STANDARD ANALYTICAL PROCEDURES
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NEW HPLC CLEANUP AND REVISED EXTRACTION PROCEDURES FOR ORGANIC CONTAMINANTS

Prepared for

The NOAA National Status and Trends Program

and

The Outer Continental Shelf Environmental Assessment Program

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CONTENTS

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PREFACE

The Environmental Conservation (EC) Division of the Northwest and Alaska Fisheries Center conducts broad-ranging research into the nature and extent of pollution and its effects on marine and estuarine fish and their habitats. While functioning within the National Marine Fisheries Service, the EC Division maintains a strong research relationship with other units of NOAA such as the Office of Oceanography and Marine Assessment of the National Ocean Service. NOAA's National Status and Trends (NS&T) Program is a good example. For 4 years the EC Division has participated in the NS&T Program, which monitors marine environmental quality at approximately 200 sites along the nation's coastlines.

Hundreds of chemical analyses are needed annually by the NS&T Program to document the quality of the marine environment and assess trends. Wherever possible, standard methods are used to minimize the analytical variability among the participating laboratories. Many of these procedures, such as those for aromatic hydrocarbons and chlorinated hydrocarbons in sediments and tissues, are relatively laborious. To expedite these analyses the EC Division's National Analytical Facility has developed a new instrumental method for the cleanup of sample extracts. The new method features (1) cleanup as effective as by former methods, (2) better precision: (3) less time required; (4) capability of monitoring the chromatographic conditions; (5) capability of being automated; and (6) less highly pure solvent required.

Through this Technical Memorandum, the new cleanup and revise extraction procedures are being made available to NS&T laboratories. The laboratory manual should also be useful to other federal, state, and local environmental programs which analyze for organic chemicals in sediments and organisms. These new procedures supersede Sections 7, 8, 10, and 11 of NOAA Technical Memorandum NMFS F/NWC-92 (MacLeod et al. 1985).

INTRODUCTION

Since its inception in 1976, NOAA's National Analytical Facility (NAF) of the Environmental Conservation Division, Northwest and Alaska Fisheries Center, has been at the forefront in developing and employing advanced methods to analyze samples from the aquatic environment for traces of toxic chemicals. These activities have focused on methods for determining anthropogenic organic compounds such as aromatic hydrocarbons (AHs) and chlorinated hydrocarbons (CHs) both in sediments and in organisms.

Most analytical methods which determine these organic compounds incorporate 3 steps:

- Removal of the organic chemical contaminants of interest (analytes) from the sample matrix by solvent(s) extraction.
- Isolation of the analytes of interest ("cleanup") from interfering compounds present in the extract, generally by one or more chromatographic techniques.
- Qualitative and quantitative instrumental analysis by gas chromatography (GC), high-performance liquid chromatography (HPLC), or mass spectrometry (MS).

The cleanup step is critical for accurate identification and quantitation of the analytes. Biogenic or other interfering compounds present in the extract can coelute with analytes of interest and prevent accurate quantitation. Some interfering compounds can also affect the reproducibility of the GC analyses by degrading the column or building up a deposit in the inlet. A variety of cleanup procedures have been developed. MacLeod et al. (1985) -published a laboratory manual detailing the use of

two gravity-flow chromatography steps for cleanup: separation on (1) silica/alumina packing and (2) a gel-permeation column.

Here, we describe a rapid, simple HPLC method, using a size-exclusion column. We developed this method to improve on the gravity-flow method by increasing efficiency, reducing costs, automating the analyses, and monitoring chromatographic conditions (Krahn et al. 1988). Improvements to the previous extraction procedures (MacLeod et al. 1985) are also described herein.

Summary of Analytical Procedures

The cleanup of sediment and tissue extracts follows the scheme shown in Figure 1, as summarized below. Sediment and tissue are extracted largely as described in MacLeod et al. (1985), then the sediment extracts are filtered through glass wool and the tissue extracts through a silica/alumina precolumn. The extracts are concentrated, and a portion of each extract is chromatographed on a 100-A size-exclusion HPLC column using methylene chloride as the mobile phase. A fraction containing AHs and CHs is collected, and the solvent volume is reduced to 250 µL. The concentrated extract is analyzed by GC with an electron capture detector (GC/ECD) to determine CHs and with a flame ionization detector (GC/FID) or mass spectrometer (GC/MS) to determine AHs. For quality assurance, sample sets are run so that in a set of 12 analyses there are (1) a blank, (2) a sample of reference material or a spiked blank, and, frequently, (3) a replicate of one of the samples.

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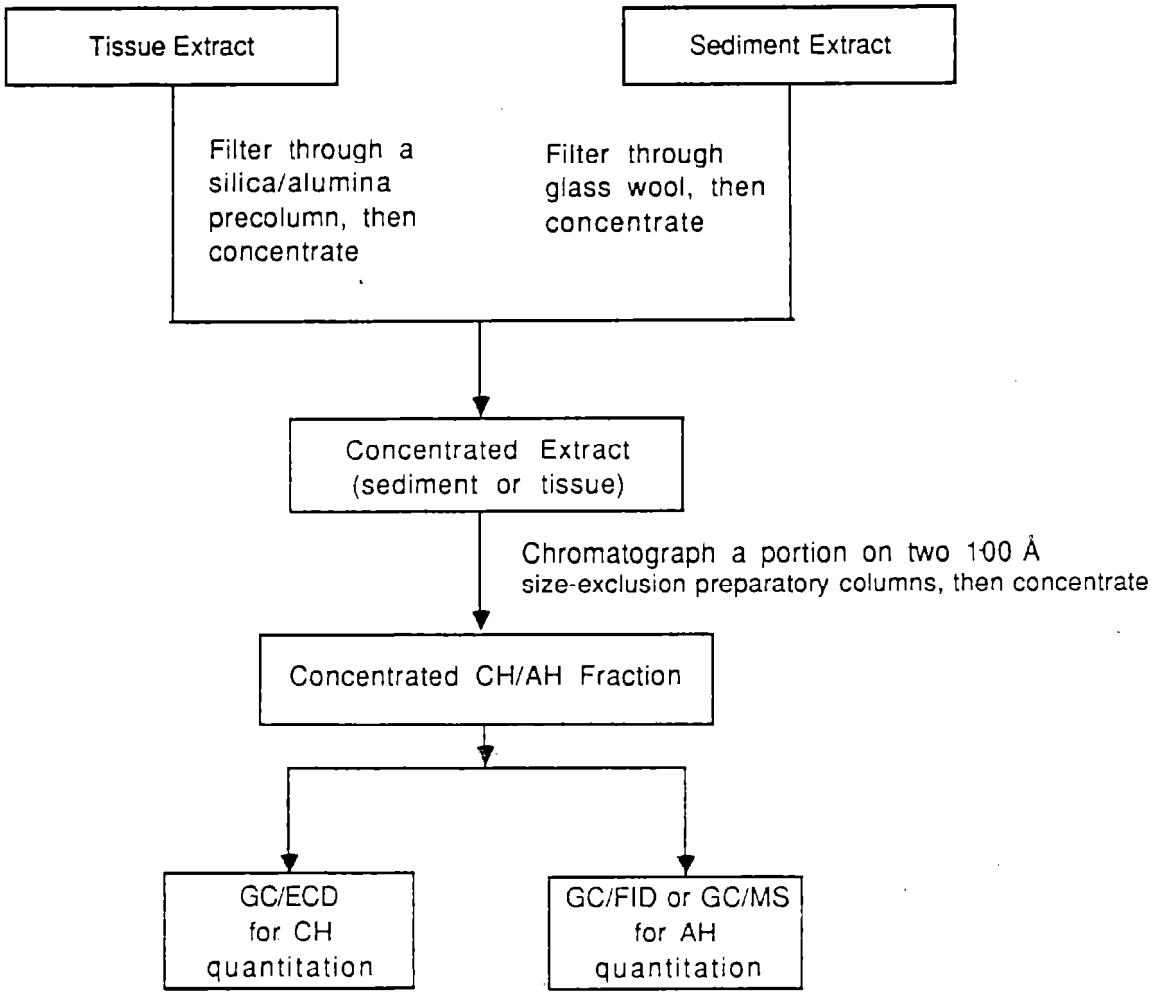

Figure 1. Flow diagram of extract cleanup.

