ORIGINAL

Battelle Duxbury Operations
Standard Operating Procedures

for

PRE-EXTRACTION TISSUE PROCESSING

1.0 OBJECTIVE

ob,iect;ve of document is 10 define procedures for),rior to lhe Processing of tissues but is not limited to, handling. fLlleting, homogenizing of for Ihis

It important thaI samplc contaminant fr"rn collected may a wide range of levels *(i.e.,* multiple of magnitude). Precautions must be taken to against c(}nlarnination of during ailiahoratory activities.

2.0 PREPARATION

2.1 APPARATIIS AND MATERIALS

for fish

- Top-loading balance of weighing to 001 g (SOP 3-160)
- or Teflon spatula
- Rokr (graduated in rnrn)

Apparatus fur homogenizing tissuc

- Tekmar Tissuemizer with probes, or equivalent
- OMNI Homogenizer with prohes, or equivalent
- Teflon jar or centrifuge bottle, 500-mL

ror

- Ziploe bags
- Nitrile (jloves (underliners)
- Permanent
- ritanium and/or Steel Knives
- PTFE
- DI water

2.2 REAGENTS

Dichloromethme (DCM), pesticide or equivalent
Acctooe, pesticide grade or equivalent
Milli-Q
Laboratory-grade Soap

Laboratory-grade Soap

2.3 LABWARE PREPARATION

Unless otherwise specified in this SOP, all labware contacting samples must be cleaned according to laboratory protocols defined in SOP 5-216.

2.4 RECORD KEEPING

Samples received into the laboratory will be logged into the Sample Management Module of the Battelle's Laboratory Information Management System (LIMS). Samples will be assigned unique identification numbers according to SOP 6-007.

Tissue processing information will be documented on a standardized form (Attachment 1).

3.0 PROCEDURES

Fish will be gutted and held frozen (≤-20°C) in the field prior to shipment to the laboratory. Scaling and resection of the fillet material will be performed in a laboratory environment to ensure consistency and minimize potential sample contamination during sample preparation.

3.1 DECONTAMINATION PROCEDURES

For all tissue processing activities, the following precautions must be taken to protect against cross-contamination:

(a) Laboratory personnel should use nitrile gloves when handling fish samples (i.e., whole body fish, fish fillets). Gloves should be replaced between fish samples.

(b) All surfaces in contact with fish during handling, weighing, and resection must be cleaned thoroughly (laboratory-grade soap and distilled-deionized water) between fish samples. Alternatively, surfaces that come in contact with fish samples must be covered with solvent-rinsed aluminum foil (dull side) and replaced after each fish sample.

(c) All tissue processing apparatus (i.e., cutting boards, spatulas, forceps, knives, homogenizing probes and other laboratory apparatus that are in contact with tissue) must be handwashed by brushing thoroughly with hot, soapy water, rinsing thoroughly with hot tap water until all traces of detergent are removed, and then rinsing with deionized water.

(d) All tissue processing apparatus must be solvent-rinsed with acetone after being washed. Solvent rinsing is performed in an exhaust hood where both the inside and outside surfaces are rinsed thoroughly with acetone and air-dried before storage or use. The homogenizer probes and parts must be solvent-rinsed, dried, and stored according to SOP 3-112.

3.2 FISH MEASUREMENTS

Each fish selected for resection and analysis is measured and weighed in the laboratory according to its unique sample identification code and recorded on the tissue processing log form (Attachment 1) or the tissue composite form (Attachment 2). The total length is measured to 1 millimeter (mm) and weighed to 0.1 grams (g) for small fish and 1g for fish greater than 100g. If there is a >10 % discrepancy in the total weight or >10 millimeter difference in total length when compared to the sample custody records, the sample will be flagged and only used for analysis if there are fewer than ten adequate samples for that species. Reference SOP 5-175 for the correct measurement of total length.

3.3 FISH PROCESSING

Scaling of fish will be performed as directed by the project manager and client. See Attachment 3 for scaling and filleting diagrams.

Fillets

Unless otherwise directed by the project manager, a fillet sample will be taken from one side of a partially frozen fish, beginning directly behind the pectoral fin. The sample identification code of each fish will be verified prior to processing to ensure the appropriate fish sample has been chosen. After the fillet is removed, the remaining fish sample will be refrozen in its original labeled packaging.

When thawing fish, the laboratory should take care to ensure that any resulting liquid is not contaminated and, if necessary, is added back to the whole body portion of the sample. The fillet will be carefully cleaned to remove skin and fatty tissue. Any trimmings will be retained with the remainder of the fish.

The fish filleting should proceed as:

1- Clean knife according to Section 3.1 prior to and between each fish.
2- Fillet the fish according to the methods described by U.S. EPA (2000) and by LACSD (see Attachment 3).
3- The fish fillet should weigh at least 100g, however, if the fish tissue is limited, a minimum of 50g can be processed (contact the Project Manger if <50g of fish tissue is acquired).
4- Wrap the fillet in solvent-rinsed, tared aluminum foil.
5- Record the fillet/composite weight.
6- Place the wrapped fillet/composite in a plastic bag until homogenization takes place.
7- Be sure to properly label the fillet with a "-F". Resubmit to sample custody for log-in.
8- The remainder of the carcass, including any trimmings, will be wrapped in the original package.
9- Be sure to label the remaining carcass with a "-W". Resubmit to sample custody for log-in.
10- Clean the PTFE cutting board according to Section 3.1 prior to and between fish.
11- Change gloves between fish.

Whole Body

The project manger may identify a few samples for whole body compositing. The sample identification code

of each fish will be verified prior to processing to ensure the appropriate fish sample has been chosen. The fish compositing should proceed as:

1- Clean knife according to Section 3.1 prior to and between each composite.
2- The fish composite should weigh at least 100g, however, if the fish tissue is limited, a minimum of 50g can be processed (contact the Project Manger if <50g of fish tissue is acquired).
3- Wrap the composite in solvent-rinsed, tared aluminum foil.
4- Record the composite weight.
5- Place the wrapped composite in a plastic bag until homogenization takes place.
6- Be sure to properly label the composite with a "-C". Resubmit to sample custody for log-in.
7- Clean the PTFE cutting board according to Section 3.1 prior to and between fish.
8- Change gloves between composites.

3.4 FISH HOMOGENIZATION

Three types of fish sample may be generated in fish processing: fillet, gutted whole body, or viscera samples. Each tissue type should be homogenized thoroughly as described in the following sections. (The homogenization of fish whole body or viscera samples will be described in the QAPP or supplemental project memo).

Decontamination procedures are described in Section 3.1.

The homogenizing of fish fillets should proceed as:

1- Using a solvent-rinsed knife, cut fillets into approximately 1" length pieces.
2- Place cuttings into a certified clean glass jar (tall 500ml jar) with a Teflon cap.
3- Tissuemize the fillet pieces until sample is thoroughly homogenized (a consistent paste).
4- The homogenate should be stored in the jar and kept frozen until ready for extraction.
5- Once a day (or every 20 samples) perform a rinsate blank on the equipment used during homogenization.

The tissue samples must be ***thoroughly*** homogenized prior to aliquotting for chemical or physical characterization to ensure that a representative sub-sample is taken. Smaller volumes of viscera samples may be acceptable since viscera will only be analyzed for organic contaminants. The laboratory is responsible for notifying the project manager if smaller than expected amounts are collected.

4.0 CALCULATIONS

Not Applicable

5.0 QUALITY CONTROL

The quality control (QC) program for this project is defined in the QAPP. The following QC samples are required during tissue processing are:

1. Rinsate Blank: Rinsate blanks will be collected at a minimum of once per day or every 15 samples whichever is more frequent. Initial rinsate samples will be analyzed to determine if decontamination between samples is adequate. If potentially significant contamination is noted in the rinsates, then decontamination procedures will be re-evaluated. If the rinsates indicate no cross contamination, then future rinsates may be archived but not analyzed (unless there are questionable data). Suspension of rinsate analysis will be at the direction of the project manager after consultation with the client. Rinsates are collected by rinsing the knife and probe used to homogenize the samples with 500mL of Milli-Q water.

2. Laboratory Duplicate: A laboratory duplicate will be analyzed with each tissue batch, to ensure adequate homogenization. If the laboratory duplicate results do not meet the specified data qualify objective, the batch will be re-homogenized and re-sampled

6.0 TRAINING

Analysts performing the procedure must initially work under the supervision of the Sample Preparation Supervisor, task leader, or other designated (trained) individual. Individuals may work independently once they have satisfactorily demonstrated their ability to perform the procedure, in the opinion of the trainer, and have shown no evidence of cross contamination as demonstrated by the analysis of three rinsate blanks.

Upon successful completion of training, a training certificate (Attachment 4) will be issued. The original will be kept on file in the Quality Assurance Office.

7.0 SAFETY

As part of the above training program, the analyst will be made aware of the particular safety concerns of this procedure, including:

- Use of protective eyewear and clothing
- Proper use of fume hoods
- Location and use of laboratory safety devices; eyewashes, emergency showers, fire extinguishers, fire blankets, and first aid kits, as well as MSDS sheets.

ATTACHMENTS

Attachment 1. Example of Montrose Tissue Processing Log
Attachment 2. Example of the Montrose Tissue Composite Log
Attachment 3. Fillet instructions according to US EPA (2000)
Attachment 4. Example of Battelle Certificate of Training.

APPROVALS

APPROVALS

	Name	Date
Author		5 9-03
Quality Systems Manager		5-9-03
Project Manager		5/9/03

Attachment 1

Montrose Tissue Processing Log

Samples Relinquished by: Location from which retrieved:	Date :	Time:
Samples received by: Storage until processing initiated:	Date :	Time:
Samples Returned to Custodian: Location Stored:	Date :	Time:

BDO Sample ID	Whole Fish Weight (g)	Whole Fish Length (mm)	Date/ Initials	Suffixed BDO ID	Fillet/Composite Weight (g)	Date/ Initials
	NA	NA	NA	Rinsate Blank		
1-						
2-						
3-						
4-						
5-						
6-						
7-						
8-						
9-						
10-						
11-						
12-						
13-						
14-						
15-						

Attachment 2

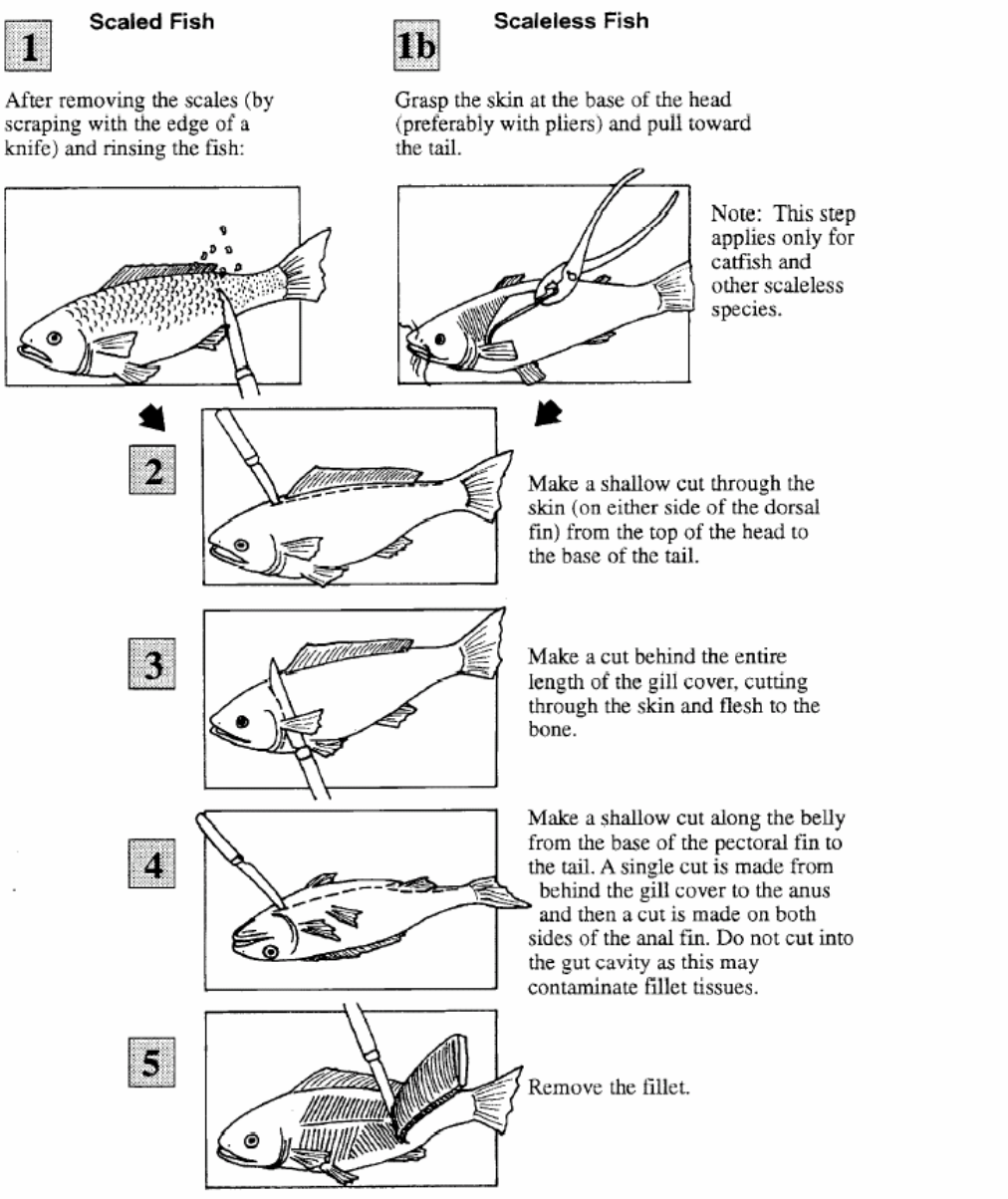
Montrose Tissue Composting Log

Samples Relinquished by: Location from which retrieved:	Date :	Time:
Samples received by: Storage until processing initiated:	Date :	Time:
Samples Returned to Custodian: Location Stored:	Date :	Time:

BDO Sample ID	Whole Fish Weight (g)	Whole Fish Length (mm)	Date/ Initials
1-			
2-			
3-			
4-			
5-			
6-			
7-			
8-			
9-			
10-			
11-			
12-			
13-			
14-			
15-			
Suffixed BDO ID:			

Attachment 3

Fillet instructions according to US EPA (2000)



Source: U.S. EPA, 1991d.

ATTACHMENT 4

Battelle Duxbury Operations

CERTIFICATE OF TRAINING

Title: **PRE-EXTRACTION TISSUE PROCESSING**

SOP#: **Montrose-001-01**

Trainee:

Instructor:

Date SOP was read: _____ (*To be completed by trainee*)

Date training was completed: _____ (*To be completed by instructor*)

APPROVED _____,
Laboratory Supervisor

Battelle Duxbury Operations
Standard Operating Procedures

for

Manual Integration Practices and Review

1.0 OBJECTIVE

This SOP describes the policies, procedures, responsibilities, and records associated with the conduct and reporting of manually integrated data.

2.0 POLICY

In an effort to assure data integrity within the laboratory and to limit liability from data that may have been inappropriately manipulated either intentionally or unintentionally, Battelle has developed a laboratory system to ensure ethical documentation, training and quality assurance practices are being conducted. Of particular concern is the fraudulent practice of using improper manual integrations by chromatographic analysts to falsify test results in initial calibrations, continuing calibrations, quality control samples and surrogate recoveries to make the control data appear to meet quality control requirements. In an effort to deter such practices, proper and improper manual integration practices have been outlined (Section 3) and the implementation of annual independent reviews by Battelle's Quality Assurance Unit (QAU) to detect and prevent improper manual integration practices by analysts has been defined. Willful failure to follow this policy shall result in disciplinary action, up to and including termination.

3.0 PROCEDURES

Manual integration of data is the process by which the automatically generated output of the data handling system of an analytical instrument (i.e. the calculation of peak area leading to a determination of concentration) is over ridden by an analyst, who adjusts a parameter used in making the determination of peak areas (U.S Environmental Protection Agency, Region V, Version 2, Memo May 11, 2001).

3.1 Proper Manual Integration Practices

All data must be integrated **consistently** in calibration standards, calibration verification standards, field samples and QC samples. Integration parameters – both automated and manual- must be technically justifiable and also adhere to valid scientific chromatographic principles. Manual integration is employed to correct improper integration performed by the data system. Examples of data system integrations that may need to be manually corrected include:

- Undetected Peaks: A shift in retention times due to matrix or compound interferences in the sample may cause a data system to exclude (or include) chromatographic peaks. Analysts must review peaks to ensure proper peak identification.

- Incorrect Peak Integration: Peaks that are very similar in retention times and quantitation ions, or are irregular in shape (i.e. split peaks), can be easily misidentified by the data system. An analyst must carefully review all peaks especially peaks that are known to have these characteristics (i.e. benzo (b) and (k) fluoranthene).
- Baseline Corrections: Highly contaminated samples can cause matrix interferences that adversely affect the baseline of target compounds.
- Peaks Identified Incorrectly: A data system's method integration parameters may not be fully optimized resulting in the misidentification of a chromatographic peak. Analysts must ensure the data system operates with minimal operator intervention and also demonstrate the methods parameters are set correctly by carefully evaluating the results of calibration standards prior to analyzing field samples.

Manual integrations are preformed by visually assessing peak symmetry and comparing the integration performed in the initial calibration with QC and field samples. On the occasion that an interfering peak is co-eluting with the peak of interest, the analyst must estimate as accurately as possible where the target peak is likely to end (or begin) and adjust the integration to reflect the correct area of the peak. The analyst must also pay particular attention to integration problems encountered for the analysis of standards or blanks, which, in theory, should be free of contamination and interfering peaks. Unusual baseline characteristics, unidentified peaks, splitting peaks, excessive tailing or other problems may indicate a need for instrument maintenance or other corrective action (SOPs 3-092 and 4-035).

3.2 Improper Manual Integration Practices

Under no circumstances should manual integration be performed solely for the purpose of making failed calibrations, surrogates or internal standards appear to meet quality control (QC) criteria. Practices such as peak shaving, peak enhancement or manipulations of the baseline to achieve these ends must never occur as this results in an improper integration rather than correcting a data system error.

3.2.1 Peak Shaving

Intentionally removing peak areas that are inconsistent with valid scientific chromatographic principles and the integration technique performed on the initial calibration and QC samples is an unacceptable manual integration practice.

3.2.2 Peak Enhancement

Improperly enhancing peak areas by combining multiple peaks to produce a value for a single compound (also known as "Mountain Ranging") that is inconsistent with valid scientific chromatographic principles and the integration technique performed on the initial calibration with QC and field samples is an unacceptable manual integration practice. While peaks at low concentrations combined with a noisy baseline may result in the appearance of "mountain ranging" even when the intent may be unintentional, data must be rejected if the chromatogram is not clear enough that "mountain ranging" can be conclusively ruled out.

3.2.3 Baseline Manipulation

Artificially dropping a baseline to increase area amount (also known as "Boat Anchoring") that is inconsistent with valid scientific chromatographic principles and the integration technique performed on the initial calibration with QC and field samples is an unacceptable manual integration practice.

3.3 General Peak Identification Practices

There are some basic peak identification rules that should be followed across all analytical instruments.

- Peak must be assessed against a signal-to-noise of approximately 3:1.
- Peak retention time is critical in peak identification (though shifting of retention times can happen depending on the sample matrix). For GC/MS analysis, the Primary Ion identification is critical in peak identification (secondary and tertiary ions are less critical in SIM methods).
- Peak shape is critical in identifying possible interference and/or degradation.
- Chemical reasonableness should be used when evaluating the data. This includes historical handling of analytes, known co-elutions, pattern recognition, and analyst's discretion. However, do not force a peak to be selected if the basic response is not met.

3.4 Documentation

Manual integration is employed to correct an improper integration performed by the data system and must always include a qualifier clearly stating that a manual integration was performed (typically denoted using "m"). In addition, the analyst that performed the manual integration must be identified on the documentation by having them initial and date the instrument report. Alternatively, when a data system is limited in its ability to qualify manual integrations such as in the GC/ECD X-Chrome Data System® it is the responsibility of the analyst to clearly document on both the instrument reports and Miscellaneous Documentation Form when data has been manually integrated. Please note, however, that due to the complex nature of environmental samples analyzed by GC/ECD, all peaks are visually reviewed and manually integrated by the analyst.

