Burlington SOP BRL SOP-00423 / 5 **Burlington**

PAH Compounds by HRGC HRMS in Food Products, Sediment and Water

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Insert Text

- BRL SOP-00423 rev 5
- PAH Curve - example

Associated Documents

Document Control Documents

- Burlington FCD BRL FCD-00002 : Data Review Checklist
- Burlington FCD BRL FCD-00003 : Organic Control Chart Out of Control Checklist
- Burlington FCD BRL FCD-00052 : MDL Template for Multi-Analyte Tests - NELAC
- Burlington SOP BRL SOP-00003 : GPC Cleanup
- Burlington SOP BRL SOP-00004 : SOXHLET EXTRACTION
- Burlington SOP BRL SOP-00005 : Separatory Funnel and Rolacell Extraction -Organics from H2O Samples
- Burlington WI BRL WI-00011 : Review and Validation of Analytical Data
- Campobello WI CAM WI-00001 : Control Charting
- Campobello WI CAM WI-00005 : Chemistry Method Validation
- Campobello WI CAM WI-00025 : Determining Method Detection Limits
- Campobello WI CAM WI-00121 : Maxxam Lab QM Supplement to Meet Requirements of DoD QSM Version 4.1
- Corporate WI Publish to All COR WI-00010 : Corporate Procedure for Nonconformances

- Corporate WI Publish to All COR WI-00011 : Corporate Procedure for Corrective and Preventative Actions
- Corporate WI Publish to All COR WI-00012 : Definitions
- Corporate WI Publish to All COR WI-00018 : Determination of Estimation of Analytical Uncertainty of Measurement
- Corporate WI Publish to All COR WI-00040 : Corporate Procedure for Policy Deviation Forms
- Corporate WI Publish to All COR WI-00044 : Manual Integration of Chromatographic Peaks
- Corporate WI Publish to All COR WI-00049 : Continuing Calibration Verification (CCV) Acceptance Criteria

Reason for Change

Date of Change March 6, 2007 **Version** 1
Change Made Initial release in the QSI

Date of Change February 5, 2009 **Version** 2
Section Changed 10.2
Change Made 10.2 Corrected typographical error by removing PCB and listing PAH as the analytes of interest.

Date of Change May 4, 2009 **Version** 3
Section Changed title, 4.0, 6.6, 9.0, 11.2, 13.0,
Change Made Title: added sediment and water
4.0: removed references to internal SOPs (they are listed in the Associated Documents)
6.6 added reference to sample disposal SOP: CAM SOP-00101
9.0 revised the paragraph to refer to Maxxam's current practice
11.2 added FMS PLE to instrumentation list
13.0 added sample preparation for sediment and water and alternate sample preparation using PLE

Date of Change July 15, 2009 **Version** 4
Section Changed 2.0, 12.4.6, 12.4.7, 12.4.11-tables 1-6, all changes highlighted
Change Made 2.0 added 18 new analytes with corresponding quant. mass ions and added monoisotopic atomic mass of S.
12.4.6 & 12.4.7 added two new surrogate stds.: 2,6-Dimethylnaphthalene-d12 and Perylene-d12
12.4.11 Table 1 added d12- 2,6-Dimethylnaphthalene
12.4.11 Table 2 added 21 new analytes with corresponding Surrogate stds.
12.4.11 Table 3 added 2 new recovery standards
12.4.11 Table 4 added 19 new calibration stds and 3 new surrogates stds.
12.4.11 Table 5 added 17 new natives in matrix spike soln.
12.4.11 Table 6 added 3 new concentrations of labeled Stds in sample extracts.

Date of Change August 5, 2010 **Version** 5
Section Changed 4.2, 4.5, 9.1, 9.2, 9.3, 11.2.2, 12.4.7, 12.4.7, 12.4.8.1, 12.4.9.1, 13.4, 13.9, 15, 16, 18
Change Made 4.2Methods USEPA 1625 and SW846 8270 cited as primary references.
4.5 updated reference section by listing additional reference methods used in this SOP
9.1 + 9.2 redefined correlation between MDL and detection limit, as well as Maxxam's RDL and LOQ, added performance of quarterly LOD and LOQ
9.3 clarified that the lab does not blank correct sample data for bias
11.2.2, removed 60M Column, optimized GC conditions
12.4.4, 12.4.7, 12.4.8.1, 12.4.9, 13.4 and 13.9, changed iso-octane to toluene
12.4.7 replaced "acetone" with "iso-octane or nonane". Additional steps added if GPC clean-up is employed
15.0 QC Table, added note to method blank criteria & corrective actions for DoD related projects
16.0 entire section updated to reflect current practice on reporting / documentation and harmonize with other HRMS SOPs
18.0 added table with % Recoveries based on EPA 1625 and 8270

Approval Status Table

Approver Source **Role for Approvers** **Parallel-Everybody** Segment

Approver	Action	Date	Comment
Edmond McNeil	Approve	August 5, 2010 3:20 PM GMT-4	
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PAH Compounds by HRGC/ HRMS in Food Products, Sediment and Water

1.0 Introduction

PAHs are extracted with organic solvents, usually dichloromethane (DCM) using a Soxhlet extractor for food products and sediments and by liquid-liquid extraction for water. Following extraction, the samples are cleaned up and passed through a series of columns that remove, by reaction, selective adsorption and size exclusion, as the bulk of the organic matrix is co-extracted with the target compounds. The resulting fraction is concentrated to a known volume, usually 500uL, for analysis. Qualitative/quantitative analysis for PAHs is performed using High Resolution Capillary Gas Chromatography (HRGC) with High Resolution Mass Spectrometry (HRMS).

1.2 Interferences

Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the total ion current profiles. All of these materials must be routinely demonstrated to be free from any interferences > method RDL under the conditions of the analysis by running laboratory method blanks. In the case of solid food (e.g. meat, fish) samples, it is especially important to separate the PAHs of interest in the method from the presence of proteins and lipids in solid food (eg. meat, fish). This is done by size exclusion, hence the reason for the use of GPC, gel permeation chromatography. In addition, the PAHs need to be separated from compounds, which are similar to them that could give false positives. Examples of such compounds are azo-arenes and aminoazo-arenes. These are usually removed by the basic alumina clean-up.

2.0 Scope

2.1 This method is to be used to measure the presence Polycyclic Aromatic Hydrocarbons (PAHs) in food, sediment and water by HRGC/HRMS (high-resolution gas chromatography/ high-resolution mass spectrometry) down to trace levels in the ppb or ng/g range.
Quantification mass ions for PAHs by HRMS are listed below:

Quantification mass ions for PAHs by HRMS

Parameter	Accurate Mass	Ion type	CAS No.
1,2,3,4-Tetrahydronaphthalene	132.0939	A	119-64-2
Naphthalene	128.0626	A	91-20-3
PFK	130.9920	Lock	
Naphthalene-d_8	136.1128	IS	1146-65-2
2-Methylnaphthalene	142.0782	A	91-57-6
1-Methylnaphthalene	142.0782	A	832-69-9
2-Chloronaphthalene	162.0236	A	91-59-8
1-Chloronaphthalene	162.0236	A	90-13-1
2-Methylnaphthalene-d_{10}	152.1410	IS	N/A
2,6-Dimethylnaphthalene	156.0939	A	581-42-0
2,3,5-Trimethylnaphthalene	170.1096	A	2245-38-7
2,6-Dimethylnaphthalene-d_{12}	168.1683	IS	N/A
Acenaphthylene	152.0626	A	208-96-8
Acenaphthylene- d_8	160.1128	IS	93951-97-4
Acenaphthene	154.0782	A	83-32-9
Acenaphthene-d_{10}	164.1410	RS	15067-26-2
PFK	169.9888	QC	
Fluorene	166.0782	A	86-73-7
Fluorene- d_{10}	176.1410	SS	N/A

Dibenzothiophene	184.0347	A	136-65-0
Phenanthrene	178.0782	A	84-01-8
Phenanthrene- d_{10}	188.1410	IS	1517-22-2
Anthracene	178.0782	A	120-12-7
Anthracene- d_{10}	188.1410	AS	1719-06-8
2-Methylanthracene	192.0939	A	613-12-7
1-Methylphenanthrene	192.0939	A	832-69-9
Fluoranthene	202.0782	A	206-44-0
Fluoranthene- d_{10}	212.1410	IS	93951-69-0
Pyrene	202.0782	A	129-00-0
PFK	204.9888	QC	
Pyrene- d_{10}	212.1410	RS	1718-52-1
9,10-Dimethylanthracene	206.1096	A	781-43-1
Benzo[a]fluorene	216.0939	A	238-84-6
Benzo[b]fluorene	216.0939	A	243-17-4
Benzo[a]anthracene	228.0939	A	56-55-3
Benzo[a]anthracene- d_{12}	240.1692	IS	1718-53-2
Chrysene	228.0939	A	218-01-9
Chrysene- d_{12}	240.1692	IS	1719-03-5
PFK	230.9856	LOCK	
Terphenyl- d_{14}	244.1974	SS	1718-51-0
5-Methylchrysene	242.1096	A	3697-24-3
7,12-Dimethylbenzo[a]anthracene	256.1249	A	57-97-6
Benzo[b,j]fluoranthene	252.0939	A	205-99-2, N/A
Benzo[b]fluoranthene- d_{12}	264.1692	IS	N/A
PFK	254.9856	QC	
Benzo[k]fluoranthene	252.0939	A	207-08-9
Benzo[k]fluoranthene- d_{12}	264.1692	IS	93952-01-3
Benzo[e] pyrene	252.0939	A	192-97-2
Benzo[e]pyrene- d_{12}	264.1692	RS	N/A
Benzo[a]pyrene	252.0939	A	50-32-8
Benzo[a]pyrene- d_{12}	264.1692	IS	63466-71-7
Perylene	252.0939	A	198-55-0
Perylene- d_{12}	264.1692	IS	1520-96-3
Benzo[g,h,i] perylene	276.0939	A	191-24-2
Benzo[g,h,i]perylene- d_{12}	288.1692	IS	93951-66-7
Indeno[1,2,3-cd]pyrene	276.0939	A	193-38-5
Indeno[1,2,3-cd]pyrene- d_{12}	288.1692	IS	N/A
Dibenz[a,h]anthracene	278.1096	A	53-70-3
PFK	268.9824	LOCK	
Dibenz[a,h]anthracene- d_{14}	292.1974	IS	N/A
Anthanthrene	276.0939	A	191-26-4
Dibenzo[a,e]pyrene	302.1096	A	192-65-4
Coronene	300.0939	A	191-07-1

Monoisotopic atomic masses used:

H=1.007825
C=12.000000
^{2}H=2.014102
S= 32.9721

Descriptions of Ion Types:

A	**Analyte**
IS	Internal standard
LOCK	Lock mass ion
QC	Quality control check ion
SS	Surrogate standard
AS	Alternate standard
RS	Recovery standard
PFK	Perfluorokerosene (HRMS reference gas)

2.2 This procedure is to be followed by the laboratory technicians for all PAH analyses done in solid food (e.g. meat, fish), sediment and water matrices. Variations relevant to an unusual or specific matrix must be documented and must be consistent with the reference methods quoted above or with methods specified by the client. The analyst may modify the extraction procedure or instrumental method to improve chemical separations or to minimize costs, provided that the method performance criteria are met, and provided that any such modifications are documented and approved by the supervisor.