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Section 1

MATERIALS

Disclaimer: Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

Note: All chemicals and solvents must be of the highest purity available.

A. **Solvents**

hexane, nonspectro	Burdick and Jackson, distilled in glass
methylene chloride	Burdick and Jackson, distilled in glass

B. **Reagents**

sodium sulfate	Reagent Grade, anhydrous granular
copper	Reagent Grade, fine granular
alumina	Sigma F-20, 80-200 mesh
silica gel	Amicon No. 84080
sand	Ottawa, MCB, kiln-dried, 30-40 mesh

C. Miscellaneous

boiling chips	Teflon, Norton Chemplast, Chemware

D. **Standards** - Those from the National Bureau of Standards (NBS) are so designated; others are prepared in this laboratory (NAF).

Instrument calibration

HPLC-AH/CH calibration-check solution (NAF)

biphenyl (-3 ng/pL)

4,4'-dibromooctafluorobiphenyl (-1.5 ng/µL) (DOB)

perylene (-1.5 ng/µL)

GC calibration-check solutions (NAF)

AHs (~ 5 ng/µL); see list in Table 1

CHs (~ 0.1 ng/µL); see list in Table 1

COP (~ 5 ng/µL); see list in Table 1

Internal Standards (I-Stds) for analyte quantitation by GC or GUMS

AH I-Std (NBS, 100 µL added to each sample before extraction and to each AH analyte-calibration solution):

naphthalene-d8 (~50 ng/µL)

acenapthene-dl0 (~50 ng/µL)

perylene-dl2 (~50 ng/µL)

CH I-Std (NAF, 100 µL added to each sample before extraction and to each CH analyte-calibration solution):

4,4'-dibromooctafluorobiphenyl
(DOB, -1 ng/µL)

COP I-Std (NAF, 100 µL added to each sediment sample before extraction and to each COP analyte-calibration solution):

5a-androstan-17B-ol (-50 ng/µL)

Spike solutions

PAH Std solution (NBS, 750 µL added to each reagent spike before extraction and to each AH analyte-calibration solution):

AHs (~ 6 ng/µL); see list in Table 2

Pesticides Std solution (NBS, 500 µL added to each reagent spike before extraction and to each CH analyte-calibration solution):

PESs (~ 0.2 ng/µL); see list in Table 2

PCB Congeners Std solution (NBS, 500 µL added to each reagent spike before extraction and to each CH analyte-calibration solution):

PCBs (-0.2 ng/µL); see list in Table 2

COP Spike solution (NBS, 100 µL added to each sediment reagent spike before extraction and to each COP analyte-calibration solution):

coprostanol (-50 ng/µL)

Recovery I-Stds

HPLUI-Std (NAF, 100 µL added to each extract just prior to HPLC cleanup and to each AH and CH analyte-calibration solution):

phenanthrene-dl0 (~50 ng/µL)

1,2,3-trichlorobenzene (~1 ng/µL)

COP HPLUI-Std (NAF, 100 µL added to each sediment extract just prior to HPLC cleanup and to each COP analyte-calibration solution):

benzo[e]pyrene-dl2 (~50 ng/µL)

HMB GUI-Std (NBS: 30 µL added to each concentrated AH/CH fraction and to each AH and CH analyte-calibration solution; 10 µL added to each concentrated COP fraction; and 30 µL added to each COP analyte-calibration solution):

hexamethylbenzene (~80 ng/µL)

TCMX GC/I-Std (NBS, 50 µL added to each concentrated AH/CH fraction and to each CH analyte-calibration solution):

tetrachloro-m-xylene (~2 ng/µL)

E. **Purified gases**

nitrogen	Prepurified Grade
helium	Prepurified Grade

F. **Tube heater** 'Modified Kontes: aluminum inserts bored to fit the l-mL tube tip; glass shroud

G. Steam table Precision Scientific, 8-hole, 120 v AC, 2 kw

H. **Vortex Genie** American Scientific Products, Model S8223

I. **Syringes** Hamilton, 50-PL through l,OOO+L sizes (optional, VWR Scientific, Digital Microdispenser, 100~PL)

Table 1. Analytes in GC calibration-check solutions

AH GC plibmtion-check	**CH GC &brationcheck**
naphthalene	hexachlorobenzene
2-methylnaphthalene	lindane
1-methylnaphthalene	heptachlor
biphenyl	aldrin
2,6-dimethylnaphthalene	heptachlor epoxide
acenaphthene	alpha-chlordane
fluorene	trans-nonachlor
phenanthrene	dieldrin
anthracene	mirex
1-methylphenanthrene	o,p'-DDE
fluoranthene	p,p'-DDE
pyrene	o,p'-DDD
benz[a]anthracene	p,p'-DDD
chrysene	o,p'-DDT
benzo[e Jpyrene	p,p'-DDT
benzo[a]pyrene	2, 4'-dichlorobiphenyl
perylene	2, 5, 4'-trichlorobiphenyl
dibenz[a,h]anthracene	2, 4, 2', 4'-tetrachlorobiphenyl
naphthalene-d8	2, 4, 5, 2', 5'-pentachlorobiphenyl
acenaphthene-dl0	2, 4, 5, 2', 4', 5'-hexachlorobiphenyl
perylene-dl2	2, 3, 4, 5, 2', 4', 5'-heptachlorobiphenyl
hexamethylbenzene	2, 3, 4, 5, 2', 3',4', 5'-octachlorobiphenyl
	2, 3, 4, 5, 6, 2', 3',4', 5'-nonachlorobiphenyl
COP G-C @ibrationcheck	4, 4'-dibromooctafluorobiphenyl (DOB)
coprostanol	
5cl-androstan-17p-ol	
benzo[e]pyrene-d12	
hexamethylbenzene	

=..

Table 2. Analytes in spike solutions

PAH Std

naphthalene
2-methylnaphthalene
1-methylnaphthalene
bip henyl
2,6-dimethylnaphthalene
acenaphthylene
acenaphthene
2,3,5-trimethylnaphthalene
fluorene
phenanthrene
anthracene
1-methylphenanthrene
fluoranthene
pyrene
benz[a]anthracene
chrysene
benzo[b]fluoranthene
benzo[k]fluoranthene
benzo[e]pyrene
benzo[a]pyrene
perylene
indeno[1,2,3cdlpyrene
dibenz[a,h]anthracene
benzo[ghilperylene

hexachlorobenzene
lindane
heptachlor
heptachlor epoxide
aldrin
dieldrin
a-chlordane
trans-nonachlor
mirex
o,p'-DDE
p,p'-DDE
o,p'-DDD
p,p'-DDD
a,~'-DDT
p,p'-DDT

Table 2. Continued.

PCB Congeners Std
2, 4-dichlorobiphenyl
2, 4, 4'kichlorobiphenyl
2, 2, 5'kichlorobiphenyl
2, 3, 2', 5'-tetrachlorobiphenyl
2, 5, 2', 5'-tetrachlorobiphenyl
2, 4, 3', 4'-te trachlorobiphenyl
2, 4, 5, 2', 5'-pentachlorobiphenyl
2, 3, 4, 3', 4'-pentachlorobiphenyl
2, 4, 5, 3', 4'-pentachlorobiphenyl -
2, 3, 4, 2', 3', 4'-hexachlorobiphenyl
2, 3, 4, 2', 4', 5'-hexachlorobiphenyl
2, 4, 5, 2', 4', 5'-hexachlorobiphenyl
2, 3, 4, 5, 2', 3', 4'-heptachlorobiphenyl
2, 3, 4, 5, 2', 4', 5'-heptachlorobiphenyl '
2, 3, 5, 6, 2', 4', 5'-heptachlorobiphenyl
2, 3, 4, 5, 6, 2', 3',4'-octachlorobiphenyl
2, 3, 4, 5, 6, 2', 3',4', 5'-nonachlorobiphenyl
decachlorobiphenyl

Section 2

SEDIMENT EXTRACTION

A. Equipment List

Note: wash all glassware and materials contacting the sample or extract with methylene chloride.