Additional documentation criteria may be required to meet specific project criteria including producing graphic instrument print-outs of the manually integrated peak or the initialing of instrument reports by a peer reviewer. Project-specific manual integration criteria must be outlined in the Quality Assurance Project Plan.

3.5 Review

Manual integrations are reviewed by an analyst and may be spot checked by a secondary reviewer (SOP 6-027). An independent review may be conducted by QAU. In addition, project-specific criteria may require that a 3rd party validation of the data be preformed. All project specific requirements must be

outlined in the QAPP.

4.0 CALCULATIONS

Not applicable

5.0 QUALITY ASSURANCE

5.1 Laboratory Inspections

Laboratory inspections of manually integrated data will be conducted at least annually by QAU. This is to ensure an independent, unbiased, and rigorous review of manual integration procedures in order to preserve data quality. The appropriate Project Manager or Laboratory Supervisor will be notified prior to the inspection to ensure personnel are available to answer questions during the inspection and to avoid scheduling conflicts. Unannounced inspections will be conducted only if a problem is suspected. Laboratory inspections will also be conducted at the request of management (Resource Managers and Laboratory Manager).

5.2 Reporting

Once the inspection is complete, the auditor prepares an inspection report that summarizes the results of the inspection. Any deficiencies are described and corrective actions are recommended. General and project-specific issues are identified as such and discussed separately in the inspection report.

The reporting procedures for project inspections are identical to those described in SOP 4-015. Outstanding issues are resolved through discussions with Laboratory, Project, or the Resource Manager, as necessary. All original audit reports are maintained in the QAU files.

6.0 RESPONSIBILITIES

It is the responsibility of each analyst to have read and understood the manual integration policy outlined in this SOP, to have read Battelle's *Standards of Business Ethics and Conduct* and to have been trained by an experienced analyst before manually integrating laboratory data. All training documentation must be submitted to the QAU for permanent filing (Attachment 2)

It is the responsibility of the Quality Assurance inspector to prepare for each inspection. Before conducting an independent review of data integrity within the laboratory, the QAU inspector must have read and understood the manual integration policy outlined in this SOP and have been trained in or have had previous experience in GC chromatographic techniques and the operation of the acquisition software used to conduct manual integrations.

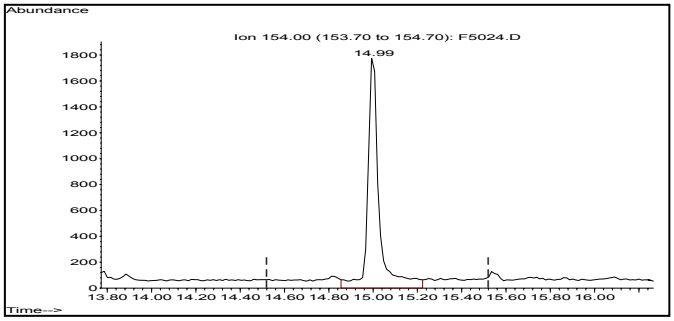
ATTACHMENTS

1. Examples of Improper Integrations
2. Example of Certificate of Training

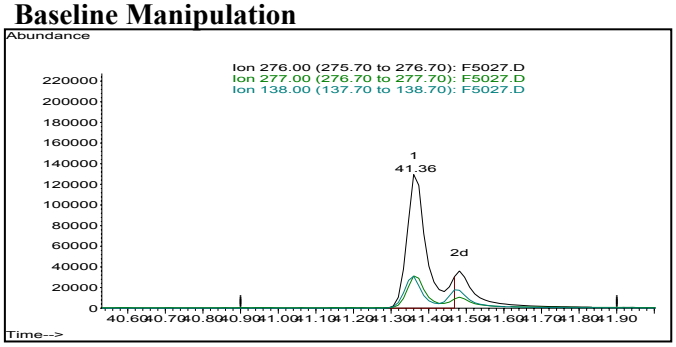
APPROVALS

Author		10-22-02
Technical Reviewer		10/22/02
Quality Systems Manager		10-25-02
Laboratory Manager		10/25/02

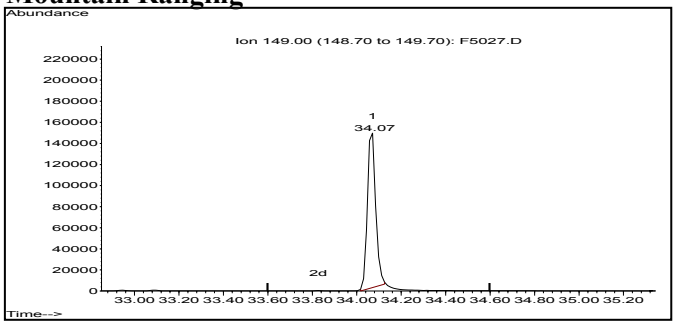
ATTACHMENT 1
Examples of Improper Manual Integrations



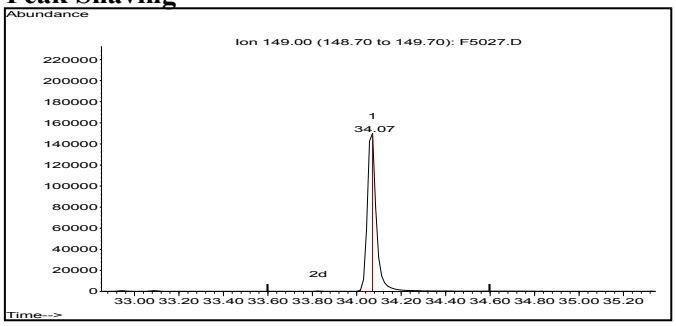
Baseline Manipulation



Mountain Ranging



Peak Shaving



Peak Shaving

Attachment 2
Record of Training
for

MANUAL INTEGRATION PRACTICES AND REVIEW

The above mentioned SOP is relevant to your work. Your signature below attests to the fact that you have read this SOP and understand the requirements associated with this procedure.

Name (Printed)	Signature	Date

Battelle
Applied Coastal and Environmental Services
Standard Operating Procedure

For

HPLC (GPC) CLEANUP OF SAMPLE EXTRACTS FOR SEMIVOLATILE ORGANIC POLLUTANTS

Summary of Changes in this Version:
The calibration solution has been updated. An additional column set has been added for use in processing tissues. References to the Foxy n fraction collector have been removed and replaced with the Waters Fraction Collector Ill. Calibration procedures have been modified. Collection windows have been removed from the SOP and will be posted with each individual HPLC system. New routine maintenance steps have been added. A new inline filter has been added to the system.

1.0 OBJECTIVE

Gel-penneation chromatography (GPC) is a size exclusion cleanup procedure used for the elimination of lipids, polymers, proteins, natural resins, cellular components, steroids, and other high molecular weight compounds from the sample extracts. GPC is appropriate for both polar and non-polar analytes; therefore, it can be effectively used to clean extracts containing a broad range of analytes (EPA, 1994).

GPC, operating on the principal of size exclusion, will not usually remove interference peaks that appear in the chromatogram since the molecular size of these compounds is relative similar to the target analytes. Separation cleanup techniques, based on other molecular characteristics (i.e., polarity), must be used to eliminate this type of interference (EPA, 1994).

This SOP focuses on sample cleanup using GPC in combination with a High Pressure Liquid Chromatography (HPLC) system. Other columns and instrumental conditions may be used for particular applications once acceptable perfonnance has been demonstrated.

2.0 PREPARATION

2.1 APPARATUS AND MATERIALS

- HPLC System
    - HPLC Pump (Waters 1515, or equivalent)
    - HPLC Autosampler (Waters 717, or equivalent)
    - HPLC Ultra violet (UV) detector, set to 254 nm (Waters 2487, or equivalent)
    - Recorder or integrator (Waters Breeze software system, or equivalent)
    - HPLC Fraction Collector (Waters Fraction Collector Ill, or equivalent)
- GPC Columns (Detailed below)
    - Waters and Sediments
        - Phenogel, 21.2 rom x 50 rom guard column (phenomenex)
        - Phenogel, 21.2 rom x 300 rom column (phenomenex)
    - Waters, Sediment, and Tissues
        - Envirogel, 4.6 rom x 30 rom guard column (Waters)
        - Envirogel, 19 rom x 150 rom column (Waters)

- Envirogel19 mm x 300 mm (Waters)

- Consumables
  - Glass screw cap vials, 25 mL, 40 mL, 60 mL with Teflon lined caps
  - Glass Autosampler vials, 4 mL
  - 4 mL vial caps, screw closure, with septa (Waters)
  - Glass Autosampler vials, 1.2 mL (Waters)
  - 1.2 mL vial, Teflon lined, crimp caps (Waters)
- Trident in-line filter cartridge holder (Restek)
  - 4 mm, 2.0 µm cap frits (Restek)
- Large glass funnels
- 0.1 µm glass fiber filters (Whatman)

2.2 REAGENTS

- Dichloromethane (DCM), pesticide grade or equivalent
- Isopropyl alcohol
- Helium, for degassing solvent
- Glycerol

Analytes for the retention time window standard

- Corn Oil, purchased locally
- bis(2-ethylhexyl) phthalate [117-81-7]
- Methoxychlor [72-43-5]
- Perylene [198-55-0]
- Sulfur [63705-05-5]

2.3 Retention Time Window Standard Preparation

The retention time standard will be prepared as follows, in DCM:

Compound	ng/µL
Corn Oil	25,000
Bis(2-ethylhexyl)phthalate	1,000
Methoxychlor	200
Perylene	20
Sulfur	80

Elemental sulfur is not readily soluble in DCM, but is soluble in wann corn oil. Weigh out and heat the corn oil first. Add the sulfur to the corn oil to dissolve prior to the addition of the remaining analytes. Allow the solution to come to room temperature prior to use as the corn oil may precipitate out of solution at lower temperatures.

The HPLC retention time window standard used for the determination of retention times is stored at 4°C and assigned an expiration date of 3 years.

2.4 GPC Columns

Sediment, Tissue and Waters:
Sediment, tissue and water extracts can be run on the Waters Envirogel column setup. Three

Envirogel columns are run in series; a 4.6 rom x 30 rom guard column, a 19 rom x 150 rom secondary column, and a 19 rom x 300 rom tertiary column. These columns are designed for high volume, high lipid injections and can handle the higher capacity lipids involved with tissue extractions. Samples run on this column set will be concentrated to 3000 µL of which 1800 µL will be injected onto the column. This column set is compatible with all sample matrices.

Sediment and Water:
Sediment and water extracts can be run on the Phenomenex column setup. Two Phenogel columns are run in series; a 21.2 rom x 50 rom guard column followed by a 21.2 rom x 300 rom column. These columns are only compatible with sediment and water matrices with a maximum injection volume of 600 µL Samples run on this column set will be concentrated to 1000 µL of which 600 µL will be injected onto the column.

2.5 LABWARE PREPARATION

Reusable lab ware contacting samples must be washed, baked and solvent rinsed according to SOP 5-216. Consumable glassware must be baked prior to use.

2.6 RECORD KEEPING

An HPLC Sample Fractionation Log Form (Attachment 1) will be filled out and kept in the HPLC logbook for each HPLC sequence. HPLC sequences (Attachment 2) are recorded electronically using the HPLC software (Breeze or equivalent); this sequence will be printed and also kept in the HPLC logbook .Refractionated samples are assigned the suffix "-R" to distinguish them from the original sample (SOP 6-007). The Extract **ill** recorded must agree with that used in the Sample Preparation Records, including the extract number if applicable (for example - XOOOI-P(2».

The HPLC Sequence will be completed for each set of fractionations/analyses by adding the results of the calibration (i.e., retention times of calibration analytes and fractionation window(s» to the printed copy. Column type, injection volume, flow rate, mobile phase, any automatic valve switching times, and initial pump pressure. System maintenance (e.g., column replacement, replacement of tubing, unclogging of system plugging, cleaning of detector) will be recorded on the appropriate forms in the appropriate HPLC Instrument Maintenance Logbook (Section 6.0).

3.0 PROCEDURE

3.1 GENERAL

Samples are extracted, or otherwise prepared, according to procedures specified in the other Battelle SOPs, run through a rapid filtering clean-up, concentrated, and submitted for HPLC cleanup. No samples will be run on the HPLC without a filtering step (detailed in the extraction SOPs) to ensure that particulates are not injected onto the instrument. All HPLC systems will have a 2.0 µm frit in-line prior to the guard column; this filter must be changed prior to each run. The retention time window is determined using the above retention time window standard. The extract is injected on the HPLC, the UV response monitored, and the analyte fraction(s) collected, concentrated and submitted for further processing / instrumental analysis. The specific collection window used will be based on the target analyte list.

3.2 **HPLC Initialization**

- Fill the HPLC reservoirs with DCM by filtering the DCM through a 0.1 glass fiber filter in a glass funnel. Reservoirs should be filled *prior* to starting the HPLC pumps.
- De-gas DCM with a steady flow of Helium through a stainless steel bubbling sinker for approximately 5 minutes.
- Disassemble the Trident in-line filter cartridge holder and remove the frit cap. Replace the frit cap with a new frit and reassemble the filter cartridge.
- Change the waste container as necessary.
- The HPLC system must be purged and primed (according to the manufacturer's guidelines) prior to continuing. The system should be run a minimum of 1 hour prior to calibration.
- Visually inspect the lines to ensure that all seals are tight and there are no apparent leaks.

3.3 **PRE-HPLC SAMPLE PREPARATION**

- All extracts must be filtered prior to HPLC to ensure that particulates are not injected onto the HPLC.
- Extracts are concentrated to approximately 1000 µL (3000 µL for the Envirogel HPLC columns) by appropriate methods and transferred to the appropriate autosampler vial.
- Measure the sample volume to the nearest 10 and record the volume on the HPLC Dilution Documentation Form in the Sample Preparation Management module in LIMS (refer to Sample Preparation Management Training Manual). Sample extracts should be loaded onto the HPLC on the same day that the volume is recorded to ensure that minimal extract evaporation will occur.
- *Extract viscosity must not exceed the viscosity of* 1:1 *glycerol/water. All extracts that exceed this viscosity must be diluted.*

3.4 **HPLC FRACTIONATION TIME DETERMINATION**

- Run two separate injections of the retention time window standard [followed by a solvent (DCM) blank] on the system. Refer to Attachment 3 for elution order.
- Compare the retention time (RT) results of all compounds between the two injections. Retention times must be within 0.1 seconds to continue.
- All peaks must be symmetrical and not overload the detector.
- The following minimum baseline resolutions must be achieved:
    - 85% resolution between Com oil and bis(2-ethylhexyl)phthalate must be achieved.
    - 85% resolution between bis(2-ethylhexyl)phthalate and methoxychlor must be achieved.
    - 85% resolution between methoxychlor and perylene must be achieved.
    - 90% resolution between perylene and sulfur must be achieved.

The above criteria must be met in order to continue. If these criteria are not met, corrective action must be taken prior to proceeding; this corrective action can include, but is not limited to, verifying the flows, cleaning the detector, and re-injecting the RT standard. Ultimately, a service call will be placed if the instrument does not provide reproducible RT data. Collection windows for standard analytes are posted for each HPLC system based on the RT of methoxychlor and *sulfur, for example, the collection window for pesticide/PCB/PAH without methoxychlor could start at Methoxychlor + 0.3 minutes and end at Sulfur - 0.5 minutes.* Windows for non-standard target analytes will be determined on a project specific need.

3.4.1 Initial Determination of Collection Windows

The initial collection windows are calibrated as follows:

- Inject two separate aliquots of DCM; each containing the target analytes and surrogate compounds of interest
- Collect multiple fractions from each aliquot injected:
    - Collect a minimum of 3, 15 second aliquots starting at the Methoxychlor RT and working backwards
    - Collect from the Methoxychlor RT to the Perylene RT
    - Collect a minimum of 3, 15 second aliquots starting at the Perylene RT and working forwards
- Run each fraction by the appropriate analytical method
- The collection window will be determined by reviewing the analytical data and which fractions the target analytes were collected in

3.5 SAMPLE FRACTIONATION/CLEANUP

- Label the fraction collector tubes and load into fraction collector.
- Load sample extract vials into autosampler rack and place rack into injector.
    - Include an HPLC fractionation retention time window standard and solvent blank for analysis every 12 hours.
- Check that the HPLC pump, autosampler, and fraction collector programs are correct.
- Ensure that the fraction collector is programmed for the appropriate collection tray (28 mm collection tray).
- Ensure that the appropriate windows and request time have been entered into the collector.
    - The request time is the amount of time the sample will be collected in each vial, this should match the total collection time if one vial is used for each sample.
- Ensure that the distance between the collection vials is set to a height that will allow clearance of the fraction collector arm, but not exceed a safe drop distance for collecting the sample.
- Ensure that the collector is set to "Multi" (multiple injections) with the "skip" function turned off.
- The fraction collector will start automatically when the first sample has been injected. The collector can be aborted by hitting the "End" button twice.

3.6 POST HPLC VERIFICATION

Using a reference vial, visually check the volumes remaining in the autosampler vials as soon as the analysis sequence is completed. If the volume appears to be significantly different from that expected, measure and record all volumes and adjust the HPLC Injection Volume form, as appropriate. Identify and resolve the injection problem.

3.7 REFRACTIONATION OF SAMPLES

Should the fractionated sample not be usable for final instrumental analysis (e.g., pump shut off in the middle of a run), the unfractionated portion of the original extract can be used for HPLC processing. The unfractionated portion is brought to the same volume as the initial extract with DCM (the exact volume indicated on an HPLC Dilution Documentation Form located in the Sample Preparation Records in LIMS),

and the sample analyzed/fractionated as described in previous sections. Unusable sample fractionations will be indicated on the HPLC Sample Fractionation Log Form, explained, dated, and initialed. Refractionations will be indicated as such on the new HPLC Sample Fractionation Form that is prepared, and the number of the refractionation will also be documented according to SOP 6-007 (e.g., "-Rl": first refractionation; "-R2": the second time the samples are refractionated).

3.8 RETENTION OF THE NON-FRACTIONED SAMPLE EXTRACT

The remaining non-fractionated split will be stored refrigerated (4°C) until the final data has been accepted by the client. Once the data has been accepted, the non-fractionated extract will be disposed of in the appropriate waste stream.

4.0 CALCULATIONS

Determination of fractionation window was discussed in Section 3.3. The fraction of the sample processed through the HPLC is calculated and recorded on the HPLC Dilution Documentation Form using the following equation.

First Fractionation: [(IV()/(EV\)] I 100%

Re-fractionation: [1- «IV\+AV)IEV\)] I [(IVR)/(EV$_R$)] I 100%

Where:

IV\ = Sample injection volume (µL) in original/first fractionation
EV\ = Pre-HPLC sample extract volume (µL) in original/first fractionation
AV = Autosampler volume difference between aspiration and dispensing (generally 0 µL Waters system).
IV$_R$ = Sample injection volume (µL) in re-fractionation
EV$_R$ = Pre-HPLC sample extract volume (µL) in re-fractionation

Baseline resolution:

%R= 100 * (I-AlB)

R = Resolution in percent
A = Height of the valley between two peaks
B = Height of the smaller peak

5.0 QUALITY CONTROL

To ensure the integrity of the cleanup process, all preparation batch QC samples, including the procedural blank (PB); will be processed through the HPLC/GPC cleanup system using the same method as the associated authentic field samples.

1. Quality control methods for this procedure will consist of establishing the RTs of the fractionation window calibration analytes before each batch of samples are run by multiple injections of an HPLC retention time window standard until two analyses result in RT differences of less than 0.1 minutes. The average RTs from these analyses will be used to program the fraction collector, please refer to the Waters Fraction Collector III manual for instructions on programming the collector. The standard will be analyzed every 12 hours to check the RTs of the calibration compounds. A solvent blank must be

run after each injection of the calibration solution.

2. After major HPLC system changes (e.g., column(s) from different manufacturer, new column dimensions installed, new dimension or length of lines/tubing), or when performance dictates, a fraction collection calibration will be performed to determine the size of the fractionation window needed to collect >95% of the first and last eluting target analytes. This calibration will be conducted by fractionating an injection of the matrix spike target solution, collecting small fractions (e.g., 0.5 minutes) from several minutes before to several minutes after the HPLC RTs of these compounds (a single larger window can be collected between the two RT marker analytes), and analyzing the fractions by the appropriate instrumental method.

