Polycyclic Aromatic Hydrocarbon (PAH) Analysis in Fish by GC/MS Using Agilent Bond Elut QuEChERS dSPE Sample Preparation and a High Efficiency DB-5ms Ultra Inert GC Column

Application Note

Food Testing and Hydrocarbon Processing

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Abstract

This application note details a quick and effective analytical method for the determination of low and trace level polycyclic aromatic hydrocarbons (PAHs) in fish samples as an alternative to procedures involving more time consuming and complex techniques. A simplified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method with dispersive solid phase extraction (dSPE) provided sufficient sample matrix cleanup while preserving low level analyte detection. A pressure controlled tee was installed post column to implement the use of automated backflush to diminish residual sample carryover and reduce instrument cycle times.

The Agilent J&W DB-5ms Ultra Inert (UI) 20 m × 0.18 mm , 0.18 µm column effectively resolved the 16 targeted PAHs. A GC/MS method employing selective ion monitoring (SIM) was calibrated at the 10, 25, 50, 100, 250, 500, and 1000 ng/mL PAH levels, yielding excellent linearity and reproducibility. The spiked levels for recovery determinations were 25, 250, and 500 ng/mL. Recoveries ranged from 80% and 139% with RSD below 6%.

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Introduction

Oil spills can introduce massive amounts of crude oil and the myriad of components found in the oil, including Polycyclic Aromatic Hydrocarbons (PAHs). PAHs are a broad class of molecules that are well known, well characterized, and a worldwide focal point for regulations as both food contaminants and environmental pollutants [1-2]. PAHs have known toxicity and in some cases have been implicated as carcinogens. These molecules are persistent in the environment and bio-accumulate in fatty tissue of fish species throughout the food chain [3]. Fortunately, from an analyst's prospective, there are historical accounts of large oil spills and their impact on aquatic species and the environment that serve as case studies for investigation of future spills [4].

When large scale oil spills occur, regulatory agencies spring to action to minimize the risk of human exposure, contain the leak at its source when possible, and protect the environment as much as feasible. In the US, the National Oceanic and Atmospheric Administration (NOAA) in coordination with the US Food and Drug Administration (FDA) and other federal and state agencies set policies for both fishery closure and the reopening of affected fisheries. As part of the reopening process for a closed fishery NOAA and the FDA have established testing procedures for PAH analyses as a requirement to reopen a fishery. Levels of concern for eight targeted PAHs and their alkylated homologues have been defined for finfish, oysters, shrimp and crab species. The established levels of concern range from a high of 233 ppm for anthracene/phenanthrene in shrimp and crabs to a low of 0.03 ppm in finfish for benzo(a)pyrene [5].

Analysis at these levels can be reliably achieved using a capillary GC/MS approach. Resolution of anthracene and phenanthrene can also be routinely achieved using an Agilent J&W DB-5ms column and appropriate instrument conditions. Use of the Ultra Inert column in this case assures less interaction between the column and the sample matrix.

Sample preparation to execute the NOAA method has historically been labor intensive involving two successive steps of preparative chromatography. In this note the use of a QuEChERs (Quick Easy Cheap Effective and Rugged) approach to sample preparation is presented for use as a screening method for PAHs. Typically sample preparation using the NOAA method takes 12-14 hours per sample. Using a QuEChERs sample preparation approach, up to 60 samples can be processed in one eight-hour shift [6]. Sample preparation time savings are substantial for the QuEChERs approach enabling higher laboratory throughput for the screening of PAHs in seafood.

The DB-5ms column has been widely used for US-EPA PAHs and offers suitable resolution of the sixteen targeted PAHs investigated by this method. The high efficiency DB-5ms Ultra Inert column was chosen for this application because of its added benefits for trace level analysis in more complex samples matrixes while retaining the same selectivity [7]. A 0.18 mm id or high efficiency GC column can also help by providing faster sample analysis than a 0.25 mm id column typically used for GC/MS analysis .[8-9].

