NOAA Technical Memorandum NMFS-NWFSC-59

Extraction, Cleanup, and Gas Chromatography/Mass Spectrometry Analysis of Sediments and Tissues for Organic Contaminants

March 2004

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EXECUTIVE SUMMARY

This document describes the analytical methods developed and used by the Environmental Conservation Division of the National Marine Fisheries Service's Northwest Fisheries Science Center for the analysis of sediment and tissue samples for nanograms per gram concentrations of selected aromatic hydrocarbons and chlorinated hydrocarbons. Detailed descriptions are presented for sample extraction, cleanup by silica/alumina columns and size-exclusion high-performance liquid chromatography, and analysis by gas chromatography/mass spectrometry, as well as the calculation of analyte concentrations and important quality assurance measurements. Detailed descriptions are also given for determining the percent dry weight of tissues and sediments, and for determining the percent total extractable content of tissues. Information pertaining to the necessary laboratory supplies and instruments is provided. In addition, to facilitate use of this document by other laboratories, the step-wise procedures are presented in a descriptive manner.

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1. INTRODUCTION

Various environmental studies have involved the analyses of marine sediments and tissues for toxic contaminants such as chlorinated pesticides, polychlorinated biphenyls, and aromatic hydrocarbons. High quality data with documented quality assurance is needed for such analyses in order to draw valid conclusions and reach appropriate decisions; for example, in damage assessment situations, for policy making, and to facilitate comparisons of the results with those of other studies. New techniques are continually sought and evaluated to further optimize the analytical accuracy, precision, sensitivity, robustness, efficiency, and safety. The Environmental Assessment Program of the Environmental Conservation Division of the National Marine Fisheries Service's Northwest Fisheries Science Center (NWFSC) develops and employs advanced methods for analyzing marine environmental samples for organic contaminants at part-per-billion concentrations. As improvements to procedures are validated, they are incorporated into the analytical protocols that are used for a multitude of projects in which selected aromatic hydrocarbons (AHs, Table 1) and chlorinated hydrocarbons (CHs, Table 2) are quantitated in tissues and sediment. This document includes the latest revisions to the methods described in Sloan et al. (1993) and Krahn et al. (1988), which updated the methods of MacLeod et al. (1985). Detailed descriptions of our current procedures are provided for complete documentation as well as for ease of use by other laboratories.

Several changes have been made to the earlier methods to improve the analyses of samples, which involve three steps: 1) extraction, 2) cleanup by silica/alumina columns and size-exclusion high-performance liquid chromatography (SEC HPLC), and 3) quantitation of CHs and AHs by gas chromatography/mass spectrometry (GC/MS). One of the major procedural modifications is the extraction of samples using an Accelerated Solvent Extractor (ASE), which reduces time, labor, solvent use, hazardous waste, and potential exposure of analysts to extraction solvent (in our case, dichloromethane) compared to our previous method. This ASE method provides an exhaustive extraction of organic compounds from various matrices while excluding water; thus both AHs and CHs are quantitatively recovered in a single extract, and the percent total extractables (lipids) in tissues can be gravimetrically determined from a portion of the ASE extract. Another major modification is the use of GC/MS selected-ion monitoring (SIM) (Burrows et al. 1990) in place of GC/electron-capture detection for CH analyses; thus the concentrated, cleaned-up extracts are analyzed for both AHs and CHs (separately) using the same GC system and configuration, as well as similar operating conditions. As MS instrumentation has improved, the sensitivity of GC/MS SIM for CH detection has approached that of electron-capture detection while being more selective, thus reducing interferences. Alterations made to the GC/MS system that allow greater instrument stability and accuracy include replacing the electron ionization filaments with chemical ionization filaments in order to use a higher source temperature, using a cool on-column injection system in the GC in place of a splitless injection system, and adding a guard column before the analytical column. Quantitation has also been optimized by using point-to-point calibration, which provides a better data fit over the entire range of GC/MS calibration standards than would be given by a single equation, such as linear, weighted linear, or quadratic regression.

Table 1. Aromatic hydrocarbon analytes.

Naphthalene
1-Methylnaphthalene
2-Methylnaphthalene
Biphenyl
2,6-Dimethylnaphthalene
Acenaphthylene
Acenaphthene
2,3,5-Trimethylnaphthalene
Fluorene
Dibenzothiophene
Phenanthrene
Anthracene
1-Methylphenanthrene
Fluoranthene
Pyrene
Retene
Benz[*a*]anthracene
Chrysene + Triphenylene [*]
Benzo[*b*]fluoranthene
Benzo[*j*]fluoranthene + Benzo[*k*]fluoranthene [*]
Benzo[*e*]pyrene
Benzo[*a*]pyrene
Perylene
Indeno[1,2,3-*cd*]pyrene
Dibenz[*a,h*]anthracene + Dibenz[*a,c*]anthracene [*]
Benzo[*ghi*]perylene

[*] These analytes are quantitated and reported as the sum of their concentrations because they co-elute during GC/MS analysis.

Table 2. Chlorinated hydrocarbon analytes including polychlorinated biphenyl (PCB) congeners (by IUPAC number).

PCBs	**Other chlorinated hydrocarbons**
PCB 17	2,4'-DDD
PCB 18	4,4'-DDD
PCB 28	2,4'-DDE
PCB 31	4,4'-DDE
PCB 33	2,4'-DDT
PCB 44	4,4'-DDT
PCB 49	Hexachlorobenzene
PCB 52	alpha-Hexachlorocyclohexane
PCB 66	beta-Hexachlorocyclohexane
PCB 70	gamma-Hexachlorocyclohexane
PCB 74	Aldrin
PCB 82	Dieldrin
PCB 87	Endosulfan I
PCB 95	Endosulfan II
PCB 99	Endosulfan sulfate
PCB 101 + PCB 90 [*]	Mirex
PCB 105	Heptachlor
PCB 110	Heptachlor epoxide
PCB 118	Oxychlordane
PCB 128	*trans*-Chlordane
PCB 138 + PCB 163 + PCB 164 [*]	*cis*-Chlordane
PCB 149	*trans*-Nonachlor
PCB 151	*cis*-Nonachlor
PCB 153 + PCB 132 [*]	Nonachlor III
PCB 156	
PCB 158	
PCB 170 + PCB 190 [*]	
PCB 171	
PCB 177	
PCB 180	
PCB 183	
PCB 187 + PCB 159 + PCB 182 [*]	
PCB 191	
PCB 194	
PCB 195	
PCB 199	
PCB 205	
PCB 206	
PCB 208	
PCB 209	

[*] These analytes are quantitated and reported as the sum of their concentrations because they co-elute during GC/MS analysis.

The sample preparation and analyses are summarized in Figure 1. Sediment and tissue samples are mixed with sodium sulfate and magnesium sulfate, then extracted using an ASE. Extracts of tissues, such as marine mammal tissues, may be split into two portions: one for isolation and quantitation of the analytes, and one for determining the percent total extractables (i.e., mostly lipids). The portions of extracts to be analyzed for AHs, CHs, or both are filtered through gravity-flow silica/alumina columns. The filtered sample extracts are then concentrated, and a portion of each filtered extract is chromatographed on a SEC HPLC column, using dichloromethane as the mobile phase, to collect a fraction containing AHs and CHs. The solvent is “exchanged” to isooctane while the fraction is reduced to a final volume of approximately 100 µL. The concentrated AH/CH fraction is then analyzed by GC/MS SIM to measure AHs, CHs, or both. Analytes are quantitated relative to extraction internal standards (also known as surrogate standards) using multiple concentration levels of GC/MS calibration standards. The concentrations of analytes in the samples are reported on a nanogram (ng) per gram (g) wet weight or dry weight basis.

Samples are typically analyzed in batches of 12 to 14 samples. Quality assurance (QA) measures are incorporated into each batch according to the Environmental Assessment Program Quality Assurance Plan (NMFS unpubl. data). Each batch of samples includes a method blank, a sample of a Standard Reference Material that has certified concentrations for many analytes, and as needed, replicate samples. Internal standards are added to the samples before extraction to monitor and account for any losses during sample preparation. HPLC internal standards are added before sample cleanup to account for the amount of total extract that is chromatographed by HPLC, and GC/MS internal standards are added before GC/MS to measure the recovery of the extraction and the HPLC internal standards. Typical goals for QA measurements are provided in Section 7.

Samples are prepared in fume hoods to minimize exposure of the analyst to solvent vapors, and personal protective equipment (e.g., rubber gloves, face shield, rubber apron) is worn by the analyst whenever concentrated acids are used. Additional laboratory safety practices are followed according to the NWFSC Chemical Hygiene Plan and the NWFSC Chemical Waste Management Guide. Analysts at other institutions would follow safety practices according to their in-house chemical hygiene and chemical waste management guidelines.

