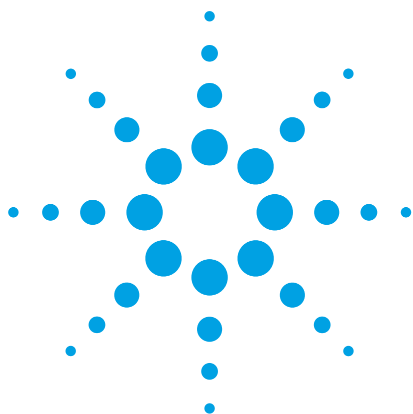

PAH Analysis in Salmon with Enhanced Matrix Removal

Application Note

Food Testing & Agriculture, Environmental

Authors

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) consist of fused benzene ring systems that resist degradation. They can be introduced to aquatic species by accumulation in the environment and cooking methods that use smoke. Analysis of PAHs in complex, high-fat food matrices can often present challenges as coextracted matrix hinders accurate quantitation in the form of interferences, matrix effects, and accumulation in the analytical flow path. Agilent Bond Elut Enhanced Matrix Removal—Lipid (EMR—Lipid) is the next generation of sample preparation products, and is used in convenient, dispersive solid phase extraction (dSPE) for highly selective matrix removal without impacting analyte recovery. This work demonstrates the effectiveness of this sample preparation methodology in the analysis of PAHs in salmon. The method delivers excellent accuracy (84 to 115%) and precision (RSD = 0.5 to 4.4%) for all 15 PAH compounds at all levels, providing a fast, robust, and effective analysis in high-fat samples.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous contaminants in the environment and may come from petrogenic or pyrogenic origins. They are composed of hydrogen and carbon arranged in two or more fused benzene rings, and can have substituted groups attached to one or more rings [1]. Concerns about PAHs arise from their persistence in the environment and known toxic, mutagenic, and carcinogenic effects on mammals for some of them [2]. Contamination of seafood can occur from accumulation of petroleum constituents in water sources and from cooking processes that introduce PAHs as combustion byproducts in smoke [3,4]. For these reasons, it is essential that analysts have robust and efficient methods for detecting contaminant PAHs at levels of concern.

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Detection of PAHs at low levels can be accomplished using GC/MS coupled with a robust and effective sample preparation method. Common preparation protocols include Soxhlet extraction [5], sonication assisted extraction [6], and pressurized solvent extraction [7]. Preparation can be coupled to cleanup procedures such as solid phase extraction [8] or gel permeation chromatography [9]. To overcome these labor-intensive and time-consuming techniques, protocols based on Quick, Easy, Effective, Rugged, and Safe (QuEChERS) [10,11] have also been implemented with good success [12,13,14]. Sample preparation is increasingly important for complex food samples, especially those high in lipids, as coextracted matrix has deleterious effects on analysis in the form of interferences, matrix effects, and accumulation in the analytical flow path.

Agilent Bond Elut Enhanced Matrix Removal—Lipid (EMR—Lipid) is a novel sorbent material that selectively removes major lipid classes from sample extracts without unwanted removal of analytes of interest. Removal of lipid species is especially important for techniques such as QuEChERS, which coextract large amounts of matrix with the target analytes. Traditionally, C18- and PSA-based sorbents were used for cleaning high-fat samples during a dispersive solid phase extraction (dSPE) step. However, these sorbents often fail to achieve adequate sample cleanup, and can exhibit nonselective interactions with analytes. This work investigates the sample preparation and analysis of 15 PAHs in salmon using a simple and effective workflow, delivering adequate cleanliness with EMR-Lipid as well as excellent accuracy and reproducibility on the GC/MS.

Experimental

Analysis was performed on an Agilent 7890 GC and an Agilent 5977 MSD equipped with multimode inlet (MMI), with an Agilent 7693 Automatic Liquid Sampler, and capillary flow technology for column backflushing. Table 1 shows the instrumental parameters, and Table 2 shows consumables and other equipment used in this work.

