Analysis of PCBs, Pesticides, PAHs, and Flame Retardants in Air and Precipitation Samples

IADN Project

Sample Preparation Procedure

Prepared by

Team IADN

School of Public and Environmental Affairs

Indiana University

Bloomington, Indiana 47405

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INTRODUCTION

This document describes the detailed laboratory procedure for extraction and chromatographic cleanup of air and precipitation samples collected for the Integrated Atmospheric Deposition Network (IADN) from six sampling stations near the Great Lakes. It includes routine operation for cleaning glassware and pre-cleaning sampling media such as XAD-2, quartz fiber filter (QFF), and laboratory chemicals. The procedure requires meticulous attention and extreme care at each step to avoid interference caused by contaminants in the solvents, sampling matrix, and reagents. These methods are strictly followed in the Environmental Chemistry Laboratory, School of Public and Environmental Affairs, Indiana University, Bloomington, Indiana. Any deviation from the procedure is documented in the laboratory notebooks.

Laboratory personnel are often required to handle chemicals and standards, which may be toxic and carcinogenic. Proper safety protection should be taken to handle these chemicals. Indiana University offers a training program for laboratory safety rules and personal protection. All laboratory employees are required to take this training.

The target compounds in this project are 84 polychlorinated biphenyl (PCB) congeners, 23 organochlorine pesticides (OCs), 16 polycyclic aromatic hydrocarbons (PAHs), and 47 flame retardants (FRs), including 36 polybrominated diphenyl ether (PBDE) congeners, 11 non-PBDEs, and 18 organophosphate esters.

A complete list of all compounds is given in Table 1.

TABLE 1. TABLE OF ANALYTES

PCBs	
4+10	89
7+9	101
6	99
8+5	119
19	83
11	97
12	81
13	87
18	85
15+17	77
16	110
32	135+144
26	123
31	149
28	118
33	114
53	131
22	132+153+105
45	163+138
52	126
49	128
47	167
48	174
44 *	202+171
37	156
42	172
41+71	180
64	199
100	169
74	170+190
70+76	201
66	207
95	194
91	205
56+60	206
92+84	

Other
Total suspended particles

Pesticides
HCB
alpha-HCH
beta-HCH
gamma-HCH
Heptachlor epoxide
alpha-Chlordane
gamma-Chlordane
Oxychlordane
trans-Nonachlor
Endosulfan I
Endosulfan II
Endosulfan Sulfate
p,p'-DDT
p,p'-DDE
p,p'-DDD
o,p'-DDT
o,p'-DDD
Aldrin
Endrin
Dieldrin
Octachlorostyrene
Methoxychlor *

PAHs
Fluorene
Phenanthrene
Anthracene
Fluoranthene
Pyrene
Retene
Benz[a]anthracene
Triphenylene+Chrysene
Benzo[b]fluoranthene
Benzo[k]fluoranthene
Benzo[e]pyrene
Benzo[a]pyrene
Indeno[1,2,3-cd]pyrene
Dibenz[a,h]anthracene
Benzo[ghi]perylene
Coronene

nBFRs
HBB : Hexabromobenzene
TBE : 1,2-bis(2,4,6-tribromophenoxy)ethane
syn-Dechlorane Plus
anti-Dechlorane Plus
EHTBB : 2-ethylhexyl-2,3,4,5-tetrabromobenzoate
BEHTBP : bis(2-ethylhexyl)tetrabromophthalate
PBBZ : 1,2,3,4,5-Pentabromobenzene
pTBX : 2,3,5,6-Tetrabromo-p-xylene
PBEB : Pentabromoethylbenzene
DBDPE : Decabromodiphenylethane
HBCD : 1,2,5,6,9,10-hexabromocyclododecane

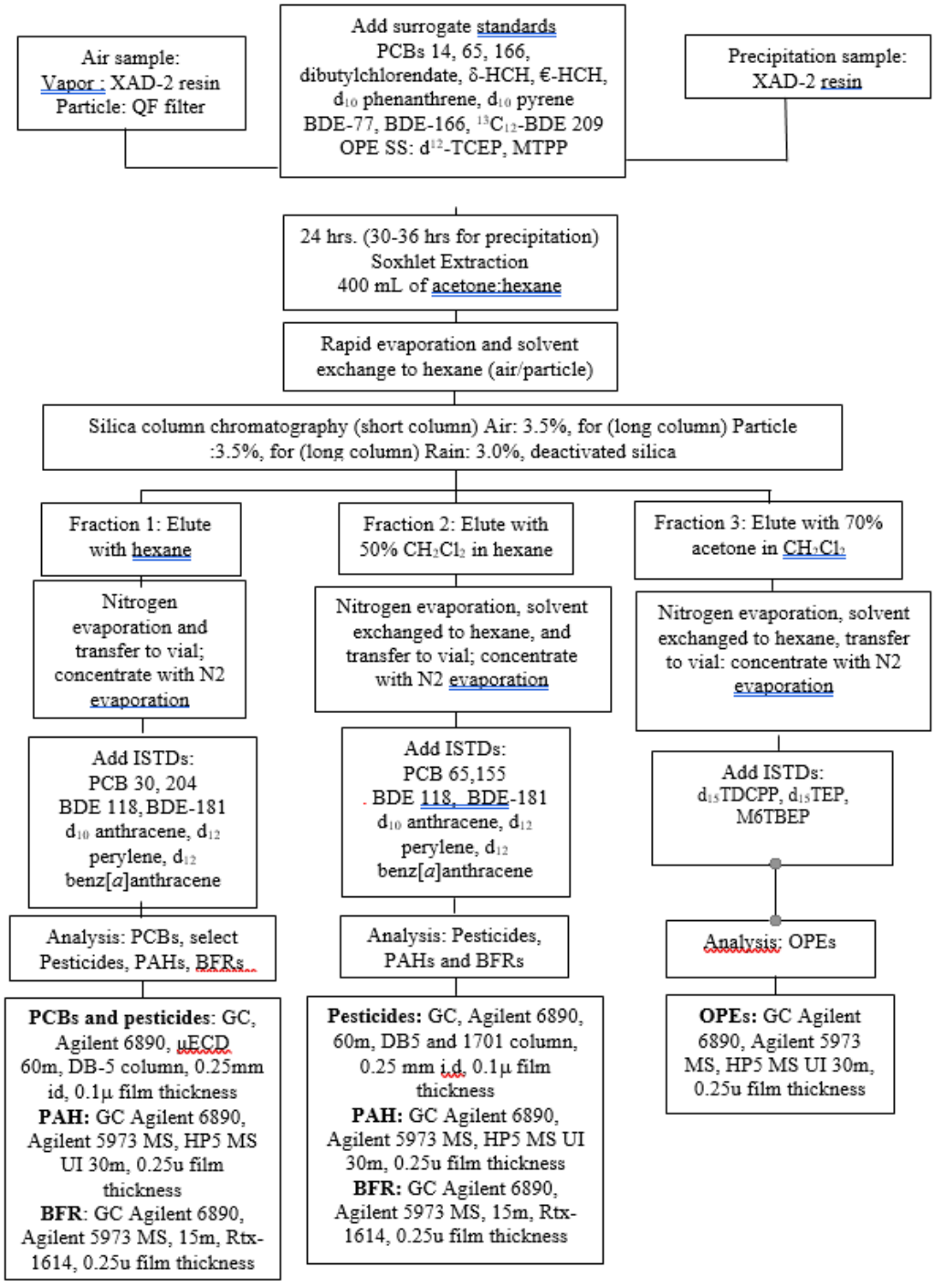
Organophosphate Esters (OPEs)
TEP
TCEP
TIPRP
TPRP
TCIPP
V6
TDCIPP
TPHP
TDBPP
TNBP
TBOEP
2IPPDPP
RDP
TOTP
4IPPDPP
TMTP+TPTP
EHDP
TPEP
B4IPPPP
BPADP
TDMPP
TIPPP
TBPP
TEHP

PBDEs	
BDE-7 *	BDE-153
BDE-10 *	BDE-154 + BB-153
BDE-15	BDE-156+169 *
BDE-17	BDE-180 *
BDE-28	BDE-183
BDE-30 *	BDE-184 *
BDE-47	BDE-191 *
BDE-49	BDE-196 *
BDE-66	BDE-197
BDE-71 *	BDE-201
BDE-85	BDE-203
BDE-99	BDE-204 *
BDE-100	BDE-205 *
BDE-119 *	BDE-206
BDE-126 *	BDE-207
BDE-138 *	BDE-208
BDE-139	BDE-209
BDE-140	

Meteorological
Temperature
Wind speed speed
Wind direction direction
Solar radiation
Relative humidity
Barometric pressure pressure

*** = not reported**

FLOW CHART 1. DIAGRAM OF SAMPLE PREPARATION



I. CLEANING: GENERAL LABWARE

General Cleaning Supplies:

Micro cleaning solution (Micro-90, International Products Corporation)

Glassware washing brushes

Deionized (DI) water, Barnstead, E-Pure series 1090, 4 module water system

Muffle furnaces: Thermolyne 30400

Ultra sonicator

Aluminum foil

Solvents: dichloromethane, hexane (Omnisolv, EMD)

Teflon squirt bottle with solvents

Beakers

Kimwipes

Procedure

1. **Glassware**

Wash general glassware like soxhlet extractors, round bottom flasks, beakers, pear shaped flasks, centrifuge tubes, separatory funnels, etc. thoroughly with micro-90 soap and water using brushes.

Rinse glassware with tap water and with organic free DI water from E-Pure system. DI water system should be turned on and 2 liters drawn off before use. Check resistivity. Change cartridges when resistivity gets to 2.

Dry the glassware at room temperature.

Cover all open ends with foil. Always use dull side of the foil towards glassware.

Muffle glassware in furnace at 5000C for 8 hours.

Allow glassware in furnace to cool to 1000C (usually it takes 10-12 hrs) before removing from furnace.

Store them in cabinets.

The volumetric flasks and the volumetric pipettes are not muffled. Volumetric flasks are cleaned with soap and water then ultrasonicated with dichloromethane 3 times, 15 minutes each time. Volumetric pipettes are initially solvent rinsed and then ultrasonicated with dichloromethane 3 times, 15 minutes each time.

