Determination of PAH in Seafood:
An Optimized Procedure for LC–Fluorescence Screening and GC–MS(MS) Confirmation

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Gulf of Mexico Seafood

PAH in Seafood

- Significant interest in analytical methods following Gulf of Mexico oil spill
- Need for rapid and sensitive analysis for fish and shellfish

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Cover story:
Analyzing Gulf Seafood

UPPER LIMITS

Levels of concern set for polycyclic aromatic hydrocarbons in Gulf seafood

CHEMICAL[a]	LEVELS OF CONCERN (PPM) SHRIMP/CRABS	OYSTERS	FINFISH
NON-CANCER CAUSING[b]			
Naphthalene	123	133	32.7
Fluorene	246	267	65.3
Anthracene & phenanthrene	1,846	2,000	490
Pyrene	185	200	49.0
Fluoranthene	246	267	65.3
CANCER POTENTIAL[b,c]			
Chrysene	132	143	35.0
Benzo[k]fluoranthene	13.2	14.3	3.5
Benzo[b]fluoranthene	1.32	1.43	0.35
Benzo[a]anthracene	1.32	1.43	0.35
Indeno[1,2,3-cd]pyrene	1.32	1.43	0.35
Dibenzo[a,h]anthracene	0.132	0.143	0.035
Benzo[a]pyrene	0.132	0.143	0.035

a Includes alkylated homologs: C1, C2, C3, C4 naphthalenes; C1, C2, C3 fluorenes; and combined C1, C2, C3, C4 anthracenes/phenanthrenes. **b** Target compounds and cancer potential based on Agency for Toxic Substances & Disease Registry. **c** Based on a 1-in-100,000 increase in lifetime upper-bound cancer risk adjusted for exposures expected to last five years. For samples containing any of the last seven compounds, the sum of the individual ratios of the detected levels to the levels of concern cannot exceed 1. **SOURCE:** Food & Drug Administration

FDA Limits of Concern (from C&EN)

Some Quotes From the C&EN Article

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- "The accepted NOAA method for PAH detection uses gas chromatography along with mass spectrometry"
  - "the method requires extensive sample cleanup"

- "FDA wanted a simpler approach with higher sample throughput"
  - "In the adapted method, PAHs are extracted from pulverized seafood through a modified QuEChERS (quick, easy, cheap, effective, rugged, and safe) sample prep"
  - "The extracts are filtered but don't require further cleanup for LC/Fluorescence analysis"
  - "Testing times were reduced from about a week to two days"
  - "Samples found positive for a target PAH were required to undergo confirmatory testing using the (NOAA) GC/MS method"

NOAA Method (for GC-MS)

- Weigh sample into ASE (accelerated solvent extraction) vessel
- Perform ASE using methylene chloride
- Evaporative concentration (Kuderna-Danish)
- Cleanup on Silica/Alumina
- Evaporative Concentration to final volume

About 1 day of Sample Prep

QuEChERS Approach For LC Method*

- Weigh sample into centrifuge tube
- Add acetonitrile
- Add QuEChERS Salts (From DisQuE tube)
- Shake well (for at least 1 minute)
- Centrifuge
- Remove aliquot of supernatant and analyze by LC-Fluorescence

About 1 hour of Sample Prep

*LC-Fluorescence method details - Tuesday Afternoon @ 1:30
"Ensuring Seafood Safety with Rapid Screening for Polyaromatic Hydrocarbons Using LC–Fluorescence", Joe Romano, Waters Corporation

LC-Fluorescence Analysis

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LC-FL Chromatogram of oyster sample spiked at 10.0 µg/g