2.3 This method is designed for use by analysts or technicians experienced in laboratory extraction of PAH from tissue matrices in particular, solid food (e.g. meat, fish) as well as sediment and water, and skilled in HRGC/HRMS instrumental analysis. It should be noted that PAHs are very common in the environment, especially in solid food (e.g. meat, fish), cooked processed or otherwise. In mammalian cells, PAHs undergo metabolic activation to form diol epoxides that bind covalently to cellular macromolecules such as DNA; thus, causing DNA replication and mutations that can and do initiate the carcinogenic process.

2.4 The native PAHs cover a linear range from 10 to 500 pg/µL.

3.0 Definitions

3.1 Refer to COR. WI-00012.

4.0 Reference Method

4.1 Test Codes

4.1.1 PAHHR-PT, the laboratory test code for the analysis of PAHs in food (meat, fish).

4.1.2 Other related test codes may be created as necessary, for example PAHHR-S (for sediments) and PAHHR-W (for water).

4.2 Primary References

4.2.1 SW-846 no. 3540C, Soxhlet Extraction, 3510C, Separatory Funnel Liquid-Liquid Extraction, 3640A Gel Permeation Cleanup, 3610B Alumina Cleanup and CARB 429. Although CARB 429 is an air method for the analysis of PAHs in stack emissions, it is only the instrumentation and quantitation portion that is used as a reference for this method.

4.2.2 Appendix A to Part 136 Method 1625 rev. B, Semi volatile Organic Compounds by Isotope Dilution GC/MS; SW 846 no. 8270C, Semi volatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS).

4.4 Deviations

4.4.1 The Soxhlet Extraction method referenced above is largely designed for sediments so the only modification is the matrix, solid food (eg. meat, fish). %Lipids instead of % moisture is measured instead, for example, while the overall extraction remains the same.

4.5 Additional References

4.5.1 Refer to QSI Associated Documents of this SOP.

4.6 Instrument Manuals

4.5.1 Routine Maintenance Log Books are available at the instruments and detail the frequent and infrequent actions needed to maintain the instrumentation in good working order.

4.5.2 Software and hardware manuals are also available at the instruments. The software users manuals are called Masslynx 4.0 Users Manual and Opus version 3.6X Acquisition Software Manual. The hardware manuals include list of parts, diagrams

and schematics and are available at the respective instruments and are put out by MicroMass and / or Waters.

REFERENCE LITERATURE is available on line. The SW 846 methods are available at http://www.epa.gov/sw-846/main.htm.

5.0 Regulatory Criteria

5.1 The Canadian Food Inspection Agency (CFIA) in Canada, and the United States Food and Drug Administration (USFDA) in the United States will set out regulatory requirements for this test. Other regulatory requirements are dependant upon given jurisdictions.

6.0 Safety & Disposal

General Safety requirements for laboratory work are provided by the Maxxam Safety Officer; including Safe Work procedures, Safety Manual and Safety Policy. MSDS's are reviewed during analyst training. Chemical wastes and sample disposal protocols are described in the Health and Safety database.

6.1 The following safety considerations are excerpts from EPA Method 1613 which is a determative method for dioxin and furan, but the safety and disposal considerations listed here are equally applicable to PAHs.

6.2 The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined. Therefore, each chemical compound should be treated as a potential health hazard and exposure to these compounds should be limited.

6.2.1 The laboratory should purchase dilute standard solutions of the analytes. If primary solutions must be prepared from the neat compounds, they must be prepared in a fume hood, and a NIOSH approved toxic gas respirator shall be worn when high concentrations are handled.

6.2.2 The laboratory maintains a file of MSDS's (Material Safety Data Sheet) and these are available to the analyst at any time.

6.2.3 PAHs and samples, which may contain these compounds, must be handled in a well-ventilated, controlled access laboratory.

6.2.4 In HRMS lab the work areas are generally assigned to low, regular or high level matrices. All glassware and tools for each area are appropriately labelled and only used for PAHs. Plastic absorbent paper may be used to line the bench tops. This paper may trap contaminants it is essential that care be taken to dispose of regularly.

6.2.5 Handling of dilute solutions normally used in analytical work presents no inhalation hazards except in the case of an accident.

6.3 All personnel should use protective equipment, including disposable gloves, aprons or lab coats, and safety glasses or masks. All employees are expected to be familiar with the ***Maxxam*** Safety manual, and all employees are provided with a copy of the manual upon hiring.

6.3.1 When handling samples suspected of containing high levels of PCDDs, PCDFs, PCBs or PAHs, an additional set of gloves may be worn beneath the latex gloves.

6.3.2 Full-face protection is necessary when working with exposed samples or pure analytical standards.

6.3.3 During analytical procedures, which may give rise to dust or aerosols, personnel should wear respirators equipped with activated carbon filters.

6.3.4 Workers must be careful to remove contaminated gloves and clothing without contacting the exterior surfaces.

6.4 Personal Hygiene

6.4.1 Workers must thoroughly wash their hands and forearms, scrubbing with mild soap, before breaks (coffee, lunch, shift change).

6.5 The effluent vapours from the GC and roughing pumps on the GC/MS should pass through either a column of activated charcoal or be bubbled through a trap containing oil or high-boiling alcohols.

6.6 Waste Disposal

Note: For International Soil Samples follow CAM SOP-00101, Disposal of Samples, Section: 8.5.

6.6.1 It is important to minimize contaminated waste. Plastic bag liners should be used in waste cans.

6.6.2 Janitors and other personnel must be trained in the safe handling of waste.

6.6.3 Additional information concerning waste disposal can be found in CAM WI-00018 – *WASTE DISPOSAL.*

6.6.3.1 Waste that is considered to be a biohazard must be disposed of accordingly.

6.7 Sample Disposal
In HRMS lab all samples are stored in the designated fridge.

6.7.1 All samples that are not beyond hazards levels are disposed as described in CAM WI-00018 – *WASTE DISPOSAL.*

6.7.2 All samples that are beyond hazards level are properly stored in sealed drums onsite, then removed and stored in a proper hazard facility offsite.

6.7.2.1 A record of samples stored are kept by the HRMS department

6.7.2.2 When a Hazardous Storage company, a proper waybill and chain of custody pick up samples is completed with a copy kept by the lab.

6.8 Decontamination

6.8.1 Personnel should use any mild soap with plenty of scrubbing action to clean any exposed skin.

6.8.2 Soaking in **Extran 300** Detergent or a similar laboratory cleaning solution may clean glassware, tools and surfaces. Washing with detergent and water follows this.

6.8.3 Clothing known to be contaminated should be collected in plastic bags. The persons who convey the bags and launder the clothing should be advised of the hazard and trained in proper handling. Clothing may be put into a washer without contact to personnel, if the launderer knows of the potential problem. The washer should be run through a cycle before being used again for other clothing.

6.9 Wipe/Swab Tests

6.9.1 A useful method of determining cleanliness of work surfaces and tools is to wipe the surface with a piece of filter paper or medical gauze (which has been dampened with solvent).

6.9.2 Extraction and analysis of the swab or wipe sample indicates acceptable cleanliness at less than 0.1 ug per wipe.

6.9.3 More than 10ug per wipe indicates an acute hazard and requires prompt cleaning before further use of the equipment or workspace. This may also indicate that unacceptable work practices have been employed

7.0 Sample Handling, Preservation, Holding Times

MATRIX	Sample Container	Mass (g)/ Volume (mL)	Holding Time	Storage Conditions	Preservation
Solid food	125-500 mL Plastic jar Teflon lined caps recommended Glass shatters easily at –20C, but can be used, and amber is recommended.	30	14 days	-20ºC Dark	None
Sediment	125-500 mL Amber wide jar Teflon lined caps recommended	30	7 days	4ºC Dark	None
Water	1L Amber Boston Round Bottle	1000	7 days	4ºC Dark	0.008% Sodium Bisulphate

MATRIX	EXTRACTION HOLDING TIME (DAYS)	ANALYSIS HOLDING TIME (DAYS)
Solid food	14	40
Sediment	7	40
Water	7	40

8.0 Interferences

In addition to that which was mentioned in section 1.2, the following is also done in order to minimize interferences:

8.1 Glassware must be scrupulously cleaned. Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. Clean as per BRL WI-00006. Certain PAH homologues have a tendency to adhere to glassware and are hard to clean. Solvent rinses with acetone and pesticide quality hexane are necessary. Thorough rinsing with such solvents usually eliminates PAH interference. After drying, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

8.2 Raw data from all blanks, samples, and spikes are evaluated for interferences. Determine if the source of interference is from sample preparation and/or cleanup and take corrective action to eliminate the problem.

8.3 Contamination by carryover can occur whenever high-concentration and low-concentration samples are sequentially analyzed. To reduce carryover, the sample syringe must be rinsed with solvent between sample injections. Whenever an unusually concentrated sample is encountered, the sample immediately following should be re-analyzed once the system is proven to be suitable (ie. background < RDL). In addition, any dilutions required could also be analyzed in this sequence.