The GC/MS system used was also equipped with backflush capability. This capability enables faster instrument cycle time by backflushing late eluting matrix components back through the inlet purge valve. Long bake out times between injections are avoided by using this technique. Backflushing has the additional benefit of increasing the time intervals for source cleaning effectively clearing deleterious matrix components from the system [10-11].

Experimental

An Agilent 7890 GC and Agilent 5975B GC/MS System equipped with a multimode inlet (MMI) and Agilent 7693 automatic liquid sampler were used for this series of experiments. The GC was also fitted with a pressure controlled tee (PCT) post-column for automated backflush. Table 1 lists the chromatographic conditions used for these analyses. Table 2 lists flow path consumable supplies used in these experiments.

Table 1. Chromatographic Conditions

GC/MSD	Agilent 7890 GC/Agilent 5975B GC/MS System
Sampler	Agilent 7693 automatic liquid sampler, 5.0 µL syringe (p/n 5181-1273)
PCT Device	Purged Ultimate Union (p/n G3186-60580)
Carrier	Helium, constant flow 1.7 mL/min
Restrictor	0.7 m × 0.15 mm id deactivated silica tubing
PCM 1	3.8 psi constant pressure
MMI	0.5 µL splitless; 320 °C, Purge flow 50 mL/min at 0.8 min Gas saver 30 mL/min at 2 min
Column	Agilent J&W DB-5msUI 20 m × 0.18 mm, 0.18 µm (p/n 122-5522UI)
Oven	50 °C (0.4 min), 25 °C/min to 195 °C (1.5 min), 8 °C/min to 265 °C, 20 °C/min to 315 °C (1.25 min)
Postrun backflush	7 min at 315 °C, backflush pressure 70 psi, 2 psi inlet pressure during backflush
MSD	340 °C transfer line, 340 °C source, 150 °C quad

Table 2 Flow Path Supplies

Vials:	Amber screw top glass vials (p/n 5183-2072)
Vial Caps:	Blue screw caps (p/n 5182-0717)
Vial inserts:	100 µL glass/polymer feet (p/n 5181-8872)
Syringe:	5 µL (p/n 5181-1273)
Septum:	Advanced green (p/n 5183-4759)
Inlet liners:	Deactivated dual taper Helix liner (p/n G5188-5398)
Ferrules:	0.4 mm id short; 85/15 vespel/graphite (p/n 5181-3323)
PCT fittings:	Internal nut (p/n G2855-20530)
PCT ferrules:	SilTite ferrules, 0.25 mm id (p/n 5188-5361)
20x magnifier :	20x Magnifier loop (p/n 430-1020)

Reagents and Chemicals

All reagents and solvents were HPLC or Ultra Resi grade. Acetonitrile (ACN) was from Honeywell (Muskegon, MI, USA), and acetone was from VWR International (West Chester, PA, USA). The 16-component PAH standard used was obtained from Agilent (p/n 8500-6035).

Solutions and Standards

The PAH stock standard solution (502 µg/mL of 16 polyaromatic hydrocarbons) was diluted in acetone to yield spiking solutions of 0.5,1, 5, 10 and 50 µg/mL. The spiking solutions were used to prepare the calibration curves in the matrix blank extract by appropriate dilution.

Sample Preparation

A red snapper fish sample was purchased from a local grocery store. The fish was chopped into small cubes and frozen at –80 °C overnight. The samples were then comminuted thoroughly to achieve sample homogeneity. The sample extraction method used the QuEChERS method followed by dSPE [5]. Figure 1 illustrates the sample preparation procedure graphically in a flow chart.