2. **Stainless Steel Tools**

Wash forceps, spatulas, stainless steel air cartridges, and aluminum cartridge rings with micro-90 soap and water using brushes.

Rinse well first with tap water and then with DI water from the E-Pure system. DI water system should be turned on and 2 liters drawn off before use.

Dry at room temperature for a minimum of 2 hours.

Put in a drying oven overnight at 1000 C.

Rinse with dichloromethane.

Wrap each tool separately in multiple foils, shiny side towards the outside.

Store them in drawers.

Rinse with dichloromethane before use.

Air sampling cartridges and screen meshes are wrapped in aluminum foil (shiny side out) and muffled in the furnace at 5000C for 8 hours before storing.

*Aluminum rings cannot be muffled and must be solvent rinsed with dichloromethane, wrapped in foil (shiny side out), and stored in drawers.

3. **Amber glass vials and Pasteur pipettes**

Put the pipette or the vials in beakers and cover beakers with foil. Always use dull side of the foil towards glassware.

Muffle beakers containing vials or pipettes in furnace at 5000C for 8 hours.

Cool glassware in furnace to 1000C (usually next morning); remove from oven. Insert clean Teflon liners (see below) into vial caps.

Cap the vials within 24 hours and store in a beaker covered with foil.

4. **Teflon liners**

Place Teflon liners in glass beaker; cover with dichloromethane.

Ultra-sonicate for 15 minutes. Drain dichloromethane.

Repeat 2 more times.

Put the beaker in a drying oven for 2 hours at 700C.

Store in sealed jar (covered with foil, lid screwed on).

5. **Microdispenser capillaries, GC vials, and stainless N_2 blow down needles**

a. Microdispenser capillaries

Before using rinse with dichloromethane and air dry.

b. GC autosampler vials

Place the vials in beakers. Cover with Al foil. Always use dull side of the foil towards glassware.

Muffle in furnace at 5000C for 8 hours.

Dispose of them after use.

c. Stainless Steel N2 blow down needles

Place needles in a clean beaker and cover with dichloromethane. Cover loosely with foil. Always use dull side of the foil towards glassware.

Sonicate needles for 10 minutes.

Drain solvent and repeat twice more.

Drain all solvent and transfer needles to clean beaker. Cover beaker with foil and store them for future use.

Just before use, squirt dichloromethane solvent through these needles.

Pass nitrogen through them for 10 minutes before use.

6. Teflon Stopcocks and Lids for Sample Jars

Wash stopcocks with micro-90 soap and water. Rinse with DI water from E-Pure system. Make sure the stopcock adjuster on the side is turned to the open position (vertical).

Lids are wiped with a damp Kimwipe soaked in tap water, and then are wiped with a damp Kimwipe soaked in DI from E-Pure system.

Air dry on Kimwipes.

Rinse the Teflon stopcocks (without washers) with dichloromethane.

Store the stopcocks in muffled jars.

Place the clean lids on muffled sample jars or wrap them in foil, shiny side out.

II. PRECLEANING: SAMPLING MEDIA AND CHEMICALS

1. Glass Wool

Supplies

Beaker (1 Liter)

Glass wool

Scissors

Muffle furnace

Procedure

Cut glass wool into 2" pieces.

Put them in muffled beaker.

Cover with foil. Always use dull side of the foil towards glassware.

Muffle in furnace at 5000C for 8 hours. Cool furnace down to 1000C.

Store on a shelf in a beaker covered with foil.

2. Teflon Boiling Chips

Supplies

Soxhlet extractor

Condenser

Sample jar and lid

500 mL round bottom flask

Boiling chips

Dichloromethane

Dichloromethane in squirt bottle

Methanol in squirt bottle

Cork ring for round bottom flask

Variable autotransformer

Heating mantle for either 1 liter or 500 mL round bottom flask

Drying oven

Procedure
Day 1

Thoroughly rinse the inside of the condenser and outside joint with solvent from squirt bottles: first with methanol, then with dichloromethane.

Put 10 to 12 boiling chips in round bottom flask. Add 350 mL of dichloromethane to flask.

Place Teflon boiling chips to be cleaned in soxhlet extractor with glass wool plug at the bottom.

Assemble flask, soxhlet, and condenser.

Turn on heater to give proper boiling (set variac to 48 for DCM or the heating mantle to dial 3).

Turn on cold water for condenser.

Extract for 18 to 24 hours.

Day 2

Turn heat off and cool it down for 15 to 30 minutes.

Turn off condenser water.

Drain as much solvent from soxhlet as possible.

Place boiling chips in muffled sample jar; cover loosely with foil. Always use dull side of the foil towards glassware.

Put sample jar in drying oven at 700C with Al foil cover cracked open.

Label sample jar with date cleaned and store the jar on shelf (covered with foil, lid screwed on).

3. SODIUM SULFATE

Supplies
Sodium sulfate (anhydrous, granular, mesh 12-60, Fisher Scientific)

Muffled sample jar, muffled beaker

Aluminum foil

Muffle furnace

Desiccator

Procedure
Do this each time silica gel is muffled for column work.

Put some sodium sulfate in a muffle beaker.

Cover with Aluminum foil with the shiny side out.

Crack open the foil before placing in muffle furnace for approximately 24 hours at 3000C.

Reduce the temperature to 1000C after the sodium sulfate has been muffled for approximately 18 hours.

Once the temperature reaches 2500C crack open muffle furnace, then when the temperature reaches 1500C take out the sodium sulfate and cover jar completely with the Aluminum foil.

Allow to sit on bench to cool until warm, approximately 30 minutes. You should be able to touch the bottom without having to remove your hand.

Once cool, transfer sodium sulfate to a jar, cover it with a lid, label with date cleaned and put it in a desiccator for storage.

4.XAD-2: (Supelpak -2SV, catalog #13674-U, 20-60 mesh size, pore diameter 90Å, Sigma-Millipore)
Supplies
Soxhlet extractor and condenser 71/60 and 29/42 joints

One liter round bottom flasks with 24/40 joint

Glass stoppers (24/40 joint)

1 or 2 liter beakers

Adapter to convert 29/42 to 24/40

Boiling chips

Dichloromethane

Hexane

Methanol

Acetone

HPLC grade water: EMD, Omni Solv

Squirt bottle

Methanol in squirt bottle

Foil

Glass wool

Cork rings

Heating mantle for 1-liter flask

Variable autotransformer

XAD-2

Procedure
i) Dry XAD-2 for air sample cartridges:

Day 1

Place XAD-2 in soxhlet extractor plugged with glass wool.

Rinse with small amount of methanol 3 times to remove water.

Add 800 mL of methanol to 1-liter flask.

Add about 20 boiling chips to flask.

Assemble flask/soxhlet/condenser.

Turn on heater to give proper boiling (set variac to 70 for methanol).

Turn on cold water for the condensers.

Cover soxhlet and flask with foil.

Extract with methanol for 24 hours.

Day 2

Turn heater off. Cool down for 15 to 30 minutes.

Flush as much methanol from soxhlet as possible.

Add 800 mL acetone to 1-liter flask.

Add about 20 boiling chips to a new flask.

Turn on heater (set variac to 55 for acetone).

Cover soxhlet and flask with foil.

Extract with acetone for 24 hours.

Day 3

Follow the procedure of Day 2 but use hexane as solvent. Use a new flask. Set variac at 50.

Extract for 24 hours.

Day 4

Follow the procedure of Day 2 but use acetone:dichloromethane (70:30 by volume). Set variac at 55.

Extract for 48 hours.

Day 5

Follow the procedure of Day 2 but use dichloromethane as solvent. Use a new flask. Set variac at 48.

Extract for 24 hours.

Day 6

Turn off heater; cool 15 to 30 minutes.

Flush as much dichloromethane as possible from soxhlet as possible.

Pour XAD-2 in a large funnel plugged with muffled glass wool and partially cover with Al-foil. Allow remaining Dichloromethane to filter into a muffled 1 L beaker until funnel stops dripping. Get rid of dichloromethane and transfer XAD-2 to a clean 2 L beaker.

Dry XAD-2 in a drying oven at 700C overnight.

Store in amber bottle in freezer at -20□C for up to three months.

Keep subsample in separate jar for checking lab blank and matrix spike.

ii) Wet XAD-2 for precipitation sample cartridges:

Day 1

Add 800 mL of methanol to 1-liter flask.

Add about 20 boiling chips to flask.

Assemble flask/soxhlet/condenser.

Turn on heater to give proper boiling (set variac to 70 for methanol).

Turn on cold water for condenser.

Cover soxhlet and flask with foil.

Extract with methanol for 24 hours.

Day 2

Turn heater off. Cool them down for 15 to 30 minutes.

Flush as much methanol from soxhlet as possible.

Add 800 mL acetone to 1-liter flask.

Add about 20 boiling chips to a new flask.

Turn on heater (set variac to 55 for acetone).

Cover soxhlet and flask with foil.

Extract with acetone for 24 hours.

Day 3

Follow the procedure of Day 2 but use hexane as solvent. Use a new flask.

Set variac at 50.

Extract with hexane for 24 hours.

Day 4

Follow the procedure of Day 2 but use acetone:dichloromethane (70:30 by volume) as solvent.

Set variac at 55.

Extract for 48 hours.

Day 5

Follow the procedure of Day 2 but use methanol as solvent. Use a new flask.

Set variac to 70.

Extract for 24 hours.

Day 8

Turn off heater; cool 15 to 30 minutes.

