# Analysis of Parent and Alkylated Polynuclear Aromatic Hydrocarbons, Selected Heterocyclic Compounds, Steranes, Triterpanes and Triaromatic Steroids by GC / MS – SIM

References: Federal Register 2003, 40 CFR, Chapter I – EPA. Part 300: National Oil and Hazardous Substances Pollution Contingency Plan, Appendix C to Part 300, Chemical Analysis of Oil Composition, May 28, 2003.

USEPA, Method 8270D Semivolatile Organic Compounds by Gas Chromatography / Mass Spectrometry (GC/MS)" in Test Methods for Evaluating Solid Waste, SW846, Third Edition (USEPA Office of Solid Waste and Emergency Response, Washington, DC, September 1994).

ASTM, Method D7363 - 07 Standard Test Method for Determination of Parent and Alkyl Polycyclic Aromatics in Sediment Pore Water Using Solid-Phase Microextraction and Gas Chromatography/Mass Spectrometry in Selected Ion Monitoring Mode.

## 1. Scope and Application

**Matrices:** Extracts from water, soil, sediment, tissue, sludges and petroleum products.

**Definitions:** Refer to Alpha Analytical Quality Manual.

This method is applicable to the analysis of sample extracts for parent and alkylated polynuclear aromatic hydrocarbons (PAHs), selected heterocyclic compounds, steranes, triterpanes and triaromatic steroids by gas chromatography / mass spectrometry with selected ion monitoring (GC/MS-SIM). Target analytes listed in Tables IA and IB are determined and measured in the concentration range of 10 to 20,000 parts per trillion (ng/L) for water samples, 1 to 2,000 parts per billion (ug/Kg) for soil and sediment samples, 2 to 4,000 parts per billion (ug/Kg) for tissue samples, and 2 to 4,000 parts per million (mg/Kg) in petroleum product samples. Analytes detected over these ranges will be diluted and re-analyzed for accurate quantitation. Lower detection limits can be achieved if large volume injection (LVI, from 1uL to 50uL volume injections) techniques are employed. This technique requires client and project specific requests.

This method is intended to assist in the identification or "fingerprinting" of source material against a potentially contaminated site and can also provide information to assist in the identification of petrogenic or pyrogenic contamination.

The data report packages present the documentation of any method modification related to the samples tested. Depending upon the nature of the modification and the extent of intended use, the laboratory may be required to demonstrate that the modifications will produce equivalent results for the matrix. Approval of all method modifications is required by one or more of the following laboratory personnel before performing the modification: Area Supervisor, Department Supervisor, Laboratory Director, or Quality Assurance Officer.

This method is restricted to use by or under the supervision of analysts experienced in the operation of the GC/MS and in the interpretation of GC/MS data. Each analyst must demonstrate the ability to generate acceptable results with this method by performing an initial demonstration of capability, analyzing a proficiency test sample and completing the record of training.

After initial demonstration, ongoing demonstration is based on acceptable laboratory performance of at least a quarterly laboratory control sample or acceptable performance from an annual proficiency test sample. A major modification to this procedure requires demonstration of

performance. The identification of major method modification requiring performance demonstration is directed by the Quality Assurance Officer and/or Laboratory Director on a case-by-case basis.

# 2. Summary of Method

An aliquot of a well mixed, homogeneous aqueous, solid, tissue or petroleum sample is accurately measured or weighed for sample preparation (Generally, 1L of water sample, 15-30g of soil, sediment or tissue sample, and 0.1g of petroleum sample). Please refer to the appropriate Alpha Analytical SOPs for extraction methods and sample preparation information:

- Method 3510C – *Extraction of Water Samples by Separatory Funnel* (OP-001),
- *Tissue Preparation and Homogenization* (OP-003) and *Tissue Extraction* (OP-018)
- *Shaker Table Extraction* (OP-013)
- *Organic Waste Dilution* (OP-021)

Water, soil/sediment, tissue and petroleum samples are spiked with surrogate compounds and extracted using methylene chloride. Sample extracts are concentrated and preliminarily screened for oil content following Alpha Analytical SOP *Gravimetric Determination* (OP-017). Gravimetric screening is essential at times to ensure the analytical equipment, as well as the cleanup columns, are not overloaded with oil laden samples. Samples may be cleaned by *Alumina Column Cleanup* (OP-009), or they may then be exchanged into hexane for optional cleanup and/or fractionation into saturated (F1) and aromatic (F2) fractions prior to analysis. See the SOP *Silica Fractionation and Cleanup* (NF02-001) for additional sample cleanup information and details. After cleanup, the extracts are concentrated to an appropriate final volume based on oil content as determined by gravimetric weighing and spiked with internal standards for GC/MS-SIM analysis.

Analytes are introduced into the GC/MS by injecting a known volume of the calibration standards, quality control samples, and sample extracts into the GC equipped with a narrow-bore capillary column. The GC column is temperature programmed to separate the analytes, which are then detected with a mass spectrometer operating in the selective ion mode. Identification of target analytes is accomplished by comparing retention times and mass spectra with the retention times and electron impact spectra of the calibration standards. Concentrations are determined using mean relative response factors from a multi-level calibration curve. Response factors for target analytes and surrogate compounds are determined relative to the internal standards. Multi-component analytes (alkylated PAHs) are assigned the response factors of their unsubstituted, parent compounds. Sterane and Steroid compounds are assigned the response factor of the compound 5B(H)-Cholane. Triterpane compounds are assigned the response factor of the compound 17A(H), 21B(H)-Hopane.

## 2.1 Method Modifications from Reference

The continuing calibration verification %D for each calibrated PAH must be below 25%, with no more than 10% of all compounds greater than 25% but less than 35%. Each CCV must be analyzed within 24 hours of the previous CCV.

The surrogate recovery limits are 50%-130%.

The duplicate RPD limit is 30%.

The PFTBA tuning is done once before each initial calibration.

The internal standard compounds used for this method are Acenaphthene-d10 and Chrysene-d12.

# 3. Reporting Limits

Reporting limits for individual alkylated PAH and biomarker compounds are 2 mg/Kg (ppm) for petroleum products, 0.667and 1.0 µg/Kg (ppb) for soil/sediment samples per project requirments, 2.67 µg/Kg (ppb) for tissue samples, and 10ng/L (ppt) for water samples.

# 4. Interferences

Contaminants in solvents, reagents, glassware, and other sample processing hardware may cause inferences that lead to discrete artifacts and/or elevated baselines in the ion current profiles. Demonstrate that all of these materials are free from interferences under the conditions of the preparation and analysis by extracting and analyzing a laboratory method blank with each batch of up to 20 samples.

Contaminants coextracted from the sample may cause matrix interferences. The extent of matrix interferences will vary considerably from sample to sample, depending upon the nature of the environment being investigated. An interference which is unique to SIM techniques can arise from the presence of a coeluting compound which contains the quantification mass ion. This event results in a positive interference to the reported value for the compound of interest. This interference is controlled to some degree by acquiring data for a confirmation ion. If the ion ratios between the quantification ion and the confirmation ion are not within the specified limits, then interferences may be present. Quantification and confirmation ions should agree within +/- 20% of the calibration standard ion ratios. However, the stability of confirmatory/primary ion abundance ratios may decrease as the IDL is approached. Analysts must apply judgment in evaluating apparent interferences.

The presence of a large amount of a single alkyl homolog group without the presence of the other related groups may be indicative of an interference. For example, the presence of an apparent $C_2$ - Naphthalenes in the absence of $C_1$ - Naphthalenes or $C_3$ - Naphthalenes may be an analytical interference. The analyst should use the spectrum ions and pattern recognition when determining whether to select a homolog group.