Samples containing 3.0 grams of fish were weighed into centrifuge tubes. QC samples were spiked with an appropriate amount of PAH spiking solution to yield QC samples with concentrations of 25, 250, and 500 ng/mL. Each sample received 12.0 mL aliquot of deionized water and 15 mL aliquot of ACN. Two ceramic bars (p/n 5982-9313) were added to each sample to aid in sample extraction. The samples were vortexed for 1 minute. An original Agilent Bond Elut QuEChERS extraction salt packet (p/n 5982-6555) containing 6 grams of $MgSO_4$ and 1.5 grams sodium chloride was added to each centrifuge tube. The capped tubes were shaken on a Geno Grinder at 1500 rpm for 1 minute. The samples were centrifuged at 4000 rpm for 5 min.

An 8 mL aliquot of the upper layer was transferred to an Agilent Bond Elut QuEChERS fatty sample dispersive SPE 15 mL tube (p/n 5982-5158). The dSPE tube was vortexed for 1 minute and then centrifuged at 4000 rpm for 5 minutes to complete the sample extraction. The liquid from the dSPE tube was transferred to a GC vial and analyzed by SIM GC/MS using the chromatographic conditions listed in Table 1.

Extractions of water and acetonitrile aliquots were prepared in the same manner as the samples and served as a reagent blanks.

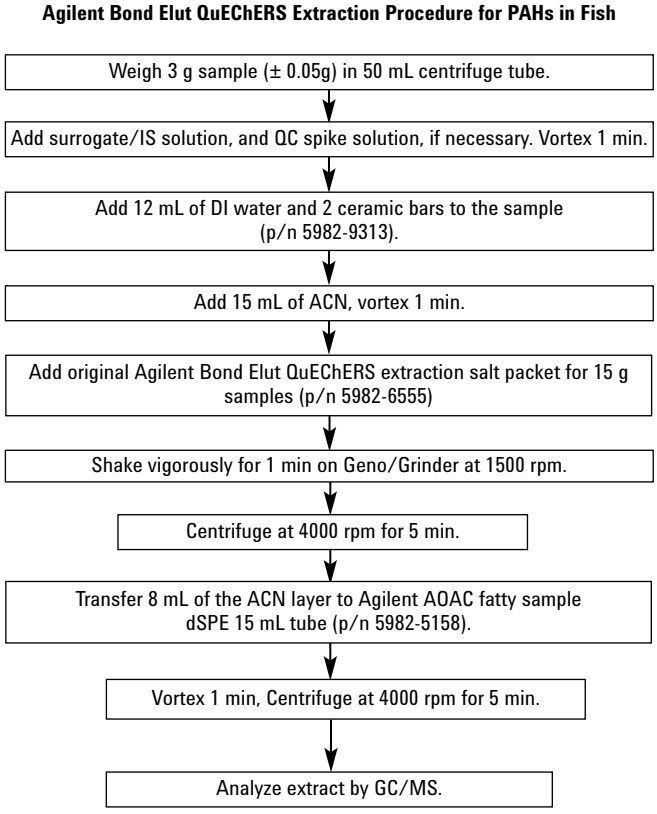

Figure 1. Flow chart of the Agilent Bond Elut QuEChERS modified extraction procedure for fish sample.

Discussion of Results

The sixteen PAH analytes were resolved on the Agilent J&W DB-5msUI 20 m × 0.18 mm, 0.18 µm analysis column in less than 20 minutes. Figure 2 shows the separation of a 500 ng/mL PAH standard solution. Quantitative GC/MS analysis of the target PAHs was performed using selective ion monitoring (SIM), which gave excellent sensitivity down to 10 ppb for all the analytes.

The performance of the DB-5msUI high efficiency column yielded excellent linearity and recovery over the calibration range of this study. The linearity of the column as defined by the R^2 values of the PAH standard curve ranged from 0.9988-0.9999. The individual PAH analyte values are shown in Table 3. Excellent signal-to-noise ratios were also seen for the lowest calibration standard on the column as shown in Figure 3. The method limit of quantitation (MLQ) of 10 ppb for benzo[a]pyrene is well below the level of concern of 30 ppb set by the NOAA and FDA.

