# U.S. Army Corps of Engineers

New England District
Concord, Massachusetts

# QUALITY ASSURANCE PROJECT PLAN

## Volume III
## Appendices B, C, D

8 January 1999 (DCN: GEP2-123098-AAET)

Revised May 2003 (DCN: GE-022803-ABLZ)

## Environmental Remediation Contract
## General Electric (GE)/Housatonic River Project
## Pittsfield, Massachusetts

Contract No. DACW33-00-D-0006



WESTON SOLUTIONS

# QUALITY ASSURANCE PROJECT PLAN, FINAL (REVISED 2003)

# ENVIRONMENTAL REMEDIATION CONTRACT GENERAL ELECTRIC (GE) HOUSATONIC RIVER PROJECT PITTSFIELD, MASSACHUSETTS

## Volume III—Appendices B, C, and D

**8 January1999 (DCN: GEP2-123098-AAET)**
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Contract No. DACW33-00-D-0006

Prepared for

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# APPENDIX D

# STANDARD OPERATING PROCEDURES—TISSUE ANALYSES

# SOP-9727
# DETERMINATION OF PERCENT LIPID IN BIOLOGICAL TISSUE

Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURES SOP-9727

# DETERMINATION OF PERCENT LIPID IN BIOLOGICAL TISSUE

This document presents the procedures, materials, and quality control used in the performance of the above analysis.

Quality Assurance Manager

6/30/98
Date

Author/Revisions By: C. Veltman, C. Horan, Y. Qian

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# DETERMINATION OF PERCENT LIPID IN BIOLOGICAL TISSUE

## 1.0 PURPOSE

This document provides the procedures for the determination of percent lipid content in tissue samples which are used by staff of the Geochemical and Environmental Research Group (GERG) of the College of Geosciences at Texas A&M University.

### 1.1 SUMMARY OF METHOD

The percent lipid content (wt/wt basis) of a tissue is defined as the percent of material extracted from biological tissue with methylene chloride.

A tissue sample is extracted with methylene chloride in the presence of sodium sulfate. An aliquot of the extract is removed for lipid determination, filtered and concentrated to a known volume. A subsample is removed, the solvent is evaporated, the lipid residue weighed, and the % lipid content is determined.

### 1.2 APPLICABILITY

#### 1.2.1 Matrix

The procedures described in this SOP are applicable to all tissue types. Certain tissues with high lipid content (>40%) , such as blubber or turtle eggs may require special treatment.

#### 1.2.4 Reporting Units

The lipid content in the tissue extract is reported as a %, on a dry weight basis

## 2.0 SAFETY

2.1 The hazards, toxicity or carcinogenicity of each compound or reagent used in GERG's standard operating procedures have not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory maintains Material Safety Data Sheets (MSDS) which contain information regarding the safe handling of chemicals used at GERG's facilities. A reference file of MSDS is available to all personnel involved with these materials. All laboratory personnel should direct any questions regarding safety issues to their supervisors or the Safety Officer.

## 3.0 QUALITY CONTROL

3.1 Process all quality control samples in a manner identical to actual samples. Use Standard Laboratory Practice when filling out all paperwork. Use black waterproof ink. When correcting an entry, use one single line through the incorrect entry, date and initial change. Write a large letter Z through all the empty sample lines, date and initial.

3.2 Prepare a method blank with every set of 20 or less samples. The QC acceptance criteria for the lipid weight (in mg) of the method blank is ≤0.005 mg.

3.3 Prepare a duplicate with every set of 20 or less samples. Calculate the relative percent difference (RPD) for the % lipid values for the duplicates. The RPD should agree within ± 25%. If the RPDs are not within specifications, reweigh the sample extracts. If the RPDs still are not within specifications, the samples may need to be re-extracted for % lipid. Notify the supervisor before proceeding.

3.4 Note in the Comments section of the Laboratory Sample Lipid Logbook if anything out of the ordinary happened to the extract or if there are unusual sample/extract characteristics (i.e., cloudy, etc.). Note if all the lipid extract is used or if multiple weighing were performed.

## 4.0 APPARATUS AND MATERIALS

### 4.1 Glassware and Hardware

Electrobalance: Cahn Electrobalance or equivalent, with an accuracy of 0.001 mg, set on the 250 mg - 1 µg scale

Hot plate: set on low heat

Micropipette: 100 µL

Rotoevaporator: Brinkmann Rotovapor R110, or equivalent

Vortex mixer

Aluminum foil: heavy duty

Flat bottom flasks: 125 mL capacity, borosilicate glass

Funnels, powder: 65 cm, borosilicate glass

Glass fiber filters: 11.0 cm, Whatman GF/C or equivalent

Graduated cylinders: 25 mL and 500 mL capacity, borosilicate glass

Pasteur pipettes: 1 mL, disposable, glass

Syringe: 1 mL and 2.5 mL volumetric

Tweezers: stainless steel, anti-magnetic

Vials: 7 dram capacity, borosilicate glass, with Teflon-lined, solvent rinsed caps

As described in the GERG's SOP, all glassware is washed and then solvent rinsed or combusted at 440°C for 4 hours.

## 5.0 Consumable Materials

Sodium sulfate: granular, anhydrous, Sigma S6264 ACS reagent grade or equivalent, combusted at 440°C for 4 hours; stored at 120°C and cooled to room temperature in a desiccator before use.

Methylene chloride: Burdick and Jackson pesticide grade or equivalent, lot tested.

## 6.0 PROCEDURE

**6.1** Based upon the Laboratory Tissue Sample Logbook benchsheet, identify the sample extracts which require % lipid determination. Label 125 mL flasks with the Laboratory Lipid Sample Logbook benchsheet number, Preparation IDs, and the Sample IDs. Write "Lipid" on each 125 mL flask.

**6.1.1** Verify that the solvent level in each 500 mL flask containing the sample extracts has been marked with a solvent resistant pen prior to removing the lipid aliquot. Later, when all of the extract has been transferred from the 500 mL flask, this mark is used to measure the total extract volume (see Section 6.15).

**6.2** Mix the tissue extract by swirling and remove a 20 mL aliquot of the tissue extract using a 25 mL graduated cylinder. Pre-wet a glass fiber filter containing approximately 10 g of anhydrous sodium sulfate with methylene chloride and filter the 20 mL aliquot. Rinse the graduated cylinder three times with 3-5 mL of methylene chloride, pouring the rinses through the filter. Then rinse the filter funnel three times with 3-5 mL of methylene chloride.

**6.2.1** Prepare a method blank and a duplicate with every sample set. Use 20 mL of the extraction method blank, filtered as a sample, as the lipid method blank. Use 20 mL of the sample extracts selected for the extraction batch original/duplicate to serve as the original/duplicate required for this procedure. If no original/duplicate is included, use the extraction batch MS/MSD for the for the lipid duplicate QC required in this procedure.

6.2.2 Record the total number of mLs removed from each extract on the appropriate Laboratory Sample Lipid Logbook benchsheet. If more then one aliquot is used, write a note of explanation in the "Comment" section of the benchsheet.

6.2.3 Note any unusual extract characteristics (i.e., cloudy) on the appropriate benchsheet in the comment section.

6.3 Rotoevaporate the filtered aliquot to near dryness. Quantitatively transfer the residue with methylene chloride to a 7-dram graduated lipid vial, labeled with the Laboratory Sample Logbook benchsheet number, Preparation ID and sample ID. The vial cap is labeled with the Laboratory Sample Logbook benchsheet number and the Preparation ID. Place the vials in the hood and allow the solvent to evaporate to dryness by loosely capping the vials. Alternatively, the solvent may be evaporated under nitrogen.

6.4 Rinse a 1 mL syringe three times with methylene chloride. Accurately add 1.0 mL of methylene chloride to the dry lipid sample with the 1 mL syringe.

6.4.1 For samples known to have a high lipid content (such as egg, liver, oil, gonads, and blubber), if more than 0.5 g was extracted, it is necessary to adjust the final lipid volume to a total of 2 mL to completely dissolve the lipid. The final volume for these high lipid content extracts should be measured with a 2.5 mL syringe after the lipid dissolution.

6.4.2 If the final lipid volume is adjusted to other than 1.0 mL, use the measured value for the total vial volume in Equation 1.

6.5 Replace the cap and place the vial on the vortex mixer. The mixer should be set at 5 and the sample vortexed for 10 seconds. Let the vial sit for about 10 minutes while calibrating the micropipette and preparing for weighing (see Sections 6.6 and 6.7).

6.6 Calibrate the 100 µL micropipette according to the manufacturer's instructions. Initial and date the micropipette calibration logbook.

Calibrate the electrobalance with the authorized 200 mg weight according to the Calibration Logbook instructions. Initial and date the electrobalance calibration logbook, located near the instrument.

6.7 Always rinse the corrugated aluminum foil support with methylene chloride prior to use. Place it on the hot plate for about one minute to allow the with methylene chloride to evaporate. Using tweezers, place a small piece (~1 cm x 2 cm) of glass fiber filter on the corrugated aluminum foil support on the hot plate for at least 5 minutes. Transfer the filter to the electrobalance weighing pan and close the door. When the reading is stable, press TARE. Place the filter back on the hot plate.

6.8 Rinse a 100 µL micropipette at least 5 times with methylene chloride, using a separate container to collect methylene chloride after rinsing.

6.9 Vortex the lipid vial again for 10 seconds. If the lipid sample is not completely dissolved, note this in the comment section. Using the rinsed micropipette, remove 100 µL from the 1 or 2 mL lipid extract and slowly dot the extract onto the warm filter. Tightly cap the 7 dram vial. Rinse the micropipette again at least 5 times with methylene chloride before reusing or storing.

6.10 When the solvent has evaporated from the filter (wait at least 6 minutes), use tweezers to place the filter on the weighing pan and close the door. When the reading is stable, record the weight (in mg) in the "Lipid wt." column on the Laboratory Sample Lipid Logbook benchsheet.

6.11 For every fifth sample extract, remove the filter from the scale and replace it on the corrugated aluminum foil support the hot plate for about three more minutes. Place the filter on the weighing pan again and when the reading is stable, record the weight (in mg) in the "Lipid wt." column. If the second weight is within ± 0.02 mg of the first weight, use this weight to proceed with calculations.

6.11.1 If the weight change exceeds ± 0.02 mg, continue heating and weighing until a stable weight is achieved, using the final weight for calculations. In addition, it may be necessary to re-heat and reweigh all other lipid extracts in the same extraction batch if this

extract does not demonstrate a stable lipid weight after three weighing activities. Notify your supervisor if this occurs.

**6.11.2** In order to track multiple weighing (or weighing at a later date), it is recommended that a single line be drawn on the lipid label each time a 100 µL aliquot is removed.

**6.12** Locate the empty 500 mL flat-bottomed flasks in the laboratory for the appropriate sample set. Fill each flask with tap water to the previously marked line, and measure the volume with a 500 mL graduated cylinder. Record the volume (in mL) in the "Total Extract Volume" column on the Laboratory Sample Lipid Logbook benchsheet. Transfer the wet weight of each extracted sample from the Laboratory Sample Logbook benchsheet to the Laboratory Sample Lipid Logbook benchsheet under "Wet SMPL Wt (g)".

**6.13** Calculate the % lipids in the extract on a dry weight basis according to Equation 1. Date and initial the Lipid Calculations box on the Laboratory Sample Lipid Logbook benchsheet.

**6.14** Using Equation 2, calculate the RPD for the % lipid values for the original and duplicate samples (or MS/MSD). The RPDs should agree within ± 25%. If the RPD is not within ± 25%, reweigh the original and duplicate sample extracts. Note in the comments section of the Laboratory Sample Lipid Logbook benchsheet if multiple sample aliquots are weighed. If the RPDs still are not within specifications, all the samples in the extraction batch may need to be re-extracted. Notify the supervisor when this occurs before proceeding.

**6.15** Loosen the caps on the 7 dram vials, let the solvent evaporate, and re-tighten the caps. Place the vials in the appropriate labeled box and store in the designated location in the laboratory.

## 7.0 CALCULATIONS

Equation 1 % Lipid (dry weight basis)

$$\text{Dry Weight Sample} = \text{Wet Weight (g)} \times \frac{\%\ \text{dry}}{100}$$

$$\%\ \text{Lipid} = \frac{\text{lipid wt (mg)}}{\text{dry wt. sample (g)}} \times \frac{\text{total vial volume}}{0.1\ \text{mL}} \times \frac{\text{total ext. vol. (mL)}}{\text{aliquot vol. (mL)}} \frac{\text{g}}{1000\ \text{mg}} \times 100$$

$$\%\text{Lipid} = \frac{(\text{lipid wt.})(\text{total ext. vol.})}{(\text{sample dry wt.})(\text{lipid ext. vol.})}$$

Equation 2 Relative Percent Difference (RPD) for Duplicates

$$\%\text{RPD} = \frac{[\text{Duplicate 1 value} - \text{Duplicate 2 value}]}{[\text{Duplicate 1 value} + \text{Duplicate 2 value}]/2} \times 100$$

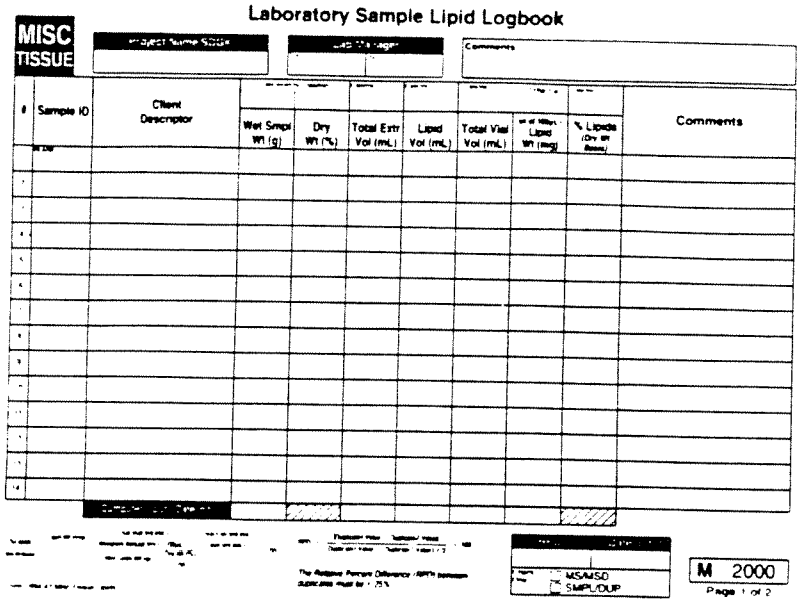
## 7.0 REPORTING AND PERFORMANCE CRITERIA

**7.1** Make one copy of the completed Lipid Logbook benchsheet and place it in the appropriate folder.

**7.2** Add the QC performance results for RPD to the control chart unless this is being done by data managerial portions of the laboratory.

## 8.0 EXAMPLE FORMS

**8.1** Lipid Logbook Benchsheet


Laboratory Sample Lipid Logbook

MISC TISSUE

| # | Sample ID | Client Descriptor | Wet Smpl Wt (g) | Dry Wt (%) | Total Extr Vol (mL) | Lipid Vol (mL) | Total Vial Vol (mL) | Lipid Wt (mg) | % Lipids | Comments |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | |

MS/MSD
SMPL/DUP

M 2000

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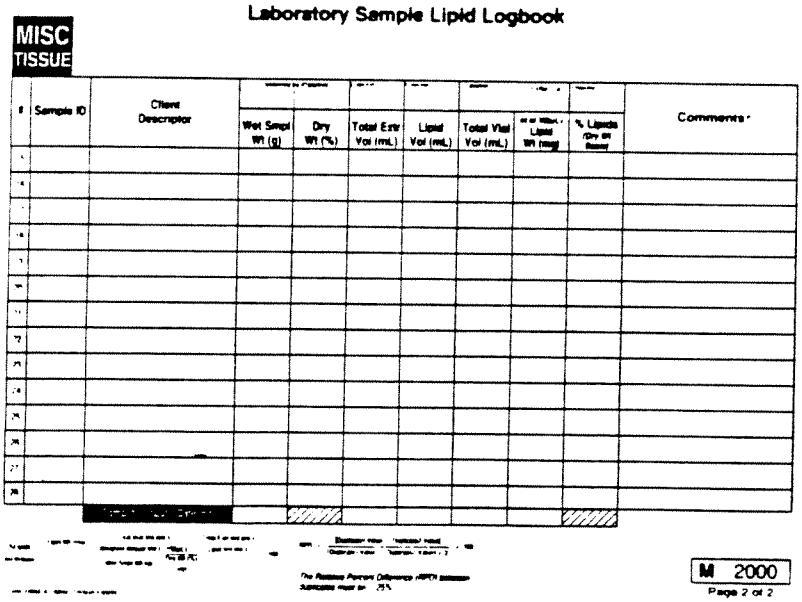
Laboratory Sample Lipid Logbook

MISC TISSUE

| # | Sample ID | Client Descriptor | Wet Smpl Wt (g) | Dry Wt (%) | Total Extr Vol (mL) | Lipid Vol (mL) | Total Vial Vol (mL) | Lipid Wt (mg) | % Lipids | Comments |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | |

M 2000

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Figure 8.1 Lipid Logbook Benchsheet.

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# SOP-9807
# EXTRACTION OF BIOLOGICAL TISSUES FOR THE ANALYSIS OF POLYNUCLEAR AROMATIC HYDROCARBONS, POLYCHLORINATED BIPHENYLS, AND ORGANOCHLORINE PESTICIDES

Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURES SOP-9807

# EXTRACTION OF BIOLOGICAL TISSUES FOR THE ANALYSIS OF POLYNUCLEAR AROMATIC HYDROCARBONS, POLYCHLORINATED BIPHENYLS, AND ORGANOCHLORINE PESTICIDES

This document presents the procedures, materials, and quality control used in the performance of the above preparation activities.

Grace E Ekman
Quality Assurance Manager

10/27/98
Date

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# EXTRACTION OF BIOLOGICAL TISSUES FOR THE ANALYSIS OF POLYNUCLEAR AROMATIC HYDROCARBONS, POLYCHLORINATED BIPHENYLS, AND ORGANOCHLORINE PESTICIDES

## 1.0 PURPOSE

This document describes the procedures used by the Geochemical and Environmental Research Group (GERG) of the College of Geosciences at Texas A&M University for the extraction of biological tissue samples prior to the analyses of polynuclear aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and organochlorine pesticides (OCs).

### 1.1 Summary of Method

Analysis of organic contaminants at trace level (parts per billion) in biological tissue matrices requires the extraction, isolation and purification of the target contaminants from their host matrices. This standard operating procedure provides a method to prepare tissue samples for quantitative determination of PAHs, PCBs, and OCs. Sample aliquots are extracted with methylene chloride using a Tissumizer. The extracts are then concentrated and purified with various chromatographic techniques prior to instrumental analysis.

### 1.2 Applicability

#### 1.2.1 Matrix and analytes

A variety of tissues and organisms can be extracted and processed using this extraction method. Some of these tissues include: livers, eggs, fish, crabs, clams, mussels, oysters and various other biological tissues. In addition to PAHs, PCBs, and OCs, other contaminants, such as organophosphorus pesticides, may be extracted by this method from tissue matrices.

#### 1.2.2 Interference

Method interferences that lead to false positives in instrumental analysis may be caused by lipid materials or other contaminants in solvents, reagents, glassware, and other sample processing hardware. Co-extracted biogenic materials that cause

interferences in the analysis of tissue extracts are removed prior to instrumental analysis by silica/alumina purification and gel permeation chromatography. All materials used in this method are routinely analyzed and demonstrated to be free from interferences by processing method blanks throughout the extraction and analytical procedures.

## 2.0 GENERAL SAFETY

The hazards, toxicity or carcinogenicity of each compound or reagent used in GERGs standard operating procedures have not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory maintains Material Safety Data Sheets (MSDS) which contain information regarding the safe handling of chemicals used at GERG's facilities. A reference file of MSDS is available to all personnel involved with these materials. All laboratory personnel should direct any questions regarding safety issues to their supervisors or the Safety Officer.

## 3.0 QUALITY CONTROL REQUIREMENTS

All QC acceptance criteria are evaluated within an analytical batch. Therefore, failure of one QC sample does not necessarily fail an entire analytical batch.

### 3.1 Surrogates

Surrogates are used to monitor method performance and are added to all samples prior to extraction. If the recovery of the surrogates in an analytical sample does not meet the QC acceptance criteria (see Instrumental SOP) and sufficient sample is available, the sample may be re-extracted. If there is not sufficient sample for re-extraction, data may be reported and properly qualified.

### 3.2 Matrix Spike

Matrix spike and/or matrix spike duplicate (MS/MSD) samples are routinely used with every 20 samples or with every sample set, whichever is more frequent. The spike recoveries of the MS/MSD are used to estimate analytical accuracy and precision in the presence of the matrix. The MS/MSD samples are spiked with known amount of target compounds and extracted in the same batch of other samples.

### 3.3 Method Blank

The method blank is used to determine if samples are contaminated during the extraction procedure. A method blank is prepared with every 20 samples or with every sample set, whichever is more frequent. If blank concentrations for any target component are above the QC acceptance criteria or if interference is present which affect the analysis or quantification of target compounds, samples included in that sample set should be re-extracted and reanalyzed. If insufficient sample is available for sample re-extraction, the data can be appropriately qualified and reported.

### 3.4 Standard Reference Material (SRM)

A certified Standard Reference Material (SRM) may be used with an extraction batch to monitor the accuracy of the analytical procedure. SRM 1941a from National Institute of Science and Technology (NIST) is currently used at GERG for the analyses of PAHs, PCB, and OCs in tissue samples.

### 3.5 Duplicate (DUP)

The duplicate (DUP) is a replicate sample that is used to evaluate sample homogeneity and analytical precision. A duplicate is prepared with every 20 sample or with every sample set when requested by the client. The QC acceptance criteria are provided in the instrumental SOPs.

### 3.6 Miscellaneous QC samples

Various other QC samples can be analyzed when requested by the client. These include the laboratory blank spike (LBS) and laboratory blank spike duplicate (LBSD) which may be used when there is either limited sample or a difficult matrix.

## 4.0 APPARATUS AND MATERIALS

### 4.1 Glassware and Hardware

All glassware is cleaned according to GERG SOP. In general, glassware is washed with micro detergent and rinsed with tap water. After air drying, glassware is wrapped with aluminum foil and "combusted" in a muffle furnace at 440°C for 4 hours.

Glassware is stored in a clean environment to prevent the accumulation of dust or other contaminants.

After initial micro detergent washing and tap water rinsing, solvent rinses with methanol followed by methylene chloride may substitute the muffle furnace heating for glassware when determined appropriate by the analyst. Solvent rinsing should always be used for "non-combustible" glassware.

The following materials are needed to perform tissue extraction activities.

Centrifuge Bottles: 200 mL, Pyrex, Kimax or equivalent.

Micro Pipettes: 20 µL, 50 µL, 100 µL, and 250 µL.

Disposable 1 mL Pasteur Pipettes.

Large Glass Funnels, 80 mm.

Flat Bottom Flasks: 125, 250 and 500 mL capacity, Kimax, Pyrex, or equivalent.

Snyder Column: 3-ball column.

Kuderna-Danish Concentrating Tubes: 25 mL graduated; Reliance Glass Works (RGW) or equivalent.

Chromatographic Column: 300 mm long x 10 mm ID column with Teflon stopcock, RGW or equivalent.

Sample Vials: 2 mL pre-cleaned amber vials with Teflon lined screw caps.

Labeling tapes

Tweezers, scissors, and a metal scoops.

Graduated Cylinders: 25 mL and 500 mL.

Assorted Beakers

### 4.2 Instrumentation

Water Bath: LAB-LINE Aquabath Model R18010 or equivalent.

Tissumizer: Pro Scientific Model PRO250 or equivalent.

Balance: Sartorius Excellence Model E2000D or equivalent.

Electrobalance: Capable of measuring to 0.001 mg, Cahn or equivalent.

Nitrogen Evaporation Unit

Centrifuge: Beckman Model TJ-6 or equivalent.

Dessicator: Portable glass dessicator, containing silica gel desiccant.

Rotary-evaporator: Buchi, model R111 or equivalent, equipped a low temperature water recirculator.

## 5.0 REAGENTS AND CONSUMABLE MATERIALS

### 5.1 Reagents

Reagent Water: HPLC Grade water; B&J; Cat. 365-4 or equivalent.

Sodium Sulfate: ACS Grade Granular, anhydrous. Combusted at 440°C for 4 hours in a beaker covered with aluminum foil. This sodium sulfate is stored at 130°C until ready to use. At time of use, remove from the oven and let cool in a glass dessicator.

**Solvents**: All solvents are pesticide grade quality or better.

Methanol: B&J; Cat. 230-4 or equivalent.
Methylene Chloride: B&J; Cat. 300-4, or equivalent.
Pentane: B&J; Cat. 312-4 or equivalent.
Hexane: B&J; Cat. GC60393-4 or equivalent.

### 5.2 Analytical Standards

Preparation of the surrogate spiking solution, matrix spike solutions, and the GC internal standard solution is provided in the GERG SOP for preparation of analytical standards.

### 5.3 Miscellaneous Material

**Teflon Boiling Chips**: Solvent rinsed. Rinse the chips in a beaker three times with methanol then three times with methylene chloride before use. Drain the solvent rinses into an appropriate waste container after use.

**Glass Wool**: Combust at 440°C for four hours in a covered beaker.

**Latex Gloves**: Various sizes, powder free

## 6.0 EXTRACTION PROCEDURES

### 6.1 Tissumizer Preparation

**6.1.1** Each probe must be disassembled and washed with micro detergent and water thoroughly before and after use. Once the probe is properly cleaned and assembled, attach it to the Tissumizer and sequentially rinse the probe by turning on the Tissumizer and rinsing the probe with water, methanol, and methylene chloride in 200 mL centrifuge bottles.

**6.1.2** If the last methylene chloride rinse turns cloudy, rinse again with methanol, then methylene chloride. The cloudiness is an indication of water remaining within the probe.

### 6.2 Percent Dry Weight Determination

**6.2.1** Remove a separate 0.5-1 gram aliquot of the homogenized tissue composite. Place it in a tared weighing container, and weigh. Dry the tissue for at least 24 hours at 65°C, allow it to cool in a desiccator, and then re-weigh. Record the weight.

6.2.2 Reheat the sample to 65°C for at least 2 hours, again cool in a desiccator for 30 minutes to room temperature, and weigh. Record the weight.

6.2.3 If the difference between the first and second weight of the dried sample is less than 0.02 grams, calculate the % dry weight according to GERG's SOP for % dry weight determination, using the second weight. If the difference is greater than 0.02 grams, continue the heating, cooling and weighing process until the difference in the last two weights is less than 0.02 grams, and calculate the % dry weight based on the last weight.

## 6.3 Extraction Setup and Weighing

6.3.1 Weigh an aliquot of homogenized tissue samples into labeled 200 mL centrifuge bottles.

6.3.1.1 Prepare a method blank in a 200 mL centrifuge bottle, which has no sample but does have all other reagent, solvent and preparatory activities performed upon it.

6.3.2 Based upon the information on the Organic Analysis Request Form, add appropriate amount of the spiking solutions to the appropriate QC sample containers (e.g., LBS, MS/MSD, etc.). A spike witness must observe this activity and initial the benchsheet.

6.3.3 Based upon the information on Organic Analysis Request Form, add appropriate amount of surrogate standards to each sample, including all QC samples. A spike witness must observe this activity and initial the benchsheet.

6.3.4 Add 100 mL of methylene chloride to each sample.

6.3.5 Add 40 mL of combusted, room temperature sodium sulfate to each sample immediately before tissumizing.

6.3.6 Macerate the tissue with tissumizer for three minutes. Make sure the sodium sulfate and the sample are free flowing in the bottle.

6.3.7 Place a large funnel on top of a labeled 500 mL flat bottom flask. Plug the stem with glass wool and add about 2 inches of room temperature sodium sulfate. Wet the sodium sulfate with methylene chloride.

6.3.8 Decant the methylene chloride extract from the macerated sample into the funnel in the labeled 500 mL flat bottom flask.

6.3.9 Add another 100 mL methylene chloride to the centrifuge bottle and repeat steps 6.3.6-6.3.9 two more times (a total of three methylene chloride extractions).

6.3.10 Rinse the centrifuge bottle with 10-15 mL methylene chloride and decant the rinsate into the funnel. Rinse the sodium sulfate in the funnel with methylene chloride.

## 6.4 Determination of % Lipids

6.4.1 After the sample has been completely extracted and filtered into the 500 mL flask, draw a line to mark the volume of the extract. This volume is later determined for use in the calculation of the % lipid content.

6.4.2 Measure out approximately 20 mL of extract into a 25 mL graduated cylinder. Record the actual volume removed for lipid determination in the Lipid Logbook.

6.4.3 Place a funnel with glass filter paper on top of a labeled 125 mL flat bottom flask.

6.4.4 Add combusted, room temperature sodium sulfate to the funnel and pour the 20 mL of extract into it.

6.4.5 Rinse the graduated cylinder three times with methylene chloride and pour the rinsate into the funnel.

6.4.6 Put a ground glass stopper on the 125 mL flask.

6.4.7 Refer to GERG's SOP to complete the % lipid determination procedure. Determine the RPD and verify that it meets QC acceptance criteria.

## 6.5 Extract Concentration and solvent exchange

6.5.1 Add 2-4 solvent cleaned Teflon boiling chips to the remaining sample extract in the 500 mL flask.

6.5.2 Place a three ball Snyder column on top of the 500 mL flask.

6.5.3 Place the flask on a hot water bath (55-65°C) and concentrate the extract to about 10-15 mL.

6.5.4 Take the flask off the water bath, cool, and rinse down the Snyder column with methylene chloride.

6.5.6 Transfer the concentrated extract to a Kuderna-Danish (K-D) concentrating tube by decanting. Rinse the flask three times with methylene chloride and add the rinsate to the concentrating tubes.

6.5.7 Add 1-2 pieces of cleaned boiling chips to the K-D concentrating tube and place the concentrating tube on a water bath (55-65°C).

Concentrate the extract to about 2 mL, gradually adding small quantities of hexane. Let the extract boil until the extract is completely in 2 mL of hexane and all the methylene chloride is gone. The extract will gradually stop boiling by this time and the top of the K-D concentrating tube becomes opaque.

6.5.8 After the methylene chloride is completely boiled off, take the concentrator tubes off the water bath and cap the concentrator tube with a glass stopper.

The sample extracts are now ready for further purification.

## 7.0 DOCUMENTATION

All of the following documents will be included in a manila folder with the date, sample logbook page number, sample information. Proper documentation for a set of samples leaving the extraction laboratory includes:

7.1 A copy of the extraction logbook benchsheet

7.2 Chain-of-custody transfer form

7.3 Organic Analysis Request Form

7.4 Sample Information Sheets

7.5 Copy of Dry Weight Logbook Page

7.6 Copy of % Lipid Logbook Page

# SOP-9808
# SEPARATION OF POLYCHLORINATED BIPHENYLS FROM ORGANOCHLORINE PESTICIDES BY SILICA GEL COLUMN CHROMATOGRAPHY

Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURE SOP-9808

# SEPARATION OF POLYCHLORINATED BIPHENYLS FROM ORGANOCHLORINE PESTICIDES BY SILICA GEL COLUMN CHROMATOGRAPHY

This document presents the procedures, materials, and quality control used in the performance of the above preparation activities.

Susie E Ekman
Quality Assurance Manager

10/27/98
Date

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# SEPARATION OF POLYCHLORINATED BIPHENYLS FROM ORGANOCHLORINE PESTICIDES BY SILICA GEL COLUMN CHROMATOGRAPHY

## 1.0 PURPOSE

This document provides the procedures that may be used for extract purification prior to analysis for polychlorinated biphenyls (PCBs), organochlorine pesticides (OCs), including toxaphene, which are used by the Geochemical and Environmental Research Group (GERG) of the College of Geosciences at Texas A&M University.

### 1.1 Summary

Congener specific PCB measurement and organochlorine pesticide determination in the extracts of tissue, sediments, and other types of matrices obtained by organic solvent extraction may contain interfering materials. These non-desirable compounds in the extract may prevent accurate identification and quantification of the target compounds at trace level (sub parts per billion) in an extract. Silica gel column chromatography is used to remove such interfering materials.

The extract purification steps using silica column chromatography described in this standard operating procedure (SOP) will isolate PCBs from OCs and/or toxaphene in the concentrated sample extract. The fraction containing the desired target compounds is collected and concentrated prior to analysis. Additional purification by charcoal column chromatography may be required to further isolate selected PCB congeners.

### 1.2 Application

Analysis of extracts for PCB congeners, OCs, and/or analysis of toxaphenes may require the use of these procedures. In addition, these procedures may be applicable to other types of analysis, such as aliphatic hydrocarbons, upon verification. Optimal results may be achieved by combining the procedures described in this SOP with other types of purification procedures, such as gel permeation chromatography or silica/alumina column chromatography.