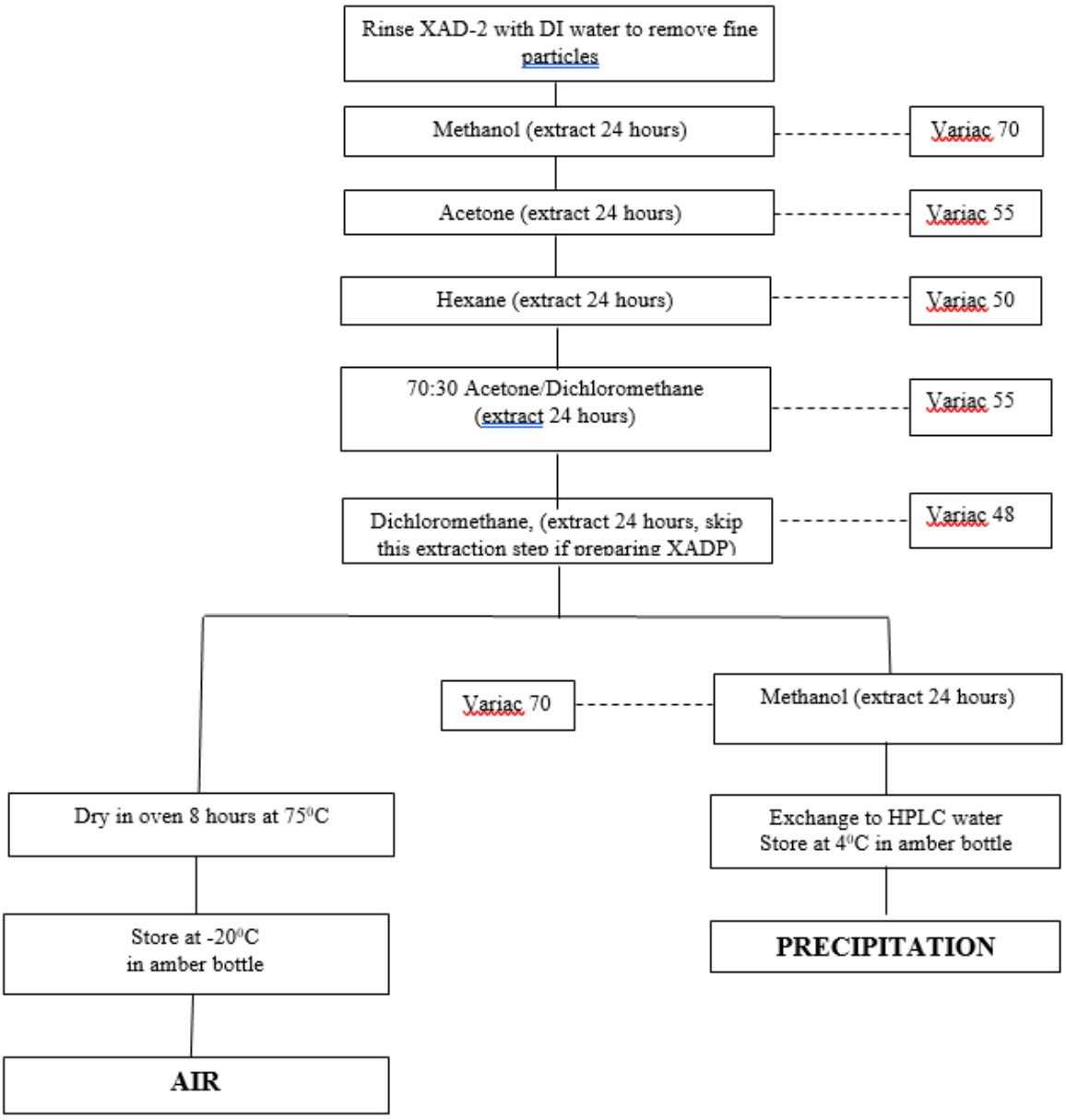
Flush as much methanol from soxhlet as possible.

Rinse XAD-2 with EMD HPLC grade water until XAD-2 does not smell of any solvent.

Store clean XAD-2 in an amber bottle at 40C. It can be used up to three months.

Keep subsample in separate jar for checking lab blank and matrix spike after making a column and drying it for extraction use.

FLOW CHART 2. DIAGRAM OF XAD-2 PRECLEANING



5. Silica and quartz fiber filters (QFF)

i) Silica: Silica gel sorbent 100-200 mesh 60 Å, EchoChrom, MP biomedical silica. It has been determined that the silica is adequately cleaned during the activation process therefore no additional processing is necessary.

ii) Quartz fiber filters (QFF): 0.3 micron, Tisch Environmental

Wrap up each QFF by aluminum foil separately, shiny side out.

Muffle at 4500C for 6 hours.

After cooling put them individually in plastic bag and store them in freezer at -200C.

iii) Glass Fiber Filters (GFF): 1.0 micron, VWR

Wrap up each GFF by aluminum foil separately shiny side out.

Muffle at 4500C for 6 hours.

After cooling put them in plastic bag and store them in freezer at -200C.

III. PREPARATION OF SAMPLING CARTRIDGES

1. Precipitation Columns for MIC sampler

Supplies

Glass rain columns: Chromatographic columns (Ace Glass Inc. 5820-16)

15 mm threaded Teflon plugs

"O" rings for the Teflon plugs

Teflon adapter with valves

Muffled glass wool

Water: EMDSolv grade

Beakers

Tweezers

Aluminum foil

Stand and clamp

Pre-cleaned wet XAD-2: Supelpak -2SV, 20-60 mesh size, pore diameter 90Å

Procedure:

Attach the column to a clamp stand so that the red arrow points up.

Attach the Teflon valve at the bottom end to control the flow.

Pack glass wool to about 1/4".

Pour water in to check and adjust the flow.

Fill the column with wet XAD-2 (11-14 cm. in length) and let it settle. Tap the column gently to get better packing. Never let the XAD-2 get dry.

Put another plug of glass wool on the top.

Put water on the top of the column and screw in the Teflon plug with "O" ring.

Turn it upside down, take the adapter valve off and put another Teflon plug in place of the valve.

Make sure that the o-ring on the Teflon plug makes a good seal.

Cover it first with Aluminum foil and then with bubble wrap.

Store them at 40 C until shipping.

Make one extra column for laboratory blank and matrix spike.

2. Quartz Fiber Filter for High-vol Air Samplers

Supplies

Quartz fiber filter: Whatman 8x10 inch, QM-A

Humidity chamber: Lab Line Descicab No 1477 with saturated solution of Lithium.

Nitrate to maintain 50% relative humidity.

Balance: Mettler AE50 with a filter chamber and a hanger underneath.

Muffle furnace

Gallon size plastic Ziploc bags

Aluminum foil

Tweezers

Procedure

Wrap quartz fiber filters with aluminum foil and make sure that the sides are not damaged.

Heat the wrapped filters at 4500 C for 6 hours in the muffle furnace.

Store them at -200 C.

Take them out of the freezer 48 hours before shipping and put them in the designated humidity chamber for 24 hours, with the aluminum foil slightly open.

After it has been equilibrated with 50% humidity for 24 hours, put a filter ID on the upper right-hand corner of the filter with a pencil. Put it into the filter chamber of the balance, using tweezers, and take the weight. Take three weights to get a good average.

Record the filter ID and the initial weight in the filter book.

Wrap the filter again in the same foil. Write the filter ID on the aluminum foil with a marker.

Put the filter in Aluminum foil in a Ziploc plastic bag and store it at -200 C until shipping.

Calibrate the balance with a set of external weights ranging from 2mg to 200mg once a month. Check the internal calibration once every two weeks. Company calibration is done annually.

Avoid touching the filter. Always use tweezers.

3. XAD-2 Cartridges for Hi-vol Air samplers

Supplies

Pre-cleaned dry XAD-2

Stainless steel cartridges, wrapped in aluminum foil and muffled

Screens, wrapped in foil and muffled

Aluminum rings for the cartridges, solvent cleaned and wrapped in foil (Do not muffle the aluminum rings)

Tweezers

Tin cans

Teflon tape

Black electric tapes

Procedure

Take a muffled stainless-steel cartridge and carefully un-wrap and remove the foil.

Put a screen and retainer ring at one end. Pour 40-42g of pre-cleaned XAD-2. Put another screen and retainer ring on the other end. Check to make sure no XAD-2 is leaking. Always handle the screens with tweezers to avoid contamination.

Wrap the XAD-2 cartridge in the same foil it was muffled in. If necessary, use some extra foil. Place the whole cartridge in a tin ointment can rinsed with solvent. Seal the cover first with Teflon tape and then with black electrical tape.

Store them at -20o C until shipping.

Record the batch number of the XAD-2 used for making the cartridges in the sampling protocol book.

IV. SAMPLE HANDLING AND STORAGE

1. Air vapor samples or XAD-2 cartridges

Check the sample packaging and the integrity of the samples very carefully. If it is not done properly write them down in Field Data Sheet and in sample log file.

Unwrap the aluminum foil carefully.

Unscrew the retainer nut and remove the screen with clean and solvent rinsed tweezers.

Transfer all XAD-2 into a previously cleaned and muffled glass jar.

Put the cap tightly after covering the jar with aluminum foil.

Label the jar with sample ID (Site_Sampler#_Sample type_Date): eg. SH 01C 031230.

Store in the freezer at –200C until analysis.

Sign and date the field data sheet and write comments, if any. File the field data sheets in folders.

Enter the field data in Laboratory log file.

File site visit data sheet on weekly basis.

If the samples cannot be transferred immediately, they can be stored in refrigerator (40C) temporarily.

2. Air particle sample or quartz fiber filter

Check the sample packaging, sample integrity, and write comments in Field Data Sheet and in the sample log file.

Unwrap the filters slightly and place in the humidity chamber (Boekel Scientific) for approximately 24 hours.

Sign, date, and file the field data sheet with comments.

File site visit sheet.

Enter field data into sample log file.

Next day take the final weight along with the sample ID code and record alongside the corresponding filter ID numbers in filter folder.

Rewrap the filters in the foil, put in a plastic bag and store at -200 C in a freezer until extraction.

Subtract the initial weight from the final weight and record the total suspended particle (TSP) of the filter in micrograms per meter3. Enter this information in sample log file.

If the samples cannot be put in the humidity chamber immediately, they can be stored in cold room (100C) temporarily.

3. Precipitation Column

Check the sample packaging and the integrity of the samples very carefully. If it is not done properly write them down in the Field Data Sheet and sample log file.

Unscrew the bottom Teflon cap and put the Teflon valve on the bottom side.

Clamp the column securely.

Put a drain jar underneath the column.

Drain extra water from the column.

Aspirate all water out of the column (15 minutes per sample).

By gentle tapping transfer all XAD-2 in a clean pre muffled jar.

