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

IADN Project

Sample Preparation Procedure

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TABLE OF CONTENTS

Flow-Charts **Page**

INTRODUCTION

This document describes detailed laboratory procedure for extraction and chromatographic cleanup of air and precipitation samples collected for the Integrated Atmospheric Deposition Network (IADN) from 5 Great Lakes stations. It includes routine operation for cleaning glassware and precleaning traps like XAD-2, Quartz fiber filter (QFF), and laboratory chemicals. The procedure needs 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 notebook.

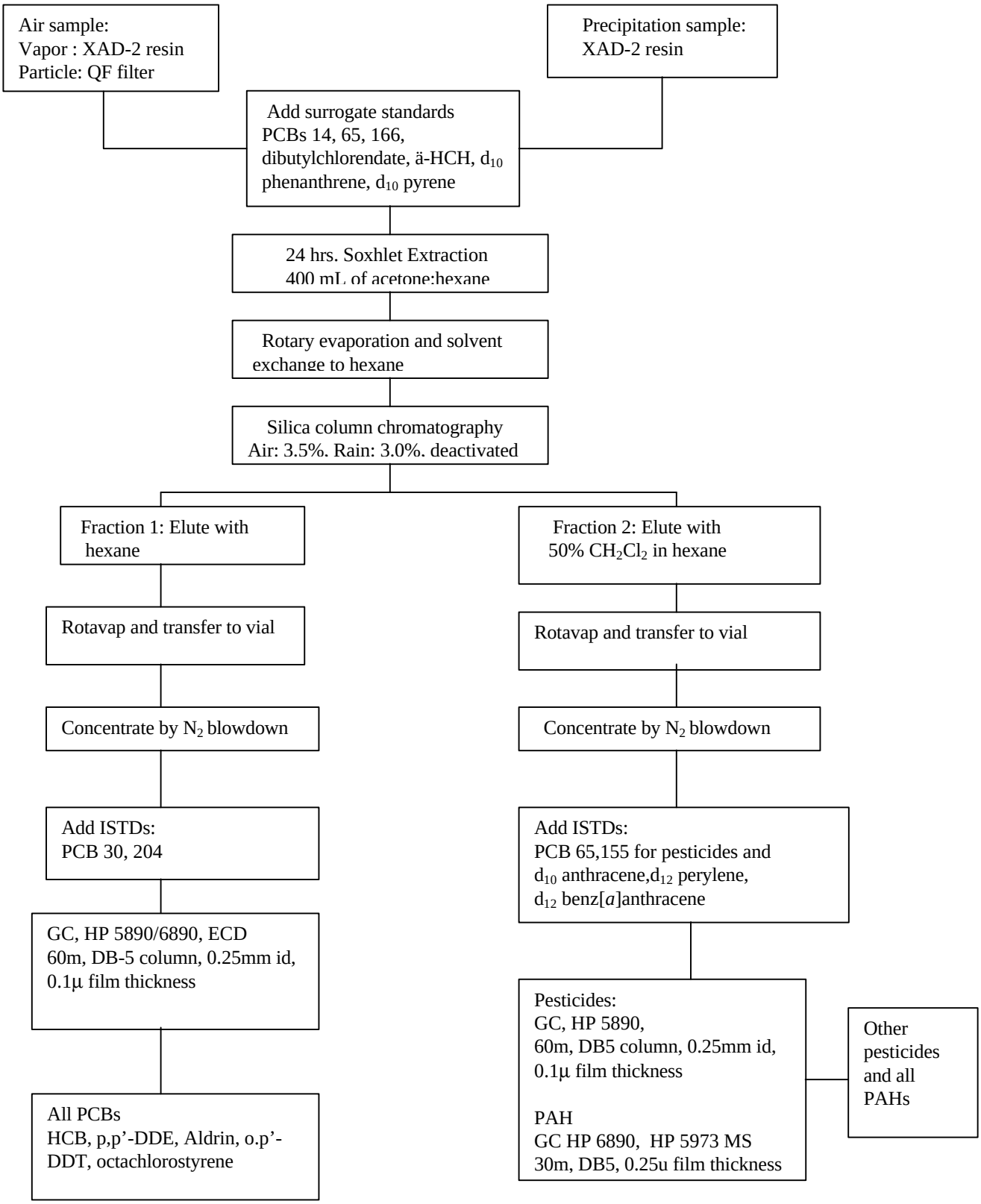
Laboratory personnel are often required to handle chemicals and standards, which may be toxic and carcinogenic. Proper safety protection should be taken to handle these chemicals. Indiana University offers a training program for laboratory safety rules and personal protection. All laboratory employees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

The compounds that are analyzed in this project are total PCBs and 109 individual PCB congeners, 22 organochlorine pesticides, and 16 polycyclic aromatic hydrocarbons. A complete list of all compounds is given in Table 1.

Table 1: List of Analytes

PCBs		**22 Pesticides**
1	87	HCB
3	85	alpha-HCH
4+10	136	beta-HCH
7+9	77	gamma-HCH
6	110	heptachloroepoxide
5+8	82	alpha-chlordane
19	151	gamma-chlordane
12	135+144	oxychlordane
13	107	*trans*-nonachlor
18	123+149	methoxychlor
17+15	118	endosulfan I
24	134	endosulfan II
27	114+131	endosulfan sulfate
16	146	p,p'-DDT
32	105+135+153	p,p'-DDE
29	141	p,p'-DDD
26	137+176	o,p'-DDT
25	130	o,p'-DDD
31	163+138	aldrin
28	158	endrin
33	129	dieldrin
53	178	Octachlorostyrene
51	175	
22	187+182	**16 PAHs:**
45	183	fluorene
46	128	phenanthrene
52	167	anthracene
43	185	fluoranthene
49	174	pyrene
47	177	retene
48	202+171	benz[*a*]anthracene
44	156	chrysene
37	173	benzo[*b*]fluoranthene
42	157+200	benzo[*k*]fluoranthene
41+71	172	benzo[*e*]pyrene
64	197	benzo[*a*]pyrene
40	180	indeno[1,2,3-*cd*]pyrene
100	193	dibenz[*a*,*h*]anthracene
63	191	benzo[*ghi*]perylene
74	199	coronene
70+76	170+190	
66	198	**Others**
95	201	Total suspended particles
91	203	
56+60	196	
84+92	189	**Meteorological**
89	208+195	Temperature
101	207	Wind speed
99	194	Wind direction
119	205	Solar radiation
83	206	Relative humidity
97	209	Precipitation
81	Total	Barometric pressure

Summary of sample preparation



I. CLEANING: GENERAL LABWARE

General Cleaning Supplies

Micro cleaning solution (Micro-90, International Products Corporation)
Dish washing brushes
Deionized (DI) water, Millipore, Milli-Q water system
Muffle furnace, Thermolyne 30400
Ultra sonicator
Aluminum foil
Solvents: dichloromethane, hexane
Squirt bottle with solvents
Beakers

Procedure

1. Glassware

Wash general glassware like soxhlet extractor, round bottom flasks, beakers, pearshaped flasks, centrifuge tubes, separatory funnels etc. thoroughly with soap and water using brushes.
Rinse glassware with hot tap water and with organic free DI water from Milli-Q system.
Dry glassware in air overnight.
Cover all open ends with foil. Always use dull side of the foil towards glassware.
Muffle glassware at 450^0C for 6 hours.
Allow glassware to cool to 100^0C before removing from furnace.
Store in cabinets.