# 5. Health and Safety

The toxicity or carcinogenicity of each reagent and standard used in this method is not fully established; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. A reference file of material safety data sheets is available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available in the Chemical Hygiene Plan.

All personnel handling environmental samples known to contain or to have been in contact with municipal waste must follow safety practices for handling known disease causative agents.

All relevant Material Safety Data Sheets (MSDSs) are kept alphabetically in the centrally located file storage.

# 6. Sample Collection, Preservation, Shipping and Handling

## 6.1 Sample Collection

Aqueous samples are collected in 1L amber glass bottles.

Soil/sediment and tissue samples are collected in glass soil jars. For soil/sediment matrices the amount of sample needed is 5-30 grams, for tissues 5-15grams, and for petroleum products the amount of sample needed is 0.1 grams.

Additional sample is needed (approximately 3X the minimum amount) if MS/MSD analyses are to be performed.

## 6.2 Sample Preservation

Aqueous samples are stored without preservative at 4°C. Soil/sediment and tissue samples are stored at 4°C, or if desired, frozen.

## 6.3 Sample Shipping

Refer to the Sample Receipt and Login SOP/01-01 for sample shipping requirements.

## 6.4 Sample Handling

The hold time for this method is 7 days for the extraction of aqueous samples and 14 days for the extraction of soil/sediment and tissue samples. There is no extraction holding time applied to petroleum product samples. If sediment or tissue samples are frozen, this suspends the holding time until removal from the freezer. All extracts must be analyzed within 40 days of the extraction date.

# 7. Equipment and Supplies

**7.1 Gas Chromatograph:** Model Agilent/HP6890 or equivalent. The instrumentation includes a temperature-programmable gas chromatograph and all required accessories including syringes, analytical columns, and gases. The injection port is designed for split or splitless injection onto a capillary column. The injection port includes a Phenomenex drilled uniliner with a hole on the top and contains a small plug of silanized glass wool. The injector port will require maintenance on an as needed basis if degradation or contamination is apparent.

**7.2 Column:** Restek or Phenomenex 60-m x 0.25 mm ID, 0.25 um film thickness, fused-silica capillary column with RTX-5 or ZB-5 bonded phase, or equivalent.

**7.3 Mass Spectrometer** – Agilent/HP5973, or equivalent. The mass spectrometer must operate at 70ev (nominal) electron energy in the electron impact ionization mode and be tuned to optimize the sensitivity of the instrument to the mass range being monitored (30 - 550 amu). The GC capillary column is fed directly into the ion source of the mass spectrometer. The source will require cleaning and/or filament replacement on an as needed basis. Please refer to the instrument hardware manual for detailed procedures, located in the laboratory next to the instrument.

**7.4 Auto sampler:** Agilent/HP 7683 series autosampler and tray, or equivalent.

**7.5 Computer:** with Windows NT version 4.0 operating software utilizing Agilent/HP Enviroquant G1701BA Version B.01.00 software, or equivalent/higher versions.

**7.6 Helium:** Ultra high purity grade (99.9999% pure).

# 8. Reagents and Standards

Use reagent grade chemicals for all reagents. Deionized (DI) water is ASTM Type II laboratory reagent grade water.

All stock standard solutions are purchased from commercial vendors as ampulated certified solutions or prepared in house from neat materials. Standards are stored according to the vendor recommendations. When an ampulated stock solution is opened, or prepared fresh from neat materials, it must be transferred into a PTFE-lined screw capped vial. All working standard solutions are stored at -10°C or less, away from light, when not in use. They are discarded after 1 year unless the vendor expiration date states otherwise or if breakdown is observed.

**8.1 Methylene Chloride,** ACS approved, Pesticide grade, see Alpha Analytical SOP Reagent, Solvent and Standard Control (G-008) for additional details regarding solvent purity.

**8.2 Hexane,** ACS approved, Pesticide grade, see Alpha Analytical SOP Reagent, Solvent and Standard Control (G-008) for additional details regarding solvent purity.

**8.3 Acetone**, ACS approved, Pesticide grade, see Alpha Analytical SOP Reagent, Solvent and Standard Control (G-008) for additional details regarding solvent purity.

**8.4 Methanol,** Purge and Trap grade, see Alpha Analytical SOP Reagent, Solvent and Standard Control (G-008) for additional details regarding solvent purity.

**8.5 Custom Mix Calibration Standard** prepared by Supelco which contains the parent PAH and Heterocyclic compounds at 1000ug/mL.

**8.6 5B(H)-Cholane (CAS# 80373-86-0) and 17A(H), 21B(H)-Hopane (CAS# 13849-96-2):** Obtained from Chiron AS Norway at 1000ug/mL. These compounds are used for calibration and quantitation of all steranes, triterpanes and triaromatic steroids, and 5B(H)-Cholane is also used as a surrogate compound.

## 8.7 Surrogates:

**8.7.1 Alternate Surrogates:** This surrogate solution had been in use from the inception of the Forensic Department, and this analytical method, until January 2011. This solution may still be used for some programs. 2-Methylnaphthalene-d10, Pyrene-d10, and Benzo(b)fluoranthene-d12 from Restek, custom mix in Methylene Chloride with a concentration of 2000ug/mL. Take 500uL of stock solution into 100mL for a low level spiking solution at 10ug/mL. Take 1250uL of stock solution into 25mL for a high level spiking solution at 100ug/mL. These solutions must be assayed for use by analysis before release to the preparation lab. All compounds must be within 10% of their true value. A manager's approval is needed for all surrogate spiking solutions prior to use. 100uL of low solution or 200uL of high solution is spiked into each QC and field sample. This amount may be adjusted to meet project specific concentrations, as needed.

**8.7.2 Surrogates:** Naphthalene-d8, Phenanthrene-d10, and Benzo(a)pyrene-d12 from AccuStandard custom mix in Methylene Chloride with a concentration of 2000ug/mL. Take 500uL of stock solution into 100mL for a low level spiking solution at 10ug/mL. Take 1250uL of stock solution into 25mL for a high level spiking solution at 100ug/mL. These solutions must be assayed for use by analysis before release to the preparation lab. All compounds must be within 10% of their true value. A manager's approval is needed for all surrogate spiking solutions prior to use. 100uL of low solution or 200uL of high solution is spiked into each QC and field sample. This amount may be adjusted to meet project specific concentrations, as needed.

**8.7.3 Biomarker surrogate:** 5B(H)-Cholane from Chiron AS Norway solution at 1000ug/mL in iso-octane. Take 1,000uL of stock solution into 100mL for a low level spiking solution at 10ug/mL. Take 1000uL of stock solution into 10mL for a high level spiking solution at 100ug/mL. These solutions must be assayed for use by analysis before release to the preparation lab. All compounds must be within 10% of their true value. A manager's approval is needed for all surrogate spiking solutions prior to use. 100uL of low solution or high solution is spiked into each QC and field sample. This amount may be adjusted to meet project specific concentrations, as needed.

**8.8 Internal Standards (IS):** Acenaphthene-d10 and Chrysene-d12 from Restek, custom mix in Methylene Chloride with a concentration of 2000ug/mL. Prepare a 250ug/mL intermediate solution by spiking 3125uL into 25mL Methylene Chloride. Then prepare a working solution by adding 2000uL to 100mL for a 5ug/mL solution. 100uL is spiked into each 1mL of QC sample or field sample, for a concentration of 500ng/uL on column.