6.0 MAINTENANCE

This procedural SOP is not intended to include a detailed description of system maintenance. Maintenance considerations and procedures are defined in each of the instrument's operations manuals located at bench level. However, a few specific items are listed below. Non-routine maintenance (e.g., pump seal, column or other hardware replacement, or dislodging of a system clog) should be recorded in the appropriate HPLC Instrument Maintenance Logbook.

HighILow Pressure. The system pressure is checked each day before samples are analyzed. Should the pressure be unacceptably high (over 500 PSI for each OPC column attached to the system; i.e. 1000 PSI for the two column setup) it may be necessary to clean or replace the pre-column. In order to determine the cause of an exceedence in pressure, the system can be started with one column in place and then adding each additional column while monitoring the pressure. A common cause of high pressure is a clogged detector cell. If the detector cell is clogged it should be removed and replaced. With time, the backpressure due to accumulations in the pre column will become unacceptably high, and the pre column will need to be replaced. It is important to replace it before the main column begins deteriorating. Columns may be cleaned by reversing the flow through the columns (maximum flow in reverse should not exceed 1 mL/min) and injecting several aliquots of toluene (this procedure should be carried out on the guard column first). The main column will last several years if the system is maintained properly. Accumulations in the main column and detector may also cause elevated pressure. Other possible causes of elevated pressure are clogged tubing at a fitting (reverse flow through tubing) and accumulations in the detector cell. Should the pressure be low (under 200 PSI) there might be a leak or it may be necessary to replace one of the HPLC pump piston seals.

Detector. Constant flow of DCM through the detector cell will cause buildup on the cell which must be periodically removed. Buildup can be removed by running isopropyl alcohol through the detector cell; this procedure should be performed at a minimum of once a month.

7.0 TRAINING

An analyst performing the procedures described in this document must initially work under the supervision of the Laboratory Manager, task leader, or another individual designated by the Laboratory Manager. Individuals may work independently once they have satisfactorily completed supervised training. Once training has been completed a Certificate of Training (Attachment 4) will be completed and filed in the Quality Assurance Unit (training files).

8.0 SAFETY

As part of the above training program, the analyst will be made aware of the particular safety concerns of this procedure, including:

- Use of protective eyewear and clothing
- Proper use of fume hoods
- Handling of hazardous chemicals and reagents
- Location and use of laboratory safety devices; eyewashes, emergency showers, fire extinguishers, fire blankets, and first aid kits, as well as MSDS sheets.

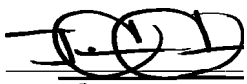
9.0 REFERENCES

U.S. EPA SW-846 Manual, Method 3640A, Revision 1, September 1994

ATTACHMENTS

Attachment 1. HPLC Sample Fractionation Log Form
Attachment 2. HPLC Sequence list
Attachment 3. Example of Approximate GPC Elution Patterns for Selected Analytes
Attachment 4. Certificate of Training

APPROVALS

	Name	Date
Author		2/16/2005
Technical Reviewer	Bryan Mann	2/16/05
Quality Systems Manager	for Bob K	2-16-05
Laboratory Manager	Nils Stein	Feb-16-2005

ATTACHMENT 1

HPLC Sample Fractionation form

				HPLC#			
Project				Batch No			
Operator				Date			
Witness				Date			
Solvent	DCM	Other		Columns	Envirogel | Phenogel | Other		
	Initial	Final					
PSI							
Routine Maintenance							
Replace Frit	| Fill Reservoirs |			Check waste | |			
RT Calibration							
Analyte	Call	Cal2	CCVI	CCV2	CCV3	CCV4	CCV5
Corn Oil							
Bis(2-ethylhexyl)phthalate							
Methoxychlor							
Perylene							
Sulfur							
Fraction Collector Program							
Window	*wlo* Methoxychlor |		*wi* Methoxychlor |		other		
	RT	*+1-*	Adjusted	M	S		
Methoxychlor							
Sulfur							
	Request time						
Comments:							

ATTACHMENT 2

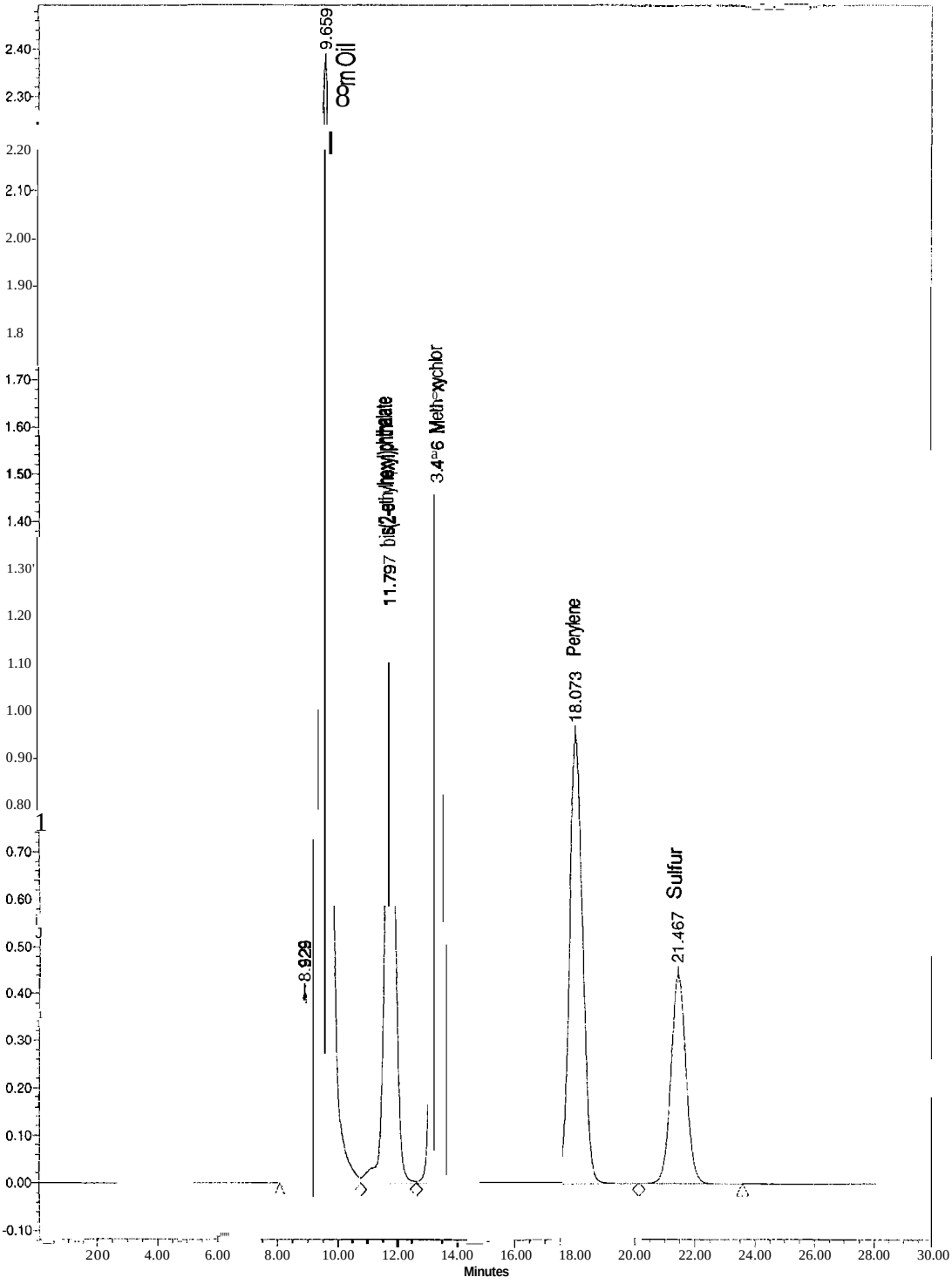
HPLC Sequence Log

Sample Set Parameters: Untitled

	Vial	Sample Name	Function	Method	Run Time (Minutes)	Inj Vol (ul)	# of Injs	Sample Weight
1	1	BF838PB-P	Inject Samples	5 191	30.00	1800.0	1	1.00000
2	2		Inject Samples				1	1.00000
3	3						1	1.00000
4	4							
5	5							
8	8							
9	9							
10	10							
11	11	GG61						
12	12	DCM						
13	13	S6907-P						
14	14							
15	15							
19	19	S6913-P	Inject	15191	30.00	1800.0	1	1.00000
20	20	S6914-P	Inject Samples	5 191	30.00	1800.0	1	1.00000
21	21	SG915--P--	InjectSamples	5 191	30.00	1800.0	1	1.00000
22	22	GG61	Inject Samples	5 191	30.00	600.0	1	1.00000
23	23	DeM	Inject	5 191	30.00	1800.0	1	1.00000

ATTACHMENT 3

HPLC Retention Time Standard



ATTACHMENT 4

Battelle
Applied Coastal and Environmental Sciences
Certificate of Training

In

***HPLC/GPC* Cleanup of Environmental Sample Extracts**

Trainee _____

Instructor _____

Date SOP Read _____

Date Training Completed _____

Supervisor Approval: _ _____

Name Date

Battelle
Applied Coastal & Environmental Services
Standard Operating Procedures

for

SOIL/SEDIMENT AND TISSUE EXTRACTION FOR SEI\1I-VOLATILE CONTAI\1INANT ANALYSIS USING THE ACCELERATED SOLVENT EXTRACTOR

Summary of changes in this version: The accelerated solvent extractor (ASE) extraction method has been modified to include a longer extraction cycle and additional static cycles. Extract cleanup has been removed from this standard operating procedure (SOP); cleanup procedures are listed as references to separate SOPs. Filtering procedures have been added for all extracts.

1.0 OBJECTIVE

ASE is an automated system for extracting organic compounds from a variety of solid matrices. The extraction solvent is pumped into an extraction cell containing the sample, which is then brought to an elevated temperature. Pressure is applied to the sample extraction cell to maintain the heated solvent in a liquid state during the extraction. After heating, the extract is flushed from the sample cell into a collection vial and is ready for further processing (see attachment 1 for processing details).

The objective of this document is to define standard procedures for extracting semi-volatile contaminants from soil/sediment and tissue matrices for analysis by gravimetric and gas chromatographic methods using an ASE extraction technique based on EPA Method 3545. The extraction procedures are also suitable for analysis of semi-volatile organic pollutants including those listed in EPA Methods 8081, 8082, 8270, etc. The method may be suitable for other analytes once acceptable extraction efficiency has been demonstrated. Generally, this procedure will be used to prepare extracts that will be analyzed after further processing using separate cleanup procedures.

Note: Soils originate on land (terrestrial environments) and are typically dry mixtures of geological and organic materials, while sediments originate from the bottom of open aqueous environments (e.g., coast, rivers, lakes) and are typically wet mixtures. Biological tissues come in a variety of forms and all will be treated similarly in this SOP.

2.0 PREPARATION

2.1 APPARATUS AND MATERIALS

- Apparatus for Extraction and Extract Concentration
  - Calibrated gas-tight microliter syringes (SOP 3-172) or pipettes (draft SOP 3-181)
  - Kudema-Danish (K-D) apparatus
    - Reservoir (250 mL or 500 mL)
    - Snyder column, three ball macro
    - Concentrator tube (10 mL or 20 mL)
  - Water bath, capable of reaching temperature of 100°C, located in fume hood

  - Nitrogen evaporation apparatus, N-Evap or equivalent, with heated water bath
  - Glass wool, muffled at 400°C for at least 4 h, cooled, stored in oven at approximately 100°C
  - Pyrex funnels (large)
- Pressurized fluid extractor
  - Dionex Accelerated Solvent Extractor (ASE) 200 with 33 mL stainless steel extraction cells
  - Dionex funnels for loading extraction tubes
  - Pre-cleaned 60 mL collection vials with Teflon septa screw caps (Environmental Sampling Supply Oakland, CA), or equivalent
  - Filter disk, stored in drying oven at 100°C (Whatman)
- Apparatus for determining percent dry weight and total extractable organics
  - Oven - drying
  - Analytical balance capable of weighing to 0.01 g (SOP 3-160)
  - Analytical micro-balance (SOP 3-004)
  - Aluminum weighing pans
  - Stainless steel or Teflon spatula
  - Gas-tight syringes, various sizes
  - Volumetric flasks, Class "A", various sizes
  - Hot plate
- Apparatus for filtering sample extracts
  - Disposable filter tubes (Applied Separations) containing a glass fiber filter. Filter tubes are rinsed with DCM prior to use.
  - 25 or 40 mL vials with Teflon lined screw caps
  - Vacuum manifold
  - Vacuum pump

2.2 REAGENTS AND SOLVENTS

- Anhydrous Sodium Sulfate, reagent grade, heated to 400°C for at least four hours, cooled and stored at room temperature in a sealed glass container.
- Acetone, pesticide grade or equivalent
- Dichloromethane (DCM), pesticide grade or equivalent
- Surrogate Internal Standards (SIS) solutions
- Internal Standards (IS) solutions
- Target analyte spiking solutions
- Silicon dioxide, chromatography grade, or equivalent, heated to 400°C for at least four hours, cooled and stored in drying oven at 100°C

2.3 LABWARE PREPARATION

All reusable glassware must be cleaned according to laboratory protocols defined in SOP 5-216, glassware is then rinsed with DCM prior to contact with samples. Pre-cleaned glassware may be used as purchased.

2.4 RECORD KEEPING

Samples will be assigned unique identification numbers and logged into the Laboratory Information Management System (LIMS) according to laboratory protocols (SOP 6-007).

Analytical data will be reported on appropriate data forms included in the sample preparation records. Information to be recorded includes wet and dry weight information, dates of extraction/processing procedures, initials of laboratory personnel performing the procedures, types and amount of internal

standards added to samples, and, if necessary, comments regarding individual samples.

A log of all samples extracted on each ASE must be kept. The information in this log should include sample ill's, date of ASE run, operator's initials, and any comments/notes made for each particular sample. This instrument logbook is assigned a tracking number and paginated according to SOP 6-039 (Attachment 2).

All samples will be managed using the LIMS from sample receipt to data reporting.

3.0 PROCEDURES

3.1 PRE-EXTRACTION PROCESSING

Decant any over-lying water from the sample *(sediments only).* All samples must be mixed well *(homogenized),* foreign objects, such as leaves, sticks and rocks, should also be removed from the sample prior to sub-sampling for extraction. Some drier sediment samples (i.e. clays) may need to be ground using a mortar and pestle prior to weighing the sample.

1. Dry weight determination
    a. Record the weight of an aluminum drying pan
    b. Place ca. 5 grams of sample on the drying pan and record the weight
    c. Place pan in drying oven overnight
    d. Record the weight of the pan and dried sample
2. In a clean beaker or pre-cleaned jar weigh out ca. 5 g of sample. Record the sample weight
3. Preparation of sample for extraction
    a. Using a clean spatula, mix the 5 g sample with ca. 5 g of Hydromatrix, the sample should mix with the Hydromatrix and no longer stick to the sides of the container. Additional Hydromatrix can be added if needed to dry the sample
    b. Cover the sample and allow to dry for ca. 1 hour
    c. After 1 hour, remix the sample and hydromatrix, larger bits of sample will be dryer and easier to break up. The sample should be broken down to roughly the same size as the hydromatrix
    d. Fortify each sample with the appropriate concentration of SIS solution, SIS concentrations are determined on a project specific basis
    e. Samples are now ready to transfer to the cells prepared in step 4
4. Preparation of ASE extraction cells
    a. Place filter disk in the bottom of each extraction cell.
    b. Using the ASE cell funnels, place ca. 2 g of clean, baked silicon dioxide in each cell
    c. Transfer sample to extraction cell and gently pack down contents with a spatula. Alternatively, a glass stopper can be used to compact the sample into the cell- it may be necessary to place a second glass filter disk at the top of the cell

Note - it is very important to pack the cell to minimize the dead volume present in a cell; excessive dead volume can cause the collection vessel to overfill.

If necessary, sample container may be rinsed with 2-3 mL of the extraction solvent. Top off extraction cell with silicon dioxide - there should be no dead volume on the top of the extraction cell.

3.2 EXTRACTION, FILTRATION, AND CONCENTRATION

The following instructions are a general guideline for the extraction procedure. Prior to the extraction of any samples the analyst should read and understand the manufacturer's instructions for the use of the ASE.

3.2.1 Sample Extraction

1. Load the labeled extraction cells onto the top of the ASE unit
2. Load clean, labeled collection vials onto the bottom tray of the ASE unit
3. Verify that the solvent reservoir is full
4. Empty or replace the solvent waste vials
5. Run the ASE rinse program a minimum of three times prior to the extraction of the first sample.
6. Use the parameters listed in table 1 for sample extraction

Table 1: Extraction parameters

Parameter	Value
Extraction Solvent	DCM [a]
Temperature	*125 DC* [b]
Pressure	2000 PSI
Heat up time	5 min
Static time	10 min
Flush volume	50%
Purge time	200 s
Static cycles	3
Rinse between samples	ON

a - Hexane, or Hexane/Acetone may be suitable for the extraction of pesticides and PCBs.
b - Extraction temperature should be lowered to *100°C* if extracting for PAH.

7. Allow extract to cool to room temperature before proceeding

3.2.2 Extract drying

All sample extracts are dried prior to continuing the extraction and cleanup procedures. Extract drying is perfonned using sodium sulfate.

1. Assemble the K-D apparatus prior to drying samples
2. Place a small glass wool plug in the end of a large glass funnel
3. Rinse the funnel and glass wool with DCM, discarding the DCM into the Drum Solvent waste steam
4. Fill the funnel with ca. ¾ full with sodium sulfate (ca. 50 g) and place over the K-D
5. Quantitatively transfer the sample extract to the K-D by pouring the extract into the funnel
6. Rinse the extract vial twice with ca. 5 mL DCM and transfer to the funnel
7. Rinse the funnel with 2 x 50 mL aliquots of DCM
8. Sample extract is now ready to concentrate

3.2.3 Extract Concentration - K-D Technique

1. Add 3-5 boiling chips to the K-D receiver and insert a Snyder column. Pre-wet the condenser column with approximately 5-mL DCM. Place the K-D apparatus in a hot water bath maintained at 60-65 ° C (monitored by an alcohol thermometer), such that the concentrator tube is partially immersed in hot water and the entire lower rounded surface of the flask is bathed in hot water vapor. At the proper rate of evaporation, the balls of the Snyder column will actively chatter, but will not flood with condensed solvent. Continue concentration until the sample volume is reduced to approximately 10 mL. Further concentrate the extract to approximately 2 mL using nitrogen evaporation techniques.
2. the Laboratory Supervisor for instructions on how to proceed if the rate of concentration significantly slows down.

Note: The water bath for the K-D is maintained at 60-65 ° C for concentrating DCM, 70-75 ° C for concentrating acetone, and a boiling bath is used for concentrating hexane or toluene.

3.2.4 Extract Concentration - TurboVap Technique (optional)

Optionally, TurboYap techniques may be used for concentrating the extracts rather than using the K-D technique.

1. Transfer sample extract to a 200 mL TurboVap tube with a 1 mL collection reservoir. TurboVap water bath temperature should be set at approximately 25 °C; nitrogen pressure should be approximately 3 to 5 psi (water bath temperature will vary with type of solvent used).
2. Follow the manufacturers' instructions for operation and maintenance. After initial TurboVap concentration the extract should be quantitatively transferred to a 4 mL vial (with DCM rinses), and concentrated using nitrogen evaporation techniques until the extract volume is 1-2 mL.

3.2.5 Extract Cleanup - Extract filtering

All Extracts must be filtered prior to *HPLC/GPC* cleanup (SOP 5-191) to remove particulates. Filtering is performed using disposable filter tubes (Applied Separations) under vacuum pressure.