The volumetric flasks and the pipets are not muffled. After cleaning with soap and water they are ultrasonicated with dichloromethane 3 times, 15 minutes each time.

2. Stainless Steel Tools

Wash forceps, spatulas, stainless steel air cartridges and aluminum nuts with soap and water.
Rinse well with hot tap water and DI water.
Dry at room temperature overnight.
Rinse with dichloromethane.
Wrap each tool separately in foil.
Store.

Air sampling cartridges and screen meshes are muffled at 450^0C for 6 hrs before storing. Al- nuts Cannot be muffled

3. Amber glass vials and Pasteur pipettes

Put the pipette or the vials in beakers and cover beakers with foil.
Muffle at 450^0C for 6 hours.
Cool to 100^0C; remove from oven. Insert Teflon liners into vial caps.
Cap the vial 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.
Place in 70°C drying oven for 2 hours.
Store in sealed jar.

5. Microdispenser capillaries, GC vials, and stainless N_2 blowdown needles

a) Microdispenser capillaries

Before using rinse with dichloromethane and air dry.

b) GC autosampler vials (reusable)

Place microvials, open end up, in a clean beaker. Cover vials with dichloromethane, making sure **NO** air bubbles remain in the microvials. Cover loosely with foil.
Sonicate microvials for 10 minutes.
Drain solvent, and repeat twice more.
Drain all solvent and transfer microvials to clean beaker; cover with foil. Muffle at 450^{o}C for 6 hours. After furnace returns to 100^{0}C (or the next morning) remove vials from furnace.
Store in sealed container.

c) GC autosampler vials and inserts (disposable)

Place the vials and the inserts in a beaker. Cover with Al-foil.
Muffle at 450^{0}C for 6 hours.
Dispose of after use.

d) Stainless N_2 blowdown needles

Place needles in a clean beaker and cover with dichloromethane. Cover loosely with foil.
Sonicate needles for 10 minutes.
Drain solvent, and repeat twice more
Drain all solvent and transfer needles to clean beaker. Cover beaker with foil.
Dry them in drying oven.

6. Teflon Stopcocks and Lids for Sample Jars

Wash stopcocks and lids with soap, tap water, and DI water.
Air dry on kimwipes.
Rinse the Teflon stopcocks (those without washers) with dichloromethane
Store the stopcocks in muffled jars.
Place the clean lids on muffled sample jars or wrap them in foil.

II. PRECLEANING: TRAPS 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
Muffle at 450^0C for 6 hours. Cool them down to 100^0C
Store

2. Teflon Boiling Chips and Sodium Sulfate

Supplies

Soxhlet extractor
Condenser
Sample jar and lid
1 liter beaker
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 inside of condenser and outside of joint with solvent in squirt bottles: first with methanol, then with dichloromethane.
Put 5 or 6 boiling chips in flask. Add 350 mL of dichloromethane to flask.
Place Teflon boiling chips or sodium sulfate 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 40-45).
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 and sodium sulfate in muffled sample jar; cover with foil.
Place boiling chips or sodium sulfate in a drying oven at 70°C approximately for 2 hours.
Cover the jars with lids.
Store the boiling chips on shelf.
Store sodium sulfate in a desiccator.

3. XAD-2

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)
One liter beaker
Beakers
Adapter to convert 29/42 to 24/40
Boiling chips
Dichloromethane
Hexane
Methanol
Acetone
HPLC grade water: EM Science
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
Rinse XAD-2 with tap water several times, stirring to remove the foam and the small particles.
Use kimwipes to remove the foam.
Place XAD-2 in soxhlet extractor plugged with glass wool.
Rinse with small amount of methanol 3 times to remove water.
Add 500 mL of methanol to 1 liter flask.
Add about 20 boiling chips to flask.
Assemble flask/soxhlet/condenser apparatus.
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 500 mL acetone to 1 liter flask.
Add about 20 boiling chips to 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 dichloromethane.
Set variac at 48.
Extract for 24 hours.

Day 5
Follow the procedure of Day 2 but use hexane as solvent. Use a new flask.
Set variac at 50.
Extract for 24 hours.

Day 6
Follow the procedure of Day 2 but use acetone:hexane 50:50 (vol:vol) as solvent.
Set the variac at 45
Extract for 24 hours.

Day 7
Turn off heater; cool 15 to 30 minutes.
Flush as much acetone/hexane from soxhlet as possible.
Pour XAD-2 in a beaker and dry in an oven at 70°C for 6 hours.
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

Note: Recycled XAD-2 is already free from foam and fine particles. To preclean this, omit the water rinsing and the methanol extraction steps. Start extraction with acetone and then follow the whole procedure. For new XAD-2, extraction period for each solvent can be extended to 48 hours.

ii) Wet XAD-2 for Precipitation sample cartridges:

Day 1
Rinse XAD-2 with tap water many times, stirring to remove foam and small particles.
Place XAD-2 in extractor plugged with glass wool.
Rinse with small amount of methanol 3 times to remove water.
Add 500 mL of methanol to 1 liter flask.
Add about 20 boiling chips to flask.
Assemble flask/soxhlet/condenser apparatus.
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 500 mL acetone to 1 liter flask.
Add about 20 boiling chips to 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
Set variac at 50.
Extract with hexane for 24 hours.

Day 4
Follow the procedure of Day 2 but use dichloromethane as solvent. Use a new flask.
Set variac at 48.
Extract for 24 hours.

Day 5
Follow the procedure of Day 2 but use hexane as solvent
Set variac at 50
Extract for 24 hours.

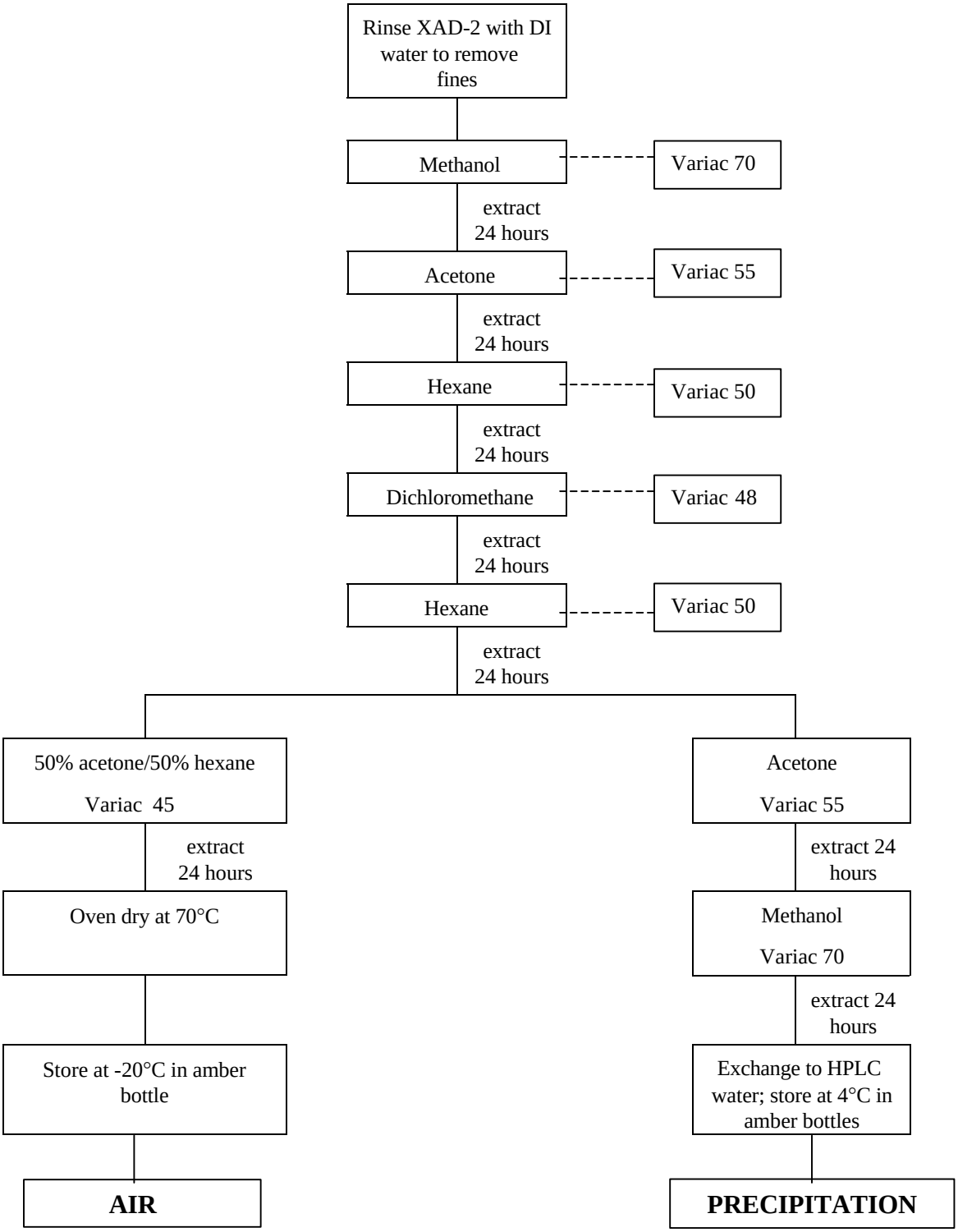
Day 6
Follow the procedure of Day 2 but use acetone as solvent. Use a new flask.
Set variac at 55
Extract for 24 hours.