## 2.0 SAFETY

The hazards, toxicity or carcinogenicity of each compound or reagent used in GERG's standard operating procedures have not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory maintains Material Safety Data Sheets (MSDS) which contain information regarding the safe handling of chemicals used at GERG's facilities. A reference file of MSDS is available to all personnel involved with these materials. All laboratory personnel should direct any questions regarding safety issues to their supervisors or the Safety Officer.

## 3.0 QUALITY CONTROL

3.1 Process QC extracts (Blank, Matrix Spike, Duplicate, etc.) in an identical manner as the other extracts.

3.2 Note in the Comments section of the Laboratory Sample Logbook if anything out of the ordinary occurred to the extract or if there are unusual extract characteristics (cloudy, precipitate present, etc.).

3.3 Use Standard Laboratory Practice when filling out all paperwork. Use black waterproof ink. When correcting an entry, use one single line through the incorrect entry, date and initial change. Write a large Z through all the empty sample lines, date and initial.

3.4 Observe good, clean, and safe laboratory practice.

## 4.0 APPARATUS AND MATERIALS

### 4.1 Glassware and Hardware

The following laboratory glassware and hardware are needed to perform the sample purification and separation using silica column chromatography. All glassware is pre-cleaned according to the GERG SOP.

**Balance**: Top loading with an accuracy of 0.001 gram

**Beakers**: 40 mL and 300 mL capacity, borosilicate glass

**Chromatographic Columns**: 30 cm x 13 mm (i.d.) with 250 mL reservoir and Teflon stopcock.

**Desiccator** with desiccant.

**Flat Bottom Flask**: 250 mL, combusted at 440°C for 4 hours

**Funnels**: Borosilicate glass, assorted sizes

**Glass rod**: 40 cm x 0.7 cm

**Pyrex Glass Wool**: Combusted at 440°C for 4 hours.

**Graduated Cylinders**: 250 mL and 2000 mL capacity.

**Muffle Furnace**: Temperature programmable up to 900°C

**Oven**: Temperature programmable up to 200°C

**Pasteur pipettes:** 1 mL, disposable, combusted at 440°C for 4 hours

**Rolling flask:** 1000 mL capacity with interior studs to facilitate mixing

**Roller-Table:** Lortone, Inc., Model NF-1 or equivalent

**Spatula**: Stainless steel with a scoop at one end

**Stoppers**: Ground glass, 24/40 and 19/22, borosilicate glass

**Forceps**: Stainless steel

### 4.2 Reagents and Consumable Materials

**Anhydrous Sodium Sulfate**: J.T. Baker; Cat. #3891-05; reagent grade or equivalent; granular powder. Combusted at 440°C for 4 hours and stored at 130°C

**Chromatographic Silica Gel**: Aldrich; Cat. # 21,447-7 or equivalent; Silica gel, grade 923, 100-200 mesh; activated at 170°C for at least 24 hours and stored at 170°C

**Purified water**: HPLC grade or equivalent; Mallinkrodt; Cat. #6795.

**Sand**: White quartz, -50 to +70 mesh; Sigma, Cat. #S-9887; combusted at 440°C for 4 hours.

**Silica Gel**: Desiccating, indicating 16-18 mesh; J.T. Baker; Cat. #3401-05.

**Solvents**: All solvents except hexane are pesticide grade or equivalent.

Methylene chloride; Burdick & Jackson; Cat. #300-4..
Methanol; Burdick & Jackson; Cat. #230-4.
Pentane; Burdick & Jackson; Cat. #312-4.
Hexane; Burdick & Jackson; Cat. GC60393-4; GC/GC-MS grade or equivalent.

## 5.0 PROCEDURE FOR PREPARING COLUMNS

### 5.1 Documentation Required

Obtain a copy of the Organic Analysis Request Form and check the Laboratory Logbook Benchsheet (QC batch) for the extracts to be purified. Verify that the QC batch of extracts require silica column separation.

### 5.2 Preparation for Column Setup

**5.2.1** Remove the sodium sulfate and silica gel from their respective storage oven and place them in a desiccator to cool to room temperature.

**5.2.2** Remove combusted sand from the 130°C oven and let it sit on the bench to cool to room temperature.

**Note**: When taking out materials from the oven, wear heat protective gloves or use large forceps capable of handling heavy objects. The beakers containing sand, silica, and sodium sulfate are heavy (up to 1000 grams),

and are hot. Make sure all the beakers are properly covered with aluminum foil.

### 5.2.3 Preparation of Deactivated Silica

**5.2.3.1** Clean and calibrate the balance prior to use according to the instructions in the balance calibration logbook.

**5.2.3.2** Place an empty mixing flask and funnel on the balance and tare the weight until the balance reading is 0.000 g.

**5.2.3.3** Weigh the silica into the flask. The total amount of silica weighed is calculated as the number of samples (including QC samples) x 20 grams. Generally, extra silica is weighed (at least 20 g) in case additional adsorbent is needed when building columns.

**5.2.3.4** Deactivate the silica by adding 2% (w/w) of HPLC water to the silica flask while the flask is still sitting on the balance.

The amount of water added is calculated by multiplying the total weight of silica x 0.02 (g). For example, if 171.2 g of silica is in the flask, 3.4 g of water (171.2 x 0.02 = 3.4) is added to the flask. Tare the weight of the silica, then add the calculated amount of water to the flask.

**5.2.3.5** Clamp two mixing flasks together at the joint and place them on the roller table. Turn on the roller table and let the chemical mixes for one hour to thoroughly mix and deactivate the silica.

### 5.2.4 Preparation of Pentane/Methylene Chloride Solutions

**5.2.4.1** The pentane/methylene chloride (50/50, v/v) solution is prepared by mixing 2 L pentane with 2 L methylene chloride. Use a 4 L labeled mixing bottle and a large graduated cylinder to measure the solvents.

**5.2.4.2** Measure 2 L pentane in the graduated cylinder and pour the pentane into the mixing bottle.

**5.2.4.3** Using the graduated cylinder, measure 2 L methylene chloride and add it to the mixing bottle. Seal the mixing bottle and mix the solvents by shaking the bottle. Shake again immediately prior to use.

**5.2.4.4** The 2% (v/v) methylene chloride in pentane is prepared by adding 80 mL of methylene chloride to 4 L of pentane. After adding the methylene chloride to the pentane bottle, seal and mix the solvent by shaking.

## 5.3 Column Setup

**5.3.1** Clamp the glass chromatography columns (30 cm x 13 mm with a Teflon stopcock and a 250 mL reservoir) onto the column rack located in the hood. The number of columns should be the same as the number of extracts.

**5.3.2** Put a 250 mL waste jar under each column to collect waste solvent.

**5.3.3** Open the stopcock. Using labeled squeeze bottles, rinse the columns three times with approximately 5 mL of methanol each time followed by three 5 mL of rinses using methylene chloride. Make sure that the rinsing solvents completely coat and rinse the inside of the column.

**5.3.4** Rinse the tips of a pair of forceps and scissors with methylene chloride. Cut a plug of glass wool with the scissors. Place the glass wool into the column using the forceps. Rinse a glass rod with methylene chloride and pack the glass wool into the bottom of the column with the glass rod.

**5.3.5** Close the stopcock, fill the column with approximately 30 mL of methylene chloride and place a funnel on top of the column.

5.3.6 Add about 2 cm of combusted sand (about 1-1/2 metal scoop) to each column through the funnel using a stainless steel scoop pre-rinsed with methylene chloride.

5.3.7 Place a 40 mL beaker on a clean calibrated balance. Tare the weight.

5.3.8 Weigh 20 g (±0.1 g) of deactivated silica.

5.3.9 Add methylene chloride to the silica in the weighing beaker. Mix well with a glass rod or stainless steel spatula to make a slurry, making sure no visible air bubbles are present.

5.3.10 Pour the silica into the column through the funnel. Rinse the funnel and column reservoir with methylene chloride, making sure all of the silica settles into the bottom of the column.

5.3.11 Add approximately 2 cm of sodium sulfate to the column using a stainless steel scoop (about 1 and one-half scoops). If necessary, rinse the column reservoir with methylene chloride to make sure all the sodium sulfate settles onto the column.

5.3.12 Open the stopcock and drain the solvent to the surface of sodium sulfate.

5.3.13 Add 50 mL of pentane and drain the pentane to the surface of sodium sulfate. Close the stopcock.

## 5.4 Extract Separation Using Silica Column

5.4.1 Rinse the outside of the column tip (below the stopcock) into the waste jar using methylene chloride.

5.4.2 Replace the waste collecting jar with a 250 mL flat bottom flask. Discard solvent waste into appropriate waste container. Each flask should be appropriately labeled with extraction QC batch

number, fraction type (PCB or OC), and extract ID number. The first flask is used to collect PCB fraction.

**5.4.3** Transfer the extracts from concentrating tubes to the column using disposable pipettes. The extracts should already be concentrated and solvent exchanged into about 1-2 mL of hexane.

**5.4.4** Open the stopcock and let the extract drain to the top surface of the column.

**5.4.5** Close the stopcock.

**5.4.6** Rinse the extract concentrating tube using 1 mL of pentane and add the rinsing solution into the column with the same disposable pipettes.

**5.4.7** Open the stopcock. Let the extract rinse drain to the surface of the column.

**5.4.8** Close the stopcock. Repeat Steps 5.4.6 through 5.4.8 with two additional pentane rinses of the concentrating tube.

**5.4.9** Add 150 mL of 2% methylene chloride/pentane solution to each column using a glass funnel. Pour the solvent slowly so that the surface of the column is not disturbed.

**5.4.10** Open the stopcock and drain at a flow rate of 1 mL/min. The flow rate is controlled by adjusting the stopcock. Collect the 2% methylene chloride/pentane fraction in the 250 mL flat bottom flask labeled as the PCB fraction. Do not let the column run dry.

**5.4.11** After all of the 150 mL of 2% methylene chloride/pentane has drained to the top of the column and is collected in the 250 mL flat bottom flask, close the stopcock. Add 120 mL of pentane/methylene chloride (50/50) solution to the column using a graduated cylinder. Replace the collection flask with another flask labeled as OC fraction. Collect the 120 mL of

pentane/methylene chloride solution at a flow rate of about 1 mL/min. Close the stopcock when the column runs dry.

**5.4.12** Remove the collecting flask and cap it with a glass stopper. The collected extracts are then transferred to the laboratory area where the extract concentration step can be performed.

**5.4.13** Sign the Laboratory Sample Logbook in the appropriate place with date and analyst's initial.

**5.4.14** Dismantle the column by pouring out the silica from the column into a specified chemical waste container. Remove the glass wool plug from the column and discard the glass wool. Place the glass column in the cleaning area so that the column can be cleaned according to GERG SOP.

## 6.0 DOCUMENTATION

All of the following documents will be included in a manila folder with the date, sample logbook page number, sample information. Proper documentation for a set of samples leaving the extraction laboratory includes:

**6.1** A copy of the extraction logbook benchsheet

**6.2** Chain-of-custody transfer form

**6.3** Organic Analysis Request Form

**6.4** Sample Information Sheets

**6.5** Copy of Dry Weight Logbook Page

**6.6** Copy of % Lipid Logbook Page

# SOP-9810
# QUANTITATIVE DETERMINATION OF CHLORINATED HYDROCARBONS BY GAS CHROMATOGRAPHY/ELECTRON CAPTURE DETECTION

Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURES SOP-9810

# QUANTITATIVE DETERMINATION OF CHLORINATED HYDROCARBONS BY GAS CHROMATOGRAPHY/ELECTRON CAPTURE DETECTION

This document presents the procedures used in the performance of the above analytical procedures.

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# QUANTITATIVE DETERMINATION OF CHLORINATED HYDROCARBONS BY GAS CHROMATOGRAPHY/ELECTRON CAPTURE DETECTION

## 1.0 PURPOSE

This document provides the procedures used by the staff of the Geochemical and Environmental Research Group (GERG) of the College of Geosciences at Texas A&M University for the quantitative determination of chlorinated hydrocarbons in sample extracts using gas chromatography/electron capture detection (GC-ECD).

### 1.1 Applicability

The instrumental procedures described in this document are applicable to the quantitative analysis of extracts obtained from water, sediment, soil, tissue, and other sample matrices after appropriate extraction and purification.

A wide variety of chlorinated hydrocarbons can be determined with the GC-ECD by using specific extraction and purification procedures with appropriate surrogates and/or internal standards, specialized calibration standards, and particular chromatographic method programs. Current analytical efforts include simultaneous determination of various organochlorine pesticides (OCs) and polychlorinated biphenyls (PCBs) as well as completely separate analysis of these analytes. With minor modifications, these analyses may include specific additional analytes such as terphenyls or toxaphene. Analysis of specific planar PCBs can also be performed. Using the appropriate standards, other organochlorines not identified in this SOP having similar extraction and chromatographic properties could also be determined by this instrumental technique.

### 1.2 Target Analyte List

The chlorinated hydrocarbons routinely determined by this method and the surrogates used for quantitation (reference surrogates) are listed in Table 1.

### 1.3 Detection Limits

The analytical method detection limit (MDL) for routine analytes can be determined following procedures outlined in 40CFR, Part 136; Appendix B; 198-199,

(1984). For specialized analyses, a lower concentration level (LCL) may be used. The LCL represents the concentration level of the lowest calibration standard used in the analysis.

## 2.0 SAFETY

The hazards, toxicity or carcinogenicity of each compound or reagent used in GERG's standard operating procedures have not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory maintains (MSDS) which contain information regarding the safe handling of chemicals used at GERG's facilities. A reference file of MSDS is available to all personnel involved with these materials. All laboratory personnel should direct any questions regarding safety issues to their supervisors or the Safety Officer.

## 3.0 QUALITY CONTROL

The analytical quality control requirements for quantitative analysis are summarized in Table 2 with details provided in the following sections.

### 3.1 Analyte Identification Criteria

#### 3.1.1 Retention Times

The retention time of target compounds in the sample must fall within ± 4 seconds of the authentic compound in the calibration standards.

#### 3.1.2 Qualitative Identification

Qualitative identification of target compounds is based on a comparison of the retention times of the target compounds contained in the calibration standards with the target compound found in the sample extract. The retention time of the peak should be within ± 4 seconds of the average retention time for the authentic compound found in the calibration standards. The experienced analyst may use manual peak selection and baseline correction when appropriate.

## 3.2 Instrumental QC Criteria

### 3.2.1 Calibration Criteria

Calibration of the instrument for the analysis of chlorinated hydrocarbons is done as part of the analytical run. The four calibration standard mixtures are interspersed with actual samples during the GC/ECD analyses. The calibration curve is then based on these four standards. If the calibration curve has an r of 0.9950 or higher (i.e., $R^2$ of 0.9900 or higher) for all analytes present in the samples it is accepted, if not the calibration standards as well as all the samples must be reanalyzed by GC/ECD. This procedure is superior to the procedure where the instrument is initially calibrated at four points and then mid-level standards are run during the analytical run. This latter calibration only insures that mid-level samples remain in calibration. Since the ECD detector is nonlinear, a one-point check on its calibration is not as rigorous as calibration during the GC/ECD run.

The recommended standard concentrations for OC/PCB analysis are approximately 5.0, 20.0, 80.0, and 200.0 ng/mL. These concentrations must be adjusted to satisfy the requirements of different analyses, e.g., aroclor, terphenyls, or planar PCBs. The analytical calibration standard preparation is provided in the appropriate GERG SOP. The calibration standards include all parent target compounds indicated in Table 1, but do not include all analytes which can be identified and quantified by this analysis. The instrument software generates and prints out calibration data for each instrument, which are included in the report folder for each analytical activity.

### 3.2.2 Solvent Blank

A solvent blank is injected and analyzed prior to every analytical sequence to verify instrument operation and lack of contamination.

### 3.2.3 Degradation Check Solution

Prior to every analytical sequence, a solution containing selected chlorinated hydrocarbons (e.g., DDT and aldrin) that are known to degrade in the injection port under certain conditions is used to verify the "inertness" of the instrument. Degradation of these compounds in the injection port should be no higher than 15% in order to continue with the analytical run. If degradation is higher than 15%, the analytical run is stopped and the injection port cleaned.

### 3.3 Criteria for QC Samples in an Analytical Batch

The acceptance criteria for QC samples are evaluated within an analytical group (QC batch or extraction batch). Therefore, failure of one QC sample type does not necessarily cause the entire QC batch to fail.

#### 3.3.1 Method Blank (BLANK)

A Method Blank is used to demonstrate freedom from contamination in the analytical procedure, and is required for each QC batch of 20 or fewer samples.

**3.3.1.1** An acceptable method blank contains no more than two target compounds at concentrations greater than three times the corresponding MDL (or LCL). If more than two of the target compounds are found in the blank at concentrations greater than three times the corresponding MDLs, re-extraction of the entire QC batch may be required as specified in the following subsections.

**3.3.1.2** If any of the target compounds are found in the blank at greater than three times the MDL, but are not detected in the analytical samples above the MDL, the reported analytical data must be flagged, but no further action is required.

**3.3.1.3** When target compounds are present in the method blank and in the analytical samples at concentrations above three times the MDL and the concentration in the sample(s) is ten times that found in the blank, the blank must be flagged but sample data can be reported and are not flagged.

**3.3.1.4** When target compounds are present in the method blank and in analytical samples at concentrations above three times the MDL and the concentration in the samples is less than ten times that found in the blank,

the QC batch should be re-extracted and re-analyzed. If no sample remains for re-extraction, the analytical data for those target compounds in the blank and samples can be reported but must be flagged.

### 3.3.2 Laboratory Blank Spike (LBS) and Laboratory Blank Spike Duplicate (LBSD)

A Laboratory Blank Spike (LBS) may be used to estimate analytical accuracy of the method if inadequate sample is available. A laboratory blank spike may be used to demonstrate that the analytical system is in control when working with a difficult matrix or sample set. It may be required with each QC batch of 20 or fewer samples.

**3.3.2.1** A Laboratory Blank Spike Duplicate (LBSD) is used to estimate both analytical accuracy and precision and may be required for each QC batch of 20 or fewer samples.

**3.3.2.2** QC acceptance criteria for the LBS or LBSD target compound recoveries are that 80% of the target compounds should have recoveries between 40 and 130% of the spike amount.

**3.3.2.3** If the LBSD has been included, the recoveries determined from the LBS and LBSD should agree within a Relative Percent Difference (RPD) of $\leq$30%.

**3.3.2.4** If 20% or more target compounds are outside this criterion, corrective action may include recalculation and/or reanalysis, re-extraction of the sample group, and instrument maintenance or re-calibration.

### 3.3.3 Matrix Spike (MS) and Matrix Spike Duplicate (MSD)

A Matrix Spike (MS) sample is used to estimate analytical accuracy in the presence of a representative matrix and is normally required for each QC batch of 20 or fewer samples.

A Matrix Spike Duplicate (MSD) is used to estimate analytical accuracy and precision in the presence of a representative matrix and may be required for each QC batch of 20 or fewer samples.

**3.3.3.1** The, QC acceptance criteria requires that 80% of the target compound recoveries in the MS and the MSD be between 40 and 130% of the spiked amount. In computing the recovery, only valid spikes will be used. In a valid spike, the amount of target compound added is at least as much as was originally present in the sample, or the spike amount causes a 50% increase in the instrument response for an analyte.

**3.3.3.2** If a Matrix Spike Duplicate (MSD) has been included with the analytical batch, the recoveries determined from the MS and MSD samples should agree within a Relative Percent Difference (RPD) of $\leq$30%.

**3.3.3.3** If 20% or more of the target compounds are outside the QC acceptance criteria, corrective action may be indicated. Corrective action may include recalculation and/or reanalysis of the MS and MSD; re-extraction of the sample/MS/MSD group; or instrument maintenance and/or re-calibration followed by reanalysis.

### 3.3.4 Duplicate (DUP)

A sample Duplicate (DUP) is used to demonstrate sample homogeneity and analytical precision in the presence of a representative matrix and may be required with each QC batch of 20 or fewer samples.

**3.3.4.1** QC acceptance criteria requires that analyte concentrations greater than ten times the MDL (or LCL) have an average Relative Percent Difference (RPD) of $\leq$30%.

3.3.4.2 If the RPD is outside the QC acceptance criteria, corrective action may be indicated. Corrective action may include recalculation or reanalysis of the DUP and the original sample, instrument maintenance, re-calibration, or re-extraction of the analytical QC batch.

### 3.4.5 Surrogate Compound Recovery

All samples are spiked with the appropriate surrogate spiking solution to both determine the concentration of target compounds and to monitor method performance. Specialized analyses may use differing surrogates and QC recovery criteria based upon method development.

3.4.5.1 QC acceptance criteria for all surrogate compounds used during routine GC-ECD analyses requires a recovery between 40 to 120 % in each sample analyzed. The following surrogate compounds are currently used by GERG staff.

3.4.5.1.1 **Routine OC/PCB/Terphenyls Combined Analysis**: PCB 103 is used in the calculation of the concentrations of the target compounds. Recoveries of the 4,4'-dibromooctafluoro-biphenyl (DBOFB) and PCB 198 are used, in addition to PCB 103, to monitor method performance. See Section 4.1.2 for the required method modification (longer run time) when analyzing for terphenyls.

3.4.5.1.2 **Organochlorine Pesticides Analysis** only: The surrogate Epsilon-HCH (ε-HCH) is used in the calculation of the concentrations of the target compounds.

3.4.5.1.3 **Congener Specific PCBs or Aroclors** only: The surrogate PCB 103 is used in the calculation of the concentrations of the target compounds. Recoveries of the DBOFB and PCB 198 are

used, in addition to PCB 103, to monitor method performance.

3.4.5.1.4 **Planar PCBs** only: Polybrominated biphenyl (PBB) 77 is the surrogate standard for this analysis. Since this compound will interfere with the analysis of source PCB congeners, it is added as a surrogate standard just before separation of planar congenors from the non-planar PCB.

3.4.5.2 If surrogate recovery fails the QC acceptance criteria, the following corrective action will be taken:

3.4.5.2.1 The calculations are checked to ensure that there are no errors.

3.4.5.2.2 The internal standard and surrogate solutions are checked for degradation, contamination, etc., and the instrument performance is checked.

3.4.5.2.3 If the recovery of the surrogate standard used in the calculation of the concentrations of target analytes (i.e., PCB 103) is outside the upper control limit, but the instrument calibration and other surrogate standard concentrations are in control, it is concluded that an interference specific to the surrogate was present that resulted in high recovery. A second surrogate standard (i.e., either DBOFB or PCB 198) may be used in the calculation of analyte concentrations. The presence of this type of interference is confirmed by evaluation of chromatographic peak shapes.

3.4.5.2.4. If a surrogate cannot be measured because the nature and concentration of interfering

compounds in the sample, a second surrogate standard meeting criteria may be used in the calculation of analyte concentrations with the written approval of the supervisor.

3.4.5.2.5 The extract is reanalyzed if the steps above fail to reveal a problem. If reanalysis of the extract yields surrogate recoveries within the stated limits, then the reanalysis data is reported. If reanalysis does not yield acceptable recoveries, the sample will be re-extracted and re-analyzed. If reanalysis does not improve surrogate recovery, the data can be reported and are properly qualified.

## 4.0 APPARATUS AND MATERIALS

### 4.1 Gas Chromatograph/Electron Capture Detector

The analytical system includes a temperature programmable gas chromatograph (Hewlett-Packard 5890A, or equivalent). The injection port is designed for split or splitless injection and analyses are conducted in the splitless mode. A 30-m long x 0.25-mm I.D. fused silica capillary column with DB-5 bonded phase (J&W Scientific) or equivalent is used. The autosampler is capable of making 1 to 4 µL injections.

A second DB-17ht column (J&W Scientific) or equivalent may be used for confirmation of target compounds. The following subsections provide the instrument method programs routinely used for the GC-ECD analyses.

**4.1.1 Routine OC/PCB; OCs Only; or Congener Specific PCB/Aroclor Analysis:** Representative extract aliquots are injected into the capillary column of the gas chromatograph using the following conditions:

| | |
|---|---|
| Injector Temp: | 275°C |
| Detector Temp: | 325°C |

| | |
|---|---|
| Oven Temp. Program: | |
| Initial Temp: | 100°C |
| Initial Hold Time: | 1 min. |
| 1st. Ramp Rate: | 5.0°C |
| 1st. Ramp Final Temp: | 140°C |
| Hold Time: | 1 min. |
| 2nd. Ramp Rate: | 1.5°C |
| 2nd. Ramp Final Temp: | 250°C |
| Hold Time: | 1 min. |
| 3rd. Ramp Rate: | 10.0°C |
| 3rd. Ramp Final Temp: | 300°C |
| Final Hold Time: | 10 min. |
| Carrier Gas: | Helium at 1.5. mL/min. |
| Make-up Gas: | Argon/Methane (95:5) at 40 mL/min. |
| Total Run Time | 99.3 min. |

The GC oven temperature program may be modified to improve resolution.

4.1.2 **Analyses Including Terphenyls:** When the analysis of terphenyls is required, the final hold time in the above method is changed to 20 minutes, resulting in a total run time of 109.3 minutes.

4.1.3 **Planar PCB Analysis:** When the analysis of planar PCBs is required, representative extract aliquots are injected into the capillary column of the gas chromatograph using the following conditions:

| | |
|---|---|
| Injector Temp: | 275°C |
| Detector Temp: | 325°C |
| Oven Temp. Program: | |
| Initial Temp: | 120°C |
| Initial Hold Time: | 1 min. |
| Ramp Rate: | 6.0°C |
| Ramp Final Temp: | 300°C |
| Final Hold Time: | 1 min. |
| Total Run Time | 32 min. |

## 5.0 ANALYTICAL STANDARDS

A full description of the procedures summarized below which are used for the preparation of standards for organochlorine hydrocarbons analyses can be found in GERG SOP 9713.

### 5.1 Surrogate Spiking Solution

Surrogate solutions are made by weighing appropriate amounts of pure compounds into a volumetric flask and diluting to volume with hexane or an equivalent non-chlorinated solvent (or by purchase of a certified standard). Surrogates are added to the samples prior to extraction at a concentration of approximately twenty (20) times the lowest calibration level or in the case of planar PCBs, just before isolation of these compounds from the PCB bulk. If higher concentrations of the target analytes are anticipated, the surrogate concentration and the final volume of the sample extract can be appropriately adjusted.

The compounds contained in the surrogate solutions for the different analysis are listed on Table 1. The concentration of each surrogate solution requires the addition of 100 µL to the extract, leading to the appropriate surrogate final concentration in the final extract volume. All sample target compounds concentrations are quantified based on the recovery of the specified analytical surrogate.

### 5.2 Internal Standard Solutions

A solution containing tetrachloro-meta-xylene (TCMX) for use as an internal standard is prepared at a concentration of 1.0 µg/mL, based on weight, from a pure compound. The stock solution is transferred to a volumetric flask and diluted to volume with hexane or an equivalent non-chlorinated solvent. Sufficient internal standard solution is added to the extract just prior to instrumental analysis to give a final concentration of 0.100 µg/mL in the final extract volume. The amount of internal standard can be adjusted as needed to meet the requirements of different analyses, e.g., planar PCBs.

### 5.3 Spiking Solution

A solution containing selected chlorinated hydrocarbons is used to fortify blank spikes and matrix spike samples. A spiking solution is made by weighing the

appropriate amounts of pure compounds into a volumetric flask and diluting them with hexane or an equivalent non-chlorinated solvent to the working concentration. The spiking solution is added to give a final concentration of approximately 0.040 µg/mL. The amount of matrix spike can be adjusted as needed to meet the requirements of the sample being prepared or the analysis required, e.g., planar PCBs.

### 5.4 Degradation Check Solution

A solution containing selected chlorinated hydrocarbons (e.g., DDT and aldrin) that are known to degrade in the injection port under certain conditions is used to verify the "inertness" of the instrument. This solution may be purchased as a certified mixed standard requiring dilution prior to use. These solutions can also be made by weighing the appropriate amounts of pure compounds into a volumetric flask and diluting them with hexane or an equivalent non-chlorinated solvent to the working concentration.

## 6.0 INSTRUMENT CALIBRATION PROCEDURE

### 6.1 Calibration

Calibration of the GC/ECD for the analysis of chlorinated pesticides is done as part of the analytical run as indicated in section 3.2.1. The four calibration standard mixtures are prepared according to GERG SOP 9713. The standards are interspersed with actual samples during the GC/ECD analyses. The calibration curve is then based on these four standards. If the calibration curve has an r of 0.9950 or higher (i.e., $R^2$ of 0.9900 or higher) for all analytes present in the samples it is accepted. If these QC acceptance criteria are not met, the calibration standards as well as all the samples must be reanalyzed by GC/ECD. A new calibration is also required if the required retention time criteria listed in Section 3.1 are not met.

No more than eight samples are run between the interspersed calibration solutions.

## 7.0 REQUIRED SAMPLE DOCUMENTATION AND IDENTIFICATION

Copies of the following documents, if applicable, must accompany the sample extract QC batch in a labeled folder when it is delivered for analysis:

Chain-of-custody documents
Sample Information Sheet(s)
Analysis Request Form(s)
Laboratory bench sheet
Dry weight bench sheet
Percent lipid bench sheet for tissue samples
Sample Action Request Form(s)

## 8.0 SAMPLE ANALYSIS

As discussed in Section 3.2.1 calibration mixtures, actual samples and QA samples (e.g., blanks, matrix spikes, SRM) are analyzed as one analytical sequence under the conditions described in Section 4.1. Before an analytical sequence is initiated, 2 µL of hexane followed by 2 µL of the Degradation Check Solution are injected into the GC/ECD to demonstrate and document that the analytical system is free from interfering contamination and that labile target compounds are not being degraded in the system.

Sample extract injections of 1 to 4 µL are then made with an autosampling device.

If the response for peak corresponding to any of the target compounds exceeds that in the highest calibration solution, the extract requires dilution to bring the injected amount into the instrument calibration range. Depending on the required dilution, a known aliquot of the extract is removed. One hundred (100) µL of the surrogate solution are added and the volume brought to 1.0 mL. The appropriate changes are made in the amount of surrogate added and the sample is re-analyzed just for those analytes requiring dilution.

### 8.1 Qualitative Identification

For a gas chromatographic peak to be identified as a target compound, it must meet the criteria specified in Section 3.1.2.

## 8.2 Quantitative Determination

### 8.2.1 Calibration

A four point calibration curve is used to establish the response of the detector. The calibration curve is prepared using a non-linear calibration equation of the form:

$$\textbf{Eq. 1} \quad Y = \left( \frac{A_a}{A_S} \right) * C_S$$

Where Y is the modified area ratio, representing the ratio of the area of the analyte ($A_a$) to the area of the surrogate standard ($A_s$) times the amount of the surrogate standard in micrograms ($C_s$).

$$\textbf{Eq. 2} \quad Y = b_0 + b_1 x + b_2 x^2$$

For Equation 2, x represents the concentration of the target compound in the calibration standards, and $b_0$, $b_1$, and $b_2$ are the coefficients of the quadratic equation.

### 8.2.2 Determine Analyte Concentration in Extracts

For the gas chromatographic peaks that have met the qualitative identification criteria in Section 3.1.2, calculate the concentration of each target compound as follows:

A solution to this quadratic equation which will produce the amount of analyte in µg/µL in the extract is:

$$\textbf{Eq. 3} \qquad x = \frac{b_1 + \sqrt{b_1^2 - 4 b_2 (b_0 - Y)}}{2 b_2}$$

Alternatively, the following equations can be used:

$$\textbf{Eq. 4} \qquad Y = A X^B$$

**Eq. 5** $$Y = \frac{C_a}{C_{su}} = A\left(\frac{A_a}{A_{su}}\right)^B$$

where A is the slope of the regression equation, B is the polynomial coefficient for the line fit, $C_a$ is the concentration of the analyte to be measured (ng/mL), $C_{su}$ is the concentration of the surrogate standard used in the calculation of the concentration of target analytes (ng/mL), $A_a$ is the area for the analyte to be measured and $A_{su}$ is the area for the surrogate standard used in the calculation of the concentration of target analytes.

### 8.2.3 Determine the Concentration of an Analyte in the Sample

To determine the analyte concentration in the sample, multiply the analyte extract concentration by $10^6$ and then divide by the sample mass.

### 8.2.2. Determine Surrogate Recovery

The percent recovery of the surrogate standards in the sample extract are calculated using the response of the internal standard added to the final extract just prior to instrumental analyses as follows:

**Eq. 6** $$\%\,\text{Surrogate Recovery} = \frac{C_{su}}{C_{is}} * \frac{A_{is}}{A_{su}} * 100$$

Where:

$C_{su}$ = Measured Concentration of Surrogate
$C_{is}$ = Measured Concentration of Internal Standard
$A_{su}$ = Known Amount of Surrogate
$A_{is}$ = Known Amount of Internal Standard

The laboratory will take corrective action whenever the recovery of any surrogates is less than 40% or greater than 120%.

**8.2.3.** If the concentration in the final extract of any of the target compounds exceeds the upper method calibration limit, the sample extract must be diluted by an appropriate dilution factor and reanalyzed. The analyte concentrations of the target analytes in the diluted extract are calculated using the surrogate recovery of the original sample.

