RESIDUES AND TRACE ELEMENTS

# Screening and Determination of Polycyclic Aromatic Hydrocarbons in Seafoods Using QuEChERS-Based Extraction and High-Performance Liquid Chromatography with Fluorescence Detection

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**A rapid, sensitive, and accurate method for the screening and determination of polycyclic aromatic hydrocarbons (PAHs) in edible seafood is described. The method uses quick, easy, cheap, effective, rugged, and safe (QuEChERS)-based extraction and HPLC with fluorescence detection (FLD). The method was developed and validated in response to the massive Deepwater Horizon oil spill in the Gulf of Mexico. Rapid and highly sensitive PAH screening methods are critical tools needed for oil spill response; they help to assess when seafood is safe for harvesting and consumption. Sample preparation involves SPE of edible seafood portions with acetonitrile, followed by the addition of salts to induce water partitioning. After centrifugation, a portion of the acetonitrile layer is filtered prior to analysis via HPLC-FLD. The chromatographic method uses a polymeric $C_{18}$ stationary phase designed for PAH analysis with gradient elution, and it resolves 15 U.S. Environmental Protection Agency priority parent PAHs in fewer than 20 min. The procedure was validated in three laboratories for the parent PAHs using spike recovery experiments at PAH fortification levels ranging from 25 to 10 000 µg/kg in oysters, shrimp, crab, and finfish, with recoveries ranging from 78 to 99%. Additional validation was conducted for a series of alkylated homologs of naphthalene, dibenzothiophene, and phenanthrene, with recoveries ranging from 87 to 128%. Method accuracy was further assessed based on analysis of National Institute of Standards and Technology Standard Reference Material 1974b. The method provides method detection limits in the sub to low ppb (µg/kg) range, and practical LOQs in the low ppb (µg/kg) range for most of the PAH compounds studied.**

Received January 19, 2011. Accepted by AK March 7, 2011.
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DOI: 10.5740/jaoacint.11-035

Polycyclic aromatic hydrocarbons (PAHs) are naturally occurring components of crude oils and coal deposits that are widely distributed into the environment via incomplete combustion of common fuels. PAHs in crude oils occur as 2- to 6-ring compounds (1–6), including both parent compounds (unsubstituted) and alkylated compounds (alkylated homologs). Total PAH contents for crude oils are commonly in the 1–2% (w/w) range (3–6). Several of the 4- to 6-ring compounds are reasonably anticipated to be human carcinogens and/or mutagens (7–10), with benzo[a]pyrene and dibenz[a,h]anthracene having the greatest toxicity.

PAHs are occasionally released into marine environments via crude oil spills. The largest oil spill in U.S. history took place from April 20 to July 15, 2010, in the aftermath of the Deepwater Horizon (DWH) oil production platform explosion. Estimates place the amount of crude oil that flowed into the Gulf of Mexico from this event somewhere in the range of 4–5 million barrels (11, 12). When oil spills occur, seafood obtained from suspect marine waters must be monitored and analyzed for PAH levels in order to assess whether the edible tissues will be safe for harvesting and consumption. While the assessment criteria may vary on an international basis, PAH monitoring typically involves the determination of targeted parent PAH compounds and selected alkylated homologs (9, 13–17). The toxicity of the alkylated homologs and various approaches for their health hazard assessment continue to be studied (18).

GC/MS after sample extraction and cleanup is an

established method for the analysis of seafood tissue samples for select PAH compounds in the ng/g (ppb) range (17). Most of the targeted PAH compounds are also strong fluorophores, and there is an established history of fluorescence-based analysis for the screening of edible seafood for parent PAH compounds, alkylated homologs, and/or metabolites (19–27). Fluorescence-based analysis is often used as a rapid means to assess the need for additional/confirmatory analysis using GC/MS. Some of this work involves methods for the estimation of total PAHs after size exclusion chromatography, with no compound specific information obtained (21, 23, 24). In contrast, methods for the screening/determination of individual PAHs in seafood (20, 22, 25–27) are based on RP-HPLC with fluorescence detection (FLD). RP-HPLC-FLD provides good resolution of the parent PAH compounds, high selectivity, and detection limits in the sub to low ppb range (20, 22, 26, 27). RP-HPLC-FLD has also been used for the determination of PAHs in other foods, including smoked meats and cheese (20, 28), tea infusions (29), and edible oils (30–33).

Edible seafood tissues are complex analytical matrixes, and protocols developed for extraction and cleanup have typically been labor-intensive, time-consuming, and expensive. Sample preparation for the established GC/MS protocol (17) is particularly labor-intensive; the multiday procedure involves extraction with dichloromethane, gravity filtration through a silica/alumina column, filtrate preconcentration, and fractionation of the concentrated filtrate using size exclusion HPLC. Other extraction protocols for PAHs in foods (20, 22, 28) have used digestion in aqueous/alcoholic KOH followed by liquid–liquid extraction with hexane or halogenated solvents. Cleanup protocols (20, 22, 28–33) have involved open or preparative column chromatography, additional liquid-liquid partitioning, SPE, preconcentration steps, and/or solvent exchanges. In addition, stringent cleaning of glassware used in PAH analysis is often conducted to prevent environmental and/or sample to sample contamination (22, 24, 28). There has been a longstanding need to develop simpler and more rapid methods, especially given the urgency to respond after massive oil spills.

The quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction and cleanup approach was introduced by Anastassiades et al. (34) for multiresidue pesticide determination in fresh fruits and vegetables, and was later extended to fatty foods (35) and validated in a collaborative study (36). The QuEChERS approach is based on single phase extraction in acetonitrile ($CH_3CN$), followed by the addition of salts to induce water partitioning into a separate phase. Dispersive SPE with an appropriate sorbent is used for subsequent cleanup. A host of partitioning salts, including magnesium sulfate ($MgSO_4$), NaCl, sodium acetate (NaOAc), and cleanup sorbents, including primary secondary amine (PSA), $C_{18}$, and alumina, have been evaluated (34, 35). The QuEChERS approach lends itself to kits that are commercially available from several vendors as separate, disposable extraction tubes with premeasured salt packets and dispersive SPE cleanup tubes preloaded with sorbent(s). The finished $CH_3CN$ extracts are suitable for LC or GC analysis (36).

Recently, Ramalhosa et al. (26) evaluated the use of single phase $CH_3CN$ extraction with QuEChERS kits for the analysis of parent PAHs in fish, including mackerel, sardines, and seabass (26). Partitioning was conducted using 6 g $MgSO_4$ and 1.5 g NaOAc salts, and dispersive SPE cleanup tubes containing 900 mg $MgSO_4$, 300 mg PSA sorbent, and 150 mg $C_{18}$ sorbent were evaluated for cleanup. Finished extracts were analyzed using RP-HPLC-FLD analysis with a Nucleosil $C_{18}$ PAH column (Macherey-Nagel, Bethlehem, PA). After observing no significant interferences in the analyzed extracts prior to or after cleanup, the authors eliminated the dispersive SPE cleanup step. They reported recoveries ranging from 84 to 111% based on spiking experiments for 16 parent PAHs in horse mackerel, and quantification limits ranging from 0.12 to 1.90 ng/g.

Sensitivity is one of the primary concerns for PAH screening methods. The lowest levels of concern (LOC) in edible seafood are set for the most potent carcinogens, benz[a]pyrene and dibenz[a,h]anthracene, with current LOCs established by the U.S. Food and Drug Administration (FDA) in the range of 35–143 µg/kg across various seafood types (37). In this work, QuEChERS-based extraction and cleanup were evaluated in conjunction with HPLC-FLD analysis for a variety of seafood matrixes, including oysters, shrimp, finfish, and crab. The work was conducted in the wake of the DWH oil spill, which provided a ready supply of suspect seafood for use in method development and assessment phases. The approach is based on the methodologies of Ramalhosa et al. (26) and Pule et al. (27), with important extensions of these former methods. Given the need for high sensitivity, special attention was given to the study of the extraction and cleanup protocols with respect to PAH recovery, and the more difficult seafood matrixes, such as oysters and shrimp, were addressed. The methodology was extended to address approximately 20 additional key PAH components of crude oils, including the alkylated PAH homologs of naphthalene, fluorene, and phenanthrene. The reported method provides a much simplified approach for the screening of PAHs in seafood, with significant increases in throughput relative to the established GC/MS protocol (17). Method validation was conducted in each of the three participating laboratories and is presented here.

## Experimental

### *Apparatus*

Apparatus used by the three participating laboratories are grouped in each section below.

(**a**) *HPLC Agilent systems.*—Chromatographic analysis and validation studies were performed using Agilent 1100 and 1200 Series LC systems (Agilent Technologies, Santa Clara, CA), each equipped with a quaternary (Agilent 1100) or binary (Agilent 1200) pump, continuous vacuum degasser, autosampler, thermostatted column compartment, and G1321A multiwavelength fluorescence detector. Agilent Chemstation software was used for instrument control and data analysis. Calibration and/or retention data from two different Agilent 1200 systems are designated as being from Systems 1 and 2. Extract stability data from an Agilent 1100 system is presented.

(**b**) *HPLC Dionex system.*—Preliminary method development experiments and source oil analysis were conducted using a Dionex Summit LC system (Dionex Corp., Sunnyvale, CA) equipped with a quaternary pump, vacuum degasser, autosampler, thermostatted column compartment, and RF2000 single wavelength programmable fluorescence detector. Dionex Chromeleon software was used for instrument control and data analysis. Data from the Dionex system is presented in this work as being from System 3.

(**c**) *Food processors and blenders.*—Edible tissue portions were composited using these food processors with stainless steel bowls: Robot Coupe® food processor (Robot Coupe, Jackson, MS) or Retsch Grindomix (Retsch, Inc., Newtown, PA). Blenders were used for smaller tissue portions (less than approximately 125 g) as follows: Magic Bullet® blender (Homeland Housewares, Los Angeles, CA) or Waring Laboratory Blender (Waring Products, Winsted, CT).

