Application Note

Abstract

Polycyclic Aromatic Hydrocarbons (PAHs) are a large group of organic compounds found naturally in the environment. PAHs are monitored by the US Environmental Protection Agency due to their carcinogenic characteristics. In recent global events, PAHs have come to the attention for contamination in sea food samples due to the oil spills. In the oceanic environment, PAHs are bio-available via the food chain and contaminated sediments which could have the potential to bioaccumulate in the marine organism we eat.

QuEChERS is a Quick-Easy-Cheap-Effective-Rugged-Safe extraction method that has been developed for the determination of pesticide residues in agricultural commodities. Since its installment in 2003, QuEChERS has been adapted for use with many additional matrices and compound classes such as sea food and PAHs.

The aim of this project is to validate the extraction performance of the AutoMate-Q40 by monitoring Polycyclic Aromatic Hydrocarbons (PAH) extracted from seafood. The target compounds were determined by gas chromatography mass spectrometry (GC-MS).

Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) are chemical compounds that consist of fused aromatic rings. PAHs are a large group of organic compounds found naturally in the environment but can be also man-made. Since these compounds are pollutants, the United States Environmental Protection Agency (US EPA), United States Food and Drug Administration (US FDA) and National Oceanic and Atmospheric Administration (NOAA) monitor these compounds due to their mutagenic and carcinogenetic properties.[1,2]

Due to recent oil spills around the globe PAHs have found their way into our nation's seafood. These compounds can enter marine organisms through contaminated soil/sediments or through the natural food chain.

The QuEChERS extraction method is applicable for PAHs, since it offers good selectivity, and sensitivity when extracting these compounds in sea food.[3,4] The aim of this project is to evaluate the performance and versatility of the AutoMate-Q40 for the extraction of PAHS in shrimp and scallops. Gas Chromatograph coupled to a Mass Spectrometer (GC-MS) was employed for the detection of PAHs in seafood. Quantification was based on matrix-matched calibration curves.

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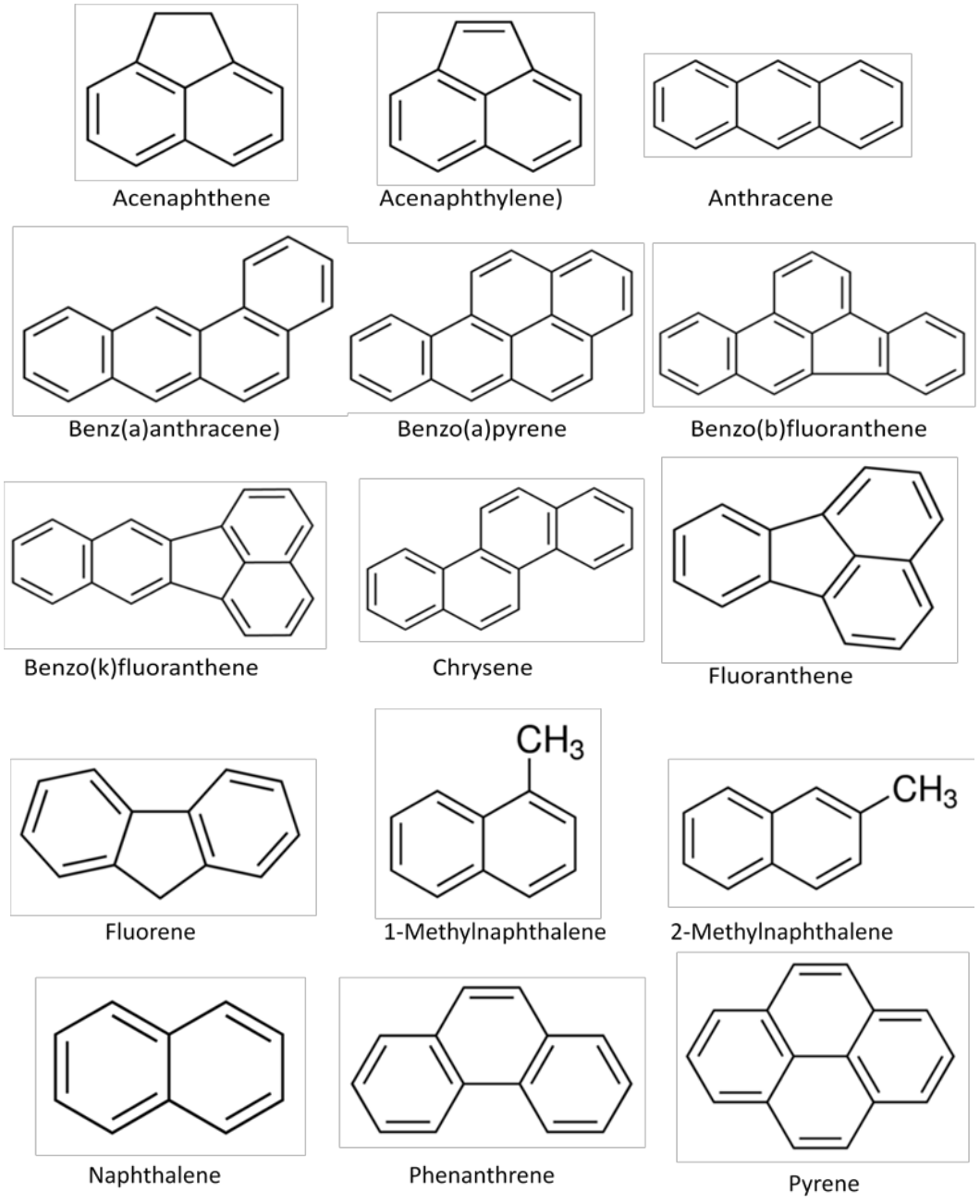
Experimental Instrument Conditions