Table 3. The R^2 Values for the PAH Calibration Standards Over the 10 ng/mL to 1000 ng/mL Range of this Study

Analyte	R^2
Naphthalene	0.9997
Acenaphthylene	0.9999
Acenaphthalene	0.9999
Fluorene	0.9999
Phenanthrene	0.9999
Anthracene	0.9999
Fluoranthene	0.9999
Pyrene	0.9998
Benz[a]anthracene	0.9993
Chrysene	0.9994
Benzo[b]fluoranthene	0.9990
Benzo[k]fluoranthene	0.9988
Benzo[a]pyrene	0.9992
Indeno[1,2,3-c,d]pyrene	0.9992
Dibenz[a,h]anthracene	0.9991
Benzo[g,h,i]perylene	0.9995

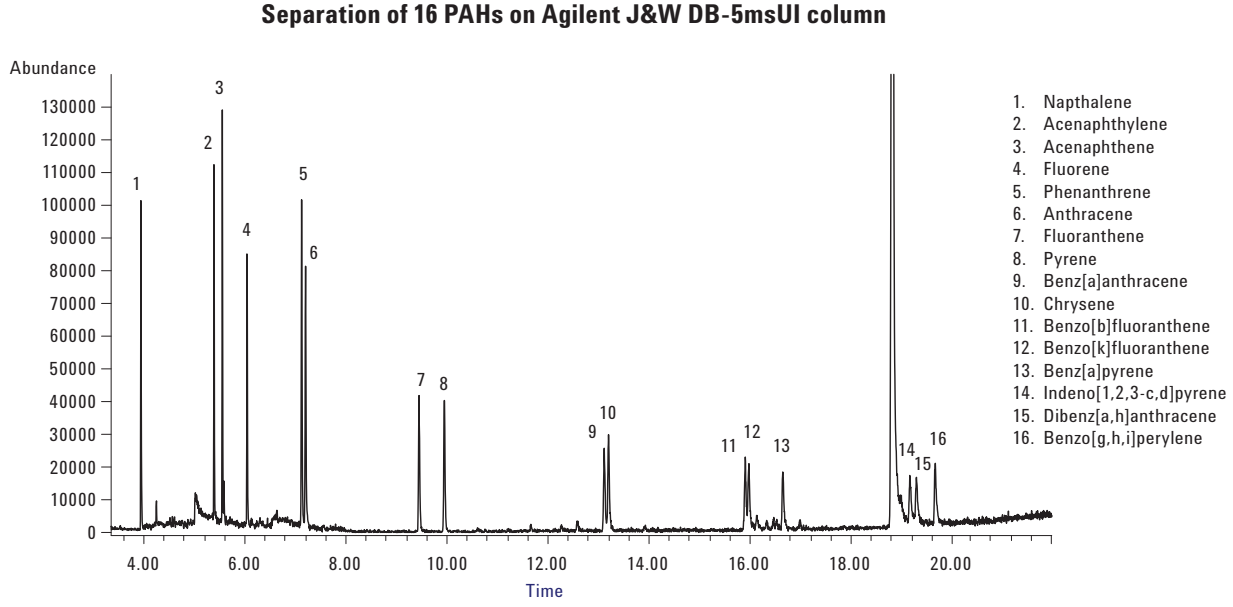

Figure 2. GC/MS chromatogram of the 500 ng/mL PAH standard prepared in sample matrix analyzed on an Agilent J&W DB-5msUI 20 m × 0.18 mm, 0.18 µm capillary GC column (Agilent p/n 122-5522UI). Chromatographic conditions are listed in Table 1.