With the help of a Pasteur pipette rinse the inside of column with acetone and collect it in the same jar.

Label the jar with sample ID. Site_sample type_ Sampler# _Collection date. Example SP 01 030508.

Store the sample in freezer at –200C until analysis.

Sign, date, and file the field data sheet.

Enter the data into sample log file.

If the samples cannot be transferred immediately, they can be stored in refrigerator (40C) temporarily.

V. EXTRACTION

1. Air samples (vapor phase and particle phase) and precipitation samples: XAD-2 cartridges, Quartz fiber filter (QFF), and XAD-2 rain columns

Supplies:

Large soxhlet extractor (55/50 and 24/40 joints)

Condenser (55/50 joint)

Round bottom flask (24/40 joint) 500 mL

Glass stopper (24/40 joint)

Beakers

Micro-dispenser (50 or 100 µl) and 1 mL pipette

Boiling chips

Acetone

Hexane

Surrogate Recovery standards: Table 2

One matrix spike vial (MS vial) with recovery standards: PCB (547.22 ng), pesticides (20 ng each), PAHs (400 ng ea), BFR (PBDE) Recovery Standard (40-200 ng/ml) and OPEs (5-100 ug/ml).

Waste solvent bottle

Cork rings (one per each 500 ml round bottom flask)

Glass wool

12" rod (glass or metal)

Large tweezers

Small tweezers

Al foil

Scissors

Heating mantle and variable autotransformer or multi-unit extraction heat

Clean XAD-2 or QFF for blank

TABLE 2. TABLE OF SURROGATE RECOVERY STANDARDS

Surrogate Standards	Concentrations
PCBs	Congener 14 : 200 ng/mL
	Congener 65 : 50 ng/mL
	Congener 166 : 50 ng/mL
PESTs	Ditubylchlorendate : 200 ng/mL
	δ-HCH : 200 ng/mL
	ε-HCH : 200 ng/mL
PAHs	d_{10}-Phenanthrene : 4 ug/mL
	d_{10}-Pyrene : 4 ug/mL
BFRs	BDE-77 : 60 ng/mL
	BDE-166 : 100 ng/mL
	$^{13}C_{12}$-BDE-209 : 80 ng/mL
OPEs	d_{12}-TCEP : 1 ug/mL
	MTPP : 1 ug/mL

Procedure

i) Setting up

One batch of samples generally include:

Regular samples: 8-15 (This usually includes field blanks and / or field duplicates)

Laboratory duplicate (usually once a month): One air vapor sample split into two equal parts in laboratory (No laboratory duplicates for filter and precipitation samples).

Laboratory blank (alternate batches): Sampling media spiked with surrogate standards.

Matrix spike (alternate batches): Sampling media spiked with recovery standards.

On the day of extraction, a unique Batch ID is assigned to a batch of samples with month, year, and sample type. Example: Batch IDs of the cartridge, filter, and precipitation samples from September 2010 will be S10C, S10F, and S10P, respectively.

Day 1

Remove standards from freezer. Standards must be at ambient temperature before use. (Ambient temperature is achieved in about 2 hours).

Thoroughly rinse inside of condenser and outside of joint with solvent in squirt bottles: first with methanol, then with dichloromethane.

Label flasks with sample IDs.

Add approximately 6 clean Teflon chips into 500 mL round bottom flask.

Pour solvent into round bottom flask: 200 mL of acetone and 200 mL of hexane (for vapor and particle only).

Vapor sample: XAD-2

Place glass wool plug at the bottom of the soxhlet extractor using large tweezers, glass or metal rod.

Carefully pour XAD-2 in soxhlet extractor. Rinse the container twice with solvent (50% acetone/50% hexane) to remove all XAD-2; pour solvent rinses into soxhlet.

Particle sample: QFF

Unwrap one QFF at a time.

Trim off the number at the corner with clean scissors rinsed with dichloromethane.

Use 2 pairs of blunt tweezers to fold one QFF; place it all the way down in soxhlet so that the top part of the QFF remains below the top level of the small siphon tube.

Label the soxhlet with the sample ID

Rinse tweezers and scissors with dichloromethane before starting the next sample.

Precipitation sample: XAD-2 column

Place glass wool plug at the bottom of the soxhlet extractor using large tweezers, glass or metal rod.

Keep a beaker with 200 mL of acetone in front of soxhlet extractor.

Carefully transfer XAD-2, and glass wool plug in the soxhlet extractor. Rinse the container twice with acetone to remove all XAD-2; Pour about 150 mL of acetone into soxhlet and let the solvent stand there for 15 min.

Hand flush the solvent. Add rest of acetone from beaker to soxhlet and flush again.

Add 200 mL of hexane to soxhlet and siphon.

Note: The precipitation samples have water in them and may not siphon on its own. Induce siphoning first 2-3 times by hand until the level of solvent in the soxhlet and in the siphon tube are the same.

Matrix spike:

Take about 20-30g of dry XAD-2 (vapor set), or muffled QFF (filter set), or 8g of wet XAD-2 (precipitation set) in a soxhlet extractor plugged with glass wool.

Add: A vial containing all recovery standards.

PCB recovery standard: complete suite of PCB congeners (547.22 ng, from S-75951 std)

Pesticide recovery standard: Calibration Reference Standard (CRS), S-8206A fortified with 3 other pesticides, all pesticides 20 ng each.

PAH recovery standard: all PAHs 400 ng each (Laboratory mix)

PBDE recovery standard: Mixture of 25 selected PBDE listed in Table 27 (40-200 ng/mL)

OPE recovery standard (used only with filter sets): all OPFRs 500 ng each (Laboratory mix)

Make sure the matrix spike vial used is recorded in the sample prep book.

The recoveries of each compound will show the extraction efficiency of that batch.

Laboratory blank

Take about 20-30g of dry XAD-2 (vapor set), or muffled QFF (filter set), or 8g of wet XAD-2 (precipitation set) in a soxhlet extractor plugged with glass wool.

Laboratory duplicate: for Chicago air vapor only

Shake the sample from Chicago in the jar to mix it thoroughly.

Weigh out the whole XAD-2.

Weigh out approximately 10g of it and carefully transfer it in a Soxhlet extractor plugged with glass wool. Weigh out another 10g of the same sample and transfer it in a second Soxhlet extractor. Label the first one as CH-02C1-yy-mm-dd and the 2nd one as CH-02C2-yy-mm-dd. Record mass in sample prep book.

ii) Spiking with Surrogate Standards

Using a 100 µL micro dispenser, spike each sample with mix surrogate standard that contain:

PCB 14: 200 ng/mL

PCB 65: 50 ng/mL

PCB 166: 50 ng/mL

Dibutylchlorendate: 200 ng/mL

δ-HCH:200 ng/mL

ε-HCH200 ng/mL

d10 phenanthrene:4 µg/mL

d10 pyrene:4 µg/mL

Using a 50 µL micro dispenser, spike each sample with:

BDE-166 100 ng/mL

BDE 77 60 ng/ml

13C12-BDE 209 80 ng/mL

d12-TCEP 1 µg/mL

MTPP 1 µg/ml

Make sure to rinse dispenser with DCM and change tip between each standard. Recovery of each surrogate standard will show the extraction efficiency of individual samples.

iii) Extraction

Day 1

Assemble flasks, soxhlets, and condensers. Place in heating mantles.

Turn on heating mantles. Set the heater at 3 and or the variac at 45.

Turn on condenser cold water on.

Cover soxhlet, top of the condenser and flask with foil.

Extract for 18 to 24 hours (30 hours for precipitation).

Day 2

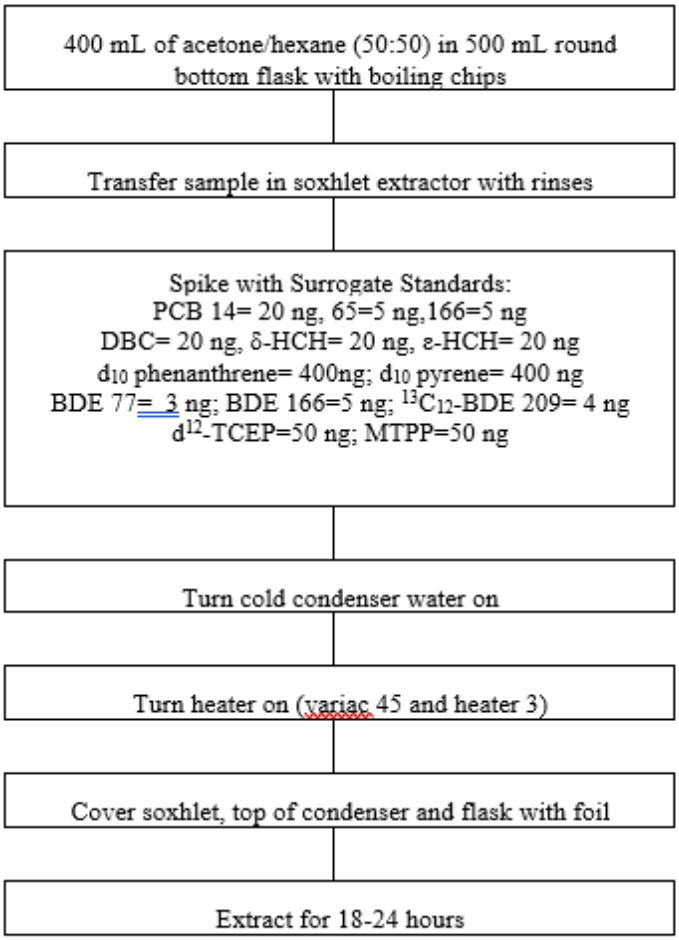
Turn the heating mantle off. Let them cool down for 30 minutes. Turn off condenser water.

Siphon off as much solvent from soxhlet extractor into flask as possible.