1. Setup vacuum manifold with disposable filter cartridges. Place 40 mL vials inside the manifold to collect rinse solvent.
2. Rinse the cartridge with ca. 20 mL of DCM under vacuum.
3. Remove 40 mL vials and dispose of rinse solvent in the appropriate waste stream.
4. Return the empty 40 mL vials to the vacuum manifold.
5. Transfer the extract to the filter cartridge, rinsing the original vial with DCM.
6. Rinse filter cartridge with ca. 15 - 20 mL of DCM (under vacuum).
7. Tum off the vacuum pump and relieve the pressure on the vacuum manifold.
8. Remove 40 mL vials from the vacuum manifold.
9. Extracts are now ready to be concentrated for *HPLC/GPC* cleanup.

3.2.6 Total Extractable Organics (TEO)

Total Extractable Organics (TEO) is determined by a gravimetric analysis of the filtered extract residue after the solvent has been evaporated. TEO on sediments may include oil weight along with other extractable organics. TEO for tissues is mostly total extractable fat.

3.2.6.1 *Gravimetric Analysis (sediments)*

1. Transfer the sample extract to a 10mL volumetric flask. *The initial volume of the sample extract may vary depending on amount of oil that may be in the extract.*
2. Record the initial volume of the sample extract.
3. Using a syringe, remove a 50 µL aliquot and place on a tared aluminum pan.
4. Heat the pan on a hot plate until the solvent has evaporated, remove from heat and allow to cool.
5. Weigh the pan and reside on a microbalance (+/- 0.001 mg), record the weight of the residue.
6. The TEO amount will be automatically calculated by LIMS using the formula in Section 4.
7. Sediment extract is now ready for further processing, please refer to attachment 1.

3.2.6.2 *Lipid Weight Determination (Tissues)*

1. Using a syringe, or volumetric glassware, measure the sample extract to 3 mL and transfer to a 4 mL vial.
2. Label and weigh an aluminum weighing pan, record the initial weight.
3. Using a syringe, remove a 150 µL aliquot of the sample extract and place on the weighing boat.
4. Place the weighing pan in a drying oven (maintained at 40 0c) for approximately 1 minute.
5. Remove from the oven and allow the pan to cool, return the pan to the oven for an additional minute if any solvent remains.
6. Weigh on a balance and record the pan and the sample extract residue.
7. The lipid amount will be automatically calculated by LIMS using the formula in Section 4.
8. Tissue extract is now ready for HPLC cleanup (SOP 5-191) if applicable; refer to attachment 1 for cleanup options.

3.2.7 Final Extract Handling

If no further processing is required, the sample extracts should be adjusted to the desired final extract volume (1000 µL unless otherwise specified in work plan) and prepared for instrumental analysis.

1. Adjust the final extract volume using N-Evap concentration. Adjust the flow of nitrogen on the N-Evap to a gentle stream - do not allow the sample to bubble or splatter, or have a large "dimple" on the surface, as this will result in the possible loss of target analytes. A small dimple is expected on the surface of the solvent. Sample extracts should be immersed in the water bath under the N-Evap unit (see batch temperature table for appropriate water batch temperature for the solvent used - Attachment 3).
2. Spike the final extract with the appropriate recovery internal standards (RIS), as listed in the work plan, and split the extract as necessary, transferring the samples to GC vials. Submit extracts to the appropriate instrument analysis facility.

4.0 CALCULATIONS

4.1 SAMPLE DRY WEIGHT DETERMINATION

Calculate percent dry weight with the following equation:

$$\%\ dry\ weight = \frac{aliquot\ dry\ wt.^{a}}{aliquot\ wet\ wt.^{a}} * 100$$

[a] corrected for pan weight

Calculate percent moisture with the following equation:

$$\% \text{ moisture} = 100 - \% \text{ dry weight}$$

Calculate sample dry weight with the following equation:

$$\text{Sample dry weight (g)} \quad \text{\% dry wt.} * \text{sample wet wt. (g)}$$

4.2 TOTAL EXTRACT WEIGHT DETERMINATION

Use the following equation to determine the total extractable organic content (TEO) weight:

$$TEO\ (mg) = \frac{\text{volume of sample extract (mL)}}{\text{aliquot vol(mL)}} * \text{aliquot wet wt. (mg)}$$

5.0 QUALITY CONTROL

Samples must be extracted in batches of 20 or fewer authentic field samples. Quality control samples accompanying each batch may include a procedural blank, laboratory control sample, matrix spike, standard reference material and/or field sample duplicate.

The QC program for each project is defined in the project work plan, which will define the type and amount of internal standards/spiking solutions to be added to the samples, the specific QC samples to be processed, any modifications to the standard QC acceptance criteria, and the corrective action required if QC results do not meet those acceptance criteria.

6.0 TRAINING

The trainee must read and fully understand the policies and procedures outlined in this SOP. The trainee will then be given a demonstration of all aspects of this SOP.

Technicians may work independently once they have satisfactorily performed the following training test:

- Complete at least four LCS samples concurrently or over a period of days that meet the ≤30 RSD acceptance criteria.

When training is completed, the trainee will be issued an Initial Demonstration of Capability Certificate (SOP 2-011) The original completed training certificate and all supporting documentation will be sent to the Quality Assurance Office.

Analysts must also demonstrate annual proficiency in the method. Section 2.5.3 of SOP 2-011 (Staff Training) outlines the acceptable performance procedures. When annual training is completed, the analyst will be issued a Demonstration of Ongoing Capability Certificate (SOP **2-011).**

7.0 SAFETY

As part of the above training program, the analyst will be made aware of the particular safety concerns of this procedure, including:

- Use of protective eyewear and clothing
- Proper use of fume hoods
- Location and use of laboratory safety devices; eyewashes, emergency showers, fire extinguishers, fire blankets, and first aid kits, as well as MSDS sheets.
- The safe use and operation of the ASE. The ASE has many moving parts and operates using a great deal of air pressure. This combination may present a threat to misplaced hands and fingers and could prove hazardous to the health of a careless operator. The operator should be cautious when the status of the instrument is other than idle. Exercise good judgment whenever operating the ASE.

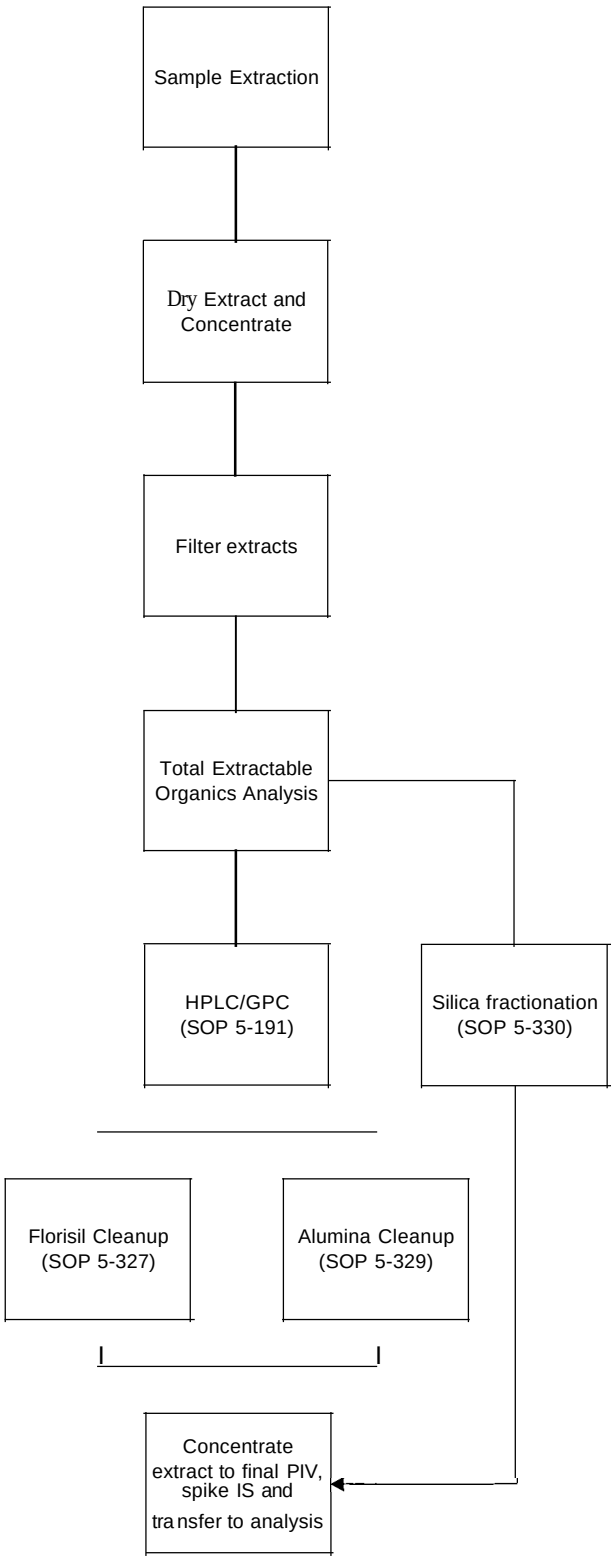
ATTACHMENTS
Attachment 1. Sample processing flow chart
Attachment 2. ASE extraction log
Attachment 3. N-Evap water batch temperature guide

APPROVALS

Author		2/15/2005
Technical Reviewer	Roxanne M. Brackett	2/15/2005
Quality Systems Manager		2-15-2005
Laboratory Manager		Feb-15-2005

ATTACHMENT 1

Sample Processing Flow Chart



ATTACHMENT 2

ASE Extraction Log

		ASE#	
Project		Batch No	
Operator		Date	
Witness		Date	
Method #		Schedule #	
Solvent	DCM	Rinse	On
(Circle one)	Other	(Circle one)	Off
Vial No.	SampleId	Vial No.	Sample Id
1.		13.	
2.		14.	
3.		15.	
4.		16.	
5.		17.	
6.		18.	
7.		19.	
8.		20.	
9.		21.	
10.		22.	
11.		23.	
12.		24.	
Comments:			

ATTACHMENT 3

N-Evap Water Bath Temperature Guide

Solvent	Boiling Point	Bath Temp	Rate (mL/min)	Time (min/200 mL)
Methylene Chloride	40°C	37°C	0.40 ml	25 min
Hexane	69°C	67°C	0.36 ml	28 min
Freon	48°C	45°C	0.48 ml	21 min
Pentane	36°C	32°C	0.42 ml	24 min
Methanol	65°C	63°C	0.25 ml	40 min
Acetone	56°C	37°C	0.35 ml	28 min

Battelle
Applied Coastal & Environmental Services
Standard Operating Procedures

for

Florisil Cleanup of Environmental Sample Extracts

Summary of changes in this version: Corrected typographical error in section 3.1.1 second bullet (1 ± 0.1 g). The bullet on dry packing Florisil columns was removed; information on slurry packing Florisil in hexane was added. An SOP revision history log has been added (Attachment 1).

1.0 OBJECTIVE

Florisil, a registered trade name of the U.S. Silica Co., is a magnesium silicate with basic properties. It is used to separate analytes from interfering compounds prior to analysis by a chromatographic method (EPA, 1996). This standard operating procedure (SOP) describes the cleanup procedure performed on sample extracts, from various matrices, using hand packed gravity fed columns or pre-packed solid phase florisil extraction (SPE) cartridges; this procedure is a modified version of EPA method 3620B. This method is suitable for sample extracts being analyzed for pesticide, polychlorinated biphenyl (PCB) and polynuclear aromatic hydrocarbons (PAH) compounds. The method may be suitable for other target analytes once acceptable efficiency has been demonstrated.

2.0 PREPARATION

2.1 APPARATUS AND MATERIALS

- Disposable 1 g Florisil columns in polyethylene (Applied Separations) – *for pesticide or PCB use only*
- Disposable 1 g Florisil columns in glass (Restek) - *for PAR, pesticide, or PCB use*
- Glass graduated cylinders, various sizes
- 25 or 40 mL vials with Teflon lined screw caps
- Vacuum manifold
- Vacuum pump
- Chromatographic columns, 10 mm id x 300 mm, Teflon stopcock
- Glass wool, muffled at 400°C for at least 4 h, cooled, stored in oven at approximately 100°C

2.2 REAGENTS

- Dichloromethane (DCM), pesticide grade or equivalent
- Hexane, pesticide grade or equivalent
- Acetone, pesticide grade or equivalent
- 9⁄1 hexane/acetone (v/v)
- Florisil, PR grade, 60 - 100 mesh
    - Activate Florisil by heating at 130°C for at least one week (168 hours) prior to use. Store activated Florisil at 130°C in foil capped Erlenmeyer flasks (or equivalent)
- Sodium sulfate, anhydrous, reagent grade,
    - Muffle at 400°C for at least 4 h, cooled, and stored in a tightly-sealed glass container at

room temperature

2.3 LABWAREPREPARATION

All reusable glassware must be cleaned according to laboratory protocols defined in SOP 5-216, glassware is then rinsed with DCM prior to contact with samples. Pre-cleaned glassware may be used as purchased.

2.4 RECORD KEEPING

Analytical data will be recorded on appropriate data forms included in the LIMS (refer to the *LIMS Sample Preparation Training Manual* for examples of reports and instructions on entering data). Information to be recorded includes column size, florisillot number, solvents used (including lot numbers), dates of processing procedures, initials of laboratory personnel who perform the procedures, extracts being processed through the procedure, and, if necessary, comments regarding individual samples.

3.0 PROCEDURES

This procedure is appropriate for extracts generated by any extraction method for PAHs, PCBs, or pesticides, the extraction procedures are outlined in separate SOPs.

3.1 EXTRACT CLEANUP - FLORISIL

Sample extracts should be at least 50% hexane, by volume, prior to the florisil cleanup procedure. This is achieved by concentrating samples to approximately 500 µL and bringing the volume to 1 mL using hexane.

3.1.1 Column Preparation (gravity columns)

- Pack tip of 10 mm column with a small plug of glass wool.
- Rinse the column with hexane, fill tip of column with enough hexane to cover the glass wool
- Using a clean, rinsed glass rod - gently pack glass wool to remove any air bubbles
- Slurry pack 1 ± 0.1 g of activated Florisil in column using hexane.
- Add approximately 2 cm of sodium sulfate.
- Rinse column with approximately 15 mL hexane, do not allow column to go dry.
- Columns are now ready for use.

3.1.2 Column Preparation (SPE Columns)

- Set up vacuum manifold with disposable 1 g Florisil columns. Place 40 mL vials inside manifold to collect rinse solvent.
- Rinse columns with approximately 20 mL of DCM prior to use. Do not allow columns to go dry after rinsing with solvent.
- Rinse columns with an additional 10 mL of 9/1 Hexane/Acetone solution. Columns will now contain the appropriate elution solvent. Do not allow the columns to go dry.
- Remove 40 mL vials from vacuum manifold and dispose of solvent into the Drum Solvent waste stream.
- Columns are now ready for use.

Note: Columns should be allowed to drip for sample extract cleanup. Vacuum pressure is not generally needed for the elution of extracts, but can be applied under low pressure. Columns should be eluted at the rate of between 3 - 5 mUminute.

3.1.3 Column Elution

- Place clean 40 mL vials under column or into the vacuum manifold.
- Transfer 1 mL extract to column, rinsing with 9/1 Hexane/Acetone solution.
- Charge extract onto column (do not allow column to dry).
- Rinse original extract vial with an additional 1 mL of 9/1 Hexane/Acetone solution
- Charge rinse onto column (do not allow column to dry).
- Elute column with 15 mL 9/1 Hexane/Acetone solution, elution is complete once solvent reaches the top of the florisil.

Concentrate the sample extract and continue processing according to the appropriate extraction procedure.

4.0 CALCULATIONS

There are no calculations associated with this procedure

5.0 QUALITY CONTROL

To ensure the integrity of the cleanup process, all preparation batch QC samples, including the procedural blank (PB), will be processed through the florisil cleanup columns using the same method as the associated . authentic field samples.

6.0 TRAINING

The trainee must read and fully understand the procedures outlined in this SOP and have documented training in the appropriate Battelle extraction SOPs. The trainee will then be given a demonstration of all aspects of this SOP.

Technicians may work independently once they have satisfactorily performed the procedures outlined in this SOP. Upon completion of training, a training certificate will be issued (Attachment 1). The original training certificate will be maintained by the Quality Assurance Unit (QAU).

7.0 SAFETY

As part of the above training program, the analyst will be made aware of the particular safety concerns of this procedure, including:

- Use of protective eyewear and clothing
- Proper use of fume hoods
- Handling of hazardous chemicals and reagents
- Location and use of laboratory safety devices; eyewashes, emergency showers, fire extinguishers, fire blankets, and first aid kits, as well as MSDS sheets.

8.0 REFERENCES

U.S. EPA SW-846 Manual, Method 3620B, Revision 2, December 1996

U.S. Food and Drug Association, Pesticides Analytical Manual (Volume 1), July 1985

ATTACHMENTS
Attachment 1: SOP Revision history
Attachment 2: Training Certificate

APPROVALS

Author		4/14/05
Technical Reviewer	Roxanne M. Brackett	4/14/05
Quality Systems Manager		4-15-05
Laboratory Manager	W.M. Stuntzner	April 14, 2005
	Name	Date

Attachment 1

Summary of Changes to SOP

Version	Summary of Changes
02	Corrected typographical error in section 3.1.1 second bullet (1 ± 0.1 g). The bullet on dry packing Florisil columns was removed; information on slurry packing Florisil in hexane was added. An SOP revision history log has been added (Attachment 1).

ATTACHMENT 2

Battelle
Applied Coastal and Environmental Sciences
Certificate of Training

In

Florisil Cleanup of Environmental Sample Extracts

Trainee _____

Instructor _____

Date SOP Read _____

Date Training Completed _____

Supervisor Approval: _ _____

Name Date

Battelle Applied Coastal and Environmental Services
Standard Operating Procedures

for

IDENTIFICATION AND QUANTIFICATION OF POLYCHLORINATED BIPHENYL CONGENERS (PCB), CHLORINATED PESTICIDES, AND PCB HOMOLOGUES BY GAS CHROMATOGRAPHY/MASS SPECTROSCOPY IN THE SELECTED ION MONITORING (SIM) MODE

Summary of changes in this version: Changed IS area count criteria to -50 to +100% of ICAL to match EPA 8270C requirement. Removed the highest two calibration levels from ICAL. Based LOC calibration on individual first and last congeners for each LOC rather than LOC area sum. Corrected text related to DDT breakdown criteria and added an equation. Added an equation to demonstrate quantification ion correction for removing the contribution from co-eluting PCB with one or two additional chlorines.

1.0 SCOPE AND APPLICATION

This Standard Operating Procedure (SOP) describes the method used for the identification and quantification of polychlorinated biphenyl (PCB) congeners, chlorinated pesticides, and PCB homologues by gas chromatography/mass spectroscopy (GC/MS) in the select ion-monitoring mode (SIM). The method described in this SOP is based on key components of the PCB congener analysis approach described in EPA Method 1668A (USEPA 1999) and the PCB homologue approach described in EPA Method 680 (USEPA 1985). Overall guidance for the method is based on EPA Method 8270C (USEPA 1996).

This SOP is applicable for the analysis of trace level PCB congeners, chlorinated pesticides, and PCB homologues in water, sediment, tissue, and other extracts. The target analytes listed in Attachment 1 are determined in the concentration range of parts per trillion (ng/L) for water samples and parts per billion (ng/g) for sediment and tissue samples. The method has been validated for the target analyte list in Attachment 1; applicability to additional analytes will be demonstrated through an initial demonstration of capability (SOP 5-295).

High resolution gas chromatography together with MS data acquisition is capable of isolating, identifying, and quantifying the congeners listed in Attachment 1. Several of the PCB congeners co-elute under the conditions presented in this method. Those with different LOC can be quantified separately by their mass spectral signatures following guidance in EPA Method 680. Co-eluting PCBs of the same LOC are reported as co-eluting pairs or triplets (Attachment 2).

2.0 METHOD SUMMARY

Polychlorinated Biphenyl (PCBs) congeners, chlorinated pesticides, and PCB homologues are separated via capillary gas chromatography, and identified and quantified using electron ionization mass spectrometry in the SIM mode. A data system interfaced to the GC/MS is used to control acquisition and to store, retrieve, and manipulate mass spectral data. This method provides specific procedures for the identification and measurement of the selected PCBs, pesticides and PCB homologues listed in Attachment 1.