Chemicals Structures

See Figure 1 for the PAHs used in this study

Figure 1 Polycyclic Aromatic Hydrocarbons (PAHs) Structures



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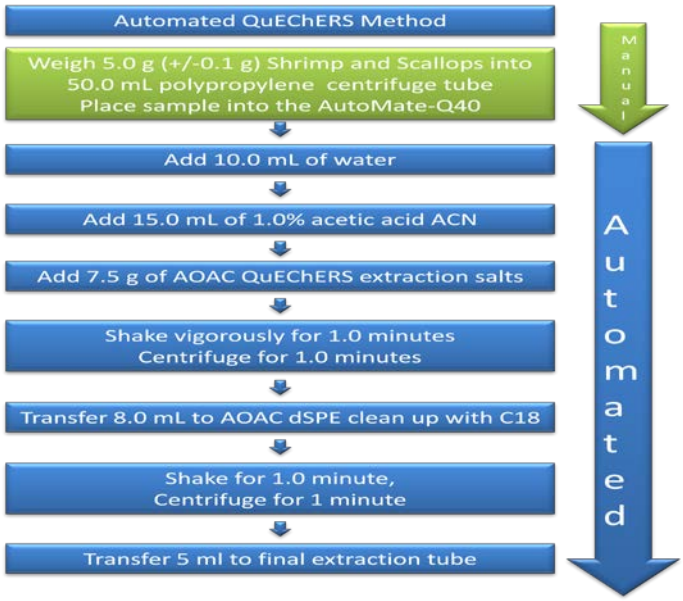
Sample Preparation

Shrimp and scallops were purchased locally in Ohio. The samples were prepared following the procedure described in the "AOAC Official Method 2007.01 Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate".[3] The samples were then stored frozen until the time of the extraction.

For this analysis, the AutoMate-Q40 used 7.5 g of AOAC QuEChERS extraction salts ($MgSO_4$ and NaOAc). The AutoMate-Q40 also used the AOAC version of $MgSO_4$ (1200.0 mg), PSA (400.0 mg) and C18 (400.0 mg) for the dSPE cleanup step. Figure 2 shows the sample preparation and extraction steps that are needed to extract the PAHs from seafood.

Figure 2 AutoMate-Q40 : Automated Extraction Parameters for Seafood



Sample Analysis

Sample analysis was conducted using an Agilent GC/MS system. For separation of the compounds of interest, a Restek 5Sil MS column was used. Table I demonstrates the optimized GC/MS analysis parameters for both the chromatographic separation and analyte transitions. Figure 3 shows the GC chromatogram spiked PAHs at 45.0 ng/g.