Day 7
Follow the procedure of Day 2 but use methanol as solvent.
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 EM Science HPLC grade water until XAD-2 does not smell of any solvent.
Store in amber bottle in a refrigerator at 4^0C up to three months.
Keep subsample in separate jar for checking lab blank and matrix spike.

Note:
For new XAD-2, extraction period for each solvent can be extended to 48 hours.
For recycled XAD-2 start extracting with acetone.

Summary of XAD-2 Precleaning Procedure



4. Silica and quartz fiber filters (QFF)

i) 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)

Wrap up each QFF by aluminum foil separately.
Muffle at 450°C for 6 hours.
After cooling put them in plastic bag and store them in freezer at -20^0C.

III. 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 ìl) and 1 mL pipette
Boiling chips
Acetone
Hexane
Surrogate Recovery standards:

STANDARDS	CONCENTRATIONS
Surrogate Standard	Congener 14: 200 ng/mL
	Congener 65: 50 ng/mL
	Congener 166: 50 ng/mL
	Dibutylchlorendate: 200 ng/mL
	ä-HCH: 200 ng/mL
	d_{10} phenanthrene: 4 µg/mL
	d_{10} pyrene: 4 µg/mL

One matrix spike vial (MS vial) with recovery standards: PCB (680.16 ng), pesticides (20 ng each), and PAHs (400 ng ea)
Squirt bottle
Waste solvent bottle
Cork rings (one per each 500ml 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

Procedure

i) Setting up

One batch of samples generally include:

a) Regular field samples: 10-12. It may include field blank and field duplicate.
b) Laboratory duplicate: One air vapor sample split into two equal parts in laboratory.
(No laboratory duplicate for filter and precipitation samples)
c) Laboratory blank: Sampling media spiked with Surrogate Standards or a
d) Matrix spike: Sampling media spiked with Recovery Standards with known amount of all compounds of interest.
(In case of precipitation samples set up both laboratory blank and Matrix spike)

On the day of extraction a unique Batch ID is assigned to a batch of sample with month, year, and sample type. Thus the Batch IDs of the cartridge and filter samples from September 98 will be S98C and S98F. The Batch ID for precipitation samples from 97 August will be AU97P.

Day 1
Remove standards from freezer. Standards **must** be at ambient temperature before using. (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 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.
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.
Rinse tweezers and scissors with dichloromethane before starting the next sample.

Precipitation sample: XAD-2 column and dry wipe
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 30g of dry XAD-2, or muffled QFF or 8g of wet XAD-2 in a soxhlet extractor plugged with glass wool.
Add:
PCB recovery standard: complete suit of PCB (680 ng, from Michael D. Mullin 94 mix)
Pesticide Recovery Standard: all pesticides 20 ng each (Laboratory mix)
PAH Recovery Standard: all PAHs 400 ng each (Laboratory mix)
The recoveries of each compound will show the extraction efficiency of that batch.

Laboratory blank
Take about 30g of dry XAD-2, or muffled QFF or 8g of wet XAD-2 in a soxhlet extractor plugged with glass wool.

Laboratory duplicate: for air vapor only
Take the weight of whole XAD-2 in a sample from Chicago or Sturgeon Point.
Weigh out approximately half of it and carefully transfer it in a soxhlet extractor plugged with glass wool.
Put the other half in another soxhlet extractor plugged with glass wool.

About 10g of XAD-2 is extracted from summer Chicago samples.

ii) Spiking with Surrogate Standards

Using a 100 µL micro dispenser, spike each sample with:

PCB 14:	20 ng
PCB 65:	5 ng
PCB 166:	5 ng
Dibutylchlorendate:	20 ng
ä-HCH:	20 ng
d_{10} phenanthrene:	400 ng
d_{10} pyrene:	400 ng

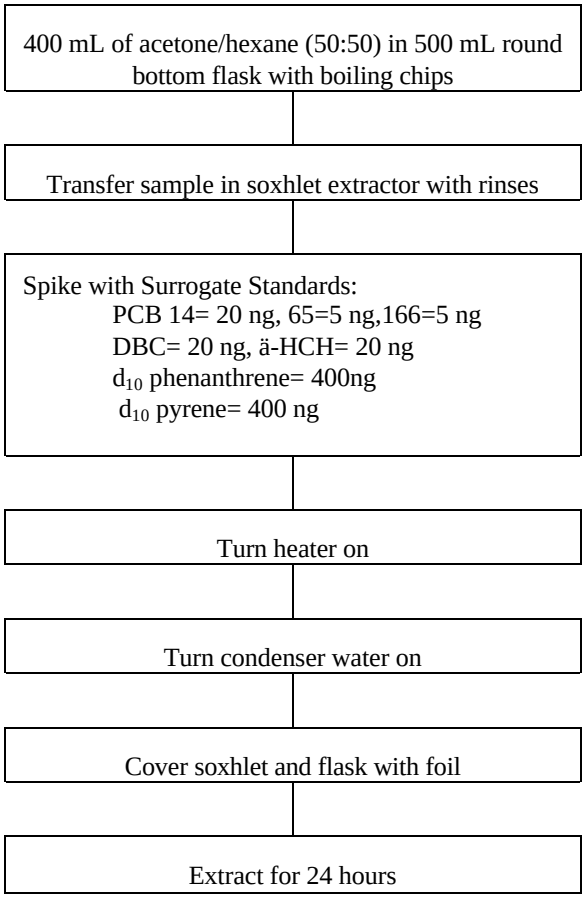
Recovery of each surrogate standard will show the extraction efficiency of individual sample.

iii) Extraction

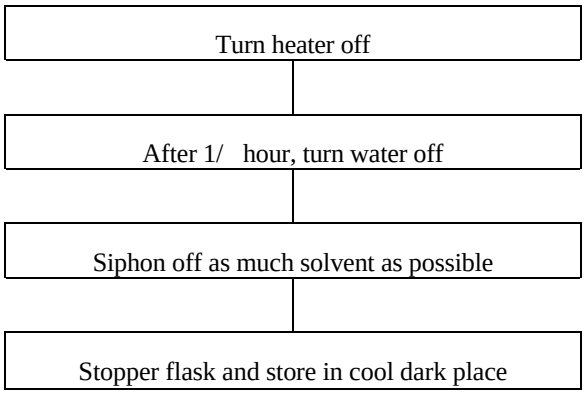
Day 1
Assemble flasks, soxhlets, and condensers. Place on heating mantles.
Turn on heating mantles. Set the heater at 3 and or the variac at 45.
Turn on condenser water on.
Cover soxhlet and flask with foil.
Extract for 18 to 24 hours.