8.4 Glassware blanks are performed with each full set of samples, if the extraction procedure used requires re-useable glassware.

9.0 Detection Limits (DL) for the target analytes.

9.1 **Detection Limit:** Detection Limit is also referred to as Method Detection Limits (MDL): an MDL is the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero. MDLs are determined by the procedure outlined in CAM WI-00005, Method Development, Revision and Validation.

Target MDLs are verified annually by spiking water/soil replicates at the CS2-CS3 levels. These samples are carried through the full extraction and cleanup procedure. MDLs are determined by multiplying the standard deviation of the results by the Student's t-Value for the number of replicates used. If Target Limits are not achieved, the exercise is repeated spiking at a lower level. An MDL study is used to determine if the target limits outlined in test methods have been achieved. These are maintained on file in the QSI MDL Module as well as at the instrument. To meet DOD requirements, these limits are then used to determine spiking levels for the Limit of Detection (LOD) verification. Each matrix is spiked two to three times the target limit (derived above) for a single analyte test or one to four times the detection limit for a multi-analyte test.

NOTE: As per CAM WI-00121, section D.1.2.1, Maxxam HRMS lab schedules quarterly LOD verifications for each respective matrix to meet DOD requirements.

Estimated Detection Limit (EDL): EDLs are sample specific and are calculated on a case specific basis for all analytes. If a signal produced is not at least 2.5 times the average background level at the expected retention time for specific isomers, the area response for the noise is calculated as follows.

$$2.5 \times \text{peak intensity of background noise} \times \frac{\text{area of internal standard}}{\text{height of internal standard}}$$

This is done for either the quantitation ion or the confirmation ion. Using theoretical ratios for the specific degree of chlorination an area for the second ion is generated. These areas are then totaled and applied to the same calculation scheme as for "positive" hits. (see Section 9). EMPCs (Estimated Maximum Possible Concentrations) are calculated whenever peaks are detected that meet all criteria except for ratios. The area in this case is recalculated (factored up or down) to meet the classical ratio and the result is flagged as an EMPC (NDR, not detected because of ratios, for Canadian Method).

9.2 Reporting Detection Limit (RDL): Reporting Detection Limit is also referred to as Limit of Quantitation (LOQ), equivalent to the Low Level Standard. RDLs are defined in the test codes in LIMS.

NOTE: As per CAM WI-00121, section D.1.2.1, Maxxam HRMS lab schedules quarterly LOQ verifications for each respective matrix to meet DOD requirements.

9.3 Measurement of Uncertainty: Refer to COR WI-00018 for Determination of Estimation of Analytical Uncertainty of Measurement procedure. MU defines an interval about a sample result that would be expected to contain a large fraction of the distribution of values that would occur through repeated analysis of similar samples using the same methodology. Uncertainty is expressed as the standard deviation of the mean of a set of data (Blank Spikes/Laboratory Control Samples). Laboratory blunders or human errors are not incorporated into the calculations.

The reported Expanded Uncertainty of an analyte is specific to the laboratory method used and incorporates laboratory procedures; physical measurements, (volume, temperature and mass), environmental variation, reagent and standard purity, sample preparation procedures (if applicable), personnel and instrumental parameters.

The most significant factors affecting the uncertainty of a measurement arise from the field-sampling program. They involve field site sample homogeneity; sampling technique; cleanliness of sampling equipment/containers; cross contamination of samples; environmental conditions; sub sampling techniques; preservation techniques; sample storage; time to submit to laboratory. These variations are not incorporated into MAXXAM's uncertainty calculations.

MAXXAM uses a coverage factor "k" (multiplier of the combined standard uncertainty) to obtain an Expanded Uncertainty. MAXXAM reports to a confidence level of 95% (k=2).

MAXXAM does not blank correct sample data for bias. The final decision on the acceptability and application of the reported data is the responsibility of the client

10.0 Glassware

10.1 Glassware Preparation and Storage

10.1.1 Soak contaminated glassware in strong detergent **Extran 300** or equivalent) for at least two hours but no longer than 24. (A bath should contain no more than .2% detergent, (200mL/100L of tap water) Scrub the glassware, rinse well with hot tap water, rinse well with reagent water and rinse three times with acetone, and allow to air dry.

10.1.2 All necessary glassware must be dedicated PAH analysis only and must be stored in a contaminant free environment.

10.2 Glassware Blank

All required re-useable glassware must be demonstrated to be free of contaminants. This is demonstrated by performing glass blank analyses along with each set of samples if re-useable glassware will be used for extraction.

10.2.1 Acceptable limits for PAHs in the glass blank are: not yet established but should be less then 200 pg per congener group

10.2.1.1 If any of the above criteria are exceeded, then re-extraction of the batch is required. If this is not possible, the senior analyst and/or manager must be notified. The detection limits may be raised, or client may need to be contacted.

10.2.2 To perform a glass blank

10.2.2.1 Pre-rinse all glassware to be used in the extraction batch and cleanup procedures.
10.2.2.2 combine all the rinsates of the final wash.
10.2.2.3 Fortify the glass blank sample with labeled PAHs and concentrate the extract to 1 mL using a roto-evaporator.
10.2.2.4 Clean up the sample (Section 12.6) and analyze for PAHs on the HRGC/MS system.

10.3 Glassware Proofing

10.3.1 After extraction of samples known to contain high levels of PAH's, a glassware proof must be analyzed and shown to be below the acceptable levels.

10.3.2 Collect all affected glassware and perform rinses.

10.3.2.1 pour a small amount of DCM into each piece of glassware. Collect all rinsates into a round bottom flask.

10.4 Disposable Glassware

10.4.1 All disposable glassware will be rinsed three times with DCM before use.

10.4.2 All disposable glassware used in this procedure is purchased from approved suppliers.

10.5 Glassware Tracking

10.5.1 All glassware in which a sample sits for a period of time (eg. flat bottom flask, seperatory funnel, erlenmeyer flask) has a letter and number designation. (L=low, R=regular, H=high.) This designation ensures the separation of different levels of glassware and allows the analyst to track trends.

10.5.2 with each set of work the analyst records the glassware and rotovaps used for each sample on a glassware tracking form. The form remains in the file with the worksheet.

10.5.3 It is the responsibility of each analyst to ensure that the glassware they are using are appropriately labeled. A glassware inventory is kept in the Q/C office.

11.0 Apparatus and Materials

11.1 APPARATUS AND MATERIAL

Laboratory Glassware and Supplies
Disposable 5 mL pipettes.
Disposable Pasteur pipettes.
Disposable 40 mL glass bottles, with Teflon R-lined lids.
These are also known as "volatiles or VOC bottles".
Disposable 15 mL, conical, centrifuge tubes.
Disposable 18 mL, round-bottom, centrifuge tubes.
80 mL, glass, chromatographic column for acid/base cleanup.
250 mL boiling flasks.
240 mL glass jars, or equivalent.
500 uL conical auto sampler vials, or similar.
Autosampler syringe-rinse containers.
600 mL Buchner funnel.
1 L side arm flasks, vacuum flasks.
Pyrex dishes or trays (drying dish).
Glass and plastic stirring rods of various sizes.
1 L amber bottles.
500 mL Erlenmeyer flasks.
300 mL glass liners for tube furnace.
2 L separatory funnels.
250mL soxhlet extraction apparatus or equivalent
500 mL boiling flask
Aluminum weighing dishes.
Filtering funnels
Graduated cylinders
Boiling chips
Volumetric flasks
Beakers
Glass Fiber filters

11.1.1 Laboratory Equipment
Analytical balance, capable of measuring to 1 mg.
Tube furnace.
Vacuum pump.
Rotary evaporator.
Nitrogen-gas evaporation unit.
Roto-rack tumbler
Drying oven, for glassware and reagents.
Centrifuge
Sonicator, or Ultra-sonic Bath.
Heating Mantles
Muffle furnace

11.2 Instrumentation:

11.2.1 VG Autospec Hi-Resolution mass spectrometer or Autospec Ultima
Hewlett Packard model 5890 Series II gas chromatograph or HP 6890 Gas Chromatograph
CTC autosampler

11.2.2 The GC/MS system conditions used for the analysis are listed below:

Column: 30M HP5 0.25 mm. ID, 0.25µm film thickness or equivalent
Injector Mode: Split/Splitless Injector in splitless mode, time 60 seconds
1.0 µL injection volume.
Injector Temperature: 300 °C

GC Temperature program: 50 °C for 1 minute
50 °C to 220 °C at 14 °C/minute
220 °C to 300 °C at 7 °C/minute
Hold at 300 °C for 40 minutes (or as required for optimization, i.e. until after the elution of dibenzo [a, e] pyrene and its three other isomers)

The mass spectrometer conditions used in the analysis are:
Electron Impact mode Electron energy 70eV
Photo Multiplier: as required V
Ionizer Temperature: 290°C
Resolution: Minimum of 8,000 must be demonstrated
Scan: SIM (total scan cycle time < 1 sec)
Lock mass ion: PFK ions are used to calibrate the SIM mass range & as QC check ions

11.2.3 FMS, Pressurized Liquid Extractor (PLE™)

12.0 Reagents and Standard Preparation

12.1 Laboratory Reagents

12.1.1 All solvents used during extraction and analysis are pesticide grade or better from Caledon, BDH or equivalent.

12.1.2 Aluminum Foil -all aluminum foil must be pre-washed before use. Pre-washed aluminium foil is prepared by washing both sides with dichloromethane.

12.1.3 Sodium sulphate is reagent grade, and in granular form. It must be pre-cleaned prior to use.

12.1.3.1 Place approximately 300 mL of sodium sulphate into a 600 mL buchner funnel, connect to a vacuum flask and pump system.
12.1.3.2 Cover the sodium sulphate with approximately 300 mL DCM.
12.1.3.3 Stir the sodium sulphate with a glass-rod and let it settle for 2 minutes.
12.1.3.4 Turn on the vacuum pump to suction off solvent.
12.1.3.5 Repeat steps 12.1.3.2 to 12.1.3.4 two more times for a total of three washings.
12.1.3.6 Remove sodium sulphate from funnel and place it in a glass or Pyrex drying dish and cover with pre-washed, perforated aluminum foil. Air dry (approximately 1 hour).
12.1.3.7 Place the dish in the oven and activate sodium sulphate overnight (approximately 12 hours) at approximately 190C.
12.1.3.8 The preceding steps must also be applied to Hydromatrix if PLE extraction is to be employed. PLE= pressurized liquid extraction.