(**d**) *Analytical balances.*—Balances used were Mettler Toledo Models PF5002-S and TR-4104D (Mettler Toledo, Inc., Columbus, OH) and Ohaus Explorer Pro (Ohaus Corp., Pine Brook, NJ). Samples were weighed to an accuracy of 0.01 g.

(**e**) *Pipettors.*—Liquid pipettors were used according to volume ranges as follows: Rainin LTS (Rainin Instrument, Oakland, CA) or Eppendorf Research (Eppendorf, Westbury, NY) for 1–10 mL tranfers; Wheaton Socorex (Wheaton Science Products, Millville, NJ) for 0.5–5 mL transfers; Rainin EDP, Biohitmline (Biohit PLC, Helsinki, Finland), and Eppendorf Reference for 100–1000 µL transfers; and Rainin EDP, Biohitmline, or Eppendorf Reference for 10–100 µL transfers. An Eppendorf Repeater Model 4780 was also used.

(**f**) *Vortex mixers.*—Vortex mixing was used at various steps in the extraction protocol as noted in the text. Vortex mixers were Vortex Genie 2 (Scientific Industries Inc., Bohemia, NY), Fisher Scientific Digital (Fisher Scientific, Pittsburgh, PA), and VWR Analog (VWR International, Radnor, PA).

(**g**) *Sample shaker.*—A Tornado II Portable Paint Shaker (Blair Equipment Co., Swartz Creek, MI) was used by one laboratory to shake samples at various points in the sample workup, including after addition of water, after addition of the $CH_3CN$ extraction solvent, and after addition of the salt partitioning packet. An empty 1 gallon paint can was fitted with a styrofoam base that accommodated ten 50 mL extraction tubes. Sample shaking was done by hand in the other two laboratories.

(**h**) *Centrifuges.*—Centrifuges were Marathon 21000R (Fisher Scientific, St. Louis, MO), Thermo IEC Centra 6PG (Thermo IEC, Needham Heights, MA), and Thermo Sorvall Legend XFR. Centrifugation was conducted for 10 min at 3000 × *g*.

### Materials and Reagents

(**a**) *Solvents and water.*—HPLC grade $CH_3CN$ and isopropyl alcohol (IPA) were obtained from Fisher Scientific. HPLC grade hexanes (95% *n*-hexane) were obtained from J.T. Baker, Inc. (Phillipsburg, NJ). Deionized (DI) water (18 Mohm) was produced using Milli-Q Gradient A-10 filtering systems (Millipore, Billerica, MA).

(**b**) *QuEChERS extraction tubes and ceramic homogenizers.*—QuEChERS 50 mL extraction tubes, AOAC **2007.01** method, the corresponding salt partitioning packets containing 6 g $MgSO_4$ and 1.5 g NaOAc, and the disposable ceramic homogenizers were obtained from Agilent Technologies.

(**c**) *QuEChERS dispersive SPE tubes.*—QuEChERS 15 mL dispersive SPE tubes containing 1200 mg $MgSO_4$ and 400 mg PSA sorbent (AOAC **2007.01** method, general fruits and vegetables) or containing 1200 mg $MgSO_4$, 400 mg PSA sorbent, and 400 mg $C_{18}$ ($C_{18}$ EC) sorbent (AOAC Method **2007.01**, general fruits and vegetables with fats and waxes) were obtained from Agilent Technologies. Dispersive SPE tubes were evaluated for sample cleanup and used only during the method development phase.

(**d**) *Syringes and filters.*—5 mL Luer lock polypropylene syringes (Becton Dickinson, Franklin Lakes, NJ) and 25 mm, 0.2 µm PTFE syringe membrane filters (Acrodisc CR, Pall Gelman Laboratory, Ann Arbor, MI) were used for filtration of $CH_3CN$ extracts after centrifugation.

### Standards and Reference Materials

(**a**) *Individual parent PAH compounds.*—Individual solution standards (200 µg/mL in methanol or methylene chloride) of naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene, and indeno[1,2,3-cd]pyrene) were obtained from Supelco (Bellefonte, PA). Benzo[e]pyrene was obtained from Supelco. Biphenyl and dibenzothiophene were obtained

from Aldrich (Milwaukee, WI). Individual standards were used to establish retention times.

(**b**) *Parent PAH stock standard mixes*.—For method development, a stock standard mix of 16 parent PAH compounds (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h] anthracene, benzo[g,h,i]perylene, and indeno[1,2,3-cd] pyrene) ranging from 10 to 50 µg/mL in $CH_3CN$ was obtained from Accustandard (New Haven, CT). For spike/recovery experiments and validation work, stock standard mixes of the same 16 parent PAH compounds at 2000 µg/mL in methylene chloride were prepared and/or obtained from Supelco and Absolute Standards, Inc. (Hamden, CT).

(**c**) *Individual PAH alkylated homologs*.—Individual standards of C1-C4 naphthalenes, C1-C3 fluorenes, and C1-C4 phenanthrenes were obtained as follows: 1-methylnaphthalene, 2-methylnaphthalene, 1,3-dimethylnaphthalene, and 2,7-dimethylnaphthalene from Acros Organics (Morris Plains, NJ); 1,6-dimethylnaphthalene, 2,6-dimethylnaphthalene, 1-methylfluorene, and 1-methylphenanthrene from Ultra Scientific (North Kingstown, RI); and 1,4-dimethylnaphthalene, 1,5-dimethylnaphthalene, and 2,3,5-trimethylnaphthalene from MP Biomedicals (Santa Ana, CA). Stock solutions (500–1000 µg/mL) of 1,2,5,6-tetramethylnaphthalene; 1,7-dimethylfluorene; 9-n-propylfluorene, 2-methylphenanthrene; 1,3-dimethylphenanthrene; 1,2,6-trimethylphenanthrene; and 1,2,6,9-tetramethylphenanthrene from Chiron AS (Emeryville, CA).

(**d**) *PAH alkylated homolog stock mix*.—A 20 component PAH compounds stock standard mix, with components ranging from 100 to 500 µg/mL in isooctane from Chiron. Alkylated naphthalenes and alkylated phenanthrenes account for 13 of the 20 components, with concentrations in the range 250–500 µg/mL. A listing of the components follows: 1-methylnaphthalene; 2-methylnaphthalene; 1,3-dimethylnaphthalene; 1,4-dimethylnaphthalene; 1,5-dimethylnaphthalene; 1,6-dimethylnaphthalene; 1,7-dimethylnaphthalene; 2,6-dimethylnaphthalene; 2,7-dimethylnaphthalene; biphenyl; phenanthrene; 1-methylphenanthrene; 2-methylphenanthrene; 3-methylphenanthrene; 9-methylphenanthrene; dibenzothiophene; 1-methyldibenzothiophene; 2-methyldibenzothiophene; 3-methyldibenzothiophene; and 4-methyldibenzothiophene. For HPLC-FLD analysis, a 30 000-fold dilution of the stock mix was made in IPA, followed by serial dilution in $CH_3CN$.

(**e**) *Standard reference material*.—Standard reference material (SRM) 1974b, Organics in Mussel Tissue, was obtained from the National Institute of Standards and Technology (NIST; Gaithersburg, MD).

(**f**) *DWH source oil*.—A 1 gallon sample of source oil from the DWH well riser was provided to FDA's Gulf Coast Seafood Laboratory in June 2010. The source oil was collected in May 2010 from aboard the Enterprise Discoverer drill ship, and was obtained directly from the MC252 well via the riser insertion tube. The source oil and all subsamples were kept refrigerated when not in use.

### *Preparation of Calibration Standards and Spiking Solutions*

(**a**) *Parent PAH calibration standard mixes*.—For calibration curves, parent PAH calibration standards mixes were prepared at concentrations of 50, 25, and 2.5 ng/mL by serial dilution of the 0.5 µg/mL parent PAH spiking solution mix (*see* part **b** below) in $CH_3CN$.

(**b**) *Parent PAH spiking solution mixes*.—Parent PAH spiking solution mixes with concentrations of 250, 5.0, and 0.5 µg/mL were prepared by serial dilution of the 2000 µg/mL parent stock standard mix in $CH_3CN$.

(**c**) *Parent PAH check standard mix*.—For calculation of spike/recovery, a parent PAH check standard mix was prepared at a dilution equivalent to the finished extracts from the spiked samples, or at 16.7 ng/mL, by serial dilution of the 250 µg/mL parent PAH spiking solution mix (see part **b** above) in $CH_3CN$.

(**d**) *PAH alkylated homolog spiking solution*.—The PAH alkylated homolog spiking solution, with concentrations of the alkylated naphthalenes and phenanthrenes ranging from 2.5 to 5.0 µg/mL, was prepared by serial dilution of the 250–500 µg/mL stock standard mix in $CH_3CN$.

### *Seafood Sample Sources and Composite Preparation*

For method development and optimization studies, seafood was obtained both from local grocery stores and harvested from Gulf of Mexico marine waters in and around the DWH oil spill. Seafood from grocery stores was obtained from the authors' three different locales (Cincinnati, OH; Newhaven, CT; and St. Paul, MN) and included fresh oysters, live blue crab, pasteurized lump crab, frozen cooked whole Dungeness crab, fresh whole shrimp, fresh cod fish, and fresh red snapper. The live blue crab was frozen after purchase. Seafood obtained from Gulf marine waters was refrigerated or frozen for shipment to the laboratories, and included whole oysters, shrimp, crab, and finfish (blue runner, gag grouper, spade fish, lady fish, Spanish mackerel, and red snapper). The oysters, crab, and finfish obtained from the Gulf waters, as well as most of the locally purchased seafood, were used in the validation studies.