**8.9 Laboratory Control Spike and Matrix Spike (LCS/LCSD/MS/MSD):** A solution of 17 priority pollutant parent PAH's from Restek, or equivalent, at 1000ug/mL. This solution is from a separate source than the calibration solutions. Prepare the spike mix by adding 250uL of the solution to 25mL of Methylene Chloride for a 10ug/mL LCS/MS/MSD spiking solution. The solution must be assayed for use by analysis before release to the preparation lab. All compounds must be within 20% of their true value. 100uL is spiked into the LCS and each designated MS/MSD field sample. This amount may be adjusted to meet project specific concentrations, as needed.

**8.10 Alaska North Slope Crude Oil (ANS):** Weigh approximately 0.5g neat oil, add 10mL each PAH and biomarker surrogates, and 200uL internal standard stock solution into 100mL of Methylene Chloride for a working solution of approximately 5mg/mL (with surrogates at 1.0µg/mL and internals at 0.5µg/mL).

**8.11 Independent Calibration Verification (ICV):** Prepared as below in Section 8.13 at 0.5ug/mL, but from a different source, lot, or vendor. Independent Check analyses must agree within 20% of their true value.

**8.12 SRM 1941b, 194 – PAH's in sediment, SRM 1974a – PAH's in Tissue and SRM 1582 PAHs' in Crude Oil:** From the National Institute of Standards & Technology (NIST). Please refer to the individual certifications for the assigned true values. These SRMs may be extracted and analyzed with sample batches as part of the overall QC evaluation if requested by the client. Other certified SRMs may be used on a project specific basis.

**8.13 Working Stock Standard:** Prepare the Working Stock Standard (all resulting concentrations at 5µg/mL) in 50mL of Methylene Chloride ($CH_3Cl_2$) as follows. (**Note:** The following is just one way an analyst may make up calibration standards. Limitations may exist that would cause the method to be adjusted. Problems with standard availability, solubility, or expiration may affect how the following 6 level calibration standards are prepared.)

| Component | Volume Added | Final Volume in ($CH_3Cl_2$) |
|---|---|---|
| Custom Supelco Mix (PAH) | 250 µL of 1000 µg/mL | 50 mL |
| 5B(H)-Cholane | 250 µL of 1000 µg/mL | 50 mL |
| Custom Dueterated Surr Mix | 125 µL of 2000 µg/mL | 50 mL |

7 Level Curve Preparation for Individual Components

| Calibration Level | Volume of Working Std. Added (5 ug/mL) | Volume of IS Stock added | Final Volume in ($CH_3Cl_2$) |
|---|---|---|---|
| Level 1 - 10 ng/mL | 200 µL | 200 uL | 100 mL |
| Level 2 - 25 ng/mL | 500 µL | 200 uL | 100 mL |
| Level 3 - 100 ng/mL | 2000 µL | 200 uL | 100 mL |

| Calibration Level | Volume of Custom PAH mix Std. Added (1000 ug/mL) | Volume of IS Stock added | Final Volume in ($CH_3Cl_2$) |
|---|---|---|---|
| Level 4 – 500 ng/mL | 25 µL | 100 uL | 50 mL |
| Level 5 - 5,000 ng/mL | 125 uL | 50 uL | 25 mL |
| Level 6 - 10,000 ng/mL | 250 uL | 50 uL | 25 mL |
| Level 7 - 20,000 ng/mL | 500 uL | 50 uL | 25 mL |

**Note**: A minimum of a 5-level curve must be analyzed, but more levels may be analyzed and evaluated.

# 9. Quality Control

The laboratory must maintain records to document the quality of data that is generated. Ongoing data quality checks are compared with established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

Quality Control (QC) samples are necessary to monitor both the sample extraction and instrument analysis procedures. The Quality Control samples described below are considered the method defaults, and are the minimum requirements, except were noted. Client and Project specific Data Quality Objectives (DQOs) supersede the requirements in this section where applicable. Client or Project specified DQOs shall be included, or referenced, in the final report to the client. Quality control exceedances are documented on the *ALKPAH Report Checklist (Form No.:103-14)*.

## 9.1 Blank(s)

A method blank must be extracted (spiked with surrogates and internal standards) and analyzed once per every 20 samples or per extraction batch, whichever is more frequent.

Acceptance Criteria: Method Blanks must not contain any individual compound at or above the concentration of the reporting limit. If a blank does contain target compounds greater than the reporting limit, they should be less than 20% of any sample results for the same compound(s).

Individual compounds may be detected in the blank below the reporting limit. Associated sample results are flagged with a "B" qualifier if the concentration of the analyte in the sample is less than 5x the concentration in the blank.

Corrective Action: If the blank does not meet the above criteria, all efforts must be made to identify and eliminate any source of contamination, and all samples associated with the contaminated blank should be re-extracted and reanalyzed. Exceptions may be made with the approval of the Department Supervisor. Any exceedances are noted in the case narrative.

## 9.2 Laboratory Control Sample and Laboratory Control Sample Duplicate (LCS/LCSD)

The laboratory control sample (LCS/LCSD) contains 17 priority pollutant parent PAH's and is from a second/separate source, to verify the accuracy of the calibration curve. The LCS is extracted along with the samples. An LCS must be extracted and analyzed once per every 20 samples or per extraction batch, whichever is more frequent.

Acceptance Criteria: All LCS/LCSD compound recoveries must be between 50-130% of the true values. The acceptable recovery QC limits are found in Section 12 for aqueous, solid, tissue, and product LCSs.

Corrective Action: If the LCS does not meet the QC limits, check to see if an analytical or spiking error has occurred. If the LCS recovery is still out of control, re-extraction of the entire extraction set may be necessary. If the samples are also associated with a matrix spike and matrix spike duplicate that are in control, re-extraction may not be necessary, as this demonstrates an isolated problem pertaining to the LCS only. Exceptions may be made with the approval of the Department Supervisor. Any exceedances are noted in the case narrative.

## 9.3 Initial Calibration Verification (ICV)

The analysis of an ICV standard must follow the initial calibration curve.

After final processing, calculate the percent recovery of each PAH by using the following calculation:

**% Recovery = Found Amount / True Value x 100**

Acceptance Criteria: All recoveries must be +/- 20% of the true values.

Corrective Action: If the initial calibration fails, perform instrument maintenance and repeat.

## 9.4 Continuing Calibration Verification (CCV)

A continuing calibration verification (CCV) standard, at the concentration of a mid-level initial calibration standard, must be analyzed at the beginning and end of every analytical sequence, and every 24 hours within the sequence, to confirm instrument stability, via response factor, for each calibrated PAH. Only the parent PAH compounds are monitored for %D and the following acceptance criteria.

Acceptance Criteria: Compare the CCV resulting response against the average response for the initial calibration for each calibrated PAH. The %D for each calibrated PAH must be below 25%, with no more than 10% of all compounds greater than 25% but less than 35%. If multiple CCVs are analyzed within an analytical sequence, each CCV must be analyzed within 24 hours

of the previous CCV. Each CCV, including the ending CCV must meet the acceptance criteria. The acceptable QC limits are found in Section 12.

Corrective Action: If the CCV does not meet the acceptance criteria for each calibrated PAH, perform instrument maintenance and repeat the continuing calibration. Re-analyze all affected samples. Reported results for the failing CCV may be "J" qualified; including all alkylated compounds quantified using the suspect response. If the failure of the suspect response appears related to a loss in MS sensitivity, instrument maintenance and repeat analysis of the continuing calibration, and all affected samples, must be performed. Exceptions may be made with the approval of the Department Supervisor. Any exceedances are noted in the case narrative.