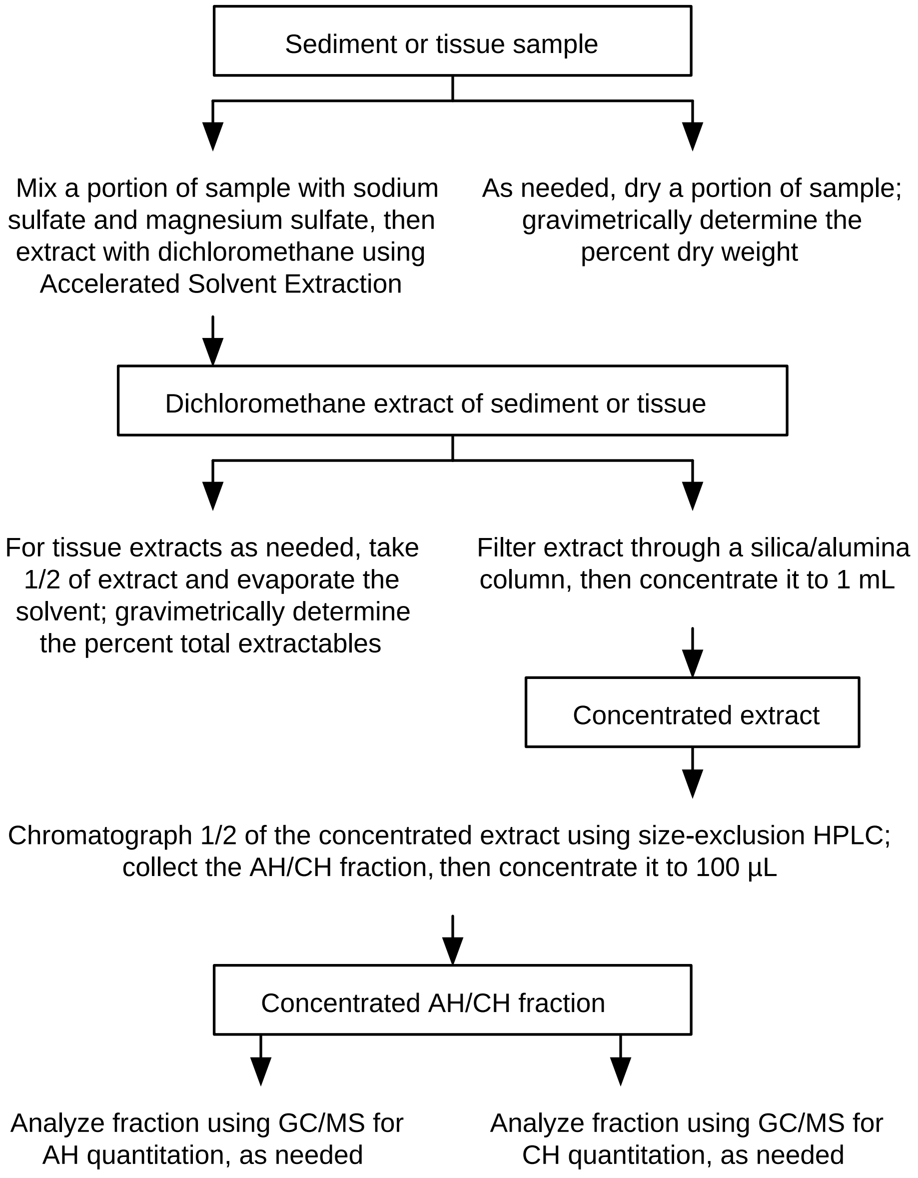

Figure 1. Flow diagram of sample preparation and analysis.

2. MATERIALS

As a convenience, equipment lists in Subsection 2.1 through Subsection 2.5 include names and locations of suppliers. These are not necessarily the preferred or sole suppliers. When no supplier is listed, the item is considered widely available.

2.1. Instruments and Accessories

2.1.1. GC/MS System

- Autosampler, Agilent 7683, with on-column injection syringe and needle guide. Agilent Technologies, Wilmington, DE
- ChemStation software, Version DA. Agilent Technologies, Wilmington, DE
- Gas chromatograph with cool on-column injection, Agilent 6890N. Agilent Technologies, Wilmington, DE
- Mass Selective Detector, Agilent 5973N. Agilent Technologies, Wilmington, DE

2.1.2. GC Gas and Accessories

- Helium, grade 5, ultra-high purity, 99.999%
- Indicating moisture trap, GMT-4-HP. Agilent Technologies, Wilmington, DE
- Indicating oxygen trap, 4004. Alltech Associates, Inc., Deerfield, IL
- Oxygen trap, disposable. 803088. Supelco, Inc., Bellefonte, PA
- Regulator, two-stage, 400 series
- Regulator, one-stage, 400 series

2.1.3. GC Column and Accessories

- Capillary column cleaving tool, 2-3740m. Supelco Inc., Bellefonte, PA
- Column nut, GC inlet, 5181-8830. Agilent Technologies, Wilmington, DE
- Column nut, MS interface, 05988-20066. Agilent Technologies, Wilmington, DE
- Column union, glass, universal, 705-0825. Agilent Technologies, Wilmington, DE
- Ferrule for inlet end of guard column, 5062-3538. Agilent Technologies, Wilmington, DE
- Ferrule for MS end of GC column, 15% graphite:85% Vespel, 1/16", 100/0-VG1, no-hole, drilled in-house. Alltech Associates, Inc., Deerfield, IL
- Guard column, deactivated fused-silica, Agilent, 10-m x 0.53-mm, 460-2535-10. Agilent Technologies, Wilmington, DE

- GC column, fused-silica, J & W Scientific DB-5, 60-m x 0.25-mm, 0.25-µm film thickness, 122-5062. Agilent Technologies, Wilmington, DE
- Gas leak detector, Leak Detective II, 20413. Restek Corporation, Bellefonte, PA
- Sealing resin for column union, polyimide, 500-1200. Agilent Technologies, Wilmington, DE

2.1.4. HPLC System and Accessories

- Autosampler, Waters 717 plus. Waters, Milford, MA
- Data system, Dynamax DA, Version 1.2B4. Rainin Instrument Co., Woburn, MA
- Filter, in-line after autosampler, 2-µm particle size, 7302. Rheodyne, Inc., Cotati, CA
- Fraction collector, Gilson FC 204. Gilson Co., Middleton, WI
- Helium for solvent degassing, Grade 5.0, 99.999%. PraxAir, Danbury, CT
- Helium sparger for solvent reservoir, 10-µm, 25312. Restek, Bellefonte, PA
- Hydrocarbon trap for helium line, Big Supelco Carb HC, 24564. Supelco, Bellefonte, PA
- Regulator for helium tank, two-stage with stainless-steel diaphragm
- Oxygen trap for helium line, Supelco Pure O, 2-2449. Supelco, Bellefonte, PA
- HPLC pump, 515. Waters, Milford, MA
- Size-exclusion column, Envirosep ABC, 350 mm x 21.2 mm. Phenomenex, Inc., Torrance, CA
- Size-exclusion guard column, Envirosep ABC, 60 mm x 21.2 mm. Phenomenex, Inc., Torrance, CA
- Solvent inlet filter for solvent reservoir, 10-µm, 59277. Supelco, Bellefonte, PA
- UV/VIS detector, Spectra 100. Spectra-Physics, San Jose, CA
- Valve, six-port, 7030. Rheodyne, Inc., Cotati, CA

2.1.5. Sample Extractor and Accessories

- Accelerated Solvent Extractor, ASE 200. Dionex, Salt Lake City, UT
- Extraction cells, 33-cc, 048764. Dionex, Salt Lake City, UT
- Nitrogen, grade 4.8. PraxAir, Danbury, CT

2.2 Solvents and Reagents

- Acetone (C_3H_6O), HPLC-UV grade. Pharmco, Brookfield, CT
- Copper, reagent grade, granular (20-30 mesh), 1720-05. J.T. Baker, Phillipsburg, NJ
- Dichloromethane (CH_2Cl_2), High Purity, 300-4. Burdick & Jackson, Muskegon, MI

- Hydrochloric acid (HCl), concentrated, reagent grade, A144-212. Fischer Scientific, Fair Lawn, NJ
- Isooctane (C_8H_{16}), Optima, 0301-4. Fischer Scientific, Fair Lawn, NJ
- Magnesium Sulfate ($MgSO_4$), anhydrous, M65-3. Fischer Scientific, Fair Lawn, NJ
- Methanol (CH_3OH), Optima, A454-4. Fischer Scientific, Fair Lawn, NJ
- Nitric acid (HNO_3), concentrated, reagent grade, A200-500. Fischer Scientific, Fair Lawn, NJ
- Sodium sulfate (Na_2S0_4), reagent grade, anhydrous, granular, S1461. Spectrum, Gardena, CA

2.3 Silica/Alumina Column Packings

- Alumina, 80-200 mesh, O537-01. J.T. Baker, Phillipsburg, NJ
- Glass wool, Pyrex 3950, 11-388. Fischer Scientific, Fair Lawn, NJ
- Sand, Ottawa, kiln-dried, 30-40 mesh, SX0075-3. EMD Chemicals, Gibbstown, NJ
- Silica, 100-200 mesh, Davisil Grade 634, S734-1. Fischer Scientific, Fair Lawn, NJ

2.4 Labware

All glassware that contacts samples or reagents is heated in a muffle furnace at 700°C for 18 hours or rinsed with acetone before use. Other labware that contacts samples or reagents is rinsed with acetone before use.

- ASE glass fiber filters, 047-017. Dionex, Salt Lake City, UT
- Balances, analytical
- Boiling chips, Teflon, AW0919120. All-World Scientific, Lynnwood, WA
- Desiccator with desiccant
- Glass rod, 1.6-cm diameter, 45-cm length
- Muffle furnace
- Ovens, drying, 50°C, 120°C, and 170°C
- Steam table
- Teflon sheeting, 2-mil thickness, for lining caps and lids
- Tube heater, 720000-0000, with glass-cylinder shroud and wire tube holder, aluminum inserts bored out to accepted 50-mL conical tubes. Kontes, Vineland, NJ
- Vial heater, column heater SP 8792 modified to hold 2-mL GC vials with a rack that allows the solvent level in the vials to be seen. Spectra-Physics, San Jose, CA
- Volume static eliminator, VSE 3000. Chapman, Hatfield, PA

- Weighing pans, aluminum, disposable, 43-mm i.d., 180-5537. All-World Scientific, Lynnwood, WA
- Weighing pans, aluminum, disposable, 75-mm i.d., 25433-020. VWR, Brisbane, CA

2.5 Disposable Glassware

Items are one-use only.

- ASE collection vials, 60-mL with cap and septa. Dionex, Salt Lake City, UT
- Bottles, amber, 250-mL, Boston round, 28-mm mouth, with Teflon-lined plastic caps
- Chromatography column, plain, custom made, 22-mm i.d. x 25-cm length. DJ's Glass Factory, San Jose, CA
- Jars, 10-ounce, 2.5-inch i.d. mouth. Aaron Packaging, Kent, WA
- Pipettes, transfer, Pasteur-style
- Tubes, centrifuge, 50-mL, 18-mm i.d. mouth, conical with Teflon-lined plastic caps
- Vials, GC, 2-mL, C4000-1W, with caps and septa, C4000-54R, and 200 µL inserts, C4010-627L. National Scientific Co., Duluth, GA
- Vials with caps and septa, HPLC, 4-mL, 99300-A, and 750-µL inserts, 200756. Sun International, Wilmington, NC

2.6. Standard Solutions and GC Conditioning Solutions

All solutions are prepared in-house using isooctane as the solvent, except the HPLC retention-time standard, which is prepared using dichloromethane. Concentrations given in parentheses are approximate; actual concentrations will vary slightly from batch to batch of the solution. The GC calibration standards and the internal standards, including GC and HPLC internal standards, are prepared such that the concentrations of the internal standards in final sample extracts analyzed by GC/MS are approximately equal to the concentrations of the internal standards in the GC calibration standards. GC conditioning solutions are used to simulate tissue or sediment samples during GC/MS performance verification and as GC/MS "warm up" samples at the beginning of a sequence of analyses.