Frozen seafood samples (oyster, shrimp, finfish, and crab) were partially thawed, and edible portions were collected. For oysters, the shells were separated, and the

contents (including liquor) were scraped from the shell and collected. For shrimp, the head, tail, and exoskeleton were removed. For finfish, the heads, tails, scales, fins, viscera, and bones were removed. If the skin was considered edible, it was also collected. For crab, the front claw was cracked, and the meat inside was removed. The top exoskeleton of the crab was removed, and the edible meat inside was removed. The crab viscera (including gills) were discarded. All of the seafood collected was homogenized using a food processor.

For finfish, homogenized tissue composites were generally made using individual fish. For oysters, shrimp, and crabs, composites were generally made using multiples from the same source or harvesting site, according to availability and/or other sampling criteria. The edible portion weights taken for compositing ranged from 90 to 300 g for oysters, 65 to 125 g for crabs, 50 to 150 g for shrimp, and 120 to 330 g for finfish. The oyster homogenate (combined meat and liquor) had a soup-like consistency. The crab and shrimp homogenates had a paste-like consistency, and the finfish homogenates were the consistency of shredded meat. Finished composites were stored in bell-type glass jars that had previously been rinsed with DI water or in whirl pack bags. The composites were refrigerated during periods of use for analysis (no more than 2 to 3 days) and then frozen for longer term storage.

### *QuEChERS-Based Extraction Procedure*

(**a**) *Optimization of sample weight and added water content in QuEChERS-based extraction.*—The effect of sample weight was evaluated for oysters and finfish, and the effect of adding water prior to extraction was evaluated for finfish. Spike/recovery experiments for parent PAHs (*see* text) were conducted in triplicate for oysters using sample weights of 5, 10, and 15 g, and a 15 mL $CH_3CN$ extraction volume. Oyster samples were fortified with 54 µL parent PAH stock mix containing 16 PAHs ranging from 10 to 50 µg/mL (*see Standards and Reference Materials*).

For finfish, spike/recovery experiments for parent PAHs (*see* text) were conducted using sample weights of 5 or 10 g, and a 15 mL $CH_3CN$ extraction volume. In addition, the effect of adding water (0, 5, or 10 mL) prior to extraction was evaluated for both sample weights. The finfish samples were fortified at two levels, using 54 µL parent PAH stock mix or 90 µL 1:10 dilution of the parent PAH stock mix. All finfish experiments were conducted in duplicate.

(**b**) *Evaluation of QuEChERS-based dispersive SPE cleanup.*—Two different dispersive SPE sorbents were evaluated for extract cleanup: PSA sorbent and PSA combined with $C_{18}$ EC (*see Materials and Reagents*). Spike/recovery experiments for 15 parent PAHs (*see* text) were conducted in triplicate for oysters, crabs, and shrimp comparing no dispersive SPE cleanup, PSA sorbent cleanup, and combined PSA/$C_{18}$ cleanup. Experiments were conducted using 5 g sample weights, no added water for oysters, 5 mL added water for crabs and shrimp, and a 15 mL $CH_3CN$ extraction volume. Samples were fortified with 54 µL parent PAH stock mix containing 16 PAHs, ranging from 10 to 50 µg/mL (*see Standards and Reference Materials*). The resulting fortification levels ranged from 110 to 540 µg/kg across PAHs, with a fortification level of 220 µg/kg for benzo[a]pyrene. Additional experiments were conducted for oysters and finfish in combination with other variables, including sample weight, extraction volume, and added water content (*see* also section **a** above). Multiple fortification levels were tested for oysters and finfish, with the overall fortification ranges across PAHs from 36 to 540 µg/kg for oysters and 10 to 540 µg/kg for finfish.

(**c**) *Finfish, shrimp, and crab.*—For extraction of finfish, shrimp, and crab, 5 g homogenized tissue composite was weighed into a QuEChERS extraction tube, and a ceramic homogenizer was added. Prior to extraction, 5 mL DI water was added, and the tube was capped, vortexed, and shaken for 1 min. For extraction, 15 mL $CH_3CN$ was added, and the tube was again capped, vortexed, and shaken for 1 min. For partitioning, the contents of one QuEChERS packet (6 g $MgSO_4$ and 1.5 g NaOAc) was added. The tube was capped, shaken vigorously for 1 min, then centrifuged (3000 × *g*, 10 min), allowing for removal of the $CH_3CN$ (upper) layer. A portion (approximately 4 mL) of the supernatant extract was filtered through a 0.2 µm PTFE syringe filter into a 4 mL amber glass vial for HPLC-FLD analysis and storage. Filtered extracts were analyzed without further dilution.

(**d**) *Oysters.*—For oysters, the same procedure was followed except that no water was added to the sample. The addition of water to homogenized oyster tissue composites was determined to be unnecessary due to the amount of water present in the native tissue.

(**e**) *SRM 1974b Organics in Mussel Tissue.*—For SRM 1974b, the procedure was identical to oysters. Due to the low levels of PAHs in the SRM, concentration of the filtered extract was necessary prior to analysis. A 1 mL volume of filtered extract was evaporated to dryness under a stream of dry air or nitrogen gas at ambient temperature, then reconstituted in 100 µL $CH_3CN$ for a 10-fold concentration.

### *HPLC-FLD Analysis*

HPLC-FLD analysis was conducted using 10 µL injections of finished extracts with the following conditions: Agilent Technologies Zorbax Eclipse PAH analytical columns (50 × 4.6 mm id, 1.8 µm particle size) fitted with Zorbax Eclipse analytical guard columns (12.5 × 4.6 mm id, 5 µm particle size); $CH_3CN$–water gradient elution with 30 min run time and 5 min re-equilibration time (*see* Table 1 for gradient conditions); flow rate, 0.8 mL/min; column thermostat, 18°C; excitation

**Table 1. HPLC mobile phase gradient conditions[a]**

| Time, min | Volume % $CH_3CN$ | Volume % water |
|---|---|---|
| 0.0 | 60 | 40 |
| 1.5 | 60 | 40 |
| 7.0 | 90 | 10 |
| 13.0 | 100 | 0 |
| 30.0 | 100 | 0 |
| 30.01 | 60 | 40 |
| 35.0 | 60 | 40 |

[a] Analysis run time 30 min with 5 min postrun re-equilibration time.

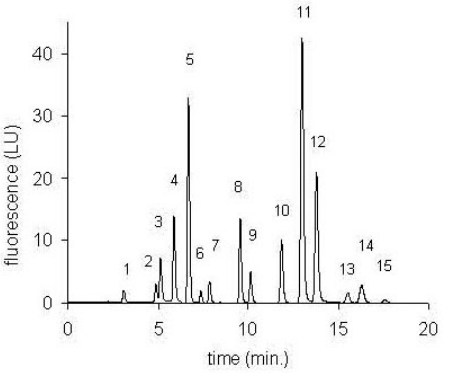


**Figure 1. HPLC-FLD chromatogram of a standard mix containing 16 parent PAHs each at a concentration 33 ng/mL. Peak labels: naphthalene (1), acenaphthylene (not observed), acenaphthene (2), fluorene (3), phenanthrene (4), anthracene (5), fluoranthene (6), pyrene (7), benzo[a]anthracene (8), chrysene (9), benzo[b]fluoranthene (10), benzo[k]fluoranthene (11), benzo[a]pyrene (12), dibenzo[a,h]anthracene (13), benzo[g,h,i]perylene (14), and indeno[1,2,3-cd] pyrene (15). Chromatogram obtained using wavelength switching.**

wavelength, 260 nm; multiple emission wavelengths, 352, 420, and 460 nm (multiwavelength with simultaneous monitoring of the three emission wavelengths); and photomultiplier tube (PMT) gain setting, 13.

Wavelength switching conditions (single wavelength monitoring with 352 nm detection from 0 to 6.35 min, 420 nm detection from 6.35 to 17.15 min, and 460 nm detection from 17.15 to 30 min) were used in method development and parent PAH spike/recovery studies. Wavelength switching conditions are only suitable for targeted parent PAH analysis, and are not applicable to general screening for PAHs and alkylated homologs.

*Note*: Mobile phase solvents require thorough degassing, both prior to use and throughout analysis, owing to the susceptibility of some PAH compounds to fluorescence quenching from dissolved oxygen. Either helium sparging or continuous inline vacuum degassing may be used.

### *Method Validation*

The entire method validation protocol for parent PAH compounds (parts **a**, **b**, **c**, and **e** below) was conducted in each of the three participating laboratories. Validation for the PAH alkylated homologs (part **d** below) was conducted in one laboratory only.

(**a**) *Parent PAH compound calibration*.—Three-point calibration curves were obtained in triplicate for 15 target parent PAH compounds (naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h] anthracene, benzo[g,h,i]perylene, and indeno[1,2,3-cd] pyrene) using the 16 parent PAH standard mix diluted to concentrations of 50, 25, and 2.5 ng/mL in $CH_3CN$. One compound in the 16 parent PAH standard mix, acenaphthylene, did not show appreciable fluorescence.

(**b**) *Parent PAH compound spike/recovery experiments*.—Spike/recovery experiments were conducted for the 15 target parent PAHs in oysters, crabs, shrimp, and finfish. Triplicate 5 g portions homogenized tissue composite were weighed into QuEChERS extraction tubes and fortified at three levels (high, mid, and low). For all four seafood matrixes, the high and mid fortification levels were 10 000 and 1000 µg/kg, respectively, which were prepared by addition of 200 or 20 µL aliquots of the 250 µg/mL parent PAH spiking solution mix. For oysters, crabs, and shrimp, the low fortification level was 50 µg kg, which was prepared by addition of 50 µL of the 5.0 µg/mL parent PAH spiking solution mix. For finfish, the low fortification level was 25 µg/kg, which was prepared by addition of 25 µL 5.0 µg/mL parent PAH spiking solution mix. Fortified seafood samples were allowed to sit for 30 min prior to extraction as described above. For mid- and high-level fortified samples, an additional dilution of finished extract (1:10 or 1:100 dilution in $CH_3CN$, respectively) was required prior to analysis to prevent saturation of the fluorescence detector.

