RESIDUES AND TRACE ELEMENTS

Compound-Specific Gas Chromatographic/Mass Spectrometric Analysis of Alkylated and Parent Polycyclic Aromatic Hydrocarbons in Waters, Sediments, and Aquatic Organisms

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Polycyclic aromatic hydrocarbons (PAHs) and their alkylated and heterocyclic analogs are ubiquitous contaminants in aquatic environments, including estuaries and marine systems. Methodology for compound-specific analysis of 63 parent, alkylated, and heterocyclic PAHs using gas chromatography/mass spectrometry (GC/MS) in both scanning and selected-ion monitoring modes has been developed and applied to sediment, natural waters and effluents, and marine organisms including oysters, mussels, and fish. Relative response factors and relative retention times for the 63 alkylated, heterocyclic, and parent PAHs compared with 6 deuterated PAHs are given. Analyses of natural sea water samples, enriched at concentrations ranging from 5 to 100 ng/L, show good accuracy (8% mean difference at the 5 ng/L level) and precision (mean RSD of 9%), and method detection limits are in the parts-per-trillion range. Results for sediments and tissues of aquatic organisms exposed to petroleum contamination demonstrate that analysis of parent PAHs alone vastly underestimates levels in sediments and tissues and the potential toxic effects of such residues in food webs. Multiple analyses of a reference tissue material show good precision (mean RSD of 15%) and accuracy (mean difference of 17%) for both alkylated and parent PAHs.

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous products of combustion of carbon-based materials. Alkylated and heterocyclic PAHs are products of diagenetic alterations of organic matter deposited in sediments and are the dominant forms of PAHs in oil and coal. As a class, PAHs and alkylated and heterocyclic PAHs are important as potential environmental contaminants from fuel combustion, energy resource exploration and production, transportation, and accidental spills. The potential adverse environmental effects of PAHs as a class have been documented extensively and include mutagenesis and carcinogenesis (1–3). Hundreds of studies document the presence and amounts of parent pyrogenic PAHs in air, water, soils, and sediments, but relatively few have focused on petrogenic alkylated and heterocyclic PAHs in water, sediments, and biological tissues.

In the late 1970s, researchers began to recognize the importance of measuring both petrogenic and pyrogenic PAHs in the aftermath of such catastrophic events as the Amoco Cadiz spill (4) or the Ixtoc oil platform explosion and fire (5). In these studies, however, necessary standards were not available to follow individual members of isomeric alkylation groups, and so, generic response factors and isomer group summations were applied. The petroleum industry has made great advances in the detailed analysis of petroleum and petroleum source rocks, using pattern recognition analysis and specific biomarker compounds analyses as tools in geochemical exploration (6). However, these compound-specific methods are, in part, proprietary, and therefore have not been applied in environmental assessments of toxicological investigations. Bence and coworkers (7, 8) used detailed analyses of alkylated and heterocyclic PAHs as part of their investigations of the aftermath of the Exxon Valdez oil spill; however, they did not report isomer-specific quantitation of residues of the spill in sediments or tissues.

Because of the relative water insolubility of members of this class of compounds (9), PAHs and their analogs have a strong tendency to sorb to soils and sediments (10) and to bioaccumulate in tissues of exposed organisms (11–14). Different members of the isomeric alkylated PAH groups that dominate petrogenic PAHs exhibit differential toxicity to benthic communities (15, 16), differential diffusion rates (17), and differential degradation rates (18). Thus, it is imperative that analytical methodology be developed that allows quantitation of as many individual isomers as is technically feasible. With the ability to assess PAH concentrations in all environmental media, rigorous toxicological evaluations or risk analyses can be performed. Analysis of residues in tissues also is especially important for protection of human food, particularly seafood.

Assessment of long-term effects of petroleum-related activities on ecosystems and food webs leading to humans requires analytical methods to quantitate individual molecular components (11, 19) that are inherent to such work. Focusing on parent pyrogenic PAHs alone may lead to misinterpretation if contributions from other petrogenic-source materials are excluded. Although alkylated and het-

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Table 1. Compound names, abbreviations, and CAS registry numbers

Compound name[a]	Abbreviation	CAS Registry No.	Compound name[a]	Abbreviation	CAS Registry No.
d_8-NAPHTHALENE (SS)	d_8-NAPH	1146-65-2	4-Methylphenanthrene	4-MP	832-64-4
Naphthalene	Naph	91-20-3	9-Methylphenanthrene	9-MP	883-20-5
2-Methylnaphthalene	2-MN	91-57-6	1-Methylphenanthrene	1-MP	832-69-9
1-Methylnaphthalene	1-MN	90-12-0	3,6-Dimethylphenanthrene	3,6-DMP	1576-67-6
2-FLUOROBIPHENYL (IS)	2-FBP	321-60-8	3,5-Dimethylphenanthrene	3,5-DMP	33954-06-2
2-Ethylnaphthalene	2-EN	939-27-5	2,6-Dimethylphenanthrene	2,6-DMP	17980-164
1-Ethylnaphthalene	1-EN	1127-76-0	2,7-Dimethylphenanthrene	2,7-DMP	1576-69-8
2,6-Dimethylnaphthalene	2,6-DMN	581-42-0	3,9-Dimethylphenanthrene	3,9-DMP	66291-32-5
2,7-Dimethyinaphthalene	2,7-DMN	582-16-1	1,6-Dimethylphenanthrene	1,6-DMP	20291-74-1
1,3-Dimethylnaphthalene	1,3-DMN	575-41-7	2,5-Dimethylphenanthrene	2,5-DMP	3674-66-6
1,7-Dimethyinaphthalene	1,7-DMN	575-37-1	2,9-Dimethylphenanthrene	2,9-DMP	17980-09-5
1,6-Dimethylnaphthalene	1,6-DMN	575-43-9	1,7-Dimethylphenanthrene	1,7-DMP	483-87-4
1,4-Dimethylnaphthalene	1,4-DMN	571-584	1,9-Dimethylphenanthrene	1,9-DMP	20291-73-0
2,3-Dimethylnaphthalene	2,3-DMN	581 40-8	4,9-Dimethylphenanthrene	4,9-DMP	66291-34-7
1,5-Dimethylnaphthalene	1,5-DMN	571-61-9	1,2-Dimethyldibenzothiophene	1,2-DMDBT	31317-1-3
Acenaphthylene	Acyl	208-96-8	Fluoranthene	Fluorant	206-44-0
1,2-Dimethylnaphthalene	1,2-DMN	573-98	1,5-Dimethylphenanthrene	1,5-DMP	66271-87-2
2-Isopropylnaphthalene	2-IPN	2027-17-0	1,8-Dimethylphenanthrene	1,8-DMP	7372-7-4
1,8-Dimethylnaphthalene	1,8-DMN	569-41-5	1,2-Dimethylphenanthrene	1,2-DMP	20291-72-9
d_{10}-ACENAPHTHENE (SS)	d_{10}-ACE	15067-26-2	9,10-Dimethylphenanthrene	9,10-DMP	604-83-1
Acenaphthene	Ace	83-32-9	Pyrene	Pyrene	129-00-0
1,6,7-Trimethylnaphthalene	1,6,7-TMN	2245 38-7	1,2,8-Trimethylphenanthrene	1,2,8-TMP	20291-75-2
Fluorene	Fluorene	86-73-7	Benzanthracene	Benz	56-55-3
Dibenzothiophene	DBT	132-65-0	d_{12}-CHRYSENE (SS)	d_{12}-CHRYS	1719-03-5
d_{10}-PHENANTHRENE (SS)	d_{10}-PHEN	1517-22-2	Chrysene	Chrys	218-01-9
Phenanthrene	Phen	85-01-8	Benzo[b]fluoranthene	BbFL	205-99-2
Anthracene	Ant	120-12-7	Benzo[k]fluoranthene	BkFL	207-08-9
4-Methyldibenzothiophene	4-MDBT	7372-8-5	Benzo[a]pyrene	BaP	50-32-8
2-Methyldibenzothiophene	2-MDBT	20928-02-3	d_{12}-PERYLENE (SS)	d_{12}-PERYL	1520-96-3
3-Methyldibenzothiophene	3-MDBT	16587-52-3	Indenot1,2,3[c,d]pyrene	Indeno	193-39-5
3-Methylphenanthrene	3-MP	832-71-3	Dibenz[a,h]anthracene	Dibenzant	53-70-3
1-Methyldibenzothiophene	1-MDBT	31317-07-4	Benzo[g,h,i]perylene	Benzoperyl	191-24-2
2-Methylphenanthrene	2-MP	2531-84-2			

[a] Compounds in all caps are surrogate standards (SS) or the internal standard (IS).

erocyclic PAHs are the dominant indicators of petrogenic impacts on the environment, most current analytical methods continue to rely on qualitative assessment of isomer patterns of alkylated PAHs and on estimates of their total group concentrations derived from response factors for parent aromatic nuclear compounds (20, 21). Often, analysis of individual isomers of alkylated and heterocyclic PAHs relies on preparative chromatography or fractionation prior to analysis and focuses on a single class of components, such as dimethylnaphthalenes (22–25). Such methods resolve target compounds well, but sample preparation is time consuming and prone to losses, and the data often are too limited for use in environmental risk assessment or mechanistic studies of toxicological effects. Sauer and Boehm (26) propose that more detailed analyses of oil spill residues is required for accurate damage assessments. The present work takes the analysis to the next level of specificity, thus further improving upon their proposal.

