PAH Analysis in Fish by GC/MS Using QuEChERS/dSPE Sample Preparation and High Efficiency GC Columns

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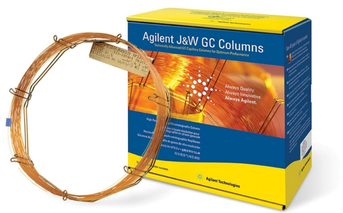
Introduction

The Deep Water Horizons disaster in the Gulf of Mexico introduced massive amounts of crude oil along with the hundreds of components in the oil including polycyclic aromatic hydrocarbons (PAHs). These molecules can be resilient in the environment and bioaccumulation in fatty tissue of aquatic species throughout the food chain.

NOAA (National Oceanic and Atmospheric Administration) in coordination with the US FDA (Food and Drug Administration) and other federal and state agencies set policy for fishery closure and re-opening of affected fisheries. Testing for PAHs is a requirement for the re-opening of a closed fishery. Levels of concern for 8 targeted PAHs (and alkyl homologues) are defined for finfish, oysters, shrimp, and crab species. These levels of concern range from 233 ppm for anthracene/phenanthrene in shrimp and crabs to 0.03 ppm in finfish for benzo[a]pyrene. Analysis at and below these levels is reliably achieved using a high efficiency capillary GC/MS system.

Sample preparation to execute the NOAA method historically has been labor intensive, taking 12-14 hours to process a sample. In this work the use of a QuEChERS/dSPE (Quick, Easy, Cheap, Effective, Rugged, and Safe/dispersive solid phase extraction) sample preparation is presented for use as a screening tool for PAHs. Sample prep with QuEChERS up to 60 samples can be processed in an 8 hour shift. Sample prep time savings translate directly to higher lab throughput.



Experimental

Chromatographic Conditions

GC/MSD:	Agilent 7890/5975B
Sampler:	Agilent 7693, 5.0 µL syringe (Agilent part #5181-1273)
PCT Device:	Purged Ultimate Union (Agilent part #G3186-60580)
Carrier:	Helium, constant flow 1.7 mL/min
Restrictor:	0.7 m x 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:	DB-5ms UI 20 m x 0.18 mm x 0.18 µm (Agilent part #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

Sample Prep

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

QuEChERS Sample Preparation Workflow

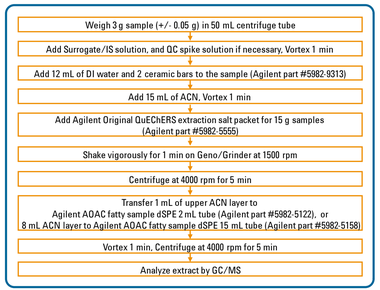
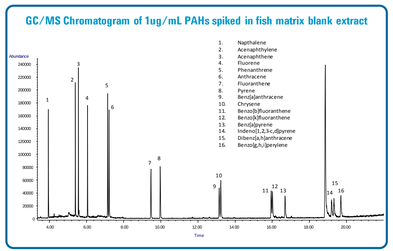


Figure 1. Flow chart of QuEChERS procedure for the determination of PAHs in fish

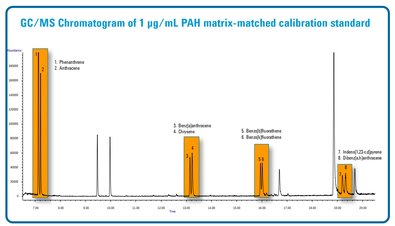
Results and Discussion

Separation of the 16 targeted PAHs on Agilent's J&W DB-5ms UI column

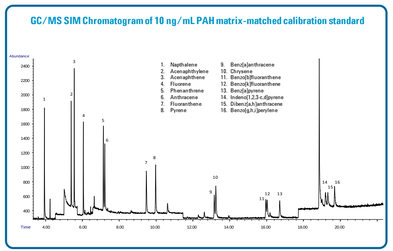


The sixteen targeted EPA PAHs were resolved on the Agilent J&W DB-5ms UI 20 m x 0.18 mm x 0.18 µm column in less than twenty minutes. The DB-5ms UI column provided satisfactory resolution for the four known critical pairs; phenanthrene/anthracene, benz[a]anthracene/chrysene, benzo[b]/[k]fluoranthene, and indeno[1,2,3-c,d]pyrene/dibenz[a,h]anthracene. Sharp, symmetrical peak shapes were obtained for the PAHs with the DB-5ms UI column.