Glassware (per sample)

250-mL bottle (amber, Boston round) with Teflon cap (Savillex, 24-mm) for the tumbler/centrifuge

500-mL bottle (amber, Boston round) with Teflon-lined cap

50-mL conical centrifuge tube with Teflon-lined cap (low-cost, disposable)

25-n& serological pipet with top sawed off (low-cost, disposable)

2-mL GC vial (for HPLC samples)

powder funnel

Solvents and Reagents (per sample)

300 mL methylene chloride (not including washes)

60 g sodium sulfate: methylene chloride-washed, dried, heated to 7000C for 18 hr, stored at 12OoC, and cooled to room temperature in a desiccator just prior to use

7.5 cc activated copper (MacLeod et al. 1985, Section 5, Part A, page 39): use immediately or store under methylene chloride for 5 1 hr

hexane (as needed)

Internal Standard (I-Std) Solutions

NBS: AH I-Std solution

NAF: CH I-Std solution
COP I-Std solution
HPLC/I-Std solution
COP HPLC/l-Std solution

Spike solutions

NBS: PAH Std solution
Pesticides Std solution
PCB Congeners Std solution
COP spike solution

Other Materials and Apparatus

1 spatula per sample

modified rock tumbler: Model NF-1, Lortone, Inc., 2856 NW Market St., Seattle, WA 98107

glass wool

masking tape

desiccator

boiling chips

1000~pL, 500-pL, 250-PL and 100~PL syringes (optional: lOO+L microdispenser)

6 ea 2-mL GC vials

Kontes tube heater: inserts modified to accept the 50-mL tubes and a glass cylinder shroud

transfer pipets (Pasteur style) with bulbs

steam table

500-n& Teflon wash-bottle (methylene chloride-filled)

centrifuge (to accommodate the tumbler/centrifuge bottles)

drying oven (12OoC)

B. **Sediment Extraction**

Note: Sediments are customarily analyzed in 12-sample sets, In addition to the 8-9 regular sediment samples in each set there are

1 blank ("reagent blank") sample

1 spiked blank ("reagent spike") sample m 1 reference sediment sample

1 field blank ("sediment blank") sample, if called for

1 duplicate sediment sample

1. Assemble the 250-mL bottles, 1 for each sample described above.
2. Prepare the field blank by washing down the empty sample container 3 times with 10 mL of methylene chloride (fresh each time), and adding- the combined washes to a, 250-mL bottle, then add 70 mL more methylene chloride. To all other bottles add 100 mL of methylene chloride.
3. To each bottle also add:
   a. 60 g of sodium sulfate
   b. 7.5 cc of activated copper
   c. 100 µL of AH.I-Std solution into the methylene chloride
   d. 100 µL of CH I-Std solution into the methylene chloride
   e. 100 µL of COP I-Std solution into the methylene chloride
4. To the spiked blank bottle also add into the methylene chloride:
   a. 750 µL of PAH Std solution
   b. 500 µL of Pesticides Std solution
   c. 500 µL of PCB Congeners Std solution
   d. 100 µL of COP spike solution
5. The blank, spiked blank, and/or field blank bottles receive nothing more.

6. Prepare 2 AH analyte-calibration solutions by adding to each of 2 GC vials:
    a. 250 µL of hexane
    b. 750 µL of PAH Std solution
    c. 100 µL of AH I-Std solution
7. Prepare 2 CH analyte-calibration solutions by adding to each of 2 GC vials:
    a. 500 µL of Pesticides Std solution
    b. 500 µL of PCB Congeners Std solution
    c. 100 µL of CH I-Std solution
8. Prepare 2 COP analyte-calibration solutions by adding to each of 2 GC vials:
    a. 800 µL of hexane
    b. 100 µL of COP spike solution
    c. 100 µL of COP I-Std solution
9. Decant the excess water from the sediment and stir it to homogenize. Discard all pebbles, seaweed, wood, crabs, etc.
10. Set aside ~ 10 g of the homogenized sediment for the Dry Weight Determination (MacLeod et al. 1985, Section 9, page 67).
11. Using a spatula and powder funnel, and being careful not to splash from the bottle, weigh 10 + 0.5 g of sediment to the nearest 0.01 g into all the tared bottles from Step 3, except the blanks. Record the weight in the log book. Store the remaining sample in a freezer.

Note: To avoid clumping and hardening of the sodium sulfate, perform Steps 12-15 immediately after adding the sediment to the bottle.

12. Cap each bottle, screwing the Teflon cap on just tight enough to prevent leakage.

Note: Do not over-tighten the cap so as to deform the seal and cause leakage.

13. Manually shake each bottle until the contents are loose.
14. Tape the cap to the bottle crosswise over the top with 2 strips of masking tape.
15. Roll each bottle 16 hr (overnight) on the tumbler at 100-250 rpm.
16. Centrifuge each bottle for 5 min at 1500 rpm.
17. Decant each extract into a labeled 500-mL bottle.
18. Add 100 mL of methylene chloide to each sample, and repeat Steps 12 through 16, except roll each bottle for 6 hr (i.e., during the day).
19. Decant the 2nd extract into the bottle from Step 17.
20. Repeat Step 18, except roll each bottle for 16 hr (i.e., overnight).
21. Decant the 3rd extract from Step 20 into the bottle from Step 19.

C. Extract Precleanup

1. Add 8-10 boiling chips to the bottle containing the combined methylene chloride extract from Step B.21.
2. Concentrate the extract on a steam table to 15-20 mL, and set it aside for Step 6.
3. Prepare a cleanup column by adding a 30- to 40-mm glass wool plug to a 25-n& pipet and tamping it down firmlv with a glass rod.
4. Wash down the pipet with - 5 mL of methylene chloride, and drain the washings into a waste container.
5. Repeat Step 4 twice, then rinse the tip of the pipet with methylene chloride.

6. Place a labeled centrifuge tube under the pipet and slowly decant the concentrated extract from Step 2 into the pipet.
7. Wash down the bottle with ~ 5 mL of methylene chloride and decant the washings into the pipet.
8. Repeat Step 7 twice.
9. Wash down the pipet wall with ~ 5 mL of methylene chloride.
10. Repeat Step 9 once.

D. **Extract Concentration**

1. Add a boiling chip to the tube from Step C. 10 and concentrate the extract to > 0.9 mL, < 1.0 mL on the tube heater.
2. Add activated copper to the tube, a few grains at a time, until no further discoloring of the copper occurs. Cover the tube and store it overnight in a refrigerator.
3. Add 100 µL each of HPLCA-Std and COP HPLC/I-Std solutions to the tube. Mix the extract on the Vortex Genie for 2 set at setting 4-6.
4. Add 100 µL of HPLC/I-Std solution to each of the AH analyte-calibration and the CH analyte-calibration solutions from Steps B.6, and B.7. Mix the solution on the Vortex Genie for 2 set at setting 4-6.
5. Add 100 µL of COP HPLC/I-Std to each of the COP analyte-calibration solutions from Step B.8; mix the solutions on the Vortex Genie for 2 set at setting 4-6.
6. Using a pipet, carefully transfer the concentrated, extract from Step 3 to a GC vial labeled EXTRACT, in addition to the sample number.
7. Proceed to Section 4 (HPLC Cleanup) with the vials from Step 6.

Section 3

TISSUE, EXTRACTION

A. Equipment List

Note: wash all glassware and materials contacting the sample or extract with methylene chloride.

Glassware (per sample, low-cost disposable, use one time only)

100-mL centrifuge tube with Teflon-lined cap

2 ea 250-mL bottles (amber, Boston round) with Teflon-lined cap

50-mL conical centrifuge tube with Teflon-lined cap

22-mm ID x 25-cm plain chromatography column, Ace Glass model 5884-998.