2.6.1. Internal Standard Solutions

- CH I-Std (internal standard for quantitating CH analytes)—contains PCB 103 at 1 ng/µL
- CH HPLC I-Std (HPLC internal standard for CHs)—contains tetrachloro-*m*-xylene (TCMX) at 1 ng/µL
- CH GC I-Std (GC internal standard for CHs)—contains tetrachloro-*o*-xylene (TCOX) at 1 ng/µL

- AH I-Std (internal standard for quantitating AH analytes)—contains naphthalene-d8, acenaphthene-d10, and benzo[*a*]pyrene-d12 at 1.7 ng/µL each compound
- AH HPLC I-Std (HPLC internal standard for AHs)—contains phenanthrene-d10 at 1.6 ng/µL
- AH GC I-Std (GC internal standard for AHs)—contains hexamethylbenzene (HMB) at 10 ng/µL

2.6.2. Calibration Standards

- AH/CH HPLC retention-time standard—contains 4,4'-dibromooctafluorobiphenyl and perylene at 1.5 ng/µL each compound
- CH GC calibration standards—contain the CHs in Table 2 (except Nonachlor III); not all compounds are contained in all levels; Level 1 at 0.001 ng/µL each compound, Level 2 at 0.003 ng/µL each compound, Level 3 at 0.01 ng/µL each compound, Level 4 at 0.03 ng/µL each compound, Level 5 at 0.1 ng/µL each compound, Level 6 at 0.3 ng/µL each compound, Level 7 at 1.0 ng/µL each compound, Level 8 at 4.0 ng/µL each compound, Level 9 at 10 ng/µL each compound, Level 10 at 20 ng/µL each compound, Level 11 at 100 ng/µL each compound, plus all levels contain PCB 103, TCMX, and TCOX at 0.3 ng/µL each compound
- AH GC calibration standards—contain the AHs in Table 1 (except Retene); Level 1 at 0.0011 ng/µL each compound, Level 2 at 0.0044 ng/µL each compound, Level 3 at 0.015 ng/µL each compound, Level 4 at 0.044 ng/µL each compound, Level 5 at 0.11 ng/µL each compound, Level 6 at 0.33 ng/µL each compound, Level 7 at 1.13 ng/µL each compound, Level 8 at 3.3 ng/µL each compound, and Level 9 at 10.5 ng/µL each compound, plus all levels contain naphthalene-d8, acenaphthene-d10, and benzo[*a*]pyrene-d12, phenanthrene-d10 at 0.5 ng/µL each compound and HMB at 3.2 ng/µL in all levels

2.6.3. Spike Solutions

- CH Spike solution—contains the CHs in Table 2 (except Nonachlor III); 0.3 ng/µL each compound
- AH Spike solution—contains the AHs in Table 1 (except Retene); 7 ng/µL each compound

2.6.4. GC Conditioning Solutions

- Sediment GC Conditioning solution—a composite of moderately contaminated sediment extracts that have been prepared for GC/MS analysis as described in Sections 3 and 4, in isooctane
- Low-Lipid Tissue GC Conditioning solution—a composite of moderately contaminated, low-lipid tissue extracts that have been prepared for GC/MS analysis as described in Sections 3 and 4, in isooctane
- High-Lipid Tissue GC Conditioning solution—a composite of moderately contaminated, high-lipid tissue extracts that have been prepared for GC/MS analysis as described in Sections 3 and 4, in isooctane

2.7 Standard Reference Materials

Standard Reference Materials (SRM) are obtained from the National Institute of Standards and Technology (NIST), Gaithersburg, Maryland.

- SRM 1974b Organics in Mussel Tissue (*Mytilus edulis*)—analyzed for quality assurance measurements in batches of low-lipid tissue samples
- SRM 1941b Organics in Marine Sediment—analyzed for quality assurance measurements in batches of sediment samples
- SRM 1945 Organics in Whale Blubber—analyzed for quality assurance measurements in batches of high-lipid tissue samples

2.8. Preparation of Silica/Alumina Column Packings

2.8.1. Silica Preparation

The silica is activated by heating it at 700°C for 18 hours, then stored at 170°C. It is allowed to cool to room temperature in a desiccator just prior to use.

2.8.2. Alumina Preparation

The alumina is activated by heating it at 120°C for 2 hours. It is allowed to cool to room temperature in a desiccator just prior to use.

2.8.3. Copper Preparation

Less than one hour before use, the copper is activated by covering it with concentrated hydrochloric acid, stirring it with a glass rod, and allowing it to stand for 5 minutes. The copper is then washed three times each with water, methanol, and dichloromethane, in that order. The copper is stored covered with dichloromethane until use to avoid contact with air.

2.8.4. Sand Preparation

The sand is soaked overnight in a 1:3 v:v mixture of concentrated nitric acid:concentrated hydrochloric acid. The sand is washed three times each with water, methanol and dichloromethane, in that order, then dried, and stored at 120°C. It is allowed to cool to room temperature in a desiccator just prior to use.

2.8.5. Glass Wool Preparation

The glass wool is prepared for use by heating it at 400°C for 18 hours. It is stored in a covered glass container at room temperature until use.

2.9. Purity Testing

New lots of reagents are tested for purity before their initial use in sample analyses. Duplicate samples of the new lot of reagent are analyzed along with duplicate samples of the lot of reagent currently in use, for comparison. For solid reagents, duplicate solvent blanks are also analyzed to demonstrate whether any impurities found are from the reagent being tested or are from the solvent or laboratory. New glassware is used during purity testing. All glassware and equipment contacting the sample are heated in a muffle furnace or rinsed with acetone before use.

2.9.1. Isooctane Purity Test

AH GC I-Std (30 µL), CH GC I-Std (30 µL), and isooctane (40 µL) are added to a labeled GC vial with an insert, and the sample is mixed thoroughly.

The sample and GC calibration solutions are analyzed for AHs and CHs using GC/MS SIM as described in Section 5 for low-lipid tissues. The GC/MS responses of any impurities that are detected at the same retention times as analytes should be smaller than the corresponding analyte responses in the lowest level of GC/MS calibration standard.

2.9.2. Dichloromethane Purity Test

Dichloromethane (200 mL) is added to a 250-mL bottle. Five boiling chips are added to the bottle, and using the steam table, the sample volume is reduced to 15–20 mL.

The sample is transferred to a 50-mL tube. No dichloromethane washes are used. A boiling chip is added to the tube, and using the tube heater, the sample is concentrated to approximately 1 mL.

AH GC I-Std (30 µL), CH GC I-Std (30 µL), and isooctane (40 µL) are added to the sample, and the sample is mixed thoroughly.

The sample is transferred to a labeled GC vial, a small boiling chip is added, and the sample is concentrated to 100 µL using the vial heater.

The sample is transferred to a labeled GC vial with an insert.

The sample and GC calibration solutions are analyzed for AHs and CHs using GC/MS SIM as described in Section 5 for low-lipid tissues. The GC/MS responses of any impurities that are detected at the same retention times as analytes should be smaller than the corresponding analyte responses in the lowest level of GC/MS calibration standard.

2.9.3. Sodium Sulfate Purity Test

Two glass-fiber filters are placed at the bottom of an ASE cell. Sodium sulfate (20 cc) is added to the labeled ASE cell. One glass-fiber filter is placed on top of the sodium sulfate in the cell.

The sample is extracted using the ASE as described in Section 3.

A boiling chip is added to the vial containing the sample, and the sample is concentrated to approximately 1 mL using the tube heater.

AH GC I-Std (30 µL), CH GC I-Std (30 µL), and isooctane (40 µL) are added to the sample, and the sample is mixed thoroughly.

The sample is transferred to a labeled GC vial, a small boiling chip is added, and the sample is concentrated to 100 µL using the vial heater.

The sample is transferred to a labeled GC vial with an insert.

The sample and GC calibration solutions are analyzed for AHs and CHs using GC/MS SIM as described in Section 5 for low-lipid tissues. The GC/MS responses of any impurities that are detected at the same retention times as analytes should be smaller than the corresponding analyte responses in the lowest level of GC/MS calibration standard.

2.9.4. Magnesium Sulfate Purity Test

The Magnesium Sulfate Purity Test is performed following the same procedure as for the Sodium Sulfate Purity Test in Subsection 2.9.3 except that magnesium sulfate (15 cc) is added to the labeled ASE cell instead of sodium sulfate.

2.9.5. Silica or Alumina Purity Test

A silica/alumina column is prepared by adding a 10 to 15-mm plug of glass wool to a chromatography column and tamping it down with a glass rod, then adding alumina (10 cc), followed by silica (20 cc), and sand (5 cc).

A solution of methanol (10% by volume) in dichloromethane (35 mL) is slowly added to the column and allowed to drain into a waste container. Then, to flush the methanol from the column, dichloromethane (35 mL) is slowly added to the column and allowed to drain into a waste container. The tip of the column is then rinsed with dichloromethane.

A labeled 250-mL bottle is placed under the column.

Dichloromethane (100 mL) is added to the column and allowed to drain into the bottle.

The sample is concentrated to 15–20 mL using a steam table, then transferred to a labeled 50-mL tube.

A boiling chip is added to the tube and the sample is concentrated to approximately 1 mL using a tube heater.

AH GC I-Std (30 µL), CH GC I-Std (30 µL), and isooctane (40 µL) are added to the sample, and the sample is mixed thoroughly.

The sample is transferred to a labeled GC vial, a small boiling chip is added, and the sample is concentrated to 100 µL using the vial heater.

The sample is transferred to a labeled GC vial with an insert.