Wang et al. (27) showed, through quantitation of specific methyl dibenzothiophenes, the usefulness of compound-specific analyses for source identification or "fingerprinting" and for investigation of biological and chemical degradation processes or weathering. Use of compound-specific analysis—particularly when supplemented by class estimates for higher alkylation groups for which few standards are available (e.g., C4- and C5-alkylphenanthrenes) but which are among the most abundant and persistent petroleum-source PAHs—allows development of accurate and realistic assessments of source impacts. Compound-specific analysis makes it possible to investigate long-term fate and transport of contaminants for use in risk assessments (18). It also allows detailed investigation of rate processes and modeling of fate and transport (17) and exposure (14), and of bioaccumulation (14, 28, 29). Because of their high relative abundance in petroleum-contaminated matrixes, several alkylated naphthalenes, dibenzothiophenes, and phenanthrene homologs were selected for method develop-

Table 2. Target analytes, retention times, mass criteria, calibration concentrations, and typical response factors for GC/MS separation of PAHs

Analyte	Retention time, min	Primary ion, *m/z*	Confirming ions, *m/z*		Typical ion ratios		Calculated standard concn, μg/mL	Typical RF[b]
			CI1	CI2	CI1	CI2		
d_8-NAPH	13.30	136					0.50	1.53E-06
Naph	13.36	128	129		10		0.50	1.11E-06
2-MN	16.26	142	141		92		0.50	1.58E-06
1-MN	16.71	142	141		94		0.50	1.62E-06
2-FBP	18.30	172					5.00	7.78E-07
2-EN	19.12	141	156		38		0.50	1.35E-06
1-EN	19.22	141	156		32		0.50	1.17E-06
2,6/2,7-DMN	19.46	156	141		75		1.00	1.63E-06
1,3/1,7-DMN	19.90	156	141		92		0.98	1.29E-06
1,6-DMN	20.01	156	141		79		0.50	1.31E-06
1,4,2,3-DMN	20.51	141	156		91		1.00	1.65E-06
1,5-DMN	20.61	156	141		87		0.50	1.51E-06
Acyl	20.80	152	153		12		0.50	1.13E-06
1,2-DMN	21.00	141	156		85		0.50	9.97E-07
2-IPN	21.07	170	155		340		0.45	3.14E-06
1,8-DMN	21.66	156	141		92		0.48	1.80E-06
d_{10}-ACE	21.76	164	165[a]				0.50	1.93E-06
Ace	21.94	153	152		48		0.50	1.38E-06
1,6,7-TMN	24.61	170	155		96		0.50	1.35E-06
Fluorene	25.21	166	165		96		0.50	1.44E-06
DBT	30.94	184	139		14		0.50	1.76E-06
d_{10}-PHEN	31.68	188					0.50	1.04E-06
Phen	31.82	178	179		15		0.50	9.56E-07
Ant	32.17	178	179		15		0.50	1.12E-06
4-MDBT	34.33	198	197		68		0.25	1.27E-06
2/3-MDBT	34.99	198	197		67		0.40	1.28E-06
3-MP	35.68	192	191		57		0.50	1.30E-06
1-MDBT	35.72	198	197		65		0.35	1.38E-06
2-MP	35.86	192	191		57		0.75	1.02E-06
4/9-MP	36.48	192	191		64		0.75	1.27E-06
1-MP	36.64	192	191		58		0.50	1.05E-06
3,6-DMP	39.36	206	191		30		0.25	1.52E-06
3,5-DMP	39.51	206	191		83		0.25	1.70E-06
2,6-DMP	39.62	206	191		17		0.25	9.03E-07
2,7-DMP	39.76	206	191		19		0.12	9.32E-07
3,9-DMP	40.17	206	191		42		0.25	1.08E-06
1,6/2,5/2,9-DMP	40.36	206	191		48		0.79	1.28E-06
1,7-DMP	40.55	206	191		30		0.28	9.57E-07
1,9/4,9-DMP	40.88	206	191		49		0.45	1.26E-06
1,2-DMDBT	40.89	212	396		23		0.40	1.56E-06
Fluorant	40.91	202	101		19		0.50	6.78E-07
1,5-DMP	41.04	206	191		65		0.20	1.33E-06
1,8-DMP	41.32	206	191		36		0.23	1.10E-06
1,2-DMP	41.82	206	191		70		0.25	1.41E-06
9,10-DMP	42.43	206	191		103		0.25	1.33E-06
Pyrene	42.46	202	101		23		0.50	7.24E-07
1,2,8-TMP	45.03	220	205		56		0.50	9.75E-07
Benz	47.78	228	226	229	23	17	0.50	7.51E-07
d_{12}-CHRYS	47.82	240					0.50	7.28E-07
Chrys	47.90	228	229	226	16	22	0.50	6.96E-07
BbFL	50.61	252	253	125	13	4	0.50	1.28E-06
BkFL	50.66	252	253	125	7	2	0.50	7.88E-07
BaP	51.38	252	253	125	19	4	0.50	1.43E-06

Table 2. *(Continued)*

Analyte	Retention time, min	Primary ion, *m/z*	Confirming ions, *m/z*		Typical ion ratios		Calculated standard concn, μg/mL	Typical RF[b]
			CI1	CI2	CI1	CI2		
d_{12}-PERYL	51.54	264					0.50	1.75E-06
Indeno	54.63	276	138		7		0.50	2.46E-06
Dibenzant	54.71	278	276	138	27	5	0.50	3.13E-06
Benzoperyl	55.47	276	138	278	8	3	0.50	3.09E-06

[a] Ion used to show interferences, rather than confirmation of peak identity.
[b] Refers to response factor or the area integrated which corresponds to a known mass (ng) of analyte measured on column in a chromatographic peak.

ment and application (15, 30–32). The high-resolution chromatographic analysis described in this paper was developed for research to assess the fate, transport, and adverse biological effects of point-source discharges of produced water (14, 15, 17) and the long-range transport of PAHs in the coastal Northwestern Gulf of Mexico (33, 34).

Techniques developed originally for relatively highly contaminated media (10 ppb to ppm range), such as sediments and effluents, were inadequate for ultratrace levels (ppt to ppb range) encountered in typical environmental samples. Thus, full-scan mass spectrometric (MS) methodology applied earlier was converted to a multiple selected-ion monitoring (SIM) method needed to detect and follow pollutants in natural waters, small sediment samples (0.5–1.0 g wet weight), and tissues from individual exposed organisms (as small as 10–20 g wet weight). Simultaneous quantitation of several herbicides, chlorinated pesticides, and polychlorinated biphenyls (PCBs) may be incorporated into the methodology to assess contributions of terrigenous inputs to aquatic systems in studies conducted in the upper San Francisco Bay estuary, the lower Mississippi River, and Gulf of Mexico coastal shelf waters along the Louisiana and Texas coasts (35).

This paper presents methodology for simultaneous determination of 63 parent, alkylated, and heterocyclic PAHs, using authentic standards. Application of the method to environmental and toxicological studies is demonstrated with examples of analyses of marine waters, whole and filtered fresh waters, and effluents using solid-phase extraction (SPE) membranes, as well as analyses of sediments and samples of tissue from exposed organisms.

Experimental

Reagents

PAH standards and internal standards were purchased from Chiron Laboratories A.S. (Troudheim, Norway) and Ultra Scientific, Inc. (Kingstown, RI). Table 1 lists compound names, abbreviations used in this paper, and CAS registry numbers. The 5 deuterated surrogate standards (Ultra Scientific) used also are listed. A sample of a certified reference standard—NIST 1974, mussel tissue—was obtained from the National Institute for Standards and Testing (Gaithersburg, MD).