2-mL GC vial (for HPLC samples)

Solvents and Reagents (per sample)

205 mL methylene chloride (not including washes)

hexane (as needed)

25 g sodium sulfate (methylene chloride-washed, dried, heated to 700º C for 18 hr, stored at 120º C, and cooled to room temperature in a desiccator just before use)

10 cc alumina (heated to 700º C for 18 hr, stored at room temperature, then activated at 120º C for 2 hr and cooled to room temperature in a desiccator just before use)

20 cc silica gel (heated to 700º C for 18 hr, stored at 170º C, and cooled to room temperature in a desiccator just before use)

5 cc sand (MacLeod et al. 1985, Section 5, Part A, page 39)

Internal Standard Solutions

NBS: AH I-Std solution

NAF: CH I-Std solution
HPLC/I-Std solution

Spike Solutions

NBS: PAH Std solution
Pesticides Std solution
PCB Congeners Std solution

Other Materials and Apparatus

1 spatula per sample

Tekmar Tissumizer with probes

desiccator

centrifuge (to accommodate the 100-mL centrifuge tubes)

boiling chips

500-mL Teflon wash-bottle (methylene chloride-filled)

l000-µL, 5OO-µL, 25O-µL and lOO-µL syringes (optional: l00-µL microdispenser)

2-mL GC vials (for analyte-calibration solutions)

steam table

modified Kontes tube heater (see Section 2)

Vortex Genie

Teflon sheeting (to line centrifuge bottle caps)

Pasteur transfer pipets with bulbs (as needed)

glass wool

glass rod

5-CC measuring spoon

B. **Extraction for CH Analysis Only** (Liver, Muscle, etc.)

Note: Tissues are customarily analyzed in 12-sample sets. In addition to the 8-9 regular tissue samples in each set there are

1 blank ("reagent blank") sample

1 spiked blank ("reagent spike") sample or 1 reference tissue sample

1 field blank ("tissue blank") sample, if called for

1 duplicate tissue sample

1. Assemble the 100-mL centrifuge tubes, 1 for each sample described above.
2. Using a spatula, and being careful to place the sample on the bottom and not the sides, weigh 3 + 0.5 g of sample to the nearest 0.01 g into the centrifuge tubes reserved for tissue samples. Record the weight in the log book.
3. Set aside ~ 0.5 g for Dry Weight Determination (MacLeod et al. 1985, Section 9, page 65) and store the remaining sample in a freezer.
4. Prepare the field blank by washing down the- empty sample container 3 times with 10 mL of methylene chloride (fresh each time) and adding the combined washings to an empty centrifuge tube. Add 5 mL more of methylene chloride to the tube. To all the other tubes add 35 mL of methylene chloride.
5. To each sample tube, blank tube, and spiked blank tube add 100 µL of CH I-Std solution into the methylene chloride.
6. To the spiked blank tube add into the methylene chloride:
    a. 500 µL of PCB Congeners Std solution
    b. 500 µL of Pesticides Std solution

7. For each set of samples prepare 2 CH analyte-calibration solutions by adding to each of 2 GC vials:
    a. 500 µL of Pesticides Std solution
    b. 500 µL of PCB Congeners Std solution
    c. 100 µL of CH I-Std solution
8. Add 25 g of sodium sulfate to each tube from Steps 4-6.
9. Macerate/extract the sample in the tube for 0.5 min with the Tissumizer at setting 80, then reduce the speed to setting 60 and continue for 1.5 min. Avoid spattering the tissue.
10. Wash down the probe with ~ 5 mL of methylene chloride, and collect the washings in the centrifuge tube.
11. Centrifuge the sample for 5 min at 2,000 rpm.
12. Decant the extract into a labeled bottle.
13. Add 35 mL of methylene chloride to the tube.
14. Repeat Steps 9-11 once.
15. Decant the extract into the bottle from Step 12.
16. Wash the sodium sulfate/sample mass by adding 10 mL of methylene chloride to the tube and mixing on the Vortex Genie for 5-10 seconds at setting 5-6.
17. Decant the extract into the bottle from Step 15, then set the bottle aside for Step D.5.

C. Extraction for AH/CH Analysis (Stomach Contents, Molluscs, etc.)

Note: Tissues are customarily analyzed in 12-sample sets. In addition to the 8-9 regular tissue samples in each set there are

1 blank ("reagent blank") sample

1 spiked blank ("reagent spike") sample or 1 reference tissue sample

1 field blank ("tissue blank") sample, if called for

1 duplicate tissue sample

1. Assemble the 100-mL centrifuge tubes, 1 for each sample described above.
2. Using a spatula, and being careful to place the sample on the bottom and not the sides, weigh 3 + 0.5 g of sample to the nearest 0.01 g into the centrifuge tubes reserved for tissue samples. Record the weight in the log book.
3. Set aside ~ 0.5 g for Dry Weight Determination (MacLeod et al. 1985, Section 9, page 65), and store the remaining. sample in a freezer.
4. Prepare the field blank by washing down the empty sample container 3 times with 10 mL of methylene chloride (fresh each time) and adding the combined washings to an empty centrifuge tube. Add 5 mL more of methylene chloride to the tube. To all the other tubes add 35 mL of methylene chloride.
5. To each sample tube, blank tube, and spiked blank tube add:
   a. 100 µL of AH I-Std solution <u>into the methylene chloride</u>
   b. 100 µL of CH I-Std solution <u>into the methylene chloride</u>

6. To the spiked blank tube add into the methylene chloride:
    a. 500 µL of PCB Congeners Std solution
    b. 500 µL, of Pesticides Std solution
    c. 750 µL of PAH Std solution
7. For each set of samples prepare 2 CH analyte-calibration solutions by adding to each of 2 GC vials:
    a. 500 µL of Pesticides Std solution
    b. 500 µL of PCB Congeners Std solution
    c. 100 µL of CH I-Std solution
8. For each set of samples prepare 2 AH analyte-calibration solutions by adding to each of 2 GC vials:
    a. 750 µL of PAH Std solution
    b. 100 µL of AH I-Std solution
    c. 250 µL of hexane
9. Add 25 g of sodium sulfate to each tube from Steps 5-6.
10. Proceed as in Steps B.9 through B.17.

D. Extract **Precleanup**

1. Prepare a cleanup column by plugging a chromatography column with glass wool (tamped down with a glass rod), then adding 10 cc of alumina, followed by 20 cc of silica gel and 5 cc of sand.
2. Slowly add 50 mL of methylene chloride to the column, and drain it into a waste container.
3. Repeat Step 2 once, then rinse the column tip with methylene chloride.
4. Place a second labeled bottle under the column.

5. Slowly decant the extract from Step B.17 into the column, and allow it to drain from the column.
6. Wash down the 1st bottle with - 5 mL of methylene chloride, and slowly decant the washings into the column.
7. Repeat Step 6 twice.
8. Wash down the column wall with 25 mL of methylene chloride, and allow the washings to drain from the column.

E. **Concentration of the Extract**

1. Add 6-8 boiling chips to the bottle containing the extract from Step D.8.
2. Concentrate the extract on a steam table to 15-20 mL, and transfer it to a labeled conical centrifuge tube.
3. Wash down the bottle with - 5 mL of methylene chloride, and add the washings to the tube.
4. Repeat Step 3 twice.
5. Add 1 boiling chip to the tube and, using the tube heater, concentrate the extract 2 0.9 mL, I 1.0 mL.
6. Add 100 uL of HPLCI-Std solution to the tube and mix the extract on the Vortex Genie for 2 set at setting 4-6.
7. Add 100 PL of HPLC/I-Std solution to each of the analyte-calibration solutions from Step B.7 or Steps C.7 and C.8 and mix each on the Vortex Genie for 2 set at setting 4-6.
8. Using a pipet, carefullv transfer the extract from Step 6 to a 2-mL GC vial labeled EXTRACT, in addition to the sample number.
9. Proceed to Section 4 (HPLC Cleanup) with vials from Step 8.

