

Determinación de hidrocarburos Policíclicos aromáticos (PAH) en marisco mediante una solución QuEChERS automatizada

Aplicación 056

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RESUMEN

Los Hidrocarburos Policíclicos Aromáticos (PAH) son un grupo de compuestos orgánicos que se encuentran en el medioambiente. Los PAH son controlados (US Environmental Protection Agency) debido a sus efectos cancerígenos, PAH y su presencia en alimentos procedentes del mar especialmente mariscos debido a los vertidos de crudo, Los PAH se introducen en la cadena trófica y tienen un poder de bioacumulación a través de los organismos marinos presentes en nuestra dieta.

El método de extracción conocido como QuEChERS (Quick-Easy-Cheap-Effective-Rugged-Safe) fue desarrollado para la determinación de residuos de pesticidas en productos vegetales y alimenticios. Desde su implantación en 2003, QuEChERS se ha adaptado a multitud de matrices incluyendo la preparación de muestra para la determinación de PAH.

La presente aplicación describe como automatizar el proceso de preparación de muestra mediante el Modelo robotizado AutoMate-Q40 en el control de Hidrocarburos Policíclicos Aromáticos (PAH) extraídos de productos marinos. La determinación final se lleva a cabo mediante Cromatografía gases masas (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.

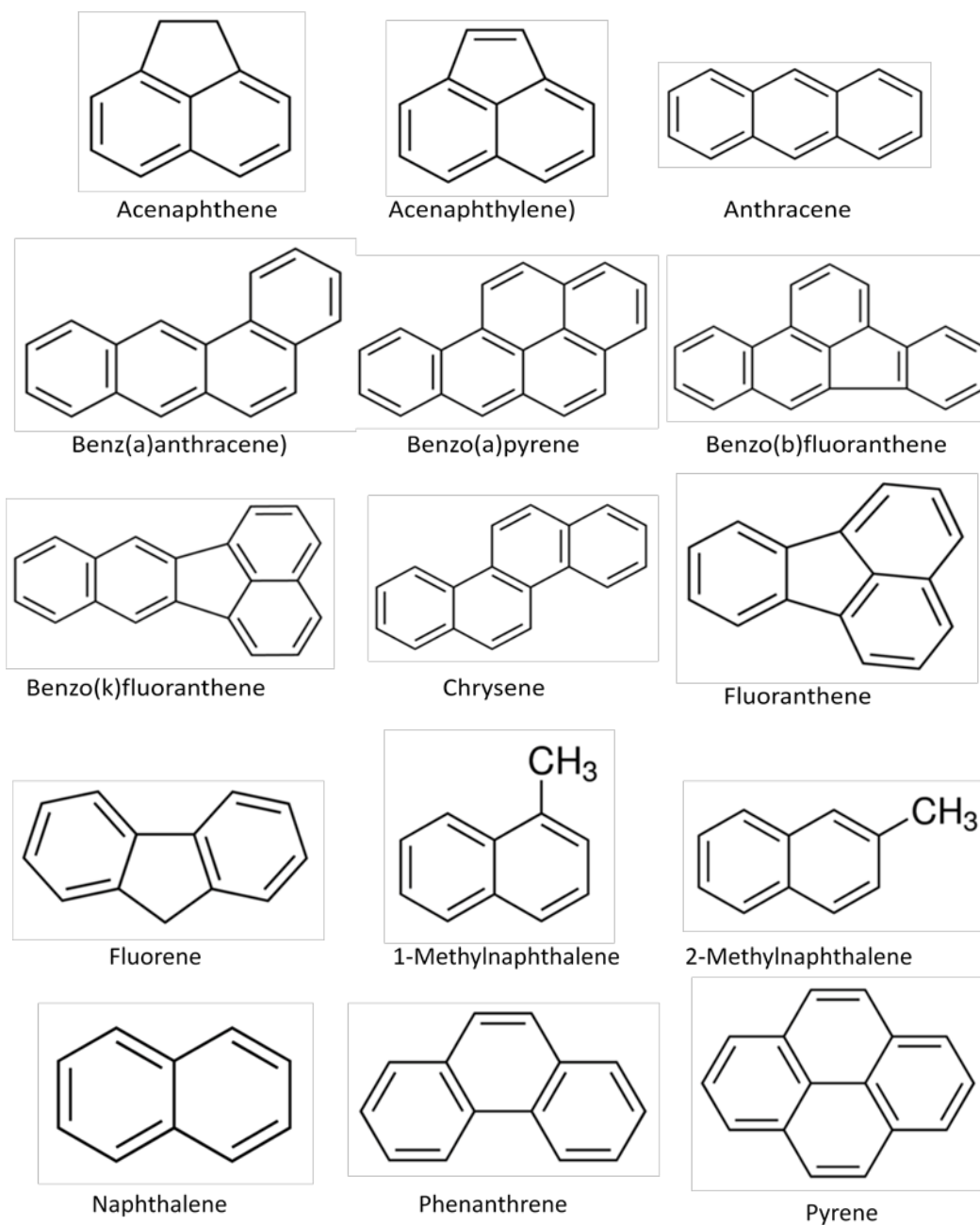


Experimental Instrument Conditions