(**c**) *Parent PAH compound method detection limit (MDL) and LOQ determination*.—MDL and LOQ for the 15 target parent PAHs were determined according to 40 *Code of Federal Regulations* (CFR) Part 136 (38) for the four seafood matrixes using a fortification level of 5 µg/kg and five replicates/matrix. For fortification, 50 µL 0.5 µg/mL parent PAH spiking solution mix was added to 5 g sample weights. Fortified seafood samples were allowed to sit for 30 min prior to extraction as described above. MDLs and LOQs were calculated as shown in Equations 1 and 2 below, where $s$ is the SD of the replicate results, $n$ is the number of replicates, and the Student's $t$-value for 99% confidence was used.

$$\text{MDL} = t_{n\text{-}1} \times s \qquad (1)$$

$$\text{LOQ} = 10 \times s \qquad (2)$$

**Table 2. HPLC-FLD calibration summary for parent PAHs in the concentration range of 0.0–50 ng/mL**

| Compound[a] | Retention time, min (RSD, %), $n$ = 9 | Linear regression values ($n$ = 3) | | |
|---|---|---|---|---|
| | | Slope | Intercept[b] | Correlation coefficient ($r^2$) |
| Naphthalene (Nph) | 3.1 (0.10) | 0.43 | 0.068 | 0.9999 |
| Acenaphthene (Ace) | 4.8 (0.07) | 0.59 | 0.062 | 1.000 |
| Fluorene (Flu) | 5.1 (0.06) | 1.6 | 0.22 | 0.9999 |
| Phenanthrene (Phe) | 5.8 (0.05) | 2.9 | 0.38 | 0.9999 |
| Anthracene (Ant) | 6.6 (0.05) | 7.0 | 1.1 | 0.9999 |
| Fluoranthene (Fla) | 7.3 (0.04) | 0.43 | 0.16 | 0.9998 |
| Pyrene (Pyr) | 7.8 (0.03) | 0.79 | 0.17 | 0.9999 |
| Benz[a]anthracene (BaA) | 9.5 (0.03) | 3.0 | 0.48 | 0.9999 |
| Chrysene (Chr) | 10.1 (0.04) | 1.2 | 0.15 | 1.000 |
| Benzo[b]fluoranthene (BbF) | 11.8 (0.08) | 2.7 | 0.82 | 0.9998 |
| Benzo[k]fluoranthene (BkF) | 12.9 (0.05) | 12 | 2.5 | 0.9999 |
| Benzo[a]pyrene (BaP) | 13.7 (0.04) | 6.4 | 1.0 | 1.000 |
| Dibenz[a,h]anthracene (DhA) | 15.4 (0.06) | 0.54 | 0.27 | 0.9997 |
| Benzo[g,h,i]perylene (BgP) | 16.1 (0.06) | 1.1 | 0.72 | 0.9996 |
| Indeno[1,2,3-cd]pyrene (IcP) | 17.3 (0.11) | 0.25 | 0.47 | 0.9960 |

[a] All data given for System 1. Regression data based on an emission wavelength of 352 nm for naphthalene, acenaphthene, fluorene, and phenanthrene; 420 nm for anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenz[a,h]anthracene, and benzo[g,h,i]perylene; and 460 nm for indeno[1,2,3-cd]pyrene.

[b] All intercept values were negative.

(**d**) *Alkylated homolog spike/recovery experiments*.—Spike/recovery experiments were conducted for alkylated naphthalenes and alkylated phenanthrenes in oysters, crabs, shrimp, and finfish based on the PAH alkylated homolog standard mix. Triplicate 5 g portions of homogenized tissue composite were weighed into QuEChERS extraction tubes. A 50 µL aliquot of the PAH alkylated homologs spiking solution was added, equivalent to fortification levels ranging from 25 to 50 µg/kg for the alkylated naphthalenes and phenanthrenes. Fortified seafood samples were allowed to sit for 30 min prior to extraction, as described above.

(**e**) *Method and solvent blanks*.—Method blanks were prepared by substituting 5 g DI water in place of sample tissue composite and performing the extraction procedure as described for oysters. Method blanks were analyzed routinely to monitor for contamination from laboratory sources. Solvent blanks ($CH_3CN$) were analyzed between samples to monitor for sample carryover.

### *Source Oil Analysis*

Approximately 10 mL DWH source oil was vortexed and mixed well just prior to sampling. Approximately 500 mg oil was transferred to a 10 mL volumetric flask, diluted to volume in hexane, and mixed well. Serial dilutions of the hexane solution were made as follows: 100 µL hexane solution taken to 10 mL in hexane (second hexane solution), then 100 µL second hexane solution mixed with 900 µL IPA (final dilution). The final dilution was analyzed using the current HPLC-FLD conditions and represented a 20 000-fold dilution of the oil.

## Results and Discussion

### *HPLC-FLD PAH Separation and Calibration*

The HPLC-FLD chromatogram for a parent PAH standard mix is given in Figure 1. Peak identifications were made based on injections of individual standards. The standard mix contains the 16 U.S. Environmental Protection Agency (EPA) priority PAHs: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[g,h,i] perylene, and indeno[1,2,3-cd]pyrene. All 16 PAHs eluted in under 20 min, and baseline resolution was obtained for all compounds except acenaphthene and fluorene (peaks 2 and 3). Acenaphthylene elutes between naphthalene and acenaphthene (peaks 1 and 2 in the Figure 1), but it is not observed in the FLD chromatogram because it has no appreciable fluorescence under the conditions used.

Three-point FLD calibration data were obtained in triplicate

**Table 3. Individual and average parent PAH recovery for oyster tissue homogenates in the fortification range 36–540 µg/kg as a function of sample weight and dispersive SPE cleanup protocol**

| | | Percent spike/recovery[a] | | |
|---|---|---|---|---|
| | | Sample weight, g | | |
| | | 5 | 10 | 15 |
| Dispersive SPE protocol | PAH compounds | *n* = 6 | *n* = 3 | *n* = 5 |
| No dispersive cleanup | Average across PAHs[b] | 93 | 84 | 78 |
| | Benzo[a]pyrene | 91 | 77 | 68 |
| | Dibenzo[a,h]anthracene | 89 | 71 | 60 |
| | Benzo[g,h,i]perylene | 85 | 63 | 54 |
| | Indeno[1,2,3-cd]pyrene | 88 | 80 | 54 |
| | | *n* = 3 | *n* = 3 | *n* = 7 |
| PSA sorbent only | Average across PAHs[b] | 92 | 85 | 78 |
| | Benzo[a]pyrene | 90 | 79 | 70 |
| | Dibenzo[a,h]anthracene | 84 | 71 | 59 |
| | Benzo[g,h,i]perylene | 80 | 62 | 52 |
| | Indeno[1,2,3-cd]pyrene | 90 | 73 | 53 |
| | | *n* = 3 | | *n* = 4 |
| PSA sorbent with $C_{18}$ EC sorbent | Average across PAHs[b] | 88 | — | 77 |
| | Benzo[a]pyrene | 83 | — | 68 |
| | Dibenzo[a,h]anthracene | 82 | — | 62 |
| | Benzo[g,h,i]perylene | 75 | — | 56 |
| | Indeno[1,2,3-cd]pyrene | 77 | — | 45 |

[a] All extractions were performed with 15 mL $CH_3CN$.

[b] Naphthalene not included in calculation of averages. PAH average recoveries represent anywhere from 10 to 14 parent PAHs.

over the range 0.0–50 ng/mL for the 15 target parent PAHs, and are summarized in Table 2 (multiwavelength detection). Retention time precision was excellent, with RSDs of 0.1% or less across all compounds. The 0.0 point was included in the regression calculations, but the regression curves were not forced through zero. A 50-fold range in the magnitude of slope values was observed across the PAHs, with the most sensitivity obtained for benzo[k]fluoranthene, anthracene, and benzo[a]pyrene. This difference in PAH response is due to the inherent differences in molar absorptivities and fluorescence quantum yields of the compounds using the current detection parameters. The calibration range was chosen to accommodate the differences in response across the 15 compounds; it was also based on a PMT setting of 13 (System 1). The actual linear range extends several times higher for many of the PAHs, and broader calibration ranges can also be obtained as needed for the most sensitive PAHs by using lower PMT setting(s). Separate experiments were conducted in which the HPLC-FLD response for the 15 parent PAHs was measured in oyster matrix extracts (matrix matched standards), and no matrix effect was observed.