The sample and GC calibration solutions are analyzed for AHs and CHs using GC/MS SIM as described in Section 5 for low-lipid tissues. The GC/MS responses of any impurities that are detected at the same retention times as analytes should be smaller than the corresponding analyte responses in the lowest level of GC/MS calibration standard.

3. SAMPLE EXTRACTION

Sediments or tissues are customarily analyzed in batches of 12 to 14 samples. In addition to the sediment or tissue samples in each batch, there are typically one method blank sample, one sample of an appropriate SRM and, as needed, one or more replicate sediment or tissue samples. Typically, the amount of sediment or low-lipid tissue analyzed is 1 to 2.5 g, depending on the expected level of contamination. The amount of high-lipid tissue analyzed is typically 1 g. If only one of the two classes of analytes (CHs or AHs) is to be quantitated in the samples, all the internal standards and the I-Std check solution for the other class of analytes should be omitted.

The volume static eliminator is used on the balances whenever weighing items, in order to reduce weighing errors caused by static charge.

3.1 Extraction

A 10-ounce jar, an ASE cell, and an ASE collection vial are labeled with the sample number.

Samples are prepared depending on the matrix type as follows:

- For sediment samples, standing water is decanted from the original sample container, and the sample is stirred to homogenize it. All pebbles, shells, biota, and other extraneous material are discarded.
- For tissue samples, the sample is homogenized in the original sample container.
- For a batch of tissue samples requiring Total Extractables Determination (Subsection 8.2), the ASE collection vial is weighed to the nearest 0.001 g, and the weight is recorded as the "ASE Vial" weight.

The sample is transferred to the labeled 10-ounce jar and weighed to the nearest 0.001 g. The weight is recorded as the "Sample Weight."

Sodium sulfate (15 cc) is added to the sample in the jar and mixed thoroughly with the sample to absorb the water in the sample, which allows greater extraction efficiency of the organic compounds. To avoid clumping and hardening of the sodium sulfate, the sample is mixed immediately after adding the sodium sulfate to the jar. The sample is mixed until it appears dry to avoid generating excessive heat in the next step, which could volatilize analytes such as the naphthalenes.

For increased absorption of water from the sample, magnesium sulfate (15 cc) is then added to the sample in the jar and mixed thoroughly with the sample.

Two glass-fiber filters are placed at the bottom of the labeled ASE cell.

The sample/drying agent mixture is transferred to the ASE cell using the ASE funnel. The bottom of the cell is tapped firmly but carefully on the counter top during the transfer to completely settle the cell contents.

The remaining cell volume is filled with sodium sulfate. Again, the bottom of the cell is tapped firmly but carefully on the counter top to completely settle the cell contents, leaving approximately 3 mm of void volume at the top of the cell.

One glass-fiber filter is placed on top of the sodium sulfate in the cell without overlapping the rim of the cell.

The procedural steps are performed depending on the type of sample batch as follows:

- For a batch of tissue samples requiring Total Extractables Determination
    - AH I-Std (150 µL) and CH I-Std (150 µL) are added onto the top filter in the cell, then dichloromethane (approximately 1 mL) is added onto the top filter in the cell to rinse the internal standards into the cell.
    - The cell threads are cleared of sample/drying agent as necessary, and the cell is capped firmly but not forcefully.
    - An AH I-Std check solution is prepared by adding isooctane (50 µL) and AH I-Std (150 µL) to a labeled GC vial.
    - A CH I-Std check solution is prepared by adding isooctane (50 µL) and CH I-Std (150 µL) to a labeled GC vial.
- For a batch of tissue or sediment samples not requiring Total Extractables Determination
    - AH I-Std (75 µL) and CH I-Std (75 µL) are added onto the top filter in the cell, then dichloromethane (approximately 1 mL) is added onto the top filter in the cell to rinse the internal standards into the cell.
    - The cell threads are cleared of sample/sulfate as necessary, and the cell is capped firmly but not forcefully.
    - An AH I-Std check solution is prepared by adding isooctane (25 µL) and AH I-Std (75 µL) to a labeled GC vial.
    - A CH I-Std check solution is prepared by adding isooctane (25 µL) and CH I-Std (75 µL) to a labeled GC vial.

The ASE cell and the ASE collection vial are loaded in the corresponding positions in the ASE carousels. Collection vials for system rinses are loaded in the rinse vial positions.

The ASE system rinse function is activated three times to flush the solvent lines.

The sample is extracted using an ASE schedule with the Rinse parameter set to [ON], and using a method with the parameter values set as below. The method parameters as set for the Accelerated Solvent Extractor would appear on screen as follows:

PREHEAT [0] min
HEAT [5] min
STATIC [5] min
FLUSH % [115] vol
PURGE [180] sec
CYCLES [2]

PRESSURE [2000] psi
TEMPERATURE [100]°C
SOL A [MeCl2] [100]%
SOL B [Other] [0]%
SOL C [Other] [0]%
SOL D [Other] [0]%

After the extraction has finished, the ASE collection vial is removed from the ASE carousel.

3.2 Extract Preparation

The procedural steps are performed depending on the type of sample batch as follows:

- For a batch of tissue samples requiring Total Extractables Determination
    - AH HPLC I-Std (150 µL) and CH HPLC I-Std (150 µL) are added to the ASE collection vial, and the contents of the vial are mixed thoroughly.
    - AH HPLC I-Std (150 µL) is added to the AH I-Std check solution, which is then mixed thoroughly.
    - CH HPLC I-Std (150 µL) is added to the CH I-Std check solution, which is then mixed thoroughly.
    - The ASE collection vial is weighed to the nearest 0.001 g, and the weight is recorded as the “ASE Vial w/Extract” weight.
    - The sample number is etched on the tab of a 75-mm i.d. aluminum weighing pan.
    - The pan is placed in a drying oven at 120°C overnight, then cooled in a desiccator for 30 minutes.
    - The weighing pan is weighed to the nearest 0.0001 g, and the weight is recorded as the “Pan” weight.
    - One half of the sample in the ASE collection vial is transferred to the pan by pressing a spatula against the rim of the ASE vial and pouring the sample down the spatula into the pan. The ASE vial is capped immediately afterward.
    - The ASE collection vial is weighed to the nearest 0.001 g, and the weight is recorded as the “ASE vial w/o TE Extract” weight.
    - The pan containing half of the sample proceeds to Total Extractables Determination as described in Subsection 8.2, and the remaining sample in the ASE collection vial proceeds to Sample Cleanup by Silica/Alumina Column Chromatography as described in Subsection 4.1.2.
- For a batch of tissue or sediment samples not requiring Total Extractables Determination
    - AH HPLC I-Std (75 µL) and CH HPLC I-Std (75 µL) are added to the ASE collection vial, and the contents of the vial are mixed thoroughly.
    - AH HPLC I-Std (75 µL) is added to the AH I-Std check solution, which is then mixed thoroughly.

- CH HPLC I-Std (75 µL) is added to the CH I-Std check solution, which is then mixed thoroughly.
- The sample in the ASE collection vial proceeds to Sample Cleanup by Silica/Alumina Column Chromatography as described in Subsection 4.1.2.

4. SAMPLE CLEANUP

4.1. Silica/Alumina Column Chromatography

A gravity-flow silica/alumina column is used for removing extraneous polar compounds from the sample.

4.1.1. Calibration of Silica/Alumina Column

Calibration is performed once per lot of silica and once per lot of alumina before their use in sample analyses.

A spiked blank is prepared by adding dichloromethane (55 mL), CH I-Std (60 µL), AH I-Std (60 µL), CH Spike (80 µL), and AH Spike (60 µL) to a labeled 250-mL bottle.

A CH analyte check solution is prepared by adding CH Spike (80 µL) and CH I-Std (60 µL) to a labeled GC vial.

An AH analyte check solution is prepared by adding AH spike (80 µL) and AH I-Std (60 µL) to a labeled GC vial.

A silica/alumina column is prepared by adding a 10 to 15-mm plug of glass wool to a chromatography column and tamping it down with a glass rod, then adding alumina (10 cc), followed by silica (20 cc) and sand (5 cc).

A solution of methanol (10% by volume) in dichloromethane (35 mL) is slowly added to the column and allowed to drain into a waste container. To flush the methanol from the column, dichloromethane (35 mL) is slowly added to the column and allowed to drain into a waste container. The tip of the column is then rinsed with dichloromethane.

A second 250-mL bottle labeled “F1” is placed under the column. The spiked blank is slowly decanted into the column, and the eluant is collected in the second bottle.

The first bottle, containing the spiked blank, is washed with dichloromethane (approximately 5 mL), the washings are slowly decanted into the column, and the eluant is collected in the second bottle. This step is repeated twice more.

Dichloromethane (35 mL) is added to the column, and the eluant is collected in the second bottle. The bottle containing the F1 fraction is then set aside.

A 50-mL tube labeled “F2” is placed under the column. Dichloromethane (10 mL) is added to the column and the eluant is collected in the tube. The tube containing the F2 fraction is then set aside.

Five boiling chips are added to the bottle containing the F1 fraction and the F1 fraction is concentrated to 15–20 mL using a steam table.

The F1 fraction is transferred to a labeled 50-mL tube.

The bottle is washed with dichloromethane (approximately 5 mL) and the washings are added to the tube. This step is repeated twice more.

A boiling chip is added to the tubes containing the F1 and F2 fractions and each fraction is concentrated to approximately 1 mL using a tube heater.

AH GC I-Std (60 µL), CH GC I-Std (60 µL), and isooctane (80 µL) are added to each fraction, which is then mixed thoroughly.

CH GC I-Std (60 µL) is added to the CH analyte check solution, which is then mixed thoroughly.

AH GC I-Std (60 µL) is added to the AH analyte check solution, which is then mixed thoroughly.

Each fraction is transferred to a labeled GC vial, a small boiling chip is added, and the fraction is concentrated to 200 µL using the vial heater.

Each fraction is transferred to a labeled GC vial with an insert.

Each fraction and analyte check solution is analyzed for AHs and CHs using GC/MS SIM as described in Section 5 to compare the recoveries of the analytes and internal standards in the F1 and F2 fractions to those in the analyte check solutions. The analytes and internal standards should completely elute in F1 and none should in F2. If any analytes elute in F2, then the volume of dichloromethane for eluting the AH/CH fraction in Subsection 4.1.2. must be increased from 35 mL to 45 mL.