Chemicals Structures

See Figure 1 for the PAHs used in this study

Figure 1 Polycyclic Aromatic Hydrocarbons (PAHs) Structures



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 (MgSO₄ and NaOAc). The AutoMate-Q40 also used the AOAC version of MgSO₄ (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



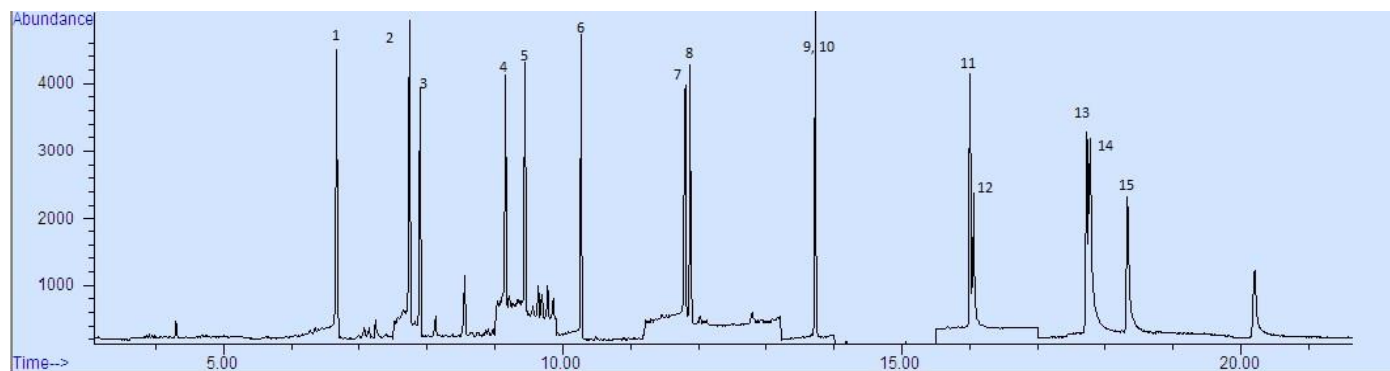
Sample Analysis

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	Acenaphthylene	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	Fluorene	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.

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
Acenaphthylene	0.998	1.000
Acenaphthene	0.999	1.000
Fluorene	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

Reproducibility and Accuracy 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
Acenaphthylene	110.08	4.77	102.20	4.51
Acenaphthene	102.32	10.45	102.47	3.86
Fluorene	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
Benzo(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

Compound	30ng/g Spike PAH	45ng/g Spike PAH		
Benzo(a)pyrene	105.28	99.76		
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
Acenaphthylene	113.83	6.78	109.44	6.03
Acenaphthene	102.91	8.42	103.24	4.42
Fluorene	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
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, February 2007, "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