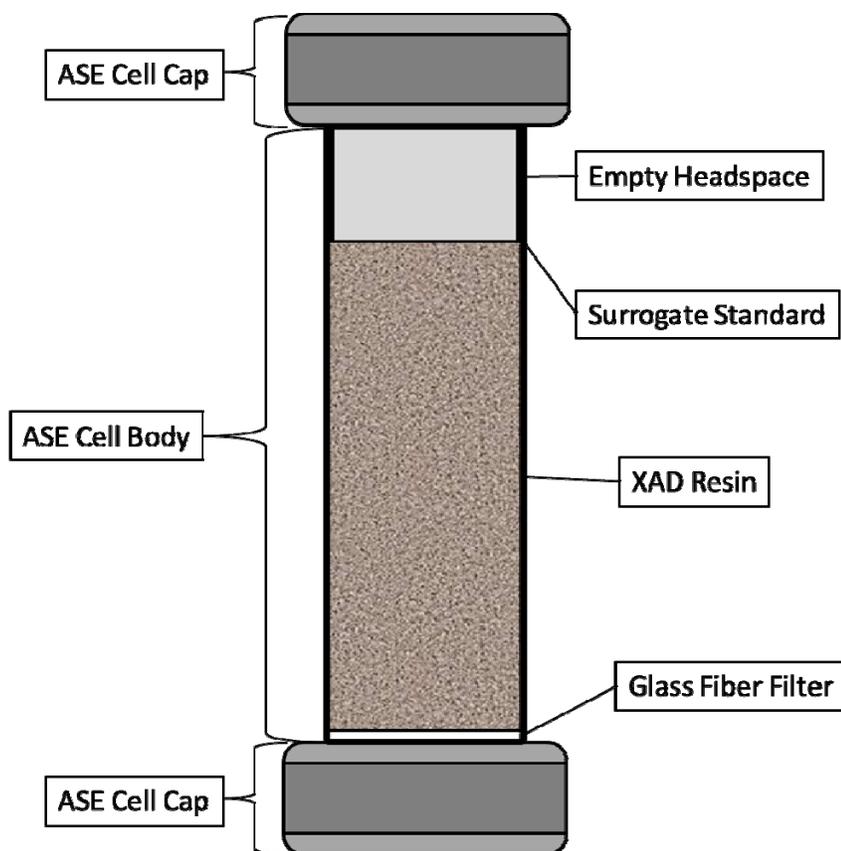
1. Thoroughly triple rinse ASE cells, caps, collection jars, and collection jar caps with septa immediately before use.
2. Screw a cap onto one end of each ASE cell.
3. Using either a long pair of tweezers or the plunger apparatus that belongs to the ASE, place a quartz fiber filter snugly at the bottom of the cap.
4. Being careful not to touch the sampling media with your gloves, carefully pour or pack the samples into ASE cells.
 - a. **For PUF:** Carefully unwrap the foil around the PUF sample and, holding onto it by the foil underneath it, fold it in half to form a "PUF taco". Grab one end of the folded PUF about an inch from the edge with a long pair of metal tweezers and, delicately, force the end of it into the ASE cell so that it stays partially in the cell when you let go of it with the tweezers. Use the tweezers to then fully stuff the PUF into the cell. Rinse the tweezers thoroughly with acetone before moving onto the next sample.
 - b. **For XAD:** Use a funnel to pour XAD into the cells. The XAD samples should be approximately 20 - 25 g, and should fill the ASE cells with about an inch of headspace remaining. If there is too much XAD to fit in one cell, you will have to weigh the sample and extract only a portion of

it, or you will have to divide the sample into two cells and combine them after extraction. Rinse the funnel thoroughly with acetone between samples.

- c. **For Quartz Filters:** Carefully unwrap the foil around the filter. The filters are often brittle and break quite easily after being stored in the freezer for an extended period of time. You will have to be creative and careful to get all of the filter material into the ASE cell without touching it. Try folding it over itself with the tweezers when possible. It may crumble, but just get all of the pieces into the cell as carefully as you can.

6.3 Surrogate Spike and Reference Creation

1. When samples are all in ASE cells, spike each sample with the same amount of surrogate standard. Allow the samples to sit open in the fume hood for a short amount of time (a few minutes) after spiking to allow the solvent to evaporate so that the PCBs in the surrogate sorb to the sampling media rather than stay in solution in hexane. At this same time, create the batch reference by spiking the same amount of surrogate into a GC vial containing ~ 0.7mL of hexane that you added to the samples. Cap the Reference immediately after adding the surrogate.
2. Screw the top caps onto the ASE cells, and make sure that both the top and bottom cap fit snugly. The cells are now ready to be extracted on the ASE. Below is a diagram of an ASE cell filled with an XAD air sample ready for extraction.
3. After creating the reference, immediately put the surrogate standard back in the freezer and again wash the syringe with dichloromethane and hexane.