4.1.2. Sample Cleanup by Silica/Alumina Column Chromatography

A silica/alumina column is prepared by adding a 10 to 15-mm plug of glass wool to a chromatography column and tamping it down with a glass rod, then adding alumina (10 cc), followed by silica (20 cc), and sand (5 cc).

The column packing is slightly deactivated for better AH recovery by slowly adding a solution of methanol (10% by volume) in dichloromethane (35 mL) to the column and allowing the solvent to drain into a waste container. To flush the methanol from the column, dichloromethane (35 mL) is slowly added to the column and allowed to drain into a waste container. The tip of the column is then rinsed with dichloromethane.

A labeled 250-mL bottle is placed under the column. The sample extract in the ASE collection vial is slowly decanted into the column, and the eluant is collected in the bottle.

The inside of the vial is washed with dichloromethane (approximately 5 mL), the washings are decanted into the column, and the eluant is collected in the bottle. This step is repeated twice more.

Dichloromethane (35 mL) is added to the column and the eluant is collected in the bottle.

Five boiling chips are added to the bottle containing the sample eluant and the sample volume is reduced to 15–20 mL using a steam table.

The sample is transferred to a labeled 50-mL centrifuge tube.

The inside of the bottle is washed with dichloromethane (approximately 5 mL) and the washings are added to the tube. This step is repeated twice more.

A boiling chip is added to the tube and the sample volume is reduced to approximately 1 mL using a tube heater.

- For a batch of sediment samples, activated copper is added to the tube a few grains at a time until no further discoloring of the copper occurs. The tube is capped and stored overnight.

The sample is transferred to a GC vial (leaving the copper in the tube in the case of sediment samples), and the volume brought to 1.0 mL either by adding dichloromethane or by evaporating the excess under a gentle stream of nitrogen gas.

The sample in the vial proceeds to SEC HPLC chromatography as described in Subsection 4.2.

4.2. Size-Exclusion High-Performance Liquid Chromatography

SEC HPLC is used for removing extraneous high molecular weight compounds from the sample. The HPLC is programmed according to Table 3. The fraction-collection time is determined immediately before chromatographing a batch of samples, as in Subsection 4.2.3. using the results from the system calibration performed in Subsection 4.2.2.

4.2.1. SEC HPLC Start Up

A 4-L bottle of dichloromethane is installed in the solvent reservoir holder, and the solvent inlet filter and helium diffuser are placed at the bottom of the bottle. The dichloromethane is degassed for approximately 10 minutes prior to SEC HPLC system operation by diffusing helium into the dichloromethane. The helium flows through a hydrocarbon trap and an oxygen trap installed in-line between the helium tank and the diffuser.

The purge valve on the pump is opened, then the pump is started in purge mode to purge the pump with the degassed solvent for 5 minutes, with the solvent flowing to the waste collection bottle. The purge valve is closed after the pump purge has finished.

Table 3. Size-exclusion high-performance liquid chromatography events.

Approximate time (min)	HPLC module	Activity
<0	Autosampler	Sample loop loaded with 500 µL of sample or calibration standard
0	Autosampler	Sample injected; signal sent to computer to start data system
0–24	Pump	Isocratic elution, 5 mL/min
1	Autosampler	Injection port rinsed; needle rinsed
14–19	Fraction collector	Fraction collected
24	Computer	Data system ends integration

The pump is started with the flow rate of the solvent set at 0.75 mL/min for purging the autosampler, then the autosampler purge mode is started. If the compression test fails, the autosampler purge is repeated.

The pump is stopped, then the flow is set to 5 mL/min and the pump is started. The pump is monitored for stable system pressure.

The wavelength on the detector is set at 254 nm, and the baseline of the UV signal is zeroed.

The fraction collector is set for no collection.

A 4-mL vial is filled with AH/CH HPLC retention-time standard and placed in the autosampler carousel.

The autosampler is programmed and initiated so that the AH/CH HPLC retention-time standard is injected and chromatographed according to Table 3, except that no fraction is collected.

The AH/CH HPLC retention-time standard is chromatographed three or more times, as necessary, to obtain three consecutive analyses having retention time stability to within + 0.05 minutes of each other for 4,4'-dibromooctafluorobiphenyl (DOB; first peak) and for perylene (PER; last peak). It may be necessary to re-zero the baseline of the UV signal at approximately 10 minutes in the first analysis of the AH/CH HPLC retention-time standard.

For each analysis of the calibration standard, the system pressure and the retention times of DOB and PER are recorded.

When the retention times of the DOB and PER peaks in the calibration standard have stabilized for three consecutive analyses, the system is ready to perform the system calibration described in Subsection 4.2.2, or the daily calibration described in Subsection 4.2.3.

The autosampler program is stopped, and the vial of AH/CH HPLC retention-time standard is removed from the autosampler tray, recapped, and set aside until needed for the daily calibration of the SEC HPLC system as described in Subsection 4.2.3.

4.2.2. SEC HPLC System Calibration

The SEC HPLC system calibration is performed only when any component of the HPLC system (e.g., pump, SEC column, length of tubing, etc.) is changed.

The SEC HPLC is started and the AH/CH HPLC retention-time standard is chromatographed three or more times, as necessary, as described in Subsection 4.2.1.

The fraction collector is programmed to collect 10 consecutive, 0.1-minute fractions starting 1 minute before the retention time of DOB, and to collect 10 consecutive, 0.1-minute fractions starting at the retention time of PER.

The fraction collector is configured with a tray for 2-mL GC vials. Twenty 2-mL GC vials labeled F1 through F20 are positioned in the fraction collector tray.

An HPLC system calibration solution is prepared by adding dichloromethane (900 µL) and AH/CH HPLC (100 µL) retention-time standard to a 4-mL HPLC vial and thoroughly mixing the contents of the vial. This vial is positioned in the autosampler tray.

The autosampler is programmed and initiated so that the HPLC system calibration solution is injected and chromatographed according to the times in Table 3, except that the fractions are collected as programmed above. The retention times of DOB and PER are recorded.

When the analysis is finished, the pump, autosampler, and fraction collector are stopped and the detector is turned off.

The remaining HPLC system calibration solution is removed from the autosampler and discarded appropriately. The GC vials containing fractions F1 through F20 are removed from the fraction collector tray.

CH GC I-Std (30 µL) and isooctane (70 µL) are added to each of the vials containing fraction F1 through F10, and the contents of each vial are mixed thoroughly.

AH GC I-Std (30 µL) and isooctane (70 µL) are added to each vial containing fractions F11 through F20, and the contents of each vial are mixed thoroughly.

A small boiling chip is added to each vial and each fraction is reduced to 100 µL using a vial heater.

Each fraction is transferred to a labeled GC vial with an insert.

The fractions F1 through F10 and the Level 5 CH GC calibration solution are analyzed by GC/MS for CHs as described in Section 5. The CH GC I-Std in the fractions is used to show that each fraction is injected and analyzed properly by the GC/MS.

The GC/MS chromatograms for fractions F1 through F10 are inspected to find the first fraction in which DOB appears, which indicates the start of the DOB elution from the SEC HPLC system.

The starting time (t1) of the DOB elution from the SEC HPLC system is determined as the starting time for the collection of the first fraction in which DOB appears.

To allow for possible retention time drift, 0.2 minute is subtracted from t1 to obtain t2, then t2 is subtracted from the DOB HPLC retention time (DOB RT) in the analysis of the HPLC system calibration standard to obtain t3 (see Figure 2).

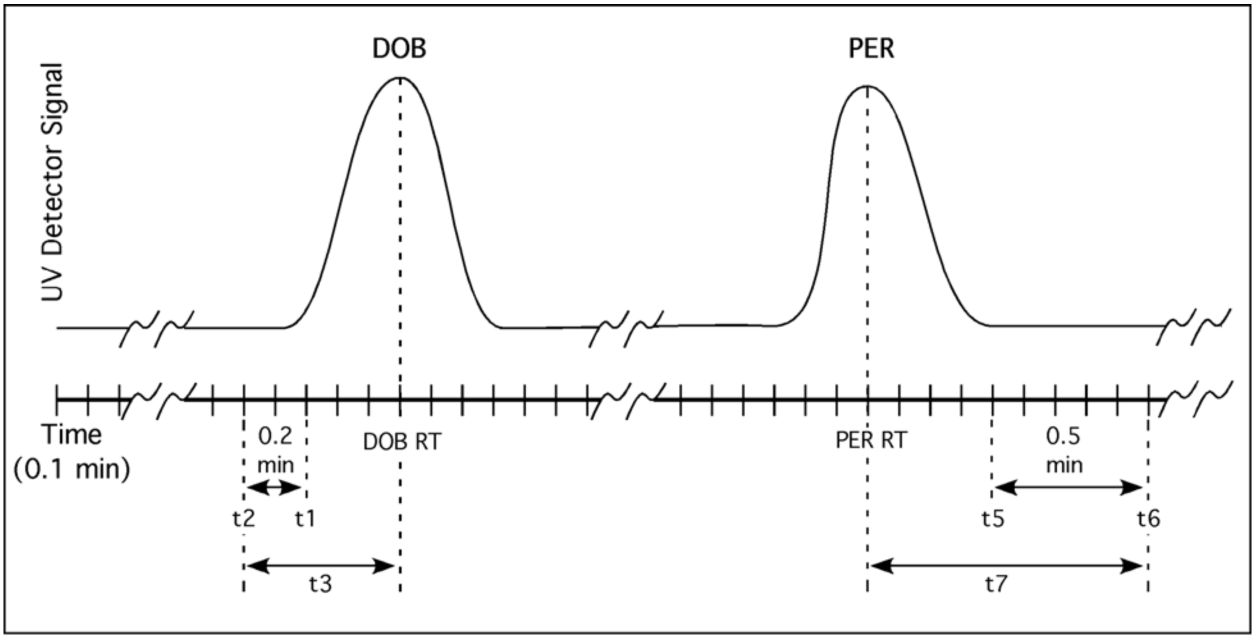

Figure 2. Size-exclusion high-performance liquid chromatograph calibration. (DOB is 4,4'-dibromooctafluorobiphenyl, PER is perylene, and RT is retention time.)