Day 2
Turn heating mantle off. Let them cool down for 30 minutes.
Siphon off as much solvent from soxhlet extractor into flask as possible.
Detach the flask and insert stopper.
Turn off condenser water.
Store the extracts in cool dark place.

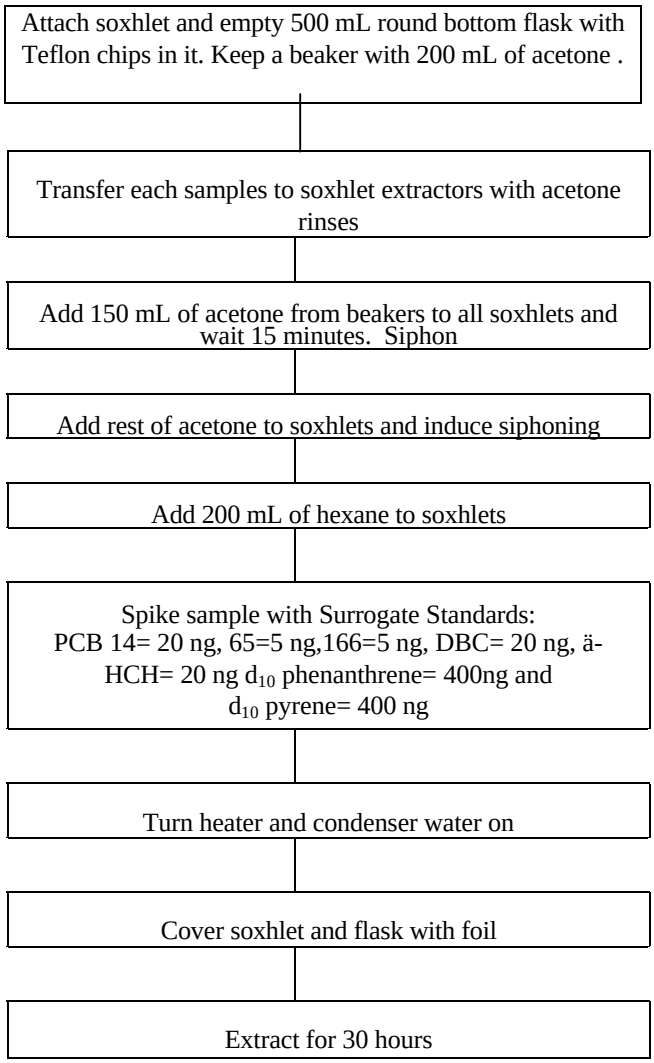
Summary of extraction of air samples
Setting up extraction



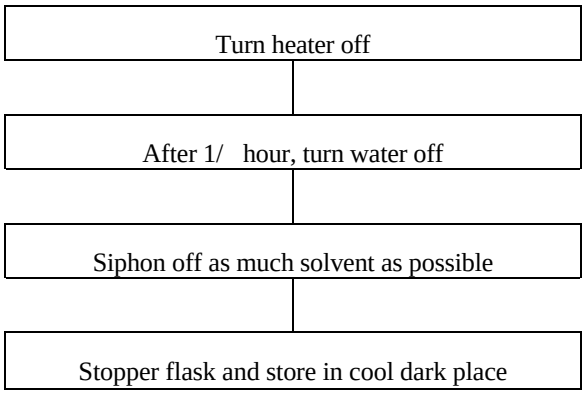
Taking extraction down



Summary of extraction of precipitation samples
Setting up extraction



Taking extraction down



IV. ROTARY EVAPORATION

1. Air (XAD-2 and QFF) Extract

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

Supplies

Splash guard with 24/40 joint
Beaker 100 or 200 mL
Waste container for used boiling chips
Hexane
Clean large forceps
Squirt bottle
Rotary Evaporator (Buchi Rotavapor, R-114)
Faucet aspirator
Chiller circulator (Neslab, Cool Flow, CFT-25)

Procedure

i) Setting up

Fill chamber with DI water.
Turn the chiller circulator on.
Set bath temperature 30^0C -35^0C.
Rinse joint of steam duct with dichloromethane or hexane.
Attach appropriate splash guard to steam duct. Clamp each joint.
Turn vacuum on with the faucet aspirator.

ii) Evaporation

Remove boiling chips from the extract with large clean forceps.
Attach flask to splashguard. Clamp joint.
Turn motor on to rotate the flask. The sample should **not** boil.
Evaporate the extract down to approximately 2 mL.
Open stopcock of the rotary evaporator to release vacuum.
Detach the flask.

iii) Solvent exchange

Add 75 mL of hexane and rotavap down to 2 mL again.
Repeat the process once more.
Rinse the splashguard with dichloromethane or hexane before next sample.

iv) Completion

Empty the receiving flask into proper waste bottle.
Turn the heater, motor, chiller, and the aspirator off.
Cover steam duct with foil

2. Rotary Evaporation and Back Extraction of Precipitation Extracts

Supplies

Splash guard with 24/40 joint
Waste container for used boiling chips
Hexane
Clean large forceps
Waste bottles
Dichloromethane in Teflon bottle
Separatory funnel
50mL Centrifuge tubes with stoppers
Pasteur pipettes
Rotary evaporator (Buchi Rotavapor, R-114)
Chiller circulator (Neslab, Cool Flow, CFT-25)