3.0 EQUIPMENT AND SUPPLIES

The following equipment is required to perform the analytical method. Equivalent apparatus and materials may be substituted if approved by the laboratory and project manager. Substitutions will be documented in the project files.

- An Agilent HP6890 Gas Chromatograph and HP5973A Mass Selective Detector *(GCIMS)* or equivalent. The *GCIMS* must be operated and maintained as described in SOP 3-092 *(Operation and Maintenance of Hewlett-Packard 5970B, 5972A and 5973A Gas Chromatograph/Mass Selective Detector (GC/MS) using Hewlett-Packard Software).*
- An Agilent HP7683Autosampier or equivalent
- Column: Agilent DB-XLB (60-m x 0.25-mm internal diameter fused silica capillary column with a 0.25-µm bonded phase) or equivalent.
- Electronic data capturing software and data analysis software (ChemStation's Enviroquant™ is a suggested software)
- Custom PCB data reduction program developed by Battelle. This macro runs inside Enviroquant™ and implements EPA Method 680 guidance for the correction of interference of co-eluting PCB congeners containing one or two additional chlorines
- Gas scrubbers for removal of moisture and impurities in the carrier gas
- 2.0-mm ill splitless goose neck glass liner or 4.0-mm splitless gooseneck glass liner (Restek, or equivalent)
- 1/4" Viton o-ring (Agilent, or equivalent)

4.0 REAGENTS AND STANDARDS

4.1 REAGENTS AND CONSUMABLE MATERIALS

4.1.1 Solvents

High quality pesticide grade hexane is the solvent for all final extracts. All sample extracts and standards should be contained in the same solvent. The effect of minor amounts of a second solvent (mixed solvents) is minimized by the internal standard method of calibration.

4.1.2 MS Tuning Solution

Perfluorotributlyamine (PFTBA), purchased from Agilent Technologies, Inc. is used neat. This solution is stored in a sealed container on the mass spectrometer. Store and use according to instrument manufacturer instructions and SOP 5-015.

4.1.3 GCIMS System Check Solution

Prepare or purchase a solution containing decafluorotriphenylphosphine (DFTPP) at a concentration approximately 5 and 4,4'-DDT at a concentration approximately 2 in hexane or cyclohexane. This solution is used to verify injection port inertness and GC column performance. Store at -10 °C or less when not in use. SOP 5-015 defines expiration dates for standards and solutions.

4.2 PCB AND PESTICIDE SOLUTIONS

PCB and Pesticide Calibration Standards
Calibration standards must contain all individual target compounds, surrogates and internal standards. If LaC are targets of interest, the analysis of LaC in samples will quantify all PCB congeners of the same level of chlorination regardless of whether all the congeners are contained in the calibration solutions.

Primary stock solutions that contain the target analytes are purchased and used to prepare the initial calibration (ICAL) standards, the calibration verification check standard (CCV) and the independent calibration check standard (ICC). Most standards used for this method are purchased from standard providers (e.g., Ultra Scientific, Absolute Standards and AccuStandard) in neat or solution form. Equivalent standards may be substituted if approved by the laboratory and project manager. All calibration standards for this method are made in cyclohexane. The ICC must be independent of the ICAL standards and thus should be made using either a standard from the same vendor with a lot number different from the ICAL standard or a standard from a different vendor.

Standards are to be prepared according to procedures outlined in SOP 5-027. All standards must be stored at less than -10°C in screw-cap amber bottles with Teflon liners. Standards must be stored separate from samples. All spiking solutions must be at room temperature (~20 0C) prior to use.

PCB and Pesticide Retention Time Marker Compounds
Knowledge of the retention time of certain PCB congeners and pesticides is required to determine the SIM acquisition windows, as well as to determine the peaks to be selected for the quantification of each LaC. Two concentration calibration congeners at each LaC, the congener that elutes first and the congener that elutes last, serve to define the data reduction windows for each LaC (Attachment 1). Process all data for each PCB LaC within the window established by the "first" and "last" PCB congeners. All PCB Retention Time Marker compounds are also PCB Calibration compounds.

Internal Standards
The PCBs C15(96) serves as internal standard (IS). The IS is added to each sample extract prior to analysis. The IS compound is contained in all calibration and calibration check solutions and sample extracts at a constant concentration.

Surrogate Internal Standards
The PCBs C13(36) and C17(192) are used as surrogate internal standards (SIS). The SIS are added to each sample before extraction and used to monitor extraction and processing efficiency. SIS may also be used to correct concentration data based on recovery. The SIS are contained in all calibration and calibration check solutions at a concentration equal to the PCB target analytes and are spiked into samples at a concentration representative of the PCB in the samples.

5.0 PREPARATION

5.1 SAMPLE COLLECTION, PRESERVATION, SHIPMENT AND STORAGE

All samples received by the laboratory are handled according to SOP 6-010, SOP 5-210 and the *Sample Receipt, Custody, Handling and Disposal* section defined in Battelle's Quality Assurance Manual.

5.2 SAMPLE PREPARATION

The following Battelle SOPs outline sample preparation procedures that are acceptable for this method:

- 5-307 (Extraction of Sediments/Soils/Tissues by ASE)
- 5-327 (Florisil Clean-up for Semi-Volatile Contaminants)
- 5-191 (HPLC Clean-up for Semi-Volatile Contaminants)

5.3 GAS CHROMATOGRAPHIMASS SPECTROMETER CONDITIONS

5.3.1 Gas Chromatograph Conditions

A 60-m x 0.25-mm ii) DB-XLB fused silica capillary column with a 0.25-µm bonded phase will be used for this method. A 2-mm ii) glass liner will be used for a l-uL injection volume. Substitution of a different chromatography column will result in data that are not comparable to those presented in this SOP.

Use the following GC conditions:

Injection port temperature	210 DC
Detector temperature	280 DC
Initial oven temperature	60 DC
Initial hold	1 min
Ramp 1 rate	10 DC /min to 160 DC
Ramp 2 rate	0.9 DC /min to 180 DC
Ramp 3 rate	2.0 DC /min to 220 DC
Ramp 4 rate	5 DC /min to 300 DC (hold for 15 min)
Carrier gas flow	1 mL/min (helium)

All gas chromatographs are fitted with Electronic Pressure Control (EPe). The EPC ramping program will be as follows:

Initial Pressure	30 psi
Initial Time	0.5 min
Rate 1	99 psi/min
Final Pressure	Constant Flow (lml/min)
Vacuum Compensation	On

5.3.2 Analyte and SIM Window Identification

The elution order and co-elution characteristics of the analytes listed in Attachment I and Attachment 2 have been determined through the qualitative analysis of individual compounds and solutions of multiple compounds and comparison of sample mass spectra with reference mass spectra under the gas chromatography conditions presented above. Documentation files are maintained on the Chemistry Server.

SIM windows are defined in Attachment 3. The SIM windows are defined by the retention times of selected PCB congeners and pesticides. Knowledge of these retention times is necessary to set the start and end times of the SIM ion groups. The retention times for these marker compounds are established by the analysis of a mid-level calibration standard under the gas chromatography conditions used to analyze samples before the initial instrument calibration and must be verified whenever instrument maintenance has been performed.

A mid-level calibration standard must be analyzed under the analytical conditions used for sample analysis every time gas chromatography column maintenance is performed. This includes analytical column replacement, as well as column clipping. The extracted ion profiles (EIP's) from these analyses are used to verify or reassign the proper group start and stop times of the SIM acquisition.

5.3.3 Mass Spectrometer Conditions

The mass spectrometer conditions are as follows:

Manifold Pressure	Less than 4×10^{-5} torr.
Electron Energy	70 volts (nominal)
Mass Range	SIM:Mode
Scan Time	≥ 1 and ≤ 2 s per cycle
Electron Multiplier Voltage	100V above tune value

Note: The GC/MS system check is performed under full scan conditions; Mass Range 50 - 450 amu. All other MS conditions are as presented above.

5.3.4 Mass Spectrometer Tuning

Tune the mass spectrometer with perfluorotributlyamine (PFTBA) following procedures described in SOP 3-092 prior to the analysis of analytical standards and/or samples.

5.3.5 SIM Ion Group Identification

The nine ion sets to be used for the data acquisition are defined in Attachment 3. Each ion in a group must have identical dwell times and each group must have the same number of ions to ensure that correct ion ratios are preserved. The total data acquisition time per cycle should be greater than 1 s and less than 2 s, which will ensure that a minimum of 5 SIM scans will be acquired during the elution of each compound.

6.0 PROCEDURES

Verify that the GC and MS conditions are as specified in Section 5.3.1 and 5.3.3, respectively; that the SIM windows have been identified as specified in Sections 5.3.2 and 5.3.5; and that the instrument meets tuning criteria specified in Section 5.3.4.

The sample extracts are concentrated to a predetermined pre-injection volume (PIV). Internal standards are added to the extract and the extracts are analyzed. Volumes of 1 µl of standard and sample aliquots are injected into the GC. Injections are made with an autosampler device. Because data quantification is based on an internal standard, the injection volume is not critical. If the response for any peak exceeds the working range of the system (i.e., the ICAL concentration range), the extract will be diluted and reanalyzed. Data from the diluted sample are then used to quantify only the compounds initially outside of the calibration range.

6.1 SEQUENCE OF ANALYSIS

An analysis sequence for PCB/pesticide is initiated with an acceptable initial calibration (lCAL) and continues until a CCV fails to meet acceptance criteria or another ICAL is performed. A sequence includes calibration samples, system check sample, quality control samples and authentic samples. The analysis of samples may continue as long as the GC/MS system check and the CCV results remain within criteria.

The order of calibration and analysis is as follows:

a. GCIMS System Check (DFTPP)

b. Initial Calibration (lCAL)

c. Independent Calibration Verification Check (lCC)

d. GCIMS System Check

e. Calibration Verification (CCV)

f. Authentic Samples Analysis

g. GCIMS System Check – Within 12 H of the start time of the previous system check

h. CCV - Within 12 H of the start time of the previous CCV

Repeat f, g, and h for continued analysis of QC and authentic samples as long as g and h continue to pass criteria.

6.2 GC/MS SYSTEM CHECK

Assess GC column performance and injection port inertness every 12 hours or less by monitoring the DFTPP spectrum and degradation of 4,4'-DDT to 4,4'-DDE and 4,4'-DDD. This analysis is conducted under full scan conditions (50 - 450 amu).

Inject of the system check solution and acquire the data. Review the data for the following:

- DFTPP Spectrum: Use DFTPP mass intensity criteria presented in Attachment 4. If the mass intensities do not meet all criteria, perform system maintenance, as necessary, and repeat the system check.

- % breakdown of 4,4'-DDT: The sum of the peak areas of DDE and DDD must not exceed 12% of the sum of the areas of the three constituents (DDT, DDE, and DDD) calculated as follows:

$$\%\,\text{Breakdown} = \left(\frac{A_{DDD} + A_{DDE}}{A_{DDr} + A_{DDE} + A_{DDD}}\right) x100$$

where A_X = Area count of the quantitation ion of the respective compound

6.3 CALIBRATION

Demonstration and documentation of acceptable initial calibration is required before any samples are analyzed and is required intermittently throughout sample analyses as dictated by the results of the CCV. The ICAL must be verified by analysis of an ICC immediately following the ICAL. After the ICAL and ICC are successfully performed, a CCV is required at the beginning and end of each 12-h period during which analyses are performed.

6.3.1 Initial Calibration

Select the appropriate Enviroquant™ acquisition method for this analysis. The method will establish placeholders for the IS, SIS, and target analytes and define the calibration method. Perform a minimum six-level calibration. The recommended calibration levels for calibration compounds, surrogates and internal standards are presented in Attachment 5.

Allow calibration solutions to reach room temperature. Analyze 1 µl of each level calibration standard and acquire data for the ions and SIM windows presented in Attachment 3.

Use the default Enviroquant™ non-linear quadratic model to calibrate the method working range. A minimum 6-level calibration curve is required. Do not force the line through the origin, and do not use the origin (0,0) as a calibration point. The Enviroquant™ quadratic model implements the following equation on a set of six or more data points:

$$x = \frac{-b \pm \sqrt{b^2 - 4a(y - c)}}{2a}$$

Where
a = I_{sl} coefficient
b = 2nd coefficient
c = Intercept (ratio of CAL response to IS response)
y = Result Response Ratio (response to IS response. For ICAL, y = 0
x = Calculated amount

The Enviroquant™ program assigns values for the first and second coefficient based on best fit to the calibration data. When 0,0 is not used as a data point, the Enviroquant™ program also assigns a value for the intercept.

PCB and Pesticide Target Compound Calibration
Display and review the SIM data for the quantification and confirmation ions of the ICAL solution. Confirm the auto-baselines on the quantification and confirmation ions or redraw the baselines, as necessary. Quantify each peak following procedures presented in the instrument manufacturer user's manual and Battelle SOP 4-038, *Manual Integration Practices and Review.* Confirm peak identification with retention time and quantification and confirmation ion ratios (Attachment 6).

As necessary, correct the quantification ion peak areas for known interferences from co-eluting PCB congeners containing one or two additional chlorines using the Battelle data reduction macro. The Battelle macro implements EPA Method 680 guidance quantifying and correcting for these mass interferences. Attachments 7 and 8 present quantification ion corrections the M+70 and M+35 ion interferences, respectively.

The target analytes and IS are programmed in the Enviroquant™ method developed for this SOP. Once identified, the Enviroquant™ program automatically enters the retention time, quantification ion peak areas, the correct IS, and ICAL PCB amounts (ng) corresponding to each peak. Repeat display, review, and entering of of each level of the calibration. Upon completion, the Enviroquant™ software will automatically calculate the coefficient of determination (r^2) based on best fit of the quadratic equation. The coefficient of determination must be >0.995 for each target analyte.

If this requirement is not met for all target analytes, take corrective action and repeat the calibration.

PCB Homologue Analysis Calibration
The LaC are identified as individual analytes in the Enviroquant™ method developed for this SOP. Identify and quantify the first and last eluting congener for each LaC following procedures described above. The Enviroquant™ macro sums the quantification ion peak areas of the first and last eluting congener and also sums the PCB amounts (ng) corresponding to each peak. These data (the summed peak areas and the summed amounts) are automatically entered in the Enviroquant™ macro once the first and

last congeners are identified. Repeat for each level of the calibration. The Enviroquant™ software will automatically calculate the coefficient of determination (r^2) for each LOC. The coefficient of determination must be >0.995 for each LOC.

Surrogate Calibration
The SIS are calibrated following the same method described above for target analytes.

6.3.2 Independent Calibration Check (ICC)

Verify the ICAL with an ICC immediately after the ICAL is completed. The ICC is analyzed under the same analytical conditions used in the ICAL and for the analysis of samples.

Analyze 1 of the ICC solution and acquire data. Identify peaks based on retention time and quantification and confirmation ion abundance as described above. Quantify and tabulate the peak areas following procedures presented in the instrument manufacturer user's manual and Battelle SOP 4-038. Confirm peak identification with retention time and quantification and confirmation ion ratios (Attachment 6).

ICC Internal Standard and Surrogate Performance Criteria
Confirm that the area counts of the IS and SIS are ± 15 % of the values found in the same solution in the ICAL.

The retention time windows for internal standards and surrogates are defined by the ICAL. Calculate the mean retention time for each in the ICAL. The absolute retention times of the internal standards and surrogates should not vary by more than ± 4 s from the mean.

ICC Target Analyte Performance Criteria
Upon peak selection, the Enviroquant™ software automatically calculates the concentration amounts from the quantification peak area. Calculate the percent difference (%DIFF) between the calculated amount ("found" amount) and the true amount using the following equation:

$$\%DIFF = \frac{(A_e - A_I)}{AI} * 100\%$$

AI = "true" analyte amount
A_e = "found" analyte amount

The calculated concentration for all analytes in the ICC must fall within 15% of the true value.

If the percent differences for the ICC analytes are less than 15%, then the ICAL is assumed to be valid. Proceed with the analysis of samples. If any differences exceed 15%, reanalyze the ICC. If the second ICC passes, proceed with the analysis of samples. If second ICC fails, perform and document remedial action (e.g., instrument maintenance) and reanalyze the ICC. If this ICC passes, proceed with the analysis of samples. If the ICC fails, repeat remedial activity. Depending upon the remedial action, the analyst may elect to rerun the ICC to confirm the original ICAL or perform a new ICAL.

The LOC ICAL does not need to be verified separately.

6.3.3 Calibration Verification Check (CCV)

Verify the ICAL at the beginning and end of each 12-h period during which analyses are to be perfonned. Successfully complete the GCIMS system check before running the CCV. Inject 1 μl of the appropriate CCV solution. Acquire data, quantify and assess the data as described above for the ICC.

Vary the standard concentration as follows: L3, L4, L5, L6 (Attachment 5) for consecutive CCV. Start over with L3 for a sequence containing more than four CCV.

CCV Internal Standard and Surrogate Criteria
Confinn that the area count of the IS quantification ion falls within a factor of 2 (-50% to +100%) of the mean area measured during the ICAL. If the percent difference exceeds these criteria, follow guidance described above for the ICC.

The absolute retention times of the internal standards and surrogates should not vary by more than ± lOs from the mean obtained in the ICAL.

CCV Target Analyte Performance Criteria
Calculate the percent difference (%DIFF) between the calculated amount ("found" amount) and the true amount as described above for the ICC. The calculated concentration for all target analytes in the CCV must fall within

20% of the true value for all PCB analytes in CCV
25% of the true value for all pesticides in CCV

If the %DIFF for the CCV analytes are less than above, then the ICAL is assumed to be valid. If the percent difference exceeds the above, follow guidance described above for the ICC.

The LOC ICAL does not need to be verified separately.

6.4 SAMPLE ANALYSIS

6.4.1 GC/MS Analysis

Remove the sample extracts from storage and allow them to return to room temperature before analysis. Bring the extract to a predetermined pre-injection volume (PIV) and add IS immediately preceding analysis. The PIV is 500 μl, the IS concentration is 100 nglml, and the injection volume is 1 μl.

Start sample analysis sequence with the analysis of quality control samples. Inject 1-μl of the sample and acquire mass spectral data under the same GC and MS acquisition method used to produce acceptable results during calibration.

Verify that the IS area measured in the sample extract falls within a factor of 2 (-50 to +100%) of the IS area measured during the most recent CCV, provided the concentrations are the same in the CCV and sample. If this is not met, dilute or concentrate the extract, as appropriate, and reanalyze the sample.

Examine the data for saturated ions in mass spectra of target compounds or for peak areas that have exceeded the calibration. If either has occurred, immediately dilute and reanalyze the extract. Additional IS must be added to the diluted extract to maintain the same concentration as in the calibration solutions.

Examine the method blank data first. An acceptable method blank will contain no analyte at a concentration greater than the reporting limit for the target analyte and contains no additional compounds with elution characteristics and mass spectra features that would interfere with the identification and

measurement of the target analytes. Consult with the project manager immediately if this is not the case.

6.4.2 Qualitative Identification of Target and SIS Compounds

Identify the target compounds and SIS based on retention time windows established during the calibration and the quantification and confirmation ion ratios presented in Attachment 6. Comparison of both retention times and ion ratios to reference standards is also useful in compound identification.

Retention Times
Sample and SIS peaks are identified visually using the Enviroquant™ display program. Display and review the SIM data for the quantification and confirmation ions of each peak in retention time order. The peak height of the quantification and confirmation ion must maximize in the same scan, or within one scan. Compare the sample and CCV data for each peak. Peaks are identified by retention time of the peak and peak patterns of homologue series. The compound retention times must fall within ± 4 seconds of the compound determined in the most recent calibration. For sample peak identification, the experience of the analysts should weigh heavily in the interpretation of chromatograms.

SIM Spectra
On the monitor, identify target analyte and SIS peaks that match retention times and other criteria above. Confirm the auto-baselines on the quantification and confirmation ions or redraw the baselines, as necessary. Quantify each peak following procedures presented in the instrument manufacturer user's manual and Battelle SOP 4-038. As necessary, also display the M+70 and M+35 ions to review potential mass interference from co-eluting PCB with two or one additional chlorines. Correct the quantification ion peak areas for identified interferences using the Battelle data reduction macro, which applies the following equation:

$$AQ_{(corrected)} = AQ - (AI * CF)$$

Where, AQ = area of the quantitation ion
AI = area of the interference ion
CF = correction factor from Attachment 7 or 8, as appropriate

For example: For an LOC4 congener with a co-eluting congener containing 2 additional chlorine atoms

$$AQ_{(corrected)} = AQ - (AI * 0.65)$$

Confirm the peak identification by comparison of ion ratios to those listed in Attachment 6. Ions must be present and the relative intensities after correction must agree to within ± 20 %.