Table 1. Instrumental conditions for the Agilent GC/MS system used for PAH Analysis

GC:	Agilent 7890B
Autosampler:	Agilent 7693 Automatic Liquid Sampler, 10.0 µL syringe (G4513-80220)
Injection volume:	0.5 µL
Carrier gas:	Helium, constant flow
Gas filter:	Gas Clean filter GC/MS, 1/8 in (p/n CP17974)
Inlet:	MMI, hot splitless injection mode, 320 °C
Purge flow to split vent:	50 mL/min at 0.75 min
Flow rate:	2.0 mL/min
Oven program:	70 °C for 1 min, then 25 °C/min to 195 °C with a 1.5 min hold, then 7 °C/min to 315 °C
Column:	Agilent J&W DB-5ms UI, 20 m × 0.18 mm, 0.18 µm (p/n 121-5522UI)
Restrictor:	Deactivated silica tubing, 0.65 m × 0.15 mm (p/n 160-7625-5)
Postrun backflush:	5 min at 315 °C, 70 psi during backflush
Aux. pressure:	2 psi during run, 70 psi during backflush
MSD:	Agilent 5977 MSD
Mode:	SIM
Transfer line temperature:	340 °C
Source temperature:	325 °C
Quad temperature:	150 °C
Solvent delay:	3.5 min

Table 2. Other consumables and equipment.

Vials:	Amber, screw top, glass (p/n 5190-7041)
Vial caps:	PTFE, 9 mm, screw cap (p/n 5182-0717)
Vial inserts:	Glass, 150 µL, with polymer feet (p/n 5183-2088)
Septum:	Long-life, nonstick, 11 mm, 50/pk (p/n 5183-4761)
Ferrules:	Vespel:graphite, 85:15, 0.4 mm id (p/n 5181-3323), UltiMetal Plus Flexible Metal ferrules (p/n G3188-27501)
Inlet liner:	Single taper, splitless, Ultra Inert (p/n 5190-7041)
Capillary flow technology (CFT):	UltiMetal Plus Ultimate Union (p/n G3186-60580), CFT capillary fitting (p/n G2855-20530)
Bond Elut EMR—Lipid dSPE:	1 g in 15 mL tube (p/n 5982-1010)
Bond Elut Final Polish for Enhanced Matrix Removal—Lipid:	2 g in 15 mL tube (p/n 5982-0101)

Geno/Grinder, Metuchen, NJ, USA

Centra CL3R centrifuge, Thermo IEC, MA, USA

Eppendorf microcentrifuge, Brinkmann Instruments, Westbury, NY, USA

Vortexer and multitube vortexers, VWR, Radnor, PA, USA

Bottle top dispenser, VWR, So. Plainfield, NJ, USA

Eppendorf pipettes

Sample preparation

Salmon was homogenized and weighed (5 g) into 50 mL centrifuge tubes and spiked as necessary with standards and isotopically labeled internal standards. Acetonitrile (ACN) (10 mL) was added, and the sample was mixed on a mechanical shaker for two minutes. Tubes were centrifuged at 5,000 rpm for five minutes. The supernatant (8 mL) was transferred to a 15 mL centrifuge tube containing 1 g EMR—Lipid sorbent, vortexed immediately to disperse, and then for an extra 60 seconds on a vortex table. The slurry was then centrifuged at 5,000 rpm for three minutes. The entire supernatant was decanted into a second 15 mL polishing tube containing 2.0 g salts (1:4 $NaCl:MgSO_4$), and vortexed immediately to disperse, followed by centrifugation at 5,000 rpm for three minutes. The upper ACN layer was transferred to sample vials for GC/MS analysis (Figure 1).