## 9.5 Matrix Spike/Matrix Spike Duplicate (MS/MSD)

Matrix spike samples are analyzed only if requested by the client. Matrix spike and matrix spike duplicate analyses are performed per client request. It is preferable to extract samples that have been selected specifically by the client. If none have been assigned than the laboratory analyst must choose a representative sample for each type of matrix prepared. Aqueous duplicates can only be done if the client supplies sufficient sample. These samples are also spiked with extraction surrogate.

Acceptance Criteria: All matrix spike compound recoveries must be between 50-150% of the true values. The acceptable recovery QC limits are found in Section 12 for aqueous, solid, tissue, and product MS/MSD's.

Corrective Action: If the MS/MSD do not meet the QC limits, check to see if an analytical or spiking error has occurred. If the recovery still exceeds the control limits, re-extraction of the set may be necessary. If the associated LCS is within control, re-extraction may not be necessary, as this demonstrates that there may be matrix effects on the accuracy of the affected results as evidenced by the matrix spike and matrix spike duplicate exceedance. Exceptions may be made with the approval of the Department Supervisor. Any exceedances are noted in the case narrative.

## 9.6 Sample Duplicate

A sample duplicate (DUP) is extracted per client request.

Acceptance Criteria: The QC limit is 30% RPD for target compounds found above 5 times the reporting limit. The acceptable RPD QC limits are found in Section 12 for aqueous, solid, tissue, and product duplicates.

Corrective Action: If the %RPD exceeds the 30% control limit, check to see if an analytical or spiking error has occurred. If the RPD still exceeds the control limits, re-extraction of the set may be necessary. If the associated LCS/LCSD is within control, re-extraction may not be necessary, as this demonstrates that there may be matrix effects on the precision of the affected results as evidenced by the matrix spike and matrix spike duplicate exceedance. Exceptions may be made with the approval of the Department Supervisor. Any exceedances are noted in the case narrative.

## 9.7 Method-specific Quality Control Samples

### 9.7.1 Surrogates

Acceptance Criteria: All surrogate recoveries must be between 50-130% of the true values. Surrogate recovery limits for all matrices can be found in Section 12.

Corrective Action: If the surrogate does not fall within the QC limits, check to see if an analytical or spiking error has occurred. If only one surrogate falls below the 50% recovery limit, but is above 10% recovery, re-extraction may not be necessary. If all surrogates are recovered below the 50% limit, re-extract the sample and report the re-extract results along with the original results, if re-extraction occurred beyond the holding time, and the re-extract surrogates are within the QC limits. If the surrogates are recovered below 50% in the re-extract, this confirms suspected matrix interference on the surrogates, and only the original analysis needs to be reported. If the chromatogram shows obvious matrix interference, no re-analysis or re-extraction is necessary.

### 9.7.2 Internal Standards

Internal standards are added to every field sample, QC sample, and method blank.

Acceptance Criteria: The acceptance limits are 50-200% of the internal standard response (or area) of the daily continuing calibration verification standard.

Corrective Action: If the internal standard areas fall outside the QC limits, check to see if an analytical, dilution or spiking error occurred.

- If internal standards are low, reanalyze the extract.
- If internal standards are high, the extract may have concentrated while on the instrument, then:
  - If no obvious interference is present, re-analyze the extract. If internal standards are now within the acceptance limits, report only the re-analysis, as long as the re-analysis occurred within the 40-day analytical hold time. If the re-analysis occurred outside of the 40-day analytical hold time, both the original and re-analysis must be reported. If the internal standards again are outside the acceptance limits, and either within or outside of the 40-day hold time, try re-analyzing at a 1:5 or greater dilution (see below).
  - If the chromatogram shows obvious matrix interference that cannot be avoided when integrating, a re-analysis at a 1:5 or greater dilution may be helpful in minimizing the interference while ensuring better quantitation.

Exceptions may be made with the approval of the Department Supervisor. Any exceedances are noted in the case narrative.

### 9.7.3 Standard Reference Materials (SRMs)

Standard reference materials (SRM) are available from the National Institute of Standards and Technology (NIST) and are extracted and analyzed with samples on a project specific basis. These are not used as controls, but to evaluate potential matrix effects in associated samples for the target compounds being evaluated.

Acceptance Criteria: Acceptance criteria for SRM analysis will vary from project to project depending upon client data quality objectives (DQOs). Generally, ± 35% difference (%D) based on the true certified values of the target compounds of interest, or 65% - 135% recovery, serve as advisory acceptance criteria.

Corrective Action: Repeat analysis and/or check to see if an analytical error has occurred. If the % recovery or %D still exceeds the control limits and the associated LCS/LCSD and/or MS/MSD are within control, re–extraction may not be necessary as this may be isolated to this sample as evidenced by the LCS and/or MS/MSD acceptance. Exceptions may be made with the approval of the Department Supervisor. Any exceedances are noted in the case narrative.

### 9.8 Method Sequence

Samples are prioritized for analysis by the Department Supervisor or GC/MS Team Leader based on client due date and sample analytical hold time. Samples are retrieved from the sample storage refrigerator.

The sequence is prepared and run using the Enviroquant software. Printouts of all sequences are kept in a three-ring binder for each instrument. The sequence printouts are used to document analytical runs; notations are manually added of any reruns or dilutions that will need to be performed.

- Instrument Primer
- Continuing Calibration Verification (CCV)
- Instrument Solvent blanks
- Method Blank
- Laboratory control samples (LCS/LCSD)
- Samples
- Continuing Calibration Verification (CCV)

## 10. Procedure

### 10.1 Equipment Set-up

Prior to the analysis of any standards or samples, the instrument acquisition and process methods must be set up. This includes the GC run parameters and the SIM mode acquisition ion entries into the different SIM acquisition retention time windows. The mass spectrometer must be tuned to the meet the abundance criteria for PFTBA (then DFTPP if required per client request or project specific DQOs) and an initial calibration must be analyzed to establish linearity of the instrument.

#### 10.1.1 PFTBA Manual Tuning

**10.1.1.1** Prior to initial calibration, tune the mass spectrometer using PFTBA (Perfluorotributylamine - calibration gas) to maximize the sensitivity of the instrument in the mass range of interest, 35-525 amu.

**10.1.1.2** The following PFTBA mass intensity criteria must be met:

| PFTBA Ion | Relative Abundance |
|---|---|
| m/e 69 | Base Peak with > 200,000 counts |
| m/e 219 | 30% to 60% of Base Peak |
| m/e 502 | 5% to 11% of Base Peak |

### 10.1.2 GC Instrumental Conditions

Inject an aliquot of 1uL into the capillary column of the gas chromatograph at the following conditions. Larger injection volumes (to 50uL using the Large Volume Injector, LVI) will be dictated by project specific DQOs.

| GC Parameter | Setting |
|---|---|
| Injector Temp: | 300 °C |
| Transfer Line Temp: | 300 °C |
| Initial Oven Temp: | 40 °C |
| Initial Hold Time: | 1 minute |
| Ramp Rate: | 6 °C / minute |
| Final Temperature: | 315 °C |
| Final Hold Time: | 30 minutes |
| Total runtime: | 76 minutes |
| Mode: | Splitless / Constant Flow 1.0ml/min |
| Purge: | 20 mL / minute – on at 1.0 minutes |
| MS Temperature | 300 |

### 10.1.3 Mass Spectrometer Conditions

The effluent from the GC capillary column is fed directly into the ion source of the mass spectrometer. The MS is operated in the SIM mode using appropriate retention time windows to include the quantification and confirmation ions for each PAH and Biomarker compounds as shown in Table II. For each retention time window the ions 191, 217, and 218 are included for sterane and triterpane quantification, if requested by the client.