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Section 4

HPLC CLEANUP

A. Instruments and Equipment

High-performance liquid chromatograph (HPLC)

Spectra-Physics 8000B (or equivalent) including:
- oven (optional)
- recorder-integrator
- data system
- ultraviolet (UV) detector (254 nm)
- 6-port valve, Rheodyne, model 7030
- in-line filter, Rheodyne, model 7302, 2-pm mesh
- helium degas system
  - helium, prepurified grade
  - regulator, 2-stage, stainless-steel diaphragm
  - hydrocarbon trap, Alltech Associates, no. 14634
  - 02/H20 indicating tube, Supelco, model OMI-1

Automatic sampler

Gilson 231/401 (or equivalent), capable of reproducible injections with minimal waste and negligible contamination

Automatic fraction collector

Gilson 201C (or equivalent), programmable to collect different volumes (times) of fractions and capable of collecting fractions into 50-n& conical centrifuge tubes with negligible contamination or loss

HPLC columns

preparatory, 2 used in series, Phenomenex Phenogel 100-A size-exclusion packing, 22.5 x 250 mm.

guard, Phenomenex Phenogel 100-A size-exclusion packing, 7.8 x 50 mm.

Glassware

2-mL GC vials (for autosampler)

50-mL conical centrifuge tubes with screw-tops and Teflon-lined caps (for fraction collector)

2-mL GC vial with 250-G insert (or conical GC vial), 1 for each HPLC fraction to be analyzed by GC.

Pasteur transfer pipets with bulbs

Internal Standard Solutions

NBS: HMB GUI-Std solution
TCMX GUI Std solution

Solvents

methylene chloride

hexane (as needed)

Other Apparatus

nitrogen gas (piped through only methylene chloride-washed Teflon, stainless-steel, or glass tubing)

B. **Setup of Instruments**

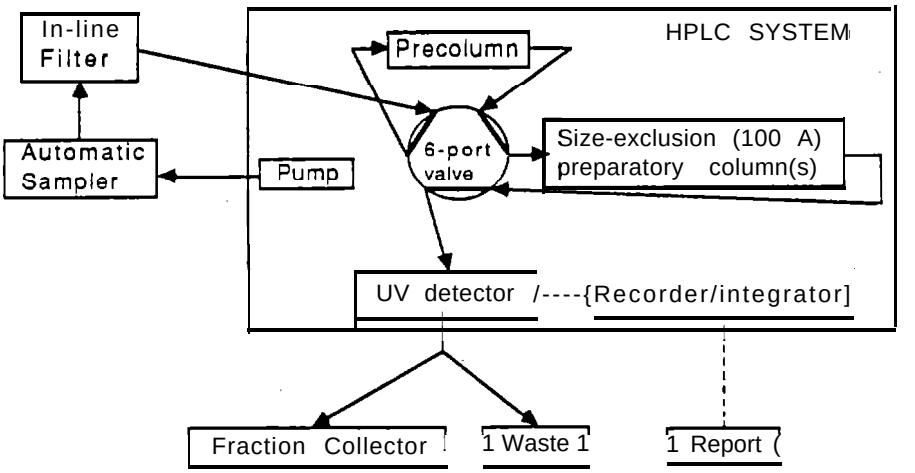
Note: The flow diagram and timetable must be adjusted for the user's particular instruments.

1. Time Table

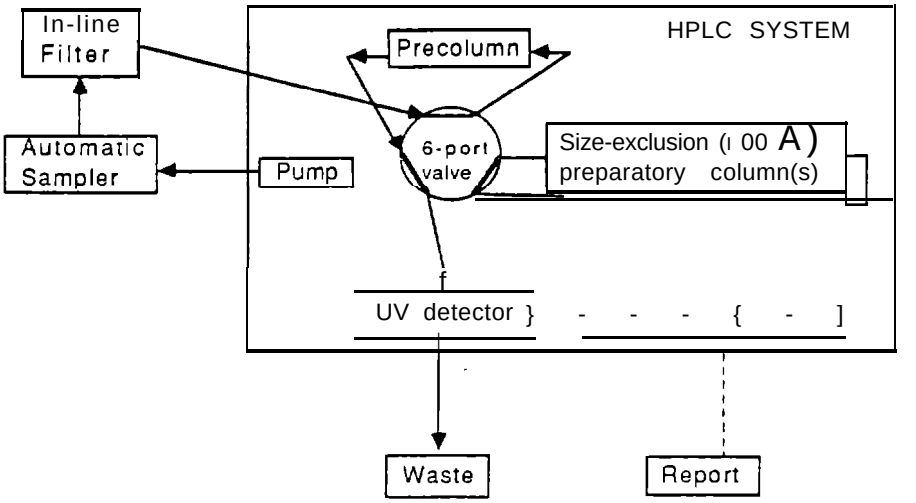
Time (min)	Instrument	Activitv/Instruction
<o	autosampler	Sample loop loaded; see 18 min below
0	HPLC	Flow = 7 mUmin; oven = 40°C; solvent = 100% methylene chloride
0	autosampler	Sample injected; signal sent to HPLC to start data system
O - 2 0	HPLC	Isocratic elution
1	autosampler	Injection port rinsed; needle rinsed
-1419	fraction collector	Fraction collected
18	autosampler	New sample picked up; loop loaded
20	HPLC	Data system ends integration

2. Setup of HPLC system

FORWARD



BACKFLUSH



arrows = solvent flow; dashed lines = electrical connections

C. Operating Parameters of Instruments

Note: These parameters pertain to NAF instruments. Users of other instruments should make appropriate adjustments.

1. Automatic sampler

   Sample size = 250 fi

   Programs (see Appendix 1)

   program 1 = standards

   program 2 = fraction collecting

   1-mL syringe

   Code #O rack

2. HPLC

   Oven temperature = 40°C

   Data system: Run time = 20 min

   Channel 1 = ultraviolet detector (254 nm)

   Chart speed = 0.5 cmmin

   Isocratic elution: 100 % methylene chloride

3. Fraction collector

   Programs (see Appendix 1)

   program 1 = standards

   program 2 = fraction collecting

   Code #24 rack to hold 50-mL conical centrifuge tubes

D. Initial and Periodic Calibration of Instrument System

1. Prepare a system-calibration solution by adding to a GC vial (a) 900 uL of methylene chloride and (b) 100 PL of HPLC calibration-check solution.

2. Select programs for each component of the HPLC instrument system (i.e., the HPLC, the autosampler, and the fraction collector) for running standards (see Appendix 1).
3. Prime the HPLC system and establish a stable flow rate and temperature (see Appendix 2, Part A).
4. Load the autosampler rack with - 6 vials of HPLC-AHCH calibration-check solution.
5. Load the fraction collector with 6 empty tubes.
6. Start the programs/instruments and run the HPLC-AWCH calibration-check solution. Consult the log from the previous day(s) to determine the retention time at which DOB has stabilized. When the system is working well, 3-4 runs of this standard should be sufficient to obtain retention time stability to within - + 0.05 min of the retention times of the previous day(s). If not, corrective maintenance is needed (e.g., install new precolumn, clean in-line filter, fix plugged lines, etc.). The peaks of the 3 calibration standards (DOB, biphenyl, and perylene) should be sharp with baseline resolution and retention times that are stable up to weeks at a time.
7. For the initial calibration, program the fraction collector to collect 10 consecutive, O.l-min fractions, starting 1 min before the retention time of DOB. Starting at the HPLUUV retention time of perylene, make a similar 1-min collection of O.l-min fractions (see Appendix 1, Part B, Program. 3).
8. Load the fraction collector rack with 20 empty GC vials.
9. Load the autosampler rack with the system-calibration solution from Step 1.
10. Start the programs/instruments.