Preparation of Standards

Authentic standards were used individually to determine the retention order of the alkylated PAH isomers and the mass spectral characteristics of the compounds. The standards include 2 isomers of methylnaphthalene (complete), 10 dimethylnaphthalene isomers (complete), 2-isopropylnaphthalene and 1,6,7-trimethylnaphthalene C3-naphthalenes (partial), 4 isomers of methyldibenzothiophene (partial), 1,2-dimethyldibenzothiophene (partial) for the class C2-dibenzothiophenes, 5 methylphenanthrenes (complete), 16 of 25 isomers of dimethyl phenanthrene (partial), and 1,2,8-trimethylphenanthrene (partial) representing the class of C3-phenanthrenes. A 10 ppm dilution in hexane containing one isomer from each standard group (e.g., a methylnaphthalene, a dimethylnaphthalene, a methylphenanthrene, a dimethylphenanthrene, etc.) was prepared and analyzed by gas chromatography (GC)/MS to determine retention order. A complete standard—containing all alkylated PAHs, parent PAH compounds (Ultra Scientific #106), and deuterated surrogate PAH standards (Ultra Scientific #108)—was prepared in dichloromethane–hexane (50 + 50) at a final concentration of 5 ppm. This complete standard was analyzed with hexamethylbenzene as an internal standard just before injection. Later, 2-fluorobiphenyl was substituted as the internal standard. After retention order was determined, chromatographic resolution of the mixture was optimized by adjusting temperature ramp rates while maintaining total analysis time at <60 min. Table 2 lists PAH target analytes, their retention times, and the mass fragments selected for quantitative analysis (primary ion) and for confirmation of identity (confirming ions) by elution order. Coeluting isomers (ring-position substitution numbers separated by a "/" mark) are also listed at the nominal retention time.

Sample Preparation

The goal for developing sample preparation methods was to allow for some steps to be performed in the field. For example, shipboard separation of marine waters into dissolved, colloidal-enriched, and suspended particulate fractions and their subsequent extraction for chlorinated hydrocarbons and selected pesticides have been described (33). A modification of the methods of MacLeod et al. (21) for sediment extractions, which could be performed on shipboard or in the laboratory, was de-

scribed by Means and McMillin (17). Sample preparation protocols for unfiltered waters, effluents, and tissue samples prior to analysis of alkylated, heterocyclic, and parent PAHs are described.

Reagent blanks, duplicates, and spiked samples were processed with each group of 10 sediment samples to monitor laboratory technique and extraction efficiency. Matrix spikes consisted of duplicate sediment samples to which ca 5 ng/g wet wt (based on an average sample size of 10 g wet wt) of the quantitative calibration standard (including all analytes) was added, along with the mixture of deuterated surrogates.

(**a**) *Waters and effluents.*—Natural waters (fresh and marine) and effluents were extracted with SPE disks (C_{18}, 47 mm, Empore; Analytichem, Harbor City, CA). Before extraction with SPE membrane, the water sample (2 L) was treated with 10 mL (0.5% of sample volume) methanol as cosolvent and 20 μL deuterated PAH (200 ng each; U.S. Environmental Protection Agency [EPA] Internal Standards Mixture, US-108 Ultra Scientific) was added. SPE disks were placed in a standard all-glass membrane filtration unit (47 mm), and a glass fiber prefilter was placed on top of the SPE disk. Before use, the SPE disks were preconditioned with 10 mL each of dichloromethane, methanol, and purified water drawn through the filter by vacuum aspiration according to manufacturer's instructions, leaving the membrane wet. The natural water sample was then applied immediately. After the sample had passed through the SPE disk, the prefilter was removed and then analytes adsorbed on the SPE disk were eluted. The prefilter was removed from the SPE disk to eliminate the contribution of hydrophobic pollutants adsorbed to the suspended particulate matter from the final analysis. A 20 × 200 mm test tube was placed inside the vacuum flask with the outlet of the filtration apparatus positioned inside the test tube. Analytes were eluted from the disk by passing 15 mL pesticide-grade dichloromethane through the membrane with application of a gentle vacuum to the vacuum flask. Then the SPE disk was removed from the apparatus, and the glass frit base was rinsed with an additional 5 mL dichloromethane. Approximately 3–5 g precleaned, anhydrous sodium sulfate was added to the sample extract to remove residual water. The extract was quantitatively transferred to a 40 mL precleaned glass vial and evaporated under a pure (99.99%, with desiccant/charcoal trap) nitrogen stream to a volume of <4 mL. The concentrate was quantitatively transferred to a 4 mL precleaned glass vial and further concentrated under nitrogen to ca 200 μL. The extract was exchanged into hexane by adding ca 2 mL hexane to the 4 mL vial and reconcentrating the sample to a final volume of 200 μL.

(**b**) *Tissues.*—Tissues were extracted with a modification of the method described by MacLeod et al. (21). Approximately 20 g defrosted, minced tissue (whole oysters or mussels, whole fish, fish filets, or fish liver) was placed in a 240 mL amber glass bottle with 100 mL pesticide-grade dichloromethane and 4 times the tissue weight of precleaned anhydrous sodium sulfate (rinsed with dichloromethane). The mixture of deuterated surrogate standards (200 ng each) was added to the bottle. The tissue was disrupted, homogenized, and extracted simultaneously with a Tekmar Tissumizer for 2 min at 24 000 rpm. The probe was rinsed with dichloromethane, with the rinse added to the sample bottle. The extract suspension was allowed to settle for 1 min. The dichloromethane extract was decanted into a glass funnel (25 × 100 mm plugged with glass wool) containing ca 30 g anhydrous sodium sulfate into a standard taper (24/40) Florence flask. The tissue suspension residue was extracted 2 additional times with 100 mL volumes of dichloromethane. After the third extraction, jar and funnel contents were rinsed with an additional 30 mL dichloromethane, which was combined with the total extract. The total extract was concentrated with a rotary evaporator (Buchi) to ca 2 mL. This concentrated extract was transferred quantitatively to a 4 mL vial and concentrated to 2 mL under a stream of ultrapure nitrogen.

Many tissue extracts contained a layer of fine white precipitate (extracted proteins and connective tissue), which was removed before silica column cleanup as follows. The extract was mixed gently but thoroughly (to keep the precipitate off the walls of the vial) with 500 μL hexane and then centrifuged for 5 min at 3000 rpm. The solvent layer was transferred to a clean 4 mL vial with a precleaned Pasteur pipette, and the protein precipitate was rinsed twice more with 1 mL hexane. All hexane extracts were combined and reduced in volume to 1 mL under a stream of ultrapure nitrogen.

Polar lipids were removed from 500 μL portions (about half) of the extract by fractionation on 200 × 10 mm columns (a solvent-rinsed 10 mL disposable glass pipettes with the top cut off to facilitate packing and sample addition) packed with 6.0 g activated 100–200 mesh Grade 923 silica between glass wool plugs. Columns were precleaned and conditioned with 10 mL dichloromethane followed by 10 mL hexane. The extract in hex-

Table 3. Instrument parameters

Parameter	Setting or specification
Gas chromatograph	
Column	DB-5, 30 m, 0.25 μm film thickness, 0.25 mm id, J&W Scientific
Carrier gas	Helium, UHP, at 40 cm/s (determined by butane injection at 100°C
Initial column temperature	50°C, held for 3 min
Temperature ramp 1	6°C/min to 120°C
Temperature ramp 2	3°C/min to 190°C
Temperature ramp 3	12°C/min to 300°C, held 14.5 min
Splitless injection port temperature	250°C
Injection port liner	Deactivated glass wool pack, Restek Corp.
Injection port purge	On at 0.5 min, 1 mL/min helium
Mass spectrometer data acquisition	
Scan range: 45–450 amu	12 ions per retention time group
Scan rate: 1.06 scans/s	15 retention time windows
	Dwell time: 30 ms/ion
	Resolution: 0.7–0.9 amu
Electron multiplier	200 V above tune value
GC/MS transfer line temperature	300°C

ane was applied to the column via a Teflon plunger 500 µL syringe (with rinsing), and the column was rinsed with 20 mL hexane. Next, analytes were eluted with dichloromethane–hexane (50 + 50). The surrogate/PAH fluorescent band was monitored with a long-wavelength (360 nm) hand-held ultraviolet lamp (Fisher Scientific, Pittsburg, PA). Collection of eluant began when the fluorescent band reached the 6 mL mark (ca one-half of the column bed) and ended when 16 mL were collected. (*Note*: For PAH analysis only, the collected volume could be reduced to 6 mL; the additional volume was required to recover chlorinated pesticides such as dieldrin.) The column effluent was collected in a 20 mL calibrated vial and concentrated to 200 µL under a stream of ultrapure nitrogen.

Reagent blanks, duplicates, and spiked samples were processed with each group of 10 samples to monitor laboratory technique and extraction efficiency. Matrix spikes consisted of duplicate tissue samples to which ca 5 ng/g wet wt (based on an average sample size of 20 g wet wt) of the quantitative calibration standard (including all analytes) was added, along with the mixture of deuterated surrogates.