7.0 Sample Extraction with the ASE 300 or the ASE 350

1. Turn on the ASE and open the nitrogen tank attached to it. Make sure that you have the appropriate pressure gauge attached to the nitrogen tank. The ASE requires at minimum 150 psi of pressure going into it in order to run. Also ensure that you have a sufficient amount of the appropriate solvents in the ASE's solvent reservoirs before beginning the extraction.
2. In the main menu, select "Load Method/Sequence" and load a method that has the following parameters. On our instrument, this is saved as Method 1.
 - a. Heat: 5 min
 - b. Static Time: 5 min
 - c. Flush Volume %: 60%
 - d. Purge Time: 200 sec
 - e. Pressure: 1500 psi
 - f. Temperature: 100 °C
 - g. Cycles: 1
 - h. Solvents: 1:1, acetone:hexane

If using the ASE 350, you can select the solvent types and ratios you want it to mix inside the extraction cell from up to 3 different solvent reservoirs at a time. If using an ASE 300, you will have to pre-mix your solvents before beginning the extraction.

3. Under the same screen, select the cell that you want the ASE to be running from.
4. Under the main menu select the "System Setup option". Under "Method Rinse", select "Yes". In the ASE 350, you can adjust this value; set the rinse volume to 5 mL. In the ASE 300 this value is not variable, but it is calibrated to rinse with 5 mL. Selecting this option rinses the lines with 5 mL of solvent between each sample. For environmental air samples, this has been shown to be a sufficient amount to prevent cross contamination in the ASE lines.
5. Load the cells and collection jars into the ASE appropriately.
6. Rinse the ASE by pressing the "Rinse" button.
7. Begin the extraction by pressing the "Start" button. When the ASE has reached the appropriate method temperature, it will begin extracting the first sample.

If extracting more than 6 samples in a batch, you can make the most of your time by first prepping, loading, and surrogate spiking the first 3 or 4 samples and beginning their extraction. While those are running on the ASE, you should have plenty of time to finish the prepping and surrogate spiking for the remaining samples. If running the ASE 350, you will have to stop the run by pressing the "Stop" button on the ASE and selecting option 1, "Abort the current run", before you can open the plastic shield door and load the final samples on the instrument. Wait until a sample and the subsequent rinse have finished before doing this. You will then have to load the method again and specify which cell you want it to begin the extraction from.

When the samples have finished running on the ASE, the collection jars can be capped with new triple rinsed septa and the sample extract (~ 100 mL) can be stored in the freezer overnight if necessary. Avoid spreading the extraction process as much as possible.

8.0 Concentration Using TurboVap II

1. Empty the ASE collection jars into freshly triple rinsed, large 200 ml TurboVap tubes. Rinse the collection jars with 5 ml of hexane and add each rinse to the appropriate TurboVap tube. Rinse the jars one time with

dichloromethane and waste the rinse. Allow the solvent to fully evaporate from the collection jars in the hood before washing with soap and water.

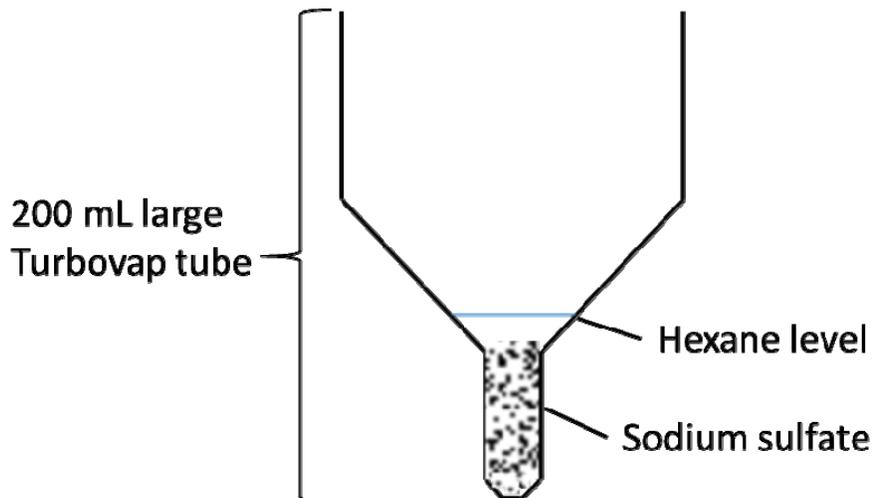
2. Concentrate the samples in the TurboVap using the sensor setting until slightly under 1 ml of sample remains.
3. Check if the samples contain water by adding a small amount of hexane to each TurboVap tube. If hexane is completely miscible with the sample, you can proceed to the section of this SOP on column cleanup. If, however, a distinct phase layer forms, or the sample turns cloudy and/or a precipitate forms at the bottom of the tube, there is water in you samples, and it must be removed before they can be run through the cleanup columns.