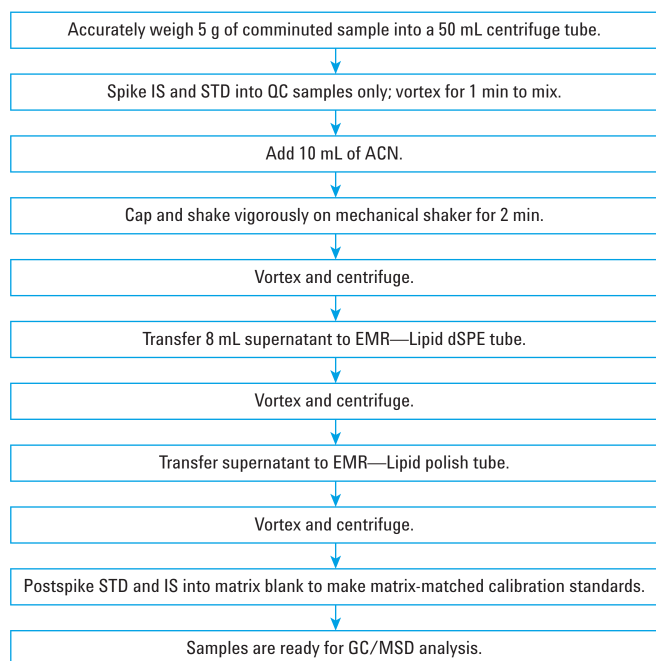

Figure 1. Sample preparation workflow for PAH in salmon using Agilent Bond Elut Enhanced Matrix Removal—Lipid before analysis by GC/MS.

Reagents and chemicals

All reagents and solvents were HPLC grade or higher. ACN was from Honeywell (Muskegon, MI, USA), and water was purified using an EMD Millipore Milli-Q Integral System (Darmstadt, Germany). PAH standards and internal standards were purchased from Ultra-Scientific as solutions (North Kingstown, RI, USA). Stock solutions were prepared at 100 µg/mL in acetone, and diluted in amber vials for working standards.

Calibration curves and quantitation

Matrix-matched calibration curves were generated over the calibration range, corresponding to 1, 10, 25, 50, 100, 250, 500, and 1,000 ng/g. Salmon blanks were carried through the entire sample preparation procedure and 950 µL of the blank extract, 25 µL standard working solution, and 25 µL stock internal standards. The internal standards were spiked into the salmon and postspiked into the matrix-matched calibration standards at 100 ng/g. All calibration curves gave exceptional linearity, with $R^2 > 0.999$ for all compounds. Salmon samples were prespiked at 25, 100, and 500 ng/g levels before extraction in six replicates. Agilent MassHunter Software was used to quantify the target analytes. Accuracy values were determined by calculating the spiked sample responses with respect to internal standards. Absolute recovery values were determined by measuring prespiked analyte response to the calibration curve without internal standard correction.

Results and Discussion

The 7890 GC and 5977 GC/MSD delivered excellent performance for the 15 PAHs and five internal standards, providing consistent results with high sensitivity. Figure 2 shows the separation achieved for the 15 PAHs on an Agilent DB-5ms UI column with a 25 ng/g prespike in salmon. The chromatogram shows baseline separation of all 15 PAHs, which is essential for accurate integration of PAH isomers phenanthrene, anthracene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, and benzo[k]fluroanthene. Some minor interferences in the chromatogram are easily separated from the peaks of interest.

Excellent accuracy and precision was achieved at 25, 100, and 500 ng/g spike levels using the optimized procedure with EMR-Lipid. Figure 3 shows that accuracy was between 84 and 115% for all analytes at all levels using isotopically labeled internal standard correction, giving RSD from 0.5 to 4.4% (Figure 4). The accuracy data are grouped into recovery ranges in Figure 5, and show that most compounds fall between 90 and 120%, with two compounds falling slightly below 90% (indo[1,2,3-cd]pyrene and benzo[g,h,i]pyrene).