Signal to Noise Ratio
A quantifiable analyte peak should exhibit a signal to noise ratio of approximately 3:1 or greater.

6.4.3 Quantification of Target and SIS Compounds

Target compounds and SIS are quantified by the internal standard method using a best fit quadratic regression from the ICAL for the quantification ions presented in Attachment 6. Apply the IS for each compound defined in Attachment 1. Upon selection of the non-linear quadratic calibration option and the identification of a peak, the Enviroquant™ software automatically calculates the compound amounts from the corrected peak areas, as described in Section 6.3. Enviroquant™ will also calculate the amount of SIS found and will calculate the SIS % recovery based on amount originally spiked into the sample. Enviroquant™ uses the following equation for calculating % recovery:

% Recovery = *(A/B)* * 100
Where:
A = Concentration quantified (ng/g)
B = Concentration spiked (ng/g)

Upon transfer of the file to LIMS, the LIMS program will calculate the target analyte concentration based on sample weight and SIS recovery, and will apply appropriate data qualifiers. Data qualifiers for the Montrose analysis are presented in the project QAPP. Review the results against calibration standards and other project samples for reasonableness.

LIMS uses the following equation for surrogate correcting the sample data:

X = A /(B / 100)

Where:
X = SIS corrected concentration (ng/g)
A = Uncorrected sample concentration (ng/g)
B = SIS recovery (%)

Note: In a diluted sample, report only those peaks that were outside the calibration range in the original analysis. All other compounds must be reported from the original (undiluted) analysis.

6.4.4 Qualitative Identification of PCB Homologue

Identify the PCB LOC homologues based on retention time of the first and last PCB eluting in each level of chlorination, the SIM ion ratios presented in Attachment 6, and characteristic peak distribution patterns.

First and Last Peak Identification
LOC peaks are identified visually using the Enviroquant™ display program. Identify the retention times of the first and last eluting PCBs. Display and review the SIM data for the quantification and confirmation ions for the first and last peaks and all peaks in between for the sample and the most recent CCV. Compare the sample and CCV data for each LOC.

The compound retention times for the first and last must fall within ±4 seconds of the compounds determined in the most recent CCV. For peak identification, the experience of the analysts should weigh heavily in the interpretation of chromatograms.

SIM Spectra
On the monitor, identify LOC patterns that match retention times and other criteria above. Confirm that the peak height of the quantification and confirmation ion of significant peaks maximize in the same scan, or within one scan of each other. Confirm the peak identification of significant peaks by comparison of ion ratios to those listed in Attachment 6. Note: Because the above target analyte analysis includes most significant PCBs in environmental samples, this data review is to confirm that there are no extraneous peaks being included in the quantitation ion extracted ion chromatogram.

Compare the peak patterns to reference samples, such as calibration or Aroclor standards to determine the reasonableness of the acquired LOC data. Environmental samples often contain altered PCB LOC patterns compared to Aroclor. Particularly in low-level samples, analyst experience identifying these patterns is essential to the correct identification ofLOC.

Signal to Noise Ratio
Because a single baseline is drawn, analyte peaks with less than a signal to noise ratio of approximately 3:1 may be acquired.

6.4.5 Quantification of PCB Homologues.

For PCB homologue quantification, it will always be necessary to manually draw a baseline. Display an individual LOC, and if relevant LOC-S1, and LOC-S2. Display a portion of the chromatogram so that the pattern takes up virtually the entire screen (x-axis) and set the signal abundance to display between 500 and 1500 area counts (y-axis). Draw a single baseline and integrate the area from the leading edge of the first eluting compound to the tailing edge of the last eluting compound in the series for the LOC, as well as LOC-Sl and LOC-S2, if relevant. Amplification of the y-axis often causes the analyst to make a decision on the correct placement of the single baseline. The baseline should split the baseline noise as much as possible, as illustrated for each LOC in Attachment 10.

PCB LOC are quantified by the internal standard method using a best fit quadratic regression from the ICAL for the quantification ions presented in Attachment 5. Apply the IS defined in Attachment 1. For quantification, follow the same procedures described above for target analytes.

6.4.6 Manual Integrations

Review all Enviroquant™ auto-integrations for best practices that are identified in Battelle SOP 4-038, *Manual Integration Practices and Review.* If an improper auto-integration is observed, manually re-draw the baseline and reintegrate the peak using proper integration techniques. Peaks that have been manually integrated will be identified on the quantification reports with an "m" notation appears adjacent to the area response.

6.4.7 Matrix Spike and Laboratory Control Sample Data

Quantify the target analytes and surrogates spiked into matrix spike (MS) and laboratory control samples (LCS) in the manner described above for target analytes in samples. Surrogate correct the MS and LCS data as described above for target analytes in samples. Calculate the % recovery of spiked compounds and surrogates in MS and LCS samples in the same manner as described above for samples.

6.5 REPORTING UNITS

Reporting units are ng/L for water and for soiVsediments and tissues.

7.0 QUALITY CONTROL

The GC/MS Facility is operated and maintained in accordance with SOP No. 3-092 and all GC/MS Facility operations are documented according to SOP No.6-OIL Analysis of quality control samples, including procedural blanks, laboratory control samples, matrix spikes, and standard reference materials (SRM) are specified in the project plan. The Quality Control acceptance criteria will follow the criteria outlined in Section 18 unless specified differently in the project plan. SOP 7-029 defines the default acceptance criteria for laboratory QC. The following quality control procedures have been adapted from relevant procedures in USEPA 1996. Key elements of the quality control program include:

1. There must be an initial calibration of the instrument as specified in Section 6.3.

2. The ICAL is verified at the beginning and end of each 12-h period during which analyses are to be performed to determine that the chromatographic system is operating properly.

3. Peak shape should be evaluated for proper peak shape and symmetry.

4. The instrumental response should be comparable to previous calibrations.

5. The system must be recalibrated if the analytical column is replaced.

8.0 DATA ANALYSIS AND CALCULATIONS

The calculations described in this SOP include

Description of Calculation	SOP Section Number
DDT breakdown	6.2
Non-linear quadratic equation	6.3
Percent difference (%DIFF)	6.3
Quantitation ion area correction	6.4
SIS % recovery	6.4
Surrogate correction of sample data	6.4

9.0 TRAINING

A training program will include all the elements presented in USEPA (2002). The trainee must read and fully understand the policies and procedures outlined in this SOP and have documented training in Battelle SOP 3-092 and 6-011. The trainee will then be given a demonstration of all aspects of this SOP.

Analysts may work independently once they have satisfactorily performed the following training test:

- Analyze an acceptable initial calibration
- Analyze at least four LCS samples concurrently or over a period of days that meet the $\leq$30 RSD acceptance criteria.

When training is completed, the trainee will be issued an Initial Demonstration of Capability Certificate (SOP 2-011). The original completed training certificate and all supporting documentation will be sent to the Quality Assurance Office.

Analysts must also demonstrate annual proficiency in the method. SOP 2-011 (Staff Training) outlines the acceptable performance procedures. When annual training is completed, the analyst will be issued a Demonstration of Ongoing Capability Certificate.

10.0 SAFETY

All GC/MS operators must be experienced with the operation and maintenance of the GC/MS system, and must have passed the training requirements presented in this SOP and SOP No. 3-092. Safety issues are described SOP No. 3-092.

11.0 POLLUTION PREVENTION

Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Staff should make every effort to use the minimum amount of sample and reagent for this analytical procedure. All samples and reagents are contained and disposed of in an appropriate manner (Section 13).

12.0 CORRECTIVE ACTION

Battelle maintains a corrective action program that is detailed in SOP 4-035. The Laboratory Manager must approve all corrective actions initiated within the laboratory. The effectiveness of corrective actions is verified by the Quality Assurance Coordinator.

13.0 WASTE MANAGEMENT

All waste streams generated within the laboratory are collected and stored in an appropriate container and disposed according to BDO SOP 5-114 or when appropriate, by other approved waste management procedures.

14.0 INTERFERENCES

Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All solvent/reagents must be analyzed initially to demonstrate they are free from interferences (SOP 5-015). Glassware must be cleaned following the procedures outlined in SOP 5-216. The use of high purity reagents and solvents helps to minimize these interference problems. Laboratory reagent blanks are analyzed routinely to demonstrate that these materials are free of interference under the analytical conditions used for samples.

Phthalate esters are common laboratory contaminants. Common flexible plastics contain varying amounts of phthalates. These phthalates are easily extracted or leached from such materials during laboratory operations. Cross contamination of clean glassware occurs when plastics are handled during extraction steps, especially when solvent-wetted surfaces are handled. Interferences from phthalates can be minimized by avoiding the use of plastics in the laboratory. The HPLC procedure used for sample preparation removes most phthalates resulting from collection, handling, or initial extraction.

Cross-contamination can occur whenever high-level and low-level samples are analyzed sequentially. Whenever an unusually concentrated sample is analyzed, the samples following it may need re-analysis if cross-contamination is suspected. The GC system may require bake-out and cleaning if the instrument has been exposed to high level samples.

For PCB analysis, interference can be caused by the presence of much greater quantities of sample components that overload the capillary column. Sample preparation techniques to minimize these interferences related to this analysis include high performance liquid chromatography (HPLC), defined in SOP 5-191, and alumina cleanup, defined in SOP 5-307. The MS detector eliminates interference by most chlorinated compounds other than PCBs. With the approach in this SOP, PCBs of the same level of chlorination that co-elute are identified and measured together. Co-eluting PCBs are only a problem if they are PCB of different LaC. Under the GC conditions in this SOP, these co-elutions have been identified. The interference problem is reduced or eliminated by rigorous application of the identification criteria and quantitation ion correction procedure described in this SOP.

15.0 DEFINITIONS

Battelle's methodology terms are consistent with the National Environmental Laboratory Accreditation Conference (NELAC) Glossary of Terms outlined in the NELAC Constitutions, Bylaws and Standards Manual. The NELAC Glossary of Terms currently being utilized by Battelle is outlined in Battelle's Quality Assurance Manual. Battelle's method detection terminology is defined in SOP 5-291 and quality control terminology in SOP 7-029.

16.0 METHOD DETECTION LIMITS

Battelle maintains a program for determining and verifying method detection limits (MDLs) and reporting limits. The policies and procedures are defined in SOP 5-291 and *Method Detection Limits* of Battelle's Quality Assurance Manual. All MDL study results are accessible within the laboratory system. All reporting limits are based on the low standard of the calibration curve, sample size, dilution factor and pre-injection volume (PIV). The MDL range for this method is presented in Table 1. The MDLs for each target analyte are presented in the project QAPP.

Table 1 Method Detection Range for Fish Tissue

Parameter Class	MDL Range	Units
PCB congener	0.117 - 0.573	ng/g
PCBLOC	0.129 -7.756	ng/g
Chlorinated pesticide	0.474 - 0.887	ng/g

17.0 METHOD PERFORMANCE

Battelle maintains a program for determining method performance. The policies and procedures are outlined in SOP 5-295 and *Method Validation and Method Detection Limits* of Battelle's Quality Assurance Manual. The Laboratory Manager must approve all new methodologies before use.

18.0 DATA ASSESSMENT AND ACCEPTANCE CRITERIA

Battelle maintains a program for assessing data and determining acceptance criteria for quality control measures. These policies and procedures are outlined in SOP 7-029 and in Battelle's Quality Assurance Manual.

Battelle also maintains a program for handling out-of-control or unacceptable data. These policies and contingencies are outlined in SOP 4-035, SOP 7-029 and *Data Validation* of Battelle's Quality Assurance Manual.

19.0 REFERENCES

Ballschmiter et al., 1992. K. Ballschmiter, R. Bacher, A. Mennel, R. Fischer, U. Riehle, and M. Swerev. Determination of chlorinated biphenyls, chlorinated dibenzodioxins, and chlorinated dibenzofurans by GC-MS. *J. High Resol. Chromatogr.* 15:260-270. April 1992.

United States Environmental Protection Agency. 1999. Chlorinated Biphenyl Congeners in Water, Soil, Sediment, and Tissue by HRGCIHRMS, Method 1668, Revision A, Office of Water, U.S. Environmental Protection Agency, Washington, DC.

United States Environmental Protection Agency. 1985. Test Methods for Determination of Pesticides and

PCBs in Water and Soil/Sediment by Gas ChromatographylMass Spectroscopy, Method 680, Physical and Chemical Methods Branch, Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio.

United States Environmental Protection Agency. 1996. Method 8270C, Semivo1atile Organic compounds by Gas ChromatographylMass Spectroscopy *(GCIMS).* Office of Solid Waste, U.S. Environmental Protection Agency, Washington DC.

U.S. EPA. 2002. National Environmental Laboratory Accreditation Conference - *Quality Systems.* July 12, 2002.

ATTACHMENTS

Attachment 1: Concentration and Retention Time Calibration Analyte List

Attachment 2: Potential Same Level PCB Co-Elutions on DB-XLB Column

Attachment 3: SIM Acquisition Ion Groups and Acquisition Windows for PCBlPesticide Analysis

Attachment 4: DFTPP Key Ions and Ion Abundance Criteria

Attachment 5: Approximate concentrations (ng/µl) of compounds in calibration solutions for SIM data acquisition - PCB and Pesticide analysis

Attachment 6: PCBlPesticide Quantification and Confirmation Ions (pCB ions and criteria from EPA Method 680, pesticides from historical data on the analysis of these compounds)

Attachment 7: Correction for Interference of PCB Containing Two Additional Chlorines (from EPA Method 680)

Attachment 8: Correction for Interference of PCB Containing One Additional Chlorine (from EPA Method 680)

Attachment 9: Summary of Changes to SOP

Attachment 10: PCB Homologue Manual Integration Screen Captures

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APPROVALS:

	Name	**Date**
Author	Wil Stenith	April 28, 2005
Quality Systems Manager	[signature]	4-28-05
Project Manager	[signature]	4-28-05

ATTACHMENT 1. Concentration and Retention Time Calibration Analyte List

Compounda, b, c, d	IS/SIS Reference	Compound	IS/SIS Reference	pound	IS/SIS Reference
Cl1(l) *	A,l	*CI6(128)*	A,2	*LOCI*	*A, I*
CIl(3) *	A,l	*CI6(138)*	A,2	*LOC2*	*A, I*
CI2(4) *	A,l	*CI6(149)*	A,2	*LOC3*	*A, I*
CI2(8)	A,l	*CI6(151)*	2	*LOC4*	*A,2*
C12(l5) *	A,l	*C16(153f*	A,2	*LOC5*	*A,2*
CI3(18)	A, I	CI6(l55) *	A,2	*LOC6*	*A,2*
C13(l9) *	A,l	*CI6(156)*	A,2	*LOC7*	*A,2*
CI3(28)	A, I	*C16(l57)*	A,2	*LOC8*	*A,2*
CI3(31)	A,l	*CI6(158)*	A,2	*LOC9*	*A,2*
CI3(37) *	A,2	*CI6(167)*	A,2	*LOC 10*	*A,2*
Cl4(44)		*Cl6(169)*	A, L		A,2
Cl4(49)	A,2	*CI7(170)*	A,2	*Oxychlordane*	A,2
CI4(52)	A,2	*CI7(177)*	A,2	*gamma-Chlordane*	A,2
CI4(54) *	A,2	*CI7(180)*	A,2	*alpha-Chlordane*	A,2
Cl4(66)	A,2	*CI7(183)*	A,2	*trans-Nonachlor*	A,2
Cl4(70)	A,2	*CI7(187)*	A,2	*cis-Nonachlor*	A,2
Cl4(74)	A,2	CI7(l88) *	A,2	*2,4'-DDE*	A,2
Cl4(77)'"	A,2	*CI7(189)* *	A,2	*4.4'-DDE*	A,2
Cl4(81)	A,2	*Cl*	,L.	*2,4'-DDD*	A,2
CI5(87)	A,2	*CI8(195)*	A,2	*4,4'-DDD*	A,2
CI5(99)	A,2	*CI8(201)*	A,2	*2,4'-DDT*	A,2
CI5(101)	A,2	CI8(202) *	A,2	*4,4'-DDT*	A,2
C15(104) *	A,L.	*CI8(203)*	A,2		
CI5(105)	A,2	CI8(205) *	A,2		
C15(l1O)	A,2	*CI9(206)* *	A,2		
CI5(114)	A,2	C19(208) *	A,2		
CI5(1l8)	A, 2	*Cll0(209)*	A,2		
CI5(1l9) d	A, 2			IS - C15(96)	A
CI5(123)	A,2			SIS - CI3(36)	I
CI5(126) *	A,2			SIS - C17(l92)	2

aAll PCB congeners are identified with the nomenclature presented in Ballschrniter et al. (1992).
b The *italicized* congeners and pesticides are the Montrose Settlement Restoration Program analytes.
c * denotes retention time calibration congener
d CI5(83) and CI5(119) co-elute. Reported as CI5(83)/CI5(119)
e CI6(153) and CI6(168) co-elute, Reported as CI6(153)/CI6(168)

ATTACHMENT 2. Potential Same Level PCB Co-Elutions on DB-XLB Column

Compound				
Cl1(l)	CI4(43)	ICI5(85)	IlcI6(128/162)	IICI6(169)
Cl1(2)	C14(44	CI5(86/109/125)	ICI6(129/158)	ICI7(170)
Cl1(3)	CI4(45)	ICI5(87)	IICI6(130)	IlcI7(171)
CI2(4/10)	CI4(46)	ICI5(88/95)	ICI6(1311133)	ICI7(172)
CI2(5)	CI4(47)		CI6(132/153/168)	IC17(173)
CI2(6)	CI4(48)	CI5(90/101)		CI7(174)
CI2(7)	CI4(49)	CI5(91)	ICI6(134)	ICI7(175/182)
CI2(8)	CI4(50)	CI5(92)	ICI6(135)	ICI7(176)
CI2(9)	CI4(51)	ICI5(93)	Cl6(136)	ICl7(177)
	CI4(52)	CI5(94)	CI6(137)	ICl7(178)
C12(11)	CI4(53)	CI5(95)	CI6(138)	ICl7(179)
CI2(12)	CI4(54)	CI5(96)	CI6(139/143)	ICl7(180)
CI2(13)	CI4(55/80)	CI5(97)	CI6(140)	Cl7(181)
CI2(14)	CI4(56)	CI5(98)	CI6(141)	
CI2(15)	CI4(57)	CI5(99)	CI6(142)	ICl7(183)
Cl3(16)	CI4(58/67)	CI5(100)		ICl7(184)
Cl3(17)		CI5(102)	CI6(144)	ICl7(185)
Cl3(18)	CI4(60)	CI5(103)	CI6(145)	ICl7(186)
Cl3(l9)	Cl4 61)	CI5 104)	CI6(146)	ICI7(187)
Cl3 20121133)	5/75)	Cl5 105)	(147/149)	ICI7(188)
		Cl5 106)	(148)	ICI7(189)
Cl3(22	l)	Cl5 107/123)		ICI7(190)
Cl3(23		Cl5 108)	Cl6(150)	C17(191)
Cl3t 24	66)		Cl6 151)	C17(l92
Cl3t 25		Cl5 110/120)	Cl6 152)	C17(l93
CI3t 26		Cl5 1111115)		C18(l94
Cl3 27	C1469	Cl5 112)	Cl6 154)	C18(l95
Cl3(28)	C1470	Cl5 113)	Cl6 155)	CI8(l961203)
Cl3(29)	CI4(71)	CI5(114)	Cl6(156)	CI8(197)
Cl3(30)	Cl4 72		CI6(157)	CI8(198)
ICl3 31)	Cl4 73	ICI5(116)		ICI8(199)
	Cl4 74,	Cl5(117)	CI6(l59)	CI8(200)
		Cl5(118)	Cl6 160/163)	C18(2011204)
Cl3 34			CI6(161)	CI8(202)
ICl3(35	CI4(77)			
Cl3 36	CI4(78)	Cl5 121)		
Cl3(37)	CI4(79)	(122)		CI8(205)
Cl3(38)			CI6(164)	C19(206
Cl3(39	CI4(81)	CI5(124)	CI6(165)	C19(207
CI4(40/68)	CI5(82)		CI6(166)	ICI9(208)
CI4(41)	CI5(83/119)	C15(126)	CI6(167)	ICI10(209)
C14(42/59)	C15(84/89)	CI5(127)		

a All PCB congeners are identified with the nomenclature presented in Ballschmiter et al., (1992),