**8.2.4** Calculate and report the Relative Percent Difference (RPD) between duplicate sample results. Document that the RPD complies with Section 3.3.4.

**8.2.5** Calculate and report the % Recovery of target compounds in the Matrix Spike (MS), Matrix Spike Duplicate (MSD), and, if analyzed, in the Laboratory Blank Spike or Laboratory Blank Spike Duplicate (LBS/ LBSD) samples.

**Eq. 7** $\%\ \text{Recovery of Spike} = (C_{ms} - C_a) / A_a * 100$

Where:

$C_{ms}$ = Measured Concentration of Analyte in the Spiked Sample
$C_a$ = Measured Concentration of Analyte in the Original Sample
$A_a$ = Known Concentration of Analyte that was added

Note: $C_a$ for a LBS/LBSD is zero by definition.

Document that the MS/MSD % Recovery complies with the criteria in Section 3.3.3, and that the LBS/LBSD complies with Section 3.3.2 criteria.

## 9.0 INSTRUMENT MAINTENANCE

### 9.1 Gas Chromatograph Maintenance

**9.1.1** The syringe is cleaned by rinsing with appropriate solvent after each injection.

9.1.2 A new injection port liner and septum are installed as needed.

9.1.3 A new injection port base plate is installed as needed.

9.1.4 One to two feet of the analytical column are removed as needed. This is necessary when there is significant tailing of the peak shapes.

9.1.5 The tanks of carrier (He) and make-up (Argon/Methane) gases are replaced when the pressure falls below 500 psi.

9.1.6 All instrument maintenance is recorded in the maintenance log for the specific instrument.

## 10.0 DOCUMENTATION REQUIRED FOR ANALYTICAL RESULTS

10.1 All injections and analytical run sequences are recorded on a computer printout kept on file at the instrument.

10.2 All information on the instrument calibration is included in the analytical folder.

10.3 For all analytical and laboratory Quality Control samples, the documentation is printed and included in the analytical folder. The compiled data report in either the GERG standard format (or other customized format requested by the client) includes Relative Percent Difference (RPD) between duplicate analyses or MS/MSD recoveries, and percent recovery of native analytes in MS/MSD and LBS/LBSD QC samples.

## 11.0 REPORTING

Data are reported to two places after the decimal point, as ng/g dry weight.

## Table 1. Chlorinated Hydrocarbons of Interest

**Surrogate Standards**

DBOFB*[1]
PCB 103*[1]
PCB 198*[1]
ε-HCH[2]
PBB 77[3]

**Internal Standards**

TCMX

**Chlorinated Benzenes**

1,2,4,5 tetrachlorobenzene*
1,2,3,4 tetrachlorobenzene*
pentachlorobenzene*
hexachlorobenzene*

**Hexachlorocyclohexane**

*alpha*-HCH*
*beta*-HCH*
*gamma*-HCH* (lindane)
*delta*-HCH*

**Other Cyclodiene Pesticides**

aldrin*
dieldrin*
endrin*

**Chlordane-related Compounds**

heptachlor*
heptachlor epoxide*
oxychlordane*
*gamma*-chlordane*
*alpha*-chlordane*
*trans*-Nonachlor*
*cis*-Nonachlor*

**DDTs and Related Compounds**

2,4'-DDE*
4,4' DDE*
2,4'-DDD*
4,4'-DDD*
2,4'-DDT*
4,4' DDT*
DDMU

**Other Chlorinated Pesticides**

pentachloroanisole*
chlorpyrifos*
mirex*
endosulfan II*
toxaphene
terphenyl

**Polychlorinated Biphenyl Congeners ****

| | | | | | | |
|---|---|---|---|---|---|---|
| 1 | 33/20 | 63 | 95/80 | 136 | 174 | 197 |
| 7/9 | 39 | 66* | 97 | 138*/160 | 175 | 199 |
| 8*/5 | 40 | 67 | 99 | 141/179 | 176/137 | 200 |
| 15 | 41/64 | 69 | 101*/90 | 146 | 177 | 201*/157/173 |
| 16/32 | 42/59/37 | 70 | 105* | 149/123 | 178 | 203/196 |
| 18*/17 | 44* | 72 | 107 | 151 | 180* | 205 |
| 22/51 | 45 | 74/61 | 110* | 153*/132 | 183 | 206* |
| 24/27 | 46 | 82 | 114 | 156 | 185 | 207 |
| 25 | 47/75 | 83 | 118* | 158 | 187* | 209* |
| 26 | 48 | 84 | 119 | 166 | 189 | |
| 28* | 49 | 85 | 128* | 167 | 191 | |
| 29* | 52* | 87*/115 | 129 | 170*/190 | 193 | |
| 30 | 53 | 91/55 | 130 | 171/202 | 194 | |
| 31 | 60/56 | 92 | 135 | 172 | 195*/208 | |

* target analytes included in the calibration mixtures.
**"/" indicates co-eluting congeners on a DB-5 column. The order of co-eluting congeners is given according to their relative contribution in common Aroclor mixtures, i.e. Aroclors 1242 and 1254.
[1] Surrogate for OC/PCB, congenor specific PCBs/Aroclor or terpheynyl analyses.
[2] Surrogate for analysis of OCs only.
[3] Surrogate for analysis of planar PCBs only.

Table 2. Summary of QC Requirements for Quantitative Analysis

| Element | Control Limit Criteria | Frequency |
|---|---|---|
| - Instrument Calibration | Minimum of 4 standards. Correlation coefficient of $\geq$0.990. | With every analytical batch interspersed with actual samples. |
| - Instrumental Blank | Instrument free of interfering contamination or perform necessary maintenance. | Prior to analysis of an analytical batches. |
| - Degradation Check Solution | Degradation of labile chlorinated compounds less than 15%. | Prior to analysis of an analytical batch |
| - Surrogate Recovery | Recovery of 40 to 120% for all surrogates. See Section 3.4.5.3 for corrective actions. | All samples. |
| - Method Blank | No more than two analytes > 3 times the MDL. See Section 3.3.1.2 for exceptions to need for re-extraction. | One per QC batch. |
| - Duplicates (if applicable) | Average RPD $\leq$30% for all analytes > 10 x LCL. See Section 3.3.4.3 for corrective action. | One per QC batch. |
| - Matrix Spike, Matrix Spike Duplicate (if applicable) | 80% of targets with a % recovery within 40 to 130%. RPD for the spike recoveries should be $\leq$30% for all analytes. See Sections 3.3.3.5 for corrective actions. | One per QC batch. |

# SOP-9811
# CHARCOAL:SILICA GEL COLUMN CHROMATOGRAPHY FOR THE SEPARATION OF PLANAR POLYCHLORINATED BIPHENYLS IN SAMPLE EXTRACTS PRIOR TO GAS CHROMATOGRAPHY-ELECTRON CAPTURE DETECTOR ANALYSIS

Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURE SOP-9811

# CHARCOAL:SILICA GEL COLUMN CHROMATOGRAPHY FOR THE SEPARATION OF PLANAR POLYCHLORINATED BIPHENYLS IN SAMPLE EXTRACTS PRIOR TO GAS CHROMATOGRAPHY-ELECTRON CAPTURE DETECTOR ANALYSIS

This document presents the procedures, materials, and quality control used in the performance of the above preparation activities.

Quality Assurance Manager _____ Date _____

Author/Revision By: G. Ekman, C. Veltman

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Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURE SOP-9811

# CHARCOAL:SILICA GEL COLUMN CHROMATOGRAPHY FOR THE SEPARATION OF PLANAR POLYCHLORINATED BIPHENYLS IN SAMPLE EXTRACTS PRIOR TO GAS CHROMATOGRAPHY-ELECTRON CAPTURE DETECTOR ANALYSIS

## 1.0 PURPOSE

This document provides the procedures used by the staff of the Geochemical and Environmental Research Group (GERG) of the College of Geosciences at Texas A&M University for the separation of planar polychlorinated biphenyls (PCBs) from other components found in sample extracts prior to further extract analysis using the gas chromatography-electron capture detector (GC-ECD) technique.

### 1.1 Summary

Assessment of the environmental levels of planar PCBs requires their measurement in samples at trace levels (parts per billion to parts per trillion). Samples are extracted, purified and concentrated as described in GERG SOPs for chlorinated hydrocarbon preparation and analysis. After GC/ECD analysis for various chlorinated hydrocarbons, the extract can be further processed. An additional surrogate, polybrominated biphenyl 77 (PBB 77), is added to the extract. Then charcoal/silica gel column chromatography is used to selectively separate this surrogate and the planar PCBs from other organic compounds in the extract, allowing quantitative recovery of the planar compounds. The second column fraction containing the desired target compounds is collected, and concentrated prior to planar PCB analysis using the GC-ECD technique.

### 1.2 Application

After completion of other analytical activities, this charcoal/silica gel column chromatography separation and purification procedure can be used for tissue, sediment and soil extracts that are to be analyzed for planar PCB target compounds. This chromatographic procedure removes the other routine organochlorine target compounds, surrogates, and internal standards found in the analyzed extract.

## 2.0 SAFETY

The hazards, toxicity or carcinogenicity of each compound or reagent used in GERG's standard operating procedures have not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory maintains Material Safety Data Sheets (MSDS) which contain information regarding the safe handling of chemicals used at GERG's facilities. A reference file of MSDS is available to all personnel involved with these materials. All laboratory personnel should direct any questions regarding safety issues to their supervisors or the Safety Officer.

## 3.0 QUALITY CONTROL (QC)

**3.1** Process QC extracts (Blank, Duplicate) in an identical manner as the other extracts. See Section 5.2.5 for specific Matrix Spike QC requirements for these extracts.

**3.2** Note in the Comments section of the Laboratory Sample Logbook if anything out of the ordinary occurred to the extract or if there are unusual extract characteristics (cloudy, difficult to transfer, etc.).

**3.3** Use Standard Laboratory Practice when filling out all paperwork. Use black waterproof ink. When correcting an entry, use one single line through the incorrect entry, date and initial change. Write a large Z through all the empty sample logbook lines, then date and initial the "Z".

**3.4** Observe good, clean, and safe laboratory practice.

## 4.0 APPARATUS AND MATERIALS

### 4.1 Glassware and Hardware

The following laboratory glassware and hardware are needed to perform the extract component separation using charcoal/silica gel column chromatography. Glassware is pre-cleaned according to GERG SOP.

**Aluminum Foil:** Pre-washed with methylene chloride

**Balance**: Top loading with an accuracy of ±0.001 gram

**Beakers**: 50 mL and 400 mL capacity, borosilicate glass

**Buchner Funnel:** 350 mL capacity

**Chromatographic Columns**: 10 mm ID x 150 mm, with a 250 mL reservoir and a Teflon stopcock

**Erlenmeyer Flask:** 500 mL

**Filter Paper:** Glass; 110 mm; Whatman; Cat. #1822-110

**Flat Bottom Flask**: 125 mL, combusted at 440°C for 4 hours

**Funnels**: Borosilicate glass, assorted sizes

**Glass rod**: 40 cm x 0.7 cm

**Glass Wool, Pyrex**: Combusted at 440°C for 4 hours, cooled and stored covered at ambient temperature

**Graduated Cylinders**: 250 mL and 1000 mL capacity

**Oven**: Temperature programmable up to 440°C

**Micropipettors:** Reusable 100µL micropipettors.

**Pasteur pipettes:** 2 mL, disposable, combusted at 440°C for 4 hours

**Nitrogen blowdown apparatus:** Thermolyne Type 12200 Dri-Bath or equivalent

**Nitrogen gas:** 99.99% pure

**Scissors**: Stainless steel

**Spatula**: Stainless steel with a scoop at one end

**Spoon:** Stainless steel balance spoon; Baxter; Cat. #S1588-1, or equivalent

**Tear-drop vials:** 2 mL glass

**Tweezers**: Stainless steel

**Waste Collection Jars:** 8 oz. Mason jars or similar containers

**Vacuum Flask:** 2000 mL

**Vials:** 40 mL glass with Teflon lined cap

### 4.2 Reagents and Consumable Materials

**AX-21 Carbon**: Anderson Development Co., Adrian, MI.

**Anhydrous Sodium Sulfate:** J. T. Baker; Cat. #3891-05; reagent grade or equivalent.

**LPS-2 Silica Gel:** Whatman; Grade 62; Cat. # 300-4 or equivalent.

**Solvents**:

Methylene chloride: B & J; Cat. #300-4; pesticide grade or equivalent.
Methanol: B & J; Cat. #230-4; pesticide grade or equivalent.
Hexane: B & J; Cat. #GC50393; GC/GC-MS grade or equivalent.
Acetone: B & J; Cat. #010-4; pesticide grade or equivalent
Toluene: B&J; Cat. #347-4; pesticide grade or equivalent
Cyclohexane: B&J; Cat. #053-4; pesticide grade or equivalent

## 5.0 PROCEDURE FOR PREPARING COLUMNS

### 5.1 Extract Documentation Required

Review a copy of the Organic Analysis Request Form, the Sample Information Sheet, and check the Laboratory Logbook Benchsheet.

**5.1.1** Verify that the extracts require charcoal/silica gel column purification using these forms.

**5.1.2** Verify that all other organochlorine analyses have been completed prior to initiating this chromatographic procedure.

## 5.2 Preparation for Column Setup

### 5.2.1 Combust Dry Materials for Column

**5.2.1.1** Combust sodium sulfate in a beaker at 440°C for four hours and cool to ambient temperature in a dessicator prior to use.

**5.2.1.2** Combust glass wool at 440°C for four hours and cool to ambient temperature prior to use.

**5.2.1.3** Combust LPS-2 silica gel at 170°C for sixteen hours. Store at 170°C, and cool to ambient temperature prior to use.

**Note:** When taking materials out of the oven, wear heat protective gloves or use large forceps capable of handling heavy objects. The beakers containing sand and sodium sulfate are heavy (up to 1000 grams), and are hot. Make sure all the beakers are tightly covered with aluminum foil prior to combusting.

### 5.2.2 Preparation of the AX-21 Carbon

**5.2.2.1** Place a glass fiber filter paper in a 350 mL Buchner funnel and 1000 ml vacuum flask apparatus.

**5.2.2.2** Pour 100 grams of AX-21 carbon powder on top of the filter paper in the Buchner funnel.

**5.2.2.3** Add 300 mL methanol to the carbon and use the vacuum pump to pull the methanol rinse through the carbon.

**5.2.2.4** Repeat the rinse two more times with methanol. When all the methanol is removed, continue the vacuum to dry the carbon for 10 seconds.

**5.2.2.5** Put the methanol washed carbon in an oven at 130°C for a minimum of 72 hours before use. Discard the methanol rinses in the appropriate waste container.

### 5.2.3 Preparation of 1:20 AX-21 Carbon/LPS-2 Silica Mixture

**5.2.3.1** Weigh 100 grams (± 1 g) of room temperature LPS-2 silica gel in a 500 mL flask. Add 5 grams of pre-washed carbon to the flask.

**5.2.3.2** Cap the flask with a piece of aluminum foil pre-rinsed with methylene chloride.

**5.2.3.3** Shake the mixture by hand until a uniform black color is achieved.

### 5.2.4 Preparation of Solvent Mixtures for Rinses and Extract Elution

To minimize waste, the quantity of 1:4 and the 9:1 solvent mixtures prepared should reflect the number of sample extracts using the correct proportions provided in the following sections.

**5.2.4.1** The 1:4 (v:v) methylene chloride:cyclohexane solvent mixture is prepared by mixing 200 mL of methylene chloride with 800 mL of cyclohexane in a 1000 mL graduated cylinder. The initial and final column rinses require a total of 60 mL of this solvent mixture. The quantity prepared should reflect a small excess of the amount required for all columns having initial and final rinses that day.

**5.2.4.2** The 9:1 (v:v) methylene chloride:toluene solvent mixture is made by mixing 900 mL of methylene chloride with 100 mL of toluene in a 1000 mL graduated cylinder. Each extract

requires the use of 30 mL for elution of the first fraction (9:1) and 30 mL for the final column rinse, or a total of 60 mL per column. To minimize waste, the quantity prepared should reflect the number of columns being used, using the correct proportions noted.

#### 5.2.5 Extract Preparation

Use of the carbon:silica gel column removes non-planar organic components from the sample extract, including extraction surrogates and the instrumental internal standard previously added for OC/PCB analysis. Therefore, a planar surrogate must be added to each sample extract and QC extract prior to loading each extract on this type of column.

The matrix spike and matrix spike duplicate (MS/MSD) extracts are typically omitted from this extract purification step. However, when the client requests an MS/MSD, a specific planar spiking solution must also be added to the appropriate samples prior to loading the extract on this type of column. The blank and duplicate QC samples only need to have the planar surrogate added.

##### 5.2.5.1 Surrogate Standard

A surrogate solution of polybrominated biphenyl 77 (PBB 77) is made by weighing an appropriate aliquot of pure material into a volumetric flask and diluting to volume with hexane. The addition of 100 µL of the surrogate standard to each extract results in a surrogate concentration of at least 10 times the MDL for the planar PCBs.

##### 5.2.5.2 Matrix Spike Solutions

The matrix spiking solution consists of the three planar PCB (77, 126, 169). The matrix spike is added to extracts at a concentration that results in a final concentration that is at least 10 times the MDL for the planar PCBs.

### 5.3 Column Construction

**5.3.1** Place each assembled column on the column rack. Without allowing the solvent bottle tips to touch the top of the reservoir, rinse consecutively with the following five solvents: methanol; acetone; methylene chloride; toluene; and hexane. Drain each solvent separately into a waste container. Make sure the solvents completely sheet and rinse the inside of the columns.

**5.3.2** Using the five-solvent rinsed tweezers, glass rod, and scissors, plug the column with a 3/4 inch by 3/4 inch room temperature combusted glass wool plug.

**5.3.3** Using a small funnel and a rinsed spoon, add 2 cm of room temperature combusted sodium sulfate to the column.

**5.3.4** Using a small beaker, weigh 2 grams of the room temperature 1:20 charcoal/silica mixture. Using a small funnel and a rinsed spoon, add the 1:20 charcoal/silica mixture to the column, pouring slowly to avoid packing charcoal/silica mixture.

**5.3.5** Using a small funnel and a newly rinsed spoon, add 2 cm of room temperature, combusted sodium sulfate to the top of the column, pouring slowly to avoid packing or displacing the charcoal/silica mixture.

**Note:** Be careful not to shake or bump the column to avoid packing the charcoal/silica mixture.

**Outline of the planar charcoal/silica gel column:**

| | |
|---|---|
| (TOP) | 2 cm small granular sodium sulfate |
| | 2 grams 1:20 charcoal/silica mixture |
| | 2 cm granular sodium sulfate |
| (BOTTOM) | glass wool plug |

## 5.4 Pre-Rinsing the Newly Constructed Column

**5.4.1** Place a waste collection jar under the column. Add 40 mL of a 1:4 (v:v) methylene chloride:cyclohexane mixture to the column reservoir. Open the stopcock fully and drain approximately 20 mL of the 1:4 solvent out of the column. The balance of the solvent is left in the column to prevent the dryness prior to use.

**5.4.1.1** The charcoal/silica gel column is now prepared for use. It may be capped with methylene chloride rinsed foil until ready for extract loading. Prior to use, the rest of this solvent is drained to the top of the sodium sulfate.

## 5.5 Extract Purification Using the Charcoal/Silica Gel Column

**5.5.1** Remove the aluminum foil caps and rinse all collection flasks three times with methylene chloride. The first fraction requires a 40 mL vial and the second fraction requires a 125 mL flat bottom flask. Rinse the inside of the aluminum foil caps with methylene chloride, and place the caps back on the flasks.

**5.5.2** Make two sets of tape labels for the 40 mL vial and the 125 mL flat bottom flasks, differentiating the two column fractions by adding "9:1" to normal identification numbers on the 40 mL vial (for fraction one). Prepare a normal label for the flat bottom flask which will be used to collect the planar PCB targets. Place these labels containing all required sample extract identification on the appropriate fraction collection flasks.

**5.5.3** Place a labeled (9:1) 40 mL vial under the valve tip. Use a combusted disposable pipette with a glass wool plug to add the concentrated sample extract in hexane (which has already been spiked with the PBB 77) to the top of the column. Open the stopcock and let the extract drain to the top of the sodium sulfate.

**5.5.4** Prepare numbered concentrator tubes containing 30 mL of the 9:1 methylene chloride solvent mixture. Using the same pipette used for the removal of the sample extract, rinse the extract vial with a small amount of a 30 ml aliquot of the 9:1 solvent. Add the rinsate

to the column. Let the rinsate drain to the top of the sodium sulfate. Rinse the extract vial and add the rinsate to the column a total of three times, letting each rinse drain to the top of the sodium sulfate before adding the next to the column.

**5.5.5** After the solvent rinses and sample extract have drained to the top of the sodium sulfate, add the remainder of the 30 mL aliquot of the 9:1 methylene chloride/toluene mixture for the elution and collection of the first (9:1) fraction.

**5.5.6** When the 9:1 fraction is completely drained, remove the 40 mL vial, put a cap on it, and replace it with the pre-rinsed 125 mL flask. Store this 9:1 fraction in the designated location until notified that the planar PCB analyses have been completed. These fractions should be disposed of with chlorinated solvent waste after analytical efforts are complete.

**5.5.7** With the stopcock fully open and without touching the lip of the solvent reservoir, add 30 mL of toluene for the elution and collection of the second fraction. When the second fraction is completely drained, remove the flask and put a stopper on it. This fraction containing the planar PCBs is now ready for concentration using the rotovap.

### 5.6 Final Column Rinses

These charcoal/silica columns may be reused for additional extracts after appropriate rinsing and storage.

**5.6.1** Place a waste collection jar under the column, add 30 mL of 9:1 methylene chloride:toluene to the column and drain to the surface of the sodium sulfate.

**5.6.2** Add 30 mL of 1:4 methylene chloride:cyclohexane to the column and drain about 20 mL into the waste jar. The balance of the solvent is left in the column to prevent the dryness prior to use. Cover the reservoir and the tip of the column with methylene chloride rinsed aluminum foil until needed for processing additional extracts for the separation of planar PCBs.

**5.6.3** To process a new extract, drain the 1:4 methylene chloride:cyclohexane stored in the column to the surface of the sodium sulfate. Repeat the procedures in Section 5.5 for the isolation of planar PCBs from extracts.

## 6.0 ROTOVAP EVAPORATION PROCESS

The rotovap bath temperature should be approximately 50°C, and should not exceed 60°C. Pre-rinse the rotovap trap before evaporation of each fraction group with five solvents (methanol, acetone, methylene chloride, toluene, and hexane) consecutively.

**6.1** The setting for the concentration of the second fraction group should be 70 mbars. Rotovap down to approximately 1 mL. In between each extract fraction, rinse the trap with methylene chloride three times and then with toluene three times.

## 7.0 FINAL NITROGEN BLOWDOWN

**7.1** Prepare 2 mL tear-drop vials by labeling them with the sample numbers. Use a permanent marker pen that will tolerate the heat from the nitrogen blowdown apparatus.

**7.2** Transfer the concentrated extract from the collection flask to the tear-drop vials using a disposable, combusted Pasteur pipette with a glass wool plug. Fill the vials to 3/4 full of the extract and collection flask rinses.

**7.2.1** Rinse each collection and concentration flask three times with about 1 mL of toluene and add the rinsate to the tear-drop vial.

**7.2.2** As the volume in the tear-drop vial is reduced, add rinsate from the extract concentration flask to the vial.

**7.3** Place new combusted disposable glass Pasteur pipettes in the nitrogen blowdown apparatus. Place a label on the pipette corresponding to its teardrop vial. These pipettes should have no glass wool in them.

**7.4** Insert the Teflon nitrogen hoses into the pipettes.

**7.5** Place the 2 mL tear-drop vials containing the sample extract on the nitrogen blowdown apparatus and turn on the heat.

**7.6** Insert the pipette into the vials, making sure the tip of the pipette does not touch the extract.

**7.7** With the individual nitrogen stream controls in the back of the hood completely off, turn on the main nitrogen line (to the left of the hood) up to 10 psi.

**7.8** Slowly adjust the individual controls up until a slight movement of the extract surface is seen.

NOTE: Each individual control valve controls six nitrogen hoses. These should be turned up slowly so that the stream does not force extract out of the vials. When turning on one controller, the other two controllers are affected and must be adjusted. Do not turn off the individual controllers until the main controller is off.

**7.9** Blow down the sample extract to approximately100 µL.

**7.10** Cap the extracts and make a final label for each with its sample identification numbers on it. Place all extract fractions in the appropriate storage area and give the extraction supervisor the completed documentation folder.

**7.11** All sample extracts are then analyzed according to the procedures described in the appropriate GERG SOP.

## 8.0 DOCUMENTATION REQUIREMENTS

Copies of following documents must accompany the sample extract set in a labeled folder before it is delivered for instrumental analysis:

Chain of Custody documents
Sample Information Sheet
Analysis Request Form
Laboratory Bench Sheet
Sample dry weight and lipid bench-sheets
Other miscellaneous information.

# SOP-9728

# QUANTITATIVE DETERMINATION OF PLANAR POLYCHLORINATED BIPHENYLS (PL-PCBs) BY ISOTOPE DILUTION HIGH RESOLUTION GAS CHROMATOGRAPHY/HIGH RESOLUTION MASS SPECTROMETRY

Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURES SOP-9728

# QUANTITATIVE DETERMINATION OF PLANAR POLYCHLORINATED BIPHENYLS (PL-PCBS) BY ISOTOPE DILUTION HIGH RESOLUTION GAS CHROMATOGRAPHY/HIGH RESOLUTION MASS SPECTROMETRY

This document presents the procedures, materials, and quality control used in the performance of the above instrumental analysis.

Quality Assurance Manager _____ Date _____

Author/Revision by: L. Chambers

# QUANTITATIVE DETERMINATION OF PLANAR POLYCHLORINATED BIPHENYLS (PL-PCBS) BY ISOTOPE DILUTION HIGH RESOLUTION GAS CHROMATOGRAPHY/HIGH RESOLUTION MASS SPECTROMETRY

## 1.0 PURPOSE AND SUMMARY

This document provides the procedures used by the staff of the Geochemical and Environmental Research Group (GERG) of the College of Geosciences and Maritime Studies at Texas A&M University for the quantitative determination of planar polychlorinated biphenyls (PL-PCBs) in sample extracts using isotope dilution high resolution gas chromatography/high resolution mass spectrometry (HRGC-HRMS).

### 1.1 Applicability

The instrumental procedures described in this document are applicable to the quantitative analysis of extracts obtained from water, sediment, soil, tissue, and other sample matrices after appropriate extraction and purification.

### 1.2 Target Analyte List and CAS Registry Numbers

The planar polychlorinated biphenyls (PL-PCBs) determined by this method and their CAS Registry numbers are listed in Table 1.

### 1.3 Retention Time References, Quantitation References, and Minimum Levels

**1.3.1** The retention time references, quantitation references, and minimum levels for determination of PL-PCBs using this method are listed in Table 2.

**1.3.2** The Minimum Level (ML) for each analyte is defined as the level at which the entire system must give a recognizable signal and an acceptable calibration point. It is equivalent to the concentration of the lowest calibration standard, assuming that all method specific sample weights, volumes, and procedures have been employed.

**1.3.3** The sample specific Estimated Detection Limit (EDL) is the concentration of an analyte required to produce a signal with a peak height at least 3 times the background signal level. An EDL is calculated for each target compound that is not detected. The quantitation software (OPUSQuan) supplied by the instrument manufacturer calculates a sample specific-EDL using an algorithm based on the criteria outlined in Section 7.9.5.1.1 of EPA Method 8290.

## 1.4 Applicable Concentration Range

The analyte concentrations in the calibration standards (PCB1-PCB7) used for instrument calibration are listed in Table 3.

## 1.5 Special Considerations

### 1.5.1 Ventilation, Respiratory Protection, Protective Clothing, Eye Protection

High purity PCB standards should be handled or transferred in an approved fume hood or with adequate ventilation. Protective gloves should be worn to prevent skin contact (Nitrile or equivalent). Safety glasses with side shields should be worn at all times.

### 1.5.2 Handling and Storage

Keep container closed and store in a cool area away from ignition sources and oxidizers. Do not breath vapor, do not get in eyes, on skin, or on clothing.

### 1.5.3 Work/Hygenic Practices

Wash thoroughly after handling. Do not take internally. Eye wash and safety equipment should be readily available when handling these standards.

#### 1.5.4 First Aid Measures

Get medical assistance for all cases of overexposure.

Skin: Wash thoroughly with soap and water.
Eyes: Immediately flush thoroughly with water for at least 15 minutes.
Inhalation: Remove to fresh air; give artificial respiration if breathing has stopped.
Ingestion: Do not induce vomiting; get immediate medical attention.

## 2.0 SAFETY

The hazards, toxicity or carcinogenicity of each compound or reagent used in GERG's standard operating procedures have not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory maintains Material Safety Data Sheets (MSDS) which contain information regarding the safe handling of chemicals used at GERG's facilities. A reference file of MSDS is available to all personnel involved with these materials. All laboratory personnel should direct any questions regarding safety issues to their supervisors or the Safety Officer.

## 3.0 QUALITY CONTROL

### 3.1 Mass Spectrometer Performance

**3.1.1** The mass spectrometer must be operated in the electron ionization mode. A static resolving power of at least 10,000 (10 percent valley definition) must be demonstrated at appropriate masses before any analysis is performed. Static resolving power checks must be performed at the beginning of each 12 hour period of operation. Corrective action (such as retuning, recalibrating, cleaning the source, checking for leaks) must be implemented whenever the resolving power does not meet the requirement.

**3.1.2** Chromatography time for PL-PCBs exceed the long term mass stability of the mass spectrometer. Because the instrument is operated in the high-resolution mode, mass drifts of a few ppm can have serious adverse effects on instrument performance. Therefore, a mass drift correction is mandatory. To that effect, a lock-mass ion is selected from the reference compound, perfluorokerosene (PFK), used for tuning the mass spectrometer. The selection of the lock-mass ion is dependent on the masses of the ions monitored (see Table 4). Any acceptable lock-mass ion at any mass between the lightest and heaviest ion in the descriptor can be used to monitor and correct mass drifts.

**3.1.3** The level of the reference compound (PFK) in the ion chamber during HRGC/HRMS analyses is adjusted so that the amplitude of the most intense selected lock-mass ion signal does not exceed 10 percent of the full scale deflection for a given set of detector parameters. The suggested PFK lock-mass ion is listed in Table 4.

**3.1.4** Documentation of the instrument resolving power is accomplished by recording the peak profile of the reference compound peaks within each descriptor. The format of the peak profile representation must allow manual determination of the resolution, i.e., the horizontal axis must be a calibrated mass scale. The result of the peak width measurement performed at the 10 percent valley definition cannot exceed 100 ppm for all reference compound peaks recorded.

**3.1.5** Total cycle time (dwell times plus switching times) for all ions within a single descriptor must be one second or less.

## 3.2 Analyte Identification Criteria

### 3.2.1 Retention Times

**3.2.1.1** For target compounds which have an isotopically labeled quantitation standard present in the sample extract, the retention time at maximum peak height of the sample components (i.e., the two ions used for

quantitation purposes listed in Table 4) must be within -1 to +3 seconds of the isotopically labeled standard.

**3.2.1.2** The ion current responses for both ions used for quantitative purposes must simultaneously reach their maxima (± 2 seconds).

**3.2.1.3** The ion current responses for both ions used for the labeled standards must reach maximum simultaneously (± 2 seconds).

### 3.2.2 Ion Abundance Ratios

The integrated ion current profiles for the two ions used for quantitation purposes must have a ratio within the range established for the homologous series to which the peak is assigned. See Table 5 for the required theoretical ion abundance ratios and their QC acceptance limits.

### 3.2.3 Signal-to-Noise Ratio

**3.2.3.1** All ion current intensities must be • 3 times noise level for positive identification of a PL-PCB compound.

**3.2.3.2** The peaks representing the native PL-PCBs in the PCB1 calibration solution must have signal-to-noise ratios greater than or equal to 3.

## 3.3 Calibration Criteria

### 3.3.1 Initial Calibration

**3.3.1.1** All seven calibration solutions listed in Table 3 (PCB1-PCB7) must be used for the initial calibration.

**3.3.1.2** The theoretical ion abundance ratios for all four native analytes and all five labeled compounds must be within the QC control limits specified in Table 5.

**3.3.1.3** The signal-to-noise ratio for the GC signals present in each Selected Ion Current Profile (SICP) must be $\geq 3$.

**3.3.1.4** The percent relative standard deviations (% RSD) for the mean relative response factors from the four unlabeled standards must not exceed 20 percent, and those for the four labeled reference compounds must not exceed 30 percent.

### 3.3.2 Calibration Verification

**3.3.2.1** Calibration verification must be performed at the beginning of each analytical sequence (a maximum of 12 hours) after successful mass resolution has been demonstrated. A calibration verification is also required at the end of a 12 hour shift or at the end of the analytical sequence, whichever is more frequent.