The value for t3 is recorded for future use in the daily calibration of the SEC HPLC (Subsection 4.2.3.).

The fractions F11 through F20 and the Level 3 AH GC calibration solution are analyzed by GC/MS SIM for AHs as described in Section 5. The AH GC I-Std in the fractions is used to show that each fraction is injected and analyzed properly by the GC/MS.

The GC/MS chromatograms for fractions F11 through F20 are inspected to find the last fraction in which PER appears, which indicates the end of the PER elution from the SEC HPLC system.

The ending time (t5) of the PER elution from the SEC HPLC system is determined as the ending time for the collection of the last fraction in which PER appears.

To allow for possible retention time drift, 0.5 minute is added to t5 to obtain t6, then the PER HPLC retention time (PER RT) in the analysis of the HPLC system calibration standard is subtracted from t6 to obtain t7.

The value for t7 is recorded for future use in the daily calibration of the SEC HPLC (Subsection 4.2.3.).

4.2.3. Daily Calibration of the SEC HPLC System

The daily calibration of the SEC HPLC system is performed immediately before chromatographing a batch of samples to determine the collection time of the AH/CH fraction.

The SEC HPLC is started and the AH/CH HPLC retention-time standard is chromatographed three or more times, as necessary, as described in Subsection 4.2.1.

Collection start and end times for the AH/CH fraction are determined using the retention times of DOB and PER from the most recent analysis of the AH/CH HPLC retention-time standard and the values t3 and t7 derived from the HPLC system calibration described in Subsection 4.2.2.

For the daily calibration, the formula for the starting time t4 for collecting an AH/CH fraction is

$$t4 = \text{DOB RT} - t3 \qquad (1)$$

where DOB RT is the DOB HPLC retention time in the most recent analysis of the AH/CH calibration standard and t3 is the value determined in Subsection 4.2.2.

For the daily calibration, the formula for the ending time t8 for collecting an AH/CH fraction is

$$t8 = \text{PER RT} + t7 \qquad (2)$$

where PER RT is the PER HPLC retention time in the most recent analysis of the AH/CH calibration standard during the daily calibration and t7 is the value determined in Subsection 4.2.2.

4.2.4. Sample Cleanup by SEC HPLC

A batch of samples is chromatographed immediately following the daily calibration of the SEC HPLC system, as described in Subsection 4.2.3.

The fraction collector is programmed to collect the AH/CH fraction for each sample in the batch, using the start and end times determined during the daily calibration of the SEC HPLC system described in Subsection 4.2.3. The starting and ending times of the collection are recorded in the HPLC logbook.

Labeled 50-mL tubes are placed in the rack of the fraction collector.

The sample obtained from the silica/alumina column chromatography cleanup described in Subsection 4.1.2 is transferred to a labeled 4-mL vial with an insert.

An HPLC blank sample is prepared by filling a labeled 4-mL vial with dichloromethane. This sample is monitored for contamination from the HPLC system.

The batch of samples and the AH/CH HPLC retention-time standard are positioned in the autosampler tray such that the HPLC blank is chromatographed at the beginning of the sequence and the AH/CH HPLC retention-time standard is analyzed in the middle and at the end of the batch.

The autosampler is programmed to sequentially chromatograph the samples in the batch plus the AH/CH HPLC retention-time standard in the middle and end of the batch.

For each analysis of the calibration standard, the system pressure and the retention times of DOB and PER are recorded. If the retention time for DOB in the middle analysis differs from the first analysis by ±0.05 minutes or more, then the starting time for collection of the fraction is changed accordingly for the remaining samples in the batch. If the retention time for PER in the middle analysis changed by ±0.05 minutes or more with respect to the retention time for DOB in the middle analysis, the collection time for the fraction is changed accordingly for the remaining samples in the batch.

After the sequence of samples and AH/CH HPLC retention-time standard has finished, the SEC guard column is backflushed for 2–3 minutes, then the pump, autosampler, and fraction collector are stopped and the detector is turned off.

The remaining sample in the HPLC vial insert is transferred back to its 2-mL GC vial and stored in a freezer.

A boiling chip is added to the 50-mL tube containing the AH/CH fraction and the fraction volume is reduced to approximately 1 mL using a tube heater.

The procedural steps are performed depending on the type of sample matrix as follows:

- For a batch of tissue or sediment samples to be analyzed for both AHs and CHs, AH GC I-Std (30 µL), CH GC I-Std (30 µL), and isooctane (40 µL) are added to the tube and the contents mixed thoroughly.
- For a batch of tissue or sediment samples to be analyzed for AHs only, AH GC I-Std (30 µL) and isooctane (70 µL) are added to the tube and the contents mixed thoroughly.
- For a batch of tissue or sediment samples to be analyzed for CHs only, CH GC I-Std (30 µL) and isooctane (70 µL) are added to the tube and the contents mixed thoroughly.

The procedural steps are performed depending on the type of sample batch as follows:

- For the check solutions in a batch of tissue samples requiring Total Extractables Determination
  - AH GC I-Std (150 µL) is added to the AH I-Std check solution, which is then mixed thoroughly.
  - CH GC I-Std (150 µL) is added to the CH I-Std check solution, which is then mixed thoroughly.
- For the check solutions in a batch of tissue or sediment samples not requiring Total Extractables Determination
  - AH GC I-Std (75 µL) is added to the AH I-Std check solution, which is then mixed thoroughly.
  - CH GC I-Std (75 µL) is added to the CH I-Std check solution, which is then mixed thoroughly.

The AH/CH fraction is transferred from the tube to a labeled GC vial. A small boiling chip is added to the vial and the fraction volume is reduced to 100 µL using a vial heater.

The fraction is transferred to a labeled GC vial with an insert.

The AH/CH fraction and the AH I-Std check solution are analyzed by GC/MS for AHs as described in Section 5. In addition, the AH/CH fraction and the CH I-Std check solution are analyzed by GC/MS for CHs as described in Section 5.

5. GAS CHROMATOGRAPHY/MASS SPECTROMETRY

Gas chromatography/mass spectrometry (GC/MS) is used to measure the amounts of the analytes and the internal standards in the samples. The GC/MS response to each compound is measured in terms of the area of the response.

5.1. GC/MS Operating Conditions

The operating conditions for the GC/MS are listed in Table 4.

The helium flows through an indicating moisture trap, an oxygen trap, and an indicating oxygen trap installed in-line between the helium regulators and the GC.

The GC/MS is checked for atmospheric leaks and the MS tune is performed or checked for stability prior to analyzing a sequence of samples and standards.

5.2. GC/MS for Quantitating AHs

The GC oven temperature is programmed according to Table 5.

Sediment samples are analyzed using scan mode, scanning all masses from 60 to 300 atomic mass units. Tissue samples are analyzed using SIM mode, scanning only the quantitation and confirmation ions during the specified time window as shown in Table 6. Scan mode provides greater ability to confirm analyte identification in the complex sediment samples, whereas SIM mode provides greater sensitivity for tissue samples that generally contain lower concentrations of contaminants. The quantitation ions for AHs in tissues shown in Table 6 are also the ions used for quantitating AHs in sediments. The time windows for SIM mode are adjusted as necessary when the GC column is changed or the guard column is trimmed.

5.2.1 Verification of GC/MS Performance for AHs

The sensitivity of the GC/MS is checked by analyzing the lowest level of AH GC calibration standard that will be used to quantitate AHs in the samples (Level 1 for tissues; Level 3 for sediments).

The stability of the GC/MS is verified by analyzing a sequence of three or more repetitions of the mid-level AH GC calibration standard that will be used (Level 4 for tissues; Level 7 for sediments). Included in this sequence are repetitions of the appropriate conditioning solution (Low-Lipid Tissue Conditioning solution for tissues, or Sediment Conditioning solution for sediments), that is, one conditioning solution before each repetition of the mid-level AH GC calibration standard. The responses of the analytes relative to the responses of their internal standard are compared among the repetitions of the mid-level AH GC calibration standard to

Table 4. Gas chromatograph/mass spectrometer operating conditions.

Parameter	**Value**
Injection volume	2 µL
Injection technique	cool on-column
Inlet temperature program	oven temperature plus 3°C
Transfer line temperature	300°C
Carrier gas	helium
Carrier gas linear velocity	30 cm/sec
Carrier gas flow	1.3 mL/min constant flow
Column	
Material	fused-silica capillary tubing
Length	60 m
Internal diameter	0.25 mm
Stationary phase	DB-5
Phase composition	5% phenyl, 95% methylpolysiloxane
Film thickness	0.25 µm
Guard column	
Material	deactivated fused silica capillary tubing
Length	10 m
Internal diameter	0.53 mm
Quadrupole temperature	150°C
Ion-source pressure	$\leq 5 \times 10^{-5}$ Torr
Ion-source temperature	300°C
Ionization mode	electron ionization
Filament	chemical ionization
Tune mode	AHs: Autotune
	CHs: Autotune, then the multiplier voltage is increased to achieve the desired sensitivity
Emission current maximum	35 µA
Scan rate	Approximately 10 to 20 scans per peak

Table 5. Gas chromatograph oven temperature program for aromatic hydrocarbons.

Parameter	Value
Initial temperature	80°C
Initial time	1 min
First rate	10°C/min to 200°C
Isothermal hold	0 min
Second rate	4°C/min to 300°C
Isothermal hold	15 min at 300°C
Total run time	53 min

Table 6. Selected-ion monitoring for quantitating aromatic hydrocarbons in tissue samples.