ATTACHMENT 3. 81M Acquisition Ion Groups and Acquisition Windows for PCB/Pesticide Analysis

Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Quantification/Confirmation Ions (m/z)								
154	256	256	258	258	292	326	326	326
152	258	258	292	292	428	324	324	324
188	292	292	290	290	326	360	360	360
190	290	290	326	326	324	362	362	362
222	326	326	324	324	360	394	394	394
224	324	324	360	360	362	396	396	396
256	360	360	362	362	298	428	428	428
258	362	362	246	246	246	430	430	430
292	246	246	318	318	79	79	464	464
290	318	318	373	373	409	409	466	466
326	185	185	375	409	407	407	498	498
324	115	115	409	79	394	464	500	500
139	139	139	407	263	396	466	409	409
141	141	141	79	235	235	235	235	235
185	373	373	212	298	237	237	237	237
115	212	212	298	394	247	247	247	247
Interference Check Ions (m/z)								
221	254	254	254	254	288	288	288	288
254	255	255	255	288	289	289	289	289
255	288	288	288	289	425	425	425	425
288	289	289	289	322	322	356	356	356
289	322	322	322	323	323	357	357	357
322	323	323	323	356	356	322	322	322
323	356	356	356	357	357	323	323	323
356	357	357	357	390	390	390	390	390
357	375	375	263	391	391	391	391	391

Window	Defined by:	~ Time (min)
1	Start of Biphenyl	O
2	Between C13(28) and C13(36)	47.4
3	Between oxych10rdane and C14(74)	55.16
4	Between 2,4'-DDE and 4,4'DDMU/C15(101)	57.3
5	Between C15(119) and C15(87)	59.15
6	Between C16(149) and C15(123)	61.76
7	Between C16(153) and C15(105)	63.5
8	Between C17(183) and C15(126)	65.7
9	Between C18(201) and C16(156)	67.3

ATTACHMENT 4. DFTPP Key Ions and Ion Abundance Criteria
(Source: EPA Method 8270C, as modified by EPA Method 680)

m/z	Relative Abundance
127	40 - 60% ofm/z 198
197	< 1% ofm/z 198
198	Base peak, 100%
199	5 - 9% ofm/z 198
275	10 - 30% ofm/z 198
365	> 1% ofm/z 198
441	Present and < m/z 443
442	> 40% ofm/z 198
443	17 - 23% ofm/z 442

ATTACHMENT 5. Recommended concentrations (ng/μl) of compounds in calibration solutions for SIM data acquisition -- PCB and Pesticide analysis

und	L1	L2	L3		L5	L6
LOCl[a]	0.002	0.005	0.02	0.05	0.1	0.24
LOC2[a]	0.002	0.005	0.02	0.05	0.1	0.24
LOC3[a]	0.002	0.005	0.02	0.05	0.1	0.24
LOC4[a]	0.002	0.005	0.02	0.05	0.1	0.24
LOC5[a]	0.002		0.02	0.05	0.1	0.24
LOC6[a]	0.002	0.005	0.02	0.05	0.1	0.24
LOC7[a]	0.002	0.005	0.02	0.05	0.1	0.24
LOC8[a]	--	0.005	0.02	0.05	0.1	0.24
LOC9[a]	--	0.005	0.02	0.05	0.1	0.24
LOClO	--	0.005	0.02	0.05	0.1	0.24
CHC[b]	0.01	0.025	0.1	0.25	0.5	1.2
IS	0.1	0.1	0.1	0.1	0.1	0.1
SIS	0.002	0.005	0.02	0.05	0.1	0.24

a Includes the individual PCB congeners identified in Attachment 1 at this LOC.
b Includes the chlorinated pesticides identified in Attachment 1.

ATTACHMENT 6. PCB/Pesticide Quantification and Confirmation Ions (PCB ions and criteria from EPA Method 680, pesticides from historical data on the analysis of these compounds)

Analyte	Quant Ion	Confirm Ion	% Confirmation Ion
Biphenyl	154	152	30
Monchlorobiphenyls	188	190	30
Dichlorobiphenyls	222	224	65
Trichlorobiphenyls	256	258	95
Tetrachlorobiphenyls	292	290	75
Pentachlorobiphenyls	326	324	65
Hexachlorobiphenyls	360	362	80
Heptachlorobiphenyls	394	396	95
Octachlorobiphenyls	430	428	90
Nonchlorobiphenyls	464	466	75
Decachlorobiphenyl	498	500	85
2,4- and 4,4- DDD and DDTs	235	237	65
2,4- and 4,4- DDE	246	318	28
a- and g- chlordane	373	375	100
cis- and trans- nonchlor	409	407	94
Oxychlordane	185	115	130
Dieldrin	79	263	25

ATTACHMENT 7. Correction for Interference of PCB Containing Two Additional Chlorines (M+70)(from EPA Method 680)

LOC Group	Quant Ion	Confirm Ion	Interference Ion	Correction Factor Fraction of Measured Interference Ion Area to be Subtracted from Quantification or Confirmation Ion	
				Quant Ion	Confirm Ion
LOC3	256	11258	254	0.99	0.33
1LOC4	292	11290	288	0.65	1.31
LOC5	326	11324	322	1.08	1.64
LOC6	360	362	356	1.61	0.71
1LOC7	394	396	390	2.25	111.23
1					

ATTACHMENT 8. Correction for Interference of PCB Containing One Additional Chlorine (M+35) (from EPA Method 680)

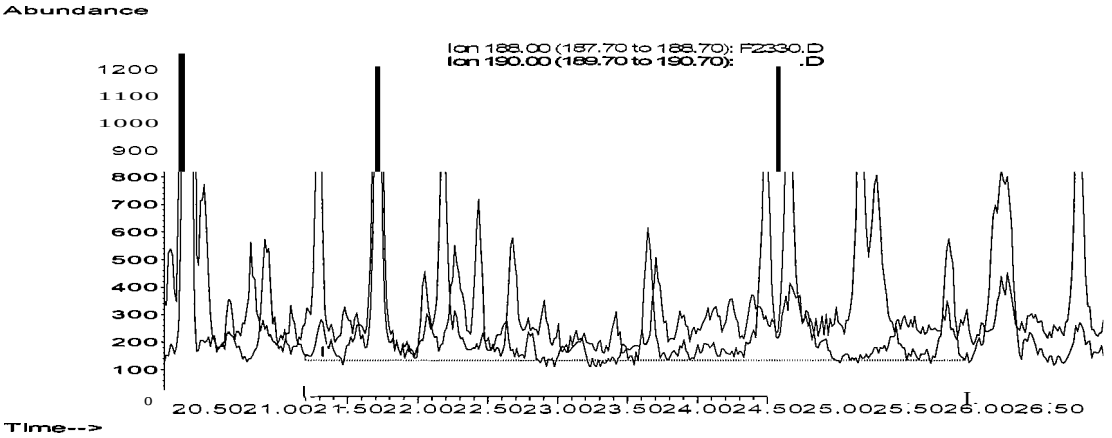
LOCGroup	Quant Ion	Confirm Ion	Interference Ion	Correction Factor Fraction of Measured Interference Ion Area to be Subtracted from Quantification or Confirmation Ion	
				Quant Ion Area	Confirm Ion Area
1 LOC2	222	11224	221	110.135	110.0
LOC3	256	11258	255	110.135	110.0
LOC4	292	11290	289	0.174	110.0
LOC5	326	11324	323	110.22	110.0
LOC6	360	11362	357	110.265	110.0
LOC7	394	11396	391	110.309	110.0
LOC8	430	11428	425	0.40	110.0

ATTACHMENT 9
Summary of Changes to SOP

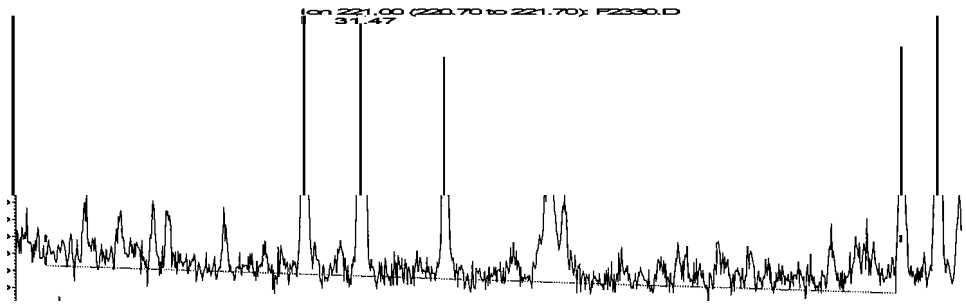
Version	Summary of Changes
02	The SIS reference for several compounds was updated in Attachment 1.
03	Implemented a Battelle Enviroquant™ macro to correct quantification ion abundance for contribution from co-eluting M+70 and M+35 PCB. Added use of a GC/MS check solution to assess system performance. Removed 13C12-4,4'DDT and C15(l03) from list of optional surrogates. Required, rather than recommended, use of the DB-XLB GC column. Modified GC conditions, which are now required, not recommended. Added use of retention time windows and removed reference to relative retention times for monitoring GC performance. Changed PCB reporting nomenclature from IUPAC to Ballschmiter et (1992). Revised SIM acquisition ion groups and windows. Changed ICAL from linear to non-linear quadratic. Clarified ICC and CCV performance criteria. Clarified method for peak and Level of Chlorination (LOC) identification and quantification.
04	All target analytes are now quantified from a single IS; C15(96). SIS concentrations in calibration solutions co-vary with target PCB analyte concentration and SIS are quantified in the same manner as target analytes. Clarified that only hexane is used as solvent for sample extracts and standards. Added text and an equation to describe the method of surrogate correction. Added text and an equation to describe the method of calculating % recovery of spiked compounds. Extracted ion chromatogram examples of the LOC quantitation have been added. A history of SOP revisions is summarized in Attachment 9.