**3.3.2.2** The isotopic ratios for all four native analytes and all five labeled compounds must be within the control limits for the theoretical ion abundance ratios specified in Table 5.

**3.3.2.3** The signal-to-noise ratio for the GC signals present in each SICP must be • 3.

**3.3.2.4** The QC acceptance criteria for the calculated relative response factors for the unlabeled standards must be within $\pm$ 20 percent of the mean values established during the initial calibration. QC acceptance criteria for the calculated relative response factors for the labeled standards must be within ± 30 percent of the mean values established during the initial calibration.

## 3.4 Criteria for QC Samples in an Analytical Batch

The acceptance criteria for QC samples are evaluated within an analytical group. Therefore, failure of one QC sample type does not necessarily cause the entire analytical batch to fail.

### 3.4.1 Method Blank (BLANK)

**3.4.1.1** A Method Blank is used to demonstrate freedom from contamination in the analytical procedure, and is required for each set of 20 or fewer samples.

**3.4.1.2** If any of the target compounds are found in the blank at greater than the Minimum Level (Table 2), re-extraction of the entire set may be required as specified in the following subsections.

**3.4.1.2.1** If any of the target compounds are found in the blank at greater than the Minimum Level, but are not detected in the analytical samples above the Minimum Level, the analytical data must be flagged, but no further action is required.

**3.4.1.2.2** When target compounds are present in the method blank and in the analytical samples at concentrations above the Minimum Level and the concentration in a sample is 10x that found in the blank, the blank must be flagged but sample data is not flagged.

**3.4.1.2.3** When target compounds are present in the method blank and in the analytical samples at concentrations above the Minimum Level and the concentration in the sample is less than 10x that found in the blank, the sample set should be re-extracted and re-analyzed. If no sample remains for re-extraction, the

analytical data for those analytes in the blank and samples must be flagged.

**3.4.1.3** Analyst discretion is used when contamination is present which does not adversely effect the overall analytical effort.

**3.4.1.4** When target compounds are present in the method blank and in the analytical samples at concentrations above the Minimum Level and the concentration in a sample is 10x that found in the blank, the blank must be flagged but sample data is not flagged. If the concentration in the sample is less than 10x that found in the blank, the sample set should be re-extracted and re-analyzed. If no sample remains for re-extraction, the analytical data for those analytes in the blank and samples must be flagged.

### 3.4.2 Laboratory Blank Spike (LBS)

**3.4.2.1** A Laboratory Blank Spike (LBS) is used to demonstrate analytical accuracy of the method, and may be required with each set of 20 or fewer samples.

**3.4.2.2** QC acceptance criteria for the target compound recoveries is 70 to 130% of the spiked amount.

**3.4.2.3** If two or more of the target compounds are outside the acceptance criteria corrective action may be indicated. Corrective action may include recalculation or reanalysis of the LBS, instrument maintenance, recalibration, or re-extraction of the analytical batch.

### 3.4.3 Matrix Spike (MS) and Matrix Spike Duplicate (MSD)

**3.4.3.1** A Matrix Spike (MS) sample is used to estimate analytical accuracy in the presence of a representative

matrix and is required for each set of 20 or fewer samples.

**3.4.3.2** A Matrix Spike Duplicate (MSD) is used to estimate analytical accuracy and precision in the presence of a representative matrix and may be required for each set of 20 or fewer samples.

**3.4.3.3** QC acceptance criteria for target compound recoveries in the MS and the MSD is 70 to 130% of the spiked amount.

**3.4.3.4** If a Matrix Spike Duplicate (MSD) has been included with the analytical batch, the results obtained from the MS and MSD samples should agree within a Relative Percent Difference (RPD) of 20%.

**3.4.3.5** The MS and MSD acceptance criteria are advisory. However, if two or more of the target compounds are outside the QC acceptance criteria corrective action may be indicated. Corrective action may include recalculation and/or reanalysis of the MS and MSD; re-extraction of the sample/MS/MSD group; or instrument maintenance and/or recalibration.

### 3.4.4 Duplicate (DUP)

**3.4.4.1** A sample Duplicate (DUP) is used to demonstrate matrix homogeneity and analytical precision in the presence of a representative matrix, and is required with each set of 20 or fewer samples.

**3.4.4.2** QC acceptance criteria for analyte concentrations greater than ten times the minimum level is a Relative Percent Difference (RPD) of 30%.

**3.4.4.3** If two or more of the target compounds are outside the QC acceptance criteria, corrective action may be

indicated. Corrective action may include: recalculation or reanalysis of the DUP and the original sample, instrument maintenance, recalibration, or re-extraction of the analytical batch.

### 3.4.5 Standard Reference Material (SRM)

**3.4.5.1** A Standard Reference Material (SRM) is used to demonstrate analytical accuracy on a reference matrix from an independent source, and may be required with each set of 20 or fewer samples.

**3.4.5.2** Currently, the SRMs available do not provide a certified or concensus concentration range for planar PCBs. Therefore, the laboratory generated QC acceptance criteria is ± 35% times (average concentration ± 2 standard deviations) of the target analyte. No more than one of the target compounds should exceed this criteria.

**3.4.5.3** The SRM acceptance criteria are advisory for planar PCBs. However, if two or more target compounds fall outside the QC acceptance criteria, corrective action may be indicated. Corrective action may include recalculation and/or reanalysis of the SRM, instrument maintenance and/or recalibration.

### 3.4.6 Labeled Compound Recovery

**3.4.6.1** All samples are spiked with the PL-PCB Labeled Compound Spiking Solution (LCSS) to monitor method performance on the sample matrix.

**3.4.6.2** QC acceptance criteria for labeled compound recovery is 40 to 135%.

**3.4.6.3** If a labeled compound recovery falls outside the acceptance criteria, but the sum of the areas of the

affected peaks is 10% or greater than the area of the corresponding peaks in the continuing calibration standards, the analytical data are flagged.

**3.4.6.5** If two or more of the labeled compounds are outside the acceptance criteria corrective action may be indicated. Corrective action may include: recalculation and/or reanalysis of the sample; instrument maintenance and/or recalibration; or re-extraction of the sample aliquot.

## 4.0 APPARATUS AND MATERIALS

### 4.1 Gas Chromatograph

**4.1.1** The Gas Chromatograph (GC) used for this analytical method is an HP5890 Series II, or equivalent. The GC has an injection port designed for capillary columns and splitless injections and is capable of temperature ramp programming with an isothermal hold. A 2 µL splitless injection is used for all extracts, blanks, calibration solutions and the performance check samples.

**4.1.2** All injections are made with a CTC-2000S programmable autosampler, or equivalent.

**4.1.3** The GC/MS interface components withstand 350°C. The GC column is fitted directly into the mass spectrometer ion source without being exposed to the ionizing electron beam.

### 4.2 GC Columns

**4.2.1** The GC analytical column used for analysis of PL-PCBs is a 60 meter J&W DB5 or DB5MS column with a 0.25 mm ID and a 0.25 µm film thickness, or equivalent.

**4.2.2** Operating conditions known to produce acceptable results with the recommended analytical column (DB5MS) are shown below:

Inject at 190°C, hold for 1 minute

Ramp at 20°C per minute to 240°C, hold for 0 minutes
Ramp at 1°C per minute to 260°C, hold for 0 minutes
Ramp at 20°C per minute to 300°C, and hold for 15 minutes

Total analysis time is approximately 40.5 minutes.

### 4.3 Detector and Data System

**4.3.1** The detector used for these analyses is a VG AutoSpec Ultima, or equivalent, utilizing 28 to 40 eV electron impact ionization, capable of repetitively monitoring a minimum of 12 exact m/z's at high resolution (≥10,000) during a period of approximately 1 second or less, and meeting all of the performance specifications outlined in Sections 3.1 and 3.2.

**4.3.2** A dedicated data system is employed to control the rapid multiple-ion monitoring process and to acquire the data. Quantitation data (peak areas and/or peak heights) and Selected Ion Monitoring (SIM) traces are acquired during the analyses and stored. The data system is capable of acquiring data at a minimum of 12 ions in a single scan. It is capable of switching to different sets of ions (descriptors) at specified times during an acquisition, and of providing hard copies of individual ion chromatograms for selected gas chromatographic time intervals. It is capable of acquiring mass spectral peak profiles and providing hard copies of peak profiles to demonstrate the required resolving power. The data system permits the measurement of noise on the baseline.

## 5.0 ANALYTICAL STANDARDS

With the exception of perfluorokerosene-H, standards are stored in the dark at < 4°C in screw-capped vials with PTFE-lined caps when not being used.

### 5.1 Mass Spectrometer Tuning Standard

Perfluorokerosene-H (high boiling PFK) is used to tune the MS prior to sample analysis. It is available from PCR, Inc., Gainesville, Florida, although equivalent material from other sources may be used.

### 5.2 Labeled Compound Spiking Solution (LCSS)

This solution contains the four $^{13}C_{12}$-labeled PL-PCB quantitation standards in nonane at the nominal concentrations listed in Table 6. Fifty (50) µL of the LCSS is diluted in an appropriate solvent (e.g., acetone, methanol, hexane) and spiked into each sample prior to extraction.

### 5.3 Precision and Recovery Standard (PAR)

The solution contains the four native PL-PCB isomers at the nominal concentrations listed in Table 6. Fifty (50) µL of this solution is diluted in an appropriate solvent and spiked into the selected Matrix Spike (MS), Matrix Spike Duplicate (MSD), and/or Laboratory Blank Spike (LBS) samples prior to extraction.

### 5.4 Internal Standard (IS)

This solution contains one $^{13}C_{12}$-labeled PCB101 isomer at the nominal concentrations listed in Table 6. Ten (10) µL of the IS are added to the final sample extract before HRGC/HRMS analysis to determine the percent recoveries for the LCSS compounds.

### 5.5 Instrument Calibration Standards (PCB1-PCB7)

These seven solutions contain the four target compounds and the five $^{13}C_{12}$-labeled quantitation and internal standards at the nominal concentrations listed in Table 3. These solutions permit the relative response factors to be calculated. The PCB4 standard is also used as the calibration verification standard.

## 6.0 INSTRUMENT CALIBRATION PROCEDURE

### 6.1 Initial Calibration

Initial calibration is required before any sample is analyzed for PL-PCBs. Initial calibration is also required if the analysis of a calibration verification standard does not meet the required criteria listed in Section 3.3.2. All seven high resolution

concentration calibration solutions listed in Table 3 must be used for the initial calibration.

**6.1.1** Tune the instrument with PFK as described in Section 3.1.1.

**6.1.2** Using the same GC and MS conditions that produced acceptable tune results, analyze a 2 µL portion of each of the seven concentration calibration solutions (PCB1-PCB7) once, and demonstrate that the following conditions are met.

**6.1.2.1** The ratio of integrated ion currents for the quantitation ions appearing in Table 5 must be within the indicated control limits set for each homologous series.

**6.1.2.2** The ratio of integrated ion currents for the ions belonging to the $^{13}C_{12}$-labeled quantitation standards and the internal standards must be within the control limits stipulated in Table 5.

Note: All ratios must be within the specified control limits simultaneously in one run. Otherwise, corrective action is necessary.

**6.1.2.3** For each SICP and for each GC signal corresponding to the elution of a target analyte and its $^{13}C_{12}$-labeled standard(s), the signal-to-noise (S/N) ratio must be greater than or equal to 3.

**6.1.4** For each injection, calculate the four relative response factors (RRF) for unlabeled target analytes [RRF(n); n = 1 to 4] relative to their appropriate quantitation standards, and the RRFs for the $^{13}C_{12}$-labeled quantitation standards [RRF(m); m = 1 to 4] relative to the internal standards according to Equations 1 and 2 (refer to Table 2).

Equation 1. $$RRF(n) = \frac{Ax * Cqs}{Aqs * Cx}$$

Equation 2. $$RRF(m) = \frac{Aqs * Cis}{Ais * Cqs}$$

where:

Ax = sum of the integrated ion abundances of the quantitation ions for unlabeled PL-PCBs,

Aqs = sum of the integrated ion abundances of the quantitation ions for the labeled quantitation standards,

Ais = sum of the integrated ion abundances of the quantitation ions for the labeled internal standards,

Cx = concentration of the unlabeled target compound in the calibration solution (in pg/µL),

Cqs = concentration of the $^{13}C_{12}$-labeled quantitation standard in the calibration solution (100 pg/µL), and

Cis = concentration of the $^{13}C_{12}$-labeled internal standard in calibration solution (100 pg/µL).

NOTE: The RRF(n) and RRF(m) are dimensionless quantities; the units used to express Cx, Cqs and Cis must be the same.

**6.1.5** Calculate the mean RRFs for the seven calibration solutions using Equations 3 and 4.

Equation 3. $$\overline{RRFn} = \left(\frac{1}{7}\right) \sum_{j=1}^{7} RRF_j(n)$$

Equation 4. $$\overline{RRFm} = \left(\frac{1}{7}\right) \sum_{j=1}^{7} RRF_j(m)$$

where:

n = a specific PL-PCB unlabeled target analyte (n = 1 to 4),

m = the $^{13}C_{12}$-labeled quantitation standard (m = 1 to 4) for that analyte, and

j = the injection number or calibration solution number (j = 1 to 7).

**6.1.6** Determine the respective percent relative standard deviations (%RSD). Document that the initial calibration meets all of the acceptance criteria outlined in Section 3.3.1.

## 6.2 Calibration Verification

Routine calibration verification must be performed at the beginning of an analytical sequence following successful MS resolution check. A routine calibration verification is also required at the end of a 12 hour analysis period or analytical sequence, whichever is more frequent.

**6.2.1** Using the same GC and MS conditions that were used for the initial calibration, analyze a 2 µL portion of the midpoint calibration solution (PCB4) and evaluate it with the following QC acceptance criteria.

**6.2.1.1** The ratio of integrated ion currents for the quantitation ions appearing in Table 5 must be within the indicated control limits set for each homologous series.

**6.2.1.2** The ratio of integrated ion currents for the ions for the $^{13}C_{12}$-labeled quantitation and internal standards must be within the control limits stipulated in Table 5.

**NOTE:** All ratios must be within the specified control limits simultaneously in one run. Otherwise, corrective action is necessary.

**6.2.1.3** For each SICP and for each GC signal corresponding to the elution of a target compound and its $^{13}C_{12}$-labeled standard(s), the signal-to-noise (S/N) ratio must be greater than or equal to 3.

**6.2.2** Referring to Table 2, calculate the concentrations of the four unlabeled target compounds, and the concentration and percent recovery of the four $^{13}C_{12}$-labeled quantitation standards in the PCB4 standard using Equations 5 and 6.

Equation 5.
$$Cx = \frac{Ax * Cqs}{Aqs * \overline{RRF}(n)}$$

Equation 6.
$$\% \text{ Recovery} = \frac{Aqs * Cis * 100}{Ais * Cqs * \overline{RRF}(m)}$$

where:

Ax = sum of the integrated ion abundances of the quantitation ions for unlabeled PL-PCBs,

Aqs = sum of the integrated ion abundances of the quantitation ions for the $^{13}C_{12}$-labeled quantitation standards,

Ais = sum of the integrated ion abundances of the quantitation ions for the $^{13}C_{12}$-labeled internal standards,

Cx = concentration of the unlabeled PL-PCBs isomers (in pg/µL),

Cqs = concentration of the $^{13}C_{12}$-labeled quantitation standard in the calibration solution (100 pg/µL),

Cis = concentration of the $^{13}C_{12}$-labeled internal standard in the calibration solution (100 pg/µL),

$\overline{RRF}(n)$ = mean relative response factor from the initial calibration for the unlabeled target analyte relative to its $^{13}C_{12}$-labeled quantitation standard [RRF(n), with n = 1 to 4], and

$\overline{RRF}(m)$ = mean relative response factor from the initial calibration for $^{13}C_{12}$-labeled quantitation standard relative to its $^{13}C_{12}$-labeled internal standard [RRF(m), with m = 1 to 4].

**6.2.3** Referring to Table 2, calculate the four RRFs for unlabeled target analytes [RRF(n); n = 1 to 4] relative to their appropriate quantitation standards (Eq. 1, Section 6.1.4), and the RRFs for the $^{13}C_{12}$-labeled quantitation standards [RRF(m); m = 1 to 4] relative to the two internal standards (Eq. 2, Section 6.1.4).

**6.2.4** Verify and document that the calibration verification meets the acceptance criteria outlined in Section 3.3.2.

## 7.0 REQUIRED SAMPLE DOCUMENTATION AND IDENTIFICATION

Copies of the following documents must accompany the sample set in a labeled folder when it is delivered to HRGC/HRMS analysis group:

Chain-of-custody documents
Sample Information Sheet(s)
Analysis Request Form(s)
Laboratory bench sheet
Dry weight bench sheet
Percent lipid bench sheet for tissue samples
Sample Action Request Form(s)

## 8.0 SAMPLE ANALYSIS

**8.1** Tune the instrument with PFK as described in Section 3.1.1.

**8.2** Inject 2 µL of the PCB4 Calibration Verification solution (Table 3) and acquire SIM mass spectral data as described in Section 3.1.1. Demonstrate and document that the criteria listed in Section 3.3.2 are met.

**NOTE:** Alternately, samples may be analyzed immediately after an initial calibration if all the criteria listed in Section 3.3.1 have been met.

**8.3** Inject 2 µL of toluene and acquire SIM mass spectral data as described in Section 3.1.1. Demonstrate and document that the analytical system is free of interfering contamination.

**8.4** Inject 2 µL of the sample extract and acquire SIM mass spectral data under the same conditions that have been established to produce acceptable results.

### 8.5 Qualitative Identification

For a gas chromatographic peak to be identified as a target compound, it must meet all of the criteria specified in Section 3.2.

### 8.6 Quantitative Determination

**8.6.1** For gas chromatographic peaks that have met all the qualitative identification criteria, calculate the concentration of the target compound using Equation 7.

Equation 7.
$$Cx = \frac{Ax * Qs}{Aqs * W * \overline{RRF}(n)}$$

where:

Cx = concentration of the unlabeled PL-PCB in pg/g (ppt),

Ax = sum of the integrated ion abundances of the quantitation ions for unlabeled PL-PCB,

Aqs = sum of the integrated ion abundances of the quantitation ions for the associated $^{13}C_{12}$-labeled quantitation standard,

Qqs = amount (in pg) of the $^{13}C_{12}$-labeled quantitation standard added to the sample immediately prior to extraction,

$\overline{RRF}(m)$ = mean relative response factor from the initial calibration for the unlabeled target compound relative to its $^{13}C_{12}$-labeled quantitation standard [RRF(n), with n = 1 to 4], and

W = weight (in grams) of the sample.

**NOTE:** If the sample matrix is water, the sample size is normally represented in liters (L), and the resulting concentration is in pg/L (ppq).

**8.6.2** Calculate the percent recovery of the four quantitation standards in the sample extract using Equation 8.

Equation 8. $$\% \text{ Recovery} = \frac{Aqs * Qis * 100}{Ais * Qqs * \overline{RRF}(m)}$$

where:

Aqs = sum of the integrated ion abundances of the quantitation ions for the $^{13}C_{12}$-labeled quantitation standards,

Ais = sum of the integrated ion abundances of the quantitation ions for the $^{13}C_{12}$-labeled internal standards,

Qqs = amount (in pg) of the $^{13}C_{12}$-labeled quantitation standard added to the sample immediately prior to extraction,

Qis = amount (in pg) of the $^{13}C_{12}$-labeled internal standard added to the sample extract in the last step of preparation,

$\overline{RRF}(m)$ = mean relative response factor from the initial calibration for $^{13}C_{12}$-labeled quantitation standard relative to its $^{13}C_{12}$-labeled internal standard [RRF(m), with m = 1 to 4].

**8.6.3** If the concentration in the final extract of any of the four target compounds exceeds the upper method calibration limit listed in Table 3, the sample extract may be diluted by an appropriate dilution factor and reanalyzed.

**8.6.4** A sample specific Estimated Detection Limit (EDL) is calculated for each target compound that is not identified. The EDL is the concentration of a given analyte required to produce a signal with a peak height of at least 3 times the background signal level. The quantitation software (OPUSQuan) supplied by the instrument manufacturer calculates a sample specific-EDL using an algorithm based on the criteria outlined in Section 7.9.5.1.1 of EPA Method 8290.

**8.6.5** Calculate and report the Relative Percent Difference (RPD) between duplicate sample results.

**8.6.6** Calculate and report the % Recovery of native analytes in the Matrix Spike (MS), Matrix Spike Duplicate (MSD), and Laboratory Blank Spike (LBS) samples.

## 9.0 INSTRUMENT MAINTENANCE

### 9.1 Gas Chromatograph Maintenance

**9.1.1** The syringe is cleaned by rinsing with appropriate solvent after each injection.

**9.1.2** A new injection port liner and septum are installed at the beginning of each new run sequence, or less frequently as required.

**9.1.3** A new injection port base plate is installed as needed.

**9.1.4** One to two feet of the analytical column are removed as needed.

**9.1.5** The tank of carrier gas (He) is replaced when the pressure falls below 500 psi.

**9.1.6** All maintenance is recorded in the "HRGC/HRMS Maintenance Log".

## 9.2 Mass Spectrometer Maintenance

**9.2.1** The high emission filament is replaced as necessary.

**9.2.2** The inner ion source assembly is cleaned and replaced as necessary.

**9.2.3** The outer ion source assembly is cleaned and replaced as necessary.

**9.2.4** The septum on the PFK reservoir is replaced as necessary.

**9.2.5** The transfer line/re-entrant assembly is disassembled, cleaned, repaired and reassembled as necessary.

**9.2.6** The rotary pump oil is changed yearly, or more frequently if indicated.

**9.2.7** The diffusion pump oil is changed as necessary.

**9.2.8** All maintenance is recorded in the "HRGC/HRMS Maintenance Log".

## 10.0 DOCUMENTATION REQUIRED FOR ANALYTICAL RESULTS

**10.1** All maintenance and repairs made to the gas chromatograph and/or the mass spectrometer are recorded in the "HRGC/HRMS Maintenance Log".

**10.2** All injections and analytical run sequences are recorded on a computer printout kept on file at the instrument.

**10.3** For all Mass Spectral Calibrations document the instrument resolving power by recording the peak profile of the reference compound peaks within each descriptor. The format of the peak profile representation must allow manual determination of the resolution, i.e., the horizontal axis must be a calibrated mass scale. (Also see Section 3.2.1.3.)

**10.4** For all Initial Calibrations the following documentation is printed and maintained for a period of at least one year.

**10.4.1** The Selected Ion Current Profile (SICP) for each ion in each calibration run (PCB1-PCB7), including any manual integrations.

**10.4.2** Listing of retention times and peak areas for all target analytes in each calibration run (PCB1-PCB7).

**10.4.3** Listing of the calculated Relative Response Factors (RRF(n) and RRF(m)) for all target analytes in each calibration run (PCB1-PCB7).

**10.4.4** Listing of the calculated Average Relative Response Factors ($\overline{RRF}$(n) and $\overline{RRF}$(m)) for all target analytes, the standard deviation and percent relative standard deviation for each $\overline{RRF}$(n) and $\overline{RRF}$(m).

**10.5** For all Calibration Verifications, the following documentation is printed and maintained for a period of not less than one year.

**10.5.1** The Selected Ion Current Profile (SICP) for each ion in the calibration run (PCB4), including any manual integrations,

**10.5.2** Listing of retention times and peak areas for all target analytes in the calibration run (PCB4),

**10.5.3** Listing of the analyte concentrations for all target analytes in the calibration run (PCB4), and

**10.6** For all toluene instrument blanks the following documentation is printed and maintained for a period of at least one year.

**10.6.1** The Selected Ion Current Profile (SICP) for each ion in the blank run demonstrating that the analytical system is free from contaminating interferences.

**10.7** For all analytical and laboratory Quality Control samples the following documentation is printed and maintained for a period of at least one year (actual retention of analytical data is determined by the contract guidelines, but shall not be less than one year).

**10.7.1** The Selected Ion Current Profile (SICP) for each ion in the analytical run, including any manual integrations,

**10.7.2** Listing of retention times and peak areas for all target compounds in the analytical run,

**10.7.3** Listing of the calculated concentrations of all target compounds, percent recovery of the $^{13}C_{12}$-labeled quantitation standards, and Estimated Detection Limits (EDLs) in the analytical run, and

**10.7.4** A compiled data report in either the GERG standard format or other customized format requested by the client. The compiled data report includes Relative Percent Difference (RPD) between duplicate analyses or MS/MSD recoveries, and percent recovery of native analytes in LBS and MS/MSD QC samples.

Table 1. Planar Polychlorinated Biphenyls Determined by Isotope Dilution High Resolution Gas Chromatography (HRGC)/High Resolution Mass Spectrometry (HRMS).

| PL-PCBs | CAS Registry | Labeled Analog | CAS Registry |
|---|---|---|---|
| PCB77 | 32598-13-3 | $^{13}C_{12}$-PCB77 | 160901-67-7 |
| PCB81 | 70362-50-4 | $^{13}C_{12}$-PCB81 | 160901-68-8 |
| PCB126 | 57465-28-8 | $^{13}C_{12}$-PCB126 | 160901-75-7 |
| PCB169 | 32774-16-6 | $^{13}C_{12}$-PCB169 | 160901-79-1 |
| | | $^{13}C_{12}$-PCB101 | 160901-69-9 |

Table 2. Retention Time References, Quantitation References, and Minimum Levels for PL-PCBs.

| PL-PCB | Retention time and quantitation reference | Minimum level[a] Water (pg/L; ppq) | Solid (pg/g; ppt) | Extract (pg/µL; ppb) |
|---|---|---|---|---|
| PCB77 | $^{13}C_{12}$-PCB77 | 10 | 1 | 0.5 |
| PCB81 | $^{13}C_{12}$-PCB81 | 10 | 1 | 0.5 |
| PCB126 | $^{13}C_{12}$-PCB126 | 10 | 1 | 0.5 |
| PCB169 | $^{13}C_{12}$-PCB169 | 10 | 1 | 0.5 |
| $^{13}C_{12}$-PCB77 | $^{13}C_{12}$-PCB101 | | | |
| $^{13}C_{12}$-PCB81 | $^{13}C_{12}$-PCB101 | | | |
| $^{13}C12$-PCB126 | $^{13}C_{12}$-PCB101 | | | |
| $^{13}C_{12}$-PCB169 | $^{13}C_{12}$-PCB101 | | | |

[a]The Minimum Level (ML) for each analyte is defined as the level at which the entire analytical system must give a recognizable signal and acceptable calibration point. It is equivalent to the concentration of the lowest calibration standard, assuming that all method-specific sample weights, volumes, and cleanup procedures have been employed.

Table 3. Concentration of PL-PCBs in Calibration Solutions.

| PL-PCB | PCB1 (pg/µL) | PCB2 (pg/µL) | PCB3 (pg/µL) | PCB4 (pg/µL) | PCB5 (pg/µL) | PCB6 (pg/µL) | PCB7 (pg/µL) |
|---|---|---|---|---|---|---|---|
| PCB77 | 0.5 | 1.0 | 5.0 | 10 | 50 | 100 | 1000 |
| PCB81 | 0.5 | 1.0 | 5.0 | 10 | 50 | 100 | 1000 |
| PCB126 | 0.5 | 1.0 | 5.0 | 10 | 50 | 100 | 1000 |
| PCB169 | 0.5 | 1.0 | 5.0 | 10 | 50 | 100 | 1000 |
| $^{13}C_{12}$-PCB77 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| $^{13}C_{12}$-PCB81 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| $^{13}C_{12}$-PCB126 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| $^{13}C_{12}$-PCB169 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Internal Standard | | | | | | | |
| $^{13}C_{12}$-PCB101 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

Table 4. Exact m/z's, m/z Types, and Elemental Compositions of PL-PCBs.

| Exact m/z[a] | m/z type | Elemental Composition | Substance[b] |
|---|---|---|---|
| 289.9224 | M | $C_{12}H_6{}^{35}Cl_4$ | TCB |
| 291.9195 | M+2 | $C_{12}H_6{}^{35}Cl_3{}^{37}Cl$ | TCB |
| 293.9165 | M+4 | $C_{12}H_6{}^{35}Cl_2{}^{37}Cl_2$ | TCB |
| 301.9626 | M | $^{13}C_{12}H_6{}^{35}Cl_4$ | TCB[c] |
| 303.9597 | M+2 | $^{13}C_{12}H_6{}^{35}Cl_3{}^{37}Cl$ | TCB[c] |
| 323.8834 | M | $C_{12}H_5{}^{35}Cl_5$ | PeCB |
| 325.8805 | M+2 | $C_{12}H_5{}^{35}Cl_4{}^{37}Cl$ | PeCB |
| 327.8775 | M+4 | $C_{12}H_5{}^{35}Cl_3{}^{37}Cl_2$ | PeCB |
| 330.9793 | Lock | $C_7F_{13}$ | PFK |
| 335.9237 | M | $^{13}C_{12}H_5{}^{35}Cl_5$ | PeCB[c] |
| 337.9207 | M+2 | $^{13}C_{12}H_5{}^{35}Cl_4{}^{37}Cl$ | PeCB[c] |
| 357.8444 | M | $C_{12}H_4{}^{35}Cl_6$ | HxCB |
| 359.8415 | M+2 | $C_{12}H_4{}^{35}Cl_5{}^{37}Cl$ | HxCB |
| 361.8386 | M+4 | $C_{12}H_4{}^{35}Cl_4{}^{37}Cl_2$ | HxCB |
| 371.8817 | M+2 | $^{13}C_{12}H_4{}^{35}Cl_5{}^{37}Cl$ | HxCB[c] |
| 373.8788 | M+4 | $^{13}C_{12}H_4{}^{35}Cl_4{}^{37}Cl_2$ | HxCB[c] |

a Exact or accurate masses used:

H = 1.007825 C = 12.00000 $^{13}C$ = 13.003355 F = 18.9984
O = 15.994915 $^{35}Cl$ = 34.968853 $^{37}Cl$ = 36.965903

b
TCB = Tetrachlobiphenyl
PeCB = Pentachlorobiphenyl
HxCB = Hexachlorobiphenyl
PFK = Perfluorokerosene

c Labeled compound

Table 5. Theoretical Ion Abundance Ratios and QC Limits

| Number of Chlorine Atoms | m/z's Forming Ratio | Theoretical Ratio | QC Limit[a] Lower | QC Limit[a] Upper |
|---|---|---|---|---|
| 4 | M/(M+2) | 0.77 | 0.54 | 1.00 |
| 4[b] | M/(M+4) | 1.60 | 1.12 | 2.08 |
| 5 | M/(M+2) | 0.61 | 0.43 | 0.79 |
| 5[b] | M/(M+4) | 0.97 | 0.68 | 1.26 |
| 6 | M/(M+2) | 0.51 | 0.36 | 0.66 |
| 6[b] | M/(M+4) | 0.65 | 0.46 | 0.85 |
| 6[c] | (M+2)/(M+4) | 1.22 | 0.85 | 1.59 |

[a] QC limits represent ±30% deviation from theoretical ion abundance ratios.

[b] Ratio is advisory only.

[c] Used for $^{13}C_{12}$-HxPCB only.

Table 6. Concentration of Stock and Spiking Solutions Containing PL-PCB Labeled Compounds.

| PL-PCB | Labeled Compound Spiking Solution (LCSS) (pg/µL)[a] | PAR Spiking Solution (pg/µL)[b] |
|---|---|---|
| PCB77 | ---- | 4.0 |
| PCB81 | ---- | 4.0 |
| PCB126 | ---- | 4.0 |
| PCB169 | ---- | 4.0 |
| $^{13}C_{12}$-PCB77 | 40 | ---- |
| $^{13}C_{12}$-PCB81 | 40 | ---- |
| $^{13}C_{12}$-PCB126 | 40 | ---- |
| $^{13}C_{12}$-PCB169 | 40 | ---- |
| | | Concentration (pg/µL) |
| Internal Standards | $^{13}C_{12}$-PCB101 | 200[c] |

[a] Spike with 50 µL; total spike = 2,000 pg (nominal).

[b] Spike with 50 µL; total spike = 200 pg (nominal).

[c] Spike with 10 µL; total spike = 2,000 pg (nominal).

# SOP-9733

# QUANTITATIVE DETERMINATION OF POLYNUCLEAR AROMATIC HYDROCARBONS BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY USING THE SELECTED ION MONITORING MODE

Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURES SOP-9733

# QUANTITATIVE DETERMINATION OF POLYNUCLEAR AROMATIC HYDROCARBONS BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY USING THE SELECTED ION MONITORING MODE

This document presents the procedures used in the performance of the above analytical procedures.

Quality Assurance Manager _____ Date _____

Author/Revision By: G. Denoux and B. Wang

# QUANTITATIVE DETERMINATION OF POLYNUCLEAR AROMATIC HYDROCARBONS BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY USING THE SELECTED ION MONITORING MODE

## 1.0 PURPOSE

This document provides the procedures used by the staff of the Geochemical and Environmental Research Group (GERG) of the College of Geosciences at Texas A&M University for the quantitative determination of polynuclear aromatic hydrocarbons (PAH) in sample extracts using gas chromatography/mass spectrometry (GC-MS).