Approximate time window	**Compounds**	**Quantitation ion, confirmation ion (atomic mass units)**
7–12 min	Naphthalene-d8	136
	Naphthalene	128, 127
	2-Methylnaphthalene	142, 141
	1-Methylnaphthalene	142, 141
12–13 min	Biphenyl	154, 153
	2,6-Dimethylnaphthalene	156, 141
13–14 min	Hexamethylbenzene	147
	Acenaphthylene	152, 151
	Acenaphthene-d10	164
	Acenaphthene	154, 153
14–17 min	2,3,5-Trimethylnaphthalene	170, 155
	Fluorene	166, 165
17–20 min	Dibenzothiophene	184, 139
	Phenanthrene-d10	188
	Phenanthrene	178, 176
	Anthracene	178, 176
20–29 min	1-Methylphenanthrene	192, 191
	Fluoranthene	202, 101
	Pyrene	202, 101
	Retene	234, 219
29–42 min	Benz[*a*]anthracene	228, 226
	Chrysene + Triphenylene	228, 226
	Benzo[*b*]fluoranthene	252, 250
	Benzo[*j*+*k*]fluoranthene[a]	252, 250
	Benzo[*e*]pyrene	252, 250
	Benzo[*a*]pyrene-d12	264
	Benzo[*a*]pyrene	252, 250
	Perylene	252, 250
42–53 min	Indeno[1,2,3-*cd*]pyrene	276, 138
	Benzo[*ghi*]perylene	276, 138
	Dibenz[*a,h*+*a,c*]anthracene[b]	278, 139

[a] Benzo[*j*]fluoranthene + Benzo[*k*]fluoranthene

[b] Dibenz[*a,h*]anthracene + Dibenz[*a,c*]-anthracene

assess the GC/MS stability. The GC/MS is considered stable if the response of an analyte relative to the response of its internal standard in a given repetition is within ±15% of the respective average for the repetitions.

5.2.2. GC/MS Analysis of Sediment Samples for AHs

One or more batches of sediment samples are analyzed in a sequence that includes one each of AH GC calibration standard Levels 3 through 6 and Levels 8 and 9 alternating with the samples, plus one Level 7 before the first sample and replicate Level 7 analyses at the middle and end of each batch. Included in this sequence is one injection of the Sediment Conditioning solution at the beginning of the sequence, which serves as a GC/MS system conditioner.

5.2.3. GC/MS Analysis of Tissue Samples for AHs

One or more batches of tissues samples are analyzed in a sequence that includes one each of AH GC calibration standard Levels 1, 2, 3, 5, 6, and 7, alternating with the samples in the sequence, plus one Level 4 before the first sample and replicate Level 4 analyses at the middle and end of each batch. Included in this sequence is one injection of the Low-Lipid Tissue Conditioning solution at the beginning of the sequence, which serves as a GC/MS system conditioner.

5.3. GC/MS for Quantitating CHs

The GC oven temperature is programmed according to Table 7.

Sediment and tissue samples are analyzed using SIM mode, scanning only the quantitation ion during the specified time window as shown in Table 8. The time windows for SIM mode are adjusted as necessary when the GC column is changed or the guard column is trimmed.

5.3.1. Verification of GC/MS Performance for CHs

The sensitivity of the GC/MS is checked by analyzing the lowest level of CH GC calibration standard that will be used to quantitate CHs in samples (Level 1 for sediments and low-lipid tissues, Level 2 for high-lipid tissues).

The stability of the GC/MS is checked by analyzing a sequence of three or more repetitions of the Level 5 CH GC calibration standard. Included in this sequence are repetitions of the appropriate conditioning solution (Low-Lipid Tissue Conditioning solution for low-lipid tissues, High-Lipid Tissue Conditioning solution for high-lipid tissues or Sediment Conditioning solution for sediments), that is, one conditioning solution before each repetition of the Level 5 CH GC calibration standard. The responses of the analytes relative to the responses of their internal standard are compared among the repetitions of the Level 5 CH GC calibration standard to assess the GC/MS stability. The GC/MS is considered stable if the response of an analyte

Table 7. Gas chromatograph oven temperature program for chlorinated hydrocarbons.

Parameter	Value
Initial temperature	1 min at 80°C
First rate	10°C/min to 150°C
Isothermal hold	0 min
Second rate	0.5°C/min to 195°C
Isothermal hold	0 min
Third rate	3°C/min to 315°C
Isothermal hold	15 min 315°C
Total run time	153 min

Table 8. Selected-ion monitoring for quantitating chlorinated hydrocarbons in tissue and sediment samples.

Approximate time window	**Compounds**	**Quantitation ion (atomic mass units)**
15–29 min	Tetrachloro-*o*-xylene	207
	Tetrachloro-*m*-xylene	207
29–35 min	alpha-Hexachlorocyclohexane	181
	Hexachlorobenzene	284
35–39 min	beta-Hexachlorocyclohexane	219
	gamma-Hexachlorocyclohexane	181
39–45 min	PCB 18	256
	PCB 17	256
45–55 min	PCB 31	256
	PCB 28	256
	PCB 33	256
	heptachlor	272
55–65 min	PCB 52	292
	PCB 49	292
	aldrin	263
	PCB 44	292
65–69 min	PCB 103	326
69–76 min	Heptachlor epoxide	353
	Oxychlordane	115
	PCB 74	292
	PCB 70	292
	PCB 66	292
	PCB 95	326
76–79 min	*trans*-Chlordane	373
79–86 min	2,4'-DDE	318
	Endosulfan I	241
	PCB 101 + PCB 90	326
	Nonchlor III	409
	cis-Chlordane	373
	PCB 99	326
	trans-Nonachlor	409

Table 8 continued. Selected-ion monitoring for quantitating chlorinated hydrocarbons in tissue and sediment samples.

Approximate time window	Compounds	Quantitation ion (atomic mass units)
86–93 min	Dieldrin	79
	PCB 87	326
	4,4'-DDE	165
	PCB 110	326
	2,4'-DDD	235
93–98 min	PCB 82	326
	PCB 151	360
	Endosulfan II	241
98–103 min	PCB 149	360
	PCB 118	326
	cis-Nonachlor	409
	4,4'-DDD	165
	2,4'-DDT	235
103–106 min	PCB 153 + PCB 132	360
	PCB 105	326
106–110 min	Endosulfan sulfate	272
	4,4'-DDT	235
	PCB 138 + PCB 163 + PCB 164	360
	PCB 158	360
110–113 min	PCB 187 + PCB 159 + PCB 182	394
	PCB 183	394
	PCB 128	360
113–115 min	PCB 177	394
	PCB 171	394
	PCB 156	360
115–121 min	PCB 180	394
	PCB 191	394
	Mirex	272
	PCB 170 + PCB 190	394
	PCB 198	430
	PCB 199	430
121–153 min	PCB 208	464
	PCB 195	430
	PCB 194	430
	PCB 205	430
	PCB 206	464
	PCB 209	498

relative to the response of its internal standard in a given repetition is within ±15% of the respective average for the repetitions.

5.3.2. GC/MS Analysis of Sediment Samples for CHs

One or more batches of sediment samples are analyzed in a sequence that includes one each of CH GC calibration standard Levels 1 through 4 and Level 6 alternating with the samples, plus one Level 5 before the first sample and replicate Level 5 analyses at the middle and end of each batch. Also included in this sequence is one injection of the Sediment Conditioning solution at the beginning of the sequence which serves as a GC/MS system conditioner.

5.3.3. GC/MS Analysis of Low-Lipid Tissue Samples for CHs

One or more batches of low-lipid tissues samples are analyzed in a sequence that includes one each of CH GC calibration standard Levels 1 through 4 and Level 6 alternating with the samples, plus one Level 5 before the first sample and replicate Level 5 analyses at the middle and end of each batch. Included in this sequence is one injection of the Low-Lipid Tissue Conditioning solution at the beginning of the sequence which serves as a GC/MS system conditioner.

5.3.4. GC/MS Analysis of High-Lipid Tissue Samples for CHs

One or more batches of high-lipid tissues samples are analyzed in a sequence that includes one each of CH GC calibration standard Levels 2, 3, and 4 and Levels 6 through 11 alternating with the samples, plus one Level 5 before the first sample and replicate Level 5 analyses at the middle and end of each batch. Included in this sequence is one injection of the High-Lipid Tissue Conditioning solution at the beginning of the sequence which serves as a GC/MS system conditioner.

6. CALCULATIONS OF RESULTS

6.1. Calculation of Analyte Concentrations

The acquired GC/MS data for the samples and calibration standards are processed using the ChemStation software to determine response areas for the analytes and internal standards. These areas are then used by a BASIC program written in-house to compute the analyte amounts in the samples (ng analyte per sample) based on point-to-point calibration. The point-to-point calibration method plots the relative response factors of the calibration standards versus their analyte areas. The linear equations generated between each pair of consecutive points are used for the calibration in order to gain a better fit over the entire range of calibration standards than would be provided by a single regression equation, such as linear, weighted linear, or quadratic regression.

For each level of calibration standard (CS), a relative response factor (RRF) is computed for each analyte relative to its internal standard (IS) as follows:

$$\text{CS RRF} = \frac{\text{(CS Analyte Concentration)}}{\text{(CS Analyte Area)}} \times \frac{\text{(CS IS Area)}}{\text{(CS IS Concentration)}} \tag{3}$$

The CH internal standard is PCB 103 for all analytes in Table 2; the AH internal standards are naphthalene-d8 for the analytes naphthalene through 2, 6-dimethylnaphthalene, acenaphthene-d10 for analytes acenaphthene through pyrene, and benzo[*a*]pyrene-d12 for analytes benz[*a*]anthracene through benzo[*ghi*]perylene as listed in Table 1.

For the analytes not contained in the calibration standards (i.e., retene and nonachlor III), the response factor of a similar analyte is used (i.e., 1-methylphenanthrene and *trans*-nonachlor, respectively).

Next, specific for each sample, a sample RRF for each analyte relative to its internal standard is computed by interpolating between the RRFs of the two consecutive calibration standards whose areas for that analyte bracket the area of the analyte in the sample as follows:

$$\text{Sample RRF} = \text{Lower CS RRF} + \frac{\text{(Sample Analyte Area – Lower CS Analyte Area)} \times \text{(Upper CS RRF – Lower CS RRF)}}{\text{(Upper CS Analyte Area – Lower CS Analyte Area)}} \tag{4}$$

where “Lower CS” is the calibration standard whose analyte area is closest to and below that of the sample’s analyte area and “Upper CS” is the calibration standard whose analyte area is closest to and above that of the sample’s analyte area.