Procedure

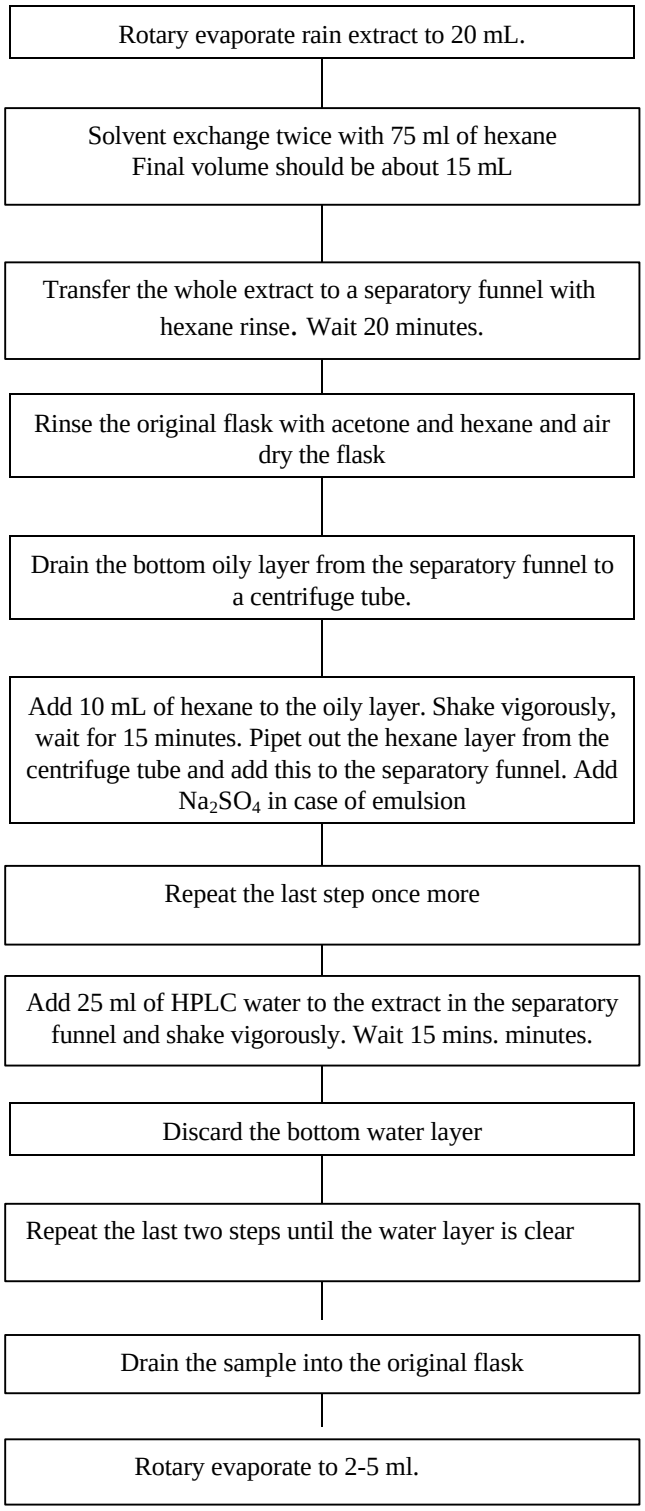
i) Evaporation

Rinse the joint of the steam duct with dichloromethane or hexane.
Attach splash guard to steam duct. Clamp each joint.
Turn vacuum on with faucet aspirator.
Remove boiling chips from the extract with large clean forceps.
Attach flask to splashguard. Clamp joint.
Turn motor on.
Turn water bath on. The temperature should be 30-35^{0}C
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 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 Na_2SO_4 and shake.
Pipet out the hexane layer from the centrifuge tube and add this to the separatory funnel.
Repeat last two steps once more.
Add 25 mL of HPLC water to the extract in the separatory funnel, shake vigorously, and let it stand for 15 minutes
Drain the bottom water layer
Repeat last two steps until the water layer is clear.
Drain the sample into the original flask
Rotary evaporate to 2-5 mL.

Rotary Evaporation and Back Extraction of Precipitation Extracts


Rotary evaporate rain extract to 20 mL.
Solvent exchange twice with 75 ml of hexane
Final volume should be about 15 mL
Transfer the whole extract to a separatory funnel with hexane rinse. Wait 20 minutes.
Rinse the original flask with acetone and hexane and air dry the flask
Drain the bottom oily layer from the separatory funnel to a centrifuge tube.
Add 10 mL of hexane to the oily layer. Shake vigorously, wait for 15 minutes. Pipet out the hexane layer from the centrifuge tube and add this to the separatory funnel. Add Na_2SO_4 in case of emulsion
Repeat the last step once more
Add 25 ml of HPLC water to the extract in the separatory funnel and shake vigorously. Wait 15 mins. minutes.
Discard the bottom water layer
Repeat the last two steps until the water layer is clear
Drain the sample into the original flask
Rotary evaporate to 2-5 ml.

V. 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, Davisil, Grade 634, 100-200 mesh, 60Å
Muffle furnace
Desiccator
Calculator
Balance
Particle mask

Procedure

i). Activation

Day 1
Place approximate amount of silica needed in a beaker. Cover the beaker with foil loosely.
Place beaker in 100°C oven, turn thermostat to 300°C; keep in oven overnight.

Day 2
Turn the oven temperature down to 100°C.
When the oven has cooled down to 250°C, crack the door of the oven open.
When the oven temperature is 150°C remove silica from the oven and make the Al-foil tightly closed.
Let it cool on the counter top until warm.
Store in a desiccator

ii). Deactivation

When silica has reached the ambient temperature (approximately 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:

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

For precipitation samples use 3% deactivation.

SHAKE WELL. Shake flask until all clumps are broken-up.
Store in desiccator overnight for equilibration.
Use deactivated silica in desiccator within three (3) days.

2. Column chromatography

Supplies: (for a 2 fraction column clean-up of one sample)

Column -1
Pear shaped flasks100 mL with 14/20 joints- 2
Glass stoppers with 14/20 joints- 2
Pasteur pipettes (9 inch and/or 5 inch):
Graduated cylinders: 50 mL and 10 mL
Beaker, 50 mL -1
Waste jar -1
Beakers, 250 mL-3
Rubber pipette bulbs
Hexane
50% hexane/50% dichloromethane
Dichlromethane
Cork rings for each 100 mL pear shaped flasks -1
Rubber hammer-1
Stainless steel spatula-1
20" rod-1
Teflon stopcock
Glass wool
3.5% or 3% deactivated silica
Sodium sulfate
Ultrasonicator

For <u>each</u> sample

Item	Air Particle (QFF)	Air Vapor (XAD-2)	Rain (XAD-2)
Amount of silica to activate/deactivate	4-6 gm	4-6 gm	4-6 gm
Column size	3.5"	3.5"	3.5"
Na_2SO_4	0.5	0.5"	1.5"
Elution volume	25 mL	25 mL	30 mL
Switching volume	4 mL	4 mL	5 mL

Procedure

i) Packing columns

Put stopcocks on columns.
Stuff glass wool (approximately 1 cm) into lower end of the 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 samples the sodium sulfate cap should be 1.5".
Clamp columns securely onto frame in ventilation hood. Place empty glass container under each column.
Close stopcocks; fill columns half full with 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 " Na_2SO_4 for XAD-2 and QFF samples, 1.5" Na_2SO_4 for precipitation samples.
Wash columns with 25 mL hexane for conditioning.
When hexane level reaches 1 cm above the top of Na_2SO_4, close stopcocks to prevent further dripping.
NEVER LET THE COLUMN RUN DRY.

ii) Fractionation

Set up

Label 100 mL pear-shaped flask for each sample for hexane and 50% dichloromethane in hexane fraction.
Place the flasks for the hexane fraction underneath the columns.
Place sample flasks in front of columns.
Place a 50 mL beaker in front of sample flask for elution solvents either hexane or 50% dichloromethane in hexane. For 1st fraction add 25 mL of hexane in beaker for air samples and 30 mL of hexane for precipitation samples.