### 1.1 Applicability

The instrumental procedures described in this document are applicable to the quantitative analysis of extracts obtained from water, sediment, soil, tissue, and other sample matrices after appropriate extraction and purification.

### 1.2 Target Analyte List

The PAHs target compounds determined by this method and the surrogate used for quantitation (reference surrogate) are listed in Table 1.

### 1.3 Method Detection Levels

The analytical method detection limit (MDL) is determined on an annual basis following procedures outlined in Federal Register (1984), Vol. 49, No. 209: 198-199. MDLs for the multi-analyte groups are estimated as twice the MDL of the parent compound.

## 2.0 SAFETY

The hazards, toxicity or carcinogenicity of each compound or reagent used in GERG's standard operating procedures have not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory maintains Material Safety Data Sheets (MSDS) which contain information regarding the safe handling of chemicals used at GERG's facilities. A reference file of MSDS is available to all personnel involved with these materials. All laboratory personnel

should direct any questions regarding safety issues to their supervisors or the Safety Officer.

## 3.0 QUALITY CONTROL

The quality control requirements for quantitative analysis are summarized in Table 2 with details provided in the following sections.

### 3.1 Mass Spectrometer Performance

**3.1.1** The mass spectrometer performance is checked daily using PFTBA according to manufacturer's tuning procedures. These procedures include the checking of peak widths, mass axis calibration, and relative abundances of masses 69, 219, and 502 against manufacturer's recommended criteria. Isotope abundances are also checked according to the manufacturer's criteria.

**3.1.2** Total cycle time (dwell times plus switching times) for all ions within a single descriptor must be one second or less.

### 3.2 Analyte Identification Criteria for Single Analyte Compounds

#### 3.2.1 Retention Times

**3.2.1.1** Qualitative identification of target compounds is based on a comparison of the retention times with the target compounds in the calibration curve with the retention time of target compounds found in the sample extract. The retention time of the compound in the extract should be within ± 4 seconds of the average retention time of the authentic compounds in the calibration standard.

**3.2.1.2** The ion current responses for ions used for quantitation and confirmation purposes must simultaneously reach their maxima (± 2 seconds).

**3.2.1.3** The ion current responses for the quantitation and confirmation ions used for the labeled standards must reach maximum simultaneously (± 2 seconds).

### 3.2.2 Qualitative Identification

The extracted ion current profiles of the quantitation ion (primary m/z) and the confirmation ion (secondary ion) for each target compound must meet the following QC acceptance criteria

**3.2.2.1** The characteristic masses of each target compound must maximize in the same scan or within one scan of each other.

**3.2.2.2** The retention time of target compounds in the sample must fall within ± 4 seconds of the retention time for the authentic compound in the calibration standards.

**3.2.2.3** The relative peak heights of the primary ion compared to the confirmation (or secondary) ion mass for a target compound should fall within ±30 percent of the relative intensities of these masses in a mass spectrum (Table 3) obtained from a reference standard of that target compound.

**3.2.2.4** A compound that does not meet secondary ion confirmation criteria may still be determined to be present in a sample after close inspection of the data by the mass spectroscopist.

**3.2.2.5** Supportive data includes the presence of the secondary ion having a ratio greater than ±30 percent of the primary ion which may be caused by an interference with the secondary ion.

**3.2.2.6** The data not meeting these criteria are reported but appropriately qualified.

## 3.3 Analyte Identification Criteria for Multiple Analyte Groups

### 3.3.1 Retention Time Windows

Prior to all analytical sequences, a reference oil solution (GERG Standard Check) is analyzed. This analysis is used to define the retention time windows for the multiple analyte groups.

### 3.3.2 Qualitative Identification

The patterns within the retention time window for the sample extract are compared to the pattern within the same window of the reference oil solution. The magnitude of the individual peaks may not be consistent but the location of the peaks within the window should be similar between the two analyses.

## 3.4 Calibration Criteria

### 3.4.1 Initial Calibration

A five-point calibration curve based on response factors is established to demonstrate the linearity of the detector. The recommended standard concentrations are approximately 20, 100, 250, 500, and 1000 ng/mL (Table 4).

**3.4.1.1** The QC acceptance criteria for linearity of the initial calibration curve requires that the percent relative standard deviation (RSD) of the response factors for each compound in the five calibration standards must be less than or equal to fifteen percent ($\leq$ 15%). If these RSD criteria are exceeded, the linearity of the initial calibration curve can be determined to be acceptable if the correlation coefficient (r) determined using linear least squares regression analyses is greater than or equal to 0.99. The instrument software generates and prints out both types of calibration data for all initial calibration determinations, which are maintained in calibration files with the raw data for that laboratory area.

### 3.4.2 Continuing Calibration Verification (CCV)

Calibration verification must be performed at the beginning of each analytical sequence. A calibration verification is also required at the end of a 12 hour shift or at the end of the analytical sequence, whichever is more frequent.

**3.4.2.1** QC acceptance criteria are based upon daily response factors for each compound which are compared to the mean response factors for the initial calibration curve. If the average daily response factors for all analytes is within ±15% of the calibration value, analyses may proceed. If, for any single analyte, the daily response factor exceeds ±25 percent of calibration value, the five-point calibration is repeated before analysis continues and all samples are re-analyzed back to last passing CCV.

### 3.4.3 GERG Standard Check

The GERG Standard Check Solution is analyzed with all analytical batches. The laboratory certified concentration range of compounds in the standard check solution has been defined as the average concentration of all previous analyses plus or minus one standard deviation.

The QC acceptance criteria for the measured concentration of the standard check compounds must be within ±25% of the laboratory certified concentration on average for all analytes greater than the LCL and not exceed 35% for any individual analyte. If, after re-analysis, the concentration for any single target compounds exceeds ±35% of the calibration value, corrective action such as instrument maintenance or a new five point calibration must be prepared prior to further analysis.

## 3.5 Criteria for QC Samples in an Analytical Batch

The acceptance criteria for QC samples are evaluated within an analytical group. Therefore, failure of one QC sample type does not necessarily cause the entire analytical batch to fail.

### 3.5.1 Method Blank (BLANK)

**3.5.1.1** A Method Blank is used to demonstrate freedom from contamination in the analytical procedure, and is required for each set of 20 or fewer samples.

**3.5.1.2** If any of the target compounds are found in the blank at greater than 3 x the MDL, re-extraction of the entire set may be required as specified in the following subsections.

**3.5.1.2.1** If any of the target compounds are found in the blank at greater than 3 x the MDL, but are not detected in the analytical samples above the MDL, the analytical data can be reported and must be flagged, but no further action is required.

**3.5.1.2.2** When target compounds are present in the method blank and in the analytical samples at concentrations above 3 x the MDL and the concentration in a sample is 10 x that found in the blank, the blank must be flagged but sample data are reportable and are not flagged.

**3.5.1.2.3** When target compounds are present in the method blank and in the analytical samples at concentrations above 3 x the MDL and the concentration in the sample is less than 10 x that found in the blank, the sample should be re-extracted and re-analyzed. If

no sample remains for re-extraction, the analytical data for those analytes in the blank and samples can be reported but must be flagged.

### 3.5.2 Laboratory Blank Spike (LBS) and Laboratory Blank Spike Duplicate (LBSD)

**3.5.2.1** A Laboratory Blank Spike (LBS) may be used to estimate analytical accuracy of the method if inadequate sample is available or if a complex matrix is present . It may be required with each set of 20 or fewer samples.

**3.5.2.2** A Laboratory Blank Spike Duplicate (LBSD) is used to estimate both analytical accuracy and precision and may be required for each set of 20 or fewer samples.

**3.5.2.3** QC acceptance criteria for the target compound recoveries are that the recovery for each target compound falls between 40 and 120%.

**3.5.2.4** If the LBSD has been included, the recoveries determined from the LBS and LBSD should agree within an average Relative Percent Difference (RPD) of $\leq$25%.

**3.5.2.5** If two or more of the target compounds are outside the QC acceptance criteria corrective action may be indicated. Corrective action may include recalculation and/or reanalysis of the LBS and LBSD; re-extraction of the sample group; or instrument maintenance and/or recalibration. A laboratory blank spike may be used to demonstrate that the analytical system is in control when working with a difficult matrix or sample set.

### 3.5.3 Matrix Spike (MS) and Matrix Spike Duplicate (MSD)

**3.5.3.1** A Matrix Spike (MS) sample is used to estimate analytical accuracy in the presence of a representative matrix and is normally required for each set of 20 or fewer samples.

**3.5.3.2** A Matrix Spike Duplicate (MSD) is used to estimate analytical accuracy and precision in the presence of a representative matrix and may be required for each set of 20 or fewer samples.

**3.5.3.3** QC acceptance criteria for target compound recoveries in the MS and the MSD is 40 to 120% of the spiked amount. In computing the QC acceptance criteria, only valid spikes will be used. In a valid spike, the amount of analyte added is at least as much as was originally present in the sample.

**3.5.3.4** If a Matrix Spike Duplicate (MSD) has been included with the analytical batch, the recoveries determined from the MS and MSD samples should agree within a Relative Percent Difference (RPD) of $\leq$25%.

**3.5.3.5** The MS and MSD acceptance criteria are advisory. However, if two or more of the target compounds are outside the QC acceptance criteria corrective action may be indicated. Corrective action may include recalculation and/or reanalysis of the MS and MSD; re-extraction of the sample/MS/MSD group; or instrument maintenance and/or recalibration.

### 3.5.4 Duplicate (DUP)

**3.5.4.1** A sample Duplicate (DUP) is used to demonstrate sample homogeneity and analytical precision in the presence of a representative matrix and may be required with each set of 20 or fewer samples.

**3.5.4.2** QC acceptance criteria for analyte concentrations greater than ten times the MDL is a Relative Percent Difference (RPD) of $\leq$25%.

**3.5.4.3** If the RPD is outside the QC acceptance criteria, corrective action may be indicated. Corrective action may include: recalculation or reanalysis of the DUP and the original sample, instrument maintenance, recalibration, or re-extraction of the analytical batch.

### 3.5.5 Standard Reference Material (SRM)

**3.5.5.1** A Standard Reference Material (SRM) is used to demonstrate analytical accuracy and may be required with each set of 20 or fewer samples.

**3.5.5.2** When requested, a standard reference material is extracted and analyzed with each batch of samples. Acceptable concentrations are defined as the range of the documented (certified or reference) concentration plus or minus the 95% confidence limits in the certification.

**3.5.5.3** The QC acceptance criteria are that 80% of the data for measured concentrations should be within ±30% of the range of the acceptable concentration for each target compound (either certified or non-certified) with concentrations greater than 10 times the MDL. Corrective action may include recalibration or re-analysis of the SRM, instrument maintenance, re-calibration, or re-extraction of the analytical batch.

### 3.5.6 Surrogate Compound Recovery

**3.5.6.1** All samples are spiked with the appropriate surrogate spiking solution to determine the concentration of

target compounds and to monitor method performance.

**3.4.6.2** QC acceptance criteria for surrogate compound recovery is 40 to 120% for all except d12-perylene. The recovery of this surrogate is advisory only.

**3.5.6.3** If surrogate recovery fails the QC acceptance criteria, the following corrective action will be taken:

a. The calculations are checked to ensure that there are no errors.

b. The internal standard and surrogate solutions are checked for degradation, contamination, etc., and the instrument performance is checked.

c. If the surrogate recovery is outside the control limits, the secondary ion may be used to check the quantitation of the surrogate. If the secondary ion is within the control limits, this recovery can be used and the data are appropriately annotated.

d. If the upper control limit is exceeded for only one surrogate, and the instrument calibration and other surrogate standard concentrations are in control, it is concluded that an interference specific to the surrogate was present that resulted in high recovery and that this interference does not affect the quantitation of other target compounds. The presence of this type of interference is confirmed by evaluation of chromatographic peak shapes. To correct for the underestimation of the analyte concentration based on this surrogate, the target compounds will be quantified using the surrogate that is chromatographically closest to the surrogate exhibiting interference.

e. If the surrogate cannot be measured because the amount and nature of interferants in the sample, the target compounds based on that surrogate will be quantified based on the closest surrogate. The surrogate recovery is appropriately qualified.

f. If the native concentration of hydrocarbons are high and require dilution for acceptable chromatographic separations, a dilution is made. A known aliquot of the extract is removed. One hundred (100) $\mu$L of surrogate are added and the volume brought to 1.0 mL. The appropriate changes are made in the dilution factor within the quantitation software and the sample is re-analyzed. There is no concentration correction based on the surrogate recovery for dilution results. The surrogate recoveries are not reported but qualified with a "D" to denote the dilution. The MDL must also be adjusted to account for any dilutions.

g. The extract is reanalyzed if the steps above fail to reveal a problem. If reanalysis of the extract yields surrogate recoveries within the stated limits, then the reanalysis data is reported. If reanalysis does not yield acceptable recoveries, the samples will be re-extracted. If re-analysis does not improve surrogate recovery, the data are reported and properly qualified.

## 4.0 APPARATUS AND MATERIALS

### 4.1 Gas Chromatograph/Mass Spectrometer

Representative aliquots are injected into the capillary column of the gas chromatograph using the following conditions:

**Injector Temp**: 300°C
**Transfer Line Temp**: 280°C

| | |
|---|---|
| **Initial Oven Temp**: | 60°C |
| **Initial Hold Time**: | 0 min. |
| **Ramp Rate**: | 12°C |
| **Final Temperature**: | 300°C |
| **Final Hold Time**: | 18 min. |

The effluent from the GC capillary column is routed directly into the ion source of the mass spectrometer. The MS is operated in the selected ion monitoring (SIM) mode using appropriate windows to include the quantitation and confirmation masses for the PAHs listed in Table 3. For all compounds detected at a concentration above the MDL the confirmation ion is checked to confirm its presence.

The analytical system includes a temperature programmable gas chromatograph (Hewlett-Packard 5890A, or equivalent). The injection port is designed for split or splitless injection and analyses are conducted in the splitless mode. A 30-m long x 0.32-mm I.D. fused silica capillary column with DB-5MS bonded phase (J&W Scientific) is used. The autosampler is capable of making 1 to 4 $\mu$L injections.

The mass spectrometer (HP 5970/72 MSD) operates at 70 eV electron energy in the electron impact ionization mode and is tuned to maximize the sensitivity of the instrument based on manufacturer specifications.

## 5.0 ANALYTICAL STANDARDS

### 5.1 Surrogate Spiking Solution

A surrogate solution is made by weighing appropriate amounts of pure compounds into a volumetric flask and diluting to volume with methylene chloride (or by purchase of a certified standard (NIST) or equivalent). Surrogates are added to the samples prior to extraction at a concentration of approximately 10 x the MDL. If higher concentrations of hydrocarbons are anticipated, the surrogate concentration can be appropriately increased.

The compounds in the surrogate solution are deuterated aromatics (Table 1). The concentration of the surrogate solution requires the addition of 100 $\mu$L to the extract, leading to a surrogate final concentration of 40 ng/mL in the final extract volume. All sample target compound concentrations are corrected for surrogate recoveries.

### 5.2 Internal Standard Solutions

A solution containing two internal standards at 20 $\mu$g/mL is prepared based on weight from a certified standard (NIST or equivalent). The stock solution is transferred to a volumetric flask and diluted to volume with methylene chloride. The internal standards are deuterated aromatics (Table 1). Sufficient internal standard solution is added to the extract just prior to instrumental analysis to give a final concentration of 40 ng/mL in the final extract volume.

### 5.3 Spiking Solution

A solution containing selected PAHs is used to fortify blank spikes and matrix spike samples. A certified solution is purchased from a commercial vendor and diluted with methylene chloride to the appropriate working concentration. Dibenzothiophene is weighed neat and added to the spiking solution to make a final concentration of about 1.0 $\mu$g/mL. The spiking solution is added to give a final concentration of approximately 10 x the MDL. If higher concentrations of hydrocarbons are anticipated, the matrix spike can be appropriately increased.

### 5.4 GERG Standard Check Solution

A solution of a laboratory reference oil is analyzed as an instrument standard check solution with each analytical batch. The concentration of oil is approximately 0.8 mg/mL. The oil is weighed into a volumetric flask and brought to volume with methylene chloride.

## 6.0 INSTRUMENT CALIBRATION PROCEDURE

### 6.1 Initial Calibration

Initial calibration is required before any sample is analyzed for PAHs. Initial calibration is also required if the analysis of a calibration verification standard does not meet the required criteria listed in Section 3.1.1.

**6.1.1** Tune the instrument with PFTBA as described in Section 3.1.1.

**6.1.2** Using the same GC and MS conditions that produced acceptable tune results, analyze a 2 $\mu$L portion of each of the five calibration

solutions once, and demonstrate that the following conditions are met.

**6.1.2.1** The ratio of integrated ion currents for the quantitation and confirmation ions appearing in Table 3 must be within ±30% the indicated percent relative abundance established for each compound.

**6.1.2.2** The ratio of integrated ion currents for the quantification and confirmation ions belonging to the surrogates and the internal standards must be within the ±30% of the relative abundance stipulated in Table 3.

Note: All ratios must be within the specified acceptance limits simultaneously in one run. Otherwise, corrective action is necessary.

**6.1.4** For each injection, calculate the relative response factors (RRF) for target compounds relative to their appropriate quantitation standards according to Equation 1.

The following formula is used to calculate the response factors (RRF) of target compounds relative to its reference surrogate in the calibration standards.

Equation 1. $RRF = (A_s C_{su})/(A_{su} C_s)$

where:

$A_s$ = Area of the quantitation ion for the target compound.
$A_{su}$ = Area of the quantitation ion for the surrogate.
$C_{su}$ = Concentration of the surrogate (ng/$\mu$L).
$C_s$ = Concentration of the target compound to be measured (ng/$\mu$L).

Note: The response factors for the multi-analyte groups are assumed to be that of the parent compound.

**6.1.5** Calculate the mean response factors ($\overline{RRF}$s) for the five calibration solutions using Equation 2.

Equation 2. $$\overline{RRF} = \left(\frac{1}{5}\right) \sum_{j=1}^{5} RRF_j$$

where:

j = the injection number or calibration solution number (j = 1 to 5).

**6.1.6** Determine the respective percent relative standard deviation for each compound in the calibration standards (%RSD) by dividing the standard deviation by the mean response factor and multiplying the result by 100. Document that the initial calibration meets all of the acceptance criteria outlined in Section 3.4.1.

### 6.2 Continuing Calibration Verification

Continuing calibration verification must be performed at the beginning of an analytical sequence following successful MS tune except when following an initial calibration. A continuing calibration verification is also required at the end of a 12 hour analysis period or at the end of the analytical sequence, whichever is more frequent.

**6.2.1** Using the same GC and MS conditions that were used for the initial calibration, analyze a 2 $\mu$L portion of the 250 ng/mL calibration solution and evaluate it with the following QC acceptance criteria.

**6.2.1.1** The ratio of integrated ion currents for the quantitation and confirmation ions appearing in Table 3 must be within the ±30% of the relative abundance established for each target compound.

**6.2.1.2** The ratio of integrated ion currents of the quantitation and confirmation ions for the surrogate and internal standards

must be within the ±30% of the relative abundance stipulated in Table 3.

**NOTE:** All ratios must be within the specified acceptance limits simultaneously in one run. Otherwise, corrective action is necessary.

**6.2.2** Calculate the daily response factor for the target compounds using Equation 1.

**6.2.3** These daily response factors for each compound are then compared to the mean response factors from the initial calibration curve. The percent difference is calculated using the following equation:

$$\text{Percent Difference} = \frac{(\overline{RRF} - RFC) \times 100}{\overline{RRF}}$$

where:

$\overline{RRF}$ = Mean response factor from initial calibration.

RFC = Response factor from current verification check standard.

The QC acceptance criteria requires that the average daily response factors for all analytes must be within ±15% of the calibration value for the analyses to proceed. If, for any single analyte, the daily response factor exceeds ±25 percent of the calibration value, the five point calibration must be repeated prior to further analysis.

## 7.0 REQUIRED SAMPLE DOCUMENTATION AND IDENTIFICATION

Copies of the following documents, if applicable, must accompany the sample set in a labeled folder when it is delivered to GC/MS analysis group:

Chain-of-custody documents
Sample Information Sheet(s)
Analysis Request Form(s)
Laboratory bench sheet
Dry weight bench sheet
Percent lipid bench sheet for tissue samples
Sample Action Request Form(s)

## 8.0 SAMPLE ANALYSIS

**8.1** Tune the instrument with PFTBA as described in Section 3.1.1.

**8.2** Inject 2 $\mu$L of methylene chloride as an instrument blank, and acquire SIM mass spectra data as described in Section 4.1. Demonstrate and document that the analytical system is free from interfering contamination.

**8.3** Inject 2 $\mu$L of the GERG Standard Check Solution and acquire SIM mass spectral data as described in Section 4.1. Demonstrate and document that the retention time windows have been established (Section 3.3) and the criteria listed in Section 3.4.3 are met.

**8.4** Inject 2 $\mu$L of the calibration verification standard (250 ng/mL) and acquire SIM mass spectral data as described in Section 4.1. Demonstrate and document that the criteria listed in Section 3.4.2.1 are met.

**8.5** Inject 2 $\mu$L of the sample extract and acquire SIM mass spectral data under the same conditions that have been established to produce acceptable results.

### 8.6 Qualitative Identification

For a gas chromatographic peak to be identified as a target compound, it must meet all of the criteria specified in Section 3.2 for single analyte compounds and Section 3.3 for multiple analyte compounds.

## 8.7 Quantitative Determination

**8.7.1** For gas chromatographic peaks that have met all the qualitative identification criteria, calculate the concentration of the target compounds using Equation 3.

Based on these response factors, sample extract concentrations for each analyte is calculated using the following formula:

Equation 3. $$C = \frac{(A_S)(C_{SU})}{(A_{SU})(\overline{RRF})\,(Sa)}$$

where:

$C$ = Concentration in sample (ng/gram or ng/liter).
$Sa$ = Sample amount (grams, liters).
$A_S$ = Area of the quantitation ion for the target compound to be measured.
$A_{SU}$ = Area of the quantitation ion for the surrogate.
$C_{SU}$ = Amount of surrogate added to each extract (ng).
$\overline{RRF}$ = Average response factor

**8.7.2** Calculate the percent recovery of the five surrogate quantitation standards in the sample extract using Equation 4.

Equation 4. $$\%\ \text{recovery} = \frac{(A_{SU} \times C_{IS})}{(C_{SU} \times A_{IS} \times \overline{RRF}_{SU})}$$

where:

$A_{IS}$ = Area of the quantitation ion for the appropriate internal standard
$A_{SU}$ = Area of the quantitation ion for the surrogate
$C_{SU}$ = ng of deuterated surrogate added to the sample
$C_{IS}$ = ng of deuterated internal standard added to the sample extract

$\overline{RRF}_{SU}$ = Average response factor for the surrogate based on the internal standard from the initial calibration.

The laboratory will take corrective action whenever the recovery of any surrogates is less than 40% or greater than 120%.

**8.7.3** If the concentration in the final extract of any of the target compounds exceeds the upper method calibration limit, the sample extract must be diluted by an appropriate dilution factor and reanalyzed. Detection limits must also be adjusted to compensate for sample dilution.

**8.7.4** Calculate and report the Relative Percent Difference (RPD) between duplicate sample results.

**8.7.5** Calculate and report the % Recovery of target compounds in the Matrix Spike (MS), Matrix Spike Duplicate (MSD), and, if analyzed, in the Laboratory Blank Spike (LBS) samples.

## 9.0 INSTRUMENT MAINTENANCE

### 9.1 Gas Chromatograph Maintenance

**9.1.1** The syringe is cleaned by rinsing with appropriate solvent after each injection.

**9.1.2** A new injection port liner and septum are installed at the beginning of each new run sequence.

**9.1.3** A new injection port base plate is installed as needed.

**9.1.4** One to two feet of the analytical column are removed as needed. This is necessary when there is significant tailing of the peak shapes.

**9.1.5** The tank of carrier gas (He) is replaced when the pressure falls below 500 psi.

**9.1.6** All instrument maintenance is recorded in the maintenance log for the specific instrument.

### 9.2 Mass Spectrometer Maintenance

**9.2.1** The emission filament is replaced as necessary.

**9.2.2** The source assembly is cleaned and replaced as necessary.

**9.2.3** The PFTBA reservoir is refilled as necessary.

**9.2.4** The transfer line/re-entrant assembly is disassembled, cleaned or repaired and reassembled as necessary.

**9.2.5** The rotary pump oil is changed yearly, or more frequently if indicated.

**9.2.6** The diffusion pump oil is changed as necessary.

**9.2.7** All maintenance is recorded in the maintenance log for the specific instrument.

## 10.0 DOCUMENTATION REQUIRED FOR ANALYTICAL RESULTS

**10.1** All injections and analytical run sequences are recorded on a computer printout kept on file at the instrument.

**10.2** For all Initial Calibrations the following documentation is printed and maintained for a period of at least one year.

**10.2.1** The Selected Ion Current Profile (SICP) for each ion in each calibration run including any manual integrations.

**10.2.2** Listing of retention times and peak areas for all target analytes in each calibration run.

**10.2.3** Listing of the calculated Relative Response Factors (RRF) for all target compounds in each calibration run.

**10.2.4** Listing of the calculated Average Relative Response Factors ($\overline{RRF}$) for all target compounds, the standard deviation and percent relative standard deviation for each $\overline{RRF}$.

**10.3** For all Calibration Verifications, the following documentation is printed and maintained for a period at least one year.

**10.3.1** The Selected Ion Current Profile (SICP) for each ion in the calibration run including any manual integrations,

**10.3.2** Listing of retention times and peak areas for all target compounds in the calibration run, and

**10.3.3** Listing of the analyte concentrations for all target compounds in the calibration run.

**10.4** For all methylene chloride instrument blanks the following documentation is printed and maintained for a period of at least one year.

**10.4.1** The Selected Ion Current Profile (SICP) for each ion in the blank run demonstrating that the analytical system is free from contaminating interferences.

**10.5** For all analytical and laboratory Quality Control samples the following documentation is printed and maintained for a period of at least one year (actual retention of analytical data is determined by the contract guidelines, but shall not be less than one year).

**10.5.1** The Selected Ion Current Profile (SICP) for each ion in the analytical run, including any manual integrations,

**10.5.2** Listing of retention times and peak areas for all target compounds in the analytical run,

**10.5.3** Listing of the calculated concentrations of all target compounds, percent recovery of the surrogates, and

**10.5.4** A compiled data report in either the GERG standard format or other customized format requested by the client. The compiled data report includes Relative Percent Difference (RPD) between duplicate analyses or MS/MSD recoveries, and percent recovery of native analytes in LBS and MS/MSD QC samples.

## Table 1. Polynuclear Aromatic Hydrocarbons of Interest.

| Compounds | I.S. Reference | Surrogate Reference | Compounds | I.S. Reference | Reference Surrogate |
|---|---|---|---|---|---|
| Naphthalene | A | 1 | Fluoranthene | B | 3 |
| $C_1$-Naphthalenes | A | 1 | $C_1$-Fluoranthenes[a] | B | 3 |
| $C_2$-Naphthalenes | A | 2 | | | |
| $C_3$-Naphthalenes | A | 2 | Pyrene | B | 3 |
| $C_4$-Naphthalenes[a] | A | 2 | $C_1$-Pyrene | B | 3 |
| Biphenyl | A | 2 | Benzo[a]anthracene | B | 4 |
| Acenaphthylene | A | 2 | | | |
| | | | Chrysene | B | 4 |
| Acenaphthene | A | 2 | $C_1$-Chrysene[a] | B | 4 |
| | | | $C_2$-Chrysene[a] | B | 4 |
| Fluorene | A | 2 | $C_3$-Chrysene[a] | B | 4 |
| $C_1$-Fluorenes[a] | A | 2 | $C_4$-Chrysene[a] | B | 4 |
| $C_2$-Fluorenes[a] | A | 2 | | | |
| $C_3$-Fluorenes[a] | A | 2 | [Benzo[b]fluoranthene Benzo[k]fluoranthene]-sum | B | 4 |
| Dibenzothiophene | A | 3 | Benzo[e]pyrene | B | 4 |
| $C_1$-Dibenzothiophenes[a] | A | 3 | Benzo[a]pyrene | B | 4 |
| $C_2$-Dibenzothiophenes[a] | A | 3 | Perylene | B | 5 |
| $C_3$-Dibenzothiophenes[a] | A | 3 | Indeno[1,2,3-c,d]pyrene | B | 4 |
| | | | Dibenzo[a,h]anthracene | B | 4 |
| Phenanthrene | A | 3 | Benzo[g,h,i]perylene | B | 4 |
| $C_1$-Phenanthrenes | A | 3 | | | |
| $C_2$-Phenanthrenes[a] | A | 3 | Specific Isomers | | |
| $C_3$-Phenanthrenes[a] | A | 3 | | | |
| $C_4$-Phenanthrenes[a] | A | 3 | 1-methylnaphthalene | B | 1 |
| | | | 2-methylnaphthalene | B | 1 |
| Anthracene | A | 3 | 2,6-dimethylnaphthalene | B | 2 |
| $C_1$-Anthracenes[a] | A | 3 | 2,3,5-trimethylnapthalene | B | 2 |
| $C_2$-Anthracenes[a] | A | 3 | 1-methylphenanthrene | B | 3 |
| $C_3$-Anthracenes[a] | A | 3 | | | |
| $C_4$-Anthracenes[a] | A | 3 | Surrogates | | |
| Internal Standards | | | Naphthalene-$d_8$ | (1) | |
| | | | Acenaphthene-$d_{10}$ | (2) | |
| Fluorene-$d_{10}$ | (A) | | Phenanthrene-$d_{10}$ | (3) | |
| Benzo (a) pyrene-$d_{12}$ | (B) | | Chrysene-$d_{12}$ | (4) | |
| | | | Perylene-$d_{12}$ | (5) | |

[a]Alkylated homologues not included in the calibration solution.

NOTE: Alkylated phenanthrenes and anthracenes, and alkylated fluoranthenes and pyrenes are quantified together as total alkylated (Cx) phenanthrene/anthracenes and total alkylated (Cx) fluoranthenes/pyrenes. Only the parent compounds and specific isomers are reported as individual compounds.