Table I GC/MS Parameters

GC Parameters	
Column	Rxi-5Sil MS Column 30 m x 0.25 mm x 0.25 µm
Oven Program	60 °C for 1 min, 15 °C/min to 295 °C for 5 min, Run Time 21.667 min
Inlet	275 C, 1.2 mL/min helium, Splitless, 40 mL/min Purge Flow
Transfer Line	240 °C
MS Parameters	
Mass Range	Selective Ion Monitoring (SIM)
Ion Source	EI, 230 °C

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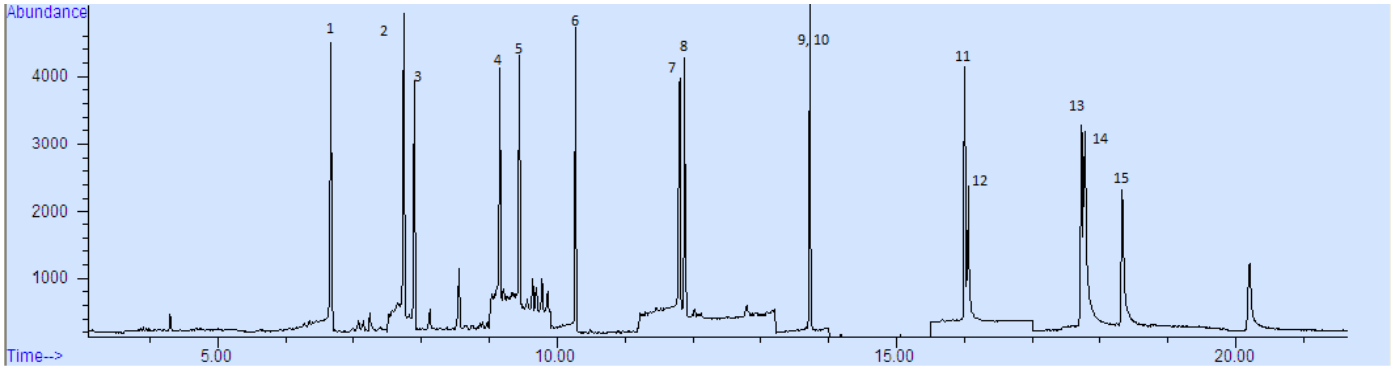
Figure 3 SIM Chromatogram of 16 PAH at 45 ng/g



1	Naphthalene	4	Acenapthylene	7	Phenanthrene	10	Pyrene	13	Benzo(b)fluoranthene
2	2-methyl-Naphthalene	5	Acenaphthene	8	Anthracene	11	Benz(a)anthracene	14	Benzo(k)fluoranthene
3	1-methyl-Naphthalene	6	Flurorene	9	Fluoranthene	12	Chrysene	15	Benzo(a)pyrene

Results

Calibration Results

Calibration standards were prepared 10-400 µL/L using matrix match blanks for both shrimp and scallops. The correlation coefficients (r2) are shown in Table II.

Table II Correlation Coefficients for Matrix Match Calibration Standards (Quadratic Regression)

Compounds	Shrimp	Scallops
	Calibration r^2	Calibration r^2
Naphthalene	0.996	1.000
2-methyl-Naphthalene	0.996	0.999
1-methyl-Naphthalene	0.996	0.999
Acenapthylene	0.998	1.000
Acenaphthene	0.999	1.000
Flurorene	1.000	1.000
Phenanthrene	0.997	1.000
Anthracene	0.995	1.000
Fluoranthene	0.997	1.000
Pyrene	0.999	1.000
Benz(a)anthracene	0.996	1.000
Chrysene	0.997	0.999
Benzo(b)fluoranthene	0.998	1.000
Benzo(k)fluoranthene	0.990	1.000
Benzo(a)pyrene	0.991	1.000

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Reproduciblilty and Accuarcy Results

Automating the QuEChERS extraction enables an easy, reliable and more reproducible extraction. The AutoMate-Q40 offers significant labor savings, while improving the reproducibility and consistency between samples.

