QUANTITATIVE DETERMINATION OF AROMATIC HYDROCARBONS USING SELECTED ION MONITORING GAS CHROMATOGRAPHY/MASS SPECTROMETRY

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ABSTRACT

Polycyclic aromatic hydrocarbons and their alkylated homologues are quantitatively determined using a gas chromatograph/mass spectrometer in selected ion monitoring mode. This method unambiguously detects low concentrations of PAH in tissues and sediments.

1.0 INTRODUCTION

A gas chromatograph/mass spectrometer (GC/MS) in selected ion mode (SIM) coupled to a capillary column is used to resolve, detect and quantitate polycyclic aromatic hydrocarbons (PAH) in tissues and sediments at parts per billion levels. Samples are injected into a temperature-programmed GC/MS, operated in splitless mode. The capillary column is a HP-5MS (60 m x 0.25 mm ID and 0.25 μm film thickness). The mass spectrometer is capable of scanning from 35 to 500 AMU every second or less and uses 70 electron volts energy in electron impact ionization mode. The data acquisition system continuously acquires and stores all data analyses.

2.0 APPARATUS AND MATERIALS

2.1 EQUIPMENT

- Gas chromatograph, split/splitless injection port and electronic pressure control
- Mass spectrometer, capable of scanning from 35 to 500 AMU, utilizing 70 electron volts of energy in impact ionization mode.
- Data acquisition system, Agilent Technologies ChemStation, capable of continuous acquisition and storage of all data during analysis, or equivalent
- Autosampler, capable of making 1 to 5 μL injections
- Capillary column, Agilent Technologies HP-5MS (60 m x 0.25 mm ID and 0.25 μm film thickness), or equivalent

- Micropipetters, calibrated, 1% accuracy, disposable tips

2.2 REAGENTS

- Dichloromethane, pesticide grade or equivalent
- Helium, 99.8% purity

2.3 STANDARDS

2.3.1 Surrogate Spiking Solution

The surrogate spiking solution is prepared from aliquots of pure compounds that are diluted with dichloromethane to a final concentration of 0.5 µg/mL. The surrogate spiking solution includes naphthalene-d_8, acenaphthalene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12} and perylene-d_{12}. Surrogate solution is added to all samples and all quality control samples prior to extraction. Surrogate compounds are resolved from, but elute in close proximity to the analytes of interest. Individual surrogate recoveries are used to correct specific analyte concentrations based on retention time.

2.3.2 Internal Standard Solution

The internal standard solution is made from aliquots of pure compounds and diluted with dichloromethane to a final concentration of 0.5 µg/mL. The internal standard solution includes fluorene-d_{10}, pyrene-d_{10}, and benzo[a]pyrene-d_{12}. The internal standard compounds are resolved from, but elute in close proximity to the analytes of interest. The internal standard solution is added to all samples and quality control samples just prior to instrument analysis. Internal standards are used to calculate relative response factors and specific analyte concentrations based on retention time.

2.3.3 Matrix Spiking Solution

Certified solutions containing 2 to 5-ring PAH compounds are purchased from commercial vendors and diluted with dichloromethane to prepare the matrix spiking solution (Table 1). The matrix spiking solution is diluted to approximately 10 times the MDL and is added to all matrix spike samples.

2.3.4 Calibration Solution

Calibrations solutions are prepared at 5 concentrations ranging from approximately 0.02 to 1 µg/mL (Table 2) by diluting commercially available certified solutions containing analytes of interest.

2.3.5 Retention Index Solutions

The mid-level calibration standard, (containing analytes at approximately 0.25 µg/mL), is used as a retention index solution to determine the retention times of unsubstituted compounds and certain substituted compounds. A crude oil/coal oil standard is used as a retention index solution for the determination of retention times for the remaining alkyl homologues. The retention index solutions are also used to evaluate instrument drift over time.

Table 1. Polycyclic Aromatic Hydrocarbons Contained in the Matrix-Spiking Solution.