12.2 Basic Alumina: 1% deactivated, purchased from suitable commercial suppliers, i.e. BDH. Store in an amber glass jar for up to 1 year at room temperature.

12.3 Solvents: Hexane, Dichloromethane (DCM) is DIG ("Distilled in Glass") grade from **Fisher or** Caledon Laboratories. Store in supplier containers at ambient temperature for up to 1 year.

12.4 Standards – All standards, spiking, calibration and recovery solutions are prepared as follows: information regarding the concentration, source, lot number, preparation date, expiry date and analyst responsible is written on the label of the bottle used to store the solutions and in the Standard and Reagent Logbook. Equivalent standards may be purchased from other suitable suppliers as available. Other volumes of a standard may be prepared by modifying the amount of stock used appropriate to the final volume required.

12.4.1 Native PAH Standards: Supplier #1 for Calibration Standards: commercially purchased standards are stored as per the manufacturer's instructions and are stable to the specified expiry date. All prepared standards are stored in a refrigerator at 2-6 °C and are stable for 1 year after preparation or until the stock standard's expiry date, if earlier. Replace sooner if there is indication of deterioration. Prepare solutions containing all of the compounds listed in Section 3.0 at the appropriate concentrations in iso-octane.

12.4.2 Stock Native PAH Mix: Supplier #1 (2000 ng/µL each): This solution is purchased from AccuStandard as a mixture of 16 PAHs (contains the 16 PAH compounds listed in EPA method 610). This solution is stored at 2-6 °C for up to 1 year after the date opened.

12.4.3 Individual Stock PAH Calibration Standards: these standards are purchased individually from Chem Service, Aldrich, Accustandard or Supelco. They represent individual additions to the standard solution of compounds of interest that are not included in the solution in Section 6.6.1 above. Individual stock solutions of neat standards are prepared at a concentration of 1000 ng/µL. These solutions are stored at 2-6 °C for up to 1 year.

For preparation of stock solutions**:** Weigh 0.0100 ±0.0002 g of the individual neat standard into a 10 mL volumetric flask and dilute to volume with DCM. This will generate a stock standard at 1000ng/uL. The weight used may vary due to the amount of stock chemical available. Other volumes of a standard may be prepared as necessary by modifying the amount of stock used appropriate to the required final volume.

12.4.4 Supplier #1 Native Intermediate Mix (10 ng/µL): with an appropriate micro-liter syringe, measure the below listed volume of each of the following solutions into a 10 mL volumetric flask, dilute to volume with toluene. Store at 2-6 °C for up to 1 year.

Compound	Conc. (ng/µL)	Volume (µL)	Final Conc. (ng/µL)
AccuStandard PAH Mix	2000	50	10
2-Methylnaphthalene	100	100	10

12.4.5 Labeled Standards: These commercially purchased standards are labeled with deuterium in order to make them as chemically similar to the native analytes as possible so that they will closely mimic the behavior of the natives during the extraction process. These compounds are stored as per the manufacturer's instructions and are stable to the specified expiry date. Prepared standards are stored at 2-6 °C and are stable for up to 1 year. Larger volumes of a standard may be prepared as necessary by modifying the amount of stock used appropriate to the required final volume. Other suppliers may be used as available.

12.4.6 Stock Labeled Standards: labeled standards are purchased as neat chemicals from Cambridge Isotope Laboratories or other suitable suppliers and are made to a concentration of 1000 ng/µL. Weigh 0.0100 ±0.0001 g of each reagent into a separate 10 mL volumetric flask and dilute to volume with DCM. These stocks are used for preparing the labeled spiking solutions as summarized below:

Surrrogate Standards

2-Methylnaphthalene-d_{10}	Fluoranthene-d_{10}	Phenanthrene-d_{10}
Benzo(a)anthracene-d_{12}	Chrysene-d_{12}	Benzo(b)fluoranthene-d_{12}
Benzo(ghi)perylene-d_{12}	Benzo(k)fluoranthene-d_{12}	Benzo(a)pyrene-d_{12}
Indeno(1,2,3-cd)pyrene-d_{12}	Fluorene-d_{10}	Dibenzo(ah)anthracene-d_{14}
Acenaphthylene-d_8	Naphthalene-d_8	Anthracene-d_{10}
2,6-Dimethylnaphthalene-d_{12}	Perylene-d_{12}	

Recovery Standards

Acenaphthene-d_{10}	Pyrene-d_{10}	Benzo(e)pyrene-d_{12}

12.4.7 Surrogate Standard Solution: stock standards are prepared as per Section 12.4.6 and are documented in the High Res Standards Logbook.

12.4.7.1 Surrogate Standards (5 and 10 ng/µL): using a micro liter syringe, transfer the following specified volume of each of the following stocks (at 1000 ng/µL) into a 10 mL volumetric flask and dilute to volume with toluene. Store at 2-6 °C for up to 1 year or to manufacturer's expiry date.

Compound	Conc. (ng/µL)	Volume (µL)	Final Conc. (ng/µL)
Naphthalene-d_8	1000	50	5
2-Methylnaphthalene-d_{10}	1000	50	5
2,6-Dimethylnaphthalene-d_{12}	1000	50	5
Acenaphthylene-d_8	1000	50	5
Phenanthrene-d_{10}	1000	50	5
Fluoranthene-d_{10}	1000	50	5
Benzo(a)anthracene-d_{12}	1000	50	5
Chrysene-d_{12}	1000	50	5
Benzo(b)fluoranthene-d_{12}	1000	100	10
Benzo(k)fluoranthene-d_{12}	1000	100	10
Benzo(a)pyrene-d_{12}	1000	100	10
Perylene-d_{12}	1000	100	10
Indeno(1,2,3-cd)pyrene-d_{12}	1000	100	10
Dibenzo(ah)anthracene-d_{14}	1000	100	10
Benzo(ghi)perylene-d_{12}	1000	100	10

Adding 10 µL of this solution to a sample prior to extraction gives a theoretical concentration of 100/200 pg/µL of internal standard compounds in the extract. For samples spiking where GPC clean-up is employed, this labeled standard can be diluted with acetone in order to facilitate dispersion of the labeled surrogates into the matrix. A dilution scheme similar to that used for the Organochlorine Pesticides can be used:

Extractors: Take 10 uL to 10 mL with acetone or 25 uL to 25 mL with acetone
Spike 1 mL / split

12.4.8 Recovery Standard: prepare a standard solution containing Acenapththene-d_{10} and Pyrene-d_{10} at 10 ng/µl and Benzo (e)-pyrene-d_{12} at a concentration of 20 ng/µL in iso-octane as documented in the High Res Stds. Logbook.

12.4.8.1. Recovery Standard Spiking Solution: take the specified volume below of the following labeled stocks, at 1000 ng/µL (see Section 6.7.1) into a 10 mL volumetric flask and dilute to

volume with toluene. Prepared standards are stored at 2-6 °C and are stable for up to 1 year. This standard is added to all samples. Adding 5 µL of this solution to a 0.5 mL extract gives a Recovery Standard concentration of 100/200 pg/µL in extract.

Compound	Conc. (ng/µL)	Volume (µL)	Final Conc. (ng/µL)
Acenaphthene-d_{10}	1000	100	10
Pyrene-d_{10}	1000	100	10
Benzo(e)pyrene-d_{12}	1000	200	20

12.4.9 Native PAH Standards: Supplier #2 for Spiking and Initial Calibration Verification (ICV): a second source of standards is used to verify the concentration of the primary calibration standards. This secondary source standard is also used to prepare native spiking solution for Blank Spikes, Matrix Spikes, and Method Spikes, to provide an additional on going crosscheck of spiking and calibration standards. A separate vial of Supelco PAH mix at 2000 ng/µL is used to prepare the spiking solution. Store at 2-6 °C to manufacturer's expiry date. Individual stocks of any required PAH's that require addition to the Supelco mix should also be prepared at 1000 ng/µL, similar to Section 6.6.3, but using neat standards from a secondary source. Note that for preparation of the Matrix Spiking Solution, individual stock solutions are also required for naphthalene and phenanthrene.

12.4.9.1 Supplier #2 Native Intermediate Mix (100 ng/µL): using a micro liter syringe, measure the below listed volume of each of the following solutions to a 10 mL volumetric flask. Note 1: the individual PAH stock solutions used for this mix are ideally secondary source stocks from the ones used for the calibration standards. Bring to volume with toluene. Store at 2 - 6°C for up to one year.

Compound	Conc. (ng/µL)	Vol. (µL)	Final Conc. (ng/µL)
Supelco PAH Mix	2000	500	100
2-Methylnaphthalene	1000	1000	100

12.4.10 PAH Initial Calibration Verification (100 pg/µL): using a micro-syringe, transfer 10 µL of the Supplier #2 Native Intermediate Mix (Sect. 6.12.1) and labeled solutions as per CS3 level instrument standard in Sect. 6.6.5, to 10 mL volumetric flask, dilute to volume with iso-octane. Transfer the standard to an amber glass vial with Teflon lined cap, store at 2-6 °C. This standard is run as a second source check of the 5-point initial calibration.

12.4.11 PAH Matrix Spiking Solution: using a micro-syringe, transfer 500 µL of the Supplier #2 Native Intermediate Mix (Section 6.12.1) to a 10 mL volumetric flask. Fortify with 200 µL of naphthalene individual stock solution at 1000 ng/µL, 50 µL of 2-methylnaphthalene individual stock solution at 1000 ng/µL, and 50 µL of phenanthrene individual stock solution at 1000 ng/µL. Dilute to volume with acetone. Store at 2-6 °C for up to one year.