Neglecting to do so will result in a reaction between the water in the sample and the silica in the cleanup column that will not allow the sample to flow through, causing a significant loss of sample and poor cleanup performance.

If processing more than 6 samples in a batch, begin concentrating samples as soon as the first 6 have completed their extraction. You will be able to completely concentrate the first 6 samples as well as take them through most of (if not all) the rest of the procedure outlined in this SOP before the subsequent 6 samples (if running a batch of 12) have finished running on the ASE. Do not, however, concentrate the first 6 samples unless you plan on finishing the entire batch. It is best practice to process all of the samples in a batch in sequence with each other.

9.0 Water Removal Using Sodium Sulfate

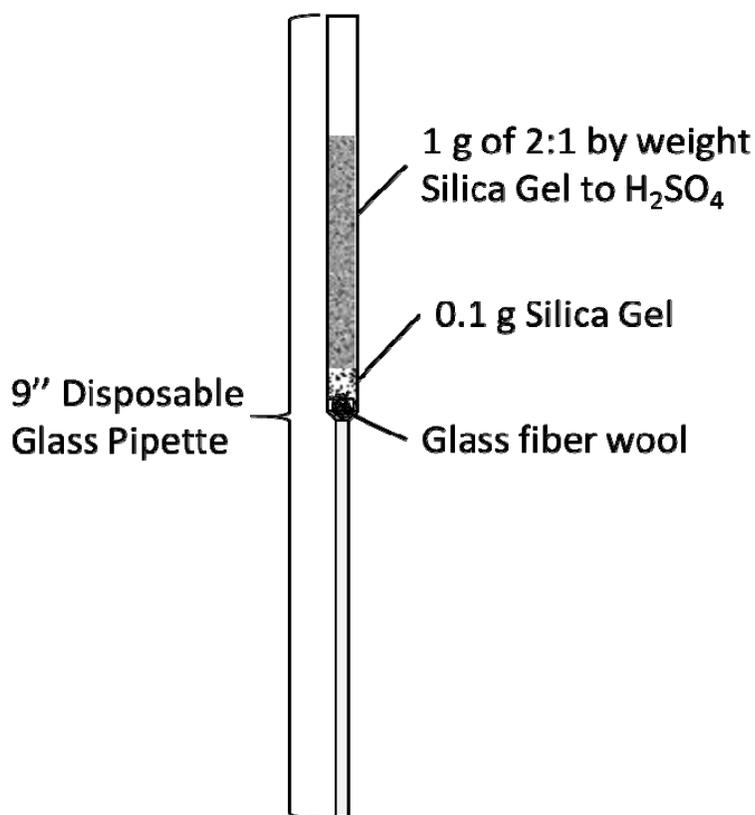
1. Add 5 ml of hexane to each sample that contains water. If there is at least one sample in the batch that contains water, be sure to add sodium sulfate to the blank, even if it does not have any water, in order to ensure that it is taken through the same steps as the most handled sample.
2. Vigorously shake the TurboVap tubes to ensure complete mixing of the water and hexane layers.
3. Add enough sodium sulfate to the TurboVap tube to absorb all of the water in the sample. The sodium sulfate will form a single semi-solid mass as it absorbs water. Add enough so that there is some free sodium sulfate remaining in the sample.
4. Concentrate excess hexane in the TurboVap. You will not be able to use the sensor setting for this, so monitor the instrument closely as the hexane will evaporate very quickly at this point. Stop the concentration when the hexane layer is approximately 5 mm above the level of the sodium sulfate, as shown in the figure below.



10.0 Column Cleanup

10.1 Column Prep

1. Using a small pair of tweezers and the tip of a 9" Pasteur pipette, gently pull apart a small amount (2-3 cm) of quartz wool and lightly pack it into a 9" Pasteur pipette. Take care not to pack the wool too tightly, as that can reduce the flow of sample through the column.
2. Using a small glass funnel, carefully add approximately 0.1 g of heat-treated silica, followed by approximately 1 g of acidified silica (2:1 sulfuric acid to heat-treated silica by weight). These masses do not need to be precise, rather the objective is to add enough acidified silica to adequately clean the samples before analysis, and enough heat treated silica at the bottom to ensure that the reaction products from the acidified cleanup do not wash through to the sample eluent. As a rough rule of thumb, add a little less than 1 cm of silica and then acidified silica until there is about 1 inch of space remaining at the top of the pipette. Below is a diagram of a cleanup column.