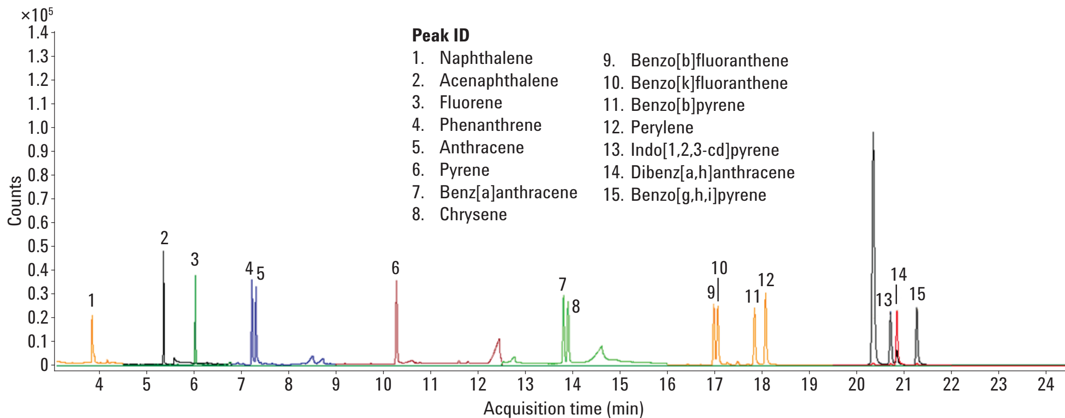

Figure 2. GC/MS SIM chromatogram of 15 PAHs from a 25 ng/g prespike in salmon.

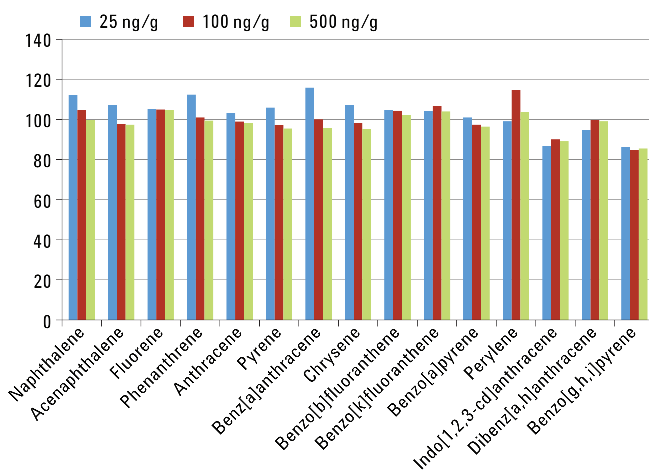

Figure 3. Accuracy results for 15 PAHs in salmon at 25 ng/g, 100 ng/g, and 500 ng/g levels.

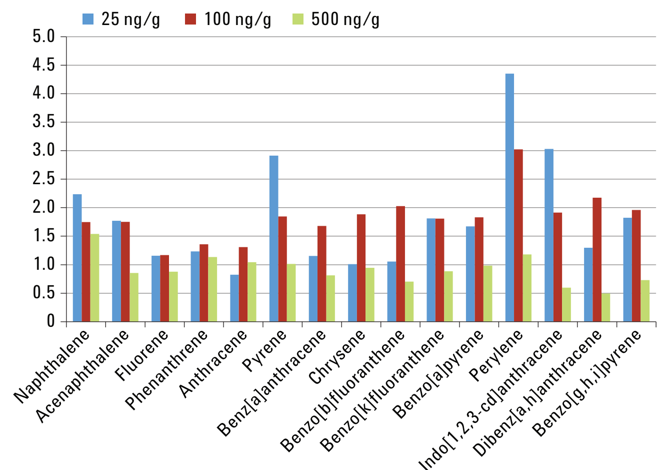

Figure 4. Precision results for 15 PAHs in salmon at 25 ng/g, 100 ng/g, and 500 ng/g levels.