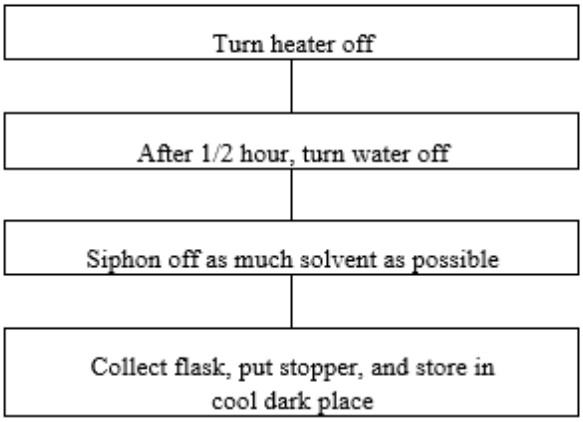
Detach the flask and insert the stopper. Store the extracts in a cool dark place.

FLOW CHART 3. DIAGRAM OF EXTRACTION OF AIR SAMPLES

Day 1

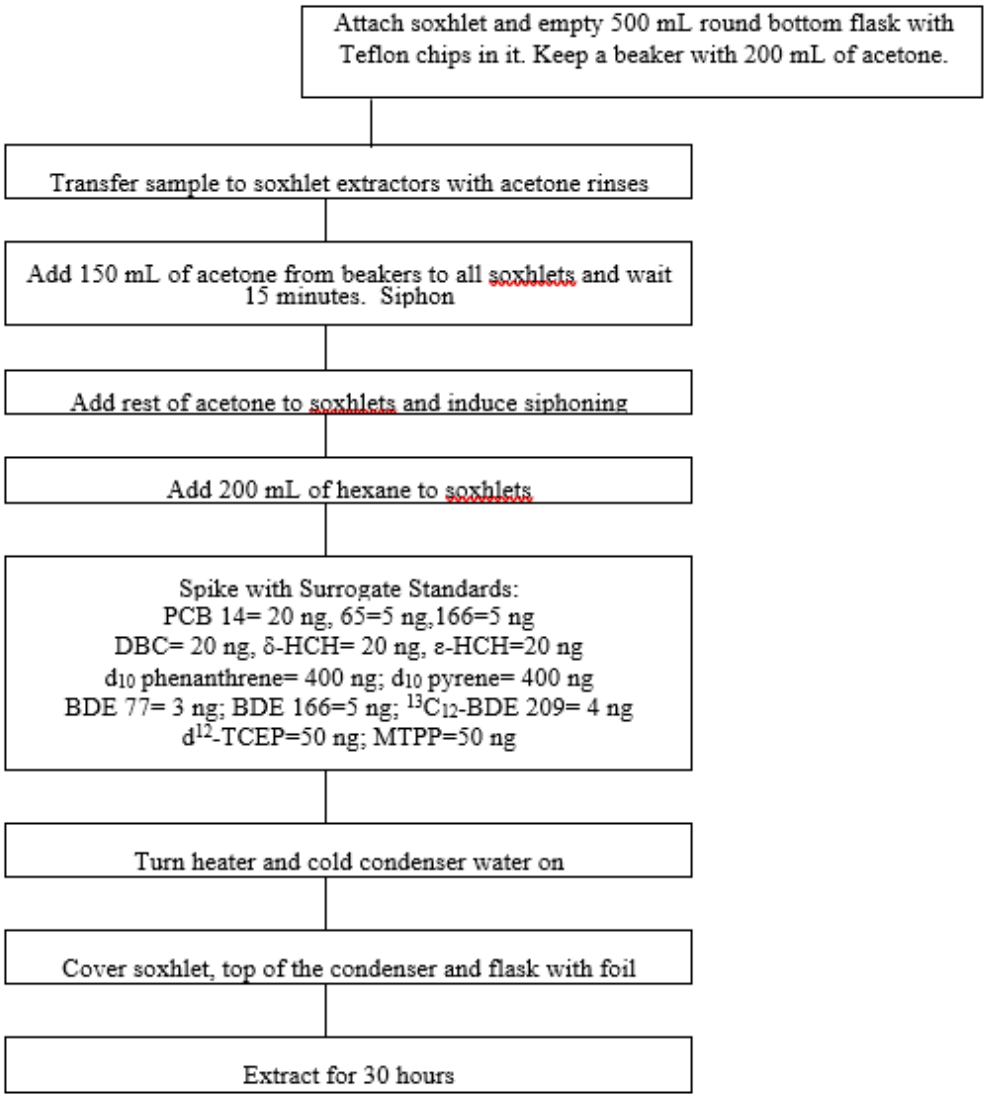


Day 2

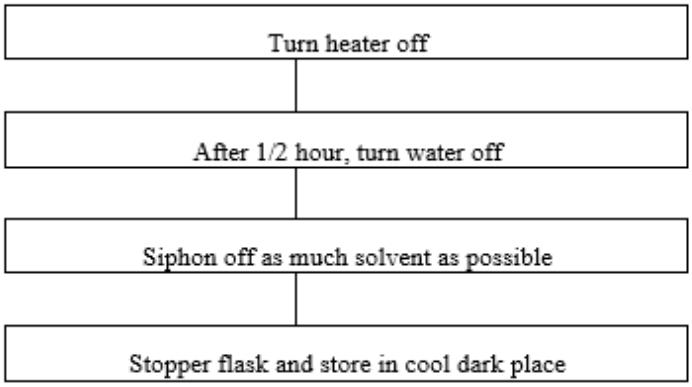


FLOW CHART 4. DIAGRAM OF EXTRACTION OF PRECIPITATION SAMPLES

Day 1



Day 2



VI. RAPID EVAPORATION

1. Air (XAD-2 extract)

After extraction, the extracts need to be concentrated and solvent exchanged to hexane before silica gel chromatography.

Supplies

RapidVap LABCONCO unit

Waste container for used boiling chips

Hexane

Clean large forceps

Squirt bottle of Dichloromethane.

600 mL RapidVap tubes

50 ml tubes and caps

Short and long disposable pipettes.

Procedure

i) Setting up

Check moisture filters if color is indicated in filter replace filter.

Power on unit.

Turn on the nitrogen tank to 20 psi.

Preheat unit, set to 30-35ºC.

Label 600 mL RapidVap tubes, place in metal rack.

ii) Evaporation

Remove boiling chips from the extract with large clean forceps.

Transfer each sample from the round bottom flask to the labeled 600 mL RapidVap tube.

Rinse round bottom flask 2 times with 5 ml of hexane and add each rinse to RapidVap tube.

Stop the unit and place tubes in the RapidVap Unit. All spaces are equal, although the spaces near the vacuum evaporate slightly quicker. This region is better suited for those with larger volumes.

Carefully close the lid and ensure rotation of 20% at the beginning to reduce chances of spillage. Watch for sample bumping (i.e. bubbling over).

iii) Time steps (temperature remains at 35ºC)

Start out with a rotation of 20%.

After 30 minutes check samples; increase rotation to 25%.

One hour after starting check samples; increase rotation to 30%.

Two hours after starting check samples; increase rotation to 35%.

Three hours after starting check samples; increase rotation to 40%.

Four hours after starting, add 25 ml hexane exchange using the hexane pump; ensure the walls are rinsed thoroughly as you slowly pump the hexane; leave rotation at 40%.

Four- and one-half hours after start check samples; increase rotation to 405-50%.

Five hours after start stop and transfer samples to 50 ml tube (~1-2 ml remain).

TABLE 3. RAPIDVAP TIME STEPS

Time	Procedure
0:00	Rotate at 20%
0:30	Check samples; increase rotation to 25%
1:00	Check samples; increase rotation to30%
2:00	Check samples; increase rotation to 35%
3:00	Check samples; increase rotation to 40%
4:00	Add 25 ml hexane exchange: ensure all walls are rinsed thoroughly as you add hexane; leave at rotation 40%
4:30	Check samples; increase rotation to 45-50%
5:00	Stop and transfer samples to 50 ml tube (~ 1.0-2.0 ml remain)

iv) Transfer

Tranfer samples from RapidVap tube to 50 ml tube and evaporating to 1 ml sample for column fractionation.

Transfer each sample using a long transfer disposable pipette to a prelabeled 50 ml tube. Ensure vessel is closed to tube during transfers to prevent loss of sample while transferring.

Rinse the walls of the RapidVap tube with four- 1 ml aliquots of hexane, then transfer to the 50 ml tube.

Add 1 ml hexane to RapidVap tube, rinse walls with long transfer pipette several times, then transfer to 50 ml tube. Repeat this step 2 times.

Concentrate to 1 ml using N-Evap. Add 10 ml of hexane from pump then concentrate to 1 ml. Repeat this step 2 times. These samples are now complete for column fractionation.

v) Completion

Shut system down in reverse order from setup.

2. Rotary Evaporation and Back Extraction of Precipitation Extracts

Supplies

Splashguard with 24/40 joint

Waste container for used boiling chips

Hexane

Clean large forceps

Waste bottles

Dichloromethane in Teflon bottle

Separatory funnel with stopcock

50mL Centrifuge tubes with stoppers

Pasteur pipettes

Rotary evaporator (Buchi Rotavapor, R-110)

Chiller circulator (Neslab, Cool Flow, CFT-25)

Kimwipes

Procedure

i) Evaporation

Rinse the joint of the steam duct with dichloromethane or hexane.

Attach splashguard to steam duct. Clamp joint.

Turn vacuum on with faucet aspirator.

Remove boiling chips from the extract with large clean forceps.

Attach flask to splashguard after rinsing the joint with dichloromethane.

Clamp joint.and turn motor on. Turn water bath on. The temperature should be 30-350C.

Turn chiller on.

The solvent should start evaporating within 2 minutes. The sample should not boil.

Concentrate the samples to about 20 mL until the two layers are separated.

Add 75 mL of hexane and rotavap down to about 20 mL. The separation mark will be clearer.

Add 75 mL of hexane again and go down to about 20 mL. The two phases should be clearly visible.

ii) Back Extraction

Transfer the whole extract to a 125 mL separatory funnel.

Rinse the original flask with 10 mL of hexane and add this to the separatory funnel. Wait 20 minutes.

Rinse the original flask with acetone and hexane into waste jar and air-dry the flask.

Drain the bottom oily layer from the separatory funnel to a 50 mL centrifuge tube.

Add 10 mL of hexane to the centrifuge tube and shake vigorously. Wait for 15 minutes.

If it forms an emulsion, add Na2SO4 and shake.