Concentrations of the proceeding solutions are given in the following tables as well as quantitation references:

Table 1:

COMPOSITION OF THE SAMPLE SPIKING SOLUTIONS

Analytes	Concentration (ng/µL)

Surrogate Standards:

d_8-Naphthalene	5
d_{10}-2-Methylnaphthalene	5
d_{12}- 2,6-Dimethylnaphthalene	5
d_8-Acenaphthylene	5
d_{10}-Phenanthrene	5
d_{10}-Fluoranthene	5
d_{12}-Benzo(a)anthracene	5
d_{12}-Chrysene	5
d_{12}-Benzo(b)fluoranthene	10
d_{12}-Benzo(k)fluoranthene	10
d_{12}-Benzo(a)pyrene	10
d_{12}-Perylene	10
d_{12}-Indeno(1,2,3,c-d)pyrene	10
d_{14}-Dibenz(a,h)anthracene	10
d_{12}-Benzo(g,h,i)perylene	10

Recovery Standards:

d_{10}-Acenaphthene	10
d_{10}-Pyrene	10
d_{12}-Benzo(e)pyrene	20

Table 2:

ASSIGNMENTS OF SURROGATE STANDARDS FOR CALCULATION OF RRFs AND QUANTITATION OF TARGET PAHs

Analyte	**Surrogate Standards:**
Native PAH	
1,2,3,4-Tetrahydronaphthalene	d_8-Naphthalene
Naphthalene	d_8-Naphthalene
2-Methylnaphthalene	d_{10}-2-Methylnaphthalene
1-Methylnaphthalene	d_{10}-2-Methylnaphthalene
2-Chloronaphthalene	d_{10}-2-Methylnaphthalene
1-Chloronaphthalene	d_{10}-2-Methylnaphthalene
2,6-Dimethylnaphthalene	d_{12-} 2,6-Dimethylnaphthalene
2,3,5-Trimethylnaphthalene	d_{12-} 2,6-Dimethylnaphthalene
Acenaphthylene	d_8-Acenaphthylene
Acenaphthene	d_8-Acenaphthylene
Fluorene	d_{10}-Phenanthrene
Phenanthrene	d_{10}-Phenanthrene
Anthracene	d_{10}-Phenanthrene
Dibenzothiophene	d_{10}-Phenanthrene
2-Methylanthracene	d_{10}-Phenanthrene
1-Methylphenanthrene	d_{10}-Phenanthrene
9,10-Dimethylanthracene	d_{10}-Fluoranthene
Benzo[a]fluorene	d_{10}-Fluoranthene
Benzo[b]fluorene	d_{10}-Fluoranthene
Fluoranthene	d_{10}-Fluoranthene
Pyrene	d_{10}-Fluoranthene
Benzo(a)anthracene	d_{12}-Benzo(a)anthracene
Chrysene	d_{12}-Chrysene
5-Methylchrysene	d_{12}-Chrysene
7,12-Dimethylbenzo[a]anthracene	d_{12}-Benzo(b)fluoranthene
Benzo(b/j)fluoranthene	d_{12}-Benzo(b)fluoranthene
Benzo(k)fluoranthene	d_{12}-Benzo(k)fluoranthene
Benzo(a)pyrene	d_{12}-Benzo(a)pyrene
Perylene	d_{12}-Perylene
Indeno(1,2,3,c-d)pyrene	d_{12}- Indeno(1,2,3,c-d)pyrene
Dibenz(a,h)anthracene	d_{14}-Dibenz(a,h)anthracene
Benzo(g,h,i)perylene	d_{12-}Benzo(g,h,i)perylene
Anthanthrene	d_{12-}Benzo(g,h,i)perylene
Dibenzo[a,e]pyrene	d_{12-}Benzo(g,h,i)perylene
Coronene	d_{12-}Benzo(g,h,i)perylene

Table 3:

ASSIGNMENTS OF RECOVERY STANDARDS FOR DETERMINATION OF PERCENT RECOVERY OF INTERNAL STANDARDS AND THE ALTERNATE STANDARD

Analyte	**Recovery Standard:**
Internal Standards	
d_8-Naphthalene	d_{10}-Acenaphthene
d_{10}-2-Methylnaphthalene	d_{10}-Acenaphthene
d_{12-} 2,6-Dimethylnaphthalene	d_{10}-Acenaphthene
d_8-Acenaphthylene	d_{10}-Acenaphthene
d_{10}-Phenanthrene	d_{10}-Pyrene
d_{10}-Fluoranthene	d_{10}-Pyrene
d_{12}-Benzo(a)anthracene	d_{10}-Pyrene
d_{12}-Chrysene	d_{10}-Pyrene
d_{12}-Benzo(b)fluoranthene	d_{12}-Benzo(e)pyrene
d_{12}-Benzo(k)fluoranthene	d_{12}-Benzo(e)pyrene
d_{12}-Benzo(a)pyrene	d_{12}-Benzo(e)pyrene
d_{12}-Perylene	d_{12}-Benzo(e)pyrene
d_{12}- Indeno(1,2,3,c-d)pyrene	d_{12}-Benzo(e)pyrene
d_{14}-Dibenz(a,h)anthracene	d_{12}-Benzo(e)pyrene
d_{12}-Benzo(g,h,i)perylene	d_{12}-Benzo(e)pyrene

Table 4:

CONCENTRATIONS OF PAHs IN WORKING GC/MS CALIBRATION STANDARDS FOR HI-RES MASS SPECTROMETRY CONCENTRATIONS (pg/µL)

	SOLUTIONS				
Calibration Standards:	**1**	**2**	**3**	**4**	**5**
1,2,3,4-Tetrahydronaphthalene	10	50	100	200	500
Naphthalene	10	50	100	200	500
2-Methylnaphthalene	10	50	100	200	500
1-Methylnaphthalene	10	50	100	200	500
2-Chloronaphthalene	10	50	100	200	500
1-Chloronaphthalene	10	50	100	200	500
2,6-Dimethylnaphthalene	10	50	100	200	500
Acenaphthylene	10	50	100	200	500
Acenaphthene	10	50	100	200	500
2,3,5-Trimethylnaphthalene	10	50	100	200	500
Fluorene	10	50	100	200	500
Dibenzothiophene	10	50	100	200	500
Phenanthrene	10	50	100	200	500
Anthracene	10	50	100	200	500
Fluoranthene	10	50	100	200	500
Pyrene	10	50	100	200	500
9,10-Dimethylanthracene	10	50	100	200	500
Benzo[a]fluorene	10	50	100	200	500
Benzo[b]fluorene	10	50	100	200	500
Benzo(a)anthracene	10	50	100	200	500
Chrysene	10	50	100	200	500
5-Methylchrysene	10	50	100	200	500
7,12-Dimethylbenzo[a]anthracene	10	50	100	200	500
Benzo(b)fluoranthene	10	50	100	200	500
Benzo(k)fluoranthene	10	50	100	200	500
Benzo(e)pyrene	10	50	100	200	500
Benzo(a)pyrene	10	50	100	200	500
Perylene	10	50	100	200	500
Indeno(1,2,3-cd)pyrene	10	50	100	200	500
Dibenz(a,h)anthracene	10	50	100	200	500
Benzo(g,h,i)perylene	10	50	100	200	500
Anthanthrene	10	50	100	200	500
Dibenzo[a,e]pyrene	10	50	100	200	500
Coronene	10	50	100	200	500
Surrogates Standards:					
d_8-Naphthalene	100	100	100	100	100
d_{10}-2-Methylnaphthalene	100	100	100	100	100
d_{12}-2,6-Dimethylnaphthalene	100	100	100	100	100
d_8-Acenaphthylene	100	100	100	100	100
d_{10}-Phenanthrene	100	100	100	100	100
d_{10}-Anthracene	100	100	100	100	100
d_{10}-Fluoranthene	100	100	100	100	100
d_{12}-Benzo(a)anthracene	100	100	100	100	100

d_{12}-Chrysene	100	100	100	100	100
d_{12}-Benzo(b)fluoranthene	200	200	200	200	200
d_{12}-Benzo(k)fluoranthene	200	200	200	200	200
d_{12}-Benzo(a)pyrene	200	200	200	200	200
d_{12}-Perylene	200	200	200	200	200
d_{12}- Indeno(1,2,3,c-d)pyrene	200	200	200	200	200
d_{14}-Dibenz(a,h)anthracene	200	200	200	200	200
d_{12}-Benzo(g,h,i)perylene	200	200	200	200	200

Recovery Standards:

d_{10}-Acenaphthene	100	100	100	100	100
d_{10}-Pyrene	100	100	100	100	100
d_{12}-Benzo(e)pyrene	200	200	200	200	200

Table 5

Concentration of Native PAHs in Laboratory Control Samples at 100% Recovery
Matrix spike solution

Compound:	Conc. in spike solution (ng/µL)
1,2,3,4-Tetrahydronaphthalene	5
Naphthalene	5
2-Methylnaphthalene	5
1-Methylnaphthalene	5
2-Chloronaphthalene	5
1-Chloronaphthalene	5
2,6-Dimethylnaphthalene	5
Acenaphthylene	5
Acenaphthene	5
2,3,5-Trimethylnaphthalene	5
Fluorene	5
Dibenzothiophene	5
Phenanthrene	5
Anthracene	5
Fluoranthene	5
Pyrene	5
9,10-Dimethylanthracene	5
Benzo[a]fluorene	5
Benzo[b]fluorene	5
Benzo(a)anthracene	5
Chrysene	5
5-Methylchrysene	5
7,12-Dimethylbenzo[a]anthracene	5
Benzo(b/j)fluoranthene	5
Benzo(k)fluoranthene	5
Benzo(e)pyrene	5
Benzo(a)pyrene	5
Perylene	5

Indeno(1,2,3-cd)pyrene	5
Dibenz(a,h)anthracene	5
Benzo(ghi)perylene	5
Anthranthene	5
Dibenzo[a,e]pyrene	5
Coronene	5

Table 6:

CONCENTRATIONS OF LABELED STANDARDS IN SAMPLE EXTRACTS (at 100% Recovery, pg/µL)

Surrogate Standards:	
d_8-Naphthalene	100
d_{10}-2-Methylnaphthalene	100
d_{12}-2,6-Dimethylnaphthalene	100
d_8-Acenaphthylene	100
d_{10}-Phenanthrene	100
d_{10}-Anthracene	100
d_{10}-Fluoranthene	100
d_{12}-Benzo(a)anthracene	100
d_{12}-Chrysene	100
d_{12}-Benzo(b)fluoranthene	200
d_{12}-Benzo(k)fluoranthene	200
d_{12}-Benzo(a)pyrene	200
d_{12}-Perylene	200
d_{12}- Indeno(1,2,3,c-d)pyrene	200
d_{14}-Dibenz(a,h)anthracene	200
d_{12}-Benzo(g,h,i)perylene	200
Recovery Standards:	
d_{10}-Acenaphthene	100
d_{10}-Pyrene	100
d_{12}-Benzo(e)pyrene	200

13.0 Sample Preparation in solid food products, sediments, water and using Pressurized Liquid Extraction (PLE ™)

Solid food products and Sediments – sample preparation

13.1 Before beginning the extraction, losses from the GPC (gel permeation clean up) should be considered. The GPC consumes 50% of the extract, so sample masses and spiking should be doubled or adjusted accordingly. For example, if typically 10uL of surrogate spiking solution is spiked onto 5g of solid food (eg. meat, fish), one should spike 20uL of surrogate solution onto 10g of solid food (eg. meat, fish). Proportions must be observed. A typical mass used for sediments is 10g.