Excellent Signal-to-Noise Ratios at Trace Levels

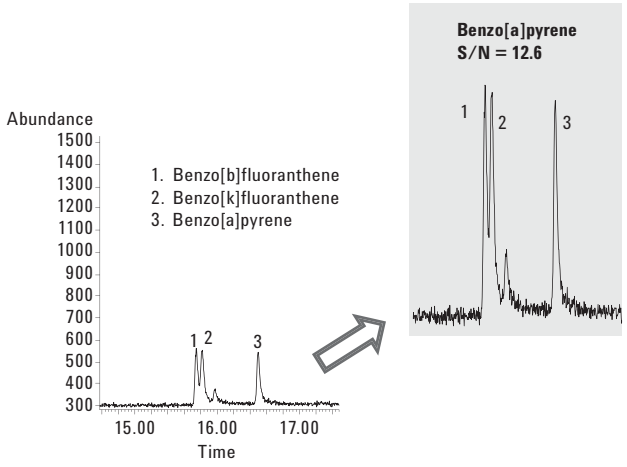

Figure 3. Enlarged view chromatogram of the benzo[a]pyrene peak in the 10 ng/mL PAH calibration standard prepared in sample matrix analyzed on the Agilent J&W DB-5msUI capillary column (p/n 122-5522UI). Chromatographic conditions are listed in Table 1.

The extraction process using the QuEChERS method followed by dispersive SPE was effective in retaining the PAHs in the spiked fish sample and providing sufficient cleanup of the sample matrix for GC/MS analysis. Figure 4 shows the separation of the extracted PAHs in a spiked fish sample on the DB-5ms UI column.

The recoveries were determined at the 25, 250, and 500 ng/mL PAH levels. Recoveries for the individual PAHs are shown in Table 4. The recoveries ranges (80% to 139%) and RSDs were excellent with the DB-5ms UI column for all PAHs investigated.

GC/MS SIM Chromatogram of Red Snapper Fish Extracts Blank Relative to Spiked Sample after Agilent Bond Elut QuEChERS Extraction and Dispersive SPE

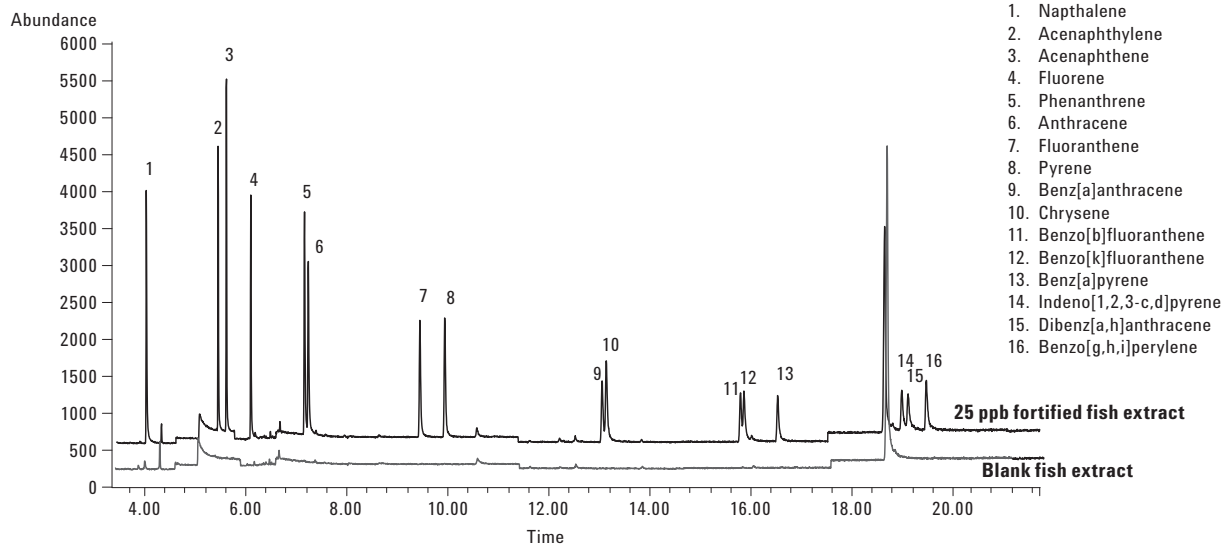

Figure 4. GC/MS SIM chromatogram of the fish extract blank and the 25 ng/mL spiked fish extract analyzed on Agilent J&W DB-5msUI capillary column (p/n 122-5522UI). Chromatographic conditions are listed in Table 1.