11. Add 50 µL of TCMX solution to each of the 1st 10 fractions collected and 50 µL of HMB solution to each of the 2nd 10 fractions collected. Label and cap the vials.
12. Analyze the 1st 10 fractions by GC/ECD for DOB and the 2nd 10 fractions by GC/FID for perylene according to Section 12, MacLeod et al. (1985). This initial calibration will not have to be repeated unless some component (e.g., length of tubing, fraction collector valve, etc.) of the system is changed.
13. Determine the difference between the start of the AWCH fraction and the HPLCUV retention time for DOB as follows (see Fig. 2):
    a. By GUECD, determine the starting time (min) for the 1st fraction in which DOB appears, t_1, Subtract 0.2 min from tl (for extra margin) to obtain t2, and enter the value for t2 as the initial "1 DRAIN" in Appendix 1, Part B, Program 2.
    b. Also record the HPLUUV retention time for DOB, t3, and the initial difference in time (min), t4, between t2 and t3.
    c. Each day, record the new HPLUUV retention time for DOB for that day, t5, Subtract t4 from t5 to redetermine the start of the AH/CH fraction, t6 (Part E , Step 7) and reenter daily this new value for "1 DRAIN" in Appendix 1, Part B, Program 2.
14. Determine the collection span of the AH/CH fraction as follows:
    a. By GUFID, determine the ending time (min) for the last fraction in which perylene appears, t7. Add 2 0.5 min to t7 (for extra margin) to obtain t8, Subtract t2 from tg to obtain tg and enter the value for t9 as the initial "1 COLL" in Appendix 1, Part B, Program 2.
    b. Also record the HPLC/UV retention time for perylene, t10, and the initial difference in time (min) tll, between t8 and t10.

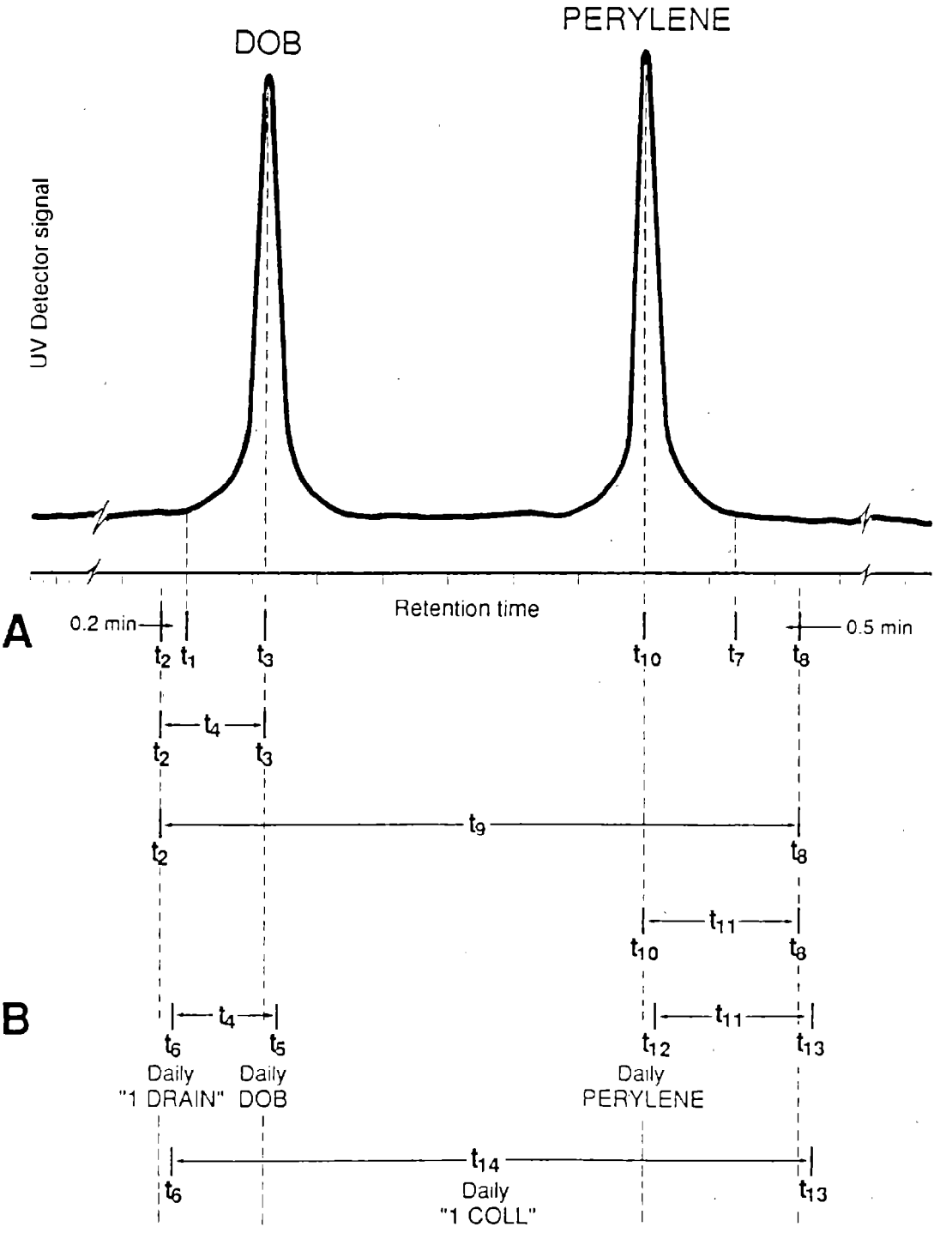


Figure 2. Examples of (A) an initial calibration of the HPLC instrument system ($t_1 \Leftrightarrow t_4$, $t_7 \Leftrightarrow t_{11}$), and (B) a daily calibration ($t_4 \Leftrightarrow t_6$, $t_{11} \Leftrightarrow t_{13}$), when the DOB and/or perylene peaks have shifted from their initial positions.

c. Only if the daily HPLUUV retention time for perylene, t12, has shifted > 0.05 min with respect to the HPLC/UV retention time for DOB, t5 (for the same day), recalculate a new collection span, as follows:

Add tll to t12 to redetermine the end of the AHXH fraction, t13.

Subtract t6 from t13 to obtain the collection span, t14 (Part E, Step 7), and enter this value for "1 COLL" in Appendix 1, Part B, Program 2.

E. **Fractionation and Collection**

1. Select programs for each component of the HPLC system (definition in Step D.2) to run the standards (see Appendix 1).
2. Prime the HPLC system and establish a stable flow rate and temperature (see Appendix 2, Part A).
3. Load the autosampler rack with - 6 vials of HPLC-AH/CH calibration-check solution.
4. Load the fraction collector with empty tubes.
5. Start the programs/intruments and run the HPLC-AWCH calibration-check solution (see Part D, Step 6 and Appendix 2, Part B).
6. Select programs for each component of the HPLC system for cleanup of sample-extracts from Section 2 or Section 3 (see Appendix 1).
7. Redetermine the start of the AH/CH fraction and, if necessary, the collection span (Part D., Steps 13.c and 14.c).
8. Edit the fraction collector program appropriately (see Appendix 1, Part B, Program 2 and Appendix 2, Part C, Steps 2 and 3).

9. Load the autosampler rack with sample-extracts, plus an HPLC-AH/CH calibration-check solution inserted after every 6 sample-extracts and also after the last sample-extract.
10. Load the fraction collector rack with empty tubes labeled to correspond with the sample numbers in the autosampler.
11. Start the programs/instruments (see Part B.l for sequence of events):
12. Monitor the following parameters:

    HPLC backpressure

    UV detector signal

    Oven temperature

    Solvent flow rate.
13. As the sample-extracts are chromatographed for cleanup, label the chromatograms, and enter the sample numbers in the HPLC log book.
14. After the sample-extracts are run, recap the sample vials, and cap the tubes containing the collected AH/CH fractions. Store the fractions in a refrigerator.
15. Proceed to Part F, G, or H.