### 10.1.4 Data Acquisition Parameters

**10.1.4.1** SIM Windows must be set up that bracket the expected retention times for each target analyte. These windows include the quantitation (primary) and confirmation ions for each parent PAH and Alkyl homolog group. To establish the expected retention time window ranges, the mid-level calibration standard must be analyzed in full scan mode. The resulting full scan analysis will dictate the windows in which the selected ions will be monitored. Depending upon the length of the analytical GC column, the time each window is selectively monitored may vary. The retention time windows must be shifted accordingly, when instrument maintenance is performed, (*i.e.,* the column is clipped).

**10.1.4.2** The “dwell” time for each window should be set to 18, and the resolution should be set to “high”.

## 10.2 Initial Calibration

**10.2.1** Before analysis of sample extracts, establish a multi-point response factor calibration curve showing the linear range of the analysis for all target analytes in Table IA and IB. Use standard concentrations of 10, 25, 100, 500, 5000, 10,000 and 20,000 ng/mL to construct the curve. See Section 8.13 for the preparation of the standard solutions for the initial calibration curve.

**10.2.2** Run a sequence with the initial calibration standards and the retention time standard.

- Create a processing method from a previous method on that instrument. Set retention time windows using a mid level standard and the retention time standard.
- Quantify and QEDIT the initial calibration standards. Update the response factors for each level of the method with these standards. Use each parent compound response for any associated homolog group. Alkylated phenanthrenes and anthracenes, and alkylated fluoranthenes and pyrenes are quantified together as total alkylated phenanthrene/anthracenes (using the phenanthrene parent response factor) and total alkylated fluoranthenes/pyrenes (using the pyrene parent response factor).

  Acceptance Criteria: 25% RSD for 90% of all target compounds, with the exception for 10% to be between 25%RSD and 35%RSD. All calibration standards must be analyzed within 24 hours.

**10.2.3** Initial Calibration Verification (ICV)

- The analysis of an ICV standard must follow the initial calibration curve.
- After final processing, calculate the percent recovery of each PAH by using the following calculation:

  **% Recovery = Found Amount / True Value x 100**

- Acceptance Criteria: All recoveries must be +/- 20% of the true values.

**10.2.4** If the initial calibration fails, perform instrument maintenance and repeat.

**10.2.5** Alaska North Slope Crude Reference Oil (ANS)

- The ANS crude oil reference standard is analyzed following each initial calibration curve. Analysis of this reference oil is to establish the integration patterns of the Alkyl PAH homolog groups, and to establish the current instrument quantitation and

confirmation ion ratios. Analysis of this standard following the initial calibration ensures the retention time windows in the SIM mode of acquisition have been set up properly.

- The analysis of the ANS standard is for reference. If instrument maintenance is performed, such as removing a significant section of the analytical column consisting of one "loop" or more, the ANS must be analyzed to update and/or confirm the SIM acquisition windows.

- The ANS reference standard is processed against the completed initial calibration curve method. See Section 11.2 for information regarding manual integration of the Alkyl PAH homolog clusters. This standard will be used for reference when processing field samples for this method, until such a time that a new ANS standard needs to be analyzed. Other project/client specific source oils may be used. These specific oils will likely display patterns that differ from ANS, but will aid analysts in pattern identification of the related field samples.

**10.2.6** ICALs are documented on the *ALKPAH Initial Calibration Checklist (103-15)*. The initial calibration must be secondary reviewed before analyzing samples.

## 10.3 Equipment Operation and Sample Processing

**10.3.1** Daily PFTBA Tuning is not required. See Section 10.1.1 for details.

**10.3.2** If the on-column concentration of any PAH compound exceeds the calibration range of 20,000 ng/mL, the sample must be diluted and re-analyzed.

## 10.4 Continuing Calibration

A continuing calibration verification (CCV) standard, at the concentration of a mid-level initial calibration standard, must be analyzed at the beginning and end of every analytical sequence, and every 24 hours within the sequence, to confirm instrument stability, via response factor, for each calibrated PAH.

**10.4.1** Quantitate and QEDIT the continuing calibration standard. **Note:** The Alkyl homolog groups may be deleted from the report. Only the parent PAH compounds are monitored for %D and the following acceptance criteria.

**10.4.2** Acceptance Criteria: Compare the CCV resulting response against the average response for the initial calibration for each calibrated PAH. The %D for each calibrated PAH must be below 25%, with no more than 10% of all compounds greater than 25% but less than 35%. If multiple CCVs are analyzed within an analytical sequence, each CCV must be analyzed within 24 hours of the previous CCV. Each CCV, including the ending CCV must meet the acceptance criteria.

**10.4.3** If the CCV does not meet 25%D and 10% of the analytes are <35%, for each calibrated PAH, the following *corrective actions* are recommended:

- Perform instrument maintenance and repeat the continuing calibration, and re-analyze all affected samples, OR,
- Qualify all results reported for the failing CCV with an appropriate qualifier, including all alkylated compounds quantified using the suspect response, and any non-detects. If the failure of the suspect response appears related to a loss in MS sensitivity,

instrument maintenance and repeat analysis of the continuing calibration, and all affected samples, must be performed.

The choice of corrective action must be made in consultation with the Department Manager, Project Manager and/or the client. The reasoning for choosing the second option must be documented in the project narrative to the client.

### 10.5 Preventive Maintenance

If performing any maintenance on any piece of equipment it must be documented in the *Instrument Maintenance Logbook* located in the laboratory specific to each instrument. Specific instrumentation service contracts or warranties differ from each instrument. See the Department Manager for specific instrument details.

**Injection Port and Column Maintenance:** Maintenance should be done when the daily CCV starts to demonstrate degradation. The type of samples analyzed will have an effect on how soon maintenance should be performed. The injection port can be cleaned using cotton swabs and DCM until no contamination is observed. Generally maintenance is performed by trimming 6 cm off the front of the column. The column is then installed into the injection port liner and the inlet nut is tightened. After cleaning the instrument must be checked for any air leaks while cooling. The first injection should be a primer to remove any active sites and to check if any of the windows need updating.

**Merlin Maintenance:** The Merlin High Pressure Microseal (Part #410 Phenomenex) should be replaced when it cannot hold back pressure. A septum may be used as a backup or for troubleshooting.

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## 11. Data Evaluation, Calculations and Reporting

**11.1** Identification of the priority pollutant PAH compounds is based on gas chromatographic relative retention times (RRTs) from the analysis of a mid-level initial calibration standard. For these compounds, manual quantitations may be performed, if necessary, by integrating the area of the quantitation ion or peak. For *alkylated* PAHs, the homolog groupings (*i.e.,* $C_3$-Naphthalenes) appear in the extracted ion current profiles (EICPs) as a cluster of isomers. Integrate peaks within the cluster by straight-line integration to the baseline, taking into account background noise in the EICPs. Reference the Alaska North Slope Crude Oil pattern book, for a cluster by cluster example of each integration for each alkylated PAH homolog group. Table II, in Section 16, lists the representative ion(s) used for quantitation and confirmation of each parent PAH and alkylated PAH homolog group.

**Note:** Manual integration is not to be used solely to meet QC criteria, nor is it to be used as a substitute for corrective action on the chromatographic system.

**11.2** From EICP of the quantification (primary) mass ions and the confirmatory mass ions, identify all target analytes according to the following criteria:

- The characteristic masses of each analyte of interest should maximize in the same, or within one scan of each other.