Loading samples and collection of first fraction

Ultrasonicate each sample in the flask and load the sample on column with Pasteur pipet.
Open stopcock and let the column drip at a rate of 1 drop per second in the pear-shaped flask.
When the sample touches the top of the Na_2SO_4, add 25 mL hexane to the top of the column and collect the first fraction

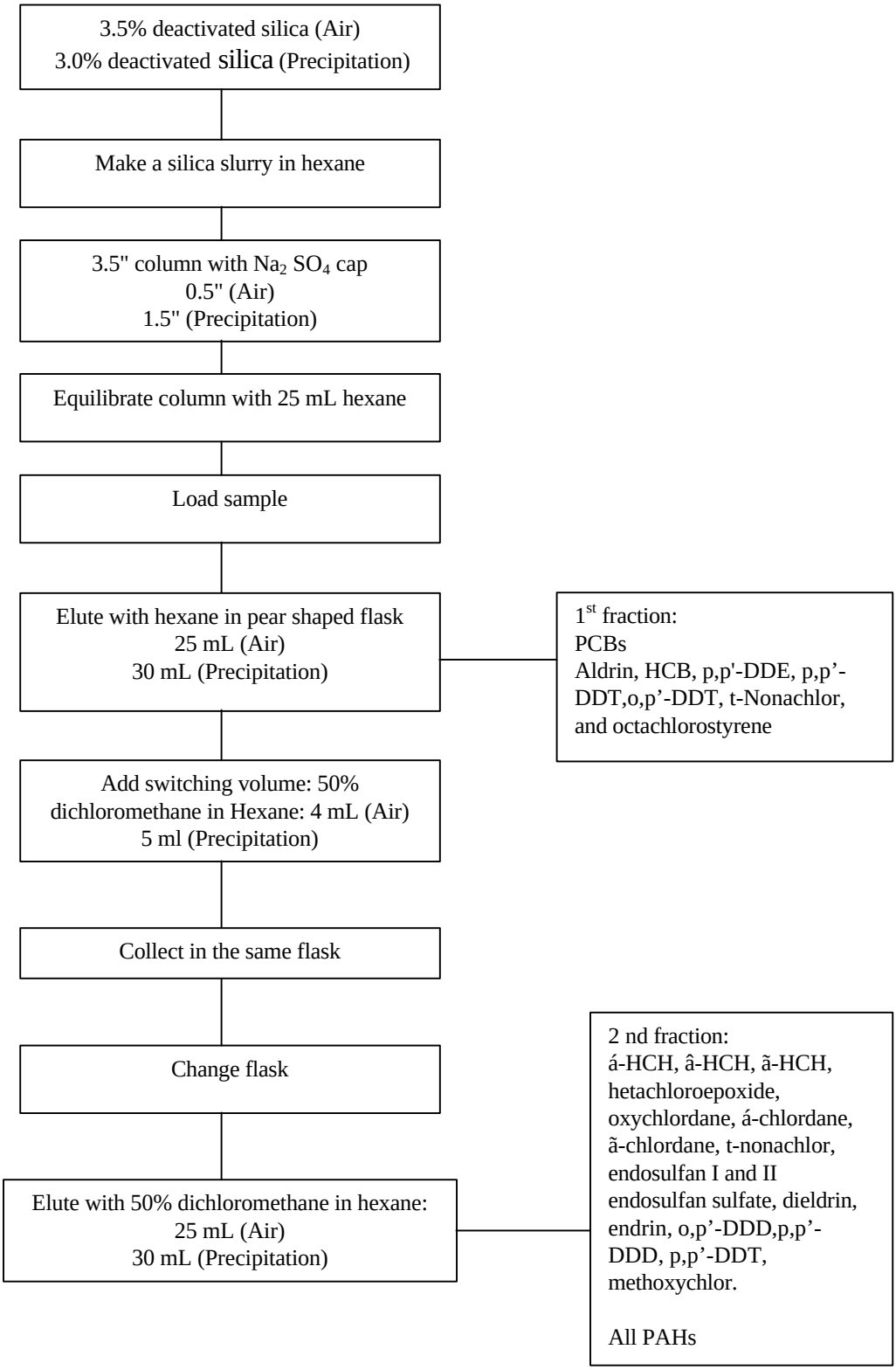
Second Fraction

After the first fraction is completely collected, add 50% dichloromethane in hexane (4mL in case of air sample and 5 mL in case of precipitation samples) on the top of the column. This is the switch volume.
After the switch volume is collected in the same flask containing hexane fraction get the pear-shaped flask and stopper it.
Put a new pear shape flask for collection of 2^{nd} fraction.
Add 25 mL (for air samples) or 30 mL (for precipitation samples) of 50% dichloromethane in hexane on the column and elute.
Once the column has stopped dripping, remove flask with second fraction, stopper it, and
Store in dark place.

iii) Clean-Up

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

Summary Flow-Chart for Silica Column Chromatography



VI. ROTARY EVAPORATION OF FRACTION 1 AND FRACTION 2

Supplies

Splash guard with 14/20 joint
Beaker 50 mL and 100 mL
Hexane
Squirt bottle
Rotary Evaporator
Faucet aspirator
Chiller circulator

Procedure

i) Setup

Fill chamber with DI water.
Turn on the chiller circulator.
Set bath temperature 30^0C -35^0C
Rinse joint of steam duct with dichloromethane.
Attach appropriate splashguard to steam duct. Clamp each joint.
Turn vacuum on with the faucet aspirator

ii) Evaporation

Attach flask to splashguard. Clamp joint.
Turn motor on to rotate the flask. The sample should **not** boil.
Evaporate sample down to approximately 1 mL
Open stopcock of rotary evaporator to release vacuum.
Detach the flask.
Hexane fraction is ready to be transferred.

iii) Solvent exchange

For 50% dichloromethane fraction, solvent exchange once with 25 mL of hexane.
Rinse splashguard with Dichloromethane before using with a different sample.

iv) Completion

Empty receiving flask into proper waste bottle as needed.
Turn off heater on rotary evaporator.
Turn motor off on rotary evaporator.
Turn chiller off
Cover steam duct with foil.

VII 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.
Using a Pasteur pipette, transfer entire sample volumetrically from flask to amber vial with 2 hexane rinses.
Close amber vial tightly, place in vial file, and store in freezer. Label the vial file with Batch ID

VIII. N_2 BLOW DOWN

Supplies

Samples in amber vials
N_2 blow down unit
Dichloromethane

Procedure

Remove all nozzle plugs from unit.
Turn on N_2 at tank and let N_2 Flush out for approximately 5 minutes.
Turn heater on LOW.
Attach clean needle to each nozzle to be used.
Place amber vials in slot; adjust N_2 flow such that there are gentle ripples in the vials.
Evaporate down all samples and all fractions to 1mL. For summer samples it may be changed to 1.5 to 2 mL especially for 50% fraction.
If the chromatograms look dirty in GC run dilute the extracts and analyze again.

Completion

Turn off N_2 at trap.
Replace the nozzle caps.
Rinse and ultrasonicate needles twice with dichloromethane.
Dry them in oven at 100°C

IX. SPIKING SAMPLES WITH INTERNAL STANDARDS (ISTD)

Supplies

Samples in 4 mL amber glass vials
Internal standards (ISTD)
Hexane
Dichloromethane
Waste containers
Microdispensers: 25, 50, and 100 µl

Procedure

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

Fraction	Compound	Sample Type	Internal standard	Spiking volume (µl)	Mass of ISTD in sample (ng)
Hexane	PCBs and pesticides	Vapor, Particle, and Precipitation	PCB 30	100	8
			PCB 204		6
50%	PAHs	Vapor, Particle, and Precipitation	d_{10} Anthracene,	50	200
			d_{12}benz[*a*]anthracene		200
			d_{12} perylene		200
50%	Pesticide	Vapor, Particle, and Precipitation	PCB 65	100	20
			PCB 155		20

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 is no air bubble.
Spike sample.
Mark each amber vial label with an appropriate color of dot: red for PCBs, blue for pesticides, and black for PAHs to denote that they have been spiked.
Rinse the dispenser with solvent
Replace glass tube used to cover plunger of microdispenser before storing.