Table 2. Summary of QC Requirements for Quantitative Analysis

| Element | Control Limit Criteria | Frequency |
|---|---|---|
| - Instrument Calibration | Minimum of 5 standards; correlation coefficient of $\geq$ 0.99 or % RSD within ± 15% for all target compounds. | Initial and after any failures of continuing calibrations. |
| - Instrument Blank | Instrument free of interfering contamination or perform necessary maintenance. | Prior to analysis of all analytical batches. |
| - GERG Standard Check | Analytes within ± 25% or average of lab certified concentration with no analytes > 35% or recalibrate. | Prior to analysis of all analytical batches. |
| - Continuing Calibration Verification (CCV) | Percent difference for all response factors within ± 15% or average of initial calibration; no single analyte greater than 25% or recalibrate and reanalyze back to last passing CCV. | After daily MS tune; once every 12 hours during the analytical sequence and at end of analytical sequence. |
| - Surrogate Recovery | Recovery of 40 to 120% for all surrogates. See Section 3.5.6 for corrective actions. | All samples. |
| - Method Blank | No analytes > 3x MDL. See Section 3.5.1 for exceptions to need for re-extraction. | One per QC batch. |
| - Duplicates (if applicable) | RPD $\leq$ 25% for all analytes > 10 x MDL. See Section 3.5.4 for corrective action. | One per QC batch. |
| - Matrix Spike, Matrix Spike Duplicate (if applicable) | % recovery within 40 to 120%. RPD for the spike recoveries should be $\leq$ 25% for all analytes. See Sections 3.5.3 for corrective actions. | One per QC batch. |
| - Standard Reference Material (if applicable) | Recovery of 80% of certified or non-certified compounds within 30% of certified range for those analytes > 10x MDL. See Section 3.5.5 for corrective action. | One per QC batch. |

Table 3. Parameters for Target Analytes.

| Compound | Quant. Ion | Conf. Ions | % Rel. Abund. of Conf. Ions |
|---|---|---|---|
| $d_8$-Naphthalene | 136 | 134 | 15 |
| Naphthalene | 128 | 127 | 15 |
| $C_1$-Naphthalenes (including isomers) | 142 | 141 | 80 |
| $C_2$-Naphthalenes | 156 | 141 | NA |
| $C_3$-Naphthalenes | 170 | 155 | NA |
| $C_4$-Naphthalenes | 184 | 169,141 | NA |
| $d_{10}$-Acenaphthene | 164 | 162 | 95 |
| Acenaphthylene | 152 | 153 | 15 |
| Biphenyl | 154 | 152 | 30 |
| Acenaphthene | 154 | 153 | 98 |
| $d_{10}$-Fluorene | 176 | 174 | 85 |
| Fluorene | 166 | 165 | 95 |
| $C_1$Fluorenes | 180 | 165 | NA |
| $C_2$-Fluorenes | 194 | 179 | NA |
| $C_3$-Fluorenes | 208 | 193 | NA |
| $d_{10}$-Phenanthrene | 188 | 184 | 15 |
| Phenanthrene | 178 | 176 | 20 |
| Anthracene | 178 | 176 | 20 |
| $C_1$-Phenanthrenes/anthracenes | 192 | 191 | NA |
| $C_2$-Phenanthrenes/anthracenes | 206 | 191 | NA |
| $C_3$-Phenanthrenes/anthracenes | 220 | 205 | NA |
| $C_4$-Phenanthrenes/anthracenes | 234 | 219,191 | NA |
| Dibenzothiophene | 184 | 152,139 | 15 |
| $C_1$-Dibenzothiophenes | 198 | 184,197 | NA |
| $C_2$-Dibenzothiophenes | 212 | 197 | NA |
| $C_3$-Dibenzothiophenes | 226 | 211 | NA |
| Fluoranthene | 202 | 101 | 15 |
| $d_{12}$-Chrysene | 240 | 236 | 30 |
| Pyrene | 202 | 101 | 15 |
| $C_1$-Fluoranthenes/pyrenes | 216 | 215 | NA |
| Benzo [a] anthracene | 228 | 226 | 20 |

NA = Not Applicable

Table 3. (Cont.)

| Compound | Quant. Ion | Conf. Ions | % Rel. Abund. of Conf. Ions |
|---|---|---|---|
| Chrysene | 228 | 226 | 30 |
| $C_1$-Chrysenes | 242 | 241 | NA |
| $C_2$-Chrysenes | 256 | 241 | NA |
| $C_3$-Chrysenes | 270 | 255 | NA |
| $C_4$-Chrysenes | 284 | 269,241 | NA |
| $d_{12}$-Benz (a)pyrene | 264 | 260 | 20 |
| Benzo [b] fluoranthene | 252 | 253,125 | 30, 10 |
| Benzo [k] fluoranthene | 252 | 253, 125 | 30, 10 |
| Benzo (e) pyrene | 252 | 253 | 30 |
| Perylene | 252 | 253 | 20 |
| $d_{12}$-Perylene | 264 | 260 | 22 |
| Benzo [a] pyrene | 252 | 253, 125 | 30, 10 |
| Indeno[1,2,3-c,d]pyrene | 276 | 277, 138 | 25,30 |
| Dibenzo [a,h] anthracene | 278 | 279, 139 | 25,20 |
| Benzo [g,h,i]pery1ene | 276 | 277, 138 | 25,20 |

NA = Not Applicable

Table 4. PAH Matrix Spike Compounds in $CH_2Cl_2$.

| Compound | Spiking Solution Concentration ($\mu$g/mL) |
|---|---|
| Naphthalene | 1.030 ± 0.10 |
| 1-Methylnaphthalene | 1.24 ± 0.5 |
| 2-Methylnaphthalene | 1.18 ± 0.04 |
| Biphenyl | 1.046 ± 0.04 |
| 2,6-Dimethylnaphthalene | 1.08 ± 0.4 |
| Acenaphthylene | 1.040 ± 0.07 |
| Acenaphthene | 1.089 ± 0.15 |
| 2,3,5-Trimethylnaphthalene | 0.99 ± 0.4 |
| Fluorene | 1.087 ± 0.08 |
| Dibenzothiophene | ~1.000 |
| Phenanthrene | 1.048 ± 0.07 |
| Anthracene | 1.169 ± 0.06 |
| 1-Methylphenanthrene | 1.04 ± 0.3 |
| Fluoranthene | 0.884 ± 0.06 |
| Pyrene | 0.881 ± 0.08 |
| Benz[a]anthracene | 7.85 ± 0.05 |
| Chrysene | 1.050 ± 0.06 |
| Benzo[b]fluoranthene | 0.785 ± 0.05 |
| Benzo[k]fluoranthene | 0.833 ± 0.12 |
| Benzo[e]pyrene | 0.840 ± 0.04 |
| Benzo[a]pyrene | 1.014 ± 0.09 |
| Perylene | 1.065 ± 0.06 |
| Indeno[1,2,3-cd]pyrene | 0.940 ± 0.07 |
| Dibenz[a,h]anthracene | 0.774 ± 0.18 |
| Benzo[ghi]perylene | 0.790 ± 0.13 |

# SOP-9804

# EXTRACTING SEDIMENT SAMPLES USING ACCELERATED SOLVENT EXTRACTOR FOR SURFACE PROSPECTING ALIPHATIC AND AROMATIC HYDROCARBON ANALYSES

Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURES SOP-9804

# EXTRACTING SEDIMENT SAMPLES USING ACCELERATED SOLVENT EXTRACTOR FOR SURFACE PROSPECTING ALIPHATIC AND AROMATIC HYDROCARBON ANALYSES

This document presents the procedures, materials, and quality control used in the performance of the above preparation activities.

Quality Assurance Manager _____ Date _____

Author/Revision By: Y. Qian

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# EXTRACTING SEDIMENT SAMPLES USING ACCELERATED SOLVENT EXTRACTOR FOR SURFACE PROSPECTING ALIPHATIC AND AROMATIC HYDROCARBON ANALYSES

## 1.0 PURPOSE

This document provides the procedures for the extraction of sediment samples using the Accelerated Solvent Extractor (ASE) and the subsequent concentration of the extracts for surface prospecting purposes which are used by the staff of the Geochemical and Environmental Research Group (GERG) of the College of Geosciences at Texas A&M University.

### 1.1 SUMMARY OF THE METHOD

This procedure uses matrix-specific extraction and analyte-specific concentration steps to allow the determination of aliphatic and aromatic hydrocarbons using total scanning fluorescence and gas chromatography (GC).

The sediment samples are weighed and placed into extraction cells. The desired solvent is placed into the solvent reservoir of the ASE 200. The method for extraction is selected and initiated. The extract is released into collection vials. The extracts are then concentrated using rotary evaporation or the Turbovap II automated concentration unit. The concentrated extract is transferred into 7 mL vials and submitted for analysis.

The analysis of surface prospecting samples are conducted according to the appropriate GERG SOP.

### 1.2 APPLICATION

**1.2.1** The extraction method described in this standard operation procedure is applicable to dried sediment samples that require measurement of aliphatic hydrocarbons and aromatic hydrocarbons for surface prospecting activities.

## 2.0 SAFETY

The hazards, toxicity or carcinogenicity of each compound or reagent used in GERG' s standard operating procedures have not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory maintains Material Safety Data Sheets (MSDS) which contain information regarding the safe handling of chemicals used at GERG' s facilities. A reference file of MSDS is available to all personnel involved with these materials. All laboratory personnel should direct any questions regarding safety issues to their supervisors or the Safety Officer.

## 3.0 QUALITY CONTROL REQUIREMENTS

Due to the nature of surface prospecting activities, the quality control samples routinely used at GERG include a method blank (BLANK) and a laboratory blank spike (LBS) per extraction batch. The client may also request duplicate analyses.

### 3.1 Method Blank (BLANK)

A Method Blank is used to demonstrate that the analytical method is free of contaminating interference. The BLANK is prepared by executing all of the specified extraction and extract purification steps except for the introduction of a sample. The BLANK is spiked with Surrogate Standard Solution (SU) and the Internal Standard (IS) at the appropriate stages of the preparation.

### 3.2 Laboratory Blank Spike (LBS)

A Laboratory Blank Spike is used to demonstrate accuracy of the method. It is prepared by executing all of the specific extraction and extraction purification steps except for the introduction of a sample. The LBS is spiked with the Surrogate Standard Solution (SU), the GERG Standard Oil, and the Internal Standard (IS) at the appropriate stages of the preparation.

### 3.3 Duplicate (DUP)

A sample Duplicate is used to demonstrate matrix homogeneity and analytical precision in the presence of a representative matrix. A DUP is prepared by executing all of the specified extraction and purification steps on replicate portions of selected sample. The DUP is spiked with the Surrogate Standard Solution (SU) and the Internal Standard (IS) at the appropriate stages of the preparation.

## 4.0 APPARATUS AND MATERIALS

### 4.1 Glassware and Hardware

The following laboratory glassware and hardware is needed to perform the extraction and concentration procedures:

Stainless steel forceps
Vials and Teflon-lined Caps: 7 mL borosilicate
Flat-bottomed Flasks: 250 mL
Collection Vials and Caps : 60 mL capacity
Beakers: 50 mL
TurboVap concentrator tubes: 200 mL with 1 mL stem, Zymark.

### 4.2 Instrumentation

Accelerated Solvent Extractor: ASE 200, Dionex
Stainless Steel Extraction Cells and Caps : 22 or 33 mL capacity, Dionex
Rotary-Evaporator: Buchi, model R110
Balance: Top Loading, 0.001 g accuracy
TurboVap II with 1 mL end-point sensor, Zymark.

## 5.0 REAGENTS AND CONSUMABLE MATERIALS

### 5.1 Reagents

**5.1.1** Hydrochloric Acid: 38%; VWR Scientific, Cat. HX0603-3 or equivalent.

**5.1.2** Solvents: Equivalent solvents from other source may be used after lot testing.

Methylene Chloride: Burdick and Jackson; Cat# 300-4, High Purity, Pesticide grade or equivalent.

Hexane: Burdick and Jackson; Cat# GC60394-4, Capillary GC/GC-MS solvent or equivalent.

Acetone: Burdick and Jackson; Cat# 010-4, High purity solvent or equivalent.

Methanol: Burdick and Jackson; Cat# 230-4, pesticide grade or equivalent.

**5.1.3** Nitrogen Gas: Compressed nitrogen.

**5.1.4** Sand: White quartz, Sigma, combusted at 440°C for 4 hours.

**5.1.5** Copper, Granular: 20-30 mesh: J.T. Baker.

**5.1.6** Glass Microfibre Filters; GF/B: 21mm; Whatman Cat# 1821-021, Combusted at 440°C for 4 hours.

## 5.2 ANALYTICAL STANDARDS

Analytical standards are prepared according to GERG SOP. When not in use, standards are stored at 4°C in a refrigerator.

### 5.2.1 Surrogate

SP-SU-XX, where X represents specific aliquot numbering.

### 5.2.2 Spike

GERG STANDARD OIL

### 5.2.3 Internal Standard

SP-IS-XXXX, where X represents specific aliquot numbering.

## 6.0 EXTRACTION PROCEDURE

### 6.1 ASE Equipment Preparation

#### 6.1.1 Preparation of Activated Copper

**6.1.1.1** Pour desired amount of granular copper into a beaker. Add enough amount of 1:1 diluted hydrochloric acid to the beaker to cover the copper. Dilute the acid in the hood by slowly adding an equal volume of acid to water with stirring. Let the copper stand in acid for approximately 2-5 minutes.

**6.1.1.2** Slowly decant the acid into the acid container. Add baking soda into another beaker. Slowly decant the acid to the beaker containing baking soda. Add water to the copper, stir, and decant the water onto the baking soda. Continue the process until the acid is neutralized (no bubbling on addition to baking soda).

**6.1.1.3** Wash the copper with methanol three times, or until the methanol wash is clear, by adding methanol into the beaker and stirring. Decant the methanol into an appropriate waste container.

**6.1.1.4** Wash the copper with methylene chloride three times, or until the methylene chloride wash is clear, by adding methylene chloride to the beaker and stirring. Decant the methylene chloride into an appropriate waste container.

#### 6.1.2 Clean Sample Extraction Cells

Disassemble the extraction cell by unscrewing the cap. Wash the extraction cell tube with soap and water using a brush. After rinsing with water, rinse the inside of the tube with acetone followed by methylene chloride.

#### 6.1.3 Cell Caps

Sonicate the cell caps in methylene chloride/methanol for 5-10 minutes. Before assembling, carefully check the inside of the caps. Make sure there is no sand, copper, sediment, or any other residual dirt inside the cap, particularly around the brown PEEK seal. If there is any, disassemble the cap and clean it.

### 6.2 Weighing Samples

#### 6.2.1 Calibrate Balance

**6.2.1.1** Tare the balance. Place a standard weight (100 g) on the weighing pan. Press CAL button. Record the weight displayed. If the weight differs from the standard by 0.005 g, re-calibrate the balance by pressing CAL button. If the reading is still out of the range of 99.995 g to 100.005 g, notify your supervisor.

**6.2.2** Assemble the extraction cell body tube and bottom cap, hand tight.

**6.2.3** Insert a combusted filter into the cell and push down with a rod. Make sure the filter is flat and covers the bottom fully.

**6.2.4** Using a funnel, add one scoop of the activated copper to the extraction cell.

**6.2.5** Place a small beaker (50mL) onto the weighing pan of the calibrated balance and tare.

**6.2.6** Add approximately 5 grams of combusted sand to the tared beaker if using 22 mL cells, or add 10 grams sand if using 33 mL cells.

**6.2.7** Tare the balance again and weigh approximately 15.0 grams of dried sample in the beaker. Mix the sample and the sand together by stirring.

**6.2.8** Pour mixture into the extraction cell through a funnel and fill the rest of the cell with sand up to about 1-2 cm below the top of the cell.

**6.2.9** Prepare a Blank and Spiked Blank sample by preparing a cell filled with sand along with activated copper and filter.

**6.2.10** Rinse a micropipette in the hood with methylene chloride at least five times. Spike all samples with appropriate amount of the Surrogate (SP-SU-XX).

**6.2.11** Rinse the micropipette five times again and spike the Spiked Blank with appropriate amount of GERG STANDARD OIL.

**6.2.12** Insert a filter on top of the sample and screw the top cap onto the cell hand tight.

## 6.3 Sample Extraction

**6.3.1** Place the assembled extraction cells onto the top cell tray on the ASE 200 in numerical order according to slots.

**6.3.2** Place labeled collection vials in the bottom collection tray to coincide with the top tray.

**6.3.3** Fill the Solvent Reservoir with hexane.

**6.3.4** Make sure the solvent waste/rinse collection vial inside the chamber of the reservoir is empty, as well as the rinse vial on the sample collection tray (R1, R2).

**6.3.5** Press ***RINSE*** button on the control panel.

**6.3.6** As the status returns to ***IDLE*** after instrumental rinse, press ***MENU*** and choose the first selection ***LOAD METHOD/SCHEDULE*** to designate the method. Enter the desired method number, press

***ENTER,*** and then press ***START.*** This will start the instrument extraction beginning with the first extraction cell.

The conditions used for the extraction of the sediment samples for surface prospecting are:

| | |
|---|---|
| Temperature | 100° C |
| Pressure | 1500 psi |
| Heating time | 4 minutes |
| Flush volume | 30% |
| Cycles | 2 |

If required, the extraction may be started from cells other than the first cell. If so desired, press ***MENU*** and choose the first selection ***LOAD METHOD/SCHEDULE*** to designate the method. Move the cursor to the third entry field and enter the vial number you wish to begin with. Move the cursor to ***METHOD NUMBER*** and enter the method number. Press ***ENTER*** . Press ***START*** and the extraction process should begin.

While the extraction is in progress, check the collected solvent volume in the sample collection vial. The vial should be more than half full if using the larger cells (33 mL) and less than to half full when using the smaller cells (22 mL). Check for leaking cells by listening to the pump action. If the pump is continuously activated while the machine is in ***STATIC,*** or if the pressure reading is constantly below the setting (1500 psi) then there is probably a leak. If this occurs, stop the extraction by pressing the ***ABORT*** button on the control panel.

## 6.4 Concentration

### 6.4.1 Rotary Evaporation

**6.4.1.1** The extraction of one full set (24 samples) will take approximately 7 hours to complete. After the completion of the extraction, remove the collection vials from the extractor. Transfer the extract to a 250 mL flat

bottom flask. Rinse the collection vial three times with hexane and combine the rinses with the extract.

**6.4.1.2** Concentrate the extract using a rotary evaporator to about 2 to 3 mL.

**6.4.1.3** Transfer the concentrated extract to a labeled 7 mL vial. Rinse the flask with hexane three times and combine the rinses with the extract.

**6.4.1.4** Using hexane, adjust the final volume in the 7 mL vial to about the shoulder of the vial (about 7 mL).

**6.4.1.5** Write the weight of the sample, batch # on the label of the 7 mL vial. The sample extract is then submitted for instrumental analysis.

### 6.4.2 TurboVap Concentration

**6.4.2.1** The sample extracts can be concentrated by an automated TurboVap unit. Transfer the extract to a 200 mL TurboVap concentrator tube.

**6.4.2.2** Rinse the sample vial with hexane three times and add the rinses to the concentrator tube. Turn on the power of the TurboVap unit. The temperature of the TurboVap is set at 50°C.

**6.4.2.3** Check the water level in the TurboVap. The water level should be just below the surface of the tube rack. If the water level is low, add water to the correct level.

**6.5.2.4** Place the concentrator tubes with sample extracts in the TurboVap. Up to 6 samples can be fitted at one time.

**6.5.2.5** Close the cover of the TurboVap and turn on the $N_2$ stream by pressing the corresponding cell button, e.g., press Cell 1 if there is a concentrator tube in the location one in the TurboVap. Pressing

the cell number again will turn off the $N_2$ stream. When the $N_2$ stream is on, a light indicator will be on for that cell.

**6.5.2.6** Check the sample extract level periodically. Take out the concentrator tube when the sample extract is concentrated to 2-3 mL. If the sample volume reaches 1 mL, the $N_2$ stream will be automatically turned off and an alarm will beep.

**6.5.2.7** Transfer the concentrated sample extract to a 7 mL vial. Rinse the concentrator tube with hexane 2-3 times and added the rinses to the vial.

**6.5.2.8** Using hexane, adjust the final volume in the 7 mL vial to about the shoulder of the vial (about 7 mL).

**6.5.2.9** Write the weight of the sample, batch # on the label of the 7 mL vial. The sample extract is then submitted for instrumental analysis.

## 7.0 DOCUMENTATION REQUIREMENTS

Sample Analysis Request Form
Sample Information Sheets
Laboratory Sample Logbook Sheets
Sample Action Request Form, when applicable

# SOP-9901

# CONFIRMATION OF ANALYTES USING GAS CHROMATOGRAPHY/MASS SPECTROMETRY WITH THE SELECTED ION MONITORING TECHNIQUE

Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURES

SOP-9901

# CONFIRMATION OF ANALYTES USING GAS CHROMATOGRAPHY/MASS SPECTROMETRY WITH THE SELECTED ION MONITORING TECHNIQUE

This document presents the procedures, materials, and quality control used in the performance of the above instrumental activities.

Quality Assurance Manager

Date

Author/Revision By: G. Ekman, M. Higginbotham, T. Wade, G. Denoux

# CONFIRMATION OF ANALYTES USING GAS CHROMATOGRAPHY/MASS SPECTROMETRY WITH THE SELECTED ION MONITORING TECHNIQUE

## 1.0 PURPOSE

This document provides the procedures used by the staff of the Geochemical and Environmental Research Group (GERG) of the College of Geosciences at Texas A&M University to confirm and quantify selected target compounds in sample extracts using gas chromatography/mass spectrometry (GC/MS) in the selected ion monitoring mode (SIM).

### 1.1 Summary of the Method

When analysis of an extract using gas chromatography techniques identifies organic analytes in high enough concentration, it is sometimes desirable to confirm the identity and concentration of such compounds using a different detector, column or analytical technique. This standard operating procedure describes the use of gas chromatography with a mass spectrometer detector (GC/MS) to confirm analytes initially determined using gas chromatography with an electron capture detector (GC/ECD), a flame ionization detector (GC/FID), or a flame photometric detector (GC/FPD).

Purified extracts from water, sediment, soil, and other sample matrices are initially analyzed for organochlorine pesticides (OCs), polychlorinated biphenyls (PCBs), aliphatic hydrocarbons, or organotins using GC/ECD, GC/FID, or GC/FPD techniques. If analytes are detected after these initial analyses at concentrations that are ten times the limit of detection for routine analyses, selected analytes may be confirmed by GC/MS analysis when required by a project.

The GC/MS operator reviews the data for those samples submitted for GC/MS confirmation analyses. The operator then obtains the original extract and prepares an extract aliquot, adding the required amount of the internal standard and surrogate(s). The extract is then analyzed using GC/MS technique to confirm the identity and concentration of selected analytes. There is no concentration correction based on surrogate recovery during GC/MS confirmation analyses since an additional known amount of internal and surrogate standards have been added during the aliquot preparation processes.

Confirmations are based on the electron impact (EI) ionization mode for mass spectrometry using SIM with three ions monitored (see Table 1) where applicable. An analyte is reported as "CONFIRMED by GC/MS" when the spectrum contains three major ions at the appropriate retention time and in the correct ratio. To report an analyte as "TENTATIVELY CONFIRMED by GC/MS" the spectrum must contain at least two these ions in the correct ratio.

If the identity of a compound cannot be confirmed or tentatively confirmed by mass spectrometry, it is assumed that the result determined using other detectors is due to an interference and the reported data is qualified as an interference.

A concentration estimate accompanies the spectral determination report. This concentration should be within an order of magnitude of the concentration reported using other detectors.

### 1.2 Applicability

GC/MS confirmation is not performed for those analytes found in an extract at concentrations less than the GC/MS instrument detection limits. In addition, GC/MS confirmation may not be feasible when limited quantities of a sample are available for preparation and analysis.

Other related organic compounds may be confirmed using this technique after appropriate instrument calibration. The full scan mode of GC-MS analysis can also be used for confirmation activities, using the same principals included in this standard operating procedure.

### 1.3 Target Analyte List

The target compounds routinely confirmed by this method and the three ions for each compound are listed in Table 1. Other related organic compounds may be confirmed using this technique after appropriate instrument calibration.

### 1.4 Quantitation Standards

The routine standards used for quantitation during GC/MS analyses is PCB 103 for pesticide and PCB confirmation activities, deuterated alkanes for aliphatic hydrocarbons, and tetrapropyl tin for organotin confirmation. Specific standards used for quantitation may vary when confirming routine and non-routine organic analytes.

### 1.5 Detection Limits

Instrument detection limits using calibration standards have been performed to verify chromatographic elution order and non-interference of major ions for target analytes.

Typical reporting limits for organochlorines and aliphatic hydrocarbons confirmation analyses using mass spectrometry are 1 µg/mL (0.1 ppm) for tissue (wet weight basis) and 1 µg/mL (0.1 ppm) for sediment/soil sample (dry weight basis) matrices. Reporting limits for the

most individual PCB congeners is 1 ng/mL (0.1 ppb) in tissue (wet weight basis) or sediment/soil (dry weight basis) sample matrices.

### 1.6 Interferences

The primary purpose for performing GC/MS confirmation is to increase the confidence that a selected analyte is actually present in a sample extract, and that the analytical result is not caused by co-elution of an interfering compound. The secondary purpose is to confirm the concentration initially determined using other detectors.

Method interferences may be caused by contaminants in solvents, reagents, and glassware. All materials utilized in this procedure are routinely demonstrated to have minimal interferences introduced during sample extraction and analytical activities by the preparation and analysis of a method blank with each QC sample batch.

Matrix interferences may be caused by naturally occurring biogenic materials extracted from the sample. The extent of matrix interferences varies considerably depending upon the nature of the sample analyzed. Matrix interferences are minimized by sample purification techniques described in detail in the appropriate GERG SOPs.

## 2.0 SAFETY

The hazards, toxicity or carcinogenicity of each compound or reagent used in GERG's standard operating procedures have not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory maintains Material Safety Data Sheets (MSDS) which contain information regarding the safe handling of chemicals used at GERG's facilities. A reference file of MSDS is available to all personnel involved with these materials. All laboratory personnel should direct any questions regarding safety issues to their supervisors or the Safety Officer.

## 3.0 QUALITY CONTROL

The quality control requirements for confirmation and quantitative analysis are provided in the following sections.

### 3.1 Mass Spectrometer Performance

**3.1.1** Prior to use, the mass spectrometer performance is checked using PFTBA according to manufacturer's tuning procedures. These procedures include the checking of peak widths, mass axis calibration, and relative abundance

of masses 69, 219, and 502 against manufacturer's recommended criteria. Isotope abundances are also checked according to the manufacturer's criteria.

**3.1.2** Total cycle time (dwell times plus switching times) for all ions within a single descriptor must be one second or less.

## 3.2 Analyte Identification Criteria for Target Compounds

### 3.2.1 Retention Times

Qualitative identification is based on a comparison of the retention times and ion current responses of the target compounds contained in reference standards with the retention times of target compounds found in the sample extract. The calibration standards provide the primary retention time information for the target compounds that are included in these standards. In addition, a PCB standard mixture may be used to establish relative retention times (RRT) for PCBs not included in calibration standards. For aliphatic hydrocarbons, a reference oil, the GERG Standard Check, is analyzed to establish the RRT for parent groups found during aliphatic confirmation analyses.

**3.2.1.1** The retention time of the compound in the extract should be within ± 4 seconds of the average retention time established for the authentic compound based upon analysis of the appropriate reference standards.

**3.2.1.2** The ion current responses for ions used for quantitation and confirmation purposes must reach their maxima within ±2 seconds of each other.

**3.2.1.3** The ion current responses for ions used for quantitation and confirmation purposes for labeled standards must reach maximum within ±2 seconds of each other.

### 3.2.2 Qualitative Identification

When possible, the multiple ion scanning in the SIM mode should include three ions for a selected analyte, preferably ions that demonstrate adequate response and are less likely to be subject to interference. The quantitation ion (primary m/z) and the confirmation ions for each target compound must meet the following QC acceptance criteria

**3.2.2.1** The characteristic masses of each target compound must maximize in the same scan or within one scan of each other.

**3.2.2.2** The retention time of target compounds in the sample must fall within ±4 seconds of the retention time for the authentic or representative compound in the reference standards.

**3.2.2.3** The relative peak heights of the three ions selected for a target compound should fall within ±30 percent of the relative intensities of those masses (see Table 1) in a mass spectrum obtained from a reference standard of that target compound run on the same instrument under the same conditions.

**3.2.2.4** The mass chromatographic peaks for the three ions selected for each target compound should be at least three times the background noise.

**3.2.2.5** The three ions should be selected from separate and unique isotopic cluster groups. If they are within a cluster group, the isotopic peaks should be in the correct proportions.

**3.2.2.6** A compound that does not meet secondary ion confirmation criteria may still be determined to be present in a sample after close inspection of the data by the mass spectroscopist. Supportive data includes the presence of the secondary ion having a ratio greater than ±30 percent of the primary ion which may be caused by an interference with the secondary ion.

## 3.3 Calibration Criteria

The most frequent confirmation requests are for organochlorine pesticides. Therefore, the organochlorine calibration procedures and quality control (QC) used for confirmation analysis are described in the following sections. Equivalent QC criteria would apply to the calibration required for confirmation of aliphatic hydrocarbons, organotins, or other non-routine analytes.

### 3.3.1 Initial Calibration

The initial calibration is based on the average response factors for injections of the multiple calibration standards prepared at various concentrations. For the organochlorines, the calibration standard concentrations can be found in the GERG SOP for organochlorine determinations using the GC-ECD technique. The organochlorine calibration standards include

all target pesticide/PCB compounds indicated in Table 1, but do not include all analytes that can be identified and quantified during this analysis.

**3.3.1.1** The QC acceptance criteria for linearity of the initial calibration curve requires that the percent relative standard deviation (RSD) of the response factors for each compound in the multi-point calibration standards must be less than or equal to fifteen percent ($\leq$ 15%). If these RSD criteria are exceeded, the linearity of the calibration curve can be determined to be acceptable if the correlation coefficient (r) determined using least squares regression analysis is greater than or equal to 0.99. The instrument software generates and prints both types of calibration data for all initial calibration determinations, which are maintained in calibration files with associated raw data for that laboratory area.

### 3.3.2 Continuing Calibration Verification (CCV)

Calibration verification must be performed at the beginning of each analytical sequence. Calibration verification is also required at the end of a 12 hour shift and/or at the end of the analytical sequence, whichever is more frequent.

**3.3.2.1** QC acceptance criteria are based upon daily response factors for each compound, which are compared to the mean response factors for the initial calibration curve. If the average daily response factors for all analytes are within ±15% of the calibration value, analyses may proceed. If, for any single analyte, the daily response factor exceeds ±25 percent of calibration value, an initial calibration is repeated before analysis continues and all samples are re-analyzed back to last passing CCV.

### 3.3.3 GERG Standard Check

The GERG Standard Check (a reference oil solution) is analyzed after the CCV with all aliphatic confirmation activities to verify instrument sensitivity and to define retention time windows.

#### 3.3.3.1 Concentration Criteria

After many analyses of the GERG Standard Check solution, concentration range of compounds in this oil have been defined as the average concentration of all previous analyses plus or minus one standard deviation.

The QC acceptance criteria requires that the measured concentration of the standard check compounds must be within ±25% of the laboratory defined concentration on average and not exceed ±35% for any individual analyte.

If, after re-analysis, the concentration for any single target compounds exceeds ±35% of the laboratory defined value, corrective action such as instrument maintenance or a new five point calibration must be prepared prior to further analysis.

### 3.3.4 PCB Standard Mix

When needed, a PCB Standard Mix is analyzed after the CCV with PCB confirmation analyses to establish the relative retention times of PCB target compounds not included in the calibration standards.

#### 3.3.4.1 Concentration Criteria

The QC acceptance criteria requires that the measured concentration of the PCB mix compounds must be within ±25% of the certified concentration on average, and not exceed ±35% for any individual analyte.

If, after re-analysis, the concentration for any single target compounds exceeds ±35% of the laboratory defined value, corrective action such as instrument maintenance or a new five point calibration must be prepared prior to further analysis.

### 3.3.5 Single Point Calibration

For certain routine and non-routine organic analytes, a single point calibration is based on the response factors for three injections in the analytical confirmation sequence of a selected mid-point calibration standard. The initial and final injections of a analysis sequence must be one of

these standards, and the third injection is interspersed with other extracts during the sequence. Verify this calibration using a separate source, e.g., a separate preparation of the standard.

**3.3.5.1** The QC acceptance criteria for the single point calibration requires that the percent relative standard deviation (RSD) of the response factors for each compound in the three injections must be less than or equal to twenty percent ($\leq 20\%$).

## 3.4 Criteria for Instrumental QC Samples

### 3.4.1 Solvent Blank

A methylene chloride solvent blank is analyzed after an initial calibration and the CCV analysis. If the solvent blank contains target compound peaks, the analytical system is out of control. The source of the contamination must be investigated, and corrective measures taken and documented before further analysis proceeds.

## 3.5 Criteria for QC Samples in an Analytical Batch

The acceptance criteria for QC samples are evaluated and determined acceptable within the initial GC/ECD, GC/FID, or GC/FPD analytical group (QC batch). However, if requested, certain QC samples, such as a duplicate or method blank, can be reanalyzed during GC/MS confirmation activities to demonstrate consistency with initial analytical results.

### 3.5.1 Surrogate Compounds and Internal Standards

During initial sample preparation, all samples are routinely spiked with the appropriate surrogate spiking solution to both monitor method performance and for determination of the concentration of target compounds.

When the extracts are prepared for GC/MS confirmation, additional known amounts of internal standard and specific surrogates are routinely added to the extract which has shown high concentrations of certain analytes. The known concentration of added internal standard or surrogate standard is used for quantitation during confirmation analyses of these extracts. The analytical result may be adjusted based upon the surrogate recovery determined during initial analysis using other detectors, and this calculation can be performed if required by the client.

## 4.0 APPARATUS AND MATERIALS

### 4.1 Mass Spectrometer System

The analytical system includes a temperature programmable gas chromatograph (Hewlett-Packard 5890A, or equivalent). The injection port is designed for split or splitless injection, and analyses are conducted in the splitless mode. The autosampler is capable of making 1 to 4 µL injections.

A mass spectrometer operating at 70 eV (nominal) in the electron impact ionization mode and tuned to maximize the sensitivity of the instrument for the mass range 10-700 amu is used for SIM analysis. The GC capillary column is inserted directly into the ion source of the mass spectrometer.

A computer system interfaced to the mass spectrometer continuously acquires and stores mass spectra throughout the duration of the chromatographic program. A mass spectrum can be obtained for each peak of interest utilizing background subtraction techniques. The computer also has software that allows searching GC/MS data files for ions of a specific mass and plotting ion abundances versus run time or scan number. The NBS 75 K Library mass spectral database is also available to the GC/MS operator.

### 4.2 Chromatographic Column

A 30-m long x 0.25-mm I.D. fused silica capillary column with DB-5MS bonded phase, or equivalent is used for routine GC/MS confirmation analyses.