Pipet out the hexane layer from the centrifuge tube and add this to the separatory funnel.

Repeat last three steps once more.

Add 25 mL of HPLC water to the extract in the separatory funnel, shake vigorously, and let it stand for 20 minutes.

Drain the bottom water layer.

Repeat last two steps until the water layer is clear.

Vigorously shake the separatory funnel again and settle.

Drain off any amount of water that forms at the bottom.

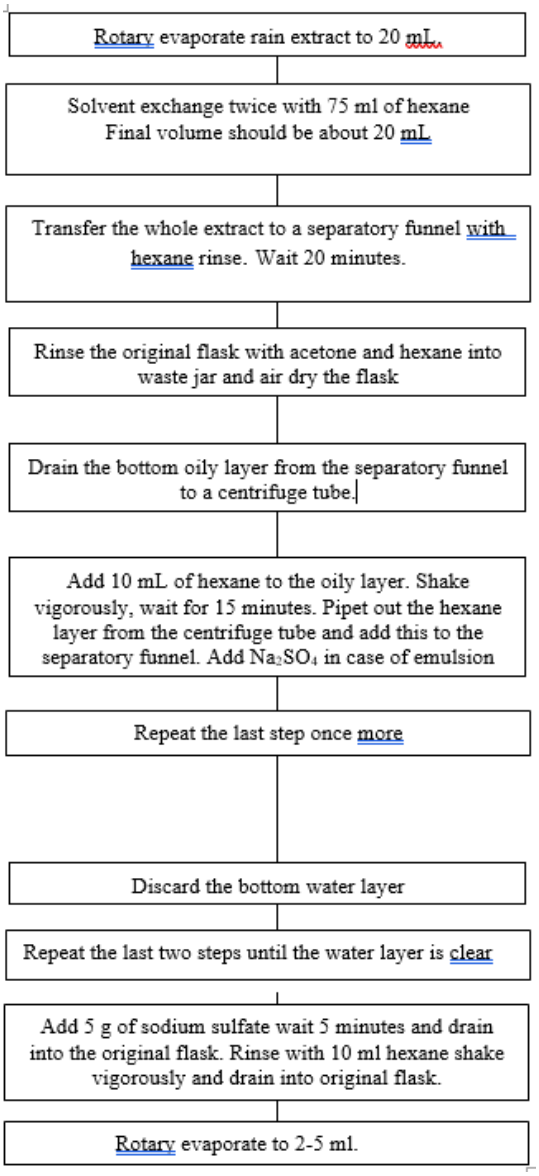
Measure out 5 grams of sodium sulfate and place in separatory funnel.

Shake vigorously and set for 5 minutes. Drain the sample into original flask.

Add 10 mls of hexane to separatory funnel and shake vigorously. Drain into original flask.

Rotary evaporate to 2-5 mL.

FLOW CHART 5. ROTARY EVAPORATION AND BACK EXTRACTION OF PRECIPITATION EXTRACTS



VII. SILICA COLUMN CHROMATOGRAPHY

1. Activation and Deactivation of silica

Supplies

Beakers

Powder funnel

Round bottom flask 250 mL with stopper and cork ring

Pipet and pipet filler

Silica gel, Echochrom MP silica, 100-200 mesh, 60Å

Muffle furnace

Desiccator

Calculator

Balance

Particle mask

Procedure

i). Activation

Day 1

Place the approximate amount of silica needed in a beaker. Cover the beaker with foil loosely.

Place the beaker in 1000C oven, turn thermostat to 3000C; keep in oven 18 hours.

Day 2

After 18 hours the oven program will go to 100C.

Crack the door of the oven open when the oven has cooled down to 250C.

When the oven temperature is 150C remove silica from the oven and make the Al foil tightly closed.

Let it cool on the countertop until warm. You should be able to touch the bottom without having to remove your hand, approximately 30 minutes.

Store in a desiccator for 2 hours to allow silica to reach ambient temperature.

ii). Deactivation

After the silica has cooled in the desiccator for 2 hours, deactivate it:

Working quickly, weigh out desired amount of silica in the round bottom flask. Stopper

the flask immediately after pouring silica.

Add 3.5% weight/volume of DI water to silica, using the following equation:

$$\frac{\%deactivation}{100\%-deactivation} = \frac{ml\ DI\ water}{weight\ of\ silica\ (grams)}$$

For precipitation samples use 3% deactivation.

SHAKE WELL. Shake flask until all clumps are broken-up.

Store in a desiccator overnight for equilibration.

Use deactivated silica in desiccator within 3 days.

2. Column chromatography for vapor samples

Supplies: (for a 3-fraction column clean-up of one sample)

For each sample:

Supelco insert

Sigma, 504394, 6 mL Glass Reaction Tube

Screw stopper from SigmaAldrich (Supelco); 57250-U; 24-Position SPE Manifold

Four 15 ml conical graduated glass tubes and stoppers (one for each fraction collected and a waste tube)

Glass column for SigmaAldrich (Supelco); 57250-U; 24-Position SPE Manifold

Deactivated silica gel, EchoChrom MP silica gel

Aluminum Foil

Glass Wool

Tray for centrifuge tubes

TABLE 4. TABLE OF COLUMN SIZE AND AMOUNT OF SILICA

Item	Air Particle (QFF)	Air Vapor (XAD-2)	Rain (XAD-2)
Amount of silica to activate/deactivate per sample	4-6 gm	1-2 gm	4-6 gm
Column size	3.5"	3.5 cm	3.5"
Na_2SO_4	0.5"	0.5 cm	1.5"
Glass wool	1 cm	0.5 cm	1 cm
Elution volume	30 ml for 1st fraction, 40 ml 2nd fraction, and 30 ml for 3rd fraction	15 mL	30 ml for 1st fraction, 40 ml 2nd fraction, and 30 ml for 3rd fraction

Procedure

i) Preparing Columns for short columns for vapor samples only

1) Clean Supelco inserts and screw stoppers for 57250-U; 24-Position SPE Manifold, by ultrasonicating three times in dichloromethane, acetone, and then methanol, each for 15 minutes. Allow to dry in fume hood or rinse with elution solvent before using.

2) Replace inserts and stoppers on the 57250-U; 24-Position SPE Manifold.

3) Prepare and label the conical graduated glass tubes for each fraction and place a screw cap on the tube. Label one conical glass tube for each sample, which does not have to be graduated, as waste.

4) Place a conical graduated tube for each column in the SigmaAldrich (Supelco); 57250-U; 24-Position SPE Manifold.

5) Put Aluminum foil down to place column glass tubes on to pack with glass wool. Pack each column with 0.5 cm of glass wool. Mark 2.5 cm from the top of the glass wool for deactivated silica. Mark 0.5 cm from mark of silica for sodium sulfate.

6) Add columns to each screw stopper. They should be secure, but not too tight that the glass breaks.

7) Rinse the 6 mL glass reaction tubes (columns) with the glass wool with hexane. Leave enough solvent to cover the media that will be added.

8) Load the media with a pipet, ensuring no air bubbles are present. Tap the top of the column with a small rubber mallet. Do not tap too hard that the column breaks or solvent splashes out of column. * Note: some air bubbles may form during the process. This is OK, and do not tap the column once the samples are loaded.

9) Add eluting solvent, (approx. 12 mL), to condition the column and elute until solvent is just above the sodium sulfate. This is collected in the waste tube. Remove the waste tube and replace with the collection tube for the first fraction.

Add sample to each prepared column, one at a time, twist the column counterclockwise quarter to half a turn to elute the sample. *Note: You can stop the column from eluting at any time by twisting the column clockwise.

Once the sample is loaded onto the column add a rinse, (3x using ~1ml elution solvent to the tube) and add that to the column.

Continue to add solvent until at least 12 ml has eluted. Then twist clockwise to stop the column, keeping the sodium sulfate and silica covered in solvent.

Move to the next sample and repeat process until all samples have completed the eluting of the first fraction. Once all the samples have the first elution completed, carefully remove the top of the SigmaAldrich (Supelco) 57250-U; 24-Position SPE Manifold and cap the full tubes that were just collected. Place the next labeled fraction tubes in the SPE Manifold to begin collecting the next fraction.

NEVER LET THE COLUMN RUN DRY.

ii) Fractionation

1) Add sample to each prepared column, one at a time, twist the column counterclockwise quarter to half a turn to elute the sample. *Note: You can stop the column from eluting at any time by twisting the column clockwise.

2) Once the sample is loaded onto the column add a rinse of hexane solvent to the tube (~1ml) and add that to the column.

3) Continue to add solvent until at least 12 ml has eluted. Then twist clockwise to stop the column, keeping the sodium sulfate and silica covered in solvent.

4) Move to the next sample and repeat process until all samples have completed the eluting of the first fraction. Once all the samples have the first elution completed, carefully remove the top of the

SigmaAldrich (Supelco) 57250-U; 24-Position SPE Manifold and cap the full tubes that were just collected. Place the next labeled fraction tubes in the SPE Manifold to begin collecting the next fraction.

5) Repeat steps 3and 4 after adding ~1 ml rinse of the 50% solvent to the corresponding tube of the sample. Continue this process until all samples have been completed for the 2nd fractionation.

6) Repeat steps 3 and 4 after adding ~1 ml rinse of the 70% solvent to the corresponding tube of the sample. Continue this process until all samples have been completed for the 3rd fractionation.

iii) Clean-Up

With a jet of air get the silica and sodium sulfate out of the column.

Use forceps to remove the glass wool or wait for the column to dry overnight and then tap out the dry silica and sodium sulfate. The silica and sodium sulfate should be treated as solid waste.

Place the Supelco inserts and screw stoppers from the SPE Manifold into a beaker and repeat the cleaning procedure Step 1 under section Preparing Columns.

3. Column chromatography for particle and precipitation samples only

Column chromatography supplies: (for a 3-fraction column clean-up of one sample)

For each sample:

Column - 1

50 mL screw cap centrifuge tubes, 3 for each sample

Pasteur pipettes (9□ inch and 5□ inch):

Graduated cylinders: 50 mL

Beaker, 50 mL - 1

Waste jar - 1

Beakers, 400 mL - 3

Rubber pipette bulbs

Hexane

50% hexane:50% dichloromethane

70% acetone:30% dichloromethane

Cork rings for each 50 mL centrifuge tubes - 2

Rubber hammer - 1

Stainless steel spatula - 1

20" rod - 1

Teflon stopcock

Glass wool

3.5% or 3% deactivated silica

Sodium sulfate

Ultrasonicator

Procedure

i) Packing Columns

Put stopcocks on columns.