Instrumental Analysis

Instrumental parameters are listed in Table 3. A Hewlett-Packard (HP) 5890A gas chromatograph equipped with a capillary column (HP DB-5) was directly interfaced to an HP 5970B mass selective detector. A temperature program for the GC oven consisting of a series of linear temperature ramps from 50° to 300°C (Table 3) optimally separates the analytes. The mass spectrometer was tuned with perfluorotributylamine (PFTBA) daily and/or after each 16 h of analysis. The instrument autotune was then manually fine-tuned to achieve at least 50–75% abundance of the ion at *m/z* 219 and at least 3% abundance of the ion at *m/z* 502 relative to the intensity of the *m/z* 69 base peak. Injector septa and injector glass liners were routinely inspected and replaced (after ca 35 injections and every analysis day, respectively) to minimize variations in analyte retention times and to optimize peak shapes. An initial calibration curve was prepared and continuing calibrations for all analytes were run at the beginning and end of each analysis group.

A multiple SIM method was developed that monitors up to 12 ions in each of 15 retention time windows, including ions selected to allow estimation of total amounts of C3- and C4-naphthalenes (*m/z* 184 and 169), C2-dibenzothiophenes (*m/z* 212 and 211), and C3-phenanthrenes (*m/z* 200 and 205) in the appropriate retention window(s). This part of the method was set up initially by full-scan GC/MS analysis of a reference South Louisiana Crude Oil (U.S. EPA-API). The reference oil was analyzed periodically to verify that the retention time windows were correct, as well as routinely after major instrument repairs and after change of columns or any column conditions.

Results and Discussion

Determination of Method Detection Limits

Detection limits for each analyte in each matrix type were estimated from statistical information derived from standard calibration curves used to determine instrumental detection limits, corrected for the concentration factor for each sample type. Triplicate analyses of a 5-point standard calibration curve was used to obtained a mean standard deviation (SD) for each analyte. This value was multiplied by a factor of 3 (36) to obtain an instrumental detection limit in units of nanograms on-column. A value of 3× SD instead of 10× SD was chosen as multiplier for mean SDs because the ratio of peak signal to background (noise) levels of the ion currents in the individual analyte mass chromatograms consistently exceeded a 5:1 ratio. The 3× multiplier maximizes the amount of numerical values reported in data sets. Although errors in absolute amounts increase as values approach the detection limit, these small numerical values frequently continue to fall in a range useful for trend analysis in some types of experimental and/or field work, where differences less than an order of magnitude are not always significant (33, 34). In this reporting system, only 2 classes of data are reported ("not detected" and numerical values), in contrast to the system outlined by Taylor (36), where 3 classes of data are reported ("not detected," "trace," and numerical values). Instrument detection limits were corrected for average concentration factors for each matrix to obtain a sample detection limit for that matrix. The nominal values of sample detection limits thus obtained are reported in Table 4 for sea water, industrial effluents, tissues, and sediments.

Chromatographic Data

Chromatographic parameters (Table 5) for 63 parent, alkylated, and heterocyclic PAHs were obtained from daily manual injections of the PAH standard mixture in scanning mode. Three alkylated and heterocyclic PAHs were added to the standard mixture at later stages of method development and are not represented in Table 5.

Verification of Method Detection Limits for Sea Water Samples

To validate these estimates in a dilute, complex environmental matrix, an experiment was conducted with natural sea water samples (from Bodega Bay, CA) spiked at 5, 10, 50, and 100 ng/L (ppt) in triplicate. Samples (2000 mL) were spiked with a standard solution containing all analytes, the internal standard, and deuterated surrogates prepared in methanol and extracted with the C_{18} SPE disk methodology described earlier. The spike concentrations corresponded to 0.1, 0.2, 1.0, and 2.0 ng on-column, respectively, on the basis of a final volume of concentrated sample extract of 200 µL and an injection volume of 2 µL. Final concentrations were calculated by using the internal standard method, with correction for recovery of the appropriate surrogate. Summary statistics for analyses of spiked samples are shown in Table 6. Aromatic PAHs were detected in triplicate samples, except for indeno[c,d]pyrene, dibenz[a,h]anthracene, and benzo[g,h,i]perylene at the lowest spiking level. Recovery of spiked components averaged 47%, which is typical for environmental samples. No differences in recoveries were observed among the 4 spike levels, indicating that breakthrough of analytes on the SPE disks was not a factor in the relatively moderate recoveries. The lower recoveries obtained for these samples may be due to partitioning of the com-

Table 4. Comparison of instrumental (LOD) and sample detection limits (SDL) for various sample matrixes estimated from standard curve data

		Estimated SDL[a], ppb			
Analyte	LOD, ng	Waters, ng/L	Effluents, ng/L	Sediments, ng/g	Tissues, ng/g
Naphthalene	0.019	0.93	1.9	0.25	0.091
2-MN	0.0073	0.36	0.7	0.098	0.035
1-MN	0.010	0.52	1.1	0.14	0.051
2-EN	0.014	0.71	1.5	0.19	0.070
1-EN	0.027	1.36	2.8	0.37	0.13
2,6/2,7-DMN	0.041	2.07	4.2	0.56	0.20
1,3/1,7-DMN	0.049	2.47	5.0	0.67	0.24
1,6-DMN	0.023	1.16	2.4	0.31	0.11
1,4/2,3-DMN	0.038	1.88	3.8	0.51	0.18
1,5-DMN	0.034	1.70	3.5	0.46	0.17
Acenaphthylene	0.020	0.98	2.0	0.26	0.095
1,2-DMN	0.027	1.34	2.7	0.36	0.13
1,8-DMN	0.012	0.60	1.2	0.16	0.058
Acenaphthene	0.021	1.04	2.1	0.28	0.10
1,6,7-TMN	0.019	0.93	1.9	0.25	0.091
Fluorene	0.020	1.00	2.0	0.27	0.098
Dibenzothiophene	0.0121	0.61	1.2	0.16	0.06
Phenanthrene	0.022	1.09	2.2	0.29	0.11
Anthracene	0.030	1.50	3.1	0.41	0.15
4-MDBT	0.013	0.66	1.4	0.18	0.065
2/3-MDBT	0.0278	1.39	2.8	0.37	0.14
3-MP	0.40	19.80	40.4	5.3	1.9
1-MDBT	0.0120	0.60	1.2	0.16	0.059
2-MP	0.035	1.77	3.6	0.48	0.17
4/9-MP	0.037	1.85	3.8	0.50	0.18
1-MP	0.023	1.17	2.4	0.31	0.11
3,6-DMP	0.017	0.84	1.7	0.23	0.082
3,5-DMP	0.033	1.67	3.4	0.45	0.16
2,6-DMP	0.011	0.54	1.1	0.15	0.053
2,7-DMP	0.0093	0.47	1.0	0.13	0.05
3,9-DMP	0.014	0.68	1.4	0.18	0.067
1,6/2,5/2,9-DMP	0.033	1.66	3.4	0.45	0.16
1,7-DMP	0.0179	0.89	1.8	0.24	0.09
1,9/4,9-DMP	0.025	1.23	2.5	0.33	0.12
Fluoranthene	0.038	1.91	3.9	0.51	0.19
1,2-DMDBT	0.025	1.26	2.6	0.34	0.12
1,5-DMP	0.0115	0.58	1.2	0.16	0.06
1,8-DMP	0.0107	0.53	1.1	0.14	0.052
1,2-DMP	0.019	0.95	1.9	0.25	0.092
9,10-DMP	0.023	1.17	2.4	0.32	0.11
Pyrene	0.037	1.87	3.8	0.50	0.18
1,2,8-TMP	0.039	1.97	4.0	0.53	0.19
Benzanthracene	0.052	2.59	5.3	0.70	0.25
Chrysene	0.046	2.32	4.7	0.62	0.23
Benzo [b] fluor	0.18	8.92	18.2	2.4	0.87
Benzo[k]fluor	0.045	2.24	4.6	0.60	0.22
Benzo[a]pyrene	0.14	6.95	14.2	1.9	0.68
Indeno[c,d]pyrene	0.17	8.72	17.8	2.3	0.85
Dibenzanthracene	0.19	9.47	19.3	2.6	0.93
Benzoperylene	0.078	3.90	8.0	1.1	0.38
Mean	0.045	2.3	4.6	0.61	0.22
Minimum	0.0073	0.36	0.74	0.098	0.035
Maximum	0.40	20	40	5.3	1.9

[a] SDL, ppb = LOD × 200 μL final volume sample amount 2× μL injected. Average sample sizes are waters, 2000 mL; effluents, 980 mL; sediments, 7.42 g; and tissues, 20.45 g.