F. Concentration of Sediment AH/CH Fractions from HBLC

1. Add a boiling chip to the tube containing the AWCH fraction and concentrate it to ≥ 0.9 mL, ≤ 1.0 mL on the tube heater.
2. Add 2 mL of hexane to the tube and concentrate the fraction to ≥ 0.9 mL, ≤ 1.0 mL.
3. Add 30 µL of HMB GC/I-Std solution and 50 µL of TCMX GC/I-Std solution to the tube, and mix the contents on the Vortex Genie for 2 set at setting 4-6,

4. Add 30 µL of HMB GC/I-Std solution to the AH analyte-calibration solutions from Section 2, Part D, Step 4. Mix the solutions on the Vortex Genie for 2 set at setting 4-6 and store them in a freezer.
5. Add 50 µL of TCMX GC/I-Std solution to the CH analyte-calibration solutions from Section 2, Part D, Step 4. Mix the solutions on the Vortex Genie for 2 set at setting 4-6 and store them in a freezer.
6. Transfer the fraction from Step 3 to a GC vial. Place this under a gentle stream of nitrogen gas, and slowly evaporate the solvent until the volume is ~ 250 µL.
7. Transfer ~ half of the fraction to a GC vial with an insert. Cap the vial, label it, and store it in a freezer.
8. Cap the vial containing the remainder of the fraction. Add an "R" (for "reserve") to the label and store the vial in the freezer.
9. Proceed to Part I.

G. Concentration of Tissue AH/CH Fractions from HPLC

1. Add a boiling chip to the tube containing the AWCH fraction and, using the tube heater, concentrate the AHKH fraction to c 0.9 mL, c 1.0 mL.
2. Add 2 mL of hexane to the tube and concentrate the fraction to ≥ 0.9 mL, ≤ 1.0 mL on the tube heater.
3. Add 50 µL of TCMX GC/I-Std solution to the tube and mix the contents on the Vortex Genie for 2 set at setting 4-6.
4. If the samples were extracted for AHs, also add 30 µL of HMB GC/I-Std solution to the tube and mix the contents on the Vortex Genie for 2 set at setting 4-6.

5. Add 50 a of TCMX GC/I-Std solution to each of the CH analyte-calibration solutions from Section 3, Part E, Step 7. Mix the solutions on the Vortex Genie for 2 set at setting 4-6 and store them in a freezer.
6. 'If the samples were extracted for AHs, also add 30 µL of HMB GC/I-Std solution to each of the AH analyte-calibration solutions from Section 3, Part E, Step 7. Mix the solutions on the Vortex Genie for 2 set at setting 4-6, and store them in a freezer.
7. Using a pipet, transfer the AH/CH fraction from Step 3 or Step 4 to a labeled GC vial, Place this under a gentle stream of nitrogen gas and slowly evaporate the solvent until the volume is either ~ 250 µL (for moderate to heavy contamination) or ~ 100 µL (for light contamination).
8. Transfer the fraction to a labeled GC vial with an insert, cap the vial, and store the vial in a freezer.
9. Proceed to Part I.

I. GC **Analysis**

The contents of the GC vial(s) are to be analyzed according to Section 12, pp. 85-110 of MacLeod et al. (1985), with the following modifications:

1. Determine the percent recovery of the HPLC/I-Std. using equation 12-2, p, 109 (MacLeod et al. 1985) and Substituting; the term HPLCYI-Std everywhere the term I-Std appears. Make no other substitutions.
2. Determine the percent recovery of the I-Std, using equation 12-2, p. 109 (MacLeod et al. 1985) and substituting; the term HPLC/I-Std everywhere the term GC/I-Std appears. Make no other substitutions.

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ACKNOWLEDGMENTS

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Appendix 1

AUTOSAMPLER AND FRACTION COLLECTOR PROGRAMS

Spectra-Physics 8000B HPLC/ Gilson Autosampler/ Gilson Fraction Collector

A. **Autosampler**

Program 1 (File 1): Runs up to 14 standards (17 min each)

Line	Command	Explanation
1	WAIT/5/0	Wait for the signal from the HPLC before continuing
2	TUBE 0/0	Needle goes into the injection port
3	DIS 0/3000/6	Rinse the loop and fill it with solvent
4	RACK CODE 0	Code for the rack that holds 2-mL GC vials
5	FOR A = 1/14	Program will run 14 standards, then go to line 33
6	TUBE A/1	Needle goes into the rinse vial
7	DIS 0/2000/9	Rinse needle by filling the vial with 2000 μL of solvent
8	HEIGHT	Raise needle out of the vial
9	IF A > 1	If the next vial is not the first in the set, execute line 10
10	PRINT 16/6	"WAIT TIME = 1600" is printed on the display
11	IF A > 1	If the next vial is not the first in the set, execute line 12
12	WAIT 1600	Wait 16 min before continuing
13	TUBE A/2	Needle goes to the standard vial

14	HEIGHT	Needle stays above the vial
15	ASPIR 0/10/1	10 μL of air is drawn into the needle
16	HEIGHT - 2	Needle goes to the bottom of the vial
17	PRINT 250/4	"ASPIR VOL = 250" is printed on the display
18	ASPIR 0/250/1	Draw 250 μL of standard into the needle
19	TUBE 0/0	Needle goes into the injection port
20	PRINT 250/5	"DISP VOL = 250" is printed on the display
21	INJECT 0	Loop is opened for loading the standard
22	DIS 0/250/1	250 μL of the standard is dispensed
23	PRINT 250/30	"LOAD VOL = 250" is printed on the display
24	IF A > 1	If the next vial is not the first in the set, execute line 25
25	WAIT/5/0	Wait for the signal from the HPLC before continuing
26	INJECT 1	Standard is injected onto the column
27	AUXIL 6/2	Signal is sent to start the HPLC data system
28	AUXIL 4/2	Signal is sent to start the fraction collector program
29	AUXIL 5/1	Instruction is sent to the fraction collector to hold until further notice
30	PRINT 250/31	"INJECT VOL = 250" is printed on the display
31	DIS 0/2000/7	Injection port is rinsed
32	NEXT A	Proceed to line 6 for the next standard
33	TUBE A/1	Needle goes to the last rinse vial
34	DIS 0/2000/9	Needle is rinsed by filling the vial with 2000 μL of solvent
35	HOME	Program ends, needle goes to rinse the station

Program 2 (File 2); Runs 14 extracts for isolation of AHs/CHs (20 min each)

Same as Program 1 except for the following:

Line	Command	Explanation
5	FOR A = 1/14	Program will run 14 samples
10	PRINT 1816	"WAIT TIME = 1800" is printed on the display
12	WAIT 1800	Wait 18 min before continuing

B. **Programmable Fraction Collector**

Program 1 (File 1): Standards; collects toxics for separate disposal

Parameter	Setting	Explanation
MODE	TIME PROG	Time program mode used in this program
RACK CODE.	**24**	Rack which holds 50-mL conical centrifuge tubes
WASTE	**0**	Not used (default 0)
INJECT	0.01	Needed to recycle
WAIT	0	Not used (default 0)
NB COLL	1	Number of fractions collected
1 DRAIN	14.5	Time from the beginning of the run to the start point for the collection of the calibration solution
1 COLL	4.0	Collect for 4 min
SAFETY	0	Not used (0 = no effect)
RINSE	0	Not used (0 = no rinse)
NB CYCLES	6	Number of cycles; instrument will repeat the same sequence beginning with injection
POSIT/CYCLE		Number of positions (tubes) covered by the collection head in each cycle

Program 2 (File 2): collects AWCH fraction

Parameter	Setting	Explanation
MODE	TIME PROG	Time program mode used in this program
RACK CODE	24	Rack which holds 50-mL conical centrifuge tubes
WASTE	0	Not used (default 0)
INJECT	0.01	Needed to recycle
WAIT	0	Not used (default 0)
N-B COLL	1	Number of fractions collected
1 DRAIN	*value for t2 or t6* [Section 4, Part D, step 131	Time from the beginning of the run to the start point for collection of the fraction
1 COLL	*value for t9 or t14* [Section 4, Part D, step 141	Collection span for the fraction
SAFETY	0	Not used (0 = no effect)
RINSE	0	Not used (0 = no rinse)
NB CYCLES	14	Number of cycles; instrument will repeat same sequence, beginning with injection
POSIT/CYCLE	1	Number of positions (tubes) covered by the collection head in each cycle