Analyte	**Spiking Solution Concentration (µg/mL)**
Decalin	1.000
Naphthalene	1.060
2-Methylnaphthalene	1.054
1-Methylnaphthalene	1.056
Benzo(b)thiophene	1.856
Biphenyl	1.060
2,6-Dimethylnaphthalene	1.060
Acenaphthylene	1.020
Acenaphthene	1.100
1,6,7-Trimethylnaphthalene	0.940
Dibenzofuran	1.000
Fluorene	1.050
Pentachlorophenol	4.000
Carbazole	1.000
Anthracene	0.801
1-Methylphenanthrene	1.050
Phenanthrene	1.060
Dibenzothiophene	1.000
Fluoranthene	1.060
Pyrene	1.060
Naphthobenzothiophene	1.000
Benz(a)anthracene	0.919
Chrysene	1.070
C30-Hopane	1.000
Benzo(b)fluoranthene	1.060
Benzo(k)fluoranthene	1.050
Benzo(e)pyrene	1.060
Benzo(a)pyrene	0.955
Perylene	0.800
Indeno(1,2,3-c,d)pyrene	0.938
Dibenzo(a,h)anthracene	0.794
Benzo(g,h,i)perylene	0.945

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Table 2. Polycyclic Aromatic Hydrocarbons Contained in Calibration Solutions and their Approximate Concentrations.

Compounds Contained in Calibration Solutions	**Level 1 (mg/mL)**	**Level 2 (mg/mL)**	**Level 3 (mg/mL)**	**Level 4 (mg/mL)**	**Level 5 (mg/mL)**
Internal Standards					
Fluorene-d_{10}	0.05	0.05	0.05	0.05	0.05
Pyrene-d_{10}	0.05	0.05	0.05	0.05	0.05
Benzo(a)pyrene-d_{12}	0.05	0.05	0.05	0.05	0.05
Surrogates					
Naphthalene-d_8	0.02	0.10	0.25	0.50	1.00
Acenaphthene-d_{10}	0.02	0.10	0.25	0.50	1.00
Phenanthrene-d_{10}	0.02	0.10	0.25	0.50	1.00
Chrysene-d_{12}	0.02	0.10	0.25	0.50	1.00
Perylene-d_{12}	0.02	0.10	0.25	0.50	1.00
5β(H)-Cholane	0.02	0.10	0.25	0.50	1.00
Analytes*					
Decalin	0.020	0.100	0.250	0.500	1.000
Naphthalene	0.021	0.106	0.267	0.531	1.061
2-Methylnaphthalene	0.021	0.104	0.263	0.524	1.047
1-Methylnaphthalene	0.021	0.105	0.265	0.527	1.053
Benzo(b)thiophene	0.037	0.184	0.464	0.928	1.856
Biphenyl	0.021	0.105	0.266	0.529	1.059
2,6-Dimethylnaphthalene	0.021	0.105	0.265	0.527	1.055
Acenaphthylene	0.020	0.101	0.255	0.508	1.016
Acenaphthene	0.022	0.109	0.276	0.548	1.097
1,6,7-Trimethylnaphthalene	0.019	0.093	0.236	0.470	0.939
Dibenzofuran	0.020	0.100	0.250	0.500	1.000
Fluorene	0.021	0.105	0.264	0.526	1.051
Pentachlorophenol	0.250	0.500	1.000	1.500	2.000
Carbazole	0.020	0.100	0.250	0.500	1.000
Dibenzothiophene	0.020	0.100	0.250	0.500	1.000
Phenanthrene	0.021	0.105	0.266	0.528	1.057
Anthracene	0.016	0.080	0.201	0.400	0.800
1-Methylphenanthrene	0.021	0.104	0.263	0.523	1.045
Fluoranthene	0.021	0.106	0.267	0.53	1.061
Pyrene	0.021	0.105	0.266	0.53	1.060
Naphthobenzothiophene	0.020	0.099	0.250	0.500	1.000
Benz(a)anthracene	0.018	0.091	0.231	0.459	0.918
Chrysene	0.021	0.106	0.267	0.532	1.064
C30-Hopane	0.020	0.100	0.250	0.500	1.000
Benzo(b)fluoranthene	0.021	0.105	0.265	0.528	1.056
Benzo(k)fluoranthene	0.021	0.105	0.264	0.526	1.052
Benzo(e)pyrene	0.021	0.105	0.265	0.528	1.056
Benzo(a)pyrene	0.019	0.095	0.238	0.477	0.954
Perylene	0.016	0.080	0.201	0.400	0.799
Indeno(1,2,3-cd)pyrene	0.019	0.093	0.236	0.469	0.937
Dibenzo(a,h)anthracene	0.016	0.079	0.199	0.396	0.793
Benzo(g,h,i)perylene	0.019	0.094	0.237	0.472	0.944