Table 5. Chromatographic data for alkylated and parent PAHs: relative retention times (RRT)[a] and relative response factors (RRF)[b] calculated by taking the ratio of the surrogate standard (SS)

Analyte	SS	Mean RRT[c]	RSD RRT, %	Mean RRF	RSD RRF, %
Naphthalene	d_8-Naph	1.005	0.012	0.80	2.2
2-MN	"	1.223	0.019	1.02	2.9
1-MN	"	1.259	0.018	1.12	3.2
2-EN	d_{10}-Ace	0.878	0.025	1.29	3.6
1-EN	"	0.883	0.012	1.45	4.1
2,6/2,7-DMN	"	0.894	0.018	0.80	3.4
1,3/1,7-DMN	"	0.915	0.010	0.67	2.3
1,6-DMN	"	0.920	0.012	0.74	3.9
1,4/2,3-DMN	"	0.943	0.010	0.87	2.9
1,5-DMN	"	0.948	0.013	0.87	3.9
Acenaphthylene	"	0.956	0.009	0.49	2.4
1,2-DMN/2-IPN	"	0.966	0.039	1.29	4.2
2-IPN	"	0.969	0.012	1.80	3.6
1,8-DMN	"	0.996	0.012	0.88	3.2
Acenaphthene	"	1.008	0.013	0.71	2.3
Fluorene	"	1.155	0.023	0.64	4.6
Dibenzothiophene	d_{10}-Phen	0.976	0.016	0.80	1.6
Phenanthrene	"	1.005	0.009	0.92	2.1
Anthracene	"	1.015	0.009	0.93	2.2
4-MDBT	"	1.081	0.014	1.26	3.4
2/3-MDBT	"	1.101	0.012	1.23	3.3
3-MP	"	1.124	0.010	1.24	4.5
1-MDBT	"	1.125	0.010	1.49	3.8
2-MP	"	1.129	0.013	1.06	3.5
4/9-MP	"	1.149	0.010	1.31	4.5
1-MP	"	1.153	0.012	1.07	7.1
4,5-DMP	"	1.178	0.032	2.46	27.9
3,6-DMP	"	1.220	0.040	1.56	7.3
3,5-DMP	"	1.223	0.040	1.98	8.3
2,6-DMP	"	1.225	0.039	0.92	7.1
2,7-DMP	"	1.228	0.042	1.08	6.5
3,9-DMP	"	1.236	0.042	1.16	7.6
1,6/2,5/2,9-DMP	"	1.240	0.041	1.44	5.7
1,7-DMP	"	1.243	0.044	1.06	7.2
1,9/4,9-DMP	"	1.249	0.042	1.47	6.5
Fluoranthene	"	1.250	0.042	0.77	7.1
1,5-DMP	"	1.252	0.043	1.69	7.6
1,8-DMP	"	1.256	0.046	1.11	8.0
1,2-DMP	"	1.264	0.048	1.49	8.1
9,10-DMP	"	1.275	0.050	1.56	9.0
Pyrene	"	1.276	0.047	0.79	10.7
Benzanthracene	d_{12}-Chrys	0.999	0.005	0.82	2.9
Chrysene	"	1.001	0.032	0.89	2.6
Benzo[b]fluor	d_{12}-Peryl	0.972	0.017	0.62	9.7
Benzo[k]fluor	"	0.974	0.020	0.58	9.2
Benzo[a]pyrene	"	0.995	0.009	0.79	3.7
Indeno[c,d]pyrene	"	1.107	0.051	1.14	25.7
Dibenzanthracene	"	1.112	0.043	1.16	24.8
Benzo[g,h,i]perylene	"	1.138	0.073	1.17	33.3

[a] RRT = RT x/RT SS.
[b] RRF = Concn x/Area x)/(Concn SS/Area SS).
[c] n = 20, data from daily calibrations over a 2-month period.

Table 6. Summary statistics for analysis of sea water samples spiked in triplicate at 4 concentrations in the parts-per-trillion range, showing mean recovery, mean calculated concentrations and standard deviations, and detection level statistics calculated from all results

	Recovery		Concentration means, ng/L					Detection limits, ng/L		
Analyte	Mean, %	SD, %	5 ppt[a]	10 ppt	50 ppt	100 ppt	Mean SD, ± ng/L	Precision, S_0	LOD, MDL	LOQ
Naphthalene	146	124	32.3	38.7	102	149	4.3	3.23	9.7	32.3
2-MN	56	27	9.0	14.9	65	124	5.7	0.61	1.8	6.1
1-MN	53	22	8.2	13.6	65	127	6.0	1.15	3.5	11.5
2-EN	34	6	3.8	8.2	55	112	6.4	2.32	6.9	23.2
1-EN	35	6	3.9	8.5	55	115	6.8	1.84	5.5	18.4
2,6/2,7-DMN	36	8	8.8	18.2	113	228	14.8	3.44	10.3	34.4
1,3/1,7-DMN	38	9	9.3	18.1	118	235	15.0	2.61	7.8	26.1
1,6-DMN	38	9	4.7	9.4	59	119	7.9	1.76	5.3	17.6
1,4/2,3-DMN	36	6	7.5	17.5	123	249	15.3	4.29	12.9	42.9
1,5-DMN	38	7	4.5	9.5	63	124	7.9	1.66	5.0	16.6
Acenaphthylene	41	8	5.1	9.9	52	106	1.2	0.16	0.5	1.6
1,2-DMN	37	7	4.3	8.5	62	124	7.9	1.93	5.8	19.3
1,8-DMN	42	8	4.5	10.7	66	131	7.8	2.18	6.5	21.8
Acenaphthene	46	10	6.7	11.8	52	103	1.4	0.63	1.9	6.3
1,6,7-TMN	43	9	5.1	10.9	69	133	8.9	1.55	4.6	15.5
Fluorene	50	10	7.2	12.7	58	109	1.5	0.34	1.0	3.4
Dibenzothiophene	51	8	2.7	5.2	24	49	0.5	0.38	1.2	3.8
Phenanthrene	70	22	8.8	17.0	54	103	2.0	2.13	6.4	21.3
Anthracene	47	7	4.4	9.1	49	98	0.6	0.38	1.1	3.8
4-MDBT	56	12	3.3	5.8	25	50	0.6	0.19	0.6	1.9
2/3-MDBT	50	9	4.0	8.0	40	78	0.6	0.43	1.3	4.3
3-MP	51	7	5.0	10.5	52	101	1.1	0.20	0.6	2.0
1-MDBT	52	9	3.8	7.4	35	70	0.8	0.44	1.3	4.4
2-MP	48	7	6.9	14.5	75	148	1.7	0.22	0.7	2.2
4/9-MP	50	8	7.2	15.2	77	151	1.8	0.07	0.2	0.7
1-MP	51	7	4.9	10.9	52	99	1.0	0.21	0.6	2.1
3,6-DMP	52	7	2.6	5.2	26	51	0.6	0.02	0.0	0.2
3,5-DMP	49	7	2.3	5.1	26	50	0.7	0.20	0.6	2.0
2,6-DMP	64	16	3.8	8.3	27	52	1.0	0.83	2.5	8.3
2,7-DMP	45	20	0.8	2.1	12	24	0.3	0.04	0.1	0.4
3,9-DMP	56	11	3.0	6.0	26	51	0.6	0.14	0.4	1.4
1,6/2,5/2,9-DMP	49	8	5.8	12.2	62	125	2.0	0.15	0.5	1.5
1,7-DMP	50	8	2.8	5.6	27	53	0.9	0.02	0.1	0.2
1,9/4,9-DMP	53	10	3.8	8.5	42	75	2.0	0.37	1.1	3.7
Fluoranthene	58	10	6.1	11.6	59	110	2.0	0.03	0.1	0.3
1,2-DMDBT	55	9	4.5	8.9	44	83	1.8	0.32	1.0	3.2
1,5-DMP	51	9	2.0	4.2	20	41	0.6	0.02	0.1	0.2
1,8-DMP	50	8	2.3	5.0	24	46	0.8	0.09	0.3	0.9
1,2-DMP	59	17	3.6	6.2	25	50	1.3	0.58	1.7	5.8
9,10-DMP	52	7	2.7	5.4	26	51	1.1	0.27	0.8	2.7
Pyrene	52	7	5.1	10.5	52	104	1.9	0.31	0.9	3.1
1,2,8-TMP	46	7	4.6	9.6	58	115	4.8	0.86	2.6	8.6
Benzanthracene	44	9	4.2	9.2	54	116	1.6	0.60	1.8	6.0
Chrysene	44	10	4.6	9.8	50	100	1.5	0.84	2.5	8.4
Benzo[b]fluor	38	12	4.2	8.7	55	131	3.7	0.66	2.0	6.6
Benzo[k]fluor	31	11	3.8	8.3	43	88	1.8	0.27	0.8	2.7
Benzo[a]pyrene	37	13	5.1	8.8	51	107	2.8	0.38	1.2	3.8
Indeno[c,d]pyrene	14	9	ND[b]	2.5	27	49	3.0	2.19	6.6	21.9
Dibenzanthracene	10	7	ND	1.8	16	32	4.9	0.38	1.1	3.8
Benzoperylene	15	9	ND	2.9	30	47	4.5	3.92	11.8	39.2
Means	47	12	5.4	9.8	50.9	99.8	3.5	0.96	2.9	9.6

[a] Indicates spike level.
[b] ND = not detected.