Stuff glass wool (approximately 1 cm into lower end of each column with 20" rod.

Measure and mark 3.5" from glass wool plug for silica packing and 0.5" for sodium sulfate cap. For rain sample the sodium sulfate cap should be 1.5".

Clamp columns securely onto frame in ventilation hood. Place an empty glass container under each column.

Close stopcocks; fill columns half full of hexane. Tap columns to get out air bubbles before packing columns.

Make slurry of hexane and deactivated silica. Pour slurry into each column. DO NOT ALLOW SILICA TO DRY OUT. Open stopcocks.

Tap columns with rubber hammer to pack silica to desired length.

Cap columns with □□□" Na2SO4 for XAD-2 and QFF samples, 1.5" Na2SO4 for precipitation samples.

Wash columns with 25 mL hexane for conditioning.

Close stopcocks to prevent further dripping when hexane level reaches 1 cm above the top of Na2SO4. NEVER LET THE COLUMN RUN DRY.

ii) Fractionation using long columns

Set up

Label 50 mL tubes for each sample for hexane, 50% dichloromethane in hexane, 70% acetone in dichloromethane fraction. Place the tubes for the hexane fraction underneath the columns.

Place sample 50 mL tubes in front of columns.

Prepare three graduated cylinders of elution solvents, one with 30 ml of hexane, one with 40 ml of 50% dichloromethane in hexane and one with 30 ml of 70% acetone in dichloromethane for each sample.

Loading samples and collection of Fraction 1

Ultrasonicate each filter sample in the flask and load the sample on column with Pasteur pipet. DO NOT ultrasonicate the precipitation samples. Once precipitation samples are loaded on the column, place the flask in the wash bin as no rinses are added to the precipitation flasks.

Open stopcock and let the column drip until the solvent in the column reaches the top of the sodium sulfate. The column should be equilibrated. When the sample touches the top of the Na2SO4, add 1 mL of hexane from the graduated cylinder to the sample flask, rinse the sample flask 3x and then add to the column. Once the rinse reaches the top of the Na2SO4, add the remainder of the 30 ml of elution solvent. Let column drip at a rate of 1-2 drops per second into the 50mL tube. Once the first fraction touches the top of the Na2SO4 ,close the stopcock on the column, remove the collection tube and cap it. The 1st

fraction is now completed. Place the 50% fraction collection tube beneath the column to begin collecting fraction 2.

Fraction 2

After the first fraction is completely collected, add 1 mL of the 50% dichloromethane in hexane to the sample tube and rinse 3x, then add this onto the top of the column. Once the rinse reaches the top of the Na2SO4 add the remainder of the 50% elution solvent. Let the column drip at a rate of 1-2 drops per second into the sample collection tube. Once the second fraction touches Na2SO4 close the stopcock on the column. The 2nd fraction is now complete. Place the 70% collection tube beneath the column to begin collecting fraction 3.

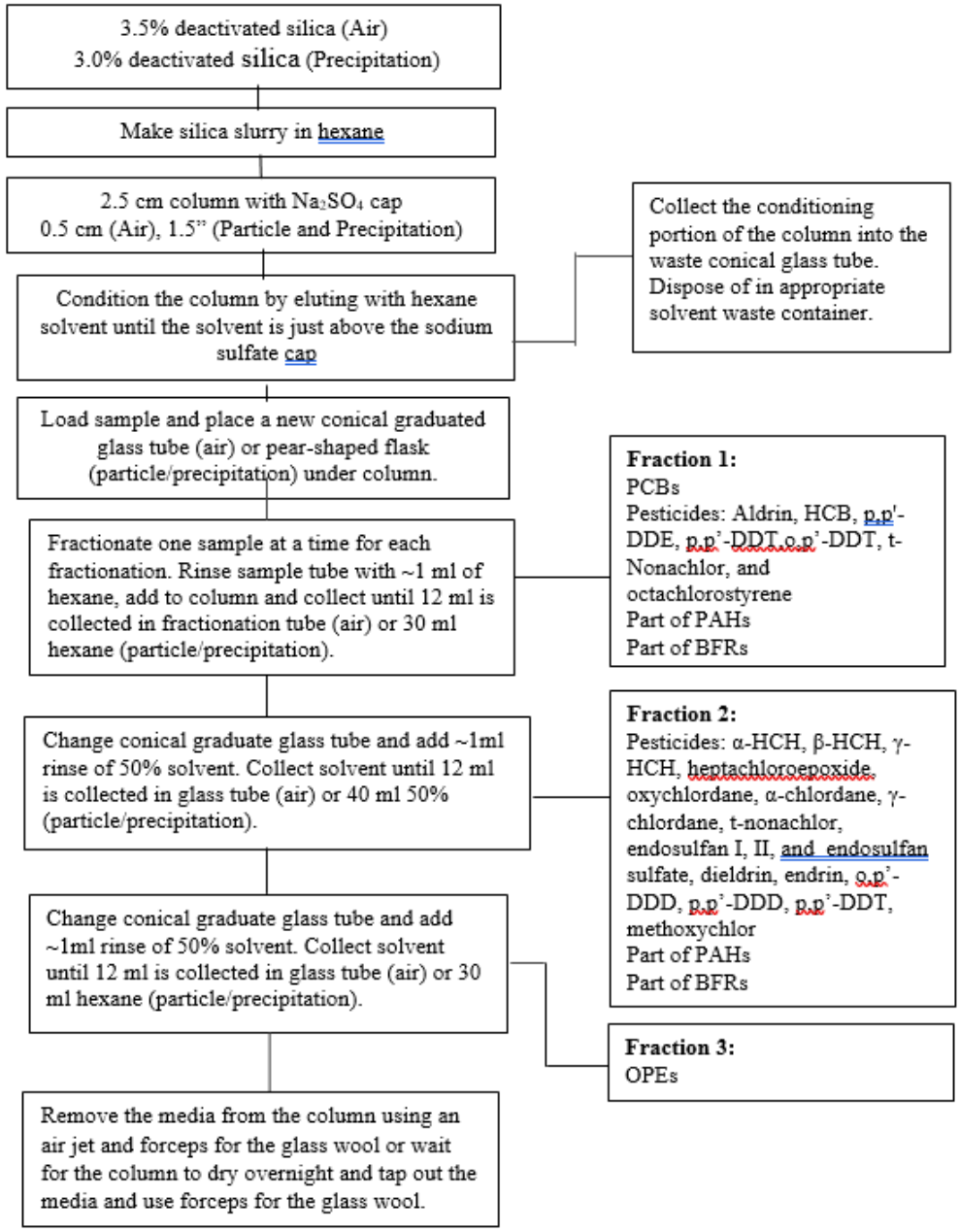
Fraction 3

After the 2nd fraction is completely collected, rinse the sample tube with 1 mL 3x of 70% acetone in dichloromethane and add this to the column. Once the rinse has reached the top of the Na2SO4 ,add the remaining 30 ml of 70% acetone in dichloromethane. Let the column drip at a rate of 1-2 drops per second into the collection tube. Allow the final 30 mL of elution solvent to drip completely into the collection tube. Remove tube with 3rd fraction, cap it, and store in dark place.

iii) Clean-Up

With a jet of air get the dry silica out of the column. Silica should be treated as solid waste.

FLOW CHART 6. DIAGRAM FOR SILICA COLUMN CHROMATOGRAPHY OF CARTRIDGE SAMPLES



iv). Re-cleaning of Fraction 1 (hexane fraction), Fraction 2 (50%), and Fraction 3 (70%)

Sometimes the chromatograms are not clean enough for correct analysis. This may be due to overloading of the silica column by the concentrated extracts. In these cases, the fractions need to be recleaned through silica column for the 2nd time.

Procedure for Fraction 1 (hexane fraction) recleaning:

Activate and deactivate the silica in the usual way.

Pack up the slurry in the same way topping it with sodium sulfate.

Directly load the Fraction 1 from the vial. Rinse the vial with 1 ml of hexane, load the rinsing on the column.

Let the column drip at the usual rate. Elute as usual and collect 12 ml in a conical graduated glass tube.

It is not necessary to collect Fraction 2.

Procedure for Fraction 2 (50% fraction) re-cleaning:

Activate and deactivate the silica in the usual way.

Pack up the slurry in the same way topping it and cap with sodium sulfate.

Load the exchanged extract from the pear shape flask on to the silica column.

Rinse tube with 1 mL 50% dichloromethane in hexane and add to column.

Elute as usual and collect 12 ml in a conical graduated tube.

It is not necessary to collect fraction 3.

Procedure for Fraction 3 (70% fraction) re-cleaning:

Transfer Fraction 3 from the vial to a centrifuge tube. Exchange the fractions to hexane by Nitrogen-Evap and solvent exchanging with 25 mL of hexane twice.

Activate and deactivate the silica in the usual way.

Pack up the slurry in the same way topping it and cap with sodium sulfate.

Load the exchanged extract from the pear shape flask on to the silica column.

Rinse tube with 1 mL 70% dichloromethane in hexane and add to column.

Elute as usual and collect 12 ml in a conical graduated tube.

VIII. NITROGEN EVAPORATION OF FRACTION 1, FRACTION 2, AND FRACTION 3

Supplies

Nitrogen-Evap Unit

Cleaned Stainless-steel needles

Hexane

Methanol

Procedure

i) Setup

Check the air and moisture traps are good by looking at the color indicators.

Attach clean Stainless-steel needles to sample locations on the N-Evap.

Slowly turn on the nitrogen tank, ensure all the valves on the regulator are open. Make sure the pressure is not too high so that the needles are blown out of the N-Evap.

Open the individual valves on the N-Evap a half turn and slowly turn the master control knob until the nitrogen is released.

Allow system to purge 5 minutes.

ii) Evaporation
Adjust the sample tray to fit the samples.