13.2 Add 60g anhydrous sodium sulphate to a 5-20g portion of a homogeneous solid food (eg. meat, fish) or sediment sample, which has already been spiked with surrogate standard, and mix thoroughly with a stainless steel spatula until free flowing. After breaking up any remaining lumps, place the solid food (eg. meat, fish) or sediment/sodium sulphate mixture in the Soxhlet apparatus on top of a glass wool plug. Add 250mL DCM plus 2mL of iso-octane keeper to the Soxhlet apparatus and reflux for 16 hours. The solvent must cycle completely through the system five times per hour.

13.3 Transfer the extract to a 250mL volumetric flask and fill to the mark with DCM. Mix well, and then remove 125mL for the determination of lipid content. Transfer the remaining 125mL, plus two 15mL DCM rinses of the volumetric flask to a flat bottom flask and rotovap to an apparent volume of 5mL.

13.3 Proceed with GPC clean-up as specified in BRL SOP-00003.

13.4 Proceed with basic alumina clean-up (deactivated 1%) employing the following steps:

Step 1 Check that the Maxxam laboratory label and the original container label have identical Client Ids
Step 2 Initial the preparation/extraction sheet in the appropriate box verifying that this check has been done
Step 3 Place a small plug of glass wool in the bottom of a disposable serological pipette and add 0.5 cm layer of sodium sulphate
Step 4 Fill to 5 mL with basic alumina (deactivated 1%)
Step 5 Add another 0.5 cm layer of sodium sulphate on the top
Step 6 Attach a Teflon tube and pre-wet the column with 20 mL of dichloromethane, discarding the rinsate
Step 7 Transfer the extract to the clean-up column quantitatively by rinsing the flask 3 times with 2 mL each DCM and applying the rinsate to the column
Step 8 Elute the column with 20 mL DCM, collecting the eluent in a 35 mL pre-cleaned vial
Step 9 Evaporate the cleaned extract to <1 mL under a gentle stream of nitrogen
Step 10 Solvent exchange the extract into toluene and make up to 0.5 mL
Step 11 Add 5 µL of the recovery standard at 10 and 20 ng/µL to the extract, cap tightly, and vortex to mix.
Step 12 **Store the extracts in the dark at room temperature until GC/MS analysis.**

Sample Preparation for water.

13.5 Before beginning the extraction, losses from the GPC (gel permeation clean up) should be considered. The GPC consumes 50% of the extract, so sample masses and spiking should be doubled or adjusted accordingly. Typically, 20uL of PAH surrogate spiking solution is spiked into 1L of water of all samples.

13.6 Preparation of Liquid Samples: prior to any liquid extractions, cleaned glassware must be thoroughly rinsed as follows. Separatory funnels are first rinsed with 100-150 ml of acetone, i.e. shake for ~2 minutes and then proceed with the DCM rinses as follows:

Step 1 Rinse sense flasks 3x times with DCM
Step 2 Prepare Allihn filters by rinsing with DCM
Step 3 Fill the Allihn filter ~1/3 to 1/2 full with anhydrous Na_2SO_4
Step 4 Rinse with 50-100 ml of DCM
Step 5 Label flasks and separatory funnels with sample number

13.7 For each sample perform the following. Samples that have no visible suspended materials do not require filtration.

Step 1 Mark the position of the meniscus on the bottle to allow the initial volume determination (Note: for River Road protocol weigh the bottles)
Step 2 Use 1L of RODI water for the method blank and 1L for the blank spike. Note: prepare one of each for every batch of 14 samples, or less, depending on the client's requirements
Step 3 Pour the sample into a 2L separatory funnel
Step 4 Add 20uL of surrogate standard .
Step 5 Spike the spiked blank, blank and matrix spike samples as follows: 20uL surrogate standard (table 5) and 20uL matrix spike (table 6).
Step 6 Add 50 ml of DCM to the sample bottle and shake vigorously for ~1 minute
Step 7 Add the DCM to the separatory funnel
Step 8 Repeat this process two more times
Step 9 Shake the separatory funnel vigorously for at least 4-5 minutes, with occasional venting
Step 10 Allow the layers to stand for ~10 minutes to achieve separation
Step 11 If necessary break up any emulsions.
Step 12 Drain the DCM through a pre-rinsed Allihn filter into a 500 ml flat bottom flask
Step 13 Extract the aqueous phase twice more with further 100 ml aliquots of DCM
Step 14 Filter each through the same Allihn filter
Step 15 Rinse the Allihn filter with ~10-15 ml of DCM
Step 16 Apply vacuum to the funnel to collect the remaining DCM
Step 17 Add 3-5 ml of iso-octane and rotary evaporate the extract to ~2 ml. Note: transfer samples directly from the flask to the first clean up column if no splits are required

Measure the sample volume by filling the sample bottle with water to the mark and pour into a 1000 ml graduated cylinder. Record the volume on the tracking sheet. Rinse separatory funnels 3x with ~100-150 ml DCM, shaking for ~2 minutes each time

13.8 Proceed with GPC clean-up as specified in BRL SOP-00003.

13.9 Proceed with basic alumina clean-up (deactivated 1%) employing the following steps:

Step 13 Check that the Maxxam laboratory label and the original container label have identical Client Ids
Step 14 Initial the preparation/extraction sheet in the appropriate box verifying that this check has been done
Step 15 Place a small plug of glass wool in the bottom of a disposable serological pipette and add 0.5

cm layer of sodium sulphate

Step 16 Fill to 5 mL with basic alumina (deactivated 1%)

Step 17 Add another 0.5 cm layer of sodium sulphate on the top

Step 18 Attach a Teflon tube and pre-wet the column with 20 mL of dichloromethane, discarding the rinsate

Step 19 Transfer the extract to the clean-up column quantitatively by rinsing the flask 3 times with 2 mL each DCM and applying the rinsate to the column

Step 20 Elute the column with 20 mL DCM, collecting the eluent in a 35 mL pre-cleaned vial

Step 21 Evaporate the cleaned extract to <1 mL under a gentle stream of nitrogen

Step 22 Solvent exchange the extract into toluene and make up to 0.5 mL

Step 23 Add 5 µL of the recovery standard at 10 and 20 ng/µL to the extract, cap tightly, and vortex to mix.

Store the extracts in the dark at room temperature until GC/MS analysis

Alternate Sample Preparation using Pressurized Liquid Extraction (PLE ™).

13.10 For details on this technique of extraction see the EPA test method SW846 #3545. "Pressurized Fluid Extraction PFE",

NOTE: IT IS HIGHLY RECOMMENED THAT THE SAMPLES BE FREEZE DRIED PRIOR TO PROCESSING. The use of sodium sulphate as a dispersant and dehydrant is detrimental as it can block the extraction vessel caps and PLE lines. Hydromatrix™ is recommended. **If sodium sulphate is to be used, use no more than 2-4g.**

13.11 Remove a portion of frozen sample (at least 2-20g) from freezer and thaw to room temperature for food samples or out of the fridge for sediment samples.

13.12 After thawing, freeze-dry using the following suggested conditions: pressure 0-100ug Hg, temperature –40C for 24 hours.

13.13 Accurately weigh 2-10.0 g of freeze dried sample in a tarred 60 mL VOC vial or other appropriate clean tarred vessel.

13.14 If the percent lipids of the sample are already known, the following sample weight strategy can be performed. For samples with higher lipid content, the less sample can be used, ie. 2.0 g for fat, 5.0 g for egg and cheese; consult with a senior analyst if you are unsure. The opposite is true for samples with lower lipid content where more samples could be taken especially if lower detection limits are desired, but again consult a senior analyst or supervisor.

13.15 Prepare the QC samples accurately, weighing 2 – 10.0 g of freeze dried an appropriate clean matrix, e.g., shrimp, ground pork, tilapia and sediment, e.g., quartz sand into a tarred, or pre-weighed clean vessel.

13.16 Fortify the QC spike samples with native spiking solution.

13.17 Fortify all samples, QC blank samples and QC spike samples with Surrogate spiking solution.

13.18 Allow samples to sit for 30 minutes.

13.19 Homogenize the freeze-dried tissue with 5-10g Hydromatrix™ or until free flowing.

13.20 Place one cap on the end of the extraction vessel on pour enough Hydromatrix™ to cover the bottom.

13.21 Pour the entire homogenized sample into the vessel. If it does not fill to the top, top up with Hydromatrix™.

13.22 Place the top cap on the vessel, and the sample is now ready to be placed into the PLE™.

13.23 Place vessel in the extractor by lifting the red handle and placing the vessel in the slot. Lower the red handle and adjust the tension against the vessel with the wrench provided. There should be just enough tension to make closing the red handle slight difficult.

13.24 Make sure that the maximum pressure setting of 1700psi is set on the front of the unit. The flow rate in the PLE™ is 35mL/min.