A precision and accuracy study was performed using the AutoMate-Q40. A 1.0 µg/mL stock PAH solution was used to fortify the seafood samples. Check standards were fortified at 10.0 and 15.0 µg/L using the AutoMate-Q40's ability to make standard additions. This translates to 30.0 and 45.0 ng/g in the final sample for seafood. These QC samples were quantified against their corresponding matrix matched calibration. The results for both shrimp and scallops can be seen in Tables III and IV.

Table III Spike Recovery in Shrimp

Compound	30 ng/g Spike PAH		45n g/g Spike PAH	
	%Recovery	%RSD	%Recovery	%RSD
Naphthalene	87.53	13.92	95.29	9.11
2-methyl-Naphthalene	97.30	19.79	91.33	3.51
1-methyl-Naphthalene	97.70	15.66	91.69	7.48
Acenapthylene	110.08	4.77	102.20	4.51
Acenaphthene	102.32	10.45	102.47	3.86
Flurorene	113.07	8.87	102.97	4.99
Phenanthrene	102.27	11.38	96.13	9.51
Anthracene	103.22	9.93	103.00	3.30
Fluoranthene	106.70	7.62	107.11	9.19
Benz(a)anthracene	115.57	22.02	100.58	18.43
Chrysene	104.12	21.11	96.73	5.01
Benzo(b)fluoranthene	99.27	10.49	98.60	5.12
Benzo(k)fluoranthene	93.95	13.35	91.89	6.19
Benzo(a)pyrene	105.28	9.75	99.76	12.49

Table IV Spike Recovery in Scallops

Compound	30ng/g Spike PAH		45ng/g Spike PAH	
	%Recovery	%RSD	%Recovery	%RSD
Naphthalene	98.06	7.57	97.04	4.08
2-methyl-Naphthalene	98.87	12.99	97.82	6.24
1-methyl-Naphthalene	94.39	3.33	98.87	3.86
Acenapthylene	113.83	6.78	109.44	6.03
Acenaphthene	102.91	8.42	103.24	4.42
Flurorene	92.14	14.18	101.67	2.27
Phenanthrene	91.27	14.78	103.09	2.24
Anthracene	105.41	9.55	100.51	6.24
Fluoranthene	97.44	9.97	104.11	2.92
Pyrene	88.51	12.39	103.69	2.34

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Benz(a)anthracene	97.16	8.08	107.11	4.04
Chrysene	96.26	6.53	94.89	4.43
Benzo(b)fluoranthene	100.04	5.65	106.53	2.24
Benzo(k)fluoranthene	94.96	5.25	93.04	2.26
Benzo(a)pyrene	106.74	5.30	105.62	2.49

Conclusion

This study demonstrates the Automate-Q40's ability to successfully process seafood samples for PAHs by the QuEChERS extraction method. By automating the liquid handling, addition of salt/buffers, sample mixing, pipetting, and liquid level sensing using the patent pending VialVision™, the AutoMate-Q40 frees the scientist from a labor-intensive extraction method and exposure to unhealthy chemicals.

The automated extraction process enables an easy, reliable and more reproducible extraction. This enables time and labor savings, while improving consistency and reproducibility of the extraction. The combined PAH spikes recoveries of 100.4%, with an average RSD of 8.14% exceed the requirement outlined for this application. These numbers indicate superb precision and accuracy thus validating the performance of the AutoMate-Q40 to adequately perform the QuEChERS extraction method for seafood.

References

1. United States Environmental Protection Agency, Polycyclic Aromatic Hydrocarbons (PAHs), Office of solid waste Washington, DC 20460, January 2008
2. U.S. EPA Method 8270D, Revision 4, February2007, “Semivolatile Organic Compounds by GasChromatography/Mass Spectrometry (GC/MS)”
3. AOCA Official Method 2007.07 Pesticide Residues in Food by Acetonitrile Extraction and Partitioning with Magnesium Sulfate. Gas Chromatography/Mass Spectrometry and Liquid Chromatography/Tandem Mass Spectrometry, First Action 2007
4. M. Anastassiades: QuEChERS a mini-multiresidue method for the analysis of pesticide residues in low-fat products

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