## 5.0 GC/MS OPERATING CONDITIONS

For organochlorine confirmation activities, representative aliquots are injected into the capillary column of the gas chromatograph using the following operating conditions:

### 5.1 Pesticide Confirmation Method

| | |
|---|---|
| **Injector Temp**: | 300°C |
| **Transfer Line Temp**: | 280°C |
| **Initial Oven Temp**: | 100°C |
| **Initial Hold Time**: | 0 min. |
| **Ramp Rate**: | 10°C/min. |
| **Temperature 2**: | 200°C |
| **Hold Time 2**: | 0 min. |
| **Ramp Rate**: | 8°C/min. |

| | |
|---|---|
| **Final Temperature**: | 300°C |
| **Final Hold Time**: | 3 min. |
| **Total Run Time** | 25.5 min. |

### 5.2 PCB Confirmation Method

| | |
|---|---|
| **Injector Temp**: | 300°C |
| **Transfer Line Temp**: | 280°C |
| **Initial Oven Temp**: | 100°C |
| **Initial Hold Time**: | 0 min. |
| **Ramp Rate**: | 10°C/min. |
| **Temperature 2**: | 200°C |
| **Hold Time 2**: | 0 min. |
| **Ramp Rate**: | 5°C/min. |
| **Final Temperature**: | 300°C |
| **Final Hold Time**: | 3 min. |
| **Total Run Time** | 33 min. |

## 6.0 INSTRUMENT CALIBRATION PROCEDURE

### 6.1 Initial Calibration

Initial calibration or calibration verification is required before any extract is analyzed for confirmation of selected target compounds. Initial calibration is also required if the analysis of a calibration verification standard does not meet the required criteria listed in Section 3.2.1.

**6.1.1** Tune the instrument with PFTBA as described in Section 3.1.1.

**6.1.2** Using the same GC and MS conditions that produced acceptable tune results, analyze a 2 µL portion of each of the calibration solutions once, and demonstrate that the following conditions are met.

**6.1.2.1** The ratio of integrated ion currents for the quantitation and confirmation ions appearing in Table 1 must be within ±30% the percent relative abundance established for each compound during reference standard evaluation.

**6.1.2.2** The ratio of integrated ion currents for the quantification and confirmation ions for the internal standards and the surrogate standards must be within the ±30% of the relative abundance

established for each compound during reference standard evaluation.

Note: All ratios must be within the specified acceptance limits simultaneously in one run. Otherwise, corrective action is necessary.

**6.1.3** For each injection, use Equation 1 to calculate the relative response factor (RRF) for each target compound relative to its reference internal standard (or surrogate if applicable) in the calibration standards.

Equation 1. $RRF = (A_s C_{IS})/(A_{IS} C_s)$

where:

$A_s$ = Area of the quantitation ion for the target compound.
$A_{IS}$ = Area of the quantitation ion for the internal standard (or surrogate if applicable).
$C_{IS}$ = Concentration of the internal standard (or surrogate if applicable), ng/µL.
$C_s$ = Concentration of the target compound to be measured (ng/µL).

**6.1.4** For each target compound, calculate the mean response factor ($\overline{RRF}$ s) for the target compounds in the calibration solutions using Equation 2.

Equation 2. $$\overline{RRF} = \left(\frac{1}{n}\right) \sum_{j=1}^{n} RRF_j$$

where:

j = the injection number or calibration solution number (j = 1 to n).

**6.1.5** Determine the respective percent relative standard deviation for each compound in the calibration standards (%RSD) by dividing the standard deviation by the mean response factor and multiplying the result by 100. Document that the initial calibration meets all of the acceptance criteria outlined in Section 3.3.1.

## 6.2 Continuing Calibration Verification (CCV)

Continuing calibration verification must be performed at the beginning of an analytical sequence following successful MS tune. Continuing calibration verification analysis is also required at the end of a 12-hour analysis period or at the end of the analytical sequence, whichever is more frequent.

**6.2.1** Using the same GC and MS conditions that were used for the initial calibration, analyze a 2 µL portion of the continuing calibration solution and evaluate it with the following QC acceptance criteria.

**6.2.1.1** The ratio of integrated ion currents for the ions appearing in Table 1 must be within the ±30% of the relative abundance established for each target compound.

**6.2.1.2** The ratio of integrated ion currents of the quantitation and confirmation ions for the surrogate and internal standards must be within the ±30% of the relative abundance established for each target compound during reference standard evaluation.

**NOTE:** All ratios must be within the specified acceptance limits simultaneously in one run. Otherwise, corrective action is necessary.

**6.2.2** Calculate the daily response factor for the target compounds using Equation 1.

**6.2.3** These daily response factors for each compound are then compared to the mean response factors from the initial calibration curve. The relative percent difference (RPD) is calculated using Equation 3.

Equation 3. $$\text{Relative Percent Difference} = \frac{(\overline{RRF} - RFC) \times 100}{\overline{RRF}}$$

where:

$\overline{RRF}$ = Mean response factor from initial calibration.

RFC = Response factor from current continuing calibration check standard.

The QC acceptance criteria requires that the average daily response factors for all analytes must be within ±15% of the calibration value for the analyses to proceed. If, for any single analyte, the daily response factor exceeds ±25 percent of the calibration value, the calibration must be repeated prior to further analysis.

### 6.3 Reference Standards

After the analysis of the CCV (or the initial mid-point standard for single point calibrations), other reference samples (PCB Mix or GERG Check Standard) are analyzed as needed for the confirmation activity. These analyses must meet the criteria provided in Section 3, and are needed to establish relative retention times for the compounds not included in calibration standards, multi-analyte groups, and peak patterns for multi-analyte groups.

## 7.0 REQUIRED SAMPLE DOCUMENTATION AND IDENTIFICATION

Copies of the following documents, if applicable, should accompany the sample set in a labeled folder when it is delivered to GC/MS analysis group:

Sample Action Request Form(s)
EXCEL printout of the Analyte Concentration Report for the Initial Run Sequence

## 8.0 SAMPLE EXTRACT ANALYSIS

**8.1** If required, complete initial calibration or single point calibration evaluations specified in Section 6.0. Acquire SIM mass spectra data using the operating conditions provided in Section 5.0. The normal injection volume for all standards and sample extracts is 2 µL.

**Note:** If organochlorine extracts have been separated into PCB and Pesticide fractions to improve GC/ECD chromatography, these extracts may need to be re-combined prior to GC/MS confirmation analysis.

**8.2** Inject methylene chloride as an instrument solvent blank, and acquire SIM mass spectra data using the operating conditions described in Section 5.0. Demonstrate and document that the analytical system is free from interfering contamination.

**8.3** Inject the calibration verification standard and acquire SIM mass spectral data using the operating conditions described in Section 5.0. Demonstrate and document that the criteria listed in Section 3.3.2.1 are met.

**8.4** If needed, inject the appropriate reference standard (PCB Mix or GERG Check Standard) and acquire SIM mass spectral data using the operating conditions described in Section 5.0. Demonstrate and document that the applicable criteria listed in Section 3.2 and 3.3 are met for these reference standards.

**8.5** Inject each sample extract and acquire SIM mass spectral data under the same conditions that have been established to produce acceptable results.

**8.6** Inject the calibration verification standard or the single point calibration standard every 10 samples or every 12-hour shift or less, and at the end of the analytical sequence, whichever is more frequent.

**8.7 Qualitative Identification**

For a gas chromatographic peak to be identified as a target compound, it must meet all of the criteria specified in Section 3.2.

**8.8 Quantitative Determination**

**8.8.1** An analyte is reported as "CONFIRMED by GC/MS" when the spectrum contains three specific ions at the appropriate retention time and relative ratios. Because certain aliphatic compounds have only two significant ions, these compounds also require an evaluation of their chromatographic pattern for confirmation analysis. These aliphatic compounds may be reported as confirmed when having two significant ions at both the appropriate retention time and relative ratio within a known chromatographic pattern.

**8.8.2** To report an analyte as "TENTATIVELY CONFIRMED by GC/MS" the spectrum must contain at least two ions at the appropriate retention time and relative ratios.

**8.8.3** If an analyte appears to be present, based on the use of gas chromatography with the electron capture, flame ionization, or flame photometric detectors, at a concentration higher than the limit of detection for mass spectrometry, and the identity of that compound cannot be confirmed or tentatively confirmed by mass spectrometry, it should be assumed that the electron capture or flame ionization result is due to an interference. The analytical report for the target analyte should be adjusted (for all samples controlled by the GC/MS sample in question) to read less

than (<) the former result. The detection limit is redefined as the apparent concentration due to the interference. The reported data for the target compound is also qualified as "Matrix Interference".

**8.8.4** Concentrations of the selected target compounds are determined based on the integrated abundance from the extracted ion current profile (EICP) of the primary characteristic ion as defined in Table 1.

Known concentrations of the internal standard or surrogate standard added to the extracts are used to quantify the selected target compounds being confirmed by GC/MS analysis. There is no concentration correction based on surrogate recovery for dilution results during GC/MS confirmation analyses. This result may be adjusted based upon the surrogate recovery during initial undiluted analysis if required by the client.

**8.8.5** For gas chromatographic peaks that have met all the qualitative identification criteria, calculate the concentration of the target compounds using Equation 4.

Equation 4.
$$C = \frac{(A_S)(C_{IS})}{(A_{IS})(\overline{RRF})(S_a)}$$

where:

$C$ = Concentration in sample (ng/gram or ng/liter).
$S_a$ = Sample amount (grams, liters).
$A_S$ = Area of the quantitation ion for the target compound to be measured.
$A_{IS}$ = Area of the quantitation ion for the internal standard or surrogate.
$C_{IS}$ = Amount of internal standard or surrogate added to each extract (ng).
$\overline{RRF}$ = Average response factor.

## 9.0 INSTRUMENT MAINTENANCE

### 9.1 Gas Chromatograph Maintenance

**9.1.1** The syringe is cleaned by rinsing with appropriate solvent after each injection.

**9.1.2** A new injection port liner and septum are installed at the beginning of each new run sequence.

**9.1.3** A new injection port base plate is installed as needed.

**9.1.4** One to two feet of the analytical column are removed as needed. This is necessary when there is significant tailing of the peak shapes.

**9.1.5** The tank of carrier gas (He) is normally replaced when the pressure falls below 500 psi.

**9.1.6** All instrument maintenance is recorded in the maintenance log for the specific instrument.

### 9.2 Mass Spectrometer Maintenance

**9.2.1** The emission filament is replaced as necessary.

**9.2.2** The source assembly is cleaned and replaced as necessary.

**9.2.3** The PFTBA reservoir is refilled as necessary.

**9.2.4** The transfer line/re-entrant assembly is disassembled, cleaned or repaired and reassembled as necessary.

**9.2.5** The rotary pump oil is changed yearly, or more frequently if indicated.

**9.2.6** The diffusion pump oil is changed as necessary.

**9.2.7** All maintenance is recorded in the maintenance log for the specific instrument.

## 10.0 DOCUMENTATION REQUIRED FOR ANALYTICAL RESULTS

**10.1** All analytical run sequences are recorded on a computer printout kept on file at the instrument.

**10.2** For all Initial Calibrations the following documentation is printed and maintained for a period of at least one year.

**10.2.1** The Selected Ion Current Profile (SICP) for each ion in each calibration run including any manual integrations.

**10.2.2** The listing of retention times and peak areas for all target analytes in each calibration run.

**10.2.3** The listing of the calculated Relative Response Factors (RRF) for all target compounds in each calibration run.

**10.2.4** The listing of the calculated Average Relative Response Factors ($\overline{RRF}$) for all target compounds, the standard deviation and percent relative standard deviation for each $\overline{RRF}$.

**10.3** For all Calibration Verifications, the following documentation is printed and maintained for a period at least one year.

**10.3.1** The Selected Ion Current Profile (SICP) for each ion in the calibration run including any manual integrations,

**10.3.2** The listing of retention times and peak areas for all target compounds in the calibration run, and

**10.3.3** The listing of the analyte concentrations for all target compounds in the calibration run.

**10.4** For all methylene chloride instrument blanks the following documentation is printed and maintained for a period of at least one year.

**10.4.1** The Selected Ion Current Profile (SICP) for each ion in the blank run demonstrating that the analytical system is free from contaminating interferences.

**10.5** For all analytical and laboratory QC samples the following documentation is printed and maintained for a period of at least one year (actual retention of analytical data is determined by the contract guidelines, but shall not be less than one year).

**10.5.1** The Selected Ion Current Profile (SICP) for each ion in the analytical run, including any manual integrations.

**10.5.2** A listing of retention times and peak areas for all target compounds in the confirmation analytical sequence.

**10.5.3** Designation of target compounds that required confirmation as either CONFIRMED by GC/MS, TENTATIVELY CONFIRMED by GC/MS, or NOT CONFIRMED and a listing of the calculated concentrations for those that were CONFIRMED by GC/MS or TENTATIVELY CONFIRMED by GC/MS.

**10.5.4** For those analytes that appeared to be present based on GC/ECD, GC/FID, or GC/FPD but cannot be confirmed or tentatively confirmed by mass spectrometry, it is assumed that the result is due to interference. For all samples controlled by the mass spectrometry confirmation sample extract in question, the GC/ECD, GC/FID, or GC/FPD report should be adjusted to read "less than" (<). The detection limit is also redefined as the apparent concentration due to the interference. Appropriate comments and/or data qualifiers are added to the data fields for that target compound.

Table 1.

| Analyte | Quantitation Ion | Confirmation Ion | Confirmation Ion |
|---|---|---|---|
| **Aliphatics** | | | |
| Deuterated Alkanes - (IS and Su) | 66 | | |
| n-alkanes | 57 | 43 | 71 |
| Pristane, Phytane | 183 | 57 | 43 |
| **PCBs** | | | |
| TCMX (IS) | 244 | | |
| PCB 103 (Su)[1] | 326 | 324 | 254 |
| PCB 198 (Su)[1] | 430 | 428 | 356 |
| PCB Chlorination Level | | | |
| $Cl_1$ | 188 | 190 | 152 |
| $Cl_2$ | 222 | 224 | 152 |
| $Cl_3$ | 256 | 258 | 186 |
| $Cl_4$ | 292 | 290 | 220 |
| $Cl_5$ | 326 | 324 | 254 |
| $Cl_6$ | 360 | 362 | 288 |
| $Cl_7$ | 394 | 396 | 322 |
| $Cl_8$ | 430 | 428 | 356 |
| $Cl_9$ | 464 | 466 | 390 |
| $Cl_{10}$ | 498 | 500 | 424 |
| **Pesticides** | | | |
| TCMX (IS)[1] | 244 | | |
| Alpha-BHC | 219 | 183 | 181 |
| Beta-BHC | 219 | 183 | 181 |
| Gamma-BHC | 219 | 183 | 181 |
| Delta-BHC | 219 | 183 | 181 |
| HCB | 284 | 142 | 249 |
| Heptachlor | 272 | 100 | 237 |
| Heptachlor-Epoxide | 353 | 355 | 81 |
| Oxychlordane | 237 | 372 | 424 |
| Gamma-chlordane | 373 | 375 | 377 |
| Alpha-chlordane | 373 | 375 | 377 |
| Trans-nonachlor | 409 | 407 | 272 |
| Cis-nonachlor | 409 | 407 | 272 |
| Aldrin | 263 | 265 | 66 |
| Dieldrin | 79 | 263 | 108 |
| Endrin | 81 | 263 | 82 |
| Mirex | 272 | 237 | 274 |
| 2,4'DDE | 246 | 248 | 176 |

Table 1. (Cont.)

| Analyte | Quantitation Ion | Confirmation Ion | Confirmation Ion |
|---|---|---|---|
| 4,4'DDE | 246 | 248 | 176 |
| 2,4'DDD | 235 | 237 | 165 |
| 4,4'DDD | 235 | 237 | 165 |
| 2,4'DDT | 235 | 237 | 165 |
| 4,4'DDT | 235 | 237 | 165 |
| Endosulfan I | 195 | 339 | 341 |
| Endosulfan II | 195 | 339 | 341 |
| Dicofol | 139 | 111 | 141 |
| PC Anisole | 280 | 264 | 237 |
| Endosulfan Sulfate | 272 | 387 | 422 |
| **Organotins** | | | |
| Tetrapropyl tin- (IS) | 118 | 120 | 116 |
| Tripropyl monohexyl tin - (Su) | 118 | 120 | 116 |
| Tetrabutyl tin | 118 | 120 | 116 |
| Tributyl monohexyl tin | 118 | 120 | 116 |
| Dibutyl dihexyl tin | 118 | 120 | 116 |
| Monobutyl trihexyl tin | 118 | 120 | 116 |

IS = Internal Standard
SU = Surrogate
1. The same surrogates are used for pesticides and PCBs.

# SOP-0008

# QUANTITATIVE DETERMINATION OF N-ALKANES, PRISTANE, PHYTANE, TOTAL RESOLVED AND UNRESOLVED COMPLEX MIXTURES, AND TOTAL PETROLEUM HYDROCARBONS

Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURES

SOP-0008

# QUANTITATIVE DETERMINATION OF n-ALKANES, PRISTANE, PHYTANE, TOTAL RESOLVED AND UNRESOLVED COMPLEX MIXTURES, AND TOTAL PETROLEUM HYDROCARBONS

This document presents the procedures used in the performance of the above analytical procedures.

Quality Assurance Manager

Date

Author/Revision By: G. Ekman, G. Denoux, J. Sericano

# QUANTITATIVE DETERMINATION OF n-ALKANES, PRISTANE, PHYTANE, TOTAL RESOLVED AND UNRESOLVED COMPLEX MIXTURES, AND TOTAL PETROLEUM HYDROCARBONS

## 1.0 PURPOSE

This document provides the procedures used by the staff of the Geochemical and Environmental Research Group (GERG) of the College of Geosciences at Texas A&M University for the quantitative determination of n-alkanes, pristane, phytane, total resolved and unresolved complex mixtures, and total petroleum hydrocarbons in sample extracts using high resolution capillary gas chromatography and the flame ionization detection (GC/FID).

### 1.2 Summary of the Method

This method was developed for the analysis of extracts from environmental samples using a high resolution capillary gas chromatograph with flame ionization detector (GC/FID). The GC/FID data is used for the quantitative determination aliphatic hydrocarbons (the normal alkanes from n-$C_{10}$ to n-$C_{32}$, pristane, and phytane) as well as several complex mixtures of hydrocarbons.

Other hydrocarbon measurements determined include the estimated values of the total unresolved complex mixture (UCM), the total resolved complex mixture (RCM), and total petroleum hydrocarbons (TPH).

The UCM concentration is determined based on the entire integrated area from n-$C_{10}$ to n-$C_{35}$, and uses the average response factor of all the n-alkanes included in the calibration standards. The UCM value represents the total integrated area of the chromatogram adjusted to remove areas of the RCM, the surrogates and internal standards, and the GC column bleed. Column bleed is defined as the reproducible baseline shift caused by the temperature of the GC oven.

The resolved complex mixture (RCM) concentration represents the sum of all the resolved hydrocarbon peaks including the n-alkanes and isoprenoids, minus all surrogate and internal standard peak areas.

The total petroleum hydrocarbons (TPH) are defined as the sum of the UCM and the RCM concentrations. It is also possible to identify a petroleum source and estimate the extent of degradation for TPH analyses by using specialized standards during calibration and in the analytical sequence.

### 1.2 Applicability

The instrumental procedures described in this document are applicable to the quantitative analysis of extracts obtained from water, sediment, soil, tissue, and other sample matrices after appropriate extraction and purification.

Oil samples, condensates, and other hydrocarbon based fluids can also be analyzed after serial dilution to reduce the concentration of hydrocarbon fluid. The fluid is quantitatively dissolved in methylene chloride. The solution is then quantitatively diluted sufficiently to yield a colorless solution prior to the addition of surrogates and the internal standard used for this analysis.

### 1.3 Target Analyte List

This method is used to quantitatively determine aliphatic hydrocarbons (AH) from n-$C_{10}$ to n-$C_{34}$, including normal alkanes, pristane, and phytane. The UCM, RCM and TPH determinations represent aliphatic, cyclic, aromatic and unsaturated hydrocarbons. The target aliphatic compounds determined by this method, as well as the deuterated surrogates and internal standards used for identification, retention time, and quantitation are listed in Table 1.

### 1.4 Method Detection Limit

The method detection limit (MDL) for aliphatic hydrocarbons in sediment is determined following the procedures outlined in Federal Register (1984), Vol. 49, No. 209: 198- 199.

### 1.5 Minimum Method Performance Criteria

The minimum method performance standard for water is the detection of 10 $\mu$g/L for the complex mixtures and 0.01 $\mu$g/L for individual alkanes and the isoprenoids, pristane and phytane. Criteria for sediments (on a wet weight basis) are 10 $\mu$g/g for the complex mixtures, and 5 ng/g for individual alkane and the isoprenoids, pristane and phytane. The minimum method performance standard for tissue (on a wet weight basis) is 10 $\mu$g/g for the complex mixtures and 10 ng/g for individual alkanes.

## 2.0 SAFETY

The hazards, toxicity or carcinogenicity of each compound or reagent used in GERG's standard operating procedures have not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory maintains Material Safety Data Sheets (MSDS) which contain information regarding the safe handling of chemicals used at GERG's

facilities. A reference file of MSDS is available to all personnel involved with these materials. All laboratory personnel should direct any questions regarding safety issues to their supervisors or the Safety Officer.

## 3.0 QUALITY CONTROL

The quality control requirements for quantitative analysis are summarized in Table 2 with details provided in the following sections.

### 3.1 Analyte Identification Criteria

#### 3.1.1 Qualitative Identification

The retention time of the target analytes must fall within ±0.5 seconds of the retention time windows established from the analysis of the calibration standards. The experienced analyst may use manual peak selection and baseline correction when appropriate.

#### 3.1.2 Reference Standard and Retention Time Index

The reference crude oil, GERG Check Standard, will be analyzed per QC batch of 20 or less samples. The results are used verify instrument performance and to define the retention times for analytical targets, since all compounds are found in this standard. The calibration solutions contain specific components for the aliphatic analysis, but do not contain all components determined.

**3.1.2.1** The laboratory certified concentration range of compounds in the standard check solution has been defined as the average concentration of all previous analyses plus or minus one standard deviation.

**3.1.2.2** The QC acceptance criteria for the measured concentration of the standard check compounds must be within ±25% of the laboratory certified concentration on average for all analytes greater than the minimum performance standard and not exceed ±35% for any individual analyte.

**3.1.2.3** If, after re-analysis, the concentration for any single target compounds exceeds ±35% of the calibration value, corrective action such as instrument maintenance or a new five point calibration must be prepared prior to further analysis.

## 3.2 Instrumental QC

### 3.2.1 Solvent Blank

An instrument solvent blank, methylene chloride, is injected prior to any analytical activity to verify that the instrument components are not contaminated.

In addition, solvent blanks are sometimes used between extract analyses to minimize carryover if high levels of hydrocarbons are present.

**3.2.1.1** The QC acceptance criteria for the instrument blank is the lack of any target compounds or interfering artifacts in this analysis. If contamination is found, the solvent blank is repeated until the contamination is gone or other instrument maintenance is performed such as replacing the column.

### 3.2.2 Initial Calibration and Calibration Checks

Prior to the analyses, a five-point calibration verifies the linearity of the detector. The GC calibration solutions are prepared by dilution of saturated hydrocarbon solutions (see Table 1) having certified purity. Five different concentrations are prepared, with one of the concentration levels near but above the method detection limit (MDL). The remaining concentrations correspond to the expected range of the sample analytes. A concentration range of 1.25 to 50 µg/mL is recommended. When operating properly, the FID detector is linear within this range.

The data acquisition software calculates the response factor and the percent relative standard deviation for each compound in the calibration standards and sample extracts. Each calibration standard is analyzed and the response factor (RF) of each alkane at each concentration level is calculated from the peak area and the concentration (see Section 9). An average response factor for each alkane and the overall average $\overline{RF}$ of all constituents is determined from the calibration data. The calibration is considered acceptable if the percent RSD for the response factor for each constituent is $< \pm 15$ %.

The initial calibration is verified by the measurement of calibration check standards at the beginning of each analytical sequence, after every 8-10 samples and at the completion of the sequence. The daily response factor for these calibration check solutions is determined. The percent difference (see Section 9) between average response factors for the initial calibration solutions and the daily response factors for the calibration check standard is calculated.

If the average percent difference of the response factors for all analytes in the calibration check standard is within ±15 percent of the corresponding calibration value, the analysis may

proceed. If the daily response factor for any individual analyte exceeds ±25 percent of the corresponding calibration value, a five-point calibration must be repeated for that compound prior to the analysis of the samples.

## 3.3 QC Criteria for Sample Extracts in the Analytical Batch

### 3.3.1 Method Blank Analysis

One method blank (also referred to as a procedural blank) is prepared with every 20 samples or with every QC batch, whichever is more frequent. The blank is prepared using all reagents and procedures for extraction, but does not contain any matrix.

The QC acceptance criteria for the method blank requires that it does not contain any target compounds at concentrations greater than three times the method detection limit (MDL). If the method blank does not meet these criteria, the analytical system is out of control and the source of the contamination must be investigated, corrective measures taken, and documented before further sample analysis.

The procedural blank extract may be reanalyzed. If the experienced analyst determines that after reanalysis of the extract contains no target analytes exceeding the stated limits, or that non-target compounds will not affect results, then the analytical data for the QC batch is reported.

If upon reinjection QC criteria are still not met, the entire QC batch may be submitted for re-extraction if sufficient sample is available. If the sample was completely consumed, the data will be reported but designated as outside the QC criteria.

In cases where an analyte is present in the blank, but the extract concentrations for that analyte are either non-detectable or are 10 times the concentration found in the blank, the data for such extracts are acceptable and can be reported.

### 3.3.2 Surrogate Compound Analysis

Prior to extraction, all samples and quality control samples are spiked with deuterated n-$C_{12}$, $C_{20}$, $C_{24}$ and $C_{30}$. The QC acceptance criteria for surrogate recovery of deuterated n-$C_{20}$, -$C_{24}$ and -$C_{30}$ is 40 to 120 %.

The following corrective actions will be taken when surrogate recovery criteria are not met:

a. Calculations are checked to assure that no errors have been made.

b. The internal standard and surrogate solutions are checked for degradation, contamination, etc., and the instrument performance is checked.

c. If the native concentration of hydrocarbons are high and require dilution for acceptable chromatographic separations, a dilution is made. A known aliquot of the extract is removed. One hundred (100) $\mu$L of surrogate are added and the volume brought to 1.0 mL. The appropriate changes are made in the dilution factor within the quantitation software and the sample is re-analyzed. There is no concentration correction based on the surrogate recovery for dilution results. The surrogate recoveries are not reported but qualified with a "D" to denote the dilution. The MDL should also be adjusted to account for any dilutions.

d. If the above steps fail to reveal a problem, the extract is reanalyzed. If reanalysis of the extract yields surrogate recoveries within the stated limits, then the reanalysis data is reported. If upon reinjection QC criteria are still not met, the sample is submitted for re-extraction if sufficient sample is available. If the sample was completely consumed, the data will be reported but designated as outside the QC criteria.

### 3.3.3 Laboratory Blank Spike (LBS) and Laboratory Blank Spike Duplicate (LBSD)

A laboratory blank spike (LBS) may be used to estimate analytical accuracy of the method for aqueous samples, if inadequate sample is available, or if a complex matrix is present. A laboratory blank spike duplicate (LBSD) is used to estimate both analytical accuracy and precision. When required, the frequency for the LBS/LBSD is one for each QC batch of 20 or fewer samples.

The LBS/LBSD contain no matrix, and are prepared by adding a standard oil solution (normally 100 µL) and surrogate standards to the extraction vessel. These spiked blanks are carried through the entire extraction process. The QC acceptance criteria for the recovery of the n-alkanes and UCM in the spike blank are 40 to 120%.

If the LBS recovery criteria are not met, check the inlet sleeve on the GC first to see if the problem is caused by a dirty sleeve resulting from high concentrations of hydrocarbons in the extracts. If the sleeve is dirty, replace it and inject a solvent blank followed by the calibration check standard. If the response factors are within ±15 percent of the corresponding calibration value, the analysis may continue.

The spiked blank extract is then reinjected on the GC. If the reinjected LBS meets the criteria, then the reanalysis data is reported. If not, the entire batch of samples are submitted for re-extraction if sufficient sample is available. If the samples were completely consumed the data will be reported but designated as outside the QC criteria.

If the LBSD is required, the recoveries determined from the LBS and LBSD should agree within an average Relative Percent Difference (RPD) of $\leq$25%.

If two or more of the target compounds are outside the QC acceptance criteria corrective action may be indicated. Corrective action may include recalculation and/or reanalysis of the LBS and LBSD; re-extraction of the sample group; or instrument maintenance and/or recalibration. A laboratory blank spike may also be used to demonstrate that the analytical system is in control when working with a difficult matrix or sample set.

### 3.4.3 Matrix Spike (MS) and Matrix Spike Duplicate (MSD)

**3.4.3.1** A Matrix Spike (MS) sample is used to estimate analytical accuracy in the presence of a representative matrix and is normally required for each QC batch of 20 or fewer samples.

**3.4.3.2** A Matrix Spike Duplicate (MSD) is used to estimate analytical accuracy and precision in the presence of a representative matrix and may be required for each QC batch of 20 or fewer samples.

**3.4.3.3** QC acceptance criteria for target compound recoveries in the MS and the MSD is 40 to 120% of the spiked amount. In computing the QC acceptance criteria, only valid spikes will be used. In a valid spike, the amount of analyte added is at least as much as was originally present in the sample.

**3.4.3.4** If a Matrix Spike Duplicate (MSD) has been included with the analytical batch, the recoveries of valid spike components determined from the MS and MSD samples should agree within a Relative Percent Difference (RPD) of $\leq$25%.

**3.4.3.5** The MS and MSD acceptance criteria are advisory. However, if two or more of the target compounds are outside the QC acceptance criteria corrective action may be indicated. Corrective action may include recalculation and/or reanalysis of the MS and MSD; re-extraction of the sample/MS/MSD group; or instrument maintenance and/or recalibration.

### 3.4.4 Duplicate (DUP)

**3.4.4.1** A sample Duplicate (DUP) is used to demonstrate sample homogeneity and analytical precision in the presence of a representative matrix and may be required with each QC batch of 20 or fewer samples.

**3.4.4.2** QC acceptance criteria for analyte concentrations greater than ten times the MDL is a Relative Percent Difference (RPD) of $\leq$25%.

**3.4.4.3** If the RPD is outside the QC acceptance criteria, corrective action may be indicated. Corrective action may include: recalculation or reanalysis of the DUP and the original sample, instrument maintenance, recalibration, or re-extraction of the analytical batch.

## 4.0 APPARATUS AND MATERIALS

### 4.1 Gas Chromatograph

A gas chromatograph with a split/splitless injection system, capillary column capability and a flame ionization detector (FID) are needed. The output from the detector is acquired and processed by an HP-Chem Station software package.

| | |
|---|---|
| **Instrument**: | Hewlett-Packard 5890 Gas Chromatograph or equivalent |
| **Features**: | Split/splitless capillary inlet system, HP- Chem Station data acquisition system or an equivalent data acquisition system |
| **Inlet**: | Splitless |
| **Detector**: | Flame ionization |

### 4.2 GC Column

A 30-m long x 0.32-mm I.D. fused silica capillary column coated with DB-5 bonded phase (J&W Scientific or equivalent) is used. Other columns with different bonded phases can be substituted as long as the performance is equivalent or better.

### 4.3 Autosampler

The autosampler used is capable of making 1-4 $\mu$L injections.

### 4.4 GC Conditions

The following GC conditions normally produce the required separations. These conditions can be modified when necessary to provide the required chromatographic separations.

**Gases**:

| | |
|---|---|
| Carrier: | Helium 2 mL/min. |
| Make-Up: | Helium 33 mL/min. |
| Detector: | Air 360 mL/min. |
| Hydrogen | 33 mL/min. |

**Temperature Program**:

| | |
|---|---|
| Injection port: | 300°C |
| Detector: | 300°C |
| Oven Program: | 60°C for 1 min. then 6°C/min. to 300°C, hold 10 min. |
| Total run time | 51 minutes |

## 5.0 ANALYTICAL STANDARDS

### 5.1 Calibration Solution for Routine Analyses

The calibration solutions are comprised of certified standards of most of the n-alkanes and isoprenoids listed in Table 1. Phytane, n-$C_{31}$, and n-$C_{33}$ are not included in the calibration solutions.

Calibration standards are prepared at five concentrations in the range of 1.25 to 50 $\mu$g/mL. Sufficient internal standard and surrogate solutions are added to all calibration standards to result in a concentration of approximately 2.0 $\mu$g/mL.

### 5.2 Calibration Solution for Special TPH Analyses

If only total petroleum hydrocarbons (TPH) are to be analyzed, then TPH standards can be used to calibrate the instrument. The calibration standards are prepared from a petroleum hydrocarbon fuel as similar as possible to the fuel suspected to be found in the samples. There should be five different concentrations, with the lowest at or below the quantitation limit and the remaining spaced out over the working range of the detector. The area under the curve should be measured over the entire range of the chromatograph corresponding to the specific type of fuel in the calibration standards, This would include diesel range organic compounds (DRO) or crude oils.

If the extent of TPH degradation is to be estimated, one or more single injections of certified TPH degradation standards should be included, to the extent available for the fuel suspected to be found in the samples. When such degradation standards are not available, the estimated degradation can also be determined by an experienced analyst, although the use of a degradation standard is preferred.