3.0 QUANTITATIVE DETERMINATION OF PAHS BY GC/MS-SIM

3.1 MASS SPECTROMETER TUNING

Prior to calibration, the MS is autotuned to perfluorotributylamine (PFTBA) using criteria established by the instrument manufacturer.

3.2 INITIAL CALIBRATION

A 5-point relative response factor (RRF) calibration curve is established for analytes of interest prior to the analysis of samples and quality control samples (Table 2). A RRF is determined, for each analyte, for each calibration level using the following equation:

$$RRF = \frac{(A_A)(C_{IS})}{(A_{IS})(C_A)}$$

Where:

A_A = the area of the characteristic ion for the analyte to be measured
A_{IS} = the area of the characteristic ion for the specific internal standard
C_A = the known concentration of the analyte in the calibration solution (µg/mL)
C_{IS} = the know concentration of the internal standard in the calibration solution (µg/mg)

The response factors determined for each calibration level are averaged to produce a mean relative response factor ($\overline{RRF_i}$) for each analyte. The percent relative standard deviation (%RSD) for the 5 response factors must be less than or equal to 15%, for each analyte.

$$\%RSD = \frac{\text{Standard Deviation of the RRFs}}{\text{Average of the RRFs}} \times 100$$

Where:

$$\text{Standard Deviation} = \sqrt{\frac{\sum_{i=1}^{n}\left(x_i - \bar{x}\right)^2}{(n-1)}}$$

Where:

x_i = each RRF value used to calculate the mean RRF
$\bar{x}$ = the mean of n values

n = total number of values (5)

3.3 CONTINUING CALIBRATION

A mid-level calibration standard is analyzed at the beginning and end of each analytical set or every 10 samples (whichever is more frequent). The daily relative response factor for each compound is compared to the mean relative response factor from the initial calibration curve and the average relative percent difference (RPD) of all analytes must be less than 25%. If the calibration check does not meet this criterion then the initial five-point calibration is repeated.

$$RPD = \frac{RRF_c - \overline{RRF_i}}{\overline{RRF_i}} \times 100$$

Where:

$\overline{RRF_i}$ = mean relative response factor from the most recent initial calibration (meeting technical acceptance criteria)

RRF_c = relative response factor from the continuing calibration standard

3.4 GC/MS-SIM ANALYSIS

The initial calibration of the GC/MS must meet the previously described criteria prior to sample analysis. Samples are analyzed in analytical sets that consist of standards, samples and quality control samples. Quality control samples are method blanks, laboratory duplicates, blank spikes, matrix spikes and standard reference materials. The type and number of quality control samples depend upon client requests and material availability. An autosampler is used to inject 1 or 2 µL of all samples, standards and QC samples into the capillary column of the GC using the following instrument conditions. Slight modifications may be necessary depending upon the analysis.

Inlet: Splitless
Carrier gas: Helium, 1 mL/min

Temperatures:
- Injection port: 300°C
- Transfer line: 290°C

Oven program:
- Initial oven temp: 60°C
- Initial hold time: 0 minutes
- Ramp rate: 7°C/min
- Final oven temp: 315°C

Final hold time: 22 minutes
Total run time: 56 minutes

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

3.5 ANALYTE IDENTIFICATION

The extracted ion current profiles of the primary m/z and the confirmatory ion for each analyte must meet the following criteria:

- The characteristic masses of each analyte of interest must be in the same scan or within one scan of each other. The retention time must fall within ±5 seconds of the retention time of the authentic compound or alkyl homologue grouping determined by the analysis of the daily calibration check or reference oil standard, respectively.
- The alkylated PAH homologue groupings (e.g. C4-naphthalene) appear as a group of isomers. The pattern of each group and the retention time window for the group is established by the analysis of a reference oil standard. Each group of alkylated homologues is integrated in its entirety and the total area response is used to determine the concentration of the entire group.
- The relative peak heights of the primary mass ion, compared to the confirmation or secondary mass ion, must fall within ±30 percent of the relative intensities of these masses in a reference mass spectrum (Table 3). The reference mass spectrum is obtained from the continuing calibration solution or the reference oil standard for the parent compounds and alkylated homologues, respectively. In some instances, a compound that does not meet secondary ion confirmation criteria may still be determined to be present in a sample after close inspection of the data by a qualified mass spectrometrist. Supportive data includes the presence of the confirmation ion, but at a ratio different then that indicated in Table 3.
- Data not meeting the criteria established in this section are appropriately qualified or re-analyzed.

Table 3. Target Analyte Parameters.

Analyte	Reference to Internal Standard and Surrogate	Quantitation Ion	Confirmation Ion	% Relative Abundance of Confirmation Ion
Fluorene-d_{10} (I -1)		176	174	85
Naphthalene-d_8 (S-1)	I-1	136	134	11
Decalin	I-1, S-1	138	96	90
C1-Decalins	I-1, S-1	152	ND	ND
C2-Decalins	I-1, S-1	166	ND	ND
C3-Decalins	I-1, S-1	180	ND	ND
Naphthalene	I-1, S-1	128	127	13
2-Methylnaphthalene	I-1, S-1	142	141	80
1-Methylnaphthalene	I-1, S-1	142	141	80
C1-Naphthalenes	I-1, S-1	142	141	ND
Acenaphthene-d_{10} (S-2)	I-1	164	162	89
2,6-Di-methylnaphthalene	I-1, S-2	156	141	90
1,6,7-Tri-methylnaphthalene	I-1, S-2	170	155	102
C2-Naphthalenes	I-1, S-2	156	141	ND
C3-Naphthalenes	I-1, S-2	170	155	ND
C4-Naphthalenes	I-1, S-2	184	169	ND
Benzothiophene	I-1, S-2	134	89	10
C1-Benzothiophenes	I-1, S-2	148	ND	ND
C2-Benzothiophenes	I-1, S-2	162	ND	ND
C3-Benzothiophenes	I-1, S-2	176	ND	ND
Biphenyl	I-1, S-2	154	152	30
Acenaphthylene	I-1, S-2	152	153	15
Acenaphthene	I-1, S-2	154	153	98
Dibenzofuran	I-1, S-2	168	139	25
Fluorene	I-1, S-2	166	165	95
C1-Fluorenes	I-1, S-2	180	165	ND
C2-Fluorenes	I-1, S-2	194	179	ND
C3-Fluorenes	I-1, S-2	208	193	ND
Pyrene-d_{10}		212	210	15
Phenanthrene-d_{10} (S-3)	I-2	188	184	15
Pentachlorophenol	I-2, S-3	266	268	70
Carbazole	I-2, S-3	167	139	10
Dibenzothiophene	I-2, S-3	184	152	18
C1-Dibenzothiophenes	I-2, S-3	198	184	ND
C2-Dibenzothiophenes	I-2, S-3	212	197	ND
C3-Dibenzothiophenes	I-2, S-3	226	211	ND
C4-Dibenzothiophenes	I-2, S-3	240	ND	ND
Phenanthrene	I-2, S-3	178	176	20
Anthracene	I-2, S-3	178	176	20
C1-Phenanthrene/anthracenes	I-2, S-3	192	191	ND
C2-Phenanthrene/anthracenes	I-2, S-3	206	191	ND
C3-Phenanthrene/anthracenes	I-2, S-3	220	205	ND
C4-Phenanthrene/anthracenes	I-2, S-3	234	219	ND
Naphthobenzothiophene	I-2, S-3	234	ND	ND
C1-Naphthobenzothiophenes	I-2, S-3	248	ND	ND
C2-Naphthobenzothiophenes	I-2, S-3	262	ND	ND
C3-Naphthobenzothiophenes	I-2, S-3	276	ND	ND
Fluoranthene	I-2, S-3	202	101	15
Pyrene	I-2, S-3	202	101	15
C1-Fluoranthene/pyrenes	I-2, S-3	216	215	ND
C2-Fluoranthene/pyrenes	I-2, S-3	230	ND	ND

Table 3. Target Analyte Parameters (Continued).