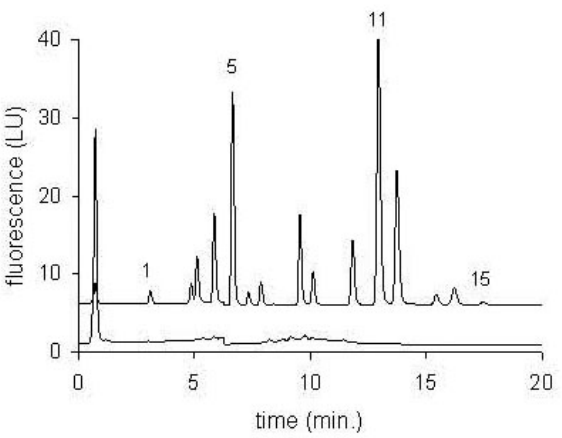


**Figure 2. HPLC-FLD chromatograms obtained from unfortified oyster tissue homogenate sample (lower trace) and sample fortified with parent PAH standard mix at 1000 µg/kg (upper trace). Peak labels as in Figure 1.**

**Table 4. Parent PAH recovery for three fortification levels in edible seafood matrixes analyzed in triplicate by three laboratories ($n$ = 9)**

| | Percent spike/recovery (RSD, %) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Oysters | | | Shrimp | | | Crab | | | Finfish | | |
| | Fortification level, µg/kg | | | Fortification level, µg/kg | | | Fortification level, µg/kg | | | Fortification level, µg/kg | | |
| PAH compound | 10000 | 1000 | 50 | 10000 | 1000 | 50 | 10000 | 1000 | 50 | 10000 | 1000 | 25 |
| Naphthalene | 90 (6.0) | 81 (7.2) | 94 (9.7) | 83 (5.9) | 78 (10) | 99 (13) | 86 (5.5) | 73 (18) | 92 (22) | 84 (3.0) | 78 (9.5) | 122 (25) |
| Acenaphthene | 94 (6.6) | 89 (4.0) | 87 (13) | 89 (3.9) | 83 (5.7) | 88 (13) | 93 (7.5) | 84 (9.5) | 82 (10) | 88 (2.5) | 84 (5.0) | 85 (4.8) |
| Fluorene | 95 (6.0) | 90 (4.6) | 91 (6.8) | 90 (3.6) | 85 (6.9) | 88 (8.6) | 94 (7.7) | 85 (6.4) | 85 (8.9) | 90 (3.3) | 86 (5.5) | 89 (6.3) |
| Phenanthrene | 94 (5.0) | 90 (5.7) | 99 (4.5) | 92 (2.2) | 87 (3.9) | 86 (5.7) | 95 (6.5) | 88 (4.8) | 87 (7.1) | 91 (4.6) | 87 (4.2) | 98 (8.7) |
| Anthracene | 93 (5.1) | 85 (5.4) | 93 (3.8) | 92 (3.1) | 87 (3.5) | 87 (7.4) | 93 (4.2) | 88 (5.0) | 86 (10) | 90 (6.9) | 86 (3.8) | 91 (6.2) |
| Fluoranthene | 94 (4.5) | 88 (5.8) | 97 (20) | 94 (1.8) | 88 (3.7) | 85 (6.6) | 95 (4.8) | 89 (4.7) | 86 (6.2) | 91 (5.2) | 86 (2.5) | 89 (3.9) |
| Pyrene | 92 (6.2) | 89 (6.5) | 96 (11) | 93 (2.9) | 86 (4.3) | 86 (7.1) | 94 (5.0) | 87 (5.5) | 87 (3.5) | 89 (5.9) | 85 (5.0) | 89 (6.4) |
| Benz[a]anthracene | 93 (5.9) | 88 (3.1) | 94 (2.7) | 94 (2.6) | 88 (2.8) | 88 (4.8) | 94 (3.0) | 89 (4.2) | 89 (3.7) | 90 (5.7) | 86 (3.9) | 88 (4.7) |
| Chrysene | 92 (5.8) | 88 (3.3) | 94 (3.4) | 96 (2.8) | 89 (3.6) | 87 (4.5) | 91 (6.7) | 89 (4.5) | 88 (3.1) | 90 (5.1) | 86 (4.2) | 87 (5.7) |
| Benzo[b]fluoranthene | 92 (7.1) | 86 (4.3) | 90 (2.8) | 94 (3.0) | 87 (3.4) | 88 (4.4) | 96 (4.7) | 90 (3.6) | 89 (3.3) | 90 (6.7) | 86 (4.1) | 88 (4.8) |
| Benzo[k]fluoranthene | 90 (6.6) | 85 (3.3) | 91 (1.9) | 94 (2.7) | 88 (3.0) | 89 (4.6) | 93 (4.0) | 89 (4.5) | 90 (2.9) | 89 (5.3) | 85 (4.1) | 89 (3.0) |
| Benzo[a]pyrene | 93 (9.6) | 80 (9.7) | 90 (3.9) | 93 (3.3) | 89 (3.9) | 89 (4.8) | 95 (4.1) | 91 (3.7) | 91 (3.6) | 86 (8.0) | 85 (9.4) | 93 (16) |
| Dibenz[a,h]anthracene | 88 (8.2) | 83 (4.0) | 88 (4.4) | 92 (3.6) | 87 (4.9) | 89 (3.9) | 90 6.7) | 89 (6.5) | 90 (1.2) | 89 (7.1) | 84 (4.2) | 87 (7.2) |
| Benzo[g,h,i]perylene | 85 (8.2) | 79 (3.7) | 87 (5.2) | 88 (3.6) | 84 (4.7) | 85 (4.2) | 87 (8.8) | 86 (6.7) | 90 (1.9) | 85 (8.4) | 81 (8.2) | 86 (11) |
| Indeno[1,2,3-cd]pyrene | 88 (10) | 81 (5.8) | 91 (7.5) | 91 (5.3) | 84 (6.9) | 87 (13) | 92 (7.4) | 90 (11) | 85 (7.0) | 86 (8.8) | 81 (10) | 78 (18) |

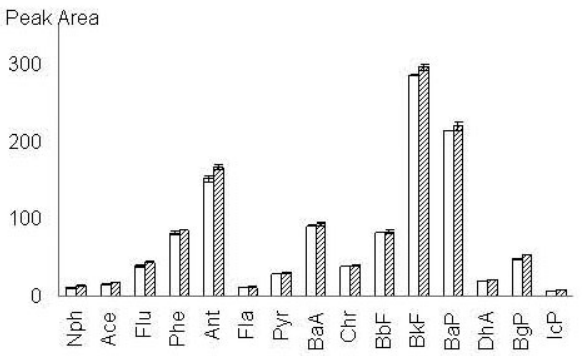


**Figure 3. PAH stability study for QuEChERS $CH_3CN$ extracts of crab spiked with 15 parent PAHs at 50 µg/kg each. Results given as PAH peak area averages for three crab extracts from original analysis (open bars) and after 28 days storage at room temperature (hatched bars). *See* Table 2 for abbreviations. Error bars represent SD for the three extracts, and they are not discernable in many cases given the high precision of the results.**

### *PAH Extraction and Sample Cleanup*

PAH extraction and dispersive SPE sample cleanup were studied for oysters, crab, shrimp, and finfish based on spike/recovery experiments using $CH_3CN$ as the extractant and two different dispersive SPE sorbents. Spiking levels ranged from 110 to 540 µg/kg across the 15 PAHs for oysters, shrimp, and crab, and from 18 to 90 µg/kg for finfish, based on the concentration range of the PAHs inherent in the stock standard mix. In particular, the benzo[a]pyrene spiking level was 220 µg/kg for oysters, shrimp, and crab, and 36 µg/kg for finfish.

Experiments were conducted without any dispersive SPE cleanup and comparing dispersive SPE cleanup based on PSA sorbent only with cleanup based on combined PSA and $C_{18}$ encapped sorbents (PSA with $C_{18}$ EC; *see* also the *Experimental* section). Experiments in which no dispersive cleanup was used showed that oysters and shrimp were more difficult matrixes for PAH extraction/recovery compared to crabs and finfish. The effects of sample weight and extraction volume were also studied for oysters and finfish, and the effect of added water content was studied for finfish.

PAH spike/recovery results are summarized in Table 3 for oyster sample weights of 5, 10, or 15 g using a 15 mL $CH_3CN$ extraction volume. The results in the table demonstrate several trends related to PAH extraction and recovery that were observed in iterative experiments with all of the seafood matrixes. PAH recoveries for experiments without dispersive SPE cleanup were comparable to recoveries in which dispersive SPE cleanup based on PSA sorbent only was used, but recoveries for cleanup using PSA sorbent combined with $C_{18}$ EC sorbent tended to be lower. The larger PAHs were observed to be both more difficult to extract from the matrix compared to the smaller PAHs, and more prone to losses associated with the dispersive SPE cleanup step, with diminishing recoveries for benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene, and indeno[1,2,3-cd]pyrene. The difficulty of extracting PAHs from the oyster matrix is clearly demonstrated in Table 3, where both average PAH recovery and recoveries for the larger PAHs decrease with increasing sample weight.

Examination of the HPLC-FLD chromatograms for peak interferences also showed no notable differences when comparing extracts prior to and after dispersive SPE cleanup. This is most likely due to the selective nature of fluorescence detection. There were also no apparent effects observed for column performance over a period of months related to the injection of extracts without cleanup. One of the three laboratories reported changing the guard column twice during this timeframe. Hence, the dispersive SPE cleanup step was eliminated from the protocol because it did not provide any certain advantages for the analysis, yet it required more time, labor, and cost. The finished protocol uses a 5 g sample weight for all of the edible seafood matrixes, with a 15 mL extraction volume. A 5 mL portion of water is added to shrimp, crab, and finfish matrixes prior to extraction. Oysters do not require the addition of water prior to extraction as there is already sufficient water in the tissue homogenate. Extracts are simply filtered after the water partioning step, and they are ready for analysis.

### *Method Validation for Parent PAH Spike/Recovery*

The method was validated for parent PAHs using fortification levels of 10 000, 1000, and 50 µg/kg for edible portions of oysters, shrimp, crabs, and finfish (low fortification level for finfish was 25 µg/kg). This 200- to 400-fold range in fortification levels was meant to represent method performance over a broad range of PAH contents, so as to address the large differences in LOC among the various carcinogenic PAH compounds. Current guidelines (35) have set the lowest LOC among carcinogenic PAHs in the range of 35–143 µg/kg (e.g., benzo[a]pyrene) across various seafood types, and the highest LOC in the range 3500–14 300 µg/kg (e.g., benzo[k]fluoranthene).