13.25 Choose the Tissue Extraction program from the computer, which will load the following extraction program on the next page:

TISSUE EXTRACTION PROGRAM FOR PLE ™

No	Time (min)	Action	Parameter	Description
1	1.2	Pump	-	Fill Extraction cell 1
2	1.2	Pump	-	Fill Extraction cell 2
3	1.2	Pump	-	Fill Extraction cell 3
4	1.0	Pump	P=1700 psi	Pressurize
5	1.0	Heat & Pump	T=90 C P=1700 psi	Pre-heat and maintain Pressure
5	1.0	Heat & Pump	T=90 C P=1700 psi	Pre-heat and maintain Pressure
6	25	Heat & Pump	T=130 C P=1700 psi	Heat and maintain Pressure
7	12	Pump	T=30 C	Cool
8	0.1	-	T=30 C P= atm	Depressurize
9	1.2	Pump	-	Flush Extraction cell 1
10	1.2	Pump	-	Flush Extraction cell 2
11	1.2	Pump	-	Flush Extraction cell 3
12	1.0	Heat & Pump	T=90 C P=1700 psi	Pre-heat and maintain Pressure
13	15	Heat & Pump	T=130C P=1700 psi	Heat and maintain Pressure
14	12	Pump	T=30 C	Cool
15	0.1	-	T=30 C P= atm	Depressurize
16	1.2	Pump	-	Flush Extraction cell 1
17	1.2	Pump	-	Flush Extraction cell 2
18	1.2	Pump	-	Flush Extraction cell 3

13.26 On the PLE computer select “single” and run step 1 above only. It may take less than 1.2 minute to fill the vessel with DCM. Observe this time adjust the times in steps 2 and 3. The reason for this is not to pre-flush the sample before the extraction can occur.

13.27 Highlight step 2 and click on "offset" in order to start the rest of the program starting on step 2.

13.28 Collect the extracts and set aside

Store the extracts in the dark at room temperature until GC/MS analysis.

13.29 Secure the extraction lines to a large jar and precede with the following Line Flush program:

LINE FLUSH PROGRAM FOR PLE ™

1	1.0	Purge	P=30 psi	Purge Nitrogen
2	1.0			Purge off

13.30 The line flush program is not performed on the sample extracts, as it is very vigorous and could purge some of the more volatile compounds away.

13.31 Now that the lines are cleared remove the sample vessels and install the surrogate sample vessels into the PLE in the same way as 13.23

13.32 Adjust the maximum pressure to 2400psi and open the "Wash PLE" program on the computer. This program cleans the inner lines of the extractor unit. Please see the following table:

WASH PLE PROGRAM FOR PLE ™

No	Time (min)	Action	Parameter	Description
1	2.0	Pump	-	Fill Extraction cell 1
2	2.0	Pump	-	Fill Extraction cell 2
3	2.0	Pump	-	Fill Extraction cell 3
4	1.0	Pump	P=2400 psi	Pressurize cell 1
5	1.0	Pump	P=2400 psi	Pressurize cell 2
6	1.0	Pump	P=2400 psi	Pressurize cell 3
7	0.1	-	T=30 C P= atm	Depressurize
8	1.0	Purge	P=30 psi	Purge Nitrogen
9	1.0			Purge off

13.33 If there are more extractions, repeat sections 13.20 through 13.32.

Automated Sample Clean-up using POWERPREP™.

13.34 A suggested clean-up scheme is listed below but is under development. The following is potentially designed to separate PAHs from commonly associated aliphatic interferences.

Step	Flow Rate, l/min	Volume, ml	Solvent	Operation
1.	10	100	DCM	Column Cond.
2.	10	75	Pentane	Column Cond.
3.	10	15	Pentane	Aliph. Fract. 1a
4.	10	10	Pentane	Aliph. Fract. 1b
5.	10	10	Pentane	Aliph. Fract. 1c
6	10	12	DCM	Solvent Exchange
7	10	25	DCM	Arom Fract. 2a
8	10	25	DCM	Arom Fract. 2b
9	10	25	DCM	Arom Fract. 2c
10	10	25	DCM	Arom Fract. 2d
11	10	50	DCM	Arom Fract. 2e
12	10	70	DCM/MeOH	Arom Fract. 2f

14.0 Analytical Determination (Procedure)

See section 11.2 for instrumentation set-up, plus the following:

14.1 Daily Start up Procedure:

Step 1 Check that the lab IDs on the extract vials are in the same order as the lab IDs listed on the run sequence

Step 2 Initial the sequence log confirming that this check has been done

Step 3 Check vacuum readings and source temperature

Step 4 Check helium supply in the gas tank at the beginning or the end of the day

Step 5 Reduce the GC temperature to 50 °C when changing the helium cylinder

Step 6 Turn off the valve control before turning off the tank and removing the regulator. This avoids getting any air in the line. Apply the same procedure for changing the air cylinder

Step 7 Use PFK to tune the instrument (min. of 8,000 resolution), check resolution and peak shape for each descriptor

Step 8 Check the peak heights for air and water; they should be much smaller than the cal gas peaks.

Step 9 Tune on 130.992 and hard copy resolution to demonstrate 8,000 resolution

Step 10 Check the column performance i.e. separation between two pairs of isomers. The following performance must be demonstrated daily:

The height of the valley between Anthracene and Phenanthrene (m/z 178)
Must be <50% of the taller of the two peaks

The height of the valley between Benzo(b)fluoranthene and Benzo(k)fluoranthene
must be < 60% of the taller of the two peaks

During initial calibration demonstrate that the instrument will achieve a minimum S/N ratio of 10:1 for all monitored ions when the lowest concentration calibration standard is injected into the GC/MS system

14.2 **Calibration:** A 5-point (10, 50, 100, 200, 500 pg/µL) calibration using the calibration standards as listed in Table 4. All data analysis is carried out using the equations listed below. The initial calibration must meet the following criteria in order to meet the method performance requirements:

14.2.1 The percent relative standard deviation (**%RSD**) for the mean relative response factors must be <30% for both the unlabelled analytes and the internal standards.

The signal to noise ratio (S/N) must be >10:1 for each ion current profile for both the unlabelled analytes and the internal standards.

14.2.2 Corrective Action: if these criteria are not met, corrective action is taken and initial calibration is repeated. Analysis of samples may not proceed until a valid calibration is established. Corrective action may indicate that an instrument tune and/or column or GC inlet maintenance is required.

14.3 **Continuing Calibration**: at the beginning of an analytical sequence of up to 12 hours, a continuing calibration check must be analyzed (if the sequence does not include a new 5-point Initial Calibration). This consists of the injection of an aliquot of the mid-point standard used in the initial calibration (see Section 8.2). The criteria for acceptance are as follows:

Measured Relative Response Factors (RRFs) of all analytes must give %RSD of ±30% of those measured in the initial calibration. If this is not met then a 5-point calibration must be run

Retention time for any internal std. must not change by more than 30 sec. from the most recent calibration

14.4 Run Format (sequence) for sample analysis:
ICAL = Initial Calibration
CCAL = Continuing Calibration

Sequence #	Sample Type		Sequence # (continued)	Sample Type
1-5	CS1-5 Calibration Standards ICAL or CS-3 CCAL		12	Sample 1 6
6	Solvent Blank (Check for carry-over)		13	Sample 1 Duplicate (if requested and sufficient sample supplied)
7	PAH Initial Calibration Verification Standard (section 6.12.2.1)		14	Sample 1 Matrix Spike (if requested and sufficient sample supplied)
8	Blank Spike #1		15	Solvent Blank (only if Matrix Spike was preceding injection)
9	Blank Spike #2		16	Sample 2

10	Solvent Blank		17	Sample n (such that this run is complete within 12 hours of the beginning of the sequence)
11	Method Blank		18	Blank
	7		19	CS3 Calibration Standard (post-run Calibration Check)

15.0 Quality Control Requirements

Summary Table of QC and Corrective Actions

QC Requirement	Frequency	Acceptable Limits	Corrective Action for Data Outside Acceptable Limits
*Method Blank	1 / batch of 20 or fewer samples	<MDL, where possible.	1. investigate possible source of contamination by checking at a minimum: instrument Spike Standard Solutions, Recovery Standards, proofing of glassware, proofing of solvent and absorbance used in clean-up 2. If positive blanks are detected at levels > 5% of sample values, the impacted samples should be repeated **Do not blank subtract.**
Matrix Spike	1 / batch of 20 or fewer samples	50-150% %RPD for MSD < 50%	1. Re-extract and rerun 2. If failure is due to matrix effect, report sample with a comment in LIMS
Laboratory Control Sample (LCS) (Blank Spike)	1 / batch of 20 or fewer samples	50-150% for air samples. See attached table for acceptable limits.	1. Re-extract and rerun
Laboratory Duplicate	1 / batch of 20 or fewer samples	50% RPD (If both values > 5X RDL)	1. Re-inject both duplicates and reanalyze. If fails again, repeat sample. 2. If failure is due to sample inhomogeneity, report sample with a comment in LIMS.
One point calibration check mid level	Beginning of every run sequence and after every twelve continuous hours of analysis	% drift must be within 15% of original calibration.	1. reanalysis of the standard 2. preparation of new standard or 3. instrument maintenance, if necessary
Internal Standard	All samples	Area within 20% of average of IS areas of initial calibration standards	1. reanalysis of the sample 2. preparation of new standard or 3. instrument maintenance, if necessary

Instrument Performance Check std (mid level CCV)	Every run sequence or 24-hour period	Absolute area count recorded. Must be within 70% - 130% of the long-term average.	1. reanalysis of the standard 3. preparation of new standard or 4. instrument maintenance, if necessary
Surrogate Recoveries	All extracted samples	50-150% for air samples. See attached table for acceptable ranges.	Re-extract or give valid explanation of qualified results.