ATTACHMENT 10
PCB Homologue Manual Integration Screen Captures

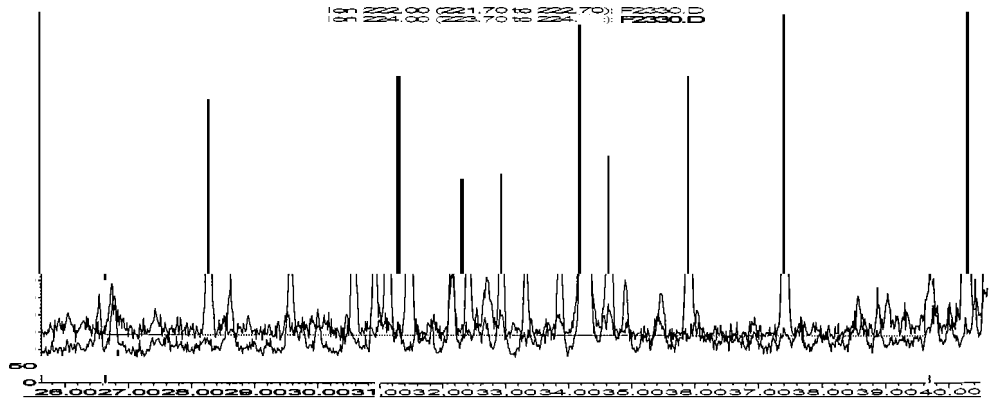
LOCI



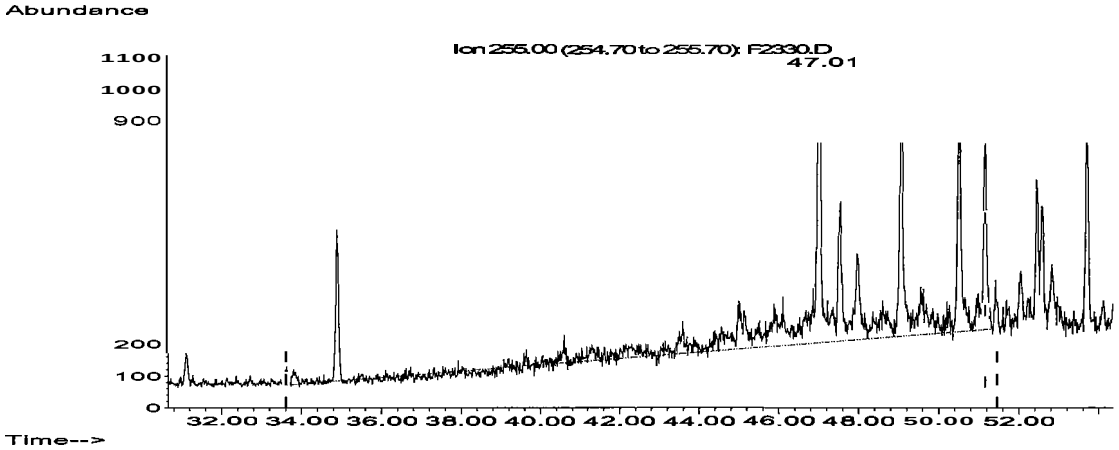
LOC2-SI



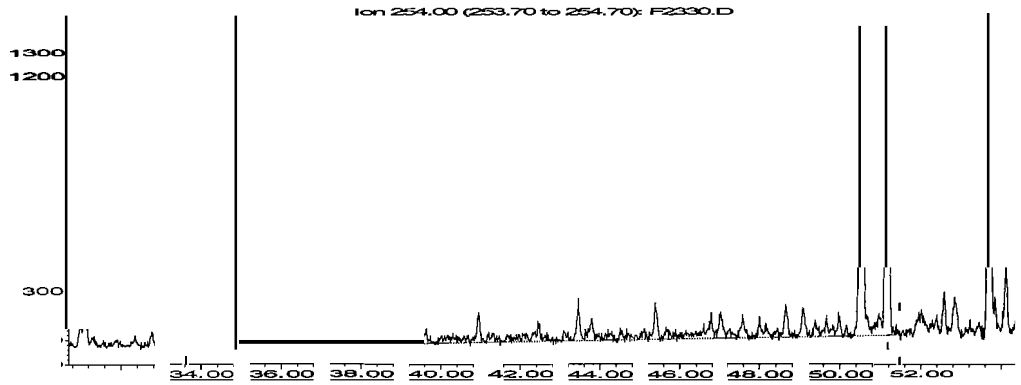
LOC2



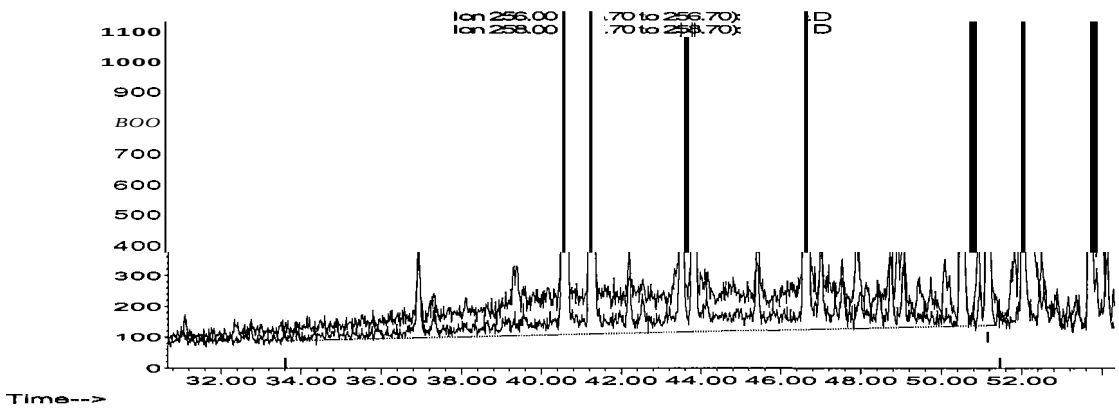
LOC3 - 81



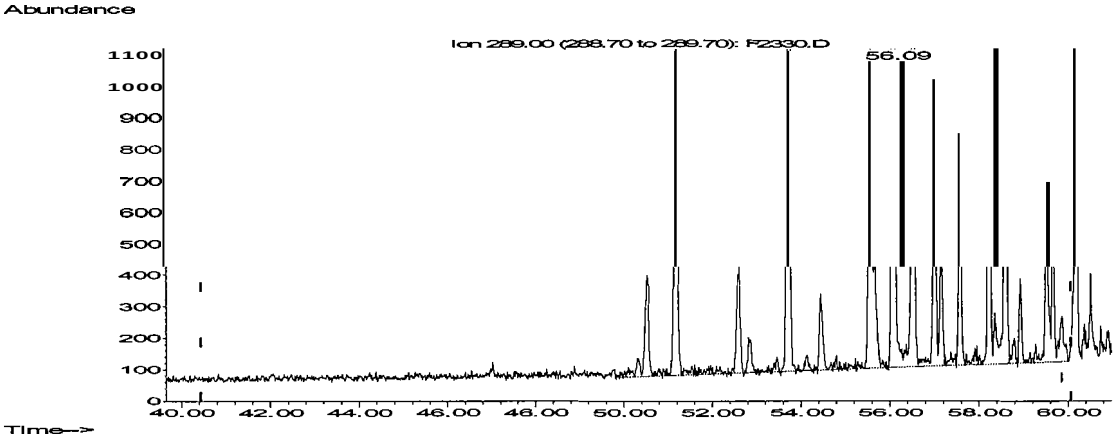
LOC3 -82



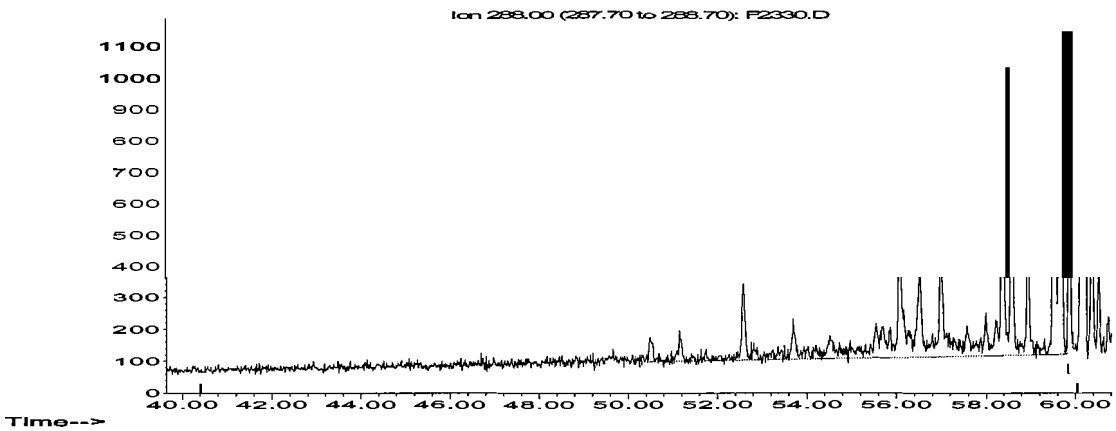
LOC3



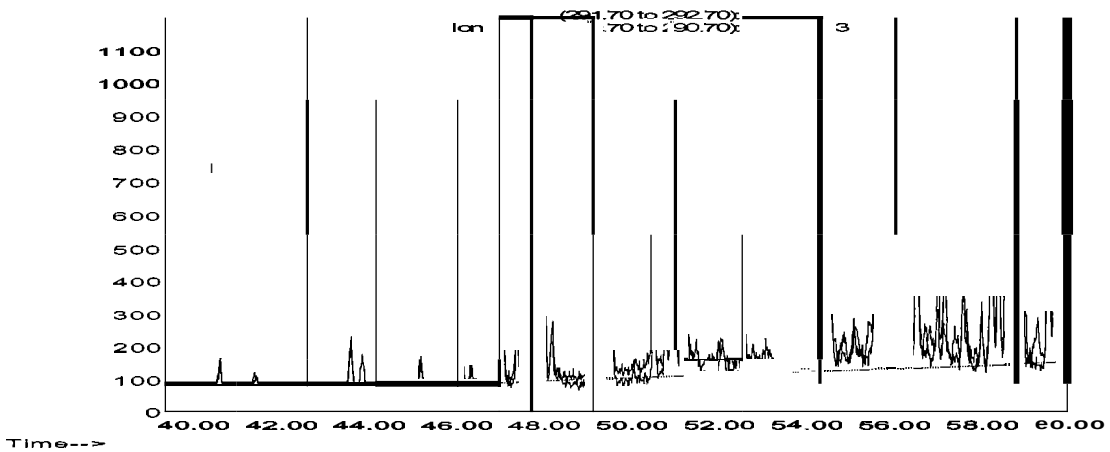
LOC4-S1



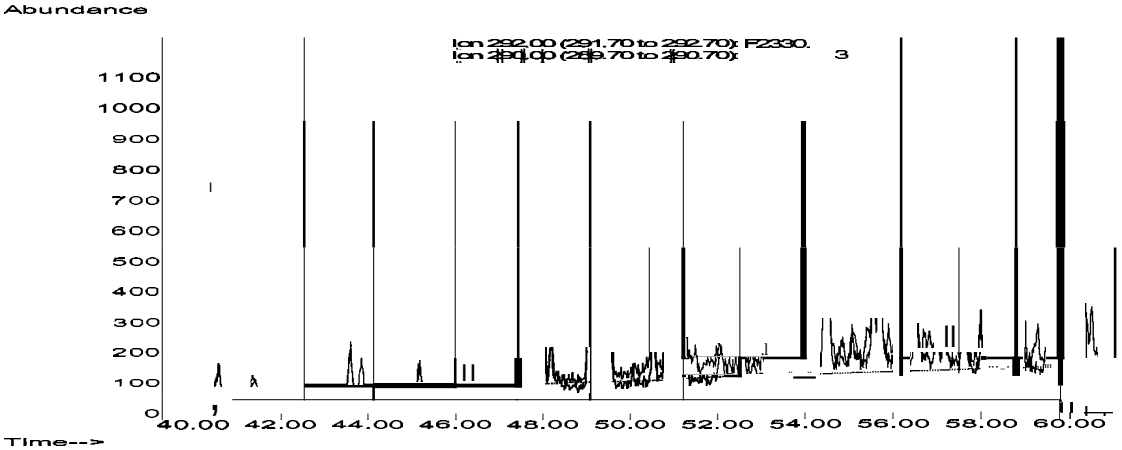
LOC4-S2



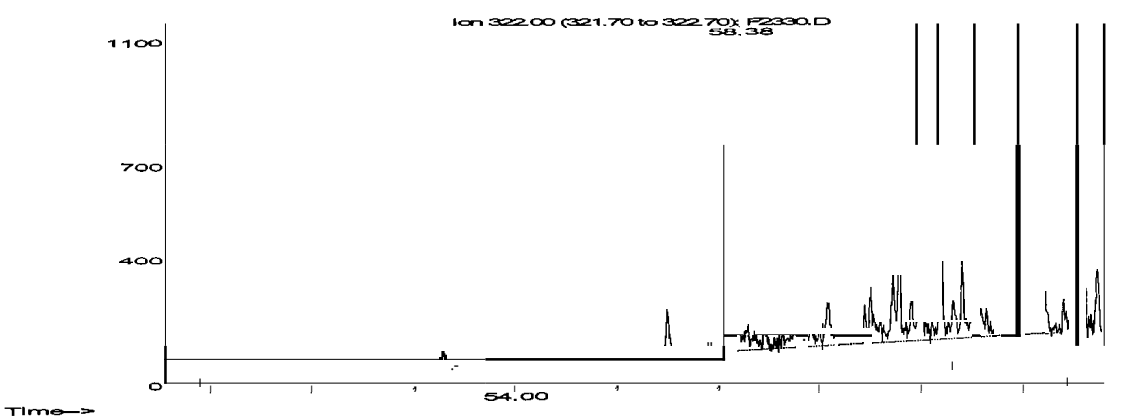
LOC4



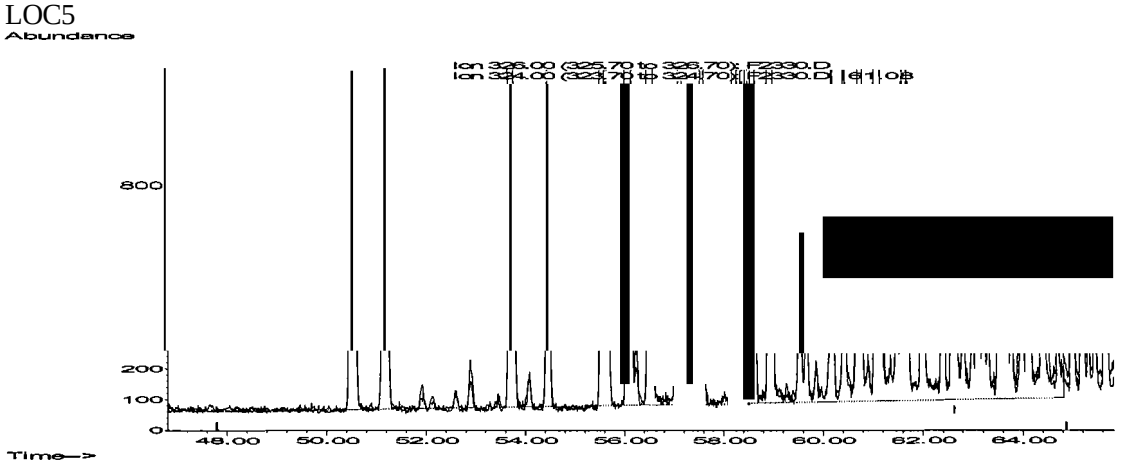
LOC5 – S1



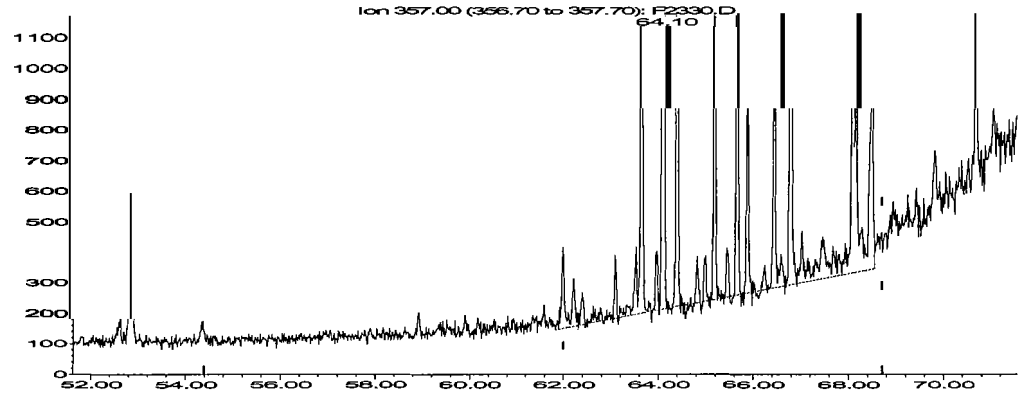
LOC5 -S2



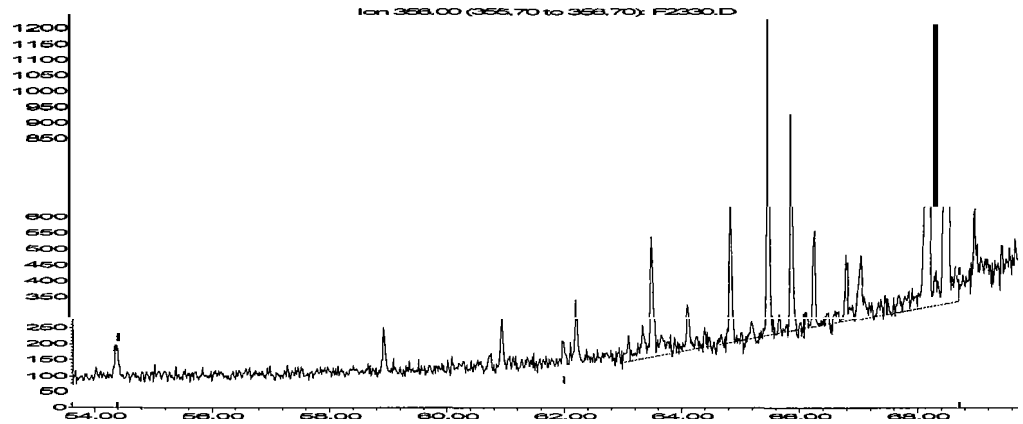
LOC5



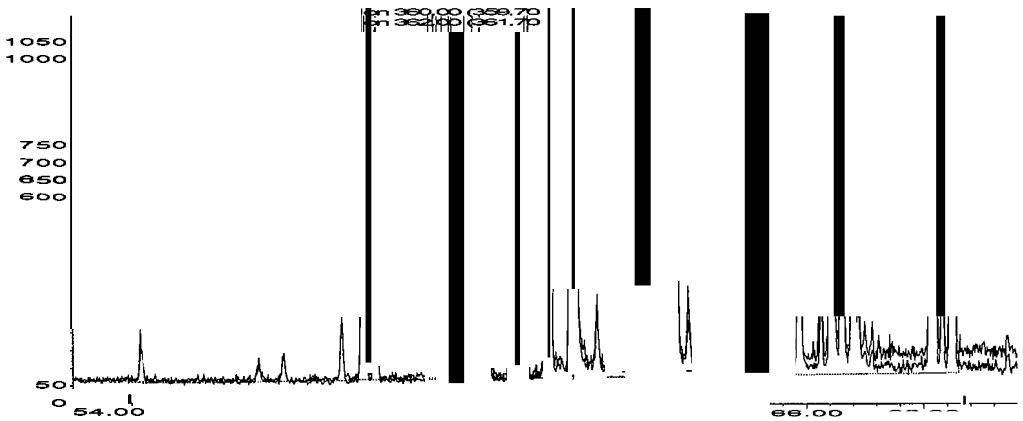
LOC6-S1



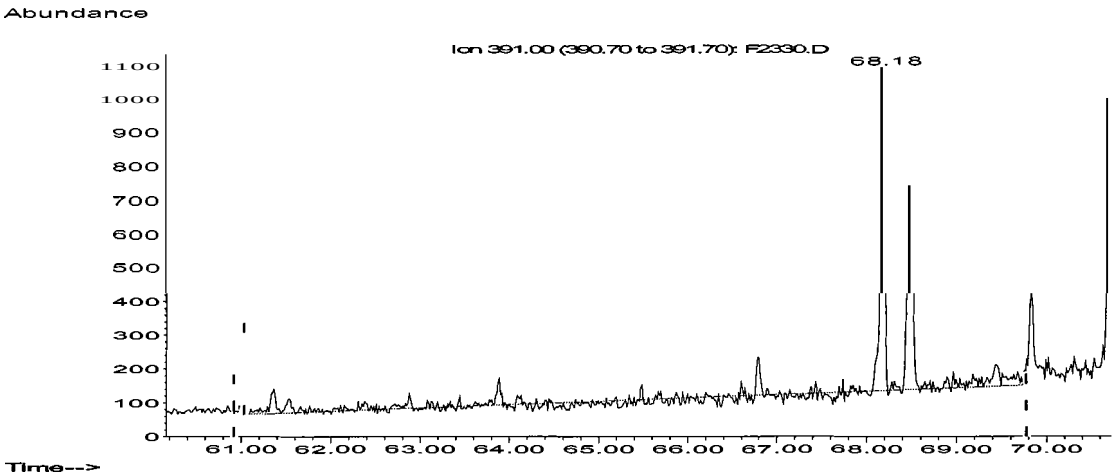
LOC6-S2



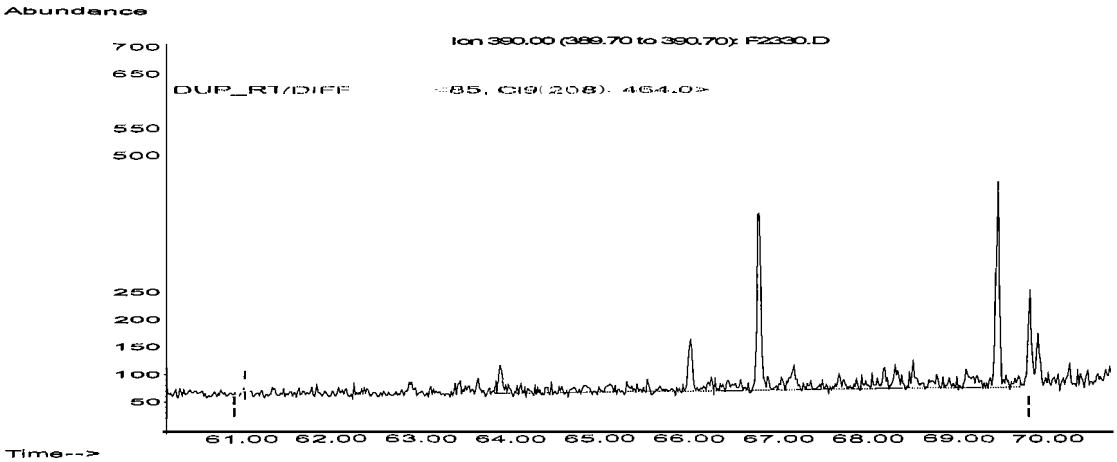
LOC6



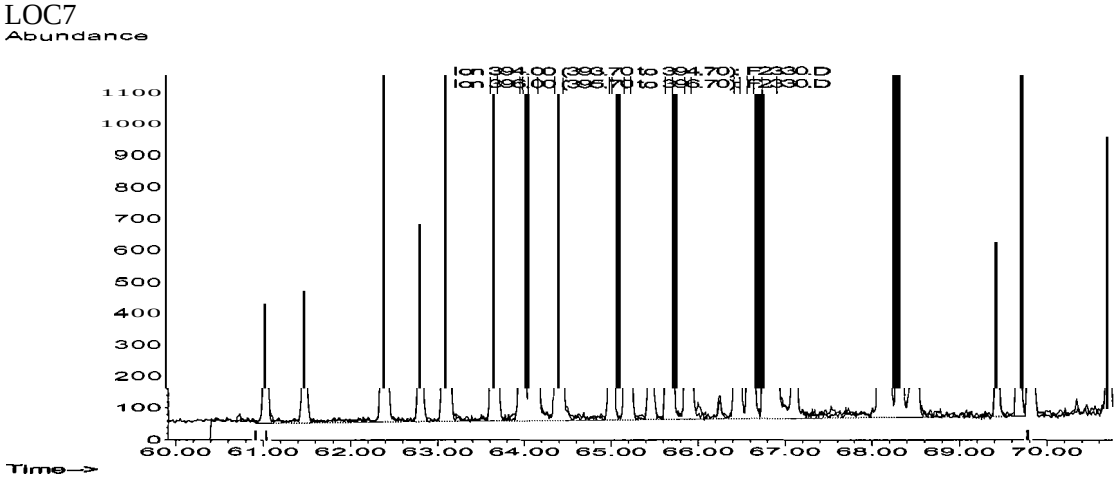
LOC7 - Sl



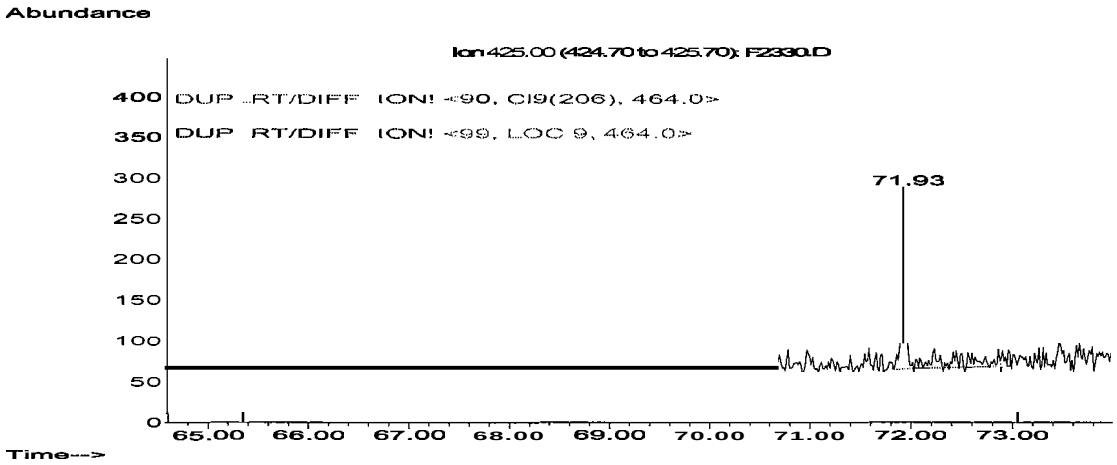
LOC7-82



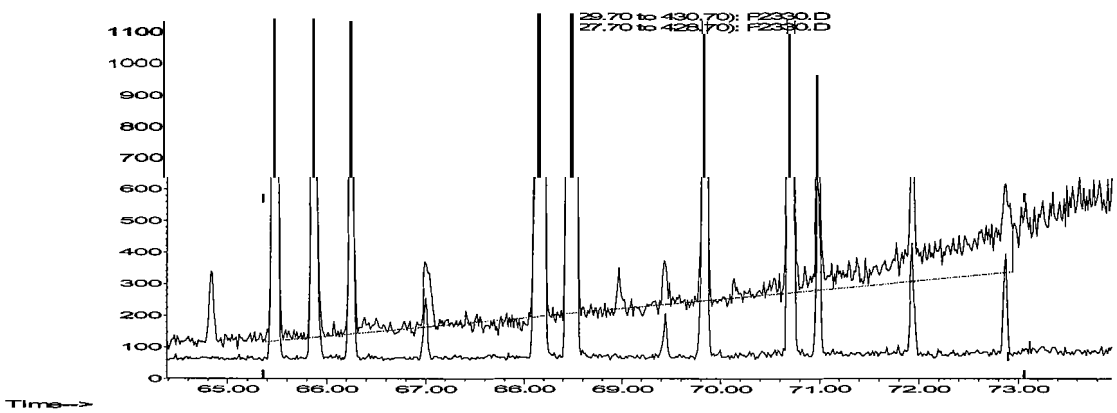
LOC7



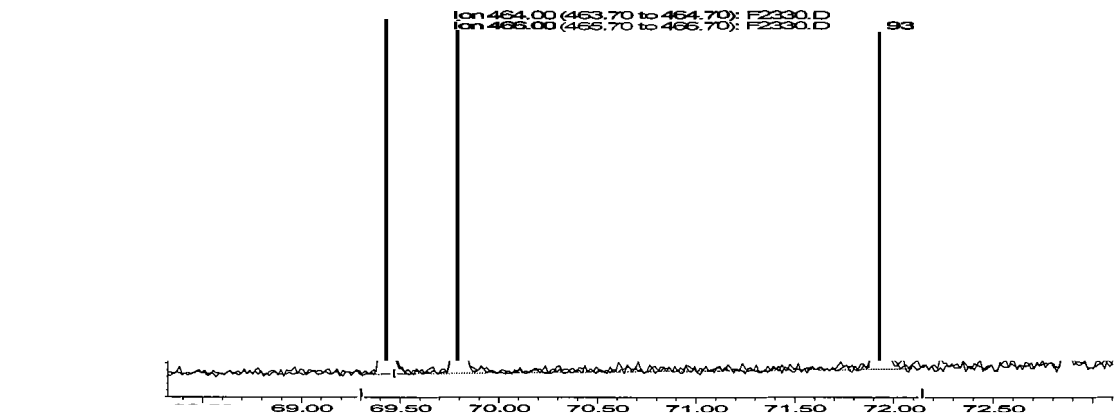
LOC8 - 81


Abundance
Ion 425.00 (424.70 to 425.70): F2330.D
DUP RT/DIFF ION! <90, Cl9(206), 464.0>
DUP RT/DIFF ION! <99, LOC 9, 464.0>
71.93
Time-->

LOC8


Abundance
29.70 to 430.70): F2330.D
27.70 to 428.70): F2330.D
Time-->

LOC9


Abundance
Ion 464.00 (463.70 to 464.70): F2330.D
Ion 466.00 (465.70 to 466.70): F2330.D
93