Analyte	Reference to Internal Standard and Surrogate	Quantitation Ion	Confirmation Ion	% Relative Abundance of Confirmation Ion
C3-Fluoranthene/pyrenes	I-2, S-3	244	ND	ND
Chrysene-d_{12} (S-4)	I-2	240	236	20
Benz(a)anthracene	I-2, S-4	228	226	30
Chrysene	I-2, S-4	228	226	30
C1-Chrysenes/Benzo(a)anthracenes	I-2, S-4	242	241	ND
C2-Chrysenes/Benzo(a)anthracenes	I-2, S-4	256	241	ND
C3-Chrysenes/Benzo(a)anthracenes	I-2, S-4	270	255	ND
C4-Chrysenes/Benzo(a)anthracenes	I-2, S-4	284	269	ND
Benzo(a)pyrene-d_{12} (I-3)		264	260	20
5b(H)-Cholane(S-6)	I-3	217	ND	ND
C29-Hopane	I-3, S-3	191	398	5
18α–Oleanane	I-3, S-3	191	412	5
C30-Hopane	I-3, S-3	191	412	5
Benzo(b)fluoranthene	I-3, S-4	252	253	30,
Benzo(k)fluoranthene	I-3, S-4	252	253	30,
Benzo(e)pyrene	I-3, S-4	252	253	30
Benzo(a)pyrene	I-3, S-4	252	253	30
Indeno(1,2,3-cd)pyrene	I-3, S-4	276	277	25,
Dibenzo(a,h)anthracene	I-3, S-4	278	279	25,
C1-Dibenzo(a,h)anthracenes	I-3, S-4	292	ND	ND
C2-Dibenzo(a,h)anthracenes	I-3, S-4	306	ND	ND
C3-Dibenzo(a,h)anthracenes	I-3, S-4	320	ND	ND
Benzo(g,h,i)perylene	I-3, S-4	276	277	25,
Perylene-d_{12} (S-5)	I-3	264	260	20
Perylene	I-3, S-5	252	253	20

ND = Not determined
(I-#) = Internal standard reference number
(S-#) = Surrogate reference number

4.0 QUANTITATION CALCULATIONS

Sample analyte concentrations are calculated based on the concentration and response of the internal standard compounds (Table 2). The equations in Section 3.2 are used to calculate the RRF of each analyte relative to the concentration and area of the internal standard in the initial calibration. Response factors for alkyl homologues are presumed equal to the response factor of the respective unsubstituted (parent) compound.

The mass (M_A) of each target analyte (ng), including alkyl homologues, is calculated using the following equation:

$$M_A = \frac{(A_A M_{IS})}{(A_{IS} \overline{RRF_i})}$$

Where:

A_A = the area of the characteristic ion for the analyte measured
A_{IS} = the area of the characteristic ion for the specific internal standard
M_{IS} = mass of internal standard added to the extract (ng)
$\overline{RRF_i}$ = the average relative response factor for the analyte from the current calibration

The concentration (C) of each target analyte in a sample (ng/g) is calculated using the following equation:

$$C = \frac{(M_A DF)}{(W)}$$

Where:

DF = the dilution factor applied to the extract

$$DF = \frac{\text{Volume of Extract (uL)}}{\text{Volume of extract used to make dilution (uL)}}$$

W = the sample weight (g)

Analyte concentrations are reported as corrected for individual surrogate recoveries. The corrections for each compound are based on the surrogates referenced in Table 3. Percent surrogate recoveries ($SU_{Recovery}$) for each surrogate are calculated using the following equation:

$$SU_{Recovery} = \frac{C_{ESU}}{C_{SU}} x100$$

Where:

C_{ESU} = calculated surrogate concentration in the extract
C_{SU} = known concentration of surrogate added to extract

Analyte concentration corrections ($C_{corrected}$) for surrogate recovery are calculated using the following equation:

$$C_{Corrected} \frac{C}{SU_{Recovery}} x\,100$$

5.0 QUALITY CONTROL

The initial calibration must pass established criteria before sample analysis can begin. All continuing calibration checks must pass established criteria for analysis to continue.