*NOTE: for DoD samples, the method blank will be considered to be contaminated if:

- The concentration of any target analyte in the blank exceeds 1/2 the reporting limit and is greater than 1/10 the amount measured in any sample or 1/10 the regulatory limit (whichever is greater);
- The concentration of any common laboratory contaminant in the blank exceeds the reporting limit and is greater than 1/10 the amount measured in any sample or 1/10 the regulatory limit (whichever is greater); or

The blank result otherwise affects the samples results as per the test method requirements or the project-specific objectives

15.1 **Control Charting:** Refer to CAM WI-00001 Control Charting procedure for generating control charts via MaxLIMS and real time (i.e., plotted manually, each time the QC sample and/or standard is analyzed). Plot the blank water matrix spike data for Benzo(a)pyrene on control chart. Update the chart immediately after the analysis of the QC point used or in the morning after an overnight run, prior to reporting of data. Monitor the chart for the development of trends. Take actions, if necessary, as defined in the SOP for Control Charting (CAM WI-00001). If an "Out of Control" point is found, terminate the analysis and resolve the problem. Note the resolution directly on the chart or control chart checklist.

15.1.1 Possible Causes of data going out of control:

- deterioration of standard or reagents
- standard, QC sample or reagent preparation error
- standard contamination
- improper sample introduction
- poor analyst technique or insufficient training
- deviation from SOP procedure
- imprecise measuring devices, i.e. pipettors, syringes

15.1.2 Corrective Actions: if "out of control" points are encountered:

- terminate the analysis, find and resolve the problem
- note the resolution directly on the chart or code the point to a reference on an "Out of Control Checklist"
- if the Blank Spike/LCS acceptance criteria is met, report the data
- if the criteria is not met, re-analyse the samples in the affected batch, re-extraction may be necessary
- if insufficient sample exists for re-extraction, flag the data in MaxLIMS comments
- if hold times will be exceeded upon re-extraction and reanalysis, flag the data in MaxLIMS comments

15.2 **Additional QC:** it may be necessary on a project by project basis to perform additional QC. This will typically be in the form of replicate injections, and possibly matrix spike and matrix spike duplicates into a portion of an extract. The exact requirements of the QC should be discussed with the MAXXAM project manager to ensure that project contractual requirements are met

16.0 Data Interpretation, Calculations and Data Reporting

16.1 Calculations

All of the calculations below are done using QuanLynx software, which is a subprogram of MassLynx version 4 Software.
The equations and definitions for all calculation done are highlighted below:

16.1.1 Relative Response Factors (RRF) for Unlabelled PAH and Surrogate Standards:

$$RRFs = \frac{As * Qis}{Ais * Qs}$$

Where:

As = Area of the response for characteristic ion of the unlabelled analyte or the Surrogate Std.
Ais = Area of the response for the characteristic ions of the appropriate Internal Std (see table 4)
Qs = Amount of the unlabelled PAH calibration analyte or Surrogate Std, injected onto the GC/MS.
Qis = Amount of the appropriate Internal standard injected onto the GC/MS (see Table 3).

16.1.2 RRF for the Determination of the Internal Standard/Alternate Standard Recovery (RRFis):

$$RRFis = \frac{Ais * Qrs}{Ars * Qis}$$

Where:
Ais = Area of the response for the characteristic ions of the appropriate Internal/Alternate std. (see table 4).
Ars = Area of the response of the characteristic ions of the appropriate Recovery Std(see table 5).
Qis = Amount of the appropriate Internal/Alternate standard injected onto the GC/MS.
Qrs = Amount of the appropriate Recovery Standard injected onto the GC/MS.

16.1.3 Mean Relative Response Factors (RRF_m):

$$RRF_m = \frac{1 * \sum_{i=1}^{n} (RRF_i)}{n}$$

Where:
RRFi = Relative response factor as calculated above.
n = Number of data points derived from the calibration.

16.1.4 Percent Relative Standard Deviation (%RSD) of Relative Response Factors:

$$\%RSD = \frac{SD}{RRF_m} \times 100\%$$

Where:
RRF_m = Mean Relative response factor as calculated above.
SD = Standard deviation of the relative response factors used to calculate the mean RRF

16.1.5 Continuing Calibration (ΔRRF):

$$\Delta RRF = \frac{RRF_c - RRF_m}{RRF_m} \times 100\%$$

Where:
RRF_m = Mean Relative response factor of a given analyte as calculated above.
RRF_c = RRF of a given analyte obtained from the continuing calibration

16.1.6 Percent Recovery of the Internal Standard (Ris):

$$Ris = \frac{Ais * Qrs}{Ars * RRFis * Qis} \times 100\%$$

Where:
Ais = Area of the response for the characteristic ions of the appropriate Internal Std.
Ars = Area of the response of the characteristic ions of the appropriate Recovery Std.
Qis = Amount of the appropriate Internal standard injected onto the GC/MS.
Qrs = Amount of the appropriate Recovery Standard injected onto the GC/MS.
RRFis = Mean relative response factor as calculated above.

16.1.7 Percent Recovery of the Surrogate Standard (Rss):

$$Rss = \frac{Ass * Qis}{Ais * RRFs * Qss} \times 100\%$$

Where:
Ais = Area of the response for the characteristic ions of the appropriate Internal Std.
Ass = Area of the response of the characteristic ions of the appropriate surrogate Std.
Qis = Amount of the appropriate Internal standard injected onto the GC/MS.
Qss = Amount of the appropriate surrogate Standard injected onto the GC/MS.
RRFs = Mean relative response factor as calculated above.

16.2 Data Validation: Refer to BRL WI-00011 Review and Validation of Analytical Data and corresponding Data Review Checklist (BRL FCD-00002) to perform a primary and a secondary analytical data review and validation prior to issuance of the Certificate of Analysis. If the data and report are acceptable the Certificate of Analysis is signed by the Project Manager and sent to the client.

16.3 Reporting (Documentation):

16.3.1. **Tracking Worksheets:** prior to sample preparation, a sample tracking / work sheet is prepared. This sheet includes details of the job number, lab sample number, client sample ID, QC samples analyzed in the batch, spiking solutions used and their concentrations, preparation date and lot #, dates of analysis and cleanup, analysts initials. A copy of the tracking sheet is maintained with analytical data.

16.3.2. **Method Deviation Records:** instances may arise in which the standard operating procedure may not be applicable to the nature of the sample and may require modifications to the normal methodology. These deviations must be discussed with the Laboratory Manager or Project Manager prior to application. If deviation is deemed appropriate, record the procedure in the QSI Policy Deviation Form and submit to the Supervisor or Operations Manager for approval (refer to COR WI-00040, Corporate Procedure for Policy Deviation Forms).

16.3.3. **Sample Deviation Records**: deviations are recorded in the MaxxLIMS job comment fields The entries listed, will appear on the Certificate of Analysis as part of the final report sent to the client. Some examples are as follows:

- analyzed past hold time
- matrix interference suspected
- sample is not homogeneous
- sample concentration too high to determine spike recovery
- diphenylether interference
- NDR—Peak does not meet ratio criteria

16.3.4. **Standard and Reagent Preparation Tracking:** record all preparations of standards and reagents in the Standard Preparation and the Reagent Preparation Logbooks. All records must contain at a minimum the following information

- Supplier Identification and Lot Number
- Date of Preparation and Expiry
- Concentration and name of Analyst who prepared the standard

16.3.5 Certificates of Analysis for Standards and Reagents: certificate of analyses are kept for all standards and reagents utilized in this SOP. These records will contain at a minimum the following information

- supplier information and Lot Number
- date received

16.3.6 Storage of Records:

16.3.6.1 Paper Records:

- raw and calculated data, including calibrations and QC results are stored chronologically in folders in the laboratory as space permits. These files are archived offsite for long term storage (5-year retention time, unless otherwise stated in the client's SOW)

16.3.6.2 Electronic Media

- data entered into LIMS is backed up daily and stored off site

17.0 Maintenance Requirements

Included all routine and non-routine requirements as per manufacturer's manual

17.1 Maintenance Tracking: analysts maintain separate logbooks or specific tracking sheets in which they include the following minimum information

17.1.1 Records of Instrument Service by the Supplier with the associated paperwork

17.1.2 Records of all maintenance performed indicating the date performed and by who

18.0 Attachments

- Example PAH curve and relevant chromatograms of samples etc., plus the instrument acquisition method.
- Table – % Recovery Limits based on US EPA 1625 and US EPA 8270

Table – % Recovery Limits based on US EPA 1625 and US EPA 8270

	USEPA 1625 Limits
Analyte	**%**
Naphthalene	**80-139**
2-Methylnaphthalene	**80-139**
Acenaphthylene	**69-186**
Acenaphthene	**79-134**
Fluorene	**81-132**
Phenanthrene	**93-119**
Anthracene	**58-174**
Fluoranthene	**71-177**
Pyrene	**76-152**
Benzo(a)anthracene	**65-168**
Chrysene	**59-186**
Benzo(b/j)fluoranthene	**32-545**
Benzo(k)fluoranthene	**59-143**
Benzo(e)pyrene	**62-195**
Benzo(a)pyrene	**62-195**
Perylene	**62-195**
Indeno(1,2,3-cd)pyrene	**23-299**
Dibenz(a,h)anthracene	**23-299**
Benzo(g,h,i)perylene	**72-160**
1-Methylnaphthalene	**80-139**
1-Methylphenanthrene	**93-119**
2,6-Dimethylnaphthalene	**80-139**
2,3,5-Trimethylnaphthalene	**80-139**
Dibenzothiophene	**79-150**
SURROGATE	**USEPA 1625 Limits**
2,6-Dimethylnaphthalene-2H12	**14-305**
2-Methylnaphthalene-2H10	**14-305**
Acenaphthylene-2H8	**23-239**
Benz(a)anthracene-2H12	**12-605**
Benzo(a)pyrene-2H12	**21-290**
Benzo(b)fluoranthene-2H12	**24-159***
Benzo(g,h,i)perylene-2H12	**14-529**
Benzo(k)fluoranthene-2H12	**11-162**
Chrysene-2H12	**13-512**
Dibenzo(a,h)anthracene-2H14	**D-227***
Fluoranthene-2H10	**20-278**
Indeno(1,2,3-c,d)pyrene-2H12	**D-171***
Naphthalene-2H8	**14-305**
Perylene-2H12	**21-290**
Phenanthrene-2H10	**24-241**

*** from Method USEPA 8270**