3. Place the pipettes into a pipette holder such that the tips can be fully inserted inside a small (50 ml) TurboVap evaporation tube. Do not place the TurboVap tubes under the columns yet. We use a custom built apparatus for this.
4. Although the components of the columns should be clean, perform a final cleaning step before the samples are added by running pure hexane through each column at least two times. One of these times, clean the outside of the columns by overfilling them with enough hexane such that hexane completely washes the outside of the columns. This way, if a column accidentally gets overfilled during the sample cleanup, or some hexane misses its target when it's getting added to the columns to elute the samples, any solvent that makes its way into the sample eluent in an unintended manner will be less likely to pick up unwanted contaminants.
5. As the hexane washes through the columns, look carefully at the section of the column that tapers from the wide section to the narrow section. More often than not, a small air bubble forms here, greatly reducing the flow of hexane through the column. If this is the case, add hexane to the column and gently push the air bubble out by lightly forcing the hexane through with a rubber pipette bulb. Be careful to remove the bulb without sucking any hexane back up through the column as you do this.

Preparing the Columns can be a bit time consuming. If you are planning to extract the samples and take them through the cleanup in the same day (which you should do if possible), make the columns immediately after all of the samples are loaded onto the ASE. Do not perform the cleaning with hexane, however, until immediately before the column cleanup step below.

10.2 Column Cleanup

1. After the columns have been washed with hexane, place a freshly triple rinsed 50 ml TurboVap evaporation tube under each column.
2. Using a 9" Pasteur pipette, transfer each sample from the 200 ml TurboVap tubes to their appropriate columns.
3. After the samples are transferred to their columns, rinse their TurboVap tubes at least three times each with ~1 ml of hexane, transferring each rinse to the cleanup column. Thoroughly rinse the sides of the tube with each hexane rinse. If you added sodium sulfate to the tube, thoroughly rinse it too. Before adding each hexane rinse to the column, allow the previous rinse to completely vacate the head space of the cleanup column pipette to avoid continually diluting a small amount of the initial sample addition; the column cleanup is more efficient the closer to a plug flow model the sample flow approximates.
4. After the samples and sample rinses have been run through the column, rinse each column by pipetting hexane until the small TurboVap tubes collect a volume of at least 10 ml.

At this point, the TurboVap tubes can be capped with the same type of triple rinsed caps and septa used for the ASE collection jars and stored in the freezer overnight if necessary.

If you mistakenly run a sample through a column without removing all of the water (it happens) and the column stops flowing, you will have to force the sample through with a pipette bulb. Fill the head space with hexane and gently force the solvent through. Repeat this until a volume of 10 ml is collected in the 50 ml TurboVap tube, concentrate the sample to slightly less than 1 mL, check for water (and remove any if necessary), and run it through a fresh cleanup column. This is a pain, so be diligent in your water removal efforts.

11.0 Final Concentration and Internal Standard Spike

1. Remove the internal standard (IS) from the freezer and allow it to come to room temperature in the fume hood.
2. Clean a gas tight syringe (usually either 25 or 100 μL) by fully rinsing it 5 times each with dichloromethane followed by hexane.
3. Concentrate the samples to 0.75-1 mL in the TurboVap II Concentration Evaporator Workstation.
4. Check that the samples are completely clean, free of any color and cloudiness after concentration. If the samples are at all cloudy or have any color, you will have to run them through cleanup columns again.
5. Transfer samples to appropriately labeled GC vials with Pasteur pipettes.
6. Spike each sample, and the reference containing the surrogate from earlier, with the same amount of internal standard. Cap the GC vials immediately after spiking with internal standard. The samples are now ready for analysis on the Triple Quad.
7. After spiking all of the samples and the reference with internal standard, immediately put the internal standard back in the freezer and again wash the syringe with dichloromethane and hexane.

If you are unable to run the samples on the triple quad, the GC vials can be capped before spiking with internal standard and stored in a freezer. It is recommended to spike the samples with internal standard as soon as possible before running them on the triple quad.

12.0 Cleaning up the Work Station

1. Rinse all reusable glass ware (ASE collection jars, large and small TurboVap tubes, etc.) with dichloromethane 1 time into the waste jug in the hood.
2. Allow dichloromethane to fully evaporate from the glassware, and subsequently soak it in warm soapy water. Do not leave the glassware soaking for over 24 hrs before thoroughly rinsing and allowing to fully air dry.
3. Empty out sampling media from the ASE cells onto a large piece of foil and allow residual solvent to evaporate before disposal in the blue barrel designated for PCB waste.
4. Allow residual solvent from all disposable glassware to evaporate in the fume hood overnight before disposing of in the blue barrel designated for PCB waste.
5. The following morning, remove all remaining waste in the hood to the designated PCB waste container in the lab.