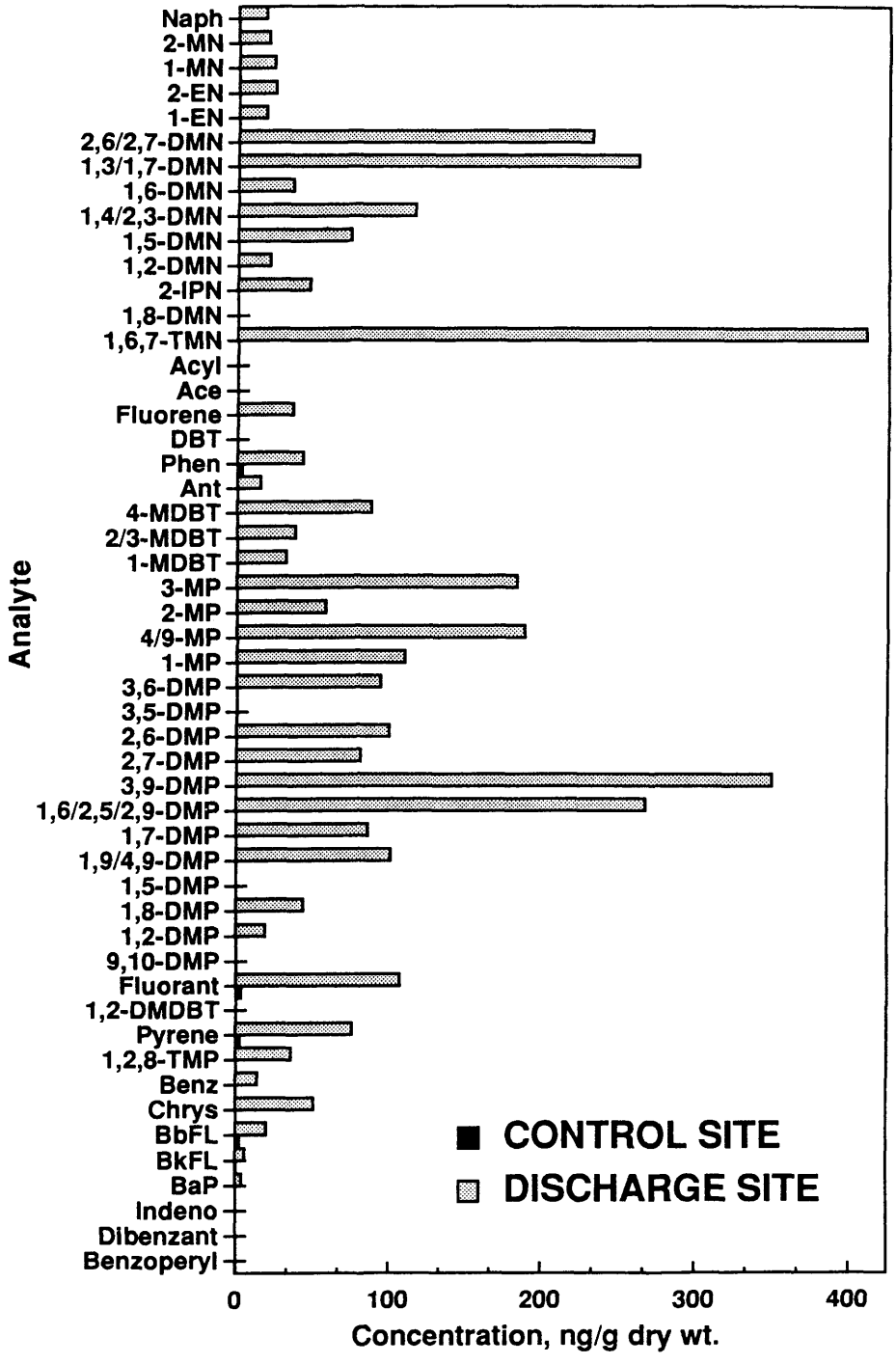

Figure 1. Alkylated and parent PAH profiles for contaminated and control site sediment samples.

pounds onto natural particulates and microparticulates (37) in natural sea water samples, which are removed on the SPE disk prefilter (particulates) or are partially unretained on the SPE disk (microparticulates). If materials retained on prefilters are extracted, then recoveries averaged >92% (data not shown). This prefilter was removed from the filtration apparatus prior to elution so that only dissolved components are measured. Mean SDs for replicate samples, averaged over all concentrations and analytes, was 3.5 ng/L. Linear regression of SD versus spiking level was used to determine SD at 0 concentration (y intercept, defined as S_0; 36). Precision (S_0), limit of detection (LOD; $3\times S_0$), and limit of quantitation (LOQ; $10\times S_0$) values (Table 6) were calculated from these data. Taylor (36) defines MDL, the method limit of detection, as $3\times S_0$, when determined from replicate analyses of samples at a minimum of 3 concentration levels. Because in the present case these calculations were performed on final concentrations in samples, the MDL calculated here also represents the LOD. When MDLs calcu-

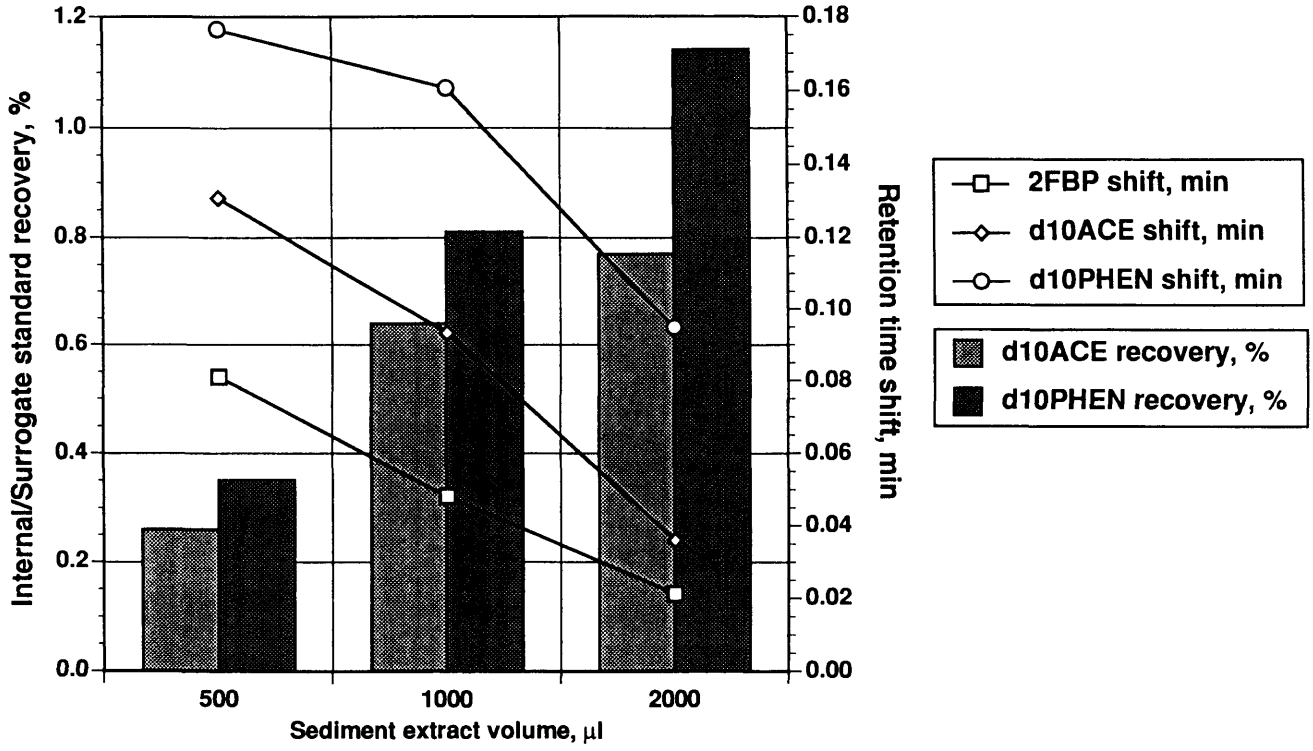

Figure 2. Inverse relationship between surrogate standard recovery and retention time shifts of selected internal and surrogate standards.

lated from the spiking experiment (Table 6) were compared with those calculated from standard calibration curve data (Table 4), the largest differences were observed for early eluting analytes, such as the naphthalenes. This observation may be due in part to the higher than expected values measured for these compounds (possibly volatile contamination) in the lowest-level spiked samples. Excluding the anomalous values for naphthalenes, the MDLs calculated from the spiking experiment averaged 1.2 times the values of MDLs calculated from standard calibration curve statistical data. This suggests that standard calibration curve data may be used reliably to estimate the MDL for compound-specific PAH analyses of dilute matrixes such as sea water samples, even though a less stringent ($3\times S_0$ versus $10\times S_0$) criterion is applied. The limits of detection reported here are consistent with other studies reporting such values for alkylated PAHs (6–8).