- The retention time should fall within ± 10 seconds of the retention time of the parent PAH from the preceding CCV. **Note:** When evaluating alkyl homolog groups, the retention time of the most intense peak within the group may not have the exact retention time of the most intense peak in the ANS reference standard. Analyst judgement and referral to each homolog groups' retention time window is essential for identification. Apply analyst judgment regarding corrective action when this criterion is not met.

- The relative peak heights of the primary ion compared to the confirmation or secondary ion masses for parent compounds should fall within ± 50 percent of the relative intensities of these masses in a the reference mass spectrum (*i.e.,* the mid-level of the initial calibration curve and/or the Alaska North Slope Crude Oil).

**Note**: The relative intensities of the primary and secondary ions may vary widely within a given group of alkyl homologs (*i.e.,* $C_3$ - Naphthalenes). Thus, the pattern of each alkyl homolog cluster, and the retention time window for the cluster, will be the primary identification criteria for alkyl homologs. In some instances, a parent compound that does not meet secondary ion confirmation criteria may still be determined to be present in a sample after close inspection of the data by the experienced mass spectrometrist. Supportive data includes the presence of the secondary ion, but ratio value greater than ± 50 percent of the primary ion, may be caused by an interference of the secondary ion.

**11.3** To calculate the ***Relative Standard Deviation*** (RSD) of all target analytes and surrogate compounds for the initial calibration use the formula below. The RSD of each target compound and surrogate must be below 25% with the exception for 10% to be between 25%RSD and 35%RSD. Additionally, use the initial six-point calibration to determine ***Relative Response Factors*** ($RRF_I$s) at each concentration level. Average the $RRF_I$s, to generate mean $RRF_I$s, for quantification of all target analytes and surrogate compounds. The $RRF_I$s are based on the internal standard compounds, and are calculated using the formula below. (The relative response factors for the continuing calibration verifications ($RRF_C$s) are calculated using the same formula). See Section 16, Table IA and Table IB, for the listing of target compounds and their associated internal standards for quantification.

$$\mathbf{RSD = SD \ / \ mean\ RRF_I \ x \ 100}$$

where:

SD = Standard deviation between the five points, for that target analyte.

$$\mathbf{RRF_I = (A_c \ x \ C_{IS}) \ / \ (A_{IS} \ x \ C_c)}$$

where:

$A_c$ = Area of the characteristic ion for the standard compound to be measured.
$A_{IS}$ = Area of the characteristic ion for the representative internal standard compound.
$C_{IS}$ = Concentration of the representative internal standard compound (ng/mL).
$C_c$ = Concentration of the standard compound to be measured (ng/mL).

**Note:** Assign the response factor of the parent compound to the alkyl homolog cluster.

**11.4** Based on the mean $RRF_I$s, calculate the ***Sample Extract Amount*** for each target analyte and surrogate in the extracts using the following formula:

$$Q_e = (A_a \times Q_{IS}) / (A_{IS} \times RRF_I)$$

where:

$Q_e$ = Sample extract amount (ng) of target analyte, from quantitation report.
$A_a$ = Area of the characteristic ion for the target analyte.
$A_{IS}$ = Area of the characteristic ion for the representative internal standard compound.
$Q_{IS}$ = Amount of internal standard compound added to each extract (ng).

**11.5** Calculate the ***Sample Concentration*** (C) for each compound by the following formula:

$$C = (Q_e / V_s) \times DF$$

where:

C = Concentration in sample (ng/L water, ug/Kg sediment/tissue, or mg/Kg product).

$V_s$ = Original volume or weight of sample extracted.

DF = Dilution factor or fraction of the original extract to which internal standard added.

**11.6** If the response of any individual target compound in a sample exceeds the linear response range, as defined by the initial calibration standards in Section 10.13, dilute the extract so that the concentrations of all individual target compounds fall within the range of the calibration curve. If that compound is also part of an alkyl homolog group, the group is considered to exceed the calibration range and thus, would also require dilution. Reported concentrations that are above the highest standard concentration in the initial calibration are qualified with an “E”. If the response of any target compound in a sample exceeds the MDL but is below the reporting limit (RL), qualify the reported concentration with a “J”. If any target compound is found in the associated sample(s) at or below 5x the concentration of the method blank, qualify the reported concentration with a “B”.

**11.7** Compare response factors for each compound in the ***Continuing Calibration Verification*** (CCV), to those of the initial calibration curve by determining the percent difference.

$$\text{Percent Difference (\%D)} = ([RRF_I - RRF_C] / RRF_I) \times 100$$

where:

$RRF_I$ = Mean response factor from initial calibration.

$RRF_C$ = Response factor from CCV.

**11.8** All results must be reported to three significant figures. All solids including soils, sediments, and sludges must be reported on a dry-weight basis. Tissue results may be reported in wet-weight depending upon client request. Petroleum results are reported “as received” or on a wet-weight basis.

**11.9** The analyst does data entry, or upload of the data, into the LIMS system. The LIMS is linked to the instrument, so the analyst must choose the sample(s) to be reported from that instrument's analytical sequence. All associated preparation and instrumental QC samples and dilutions are also chosen. Once the data/samples have been selected and with the proper QC samples, the batched data set is reported.

**11.10** The laboratory generates two types of data packages from the LIMS: "Standard" for routine projects and "CLP-like" for fully data validated projects. A standard package consists of sample results and the associated QC sample results. A CLP-like package includes all sample results, all preparation and instrumental QC results, and the associated supporting raw data. A secondary review is performed on all data.

**11.11** Procedures for data and record management must adhere to the Quality Systems Manual, other subordinate documents covering record keeping, and the *Document Control* SOP, G-016. All records shall be stored in such a manner as to be safe and accessible for at least 10 years.

**11.12** Notebooks: Laboratory notebooks are designed to accommodate the specific analysis. Instrument printouts are used to document run sequences, and each sequence printout is filed in a three-ring binder. Each sequence notebook page is numbered. If a sample requires re-analysis or re-extraction for any reason, a notation is made next to the sample entry on the sequence log. The sequence run log is permanently bound, assigned an internal ID number, and filed accordingly. Such files shall be archived so as to remain available for at least 10 years. All laboratory notebooks must follow the specifications in the *Laboratory Notebook Usage* Work Instructions, WI 108-01, and all record keeping and document control practices.

**11.13** Electronic records: All data files from computers, attached to instruments, shall be backed up daily onto the proper directory on the server. The backups shall be stored so as to be accessible for 10 years. Movement of the data files to the server is the responsibility of the primary analyst. Server backup and storage is the responsibility of the IT department.

# 12. Contingencies for Handling Out-of-Control Data or Unacceptable Data

All Alkylated PAH-SIM results are reportable without qualification if analytical holding times are met, preservation requirements (including cooler temperatures) are met, and all QC criteria defined in the table below are met. If any of the below QC parameters are not met, all associated samples must be evaluated for re-analysis. See Sections 9, 10.1, 10.2, 10.3 and 10.4 for additional QC discussion including corrective actions for any QC outliers.

| QC Parameter | Acceptance Criteria |
|---|---|
| Initial Calibration Curve | ≤ 25%D for all target analytes with exception for 10% of target analytes can be >25%, but ≤ 35% |
| Independent Calibration Verification | +/- 20% recovery of the true values |
| Continuing Calibration Verification | Analyzed every 10 samples or 24 hours, ≤ 25%D for all target analytes with exception for 10% of target analytes can be >25%, but ≤ 35% |
| Method Blank | No analyte at or above the RL, "B" qualify analyte if detected in the samples ≤ 5x the concentration in the blank |
| Laboratory Control Sample | 50-130%R for all target analytes |
| Matrix Spike / Matrix Spike Duplicate | 50-150%R for all target analytes, 30% RPD between the duplicates. |

| | |
|---|---|
| Sample / Sample Duplicate | 30% RPD between the duplicates. |
| Surrogates | 50% - 130% recovery |
| Internal Standards | 50% - 200% of the daily CCV area for the Internal Standards |
| SRM | +/- 35% D or 65% - 135% recovery |

Section 9, Quality Control, defines the corrective actions that must be taken in instances where QC outliers exist.