Table 4. Recovery and Repeatability of PAHs in Spiked Red Snapper Fish with Agilent J&W DB-5msUI Column (p/n 122-5522UI)

`	25 ng/mL fortifield QC		250 ng/mL fortifield QC		500 ng/mL fortifield QC	
Analyte	**% Recovery**	**RSD (n=6)**	**% Recovery**	**RSD (n=6)**	**% Recovery**	**RSD (n=6)**
Naphthalene	80.35	3.29	96.77	4.23	98.64	1.88
Acenaphthylene	95.28	2.30	103.36	2.80	101.02	2.27
Acenaphthalene	92.28	2.51	101.18	2.87	100.69	2.34
Fluorene	95.98	2.99	105.94	2.82	105.00	1.28
Phenanthrene	100.51	3.46	104.93	2.71	103.25	1.70
Anthracene	107.38	3.51	105.95	3.45	105.38	1.74
Fluoranthene	113.27	3.87	105.76	3.33	103.64	1.81
Pyrene	113.55	3.51	103.99	3.24	102.29	1.94
Benz[a]anthracene	129.79	3.41	101.45	3.91	100.61	3.24
Chrysene	116.75	4.01	98.55	4.17	95.95	5.61
Benzo[b]fluoranthene	131.20	3.70	98.77	4.08	98.08	3.24
Benzo[k]fluoranthene	139.45	2.52	99.13	3.98	95.31	4.54
Benzo[a]pyrene	125.30	3.68	95.33	3.89	96.82	1.80
Indeno[1,2,3-c,d]pyrene	119.51	3.47	94.57	3.23	93.71	2.55
Dibenz[a,h]anthracene	126.35	3.54	98.55	3.50	98.85	2.24
Benzo[g,h,i]perylene	114.91	4.93	97.30	3.37	95.63	1.83

Conclusions

This application note successfully shows a quick and efficient analytical method for monitoring low and trace level PAHs in fish samples suitable to address current food safety concerns. This method demonstrates the feasibility of a simplified approach for routine fish screening as an alternative to more time consuming and complex techniques.

The Agilent Bond Elut QuEChERS method followed by dSPE for fatty samples was effective at providing enough sample cleanup to avoid matrix interferences while still maintaining low level analyte detection. The simple QuEChERS extraction method allows faster sample prep, facilitating higher sample throughput. Any residual sample matrix carryover is removed through use of backflush, which eliminates the need for a bakeout cycle, and significantly reduces analytical run times.

The Agilent J&W DB-5ms UI High Efficiency column was effective at analyzing 16 PAHs in a fish matrix following sample matrix cleanup by QuEChERS and dSPE. The DB-5ms UI provided satisfactory resolution of the four known critical pairs; phenanthrene/anthracene, benz[a]anthracene/chrysene, benzo[b]/[k]fluoranthene, and indeno[1,2,3-c,d]pyrene/dibenz[a,h]anthracene. The performance of the DB-5ms UI yielded excellent linearity over the range of concentrations studied with R^2 values between 0.9988-0.9999 for the PAH compounds. Recovery and reproducibility was shown to be greater than 80% with an RSD below 6.0. The method limit of quantitation of 10 ppb for the benz[a]pyrene using this approach was significantly lower than current levels of concern.

Acknowledgements

The authors wish to thank Joan Stevens for her help and suggestions with the Agilent Bond Elut QuEChERS extraction procedure.

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Printed in the USA
January 6, 2012
Publication Number 5990-6668EN

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