Load the tubes between the spring and receptacle and allow bottom of the vial to tube to rest on the sample tray.

Adjust the master control knob to produce visible ripples on the surface of the solvent. (Do not set the flow so high that you create bubbles or splashing within the sample container.)

Allow samples to evaporate. Check periodically to avoid overconcentration or dryness.

iii) Solvent exchange
For Fraction 1, stop once there is about 0.5 mL remaining in the tube.

For Fraction 2, solvent exchange once with 2.0 mL of hexane and concentrate to 0.5 mL.

For Fraction 3, solvent exchange once with 2.0 mL of methanol and concentrate to 0.5 mL.

iv) Completion
Remove samples and place used stainless-steel needles upside down in a graduated cylinder or beaker for cleaning.

Turn off nitrogen, bleed system, and ensure all valves are closed, include the master control knob.

Place needles in a clean beaker and cover with dichloromethane.

Cover loosely with foil with dull side down.

Sonicate needles for 15 minutes.

Drain solvent and repeat twice more.

Drain all solvent and transfer needles to clean beaker.

Cover the beaker with foil and store them for future use.

IX.TRANSFER OF SAMPLES

Supplies (each sample)
Pasteur pipettes (9.□ inch and/or 5.□ inch):

Amber glass vial (4 mL) for each fraction

Beaker

Vial file for 4 mL vials

Rubber pipette bulbs

Hexane

Procedure

Label each amber vial with sample ID and fraction ID.

Transfer entire sample volumetrically from flask to amber vial with 2 hexane rinses using a pasteur pipette.

Close amber vial tightly, place in vial file, and store in freezer at -200C. Label the vial file with Batch ID.

X. NITROGEN EVAPORATION (N-EVAP)

Supplies

Nitrogen Evaporation (N-Evap) Unit

Cleaned Stainless-steel needles

Hexane

Methanol

Procedure

i) Setup

Check the air and moisture traps are good by looking at the color indicators.

Attach clean Stainless-steel needles to sample locations on the N-Evap.

Slowly turn on the nitrogen tank, ensure all the valves on the regulator are open. Make sure the pressure is not too high so that the needles are blown out of the N-Evap.

Open the individual valves on the N-Evap a half turn and slowly turn the master control knob until the nitrogen is released.

Allow system to purge 5 minutes.

ii) Evaporation

Adjust the sample tray to fit the 4 mL vials containing samples.

Load the tubes between the spring and receptacle and allow bottom of the vial to tube to rest on the sample tray.

Adjust the master control knob to produce visible ripples on the surface of the solvent. (Do not set the flow so high that you create bubbles or splashing within the sample container.)

Allow samples to evaporate. Check periodically to avoid overconcentration or dryness.

Evaporate down all samples and all fractions to approximately 1mL. For summer samples it may be changed to 1.5 to 2 mL especially for 50% fraction.

Completion

Remove samples and placed used stainless-steel needles upside down in a graduated cylinder or beaker for cleaning.

Turn off nitrogen, bleed system, and ensure all valves are closed, include the master control knob.

Place needles in a clean beaker and cover with dichloromethane.

Cover loosely with foil with dull side down.

Sonicate needles for 15 minutes.

Drain solvent and repeat twice more.

Drain all solvent and transfer needles to clean beaker.

Cover beaker with foil and store them for future use.

XI. SPIKING SAMPLES WITH INTERNAL STANDARDS (ISTD)

Supplies

Samples in 4 mL amber glass vials

Internal standards (ISTD)

Hexane

Dichloromethane

Waste containers

Microdispensers: 50 and 100 µl

Procedure

Remove internal standards from freezer; equilibrate to ambient temperature (approximately two hours).

Clean microdispenser by rinsing with dichloromethane.

Insert a new glass capillary.

Rinse the capillary with hexane twice and air dry. Draw spiking standard. Make sure that there are no air bubbles in the capillary.

Spike the sample.

Mark each amber vial label with an appropriate color of dot to denote that they have been spiked:

red for PCB

blue for pesticides

black for PAHs

purple for PBDEs

Rinse the dispenser with solvent

Replace glass tube used to cover plunger of microdispenser before storing.

TABLE 5. TABLE OF INTERNAL STANDARDS AND MASS PER FRACTION

Fraction	Compounds	Internal Standard	Concentration	Spike	Vial	Box
Fraction 1	PCB	Cong, 30, 204	30=80 ng/ml, 204=60 ng/ml	100 µl	●	✓
	PAH	d_{10}anthracene, d_{12}benz[a]anthracene, d_{12}perylene	4 µg/ml each	50 µl	●	✓
	BFR	BDE-118, BDE-181	BDE-118= 0.1 µg/ml, BDE-181= 0.2 µg/ml,	50 µl for air 200 µl for precip	●	✓
Fraction 2	Pesticide	cong. 65,155	65=20 ng/ml, 155=20 ng/ml	100 µl	●	✓ (DB-5) ✗ (1701)
	PAH	d_{10}anthracene, d_{12}benz[a]anthracene, d_{12}perylene	4 µg/ml each	50 µl	●	✓
	BFR	BDE-118, BDE-181,	BDE-118= 0.1 µg/ml, BDE-181= 0.2 µg/ml,	50 µl for air 200 µl for precip	●	✓
Fraction 3	OPEs	d_{15}TDCPP, M_6TBEP, d_{15}TEP	4 µg/ml each	100 µl	●	✓ (OPE)

XII. MAKING MICROVIALS FOR GC ANALYSIS

Supplies

Disposable microvials with inserts

Pasteur pipettes

Vial racks

Septa (vial caps)

Crimper

Procedure for preparing GC microvials

Label microvials with sample IDs and fractions. Arrange for 2 hexane blanks, 2 Calibration Standards and 1 reference standard.

Put the insert in the vial.

Using a pasteur pipette, put approximately 200 µL of each sample, hexane, appropriate Calibration Standards, and Reference Standard in the inserts.

Use different pasteur pipette for different sample and standard.

Crimp septa onto the microvials.

Load the microvials into GC or GC/MS autosampler.

TABLE 6. TABLE OF CALIBRATION STANDARDS

Fraction	Target compounds	Calibration standards
1	PCBs	S-8074A-R1, S-8074B-R1 S-8074C-R1 (0.5 to 1 ug/mL- supplied by EPA and Env. Canada) fortified with 5 pesticides
2	Pesticides	Mixed pesticide standard: 20 ng/mL each
2	PAH	Mixed PAH standard 200 ng/mL each (approximately)
1, 2	Brominated flame retardants	Mixed flame retardants standard: 2-40 ng/mL
3	OPEs	Mixed OPE standard: 2 µg/mL

XIV. SAFETY

Working in the Laboratory

Chemists working in the laboratory should follow certain safety rules:

Individual is required to wear a lab coat whenever working in the lab.

Eye protection with splash resistant safety glasses or safety goggles is required. Contact lenses are forbidden

Protective gloves should be used while handling samples or standards. Special solvent resistant gloves should be used while handling large amount of solvents.

All solvent work should be done inside fume hood.

Open shoes are not allowed in the laboratory.

Particle mask is required when using dry silica.

Generally, nobody should work alone in the laboratory. If work must be performed after hours or on the weekends inform supervisor or other laboratory personnel so that your presence is known.

Chemicals and solvents are stored in separate storage areas. One week's supply is kept in the laboratory. Solvents are stored in special solvent cabinet. Acids must be separated from bases. A rubber bucket is used to carry any chemicals.

Gas cylinders should be well secured at all times. Flammable gases are stored in separate cage.

Hands should be washed thoroughly after work. Protective hand cream "Soft guard" is available.

No food or drink is allowed in the laboratory.

In case of minor spillage get spillage kit to clean the area. A major spill requires the University Health and Safety Division to be contacted and the working area needs to be evacuated.

MSDS and safety manuals are filed in a three ring binders and kept in book case near the laboratory main entrance. MSDS for chemicals used, can be located online from IU EHS https://protect.iu.edu/environmental-health/safety-data-sheets/index.html.

All chemicals and standard should be labeled properly with scientific name, date, and initials of person to contact.

Empty chemical bottles should be flushed out with water, or, in case of liquid, allowed to evaporate under a hood before discarding.

All employees should take the Safety training offered by Indiana University.

Safety Equipment

Fume Hood

IADN sample preparation requires frequent use of solvent. Therefore, all extraction, column chromatography, standard preparation, sample transfer, nitrogen blow down and preparation of microvials should be done in the hood. It is really important to check hood from time to time to ensure that it is working properly. A flow of 80-120 linear feet per second must cross the hood.

Safety Showers

Emergency showers are in strategic areas of the laboratory to provide to provide immediate emergency protection against fire or chemical injury. It is operated by pulling the handle down. It delivers 30 gallons of water per minute. Authorized personnel check it periodically.

Eye Wash

Emergency eyewash is in the laboratory. It is operated by pulling the lever forward. It is checked every month to ensure that is functioning properly.

Waste disposal

Solvents

Label 2 containers, `CHLORINATED WASTE' and `NON-CHLORINATED WASTE'.

Containers may be empty glass bottles from solvents or 5-gallon metal cans.

When in use they are to be placed inside a fume hood with the sash pulled down.

University Health and Safety Department will pick up the waste solvent.

Label the container properly and sign it. The request for waste pick up should be done on line.

Silica

After solvent has evaporated, pour silica into a separate bottle. When the bottle is full label it. University Health and Safety will pick it up together with the waste solvent.

Teflon Boiling Chips

Place in waste container (i.e., beaker) under hood until solvent evaporates, then empty into trashcan.

Glass

Place in `Broken Glass Disposal Containers'. Close the container according to directions when the

container is full. Leave for the custodial services to pick-up or take out to the trash dumpster.

Foil

Place in trashcan.

Fiberglass wool

Place in waste container (i.e., beaker) under hood until solvent evaporates, then empty into trashcan.

Sharp objects

Sharp objects like needle and syringes should be disposed off in special container designed for sharp objects.

XAD-2 and QFF

Leave in soxhlet under hood until solvent has evaporated. Pour XAD-2 into container labeled `USED XAD-2'. Discard QF into trash can.