If non-compliant Alkylated PAH-SIM results are to be reported, the Department Manager and/or the Laboratory Director must approve the reporting of these results. The laboratory Project Manager shall be notified, and may chose to relay the non-compliance to the client, for approval, or other corrective action, such as re-sampling and re-analysis. The analyst or Department Manager performing the secondary review initiates the project narrative, and the narrative must clearly document the non-compliance and provide a reason for acceptance of these results.

# 13. Method Performance

## 13.1 Method Detection Limit Study (MDL) / Limit of Detection Study (LOD) / Limit of Quantitation (LOQ)

The laboratory follows the procedure to determine the MDL, LOD, and/or LOQ as outlined in Alpha SOP/08-05. These studies performed by the laboratory are maintained on file for review.

## 13.2 Demonstration of Capability Studies

Refer to Alpha SOP/08-12 for further information regarding IDC/DOC Generation.

### 13.2.1 Initial (IDC)

The analyst must make an initial, one-time, demonstration of the ability to generate acceptable accuracy and precision with this method, prior to the processing of any samples.

### 13.2.2 Continuing (DOC)

The analyst must make a continuing, annual, demonstration of the ability to generate acceptable accuracy and precision with this method.

# 14. Pollution Prevention and Waste Management

Refer to Alpha's Chemical Hygiene Plan and Waste Management and Disposal SOP for further pollution prevention and waste management information.

# 15. Referenced Documents

Chemical Hygiene Plan

SOP/08-05 MDL/LOD/LOQ Generation

SOP/08-12 IDC/DOC Generation

Hazardous Waste and Sample Disposal SOP (G-006)

SOP/08-01 Document Control

Logbook Useage Work Instructions, WI 108-01

# 16. Attachments

Table IA: PAH, Alkyl PAH, and Heterocyclic Target Compounds Internal Standard Reference

Table IB: Sterane and Triterpane Target Compounds Internal Standard Reference

Table II: PAH, Alkyl PAH, Sterane and Triterpane Quantification Primary and Confirmation Ions

**Table IA: PAH, Alkyl PAH, and Heterocyclic Target Compounds Internal Standard Reference**

| Compound | IS Reference | Compound | IS Reference |
|---|---|---|---|
| cis/trans-Decalin | 1 | Benzo(b)fluorene | 1 |
| $C_1$-Decalins | 1 | Pyrene | 1 |
| $C_2$-Decalins | 1 | $C_1$-Fluoranthrenes/Pyrenes | 1 |
| $C_3$-Decalins | 1 | $C_2$-Fluoranthrenes/Pyrenes | 1 |
| $C_4$-Decalins | 1 | $C_3$-Fluoranthrenes/Pyrenes | 1 |
| Naphthalene | 1 | $C_4$-Fluoranthrenes/Pyrenes | 1 |
| $C_1$-Naphthalenes | 1 | Naphthobenzothiophenes | 1 |
| $C_2$-Naphthalenes | 1 | $C_1$-Naphthobenzothiophenes | 1 |
| $C_3$-Naphthalenes | 1 | $C_2$-Naphthobenzothiophenes | 1 |
| $C_4$-Naphthalenes | 1 | $C_3$-Naphthobenzothiophenes | 1 |
| 2-Methlynaphthalene | 1 | Benz[a]anthracene | 2 |
| 1-Methlynaphthalene | 1 | Chrysene | 2 |
| Benzothiophene | 1 | $C_1$-Chrysenes | 2 |
| $C_1$-Benzo(b)thiophenes | 1 | $C_2$-Chrysenes | 2 |
| $C_2$-Benzo(b)thiophenes | 1 | $C_3$-Chrysenes | 2 |
| $C_3$-Benzo(b)thiophenes | 1 | $C_4$-Chrysenes | 2 |
| $C_4$-Benzo(b)thiophenes | 1 | Benzo[b]fluoranthene | 2 |
| Biphenyl | 1 | Benzo[j]+[k]fluoranthene | 2 |
| 2,6-Dimethylnaphthalene | 1 | Benzo[a]fluoranthene | 2 |
| Dibenzofuran | 1 | Benzo[e]pyrene | 2 |
| Acenaphthylene | 1 | Benzo[a]pyrene | 2 |
| Acenaphthene | 1 | Perylene | 2 |
| 2,3,5-Trimethylnaphthalene | 1 | Indeno[1,2,3-c,d]pyrene | 2 |
| Fluorene | 1 | Dibenz[ah]+[ac]anthracene | 2 |
| $C_1$-Fluorenes | 1 | Benzo[g,h,i]perylene | 2 |
| $C_2$-Fluorenes | 1 | | |
| $C_3$-Fluorenes | 1 | | |
| Dibenzothiophene | 1 | **Surrogate Compounds** | |
| $C_1$-Dibenzothiophenes | 1 | Naphthalene-$d_8$ | 1 |
| $C_2$-Dibenzothiophenes | 1 | Phenanthrene-$d_{10}$ | 1 |
| $C_3$-Dibenzothiophenes | 1 | Benzo(a)pyrene-$d_{12}$ | 2 |
| $C_4$-Dibenzothiophenes | 1 | 5B(H) - Cholane | 2 |
| Phenanthrene | 1 | | |
| $C_1$-Phenanthrenes/Anthracenes | 1 | **Alternate Surrogates** | |
| $C_2$-Phenanthrenes/Anthracenes | 1 | Methylnaphthalene-d10 | 1 |
| $C_3$-Phenanthrenes/Anthracenes | 1 | Pyrene-d10 | 1 |
| $C_4$-Phenanthrenes/Anthracenes | 1 | Benzo(b)flouranthene-d12 | 2 |
| Anthracene | 1 | | |
| Retene | 1 | **Internal Standards** | |
| Carbazole | 1 | Acenapthene-$d_{10}$ | 1 |
| 1-Methylphenanthrene | 1 | Chrysene-$d_{12}$ | 2 |
| Fluoranthene | 1 | | |

Note: Alkylated phenanthrenes and anthracenes, and alkylated fluoranthenes and pyrenes are quantified together as total alkylated phenanthrene/anthracenes (using the phenanthrene parent response factor) and total alkylated fluoranthenes/pyrenes (using the pyrene parent response factor).