Figure 2 provides a representative chromatogram of an unfortified oyster tissue homogenate (lower trace), and the same sample at the 1000 µg/kg fortification level (upper trace). A very broad, low-intensity peak was observed in the retention window from 6 to 15 min for the unfortified sample, which was typical for oysters. No shift in PAH retention time or loss in retention time precision was observed for any of the seafood matrixes at any of the fortification levels. Table 4 provides the PAH recovery results for the three fortification levels in the four seafood types, with each value based on

**Table 5. Retention and response factor data for related PAHs and PAH alkylated homologs**

| Compound | Retention time, min | | Relative response factor[a] |
|---|---|---|---|
| | System 3 | System 2[b] | |
| Naphthalene | 3.03 | 2.80 | 1.0 |
| 1-Methylnaphthalene | 4.17 | 3.92 | 1.2 |
| 1,4-Dimethylnaphthalene | 5.64 | 5.24 | 0.68 |
| 1,5-Dimethylnaphthalene | 5.65 | 5.23 | 2.1 |
| 1,3-Dimethylnaphthalene | 5.79 | 5.36 | 1.5 |
| 2,7-Dimethylnaphthalene | 6.21 | 5.73 | 1.5 |
| 2,3,5-Trimethylnaphthalene | — | 6.29 | 0.56 |
| 1,2,5,6-Tetramethylnaphthalene | — | 7.90 | 1.9 |
| Biphenyl | 4.03 | 3.81 | 2.3 |
| Fluorene | 5.08 | 4.72 | 4.0 |
| 1-Methylfluorene | 6.74 | 6.19 | 3.0 |
| 9-n-Propylfluorene | — | 7.12 | 3.1 |
| 1,7-Dimethylfluorene | — | 8.09 | 5.7 |
| Dibenzothiophene | 5.72 | 5.30 | 1.0 |
| Phenanthrene | 5.92 | 5.46 | 5.8 |
| 1-Methylphenanthrene | 7.63 | 7.00 | 7.3 |
| 2-Methylphenanthrene | — | 7.37 | 3.6 |
| 1,3-Dimethylphenanthrene | — | 7.89 | 8.1 |
| 1,2,6-Trimethylphenanthrene | — | 9.62 | 6.4 |
| 1,2,6,9-Tetramethylphenanthrene | — | 10.2 | 3.4 |
| Benzo[e]pyrene | 12.1 | 11.2 | 1.4[c] |
| Benzo[a]pyrene | 14.8 | 13.5 | 6.1[c] |

[a] Response factor [peak area/concentration (ppb)] calculated and ratioed to naphthalene response factor. Relative response factor given for System 2, and with λem of 352 nm for all compounds unless noted.

[b] Shorter retention times for System 2 due in part to no UV-Vis detector in system configuration, as was the case for Systems 1 and 3.

[c] Response factor with λem of 420 nm for these compounds.

triplicate experiments in the three validating laboratories ($n$ = 9). The results for naphthalene were corrected for the background signal from method blanks, and they are considered estimates. The naphthalene background levels observed in the method blanks typically fell in the range of 5 to 10 ppb (ng/mL, solution concentration basis), which is equivalent to a range of 15–30 ng/g for the 5 g sample weight and 15 mL extract volume. The vast majority of the recovery results are in range 85–99% with good precision (<10% RSD). The lowest recovery was 78% (excluding naphthalene results).

The stability of the fortified seafood extracts was assessed by one of the laboratories using the extracts with the lowest fortification level (50 µg/kg). The extracts were stored under ambient conditions in the QuEChERS tubes with no special precautions to maintain stability (benchtop storage in either racks or boxes). The triplicate extracts for each sample were reinjected after storage for 27 days (oysters), 25 days (shrimp), 28 days (crab), or 22 days (finfish). Results for the crab extracts are given in Figure 3, with the average peak areas from the three samples compared for the original analysis (open bars) and after the storage period (hatched bars). The results for crab and the other seafood matrixes showed little to no changes in peak areas across all 15 parent PAHs, indicating that the extracts were stable throughout the test period.

### *DWH Source Oil and PAH Alkylated Homolog Validation*

Alkylated homologs of the parent PAHs are known to represent a large proportion of the PAH content in crude oils, including alkylated homologs of naphthalene, fluorene, dibenzothiophene, phenanthrene, and chrysene (4–6). Oil spill sourcing studies (4) target specific alkylated PAHs, including the $C_1$- to $C_4$-naphthalenes and phenanthrenes, and the $C_1$- to $C_3$-fluorenes, dibenzothiophenes, and chrysenes. Retention data for

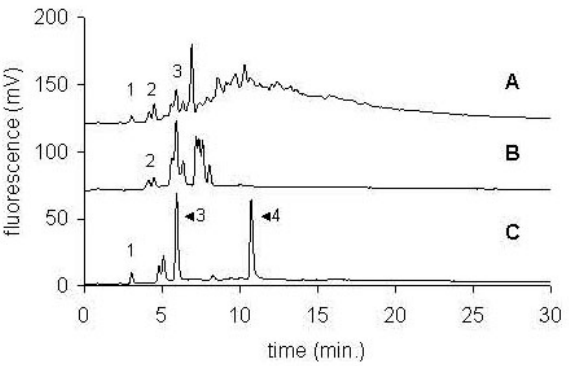


**Figure 4. 352 nm HPLC-FLD chromatograms of (A) the DWH source oil, (B) the alkylated PAH homologs standard mix, and (C) the parent PAH standard mix. Peaks corresponding to (1) naphthalene, (2) a C1-naphthalene, (3) phenanthrene, and (4) chrysene are noted. Naphthalene (1) is present in the source oil (A) and the parent PAH standard mix (C) chromatograms. A $C_1$-naphthalene (2) is present in the source oil (A) and the alkylated PAH homologs standard mix (B) chromatograms. Phenanthrene (3) is present in all three chromatograms (A, B, C).**

several alkylated homologs of naphthalene, fluorene, and phenanthrene, and other crude oil-related PAHs were obtained based on injection of the individual standards, and are given in Table 5 for two different HPLC systems (Systems 2 and 3). The data show a large amount of retention window overlap among the series of

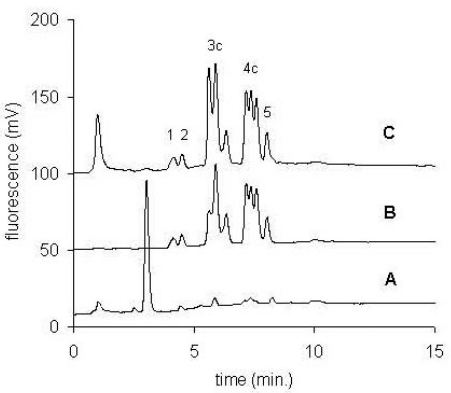


**Figure 5. HPLC-FLD chromatograms obtained for an extract of finfish (lower trace A), the PAH alkylated homolog standard mix (middle trace B), and an extract of finfish fortified with the standard mix (upper trace C). Spiking levels for the individual alkylated naphthalene and phenanthrene-based PAHs were in the 25–50 µg/kg range for finfish. The emission wavelength for detection was 352 nm. Peaks (1, 2, 5) or peak clusters (3c, 4c) used for spike/recovery calculations are noted in the figure. The large peak in the unfortified extract (A) corresponds to naphthalene. *See* also Table 6 and the text for discussion.**

naphthalenes (range 3–8 min), fluorenes (5–9 min), and phenanthrenes (6–11 min). Response factors relative to naphthalene were calculated for these PAH compounds by ratioing the peak area/concentration (ppb) for each compound to that of naphthalene. They are also given in the table (last column). The relative response factors show an equivalent to higher fluorescence response under the method conditions for most of the PAH compounds versus naphthalene. Higher responses were obtained as a whole for the series of alkylated fluorenes and phenanthrenes versus the alkylated naphthalenes.

In order to assess the DWH source oil PAH profile, a sample of the source oil was analyzed using the HPLC-FLD method. A 20 000-fold dilution of the whole oil was prepared using serial dilutions in hexane followed by IPA (*see Experimental* section). The DWH source oil chromatogram (352 nm) is given in Figure 4 (upper trace A), compared to the chromatograms for the alkylated PAH homolog standard mix (B) and the parent PAH standard mix (C). The source oil exhibits a series of closely spaced and overlapping peaks from approximately 3 to 25 min (A), then the chromatogram returns to baseline. Peaks consistent with naphthalene, a $C_1$-naphthalene, and phenanthrene (peaks 1, 2, and 3, respectively) are discernible in the source oil profile (A). Much less intensity was observed in the source oil 420 nm chromatogram (not shown), and no peaks were observed in the 460 nm source oil chromatogram. The alkylated PAH homolog standard mix contains 20 PAH compounds, including phenanthrene, dibenzothiophene, and series of $C_1$- and $C_2$-naphthalenes, $C_1$-phenanthrenes, and $C_1$-dibenzothiophenes. All 20 compounds exhibit substantial fluorescence at 352 nm, and are observed with much coelution in the corresponding chromatogram (B) in the retention window from 4 to 9 min. Only five of the 16 components in the parent PAH standard mix (naphthalene, acenaphthene, fluorene, phenanthrene, and chrysene) exhibit substantial fluorescence at 352 nm, and these are observed in the corresponding chromatogram (C). The DWH source oil is known to be a lighter crude type oil, and its composition is known to be particularly high in naphthalene- and phenanthrene-based PAHs, with lesser amounts of the fluorenes, dibenzothiophenes, and chrysenes (39). The DWH source oil chromatographic profile and high fluorescence response at 352 nm are consistent with its known composition.