Program 3 (File 3): Initial and Periodic Calibration of the System

Parameter	Setting	Exnlanation
MODE	TIME PROG + TIME	Both time and time program modes used in this program
RACK CODE	0	Rack which holds 2-mL GC vials
WASTE	0	Not used (default 0)
INJECT	0	Not used (default 0)
WAIT	0	Not used (default 0)
NB COLL	2	Number of fractions collected
1 DRAIN	adjust	Time from the beginning of the HPLC run to the start point for the collection of the first fraction. This is **1.0** min before the HPLCXJV retention time for DOB, t3 (see Section 4, Part D, Step 13.b)
1 SUB-FR	0.1	Collect 0.1 min subfractions
1 COLL	1.0	Collect subfractions for 1 min for a total of 10 subfractions
2 DRAIN	*adjust*	Time from the end of collecting the first fraction to the start point for collection of the second fraction. This is the HPLCIW retention time for perylene, t10, minus the HPLUUV retention time for DOB, t3 (see Section 4, Part D, Steps 13-b and 14.b)
2 SUB-FR	0.1	Collect 0.1 min subfractions
2 COLL	1.0	Collect subfractions for 1 min for a total of 10 subfractions
SAFETY	0	Not used (0 = no effect)
RINSE	1	Rinse for 1 min to clean out lines

NB CYCLES	1	Number of cycles; instrument Will repeat same sequence beginning from injection
POSIT/CYCLE	20	Number of positions (vials) covered by the collection head in each cycle

Appendix 2

OPERATION OF THE HPLC SYSTEM

Spectra-Physics 8000B HPLC/ Gilson Autosampler/ Gilson Fraction Collector

A. **Instrument Start-up**

1. Check the fans in the HPLC (two above the board cage and one behind the oven).
2. Install a bottle of methylene chloride in the solvent reservoir holder, and fill the autosampler dilutor reservoir.
3. Turn on the helium to 3 psi, and check for bubbles flowing through the solvent. Degas the solvent for 15 min.
4. Turn on the *W* detector.
5. Check to see that the backflush valve is in the RUN position.
6. Enter the valve corresponding to the HPLC solvent line (e.g., valve B: VB ENTER).
7. Open the column by-pass valve and the priming valve.
8. Using the syringe, prime the pump slowly to remove bubbles.
9. Close the priming valve.
10. Start the solvent flowing at 7 mL/min: F:7 ENTER.
11. Close the column by-pass valve.
12. Close the oven door, and set the temperature at 40°C: T:40 ENTER.
13. Set the *W* range to 0.04.
14. Plot the pressure: GB1 ENTER. Monitor the pressure plot for bubbles.

Note: If the graph doesn't stabilize, stop plotting: GX ENTER. Stop the flow: VX ENTER; then repeat Steps 7 -14.

15. When the plot stabilizes and bubbles are gone, calibration can begin. Stop plotting: GX ENTER. Leave the flow at 7 mL/min and the oven at 40°C.
16. Turn on the dilutor.
17. Prime the dilutor until the bubbles are out of the lines: PRIME; to stop prime when through: PRIME.

B. Daily HPLC Calibration

1. Check that the autosampler rack (code #0) is sitting properly in place.
2. Put clean, empty GC vials in the 1st row (back row), filling the slots from left to right.
3. Take 6 vials of HPLC-AH/CH calibration solution from the freezer, remove the cloth labels, and label the vials with a felt pen. Put the vials in the 2nd row, filling the slots from left to right.
4. Load the autosampler File 1: EDIT FILE 1 ENTER ENTER ENTER .
5. Put 6 conical centrifuge tubes into a rack (code # 24), positions 1-4, in the fraction collector:

1 4 5 8 9 1 2 1 3	(Numbering system
2 3 6 71011 14	for rack code # 24)

6. Load the fraction collector File 1: RESET 1 ENTER.
7. Set the W range to .04.
8. Start File 1 of the autosampler: START.
9. Start HPLC Parameter Set 1: SB1 ENTER. Note that the autosampler will put the fraction collector and the HPLC data system into run automatically. For the first run, the printer will start plotting before injection. After injection, the HPLC run light will stop blinking

(indicating that the data system is in RUN), and Run Time can be displayed on the LED: E - ENTER.

10. Adjust the *W* baseline by rotating the range balance knob until the detector level reads 1000 on the LED. To observe the detector level on the LED: D1L ENTER.
11. As the calibration solutions are run, record the retention times of 4,4'-dibromooctafluorobiphenyl (DOB; first peak) and perylene (last peak) in the HPLC log book, and label the chromatograms "AHKH calib."
12. After retention times have stabilized (3-6 runs), the system is ready to run samples. Wait until the autosampler displays "WAIT TIME = 1600," then stop the program: PAUSE HOME.
13. Allow the HPLC to finish the run, then while it is printing, reset: SX ENTER, Immediately restart the flow: F:7 ENTER, and set the oven temperature: T:40 ENTER.
14. Stop the fraction collector program: PAUSE HOME.
15. Empty the collection tubes from the fraction collector into the TOXIC WASTE receptacle.
16. Empty and discard the rinse vials from the autosampler.
17. Replace the cloth labels on the calibration solutions vials. Store the vials in the freezer.

C., Cleanup of Samples and Isolation of an AH/CH Fraction

1. Load Fraction Collector File 2: RESET 2 ENTER.
2. Edit "1 DRAIN" in File 2: EDIT ENTER. Continue pressing ENTER until "1 DRAIN" is displayed. Enter the value of t6 calculated in Section 4, Part E, Step 7.

3. Only if necessary, edit "1 COLL" in File 2 by entering the value of t-14 calculated in Section 4, Part E, Step 7. Continue pressing ENTER until "READY - HIT START" is displayed.
4. Put clean, labeled conical centrifuge tubes in positions l-6 and 8-13 of rack code # 24. Put a used tube in positions 7 and 14 for the calibration solutions.
5. Put 15 clean, empty GC vials in the 1st row of the autosampler rack code #O (left to right). Remove the cloth labels from the sample vials, and label the vials with a felt pen. Put sample vials in positions 116 and 8-13 (left to right) in the 2nd row (from the back) of the rack. Put calibration solutions in positions 7 and 14.
6. Load Autosampler File 2: EDIT FILE 2 ENTER ENTER ENTER.
7. Start Autosampler File 2: START.
8. Set the *W* detector range at *0.64* for sediment samples and 0.32 for tissue samples.
9. Start HPLC Parameter Set 2: SB2 ENTER.
10. Record the fraction collection time in the HPLC log book.
11. As the samples are run, label the the chromatograms and log the sample numbers.
12. After the samples are run, recap and relabel vials, then store them in the freezer.
13. Empty and discard the rinse vials from the autosampler.
14. Cap the collected fractions and give them to the chemist who will prepare them for GC analysis. Store the samples in the freezer if necessary.

15. Empty the collection tubes containing the fraction from the calibration runs into the TOXIC WASTE receptacle.
16. If another cycle of samples is to be run, reload the autosampler with samples as described above. Then, without turning off the HPLC, reset the autosampler: PAUSE HOME EDIT FILE 2 ENTER ENTER ENTER START.
17. Reload the fraction collector with collector tubes as described above.
18. If the new HPLCKJV retention time for DOB has changed ~0.05 min, reedit the fraction collector "1 DRAIN" value (see Step 2).
19. If the new HPLCAJV retention time for perylene has changed 2 0.05 min with respect to the new HPLC/UV retention time for DOB in Step 18, reedit the fraction collector "1 COLL" value (see Step 3).

D. **Instrument Shut-down**

1. While the last run is being plotted and the autosampler displays "WAIT TIME = 1800," send the autosampler home: PAUSE HOME.
2. After the plotting of the last run is completed, open the oven door, and stop the HPLC program: SX ENTER.
3. Start the flow at 2 mL /min: F:2 ENTER.
4. Send the fraction collector home: PAUSE HOME.
5. After 5 min, switch the backflush valve to the "BACKFLUSH" position.
6. After another 5 min, stop the flow: VX- ENTER.
7. Turn off the *W* detector.
8. Turn off the helium at the tank, not at the regulator.
9. Turn off the dilutor.
10. Turn off the ventilation fan.

11. Replace the waste bottles with empty bottles.
12. Check the recorder paper supply (add more if necessary).
13. For each set of samples run, file the labeled chromatograms in a folder.