**Table IB: Sterane and Triterpane Target Compounds Internal Standard Reference**

| Compound | IS Reference | Compound | IS Reference |
|---|---|---|---|
| C23 Tricyclic Terpane | 2 | 30,31-Trishomohopane-22S | 2 |
| C24 Tricyclic Terpane | 2 | 30,31-Trishomohopane-22R | 2 |
| C25 Tricyclic Terpane | 2 | Tetrakishomohopane-22S | 2 |
| C24 Tetracyclic Terpane | 2 | Tetrakishomohopane-22R | 2 |
| C26 Tricyclic Terpane-22S | 2 | Pentakishomohopane-22S | 2 |
| C26 Tricyclic Terpane-22R | 2 | Pentakishomohopane-22R | 2 |
| C28 Tricyclic Terpane-22S | 2 | 13b(H),17a(H)-20S-Diacholestane | 2 |
| C28 Tricyclic Terpane-22R | 2 | 13b(H),17a(H)-20R-Diacholestane | 2 |
| C29 Tricyclic Terpane-22S | 2 | 13b,17a-20S-Methyldiacholestane | 2 |
| C29 Tricyclic Terpane-22R | 2 | 14a(H),17a(H)-20S-Cholestane/ 13b(H),17a(H)-20S-Ethyldiacholestane | 2 |
| 18a-22,29,30-Trisnorneohopane-TS | 2 | 14a(H),17a(H)-20R-Cholestane/ 13b(H),17a(H)-20R-Ethyldiacholestane | 2 |
| C30 Tricyclic Terpane-22S | 2 | Unknown sterane | 2 |
| C30 Tricyclic Terpane-22R | 2 | 13a,17b-20S-Ethyldiacholestane | 2 |
| 17a(H)-22,29,30-Trisnorhopane-TM | 2 | 14a,17a-20S-Methylcholestane | 2 |
| 17a/b,21b/a 28,30-Bisnorhopane | 2 | 14a,17a-20R-Methylcholestane | 2 |
| 17a(H),21B(H)-25-Norhopane | 2 | 14a(H),17a(H)-20S-Ethylcholestane | 2 |
| 30-Norhopane | 2 | 14a(H),17a(H)-20R-Ethylcholestane | 2 |
| 18a(H)-30-Norneohopane-C29Ts | 2 | 14b(H),17b(H)-20R-Cholestane | 2 |
| 17a(H)-Diahopane | 2 | 14b(H),17b(H)-20S-Cholestane | 2 |
| 30-Normoretane | 2 | 14b,17b-20R-Methylcholestane | 2 |
| 18a(H)&18b(H)-Oleananes | 2 | 14b,17b-20S-Methylcholestane | 2 |
| 17a(H),21B(H)-hopane-C30H52 | 2 | 14b(H),17b(H)-20R-Ethylcholestane | 2 |
| Moretane | 2 | 14b(H),17b(H)-20S-Ethylcholestane | 2 |
| 30-Homohopane-22S | 2 | C26,20R- +C27,20S- triaromatic steroid | 2 |
| 30-Homohopane-22R | 2 | C28,20S-triaromatic steroid | 2 |
| Gammacerane/C32-diahopane | 2 | C27,20R-triaromatic steroid | 2 |
| 30,31-Bishomohopane-22S | 2 | C28,20R-triaromatic steroid | 2 |
| 30,31-Bishomohopane-22R | 2 | | |

**Table II: PAH, Alkyl PAH, Sterane and Triterpane Quantification Primary and Confirmation Ions**

| Compound | Quantification & Confirmation Ions | Compound | Quantification & Confirmation Ions |
|---|---|---|---|
| cis/trans-Decalin | 138, 96 | Fluoranthene | 202, 101 |
| $C_1$-Decalins | 152 | Benzo(b)fluorene | 216, 215 |
| $C_2$-Decalins | 166 | Pyrene | 202, 101 |
| $C_3$-Decalins | 180 | $C_1$-Fluoranthrenes/Pyrenes | 216, 215 |
| $C_4$-Decalins | 194 | $C_2$-Fluoranthrenes/Pyrenes | 230, 215 |
| Naphthalene | 128, 127 | $C_3$-Fluoranthrenes/Pyrenes | 244, 229 |
| $C_1$-Naphthalenes | 142, 141 | $C_4$-Fluoranthrenes/Pyrenes | 258 |
| $C_2$-Naphthalenes | 156, 141 | Naphthobenzothiophene | 234, 189 |
| $C_3$-Naphthalenes | 170, 155 | $C_1$-Naphthobenzothiophene | 248 |
| $C_4$-Naphthalenes | 184, 169, 183 | $C_2$-Naphthobenzothiophene | 262 |
| 2-Methylnaphthalene | 142, 141 | $C_3$-Naphthobenzothiophene | 276 |
| 1- Methylnaphthalene | 142, 141 | Benz[a]anthracene | 228, 226 |
| Benzothiophene | 134 | Chrysene | 228, 226 |
| C1-Benzo(b)thiophenes | 148 | $C_1$-Chrysenes | 242, 241 |
| C2-Benzo(b)thiophenes | 162 | $C_2$-Chrysenes | 256, 241 |
| C3-Benzo(b)thiophenes | 176 | $C_3$-Chrysenes | 270, 255 |
| C4-Benzo(b)thiophenes | 190 | $C_4$-Chrysenes | 284, 269 |
| Biphenyl | 154, 153 | Benzo[b]fluoranthene | 252, 253 |
| 2,6-Dimethylnaphthalene | 156, 155 | Benzo[j]+[k]fluoranthene | 252, 253 |
| Dibenzofuran | 168, 139, 169 | Benzo[a]fluoranthene | 252, 253 |
| Acenaphthylene | 152, 153 | Benzo[e]pyrene | 252, 253 |
| Acenaphthene | 154, 153 | Benzo[a]pyrene | 252, 253 |
| 2,3,5-Trimethylnaphthalene | 170, 155 | Perylene | 252, 253 |
| Fluorene | 166, 165 | Indeno[1,2,3-c,d]pyrene | 276, 138, 277 |
| $C_1$-Fluorenes | 180, 165 | Dibenz[ah]+[ac]anthracene | 278, 139, 279 |
| $C_2$-Fluorenes | 194, 179,195 | Benzo[g,h,i]perylene | 276, 277 |
| $C_3$-Fluorenes | 208, 197 | **Biomarker Compounds** | |
| Dibenzothiophene | 184, 152 | Triterpanes | 191 |
| $C_1$-Dibenzothiophenes | 198, 197 | Steranes | 217, 218 |
| $C_2$-Dibenzothiophenes | 212, 197 | Triaromatic steroids | 231 |
| $C_3$-Dibenzothiophenes | 226, 211 | **Surrogate Compounds** | |
| $C_3$-Dibenzothiophenes | 240, 225 | 2-Methylnaphthalene-$d_{10}$ | 152, 150 |
| Phenanthrene | 178, 176 | Pyrene-$d_{10}$ | 212, 211 |
| $C_1$-Phenanthrenes/Anthracenes | 192, 191 | Benzo(b)fluoranthene-$d_{12}$ | 264, 260 |
| $C_2$-Phenanthrenes/Anthracenes | 206, 191, 207 | Naphthalene-$d_8$ | 136, 134 |
| $C_3$-Phenanthrenes/Anthracenes | 220, 205 | Phenanthrene-$d_{10}$ | 188, 184 |
| $C_4$-Phenanthrenes/Anthracenes | 234, 219 | Benzo(a)pyrene-$d_{12}$ | 264, 260 |
| Anthracene | 178, 176 | 5B(H) - cholane | 217, 218 |
| Retene | 234, 219 | **Internal Standards** | |
| Carbazole | 167, 139 | Acenapthene-$d_{10}$ | 164, 162 |
| 1-Methylphenanthrene | 192 | Chrysene-$d_{12}$ | 240, 241 |

**Note:** Alkylated phenanthrenes and anthracenes, and alkylated fluoranthenes and pyrenes are quantified together as total alkylated phenanthrene/anthracenes (using the phenanthrene parent response factor) and total alkylated fluoranthenes/pyrenes (using the pyrene parent response factor).