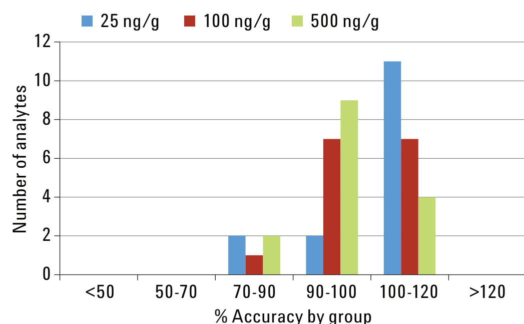

Figure 5. Grouped accuracy results for PAHs in salmon at 25 ng/g, 100 ng/g, and 500 ng/g levels.

Absolute recovery was from 62 to 98% without the use of internal standards (Table 3). Two compounds, indo[1,2,3-cd]pyrene and benzo[g,h,i]pyrene, gave recoveries slightly lower than 70%. The PAH absolute recoveries decrease with increasing molecular weight due to decreasing solubility in ACN. However, most recoveries are high and easily corrected using the internal standards. Internal standard absolute recoveries are also high as shown in Table 4. Despite the solubility limitation of ACN, this method gives good to excellent recoveries and highly reproducible results in the high-fat salmon sample.

EMR-Lipid dSPE

Salmon was chosen as a representative sample due to its high fat content relative to other seafood. The optimized procedure deviates from a typical QuEChERS protocol in several ways that streamline the workflow and take advantage of the EMR—Lipid dSPE cleanup step. First, the salmon is extracted directly with ACN without extra water or QuEChERS extraction salts. After centrifugation, the supernatant consists of ACN and a small amount of water from the sample. The supernatant is transferred to the EMR—Lipid tube for dSPE matrix removal. Finally, the dSPE supernatant is transferred to a polish tube containing 2.0 g NaCl/$MgSO_4$ (1:4) to induce phase separation. The upper ACN layer is then transferred to vials for analysis.

Table 4. Absolute recovery and precision (%RSD) for internal standards in salmon (n = 6).

	100 ng/g Spike	
Compound	**Rec.**	**%RSD**
Naphthalene-d8	87.8	1.0
Acenaphthalene-d10	93.3	0.8
Phenanthrene-d10	94.9	0.8
Chrysene-d12	87.1	1.0
Perylene-d12	86.4	3.1
Average	**89.9**	**1.3**

Table 3. List of PAHs used in this study and their accuracy, absolute recoveries, and relative standard deviations (RSDs) in salmon (n = 6).

	25 ng/g Spike			**100 ng/g Spike**			**500 ng/g Spike**		
Compound	**Acc.**	**Rec.**	**%RSD**	**Acc.**	**Rec.**	**%RSD**	**Acc.**	**Rec.**	**%RSD**
Naphthalene	112.2	86.7	2.2	104.8	89.7	1.7	99.7	85.8	1.5
Acenaphthalene	107.1	90.1	1.8	97.6	89.9	1.8	97.3	90.6	0.9
Fluorene	105.3	94.6	1.2	105.0	94.2	1.2	104.6	96.2	0.9
Phenanthrene	112.3	95.3	1.2	101.0	94.1	1.4	99.4	94.5	1.1
Anthracene	103.1	91.6	0.8	98.9	90.7	1.3	98.3	92.6	1.0
Pyrene	105.8	97.6	2.9	97.1	88.9	1.8	95.4	89.7	1.0
Benz[a]anthracene	115.8	91.2	1.2	100.1	84.7	1.7	95.8	85.7	0.8
Chrysene	107.2	83.6	1.0	98.2	83.2	1.9	95.4	85.4	0.9
Benzo[b]fluoranthene	104.8	78.3	1.1	104.3	76.1	2.0	102.2	79.2	0.7
Benzo[k]fluoranthene	104.1	78.8	1.8	106.6	77.5	1.8	104.0	80.3	0.9
Benzo[a]pyrene	101.0	74.2	1.7	97.4	71.8	1.8	96.4	74.8	1.0
Perylene	99.1	74.4	4.4	114.7	76.4	3.0	103.6	80.3	1.2
Indo[1,2,3-cd]pyrene	86.7	66.1	3.0	90.0	66.2	1.9	89.1	69.1	0.6
Dibenz[a,h]anthracene	94.7	73.9	1.3	99.7	72.2	2.2	99.0	76.2	0.5
Benzo[g,h,i]pyrene	86.4	64.7	1.8	84.7	62.3	2.0	85.6	66.3	0.7
Average	**103.0**	**82.7**	**1.8**	**100.0**	**81.2**	**1.8**	**97.7**	**83.1**	**0.9**