An acceptable method blank analysis may not contain more than two target analytes at concentrations three times greater than the MDL. This criterion does not apply if the analytes detected in the method blank are not detected in the associated samples or if the sample analyte concentrations are 10 times greater than the blank analyte concentrations. If the method blank exceeds these criteria then the analytical procedure is not in control. The source of the contamination must be investigated, and corrective measures taken and documented before further sample analysis occurs.

All samples and quality control samples are spiked with deuterated PAH surrogate compounds prior to extraction. The surrogate compounds evaluate sample matrix effects and analytical efficiencies associated with sample preparation and analysis. The recovery of surrogate compounds is monitored in each sample and quality control sample. The laboratory will take corrective action if the average surrogate recovery, with the exception of perylene-$d_{12,}$ is less than 50% or greater than 150%. The following corrective action will be taken if the above criteria are not met:

1) Calculations are checked to assure that no errors have been made.
2) The internal standard and surrogate solutions are checked for degradation, contamination, etc., and instrument performance is checked.
3) If surrogate recovery is outside the control limits, the secondary ion may be used to check the quantitation of the surrogate for matrix interference. If the secondary ion is within the control limits, this recovery is appropriately qualified.
4) If the upper control limit is exceeded for a surrogate, and the instrument calibration and surrogate standard concentration are in control, it is concluded that an interference specific to the surrogate was present that resulted in high recovery and that this interference does not affect the quantitation of other target compounds. The presence of this type of interference is confirmed by evaluation of chromatographic peak shapes. If analytes are surrogate corrected, another surrogate eluting in close proximity may be used to calculate surrogate corrected analyte concentrations and the change is appropriately documented.
5) If the surrogate cannot be measured because only a portion of the sample is analyzed, (such as sample dilution) then no corrective action is required. The surrogate recovery is appropriately qualified.
6) If the steps above fail to reveal a problem, the extract is re-analyzed, if sufficient material is available. If the sample was completely consumed, the data will be reported but designated as outside the quality control criteria.

The average recovery for all compounds in a blank spike or valid matrix spike must fall between 80% and 120%. No more than two individual analyte recoveries in a blank spike

or valid matrix spike can exceed 40-120% with the exception of biphenyl and decalin. A valid matrix spike is a matrix spike where the amount of analyte added is at least as much as was present in the sample originally. If the matrix spike is valid and the QC criteria are not met then the quality control sample failing the criteria will be re-analyzed. If the re-analyzed spike meets the criteria, then the data for the re-analyzed quality control sample are reported. If the analytes that are in violation are not present in the samples analyzed with this extraction batch, the violation is noted but no action is required. If upon re-analysis, quality control criteria are still in violation, the entire batch of samples is re-extracted and re-analyzed, if deemed necessary. If sufficient sample is unavailable for the re-extraction of the matrix-spike, a blank spike may be analyzed. The acceptable average RPD for a valid matrix spike/matrix spike duplicate or blank spike/blank spike duplicate pair is 30%. No more than two individual analyte RPDs may exceed 35%.

The average acceptable RPD between the duplicate and original sample, for analytes greater than 10 times the concentration of the MDL, is 30%. No more than two individual analyte RPD can exceed 35%.

The average RPD for target compounds in a SRM should not exceed 30% of the upper and lower bounds of the mean certified values. No more than two target analytes should deviate more than 35% from the upper or lower bounds of the mean certified values.

The method detection limit (MDL) is determined following the procedures outlined in Federal Register (1984), Vol. 49, No. 209: 198-199.