Sediments

Figure 1 presents a profile of analyte concentrations determined by SIM analysis in 2 sediment core samples: one collected 400 m downstream from a discharge of produced water at midchannel at Pass Fourchon, LA, and the other a reference "clean" sediment core collected from nearby Lake Champagne, LA. The profiles illustrate the importance of alkylated and heterocyclic PAHs compared with parent PAHs at this type of site. At sites associated with petroleum contamination, concentrations of C3-alkylated PAHs tend to be the highest among the alkylation groups (1, 7, 27). Use of a combination of individual isomer quantitation as well as class estimates for the higher alkylated PAH groups yields a relatively complete analysis of total PAHs in contaminated sediments. Analysis of such sediments for parent PAHs only, as is typically performed with EPA methods such as Method 8270, grossly underestimates the load of PAH contamination. The method described here may be adapted to include other contaminant groups by adding appropriate ions into the correct retention windows in the mass spectrometer program or by adding alkylated and heterocyclic isomeric standards as they become available. For example, estimates of total petroleum hydrocarbons (alkanes, alkenes, alicyclic hydrocarbons, etc.) may be obtained from the ion chromatogram for *m/z* 57 (17). Other classes of alkylated PAHs, such as alkylfluorenes and alkylchrysenes, also have been determined (15) by using the response factors for the parent nuclear PAHs. In some instances, it may be advantageous to include other biomarker compounds such as the hopanes (6) used by the petroleum industry for geochemical purposes.

In the case of relatively fresh inputs of petroleum discharges to sediment contaminant loading (e.g., chronic discharges or spills), SIM analysis allows simultaneous detection of parent, alkylated, and heterocyclic PAHs as well as of the normal hydrocarbons. If sediments are heavily contaminated (total PAH > 20 ppm) or extremely weathered, the sample extract typically may be diluted rather than fractionated to accommodate SIM analysis of aromatic and normal hydrocarbons while avoiding matrix effects commonly encountered with very concentrated analyte solutions, such as retention time shifts, peak broadening, and a large unresolved peak at the baseline. During development and application of the present method, shifts in certain

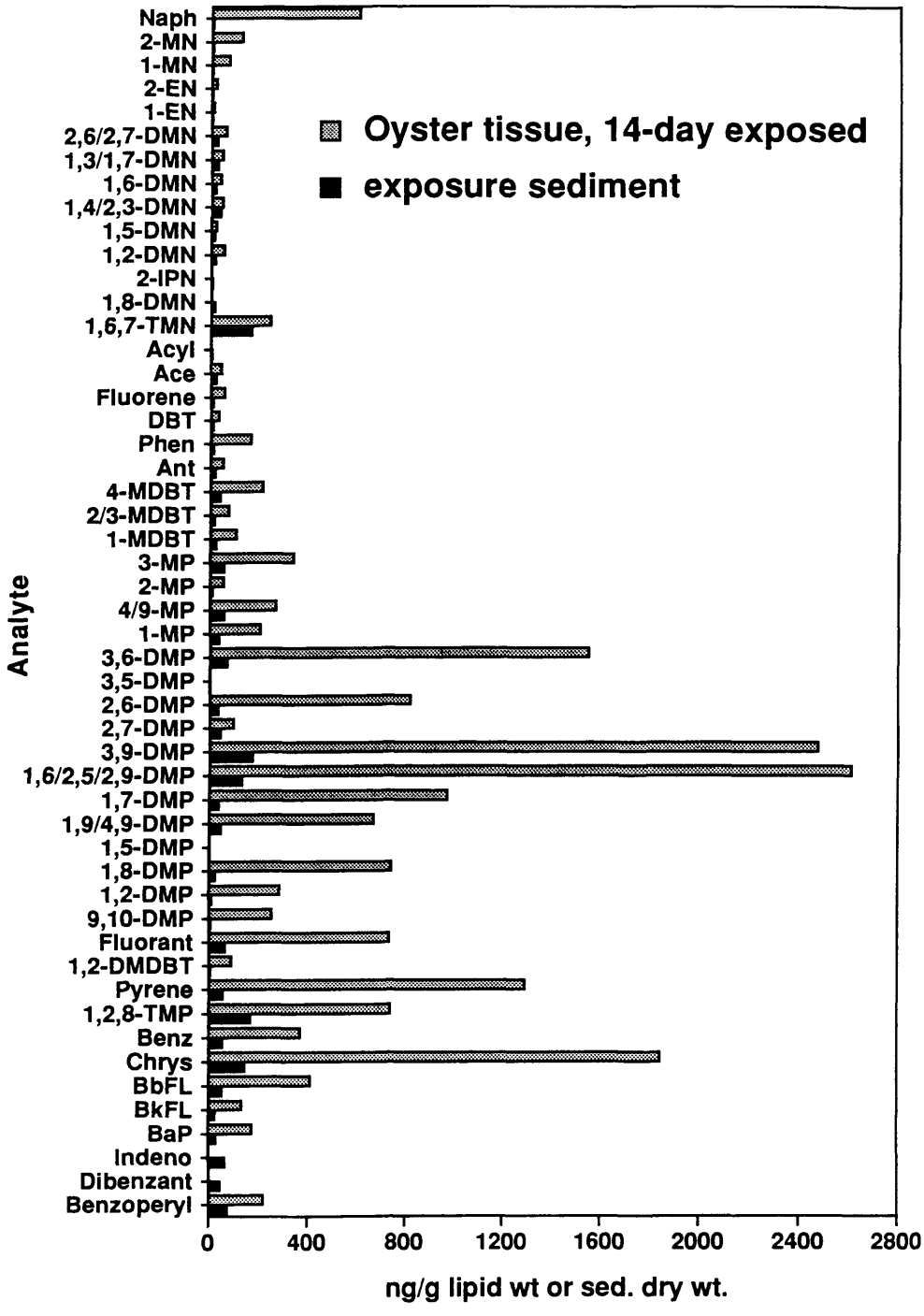

Figure 3. Alkylated and parent PAH profiles in oyster tissues exposed for 14 days to contaminated sediment compared with corresponding exposure sediment concentrations.

peak retention times were found to be useful indicators of injection port overload during analysis of samples containing multiple contaminant types (e.g., PCBs, herbicides, and PAHs), which may or may not be target analytes but which are coextracted and coinjected onto the gas chromatograph nevertheless. 2-Fluorobiphenyl and d_{10}-phenanthrene were used to evaluate whether injector overload was occurring, with retention time shifts greater than 0.02 and 0.09 min, respectively, as indicators. Figure 2 shows the inverse relationship between recovery of 2-fluorobiphenyl and 2 deuterated surrogates and retention time shifts for these analytes. Retention time shifts of these analytes were observed in many types of extracts from heavily contaminated sediments and resulted in an overall suppression of the mass spectrometer detector response, leading to erroneously low analyte concentrations. When overload indicator shifts are observed, the sample could be fractionated, di-

Table 7. Summary of QA/QC parameters of sediment and tissue analyses

Surrogate recovery	Sediments		Tissues	
	Mean recovery, %		Mean recovery, %	
d_8-NAPHTHENE	52.5		64	
d_{10}-ACENAPHTHENE	95.9		81	
d_{10}-PHENANTHRENE	113.1		86	
d_{12}-CHRYSENE	49.1		65	
2-Fluorobiphenyl	99.6		132	
d_{12}-PERYLENE	59.0		27	
Analyte	Mean difference, %	Mean spike recovery, %	Mean difference, %	Mean spike recovery, %
Naphthalene	15	54	62	90
2-MN	20	56	42	118
1-MN	23	57	46	121
2-/1-EN	20	60	20	127
2,6-DMN	47	79	30	125
2,7-DMN	32	66	30	125
1,3/1,7-DMN	23	60	28	126
1,6-DMN	23	60	31	124
1,4/2,3-DMN	18	59	5	129
1,5-DMN	NA[a]	66	NA	131
Acenaphthylene	39	70	32	98
1,2-DMN	38	61	37	128
Acenaphthene	32	69	47	104
1,6,7-TMN	25	72	14	144
Fluorene	21	75	42	108
Dibenzothiophene	26	76	8	81
Phenanthrene	23	71	29	96
Anthracene	35	73	61	94
4-MDBT	31	66	0	87
2/3-MDBT	33	70	30	77
3-MP	36	62	19	105
2-MP	31	62	25	97
4/9-MP	29	61	22	98
1-MP	32	60	18	101
3,6-/3,5-DMP	21	59	14	96
2,6-DMP	23	57	23	101
2,7-DMP	23	56	21	87
1,2-DMDBT	NA	62	23	95
3,9-DMP	21	49	24	93
1,6/2,5/2,9-DMP	25	59	27	93
1,7-DMP	21	52	17	93
1,9/4,9-DMP	22	52	20	93
1,5-DMP	NA	58	30	84
Fluoranthene	36	81	31	70
1,8-DMP	26	53	29	92
1,2-DMP	31	61	17	89
9,10-DMP	NA	53	41	92
Pyrene	34	89	22	86
1,2,8-TMP	33	119	25	135
Benzanthracene	36	59	18	108
Chrysene	34	56	18	111
Benzo(b)fluor	27	59	32	60
Benzo(k)fluor	NA	55	32	60
Benzo(a)pyrene	32	49	16	79
Indenopyrene	33	93	36	178
Dibenzanthracene	42	98	NA	122
Benzoperylene	28	96	3	112
Means	30	67	27	106

[a] NA = not applicable.