X. MAKING MICROVIALS FOR GC ANALYSIS

Supplies

Disposable microvials with inserts
Pasteur pipettes
Vial racks
Septa (vial caps)
Crimper

Procedure

Label microvials with sample IDs, 2 hexane blanks, 2 Calibration Standards and 1 performance Standard.

Using a Pasteur pipet, put approximately 200 ì L of each sample, hexane, appropriate Calibration Standards, and Performance Standard in the inserts. Use different Pasteur pipets for different sample and standard.

Fraction	Target compounds	Calibration standard
Hexane	PCBs	Mullin 94: 680 ng/mL
50%	pesticides	Mixed pesticide standard: 20 ng/mL each
50%	PAH	Mixed PAH standard 200 ng/mL each (approximately).

Crimp septa onto the microvials.

Load the microvials into GC or GC/MS autosampler or store in freezer for future use.

XI. STANDARDS

The standards are procured from Ultra Science, Inc. or AccuStandards, Inc. They are further diluted in the laboratory with Hexane to make stock and working standards.

XA. PCB Stock Standards:

1. Mullin's 94 mix: 170.8 ug/mL: Mixture of 1232,1248, and 1262 in 25:18:18. This was supplied by Environmental Protection Agency. This is a stock PCB standard for making Calibration Standard, Performance Standard, and Recovery Standard.

2. PCB Surrogate Standards

Standards	AccuStandard Ampule	Stock concentrations (in Hexane)
PCB Congener 14	100 ì g/mL in Isooctane	1 ì g/mL
PCB Congener 65	100 ì g/mL in isooctane	1 ì g/mL
PCB Congener 166	100 ì g/mL in isooctane	1 ì g/mL

3. PCB Internal Standards (ISTD)

Standards	AccuStandard Ampule	Stock concentrations (in Hexane)
Congener 30	100 ì g/mL in Isooctane	1 ì g /mL
Congener 204	100 ì g /mL in isooctane	1 ì g /mL

4. PCB calibration standard for PCBs: Used to calibrate the instrument

Compounds	Concentration (in Hexane)
Mullin 94	680.16 ng/mL
14 (Surrogate)	20 ng/mL
65 (Surrogate)	5 ng/mL
166 (Surrogate)	5 ng/mL
30 (ISTD)	8 ng/mL
204 (ISTD)	6 ng/mL
p,p'-DDE	20 ng/mL
HCB	20 ng/mL
trans-nonachlor	20 ng/mL
p,p'- DDT	20 ng/mL
o,p'-DDT	5 ng/mL
octachlorostyrene	9.38 ng/mL
Aldrin	5 ng/mL

5. PCB Recovery standard: Used for matrix spike

Compounds	Concentrations (in Hexane)
Mullin 94	680.16 ng/mL

6. PCB Performance Standard: Used for instrument calibration check

Compounds	Concentration (in Hexane)
Mullin 94	510.12 ng/mL
Cong 14 (Surrogate)	10 ng/mL
Cong 65 (Surrogate)	10 ng/mL
Cong 166 (Surrogate)	10 ng/mL
Cong 30 (ISTD)	8 ng/mL
Cong 204 (ISTD)	6 ng/mL
trans-Nonachlor	10ng/mL
HCB	10 ng/mL
p,p'-DDE	10 ng/mL

p,p'-DDT	10 ng/mL

XB. Pesticide Standards

1. Pesticide Stock Standards

A composite of 17 pesticides, US112B (2000 ì g/mL), was bought from Ultra Scientific, Inc. and used as stock pesticide standard.

Additional individual pesticide standards were also purchased from Ultra Science, Inc. or AccuStandard Inc. and diluted in the laboratory with hexane to desired concentrations. US 112B is fortified with these additional standards. Calibration standards, Performance standard and the Recovery standard were prepared from these stock standards.

US 112B ampule =2000 ì g /mL
100 ì L is diluted to 100 mL of hexane. Final concentration is 2000 ng/mL

Pesticides	Final Concentration
Aldrin	2000 ng/mL
á-HCH	2000 ng/mL
â-HCH	2000 ng/mL
ã-HCH	2000 ng/mL
ä-HCH	2000 ng/mL
p,p'-DDD	2000 ng/mL
p,p'-DDE	2000 ng/mL
p,p'-DDT	2000 ng/mL
Dieldrin	2000 ng/mL
Endosulfan I	2000 ng/mL
Endosulfan II	2000 ng/mL
Endosulfan Sulfate	2000 ng/mL
Endrin	2000 ng/mL
Endrin Aldehyde	2000 ng/mL
heptachlor	2000 ng/mL
heptachlorepoxide	2000 ng/mL
methoxychlor	2000 ng/mL

2. Pesticide Surrogate standard:

Surrogate Standards	Ultra Sc. ampule	Stock Concentrations (in Hexane)
Dibutylchlorendate	2000 ì g/mL Methanol	1 ì g/mL
ä-HCH	100 µg/mL Hexane	2 ì g/mL

3. Pesticide Internal standards: PCB Congener 65, 155

compound	Ultra/AccuStandard Ampule Conentration	stock concentration	Working concentration
Congener 65	100 ì g/ mL	1 ì g/ mL	200 ng/mL
Congener 155	35 ì g/ mL	1 ì g/ mL	200 ng/mL

4. Pesticide Calibration Standard : in Hexane
Used for calibration of the instrument for pesticide analysis in 50% dichloromethane fraction.

Compounds	Concentrations (in Hexane)
á-HCH	20 ng/mL
â-HCH	20 ng/mL
ã-HCH	20 ng/mL
ä-HCH (Surrogate)	20 ng/mL
Heptachloroepoxide	20 ng/mL
dieldrin	20 ng/mL
p,p'-DDT	20 ng/mL
p,p'-DDD	20 ng/mL
o,p'-DDD	20 ng/mL
o,p'-DDT	20 ng/mL
á-chlordane	20 ng/mL
ã-chlordane	20 ng/mL
t-nonachlor	20 ng/mL
endosulfan I	20 ng/mL
endosulfan II	20 ng/mL
endosulfan sulfate	20 ng/mL
endrin	20 ng/mL
heptachloroepoxide	20 ng/mL
oxychlordane	20 ng/mL
dibutylchlorendate(Surrogate)	20 ng/mL
cong. 155 (ISTD)	20 ng/mL
cong. 65 (ISTD)	20 ng/mL

Methoxychlor	20 ng/mL

5. **<u>Pesticide Performance Standard:</u> Used to check the Instrument Calibration**

Compounds	**Concentrations in Hexane**
á-HCH	10 ng/mL
â-HCH	5 ng/mL
ã-HCH	10 ng/mL
ä-HCH (Surrogate)	10 ng/mL
dieldrin	10 ng/mL
endrin	5 ng/mL
p,p'-DDT	10 ng/mL
p,p'-DDD	10 ng/mL
o,p'-DDD	10 ng/mL
o,p'-DDT	10 ng/mL
á-chlordane	10 ng/mL
ã-chlordane	10 ng/mL
t-nonachlor	10 ng/mL
oxychlordane	10 ng/mL
heptachloroepoxide	10 ng/mL
endosulfan I	10 ng/mL
endosulfan II	10 ng/mL
endosulfan sulfate	10 ng/mL
methoxychlor	10 ng/mL
dibutylchlorendate (Surrogate)	10 ng/mL
cong. 155 (ISTD)	10 ng/mL
cong. 65 (ISTD)	10 ng/mL