Sufficient Resolution of PAH Critical Pairs with Agilent's J&W DB-5ms UI column

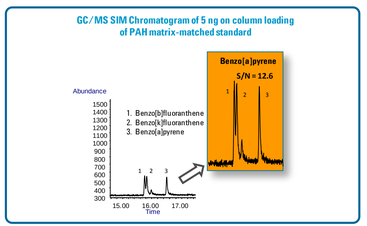


Agilent's Ultra Inert columns minimize activity improving trace analysis in complex mixtures



PAHs tend to adsorb onto active sites within the GC flow path. Minimizing column activity is critical to ensuring accurate and consistent results. The high level of inertness of Agilent's J&W DB-5ms UI column results in better peak shape for active analytes, giving better sensitivity at trace levels. The higher sensitivity translates to lower detection limits.

Excellent Signal-to-Noise Ratios at Trace Levels



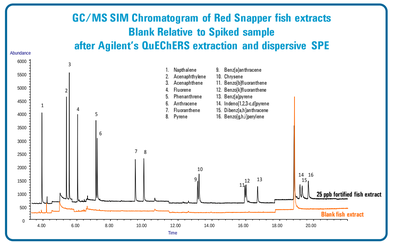
Benzo[a]pyrene is a PAH of particular interest due to its toxicity and is often used as an indicator for overall PAH contamination. Excellent signal to noise ratios were seen at the low calibration levels on the column as shown above. The method limit of quantization (MLQ) of 10 ppb for benzo[a]pyrene is well below the level of concern of 30 ppb set by the NOAA and FDA.

Results and Discussion

The extraction process using the QuEChERS method followed by dispersive SPE was effective in retaining the PAHs in the spiked fish sample and providing adequate cleanup of the sample matrix for GC-MS analysis. The figure shown below depicts the extracted PAHs in a spiked fish sample relative to the fish matrix blank 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 below. The recoveries ranges (80-139%) and RSDs were excellent with the Agilent J&W DB-5ms UI column for all PAHs investigated.

Agilent's QuEChERS Extraction Kits provide sufficient matrix cleanup while preserving low level detection



Recovery and Repeatability of PAHs in fortified Red Snapper fish with Agilent's J&W DB-5ms UI column

	25 ng/mL fortified QC		250 ng/mL fortified QC		500 ng/mL fortified QC	
Analytes	%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-cd]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

- The Agilent J&W DB-5ms UI high efficiency (0.18 mm ID) column was effective at analyzing 16 PAHs in a fish matrix
- Agilent's QuEChERS Extraction and Dispersive SPE kits provided a simple, fast, and economical method for the purification of PAHs in a fish matrix
- The performance of the J&W DB-5ms UI column yielded excellent linearity, along with good recovery and reproducibility for all sixteen PAHs

References

Polycyclic Aromatic Hydrocarbon (PAH) Analysis in Fish by GC/MS Using QuEChERS/dSPE Sample Preparation and a High Efficiency DB-5ms Ultra Inert GC Column
Agilent Publication #5990-6668EN

GC/MS Analysis of European Union (EU) Priority Polycyclic Aromatic Hydrocarbons (PAHs) using an Agilent J&W DB-EUPAH GC Column with a Column Performance Comparison
Agilent Publication #5990-4883EN

PAH Analyses with High Efficiency GC Columns: Column Selection and Best Practices
Agilent Publication #5990-5872EN

Polycyclic Aromatic Hydrocarbon (PAH) Analysis Using an Agilent J&W DB-5ms Ultra Inert Capillary GC Column
Agilent Publication #5989-9181EN

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