Table 8. Data from triplicate analysis of reference mussel tissue (NIST, SRM 1974), showing difference (%) of sample means from NIST certified/uncertified concentrations

Analyte	MDL[a], ng/g	Mean, ng/g[b]	SD, ng/g	RSD, %	NIST concn[c], ng/g	Difference, %
Naphthalene	0.18	1.6	0.058	3.5		
2-MN	0.071	1.9	0.92	48	(2.1 ± 0.5)	8
1-MN	0.10	1.2	0.22	19	(1.1 ± 0.2)	5
2-EN	0.14	ND[d]	NA[e]	NA		
1-EN	0.27	ND	NA	NA		
2,6/2,7-DMN	0.41	1.6	0.72	46		
1,3/1,7-DMN	0.49	1.3	0.29	23		
1,6-DMN	0.23	1.2	0.38	33		
1,4/2,3-DMN	0.37	0.70	NA	NA		
1,5-DMN	0.33	ND	NA	NA		
Acenaphthylene	0.19	0.69	0.021	3.1		
1,2-DMN	0.26	ND	NA	NA		
1,8-DMN	0.12	ND	NA	NA		
Acenaphthene	0.20	1.4	0.15	11		
1,6,7-TMN	0.18	2.6	0.96	37		
Fluorene	0.20	1.1	0.058	5.4	(1.5 ± 0.2)	29
Dibenzothiophene	0.24	1.6	0.23	14		
Phenanthrene	0.21	5.9	0.70	12	5.6 ± 1.4	6
Anthracene	0.30	1.2	0.40	34	0.75 ± 0.21	56
4-MDBT	0.13	4.1	0.30	7.3		
2/3-MDBT	0.26	1.8	0.23	13		
3-MP	3.9	TR[f]	NA	NA		
1-MDBT	0.084	1.1	0.12	11		
2-MP	0.35	4.4	0.87	20		
4/9-MP	0.36	3.8	0.50	13	(2.7 ± 0.6)	42
1-MP	0.23	2.4	0.29	12	(2.3 ± 0.6)	3
3,6-DMP	0.16	6.8	1.1	16	(4.2 ± 1.0)	63
3,5-DMP	0.33	ND	NA	NA		
2,6-DMP	0.11	4.4	0.55	13	(4.6 ± 0.9)	5
2,7-DMP	0.38	3.8	0.41	11	(4.3 ± 1.1)	11
3,9-DMP	0.13	13	1.7	13	(11 ± 2.0)	18
1,6/2,5/2,9-DMP	0.33	6.8	0.61	8.9	(5.8 ± 1.4)	17
1,7-DMP	0.32	5.5	0.55	10	(5.2 ± 1.1)	5
1,9/4,9-DMP	0.24	3.6	0.45	13		
Fluoranthene	0.38	35	3.2	9.1	33.6 ± 5.8	5
1,2-DMDBT	0.25	ND	NA	NA		
1,5-DMP	0.28	ND	NA	NA		
1,8-DMP	0.11	2.1	0.21	9.8		
1,2-DMP	0.19	1.6	0.36	23		
9,10-DMP	0.23	1.3	0.55	42		
Pyrene	0.37	36	3.6	10	34.1 ± 3.7	6
1,2,8-TMP	0.39	2.9	0.26	9.1		
Benzanthracene	0.51	4.5	0.40	8.9	(4.6 ± 0.4)	1
Chrysene	0.46	16	1.0	6.3	(15.3 ± 1.4)	5
Benzo[b,k]fluor	2.2	18	2.1	12	b = 6.5 ± 1.2	NA
Benzo[a]pyrene	1.4	13	1.5	12	2.29 ± 0.47	453[g]
Indeno[c,d]pyrene	1.7	TR	NA	NA	1.80 ± 0.33	NA
Dibenzanthracene	1.9	ND	NA	NA	(0.35 ± 0.01)	NA
Benzoperylene	0.77	TR	NA	NA	2.47 ± 0.28	NA
Average			0.72	16		17

[a] MDL = LOD (200 μL/(5.08 g avg sample wt × 2 μL injected).
[b] Corrected values: <Blank = ND, <MDL = TR.
[c] Noncertified SRM values are shown in parentheses.
[d] ND = not detected.
[e] NA = not applicable.
[f] TR = trace.
[g] Excluded from mean calculation.

luted, or reextracted with a smaller sample size to yield an extract that could be analyzed accurately.

Tissues

Figure 3 presents results of analysis of parent, alkylated, and heterocyclic PAHs from an experiment to measure bioaccumulation by aquatic organisms through exposures to PAH-contaminated sediments. These data were derived from a more comprehensive study of bioavailability of PAHs from sediments (14). A highly contaminated sediment, collected near the Pass Fourchon discharge site referred to above and analyzed in detail (Figure 1), was diluted with reference sediment to a final level of 25% and equilibrated for 30 days prior to exposure to oysters. (Undiluted sediment was lethal to the oysters within 7 days.) Sediment concentrations determined by this method were compared with concentrations in tissues from oysters exposed to the sediment for 14 days. Details are reported by Means et al. (14). Figure 3 also demonstrates the importance of alkylated and heterocyclic PAHs in identifying potential toxic effects on aquatic organisms, as well as in accurate risk assessments of potential food-chain transfers to humans through the food supply.

Recoveries of surrogate standards and individual PAH standard spikes in sediment and tissue samples derived from analyses of field samples are reported in Table 7. Mean recoveries of surrogate ranged from about 49 to 113% for sediment samples from the Gulf of Mexico and 27 to 132% for oyster tissue samples from a field investigation in the San Francisco Bay, CA. Overall mean differences between duplicate analyses were ±30 and ±27% for sediments and tissues, respectively, and mean spike recoveries for each component were 67 and 106% for sediments and tissues, respectively.

Table 8 presents results of triplicate analyses of the reference mussel tissue sample (SRM 1974, NIST) by the present methods. The mean relative standard deviation (RSD) of ±16% demonstrates the good precision of the methodology, while the mean deviation (±17%) from certified values demonstrate good accuracy for certified analytes. The mean deviation was calculated excluding benzo[a]pyrene, because this analyte alone showed a large positive deviation from certified values. This deviation is not due to unresolved benzo[e]pyrene, because these 2 isomers are routinely resolved under the analytical conditions. Benzo[e]pyrene typically is not reported in our analyses.

Conclusions

The compound-specific analysis of 63 alkylated, heterocyclic, and parent PAHs in water, sediments, and biological tissue samples by GC/MS was developed and applied to studies of the fate, transport, and bioavailability of PAHs in petroleum-contaminated environments. The methodology allows quantitation of PAHs in a wide range of environments, from heavily contaminated sediments (total PAHs in the ppm range) to ultratrace levels in sea water (ppt) by use of either scanning or multiple SIM MS analysis. The methodology has been field-tested and is applicable to assessments of impacts near discharge sites, studies of short-term and long-term fate and transport of these contaminants in aquatic ecosystems, assessments of impacts in benthic communities, and studies of bioaccumulation in aquatic organisms. The method for tissue analysis recently was coupled with a matrix solid-phase dispersion extraction procedure to allow analysis of very small biological samples (0.2 to 0.5 g wet weight; Means et al., in press). The inclusion of compound-specific analysis of alkylated and heterocyclic PAHs in evaluating toxicological and environmental impacts and potential risks of polluted environments, especially where the source of contamination is petroleum, is crucial because of the magnitude of their abundance compared with the 16 traditionally studied parent PAHs.

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