### 5.3 Surrogate Spiking Solution

A surrogate solution is made by weighing appropriate amounts of pure deuterated n-$C_{12}$, $C_{20}$, $C_{24}$ and $C_{30}$ compounds into a volumetric flask and diluting to volume with methylene chloride. Surrogates are added to all samples and quality control samples prior to extraction at a concentration of approximately 10 times the MDL. For routine analysis, 100 µL of surrogate is added to each sample, unless otherwise specified. If higher concentrations of hydrocarbons are anticipated, the surrogate concentration can be appropriately increased.

### 5.4 Internal Standard Solutions

The internal standard for this analysis is deuterated n-$C_{16}$. An internal standard solution is made by weighing an appropriate aliquot of pure material into a volumetric flask and diluting to volume with methylene chloride. Internal standard should be added to each sample extract prior to analysis to obtain a final concentration of approximately 2 µg/mL. For higher concentrations of target compounds in the sample, the internal standard concentration is appropriately increased.

### 5.5 Matrix Spiking Solution

The matrix spiking solution consists of 12 aliphatic solutions including n-$C_{10}$ to n-$C_{34}$ and pristane (see Table 1). These solutions are weighed into a volumetric flask and brought to volume with methylene chloride

The normal volume of matrix spike added to samples is 100 µL, resulting in a concentration approximately 10 times the MDL. For higher concentrations of target compounds in a sample, the matrix spike can be appropriately increased.

### 5.6 GERG Check Standard Solution

A solution of a laboratory reference oil, the GERG Check Standard, is analyzed with each analytical batch as a retention time standard, as well as a laboratory certified instrument check standard. The concentration of oil is approximately 0.8 mg/mL. The oil is weighed into a volumetric flask and brought to volume with methylene chloride.

## 6.0 REQUIRED SAMPLE DOCUMENTATION AND IDENTIFICATION

Copies of the following documents, when applicable, should accompany the extract set in a labeled folder when it is delivered to GC analysis group:

- Chain-of-custody documents
- Sample Information Sheet(s)
- Analysis Request Form(s)
- Laboratory bench sheet
- Dry weight bench sheet
- Percent lipid bench sheet for tissue samples
- Sample Action Request Form(s)

## 7.0 INSTRUMENT CALIBRATION PROCEDURE

### 7.1 Initial Calibration

Prior to the analyses, a five-point calibration establishes the linearity of the detector. Each calibration standard is analyzed and the average response factor ($\overline{RF}$) and percent RSD of each compound at each concentration level is calculated from the peak area and the concentration (see Section 3.3).

After the initial calibration curve has been established, no new calibration curve is required as long as the daily calibration verification using a mid-level calibration check solution meets QC criteria cited in Section 3.3.

## 8.0 EXTRACT ANALYSIS

Add the appropriate amount of internal standard (IS) to each sample and QC extract. Enter the analytical run sequence for the extracts, based upon the written run sequence. After injection of the instrument solvent blank, analyses are initiated with a calibration check, followed by the GERG Check Standard, 8 to 10 samples, and then another calibration check. This sample and calibration check sequence is repeated, always ending with a calibration check. If the daily response factor for any analyte in the calibration check standard fails to meet the criteria established in Section 3.3, check the inlet sleeve on the GC. If the problem is caused by a dirty sleeve resulting from high concentrations of hydrocarbons in the extracts, replace it and inject a solvent blank followed by the calibration check standard. If the daily response factors are all within ±25 percent of the corresponding calibration average RF value, the analysis may continue.

If the criteria are not met, the instrument is recalibrated. All samples that were injected after the calibration check standard exceeded the criteria are reinjected.

Sample injections of 1 to 4 $\mu$L are made with an autosampling device.

If, after analysis, an experienced analyst decides that insufficient chromatographic separation was obtained for an extract, the extract is diluted and reanalyzed. Dilutions are quantitatively prepared by taking a known aliquot of the extract and diluting it with methylene chloride. Dilutions of less then a 1:5 ratio do not require the addition of surrogate or internal standards. The dilution factor in the analytical software will require adjustment to reflect any additional amount of surrogate.

## 9.0 CALCULATIONS

The preferred quantitation method for analyte concentrations is based on the surrogates added prior to sample extraction.

**9.1** A specific amount of surrogate (SU) is added to each sample prior to extraction. The recovery of this surrogate is monitored in each extract by comparison the response of the internal standard (I.S.), deuterated n-$C_{16}$, that is added to the final extract just prior to analysis, as shown in Equation 1.

**Equation 1:**

$$\text{Percent SU recovery} = \frac{(A_{su} \times C_{is})}{(C_{su} \times A_{is} \times \overline{RF_{su}})} \times 100$$

where:

$A_{is}$ = Area of internal standard (deuterated n-$C_{16}$)
$A_{su}$ = Area of surrogate (deuterated n-$C_{12}$, -$C_{20}$, -$C_{24}$, -$C_{30}$)
$C_{su}$ = ng of surrogate (deuterated n-$C_{12}$, -$C_{20}$, -$C_{24}$, -$C_{30}$) added to the sample
$C_{is}$ = ng of internal standard (deuterated n-$C_{16}$ ) added to the final sample extract
$\overline{RF_{su}}$ = Average response factor for individual surrogate (deuterated n-$C_{12}$, n-$C_{20}$, n-$C_{24}$, or-n $C_{30}$) from initial calibration

### 9.2 Initial Calibration and Continuing Calibration Checks

Each calibration standard is analyzed and the response factor (RF) of each compound at each concentration level is calculated from the peak area and the concentration.

Equation 2 is used to calculate the response factors for the initial calibration of each alkane in the calibration standards relative to the surrogate standard.

**Equation 2:**

$$RF = \frac{(A_s \times C_{su})}{(A_{su} \times C_s)}$$

where:

$A_S$ = Area for an alkane analyte.
$A_{su}$ = Area for the surrogate standard (deuterated n-$C_{20}$).
$C_{su}$ = Concentration (ng/mL) of the surrogate standard (deuterated n-$C_{20}$).
$C_S$ = Concentration of the alkane analyte.

Using this data, the average response factor for each alkane in the five calibration standards ($\overline{RF}$) is determined for later use when calculating the concentration of these alkanes in sample extracts. In addition, the average of the response factors for all analytes in all calibration standards ($\overline{RF}_u$) is determined for use when calculating the UCM and RCM results.

The daily response factors, $RF_D$, for the initial calibration check and for calibration checks analyzed after every 10 to 12 extract analyses is determined using Equation 2. Then the percent difference for the response factor of each compound in the calibration check standard is calculated relative to the average response factor for compounds in the initial calibration standards using Equation 3.

**Equation 3:**

$$\text{Percent Difference} = \left( \frac{\overline{RF} - RF_D}{\overline{RF}} \right) \times 100$$

where:

$\overline{RF}$ = Average alkane response factor from initial calibration.
$RF_D$ = Daily response factor for same alkane in calibration check standard.

If the average percent difference of the response factors for all analytes in the calibration check standard is within ±15 percent of the corresponding calibration value, the analysis may proceed.

If, for any individual analyte, the percent difference of the daily response factor exceeds ±25 percent of the corresponding calibration average alkane response factor, analysis must be stopped.

1. Check the inlet sleeve on the GC first to see if the problem is caused by a dirty sleeve resulting from high concentrations of hydrocarbons in the extracts. If the sleeve is dirty, replace it and inject a solvent blank followed by the calibration check standard. If the response factors are within ±25 percent of the corresponding calibration value, the analysis may proceed.

2. If there is no problem with the sleeve, a five-point calibration must be repeated prior to continuing the analysis of the samples.

### 9.3 Individual Analyte Calculations

As shown in Equation 4, analyte concentrations are determined based on the average response factors for each alkane from the 5-point calibration solutions. The calculation uses the ratio of the concentration and area of the surrogate standards added to sample prior to extraction to the area of the target analyte in the extract.

If an individual saturated hydrocarbon found in the extract is not in the calibration solution, a response factor (RF) is estimated from the average response factors of hydrocarbons eluting immediately before and after the compound. For phytane, the $\overline{RF}$ for pristane is used. The calculation includes division by the sample weight to provide the final concentration on a ng/g basis.

**Equation 4**

$$C = \frac{(A_s \times C_{su} \times D)}{(A_{su} \times \overline{RF} \times W_s)}$$

where:

$C$ = Concentration in sample (ng/g).
$A_S$ = Area of the peak to be measured.
$A_{su}$ = Area of the surrogate standard (deuterated n-$C_{20}$).
= Amount of surrogate standard added to each extract (ng).
$\overline{RF}$ = Average response factor for an alkane based on the initial calibration.
$W_S$ = The original weight of dried sample extracted (g).

### 9.4 UCM Calculations

The average response factor, $\overline{RF}_u$, of all n-alkanes in the initial 5-point calibration curve is used for the estimated UCM concentration calculations using Equation 5.

**Equation 5**

$$UCM = \frac{(A_c \times C_s \times D)}{(A_{su} \times \overline{RF}_u \times S)}$$

where:

$A_C$ = The corrected area of the sample chromatogram, which is defined as the total area of the chromatogram minus the area of the baseline shift and minus the area of all resolved peaks including the internal standard and all surrogate standards.
$C_{su}$ = $\mu$g of surrogate standard (SU; deuterated n-$C_{20}$) added to the extract
D = Dilution factor (if any)
$A_{su}$ = Area response of the surrogate standard deuterated n-$C_{20}$
$\overline{RF}_u$ = Average of the response factors of all analytes in the initial calibration curve
S = Amount of dry sample extracted, in g, for sediment or soil

## 9.5 RCM Calculations

The estimated total resolved complex mixture (RCM; in $\mu$g/g) concentration represents the sum of all the resolved hydrocarbon peaks, minus all surrogate peak areas and internal standard peak areas. RCM is determined using Equation 6.

**Equation 6**

$$RCM = \frac{(A_r \times C_{su} \times D)}{(A_{su} \times \overline{RF}_u \times W_s)}$$

where:

$A_r$ = Total area of all resolved peaks minus (the area of the internal standard and the surrogates).
$C_{su}$ = $\mu$g of surrogate standard (su) deuterated n-$C_{20}$ added to the extract
D = Dilution factor (if any)
$A_{su}$ = Area response of the surrogate standard deuterated n-$C_{20}$
$\overline{RF}_u$ = Average response factor of all analytes in the initial calibration curve
$W_S$ = Amount of sample extracted in g (dry weight) for sediment

### 9.6 TPH Concentration and Percent Degradation

The TPH concentration is calculated as the sum of the total UCM and RCM concentrations.

The percent (%) Degradation is determined as a ratio of the TPH found in a sample as compared to the chromatogram of the degradation standard if available, or as compared to the chromatogram of the fuel calibration standards.

## 10.0 DOCUMENTATION REQUIRED FOR ANALYTICAL RESULTS

**10.1** All injections and analytical run sequences are recorded on a computer printout kept on file at the instrument.

**10.2** For all Initial Calibrations the following documentation is printed and maintained for a period of at least one year.

**10.2.1** Listing of retention times and peak areas for all target analytes in each calibration run.

**10.2.2** Listing of the calculated Average Response Factors and the daily response factors for all target compounds in each calibration standard.

**10.3** For all Calibration Verifications, the following documentation is printed and maintained for a period at least one year.

**10.3.1** Listing of retention times, daily response factors and peak areas for all target compounds in the CCV; and

**10.3.2** Listing of the analyte concentrations for all target compounds in the CCV.

**10.4** For all methylene chloride instrument blanks the following documentation is printed and maintained for a period of at least one year.

**10.4.1** The chromatograph for the instrument blank run demonstrating that the analytical system is free from contaminating interferences.

**10.5** For all analytical and laboratory Quality Control samples the following documentation is printed and maintained for a period of at least one year (actual

retention of analytical data is determined by the contract guidelines, but shall not be less than one year).

**10.5.1** Listing of retention times and peak areas for all target compounds in the analytical run, including any manual integrations.

**10.5.2** Listing of the calculated concentrations of all target compounds, percent recovery of the surrogates, and

**10.5.3** A compiled data report in either the GERG standard format or other customized format requested by the client. The compiled data report includes Relative Percent Difference (RPD) between duplicate analyses or MS/MSD recoveries, and percent recovery of native analytes in LBS and MS/MSD QC samples.

## 11.0 REPORTING

### 11.1 Reporting Units

Reporting units for individual compounds are ng/g for sediments (dry weight) and the UCM and RCM concentration estimates are reported in $\mu$g/g. For estimated TPH degradation, the reporting units are Percent (%) Degradation.

Table 1(a). Target Aliphatic Hydrocarbons (AH)

| | |
|---|---|
| n-$C_{10}$ | n-$C_{22}$ |
| n-$C_{11}$ | n-$C_{23}$ |
| n-$C_{12}$ | n-$C_{24}$ |
| n-$C_{13}$ | n-$C_{25}$ |
| n-$C_{14}$ | n-$C_{26}$ |
| n-$C_{15}$ | n-$C_{27}$ |
| n-$C_{16}$ | n-$C_{28}$ |
| n-$C_{17}$ | n-$C_{29}$ |
| Pristane | n-$C_{30}$ |
| n-$C_{18}$ | n-$C_{31}$ |
| Phytane | n-$C_{32}$ |
| n-$C_{19}$ | n-$C_{33}$ |
| n-$C_{20}$ | n-$C_{34}$ |
| n-$C_{21}$ | n-$C_{34}$ |

Table 1(b). Aliphatic Hydrocarbons in Standard Solutions.

| Deuterated Surrogate Solutions | Matrix Spike Solutions | Deuterated Internal Standard |
|---|---|---|
| n-$C_{12}$ | n-$C_{12}$ | n-$C_{16}$ |
| n-$C_{20}$ | n-$C_{15}$ | |
| n-$C_{24}$ | n-$C_{17}$ | |
| n-$C_{30}$ | Pristane | |
| | n-$C_{18}$ | |
| | n-$C_{20}$ | |
| | n-$C_{24}$ | |
| | n-$C_{28}$ | |
| | n-$C_{30}$ | |
| | n-$C_{32}$ | |
| | n-$C_{34}$ | |

NOTE: Calibration standards include all of the Table 1(a) aliphatic hydrocarbons except phytane, n-$C_{31}$, and n-$C_{33}$. Surrogate n-$C_{20}$ is normally used for quantitation

Table 2. Summary of QC Requirements for Quantitative Analysis

| Element | Control Limit Criteria | Frequency |
|---|---|---|
| - Instrument Calibration | Minimum of 5 standards; Response factor % RSD within ± 15% for all target compounds. | Initial and after any failures of continuing calibrations. |
| - Instrument Blank | Instrument free of interfering contamination or perform necessary maintenance. | Prior to analysis of all analytical batches. |
| - GERG Standard Check | Analytes within ± 25% or average of lab certified concentration with no analytes > 35% or recalibrate. | Prior to analysis of all analytical batches. |
| - Continuing Calibration Verification (CCV) | Percent difference for all response factors within ± 15% or average of initial calibration; no single analyte greater than 25% or recalibrate and reanalyze back to last passing CCV. | After initial calibration, after 8-10 extracts during the analytical sequence, and at end of analytical sequence. |
| - Surrogate Recovery | Recovery of 40 to 120% for all surrogates. See Section 3 for corrective actions. | All samples. |
| - Method Blank | No analytes > 3x MDL. See Section 3 for exceptions to need for re-extraction. | One per QC batch. |
| - Duplicates (if applicable) | RPD $\leq$ 25% for all analytes > 10 x MDL. See Section 3 for corrective action. | One per QC batch. |
| - Matrix Spike, Matrix Spike Duplicate (if applicable) | % recovery within 40 to 120%. RPD for the spike recoveries should be $\leq$ 25% for all analytes. See Sections 3 for corrective actions. | One per QC batch. |
| - Standard Reference Material (if applicable) | Recovery of 80% of certified or non-certified compounds within 30% of certified range for those analytes > 10x MDL. See Section 3 for corrective action. | One per QC batch. |

# SOP-0101

# EXTRACTION AND ANALYSIS OF PLANT PIGMENT SAMPLES USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURES

SOP-0101

# EXTRACTION AND ANALYSIS OF PLANT PIGMENT SAMPLES USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

This document presents the procedures used in the performance of the above preparation activities.

Quality Assurance Manager

Date

Author/Revision By: Yaorong Qian

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# EXTRACTION AND ANALYSIS OF PLANT PIGMENT SAMPLES USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

## 1.0 PURPOSE

This document provides the procedures which are used by staff of the Geochemical and Environmental Research Group (GERG) of the College of Geosciences at Texas A&M University for the extraction and analysis of plant pigment compounds in various samples.

## 1.1 SUMMARY OF THE METHOD

This standard operating procedure (SOP) provides a precise and accurate method to quantitatively determine pigment compounds in aqueous, vegetative, and sediment/soil matrices. Aqueous samples are collected in precleaned amber bottles and particulate materials in the water are collected on a GF/F type glass fiber filter by filtering the collected water samples. The filters are preserved in liquid $N_2$ after wrapping the samples in aluminum foil. Vegetative and other types of soil/sediment samples are collected in amber containers and preserved in liquid $N_2$. Alternatively, samples may be preserved in freezers below –20°C in the dark (e.g. wrapping in aluminum foil).

Samples are extracted with acetone in the dark overnight while maintaining the temperature below 0°C. Aliquots of the extracts are analyzed with a high performance liquid chromatography (HPLC) using a visible wavelength absorbance detector at 436 nm.

## 1.2 APPLICABILITY

### 1.2.1 Matrix

This method is applicable to aqueous, plant, and other types of solid samples.

### 1.2.2 Interferences

Discrete artifacts in the solvents, reagents, glassware, and other sample processing hardware may cause interferences and/or elevated baselines that may cause misinterpretation of chromatographic data. All materials used during the extraction procedure must be demonstrated to be free from significant interferences under the same conditions of analysis by analyzing laboratory method blanks, at a frequency of one blank per 20 samples or one with each batch if the number of samples is less than 20.

Matrix interferences result from the co-extraction of compounds other than the analytes of interest. Lipids and other types of compounds in plants, sediment/soil, and water may

interfere with the analysis of target compounds. Different detectors, columns, or analytical conditions may be used to confirm the identity of target pigment compounds.

### 1.3 Target Analyte List

Most of the target pigments are not commercially available. The retention time, calibration, and response of those pigment compounds were determined previously with pure standards obtained from individual investigators or from EPA's Office of Research and Development. The target pigment compounds determined by this method, as well as the internal standard (canthaxanthin) used for identification, retention time, and quantification are listed in Table 1.

### 1.4 Method Detection Limit

The method detection limits (MDLs) for pigments are determined following the procedures outlined in Federal Register (1984), Vol. 49, No. 209: 198- 199 (40 CFR Part 136).

The minimum method detection limit for water is 50 µg/L. Previous experience indicate that the MDLs could be as low as 5 µg/L for water samples. Vegetative, sediment/soil, and other types of matrices may have different minimum method detection limit and may need to be determined individually.

All sample processing (collection, storage, extraction) and analysis should be conducted under reduced light conditions because plant pigments are light sensitive compounds. Extracts should be analyzed within 3 days of preparation unless long-term storage stability has been demonstrated.

### 1.5 Reporting Units

Reporting units for aqueous samples for pigment analysis are nanograms per liter (ng/L) unless otherwise requested. Reporting units for solid samples are ng/g (wet weight) unless otherwise requested.

## 2.0 SAFETY

The hazards, toxicity or carcinogenicity of each compound or reagent used in GERG's standard operating procedures have not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory maintains Material Safety Data Sheets (MSDS) which contain information regarding the safe handling of chemicals used at GERG's facilities. A reference file of MSDS is available to all personnel involved with these materials.

All laboratory personnel should direct any questions regarding safety issues to their supervisors or the Safety Officer.

## 3.0 QUALITY CONTROL REQUIREMENTS

The quality control samples routinely used at GERG include a method blank (BLANK), a sample duplicate (DUP), a calibration check per batch of 20 or less samples. The number and type of QC samples may be modified to satisfy the client's requests and sample availability.

### 3.1 Qualitative Identification

The retention times of the target analytes relative to the internal standard canthaxanthin under current HPLC conditions are listed in Table 1. The relative retention times of the target analytes should fall within ±0.2 minutes of the relative retention time windows established from the analysis of the calibration standards under identical HPLC conditions. Variations of instrumental conditions, such as HPLC mobile phase compositions, temperature, column characteristics, and gradient program, would change the relative retention times of the target analytes. The experienced analyst may use manual peak selection and baseline correction when appropriate.

### 3.2 Initial Calibration and Calibration Checks

The instrument has been calibrated initially with two concentration levels of standard pigment compounds and that file has been maintained.

The data acquisition software calculates the response factor. Each calibration standard is analyzed and the daily response factor (RF) of each pigment at each concentration level is calculated from the peak area and the concentration. An average response factor for each compound ($\overline{RF}$) is determined from the calibration data.

The initial calibration is verified by the measurement of calibration check standards in each analytical batch.

If the average percent difference of the response factors for all analytes in the calibration check standard is within ±25 percent of the corresponding calibration value, the analysis may proceed. If the daily response factor for any individual analyte exceeds ±25 percent of the corresponding calibration value, a re-calibration must be performed for specific compounds prior to the analysis of the samples.

## 3.3 QC Criteria for Sample Extracts in the Analytical Batch

### 3.3.1 Method Blank Analysis

One method blank (also referred to as a procedural blank) is prepared with every 20 samples or with every QC batch, whichever is more frequent. The blank is prepared using all reagents and procedures for extraction, but does not contain any matrix.

The QC acceptance criteria for the method blank requires that it does not contain any target compounds at concentrations greater than three times the method detection limit (MDL). If the method blank does not meet these criteria, the analytical system is out of control and the source of the contamination must be investigated, corrective measures taken, and documented before further sample analysis.

The procedural blank extract may be reanalyzed. If the experienced analyst determines that after reanalysis of the extract contains no target analytes exceeding the stated limits, or that only non-target compounds present in the blank, which will not affect results, then the analytical data for the QC batch is reported.

If upon reinjection QC criteria are still not met, the entire QC batch may be submitted for re-extraction if sufficient sample is available. If the sample was completely consumed, the data will be reported but designated as outside the QC criteria.

In cases where target analyte is present in the blank, but the extract concentrations for that analyte are either non-detectable or are 10 times the concentration found in the blank, the data for such extracts are acceptable and can be reported. Extract concentrations determined for an analyte that is not 10 times the concentration found in the blank may be reported, but is flagged with a "B" to indicate possible contamination.

### 3.3.2 Duplicate (DUP)

A sample Duplicate (DUP) is used to demonstrate sample homogeneity and analytical precision in the presence of a representative matrix and may be required with each QC batch of 20 or fewer samples.

QC acceptance criteria for analyte concentrations greater than ten times the MDL is a Relative Percent Difference (RPD) of $\leq$25%.

If the RPD is outside the QC acceptance criteria, corrective action may be indicated. Corrective action may include: recalculation or reanalysis of the DUP and the original sample, instrument maintenance, recalibration, or re-extraction of the analytical batch.

## 4.0 APPARATUS AND MATERIALS

### 4.1 High Performance Liquid Chromatography

A high performance liquid chromatography (HPLC) with an autosampler, a ternary pump, and a visible wavelength detector is needed. The output from the detector is acquired and processed by LabSystem's *Xchrom* chromatographic software.

**Instrument**: Hewlett-Packard (HP) 1050 quaternary pump and HP 1050 autosampler or equivalent

**Detector**: Waters 2487 dual λ UV-Visible absorbance detector.

**Column:** A 25 cm long x 0.46 mm I.D. Spherisorb ODS-2, 5 μm, C18 column (Waters) or equivalent.

### 4.2 HPLC Conditions

The following HPLC conditions normally produce the required separations. These conditions can be modified when necessary to provide the required chromatographic separations.

Flow rate: 1 mL/min.
100% A at time 0.0 min.
100% B at 2 min.
25% B and 75% C at 22 min.
100% B at 24 min.
100%B at 25 min
100%A at 28 min.
END at 35 min.

Mobile phase A, B, and C are methanol/0.5M ammonium acetate (80/20, v/v), acetonitrile/water (90/10, v/v), and ethyl acetate, respectively.

### 4.3 Glassware and Hardware

All glassware is cleaned according to GERG SOP. Glassware is stored in a clean environment to prevent the accumulation of dust or other contaminants.

After initial micro wash and tap water rinsing, solvent rinses with methanol followed by methylene chloride may substitute the muffle furnace heating for glassware when determined appropriate by the analyst. Solvent rinsing should always be used for "non-combustible" (glassware that cannot be oven heated) glassware.

The following laboratory glassware and hardware is needed to perform the aqueous extraction and purification procedure:

**Autosampler Vials:** 0.7 mL amber autosampler vials for HPLC.
**Graduated Cylinder**: 1 or 2 L.
**Disposable Glass Pasteur Pipettes:** 1 mL.
**Micropipettor:** 20 µL, 100 µL or as required.
**Test Tubes:** 15 mL capacity or equivalent.
**Centrifuge**
**Vortex Mixer**
**Aluminum Foil**

### 4.4 Solvent and Chemicals

**4.4.1 Solvents**: Equivalent solvents from other sources may be used after lot testing.

Methanol: B&J; Cat. 230-4 or equivalent.
Acetone: B&J; Cat. 010-4, or equivalent.
Acetonitrile: B&J; Cat. 016-4 or equivalent.
Water: B&J; Cat. 365-4 or equivalent.
Ethyl acetate: B&J; Cat. GC100-4 or equivalent

**4.4.2 Ammonium Acetate**: Analytical Grade, Fisher Scientific. 0.5M aqueous solution. 77 g of ammonium acetate crystals are dissolved in 2 L of HPLC water.

### 4.5 Analytical Standards

Analytical Standards are purchased as solid crystals with certification of their purity and authenticity. When not being used, standards are stored in the dark at approximately -20°C in amber glass containers.

Chlorophyll *a*: Sigma Cat. C5753
Chlorophyll *b*: Sigma Cat. C5878
β-carotene: Sigma Cat. C4582
Canthaxanthin: Hoffman La Roche, Switzerland.

## 5.0 EXTRACTION PROCEDURES

Perform all the sample extraction and analysis tasks under reduced light conditions.

Prepare labels for the glassware needed for the samples and QC requirements for each extraction QC batch.

Transfer the filter samples (or other types of samples) into labeled 15 mL test tubes. Add 4-6 mL of acetone to each test tube.

Prepare a procedural blank sample with solvent only.

Prepare a calibration check sample with chlorophyll standard solution.

Add appropriate amount of canthaxanthin standard solution to each sample. Vortex each test tube for 30 seconds. Make sure the filter is submerged in solvent. If not, push the filter down with a clean stainless steel spatula.

Macerate the filter or the sample with a small grinder using a small stainless steel probe.

Wrap the test tube rack along with samples in aluminum foil. Label the sample rack with the benchsheet page #. Place the samples in a freezer at –20°C overnight.

After retrieving the samples from the freezer the second day, vortex each test tube for 30 seconds. Centrifuge all the samples for 2-4 minutes.

Transfer 200 µL of the acetone extract to an amber autosampler vial.

Prepare a duplicate sample for each batch by transferring the specified sample extract to an extra vial labeled "DUP" and the original sample ID..

Add 20 µL of 0.5M ammonium acetate solution to each vial. Vortex each vial after capping.

## 6.0 SAMPLE ANALYSIS

Prior to sample analysis, check the solvent levels in solvent reservoirs of HPLC system. Check the pressures of compressed $N_2$ and helium that supply the autosampler. Make sure there are sufficient solvents in the reservoirs and sufficient compressed gases (at least 200 psi for one batch of 20 samples). Check the detector wavelength (436 nm for pigment) and make sure the lamp is on.

Start HPLC pump with solvent at 100%A. Make sure the pump method # is 3 (pigment method).

Setup an analysis sequence in the computer in *Xchrom* and print a copy of the sequence. Start the run sequence in *Xchrom* (the computer will be waiting for sample injection).

Program the HPLC autosampler with # of samples. Make sure the autosampler method # is 3 (pigment method). Load the sample vials in an autosampler tray according to the sample sequence in the *Xchrom*.

Start the analysis by pressing START on the autosampler.

## 7.0 INITIAL CALIBRATION AND CONTINUING CALIBRATION CHECKS

Each calibration standard is analyzed and the response factor (RF) of each compound at each concentration level is calculated from the peak area and the concentration.

Equation 1 is used to calculate the response factors for the initial calibration of each compound in the calibration standards relative to the IS standard.

**Equation 1:**

$$RF = \frac{(A_s \, x \, C_{IS})}{(A_{IS} \, x \, C_s)}$$

where:

$A_S$ = Area for an analyte.
$A_{IS}$ = Area for the internal standard (canthaxanthin).
$C_{IS}$ = Concentration (ng/mL) of the internal standard (canthaxanthin).
$C_S$ = Concentration of the analyte.

Using this data, the average response factor for each pigment in the calibration standards ($\overline{RF}$) is determined for later use when calculating the concentration of these pigments in sample extracts.

The daily response factors for calibration checks analyzed with each batch of samples is determined using Equation 1. Then the percent difference for the response factor of each compound in the calibration check standard is calculated relative to the average response factor for compounds in the initial calibration standards using Equation 2.

**Equation 2:**

$$\text{Percent Difference} = \left( \frac{\overline{\text{RF}} - RF_C}{\overline{RF}} \right) x100$$

where:

$\overline{\text{RF}}$ = Average pigment response factor from initial calibration.
$\text{RF}_C$ = Daily response factor for same pigment in calibration check standard.

If the average percent difference of the response factors for all analytes in the calibration check standard is within ±25 percent of the corresponding calibration value, the analysis may proceed.

If, for any individual analyte, the percent difference of the response factors exceeds ±25 percent of the corresponding calibration average response factor, analysis must be stopped.

Check the HPLC guard column, and analytical column first to see if the problem is caused by a dirty guard column or aging analytical column. If the guard column or the analytical column is malfunctioning, replace it and inject a solvent blank followed by the calibration check standard. If the response factors are within ±25 percent of the corresponding calibration value, the analysis may proceed.

If there is no problem with the columns, a re-calibration for specific compounds must be repeated prior to continuing the analysis of the samples.

## 8.0 ANALYTE CONCENTRATION CALCULATIONS

The quantification method for analyte concentrations is based on the internal standard added prior to sample extraction. A specific amount of internal standard (IS) is added to each sample prior to extraction.

As shown in Equation 3, analyte concentrations are determined based on the average response factors for each compound. The calculation uses the ratio of the concentration and area of the internal standards added to sample prior to extraction to the area of the target analyte in the extract.

If an individual compound found in the extract is not in the calibration solution, a response factor (RF) is estimated from the average response factors of the compounds eluting immediately before and after the compound.

**Equation 3**

$$C = \frac{(A_S \, x \, C_{IS} \, x \, D)}{(A_{IS} \, x \, \overline{RF} \, x \, W_S)}$$

where:

$C$ = Concentration in sample (ng/g or ng/L).
$A_S$ = Area of the peak to be measured.
$A_{su}$ = Area of the internal standard (canthaxanthin).
$C_{IS}$ = Amount of internal standard added to each extract (ng).
$\overline{RF}$ = Average response factor based on the initial calibration.
$W_S$ = The original sample weight or volume extracted (g or L).

## 9.0 DOCUMENTATION REQUIRED FOR ANALYTICAL RESULTS

All injections and analytical run sequences are recorded on a computer and a printout kept on file. For all calibration verifications, all samples and all QC samples the following documentation is printed and maintained for a period at least one year (actual retention of analytical data is determined by the contract guidelines, but shall not be less than one year).

**9.1** Listing of retention times and peak areas for all target compounds in the analytical run, including any manual integration.

**9.2** Listing of the calculated concentrations of all target compounds, and

**9.3** A compiled data report in either the GERG standard format or other customized format requested by the client. The compiled data report includes Relative Percent Difference (RPD) between duplicate analyses QC samples.

Table 1(a). Target pigment compounds and the relative retention times (to internal standard canthaxanthin).

| Pigments | Relative retention time |
|---|---|
| Chlorophyllide *a* | 0.32 |
| Chlorophyll *c3* | 0.35 |
| Chlorophyll *c2* | 0.45 |
| Peridinin | 0.50 |
| 19'-butanoyloxyfucoxanthin | 0.52 |
| Fucoxanthin | 0.56 |
| 19'-hexanoyloxyfucoxanthin | 0.59 |
| Neoxanthin | 0.62 |
| Prasinoxanthin | 0.67 |
| Violaxanthin | 0.72 |
| Diadinoxanthin | 0.79 |
| Alloxanthin | 0.87 |
| Myxoxanthin (Myxoxanthophyll) | 0.89 |
| Diatoxanthin | 0.92 |
| Lutein | 0.95 |
| Zeaxanthin | 0.98 |
| Canthaxanthin (IS) | 1.00 |
| Chlorophyll *b* | 1.10 |
| Chlorophyll *a* isomer | 1.16 |
| Chlorophyll *a* | 1.19 |
| Chlorophyll *a'* | 1.22 |
| □-carotene | 1.39 |

Note 1: The retention time and relative retention time may change is HPLC conditions change.

Note 2: Zeaxanthin co-elutes with nostaxanthin under current HPLC conditions.