Recovery experiments were conducted in triplicate for the alkylated PAH homologs in oysters, crab, shrimp, and finfish based on spiking the matrixes with the alkylated PAH homolog standard mix. A single fortification level was tested, with resultant spiking levels in the 25–50 µg/kg range for the individual naphthalene- and phenanthrene-based PAHs. HPLC-FLD chromatograms (352 nm) are given in Figure 5 for an unfortified finfish sample, the alkylated PAH homolog standard mix (B), and a fortified finfish sample (C). Recovery results are given in Table 6

**Table 6. Recovery data for alkylated PAH homologs in edible seafood with fortification levels of 25–50 µg/kg**

| Peak/peak cluster No.[a] | Retention times, min | Assigned compound(s) | Recovery, % (RSD, %), $n$ = 3 | | | |
|---|---|---|---|---|---|---|
| | | | Oysters | Shrimp | Crab | Finfish |
| 1 | 4.1 | Biphenyl, 1-Methylnaphthalene | 128 (1.4) | 107 (3.5) | 120 (4.7) | 119 (1.2) |
| 2 | 4.5 | 2-Methylnaphthalene | 122 (3.6) | 103 (0.36) | 107 (8.2) | 116 (3.9) |
| 3c | 5.6, 5.9, 6.4 | Dimethylnaphthalenes, dibenzothiophene, phenanthrene | 119 (0.31) | 109 (1.0) | 108 (4.9) | 115 (2.1) |
| 4c | 7.2, 7.4, 7.6 | Methylphenanthrenes, methyldibenzothiophenes | 107 (1.7) | 114 (0.51) | 115 (2.2) | 117 (2.0) |
| 5 | 8.0 | Methylphenanthrenes | 87 (1.1) | 127 (1.5) | 119 (0.8) | 122 (0.10) |

[a] *See* also Figure 5.

and range from 87 to 128% across the four seafood matrixes with good precision. As a whole, the alkylated PAH homologs are not chromatographically resolved, and peak or peak cluster assignments are noted in both Figure 5 and Table 6. Although the alkylated PAH homologs are not chromatographically resolved, the 352 nm chromatogram may be used to calculate an estimate for total amounts of both parent and alkylated PAHs for the naphthalenes, acenaphthenes, fluorenes, phenanthrenes, dibenzothiophenes, and chrysenes. Conservative estimates (overestimates) can be made by using the calibration data based on the parent PAH compound with the lowest response, i.e., naphthalene (*see* slopes, Table 2, and relative response factors, Table 5).

### *MDL and LOQ Values*

MDL and LOQ for the 15 parent PAHs were determined for the four seafood matrixes using five replicates and a low fortification level of 5.0 µg/kg. Results given in Table 7 show sub to low ppb (µg/kg) MDLs for almost all of the PAH compounds. The MDLs are directly influenced by the fluorescence response for each of the PAH compounds under the method conditions (compare slopes from Table 2), with the highest MDLs seen for naphthalene, dibenz[a,h] anthracene, benzo[g,h,i]perylene, and indeno[1,2,3]pyrene. The fluorescence excitation and emission wavelengths in the current method are not optimized for individual PAH compounds, but they represent a compromise to allow

**Table 7. MDL and LOQ values for parent PAHs in edible seafood matrixes, µg/kg[a]**

| PAH compound | Oysters | | Shrimp | | Crab[b] | | Finfish | |
|---|---|---|---|---|---|---|---|---|
| | MDL | LOQ | MDL | LOQ | MDL | LOQ | MDL | LOQ |
| Naphthalene | 8.1 | 18 | 3.9 | 8.4 | 6.9 | 15 | 8.1 | 18 |
| Acenaphthene | 0.80 | 1.7 | 0.76 | 1.7 | 1.6 | 3.4 | 0.80 | 1.7 |
| Fluorene | 0.56 | 1.2 | 0.22 | 0.48 | 0.70 | 1.5 | 0.56 | 1.2 |
| Phenanthrene | 0.31 | 0.67 | 0.59 | 1.3 | 0.79 | 1.7 | 0.31 | 0.67 |
| Anthracene | 0.12 | 0.27 | 0.09 | 0.19 | 0.73 | 1.6 | 0.12 | 0.27 |
| Fluoranthene | 2.2 | 4.8 | 1.6 | 3.5 | 0.86 | 1.9 | 2.2 | 4.8 |
| Pyrene | 1.7 | 3.7 | 0.65 | 1.4 | 1.0 | 2.2 | 1.7 | 3.7 |
| Benz[a]anthracene | 0.65 | 1.4 | 0.46 | 0.99 | 0.56 | 1.2 | 0.65 | 1.4 |
| Chrysene | 0.70 | 1.5 | 0.42 | 0.92 | 0.60 | 1.3 | 0.70 | 1.5 |
| Benzo[b]fluoranthene | 0.28 | 0.61 | 0.53 | 1.2 | 0.54 | 1.2 | 0.28 | 0.61 |
| Benzo[k]fluoranthene | 0.25 | 0.53 | 0.09 | 0.20 | 0.38 | 0.81 | 0.25 | 0.53 |
| Benzo[a]pyrene | 0.27 | 0.59 | 0.10 | 0.22 | 0.50 | 1.1 | 0.27 | 0.59 |
| Dibenz[a,h]anthracene | 2.5 | 5.5 | 1.7 | 3.8 | 1.7 | 3.6 | 2.5 | 5.5 |
| Benzo[g,h,i]perylene | 1.6 | 3.5 | 1.2 | 2.6 | 1.1 | 2.4 | 1.6 | 3.5 |
| Indeno[1,2,3-cd]pyrene | 3.1 | 6.7 | 2.9 | 6.4 | 7.6 | 17 | 3.1 | 6.7 |

[a] Determination based on five replicates from each of the three laboratories ($n$ = 15).

[b] Crab data were obtained under wavelength switching conditions.

**Table 8. Parent PAH certified and determined amounts for NIST SRM 1974b mussel tissue**

| Compound | Certified value, µg/kg[a] | Control limits range, µg/kg[b] | Determined amount, µg/kg | | | |
|---|---|---|---|---|---|---|
| | | | Laboratory 1 | Laboratory 2 | Laboratory 3 | Overall average |
| | | | ($n$ = 3) | ($n$ = 3) | ($n$ = 3) | ($n$ = 9) |
| Naphthalene[c] | 2.43 | 1.6–3.3 | 8.86 | 5.74 | 4.26 | 6.28 |
| Fluorene | 0.494 | 0.3–0.7 | 0.42 | 0.41 | 0.41 | 0.41 |
| Phenanthrene | 2.58 | 1.7–3.5 | 2.39 | 2.57 | 1.54 | 2.17 |
| Anthracene | 0.527 | 0.3–0.8 | 0.17 | 0.36 | 0.30 | 0.28 |
| Fluoranthene | 17.1 | 11.5–23.1 | 15.3 | 16.4 | 14.8 | 15.5 |
| Pyrene | 18.04 | 12.2–24.2 | 19.0 | 15.1 | 16.9 | 17.0 |
| Benz[a]anthracene | 4.74 | 2.9–6.9 | 4.07 | 3.86 | 4.15 | 4.02 |
| Chrysene | 6.3 | 3.7–9.5 | 9.40 | 6.87 | 8.04 | 8.10 |
| Benzo[b]fluoranthene | 6.46 | 4.1–9.2 | 5.22 | 4.41 | 4.26 | 4.63 |
| Benzo[k]fluoranthene | 3.16 | 2.1–4.3 | 2.38 | 2.40 | 2.20 | 2.33 |
| Benzo[a]pyrene | 2.80 | 1.7–4.1 | 2.03 | 2.18 | 2.43 | 2.22 |
| Dibenz[a,h]anthracene[c] | 0.327 | 0.2–0.5 | 2.28 | 1.83 | 2.69 | 2.27 |
| Benzo[g,h,i] perylene | 3.12 | 2.0–4.5 | 3.01 | 2.89 | 2.78 | 2.89 |
| Indeno[1,2,3-cd] pyrene[c] | 2.14 | 1.4–2.9 | 0.64 | 0.53 | 3.20 | 1.46 |

[a] Wet mass basis (ref. 40).

[b] NOAA (ref. 17) defines the lower control limit as 0.7 × (certified value – uncertainty value), and the upper control limit as 1.3 × (certified value + uncertainty value).

[c] Certified value is below the method LOQ for these compounds, making them exempt in the NOAA criteria (ref. 17).

detection of compound groups. The higher MDL for naphthalene is also related to the lower precision obtained for this compound, which may be attributed to naphthalene background levels (ubiquitous contaminant). All of the MDLs are one to four orders of magnitude below current guidelines for LOC in seafood (*see* previous discussion), making the current method well suited for its intended use. While some of the calculated LOQ are below 1.0 ppb (µg/kg), the low ppb range is considered a practical LOQ for determination of the PAH compounds using the current method.

### *Analysis of NIST SRM 1974b Organics in Mussel Tissue*

Additional method validation was conducted using NIST SRM 1974b, a frozen mussel tissue homogenate with certified levels of 23 incurred PAH compounds (40). Each of the validating laboratories conducted analysis of NIST SRM 1974b in triplicate for 14 of the incurred PAHs corresponding to parent compounds in the present study. Given the extremely low levels of the incurred PAHs in the SRM, concentration of the filtered extract was necessary prior to analysis (*see Experimental* section). Results are summarized in Table 8 for each of the three laboratories, along with the NIST-certified values and allowable control limits established by the National Oceanic and Atmospheric Administration (NOAA) for assessment of method accuracy (17). Determined amounts for 11 PAHs fell within the NOAA control limits. Although the other three compounds (naphthalene, dibenz[a,h]anthracene, and indeno[1,2,3-cd]pyrene) did not fall within the control limits, they are considered exempt from consideration (17) because the certified values fall below the method LOQs for these compounds.

### *Method Application and Continuing Validation*

The PAH screening method has been applied to the analysis of hundreds of seafood samples obtained from multiple harvesting sites in the waters off Louisiana, Alabama, Mississippi, and Florida. Seafood samples analyzed in July and August 2010, included eastern oysters, brown shrimp, blue crab, crab hepatopancreas, and multiple finfish varieties (sand sea trout, spotted sea trout, red drum, black drum, gafftopsail catfish, Atlantic croaker, sheepshead, Spanish mackerel, southern kingfish, and flounder). Validation testing has continued with application of the method, with spike/recovery experiments conducted for every batch analysis at a fortification level of 50 ng/g for the 15 parent PAHs listed in Table 2. Generally, good recoveries were obtained across the seafood matrixes and PAHs. Observed recovery ranges across PAHs (excluding naphthalene) and by matrix were as follows: oysters ($n = 1$), 88–112%; shrimp ($n = 3$), 67–108%; crab ($n = 6$), 68–128%; and finfish ($n = 4$), 67–111%. Recoveries for naphthalene were also generally in the specified ranges, with isolated outliers.