The amount of each analyte in each sample (ng Analyte per Sample) is then calculated as follows:

$$\text{ng Analyte/Sample} = \text{Sample RRF} \times \text{Sample Analyte Area} \times \frac{\text{(Sample IS ng)}}{\text{(Sample IS Area)}} \qquad (5)$$

where "Sample IS ng" is the ng amount of internal standard added to the sample prior to extraction.

These analyte amounts for the samples are then used by a FileMaker Pro database to compute the analyte concentrations in the samples. Concentrations on a wet weight basis or a dry weight basis are calculated as follows:

$$\text{ng/g Wet Weight} = \frac{\text{ng Analyte per Sample}}{\text{Sample Weight}} \qquad (6)$$

and

$$\text{ng/g Dry Weight} = \frac{\text{ng Analyte per Sample} \times 100}{\text{Sample Weight} \times \text{\% Dry Weight}} \qquad (7)$$

where the "% Dry Weight" is determined as described in Subsection 8.1.

6.2. Calculation of Internal Standard Recoveries

The FileMaker Pro database uses the internal standard areas for the samples and calibration standards to compute the internal standard recoveries, which are calculated depending on the type of analyses, as follows:

- For CH analyses, for each sample, including the CH I-Std check solution, the percent recovery of the CH internal standard (PCB 103) is computed based on the CH HPLC internal standard (TCMX). Furthermore, the CH HPLC internal standard recovery is computed based on the CH GC internal standard (TCOX). For these calculations, the Level 5 GC calibration standard is used for the relative response factors of the internal standard.
- For AH analyses, for each sample, including the AH I-Std check solution, the AH internal standards (naphthalene-d8, acenaphthene-d10, and benzo[*a*]pyrene-d12) are computed based on the HPLC internal standard (phenanthrene-d10). Furthermore, the AH HPLC internal standard recovery is computed based on the AH GC internal standard (HMB). For these calculations, the Level 3 GC calibration standard in the case of analyses using SIM mode, or the Level 6 GC calibration standard in the case of analyses using scan mode, is used for the relative response factors of the internal standards.

The percent recovery of each internal standard (IS % Recovery) is calculated as follows:

$$\text{IS \% Recovery} = \text{CS IS RRF} \times \text{Sample IS Area} \times \frac{\text{(Sample HPLC IS ng)}}{\text{(Sample HPLC IS Area)}} \tag{8}$$

where

$$\text{CS IS RRF} = \frac{\text{(CS IS Concentration)}}{\text{(CS IS Area)}} \times \frac{\text{(CS HPLC IS Area)}}{\text{(CS HPLC IS Concentration)}} \tag{9}$$

and “Sample HPLC IS ng” is the ng amount of HPLC internal standard added to the sample.

Each HPLC internal standard percent recovery (HPLC IS % Recovery) is monitored for in-house quality assurance information and is calculated as follows:

$$\text{HPLC IS \% Recovery} = \text{CS HPLC IS RRF} \times \text{Sample HPLC IS Area} \times \frac{\text{(Sample GC IS ng)}}{\text{(Sample GC IS Area)}} \tag{10}$$

where

$$\text{CS HPLC IS RRF} = \frac{\text{(CS HPLC IS Concentration)}}{\text{(CS HPLC IS Area)}} \times \frac{\text{(CS GC IS Area)}}{\text{(CS GC IS Concentration)}} \tag{11}$$

and “Sample GC IS ng” is the ng amount of GC internal standard added to the sample.

6.3. Calculating and Reporting Lower Limits of Quantitation

The lower limit of quantitation for a given analyte in a given sample is the concentration that would be calculated if the analyte had a GC/MS response area equivalent to its area in the lowest level calibration standard used in the calibration. When an analyte is not detected in a sample or has an area that is smaller than its area in the lowest level calibration standard used, the concentration of the analyte in that sample is reported to be less than the value of its lower limit of quantitation.

6.4. Calculating and Reporting Estimated Concentrations for Analytes Exceeding the Calibration Range

When a given analyte in a given sample has a GC/MS area that is larger than its area in the highest level calibration standard used in the calibration, the analyte amount is calculated using the relative response factor of that analyte in the highest level calibration standard used. The concentration is footnoted as exceeding the calibration range and is therefore an estimate.

7. QUALITY ASSURANCE

The QA measures are incorporated into the analyses of each batch of samples according to the Environmental Assessment Program Quality Assurance Plan (NMFS unpubl. data). Specific quality assurance goals for batches and individual samples, as well as the percent of the total results that must meet the goals, depend on the project for which the samples are analyzed and the purpose of the data. Typical goals are presented here. When quality assurance goals are not met, the data may be qualified or the sample(s) reanalyzed.

7.1. GC/MS Stability

The stability of the GC/MS is evaluated using the repetitions of the mid-level GC calibration standard analyzed intermittently in the sequence of samples and other GC calibration standards. The GC/MS is considered stable if the response of an analyte relative to the response of its internal standard in a given repetition is within ±15% of the respective average for the repetitions.

7.2. Internal Standard Recoveries

The percent recoveries of internal standards in samples are considered acceptable if they are between 60% and 130%. The percent recoveries of internal standards in the I-Std check solutions are monitored for problems with the internal standard solutions.

7.3. Reference Material Analyte Concentrations

At least one sample of an appropriate NIST SRM is analyzed in each batch of samples to indicate the accuracy of the data for the entire batch. NIST provides certified concentrations and uncertainty values (for approximately 95% confidence) for many of the analytes in Tables 1 and 2. For the analytes having NIST certified concentrations, upper and lower control limits are set in accordance with those set by NIST in the Intercomparison Exercise Program for Organics in the Marine Environment. They are defined as follows:

$$\text{Upper Control Limit} = 1.3 \times (\text{Certified Concentration} + \text{Uncertainty Value}) \qquad (12)$$

and

$$\text{Lower Control Limit} = 0.7 \times (\text{Certified Concentration} - \text{Uncertainty Value}) \qquad (13)$$

The accuracy of the SRM result is considered acceptable if the determined concentration falls within the Upper and Lower Control Limits. This goal does not apply to analytes with concentrations below their limit of quantitation. The analysis of the SRM is acceptable if at least 70% of the analytes having NIST certified concentrations are within their control limits.

Analytes that do not have NIST certified concentrations, as a result, do not have QA goals for accuracy, but can be monitored and compared to NIST reference concentrations where available, or to previous in-house results.

7.4. Precision of Replicate Samples

When three or more replicate samples are analyzed, the precision of the results can be evaluated based on the percent relative standard deviation of the concentrations of each analyte. The precision is considered acceptable if the percent relative standard deviation is ≤15% for at least 90% of the analytes. For duplicate samples, this translates to a relative percent difference of ≤30% for at least 90% of the analytes.

7.5. Method Blank Contamination

A method blank is analyzed with each set of samples to monitor for contamination from laboratory sources. Ideally, analytes would not be detected in the method blank above their limit of quantitation. However, if analytes are measured in the method blank, their concentrations need to be compared to the concentrations in the sediment or tissue samples in the same batch. For each project, criteria need to be determined for contaminants in the method blank; for example, the method blank contamination might be considered acceptable if no more than five analytes are measured and the analyte concentrations in the method blank do not exceed two times the lower limit of quantitation. Also, analytes in tissue or sediment samples having concentrations between the lower limit of quantitation and four times the concentration in the method blank for the batch might be footnoted as such.

8. GRAVIMETRIC DETERMINATIONS

The volume static eliminator is used on the balances whenever weighing items, in order to reduce weighing errors caused by static charge.

8.1. Dry Weight Determination

The sample number is etched on the tab of a 43-mm i.d. aluminum weighing pan.

The pan is placed in a drying oven at 120°C overnight then cooled in a desiccator for 30 minutes.

The pan is weighed to the nearest 0.001 g, and the weight is recorded as the "Pan" weight.

The procedural steps are performed depending on the type of sample matrix as follows:

- For sediment samples, using a spatula, the sediment is stirred in its container until thoroughly homogenized. (Standing water was decanted from the sediment sample container and all pebbles, shells, biota, and other extraneous material were discarded previously in Section 3. No further decanting of excess water is done here so that the water content is the same as when the portion of sediment was taken for AH and CH analysis.) The sediment (2 ± 0.5 g) is placed into the pan, and the pan is weighed to the nearest 0.001 g. The weight is recorded as the "Pan w/Wet Sample" weight.
- For tissue samples, the pre-homogenized tissue (1 ± 0.5 g) is placed into the pan, and the pan is weighed to the nearest 0.001 g. The weight is recorded as the "Pan w/Wet Sample" weight.

The pan is placed in a drying oven at 120°C for 24 hours then cooled in a desiccator for 30 minutes.

The pan is weighed to the nearest 0.001 g, and the weight is recorded as the "Pan w/Dry Sample" weight.

The percent dry weight of the sample is determined as follows:

$$\%\ \text{Dry Weight} = \frac{(\text{Pan w/Dry Sample} - \text{Pan}) \times 100\%}{(\text{Pan w/Wet Sample} - \text{Pan})} \qquad (14)$$

8.2. Total Extractables Determination

The pan containing the sample for total extractables from Section 3 is placed on a covered rack in the hood and the solvent is allowed to completely evaporate (approximately 1–2 hours).

The pan is dried in a 50°C oven for 2 hours, then cooled in a desiccator overnight.

The pan is weighed to the nearest 0.0001g and the weight is recorded as the "Pan w/TE" weight.

The percent total extractables (% TE) content of the sample is calculated as follows:

$$\%\ \text{TE} = \frac{(\text{Pan w/TE} - \text{Pan}) \times (\text{ASE Vial w/Extract} - \text{ASE Vial}) \times 100\%}{(\text{ASE Vial w/Extract} - \text{ASE Vial w/o TE Extract}) \times \text{Sample Weight}} \qquad (15)$$

9. CONCLUSIONS

The analytical methods presented here result from research, development, and application by chemists in the Environmental Assessment Program. These methods are continuously upgraded for better accuracy, precision, sensitivity, robustness, safety, and cost effectiveness, as well as for minimizing contamination from laboratory materials and from sample-to-sample carry-over. Our productivity and data quality have increased due to the new and improved techniques and instrumentation described here. Quality assurance measures are also included, which are monitored and reported for every batch of samples. Our methods are provided here in detail for complete documentation as well as for ease of use by other laboratories.

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