6. <u>Pesticide Recovery Standard 1</u>: Used for Matrix Spike Experiment

Compounds	Concentrations in Hexane
HCB	100 ng/mL
á-HCH	100 ng/mL
ã-HCH	100 ng/mL
Dieldrin	100 ng/mL
p,p'- DDE	100 ng/mL
p,p'- DDD	100 ng/mL
p,p'- DDT	100 ng/mL
á-chlordane	100 ng/mL
ã-chlordane	100 ng/mL
trans-nonachlor	100 ng/mL

7. <u>Pesticide Recovery Standard 2</u>: Used for Matrix Spike Experiment

Compounds	Concentrations in Hexane
endosulfan I	100 ng/mL
endosulfan II	100 ng/mL
endosulfan sulfate	100 ng/mL
heptachloroepoxide	100 ng/mL
â-HCH	100 ng/mL
aldrin	100 ng/mL
oxychlordane	100 ng/mL
endrin	100 ng/mL
o,p'-DDD	100 ng/mL
o,p'-DDT	100 ng/mL
octachlorostyrene	105 ng/mL

XC. PAH Standard

1. PAH calibration standard

solvent = hexane

PAH	Concentrations (ng/mL of Hexane)
Acenaphthene	200
Acenaphthylene	200
Anthracene	200
Benz[*a*]anthracene	200
Benzo[*a*]pyrene	200
Benzo[*b*]fluoranthene	200
Benzo[*e*]pyrene	200
Benzo[*ghi*]perylene	200
Benzo[*k*]fluoranthrene	200
Chrysene	200
Coronene	200
d_{10}anthracene-ISTD	200
d_{12} perylene-ISTD	200
d_{12}benz[*a*,*h*]anthracene-ISTD	200
Dibenz[*a*,*h*]anthracene	200
Fluoranthene	200
Fluorene	200
Indeno[1,2,3-*cd*)pyrene	200
Phenanthrene	200
Pyrene	200
Retene	200
d_{10} pyrene (Surrogate)	200
d_{10} Phenanthrene (surrogate)	200

2. PAH Performance Standard
solvent = hexane

PAH	Concentrations (ng/mL of Hexane)
acenaphthene	100
acenaphthylene	100
anthracene	100
benz[*a*]anthracene	100
benzo[*a*]pyrene	100
benzo[*b*]fluoranthene	100
benzo[*e*]pyrene	100
benzo[*ghi*]perylene	100
benzo[*k*]fluoranthrene	100
chrysene	100
coronene	100
d_{10}anthracene-ISTD	100
d_{12} perylene-ISTD	100
d_{12}benz[*a,h*]anthracene-ISTD	100
dibenz[*a,h*]anthracene	100
fluoranthene	100
fluorene	100
indeno[1,2,3-*cd*]pyrene	100
phenanthrene	100
pyrene	100
retene	100
d_{10} pyrene (Surrogate)	200
d_{10} Phenanthrene(Surrogate)	200

3. <u>PAH Recovery Standard</u>: Used for Matrix Spike

Compounds	**Concentrations (ìg/mL of Hexane)**
acenaphthene	1.97
acenaphthylene	1.97
anthracene	1.97
benz[*a*]anthracene	1.97
benzo[*b*]fluoranthene	1.97
benzo[*k*]fluroanthene	1.97
benzo[*a*]pyrene	1.97
benzo[*e*]pyrene	1.91
benzo[*ghi*]perylene	1.97
chrysene	1.97
coronene	1.93
dibenz[*a*,*h*]anthracene	1.97
fluoranthene	1.97
fluorene	1.97
indeno[1,2,3-*cd*]pyrene	1.97
naphthalene	1.97
phenanthrene	1.97
pyrene	2.0
retene	1.98

4. PAH Internal Standard

Compound	Final Concentration (ì g/mL of Hexane)
d_{10}anthracene	4
d_{12}benz[a]anthracene	4
d_{12} perylene	4

5. PAH surrogate standard:

Compound	Final Concentration (ì g/mL of Hexane)
d_{10}phenanthrene	4
d_{10} pyrene	4

6. Matrix Spike Vial (MS Vial)

One MS vial is prepared from PCB Recovery standard, Pesticide Recovery Standard 1, Pesticide Recovery Standard 2, and PAH recovery Standard. This is used for spiking the matrix in the soxhlet for matrix spike recovery experiment.

Standards	Stock	MS-vial
PCB Recovery Standard	680 ng/mL	680 ng
Pesticide Recovery Standard 1	100 ng/mL	20 ng ea pesticide
Pesticide Recovery Standard 2	100 ng/mL	20 ng ea pesticide
PAH Recovery Standard	2 ì g/mL	400 ng each PAH

XII. SAFETY

1. Emergency Numbers

Name	**Telephone numbers**
IU Fire Department	911
Environmental Health and Safety	812-855-3234
Ronald A. Hites	812-855-0193 (O) 812-334-1323 (H)

2. Chemists' Telephone Numbers

Name	**Telephone Numbers**
Ilora Basu	812-855-2926 (O) 812-334-2184 (H)
Stephanie Brown	812-855-5035 (O) 812-337-1839 (H)
James M. O'Dell	812-855-5040 (O) 812-935-7197 (H)
Karen Arnold	812-856-4634 (O) 812-275-8273 (H)

3. Working in the Laboratory

Chemists working in the laboratory should follow certain safety rules:

a) Individual is required to wear a lab coat whenever working in the lab.
b) Eye protection with splash resistant safety glasses or safety goggles is required. Contact lenses are forbidden.
c) Protective gloves should be used while handling samples or standards. Special solvent resistant gloves should be used while handling large amount of solvents.
d) All solvent work should be done inside fume hood.
e) Open shoes are not allowed in the laboratory.
f) Particle mask is required when using dry silica.
g) Generally, nobody should work alone in the laboratory. If work must be performed after hours or on the weekend inform supervisor or other laboratory personnel so that your presence is known.
h) 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.
i) Gas cylinders should be well secured at all times. Flammable gases are stored in separate cage.
j) Wash your hands thoroughly after work. Protective hand cream "Soft guard" is supplied.
k) No food or drink is allowed in the laboratory.
l) 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.
m) MSDS are filed in a three ring binders.
n) All chemicals and standard should be labeled properly with scientific name, date, and initials of person to contact.
o) Empty chemical bottles should be flushed out with water, or, in case of liquid, allowed to evaporate under a hood before discarding.

4. Safety Equipment

a) 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 real 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.

b) Safety Showers

Emergency showers are located in strategic areas of the laboratory to provide to provide immediate emergency protection against fire or chemical injury. It is operated by pulling the hanging ring down. It delivers 30 gallons of water per minute.

c) Eye Wash

Emergency eyewash is located in the laboratory. It is operated by pushing the lever backward.

5. Waste disposal

Solvents
Label 2 containers, `**CHLORINATED WASTE**' and `**NON-CHLORINATED WASTE**'.
Containers may be empty glass bottles from solvents or poly jericans (10 liters or less).
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 bottle properly and sign it.

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 trash can.

Glass
Place in `Broken Glass Disposal Containers'. When containers are full, close according to directions on box; leave for janitors to pick-up or take out to the trash dumpster.

Foil
Place in trash can.

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

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.