## Summary

The HPLC-FLD method with QuEChERS based extraction is suitable for the screening and determination of both parent PAHs and their alkylated homologs in edible seafood, with validation testing conducted in three laboratories for oysters, shrimp, finfish, and crab.

$CH_3CN$ extraction provided good recoveries for 15 EPA priority pollutant parent PAHs (78–99%) and several series of alkylated PAH homologs (87–128%). Method accuracy was further demonstrated based on analysis of NIST SRM 1974b. The method provides the required sensitivity for PAH screening, with MDLs in the sub to low ppb (µg/kg) range for most of the PAH compounds. The finished method is simpler and more rapid compared to previous methods, requiring no dispersive SPE cleanup and providing resolution of the 15 parent PAHs in under 20 min. The method has been successfully applied to hundreds of samples related to the DWH oil spill, including multiple varieties of oysters, shrimp, crab, and finfish. The HPLC-FLD method is currently in use for monitoring purposes; it provides a means to assess the need for additional testing using established, yet more labor-intensive, methods.

## References


(1) *Oil in the Sea III: Inputs, Fates, and Effects* (2003) Appendix J, Committee on Oil in the Sea, Ocean Studies Board and Marine Board, National Research Council, The National Academies Press, Washington, DC, pp 253–254

(2) Environment Canada Environmental Science and Technology Centre Oil Properties Database, http://www.etc-cte.ec.gc.ca/databases/OilProperties/Default.aspx (accessed August 31, 2010)

(3) Sauer, T.C., Brown, J.S., Boehm, P.D., Aurand, D.V., Michel, J., & Hayes, M.O. (1993) *Mar. Pollut. Bull.* **27**, 117–134

(4) Wang, Z., Fingas, M., & Page, D.S. (1999) *J. Chromatogr. A* **843**, 369–411. doi:10.1016/S0021-9673(99)00120-X

(5) Saravanabhavan, G., Helferty, P.A., Hodson, P.V., & Brown, R.S. (2007) *J. Chromatogr. A* **1156**, 124–133. doi:10.1016/j.chroma.2007.04.045

(6) Deepthike, H.U., Tecon, R., Van Coten, G., Van Der Meer, J.R., Harms, H., Wells, M., & Short, J. (2009) *Environ. Sci. Technol.* **43**, 5864–5870. doi:10.1021/es900734k

(7) U.S. Department of Health and Human Services (2005) *Report on Carcinogens*, 11th Ed., Public Health Service, National Toxicology Program, http://ntp.niehs.nih.gov/ntp/roc/toc11.html

(8) Nisbet, I.C.T., & LaGoy, P.K. (1992) *Regul. Toxicol.*

*Pharmacol.* **16**, 290–300. doi:10.1016/0273-2300(92)90009-X

(9) Bolger, M., Henry, S.H., & Carrington, C.D. (1996) *American Fisheries Society Proceedings of the Exxon Valdez Oil Spill Symposium*, Anchorage, AK, **18**, 837–843

(10) Collins, J.F., Brown, J.P., Alexeeff, G.V., & Salmon, A.G. (1998) *Reg. Toxicol. Pharmcol.* **28**, 45–54. doi:10.1006/rtph.1998.1235

(11) Lubchenco, J., McNutt, M., Lehr, B., Sogge, M., Miller, M., Hammond, S., & Conner, W. (2010) http://www.restorethegulf.gov/release/2010/09/09/bp-deepwater-horizon-oil-budget-what-happened-oil (accessed January 18, 2011)

(12) Crone, T.J., & Tolstoy, M. (2010) *Science* **330**, 634. doi:10.1126/science.1195840

(13) Brown, D.W., Burrows, D.G., Sloan, C.A., Pearce, R.W., Pierce, S.M., Bolton, J.L., Tilbury, K.L., Dana, K.L., Chan, S.-L., & Varanasi, U. (1996) *American Fisheries Society Symposium Proceedings of the Exxon Valdez Oil Spill Symposium,* Anchorage, AK, **18**, 844–855

(14) Webster, L., Angus, L., Topping, G., Dalgarno, E.J., & Moffat, C.F. (1997) *Analyst* **122**, 1491–1495. doi:10.1039/a705977i

(15) U.S. Environmental Protection Agency (2000) *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories* EPA 823-B-00-007, 3rd Ed., Wahsington, DC

(16) Law, R.J., Kelly, C., Baker, K., Jones, J., McIntosh, A.D., & Moffat, C.F. (2002) *J. Environ. Monit.* **4**, 383–388. doi:10.1039/b200633m

(17) Sloan, C.A., Brown, D.W., Pearce, R.W., Boyer, R.H., Bolton, J.L., Burrows, D.G., Herman, D.P., & Krahn, M.M. (2004) *NOAA Tech. Memo*., NMFS-NWFSC-59, 47 pp

(18) Baird, J.S., Bailey, E.A., & Vorhees, D.J. (2007) *Hum. Ecol. Risk Assess.* **13**, 322–338. doi:10.1080/10807030701226277

(19) Krahn, M.M., Myers, M.S., Burrows, D.G., & Malins, D.C. (1984) *Xenobiotica* **14**, 633–646. doi:10.3109/00498258409151461

(20) Lawrence, J.F., & Weber, D.F. (1984) *J. Agric. Food Chem.* **32**, 789–794. doi:10.1021/jf00124a022

(21) Krone, C.A., Stein, J.E., & Varanasi, U. (1992) *Chemosphere* **24**, 497–510. doi:10.1016/0045-6535(92)90424-P

(22) Perfetti, G.A., Nyman, P.J., Fisher, S., Joe, F.L. Jr, & Diachenko, G.W. (1992) *J. AOAC Int.* **75**, 872–877

(23) Krahn, M.M., Ylitalo, G.M., Buzitis, J., Chan, S-L., & Varanasi, U. (1993) *J. Chromatogr.* **642**, 15–32. doi:10.1016/0021-9673(93)80073-H

(24) Hufnagle, L.C. Jr, Camarata, S.E., Ernest, D., Krone, C.A., & Krahn, M.M. (1999) *Arch. Environ. Contam. Toxicol.* **37**, 220–226. doi:10.1007/s002449900508

(25) Kelly, C.A., Law, R.J., & Emerson, H.S. (2000) *Sci. Ser. Aquat. Environ. Prot. Analyt. Meth*., No. 12, Center for Environment, Fisheries, and Aquaculture Science, Lowestoft, Suffolk, UK, 18 pp

(26) Ramalhosa, M.J., Paiga, P., Morais, S., Delerue-Matos, C., & Oliveira, M.B.P.P. (2009) *J. Sep. Sci.* **32**, 3259–3538

(27) Pule, B.O., Mmualefe, L.C., & Torto, N. (2010) *Agilent Technologies Application Note 5990-5441EN*, Santa Clara, CA

(28) Joe, F.L. Jr, Salemme, J., & Fazio, T. (1984) *J. Assoc. Off. Anal. Chem.* **67**, 1076–1082

(29) Kayeli-Sayedi, M.N., Rubio-Barrosa, S., Cuesta-Jimenez, M.P., & Polo-Diez, L.M. (1998) *Analyst* **123**, 2145–2148. doi:10.1039/a803967d

(30) Hendrikse, P.W., & Diefenbacher, A. (1991) *Pure Appl. Chem.* **63**, 1659–1666. doi:10.1351/pac199163111659

(31) Barranco, A., Alonso-Salces, R.M., Bakkali, A., Berrueta, L.A., Gallo, B., Vicente, F., & Sarobe, M. (2003) *J. Chromatogr. A* **988**, 33–40. doi:10.1016/S0021-9673(02)02059-9

(32) Barranco, A., Alonso-Salces, R.M., Corta, E., Berrueta, L.A., Gallo, B., Vicente, F., & Sarobe, M. (2004) *Food Chem.* **86**, 465–474. doi:10.1016/j.foodchem.2003.10.030

(33) Moret, S., Dudine, A., & Conte, L.S. (2000) *J. Am. Oil Chem. Soc.* **77**, 1289–1292. doi:10.1007/s11746-000-0203-5

(34) Anastassiades, M., Lehotay, S.J., Stajbaher, D., & Schenck, F.J. (2003) *J. AOAC Int.* **86**, 412–431

(35) Lehotay, S.J., Mastovska, K., & Lightfield, A.R. (2005) *J. AOAC Int.* **88**, 615–629

(36) Lehotay, S.J. (2007) *J. AOAC Int.* **90**, 485–520

(37) U.S. Food and Drug Administration (2010) *Protocol for Interpretation and Use of Sensory Testing and Analytical Chemistry for Re-Opening Oil-Impacted Areas Closed to Seafood Harvesting*, http://www. fda.gov/Food/ucm217601.html

(38) U.S. Environmental Protection Agency (2009) *Definition for the Procedure for the Determination of the Method Detection Limit in Code of Federal Regulations*, 40 CFR, Part 136, Appendix B, Washington, DC

(39) *Hydrocarbon Results for MC252 Riser Oil*, ENTRIX, Inc. Memorandum Rob Barrick to Ralph Markarian, John Dimitry, May 3, 2010, Seattle, WA, publicfiles.dep.state.fl.us/.../Oil.../Riser%20Oil%20Analysis%203May10.pdf

(40) National Institute of Standards and Technology (2003) *NIST Certificate of Analysis, Standard Reference Material 1974b, Organics in Mussel Tissue*, Gaithersburg, MD