As is typical with protocols for enhanced matrix removal, this approach takes advantage of the enhanced cleanup by using a larger sample size, which in turn improves the overall sensitivity of the method. For conventional EMR—Lipid protocols, additional water is added to activate the sorbent material before dSPE. For this optimized protocol, it was found that extra water decreased the solubility of PAHs and negatively impacted some absolute recoveries. Therefore, the supernatant from extraction was transferred directly to the EMR—Lipid tube without additional water, providing adequate cleanup for GC/MS SIM analysis. Immediate mixing after the addition of supernatant to the EMR—Lipid and EMR—Lipid polish tubes suspends the solids to ensure maximum interaction with sorbent and avoids clumping. For optimal matrix removal, extra water can be added to the dSPE, and recoveries can be effectively corrected with the internal standards to give excellent accuracy and precision.

Conclusions

This work demonstrates a fast and easy method that effectively quantitates low to high-level concentrations of PAHs in high-fat salmon samples. The workflow is as easy as QuEChERS, but implements the new EMR—Lipid dSPE sorbent to minimize fat coextractives, maximize recovery, and give a high level of precision.

Although fat content in matrices such as salmon can vary greatly, Agilent Bond Elut Enhanced Matrix Removal—Lipid is a one-size-fits-all fat removal sorbent that does not interact with analytes of interest. Fat removal is maximized by using additional water with EMR—Lipid during the dSPE step. However, in this case, more water decreases solubility of PAHs and is not desirable for PAH sample preparation. Future work will continue to optimize EMR—Lipid for challenging sample types and applications to broaden its value on current and next generation chromatographic and detection systems.

Table 5. Target analytes, retention time, target ion, and internal standard designations for GC/MS SIM method.

	GC/MS (SIM)			
Compound	**RT**	**Target ion**	**Dwell (ms)**	**Internal standard**
Naphthalene	3.89	128.0	20	Naphthalene-d8
Acenaphthalene	5.37	152.0	20	Acenaphthalene-d10
Fluorene	6.05	166.0	20	Acenaphthalene-d10
Phenanthrene	7.25	178.0	20	Phenanthrene-d10
Anthracene	7.34	178.0	20	Phenanthrene-d10
Pyrene	10.31	202.0	20	Phenanthrene-d10
Benz[a]anthracene	13.83	228.0	20	Chrysene-d12
Chrysene	13.93	228.0	20	Chrysene-d12
Benzo[b]fluoranthene	16.99	252.0	20	Perylene-d12
Benzo[k]fluoranthene	17.08	252.0	20	Perylene-d12
Benzo[a]pyrene	17.85	252.0	20	Perylene-d12
Perylene	18.09	252.0	20	Perylene-d12
Indo[1,2,3-cd]pyrene	20.72	276.0	20	Perylene-d12
Dibenz[a,h]anthracene	20.87	278.0	20	Perylene-d12
Benzo[g,h,i]pyrene	21.29	276.0	20	Perylene-d12
Internal standards				
Naphthalene-d8	3.87	136.0	20	–
Acenaphthalene-d10	5.52	162.0	20	–
Phenanthrene-d10	7.22	188.0	20	–
Chrysene-d12	13.86	240.0	20	–
Perylene-d12	18.03	264.0	20	–

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Printed in the USA
August 21, 2015
5991-6088EN

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