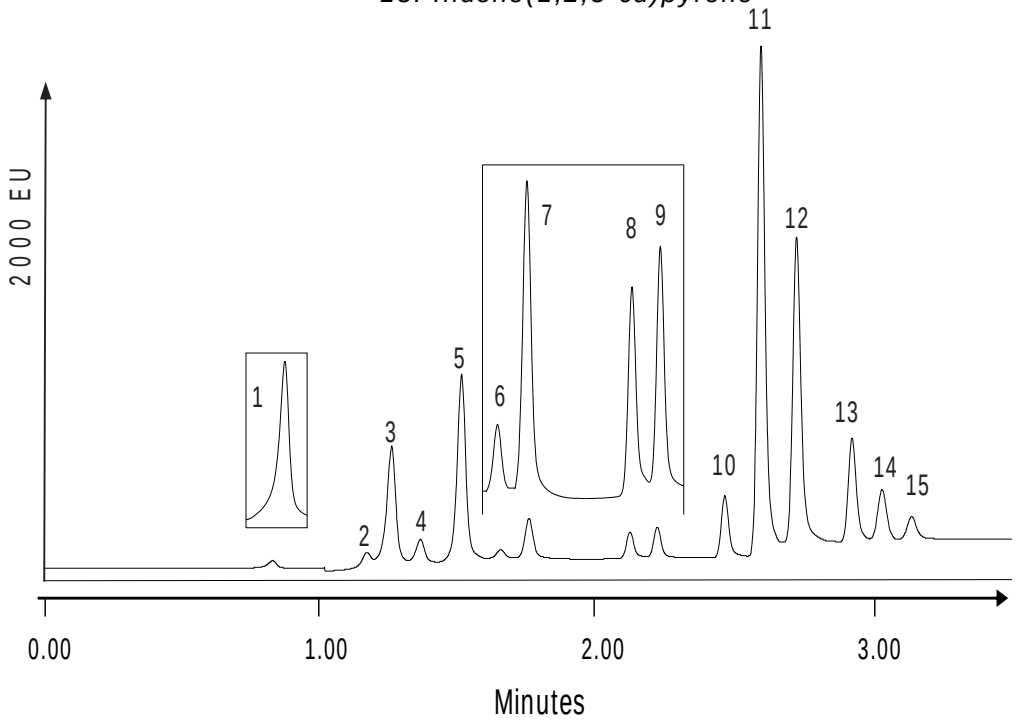
System: ACQUITY H Class- FLR with Large Volume Flow Cell
Column: Waters PAH 4.6 X 50 mm, 3µm, @ 35° C
Injection Volume: 10 µl
Sampling Rate: 20 pts/sec

Detection: Fluorescence (FL)
(using timed programmed wavelength changes)
Software: Empower™ 2
Mobile Phase A: Water
Mobile Phase B: Methanol
Mobile Phase C: Acetonitrile
Flow Rate: 2.0 ml/min

Gradient Profile:

Time (min)	Flow Rate (mL/min)	% A	% B	%C	Curve
0.00	2.0	30	70	0	
2.25	2.0	0	70	30	6
3.50	2.0	0	0	100	6
3.60	2.0	30	70	0	6

1. *Naphthalene*
2. *Acenaphthene*
3. *Fluorene*
4. *Phenanthrene*
5. *Anthracene*
6. *Fluoranthene*
7. *Pyrene*
8. *Benzo(a)anthracene*
9. *Chrysene*
10. *Benzo(b)fluoranthene*
11. *Benzo(k)fluoranthene*
12. *Benzo(a)pyrene*
13. *Dibenzo(a,h)anthracene*
14. *Benzo(g,h,i)perylene*
15. *Indeno(1,2,3-cd)pyrene*



A New Confirmation Analysis Option

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Instead of perfoming a second (ASE) extraction on a new portion of sample for GC-MS confirmation,

Why not use the QuEChERS extract for GC-MS Analysis?

Sample Preparation and Analysis Strategy
PAH Analysis in Shellfish

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Extract Shellfish
(QuEChERS)

Perform LC - Fl
Screening Analysis

PAH Detected?

Above Action Limit

SPE Cleanup of QuEChERS Extract

Below Action Limit

Done

Oasis ® HLB
Initial Cleanup and
Exchange to Hexane

Sep -Pak ® Silica
PAH Isolation
and Cleanup

Perform GC-MS(MS)
Confirmatory Analysis

SPE Prior to GC/MS(MS)
Oasis HLB for Solvent Exchange and Initial Cleanup

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Sample Pre-preparation

- Take 1 mL of the top (ACN) layer from the QuEChERs Extraction
- Add internal standards
- Dilute to 3 mL with water

This Oasis HLB SPE provides solvent exchange with no loss of volatile constituents such as naphthalene

CARTRIDGE 1

Oasis HLB
3 cc, 60 mg

Condition
1 mL acetonitrile (ACN),
1 mL 25:75 ACN/water

Load
diluted extract

Wash
1 mL 50:50 ACN/water and dry cartridge under vacuum for a few minutes

Go to Sep-Pak Silica, cartridge 2 →

SPE for Cleanup Prior to GC/MS(MS)

Certified Sep-Pak Silica

CARTRIDGE 2

Certified
Sep-Pak Silica
3 cc, 500 mg

Condition
2 mL hexane

Attach Cartridge 1 to cartridge 2 with adaptor

Wash
2 mL hexane (discard)

Install collection vessels

Elute
3 mL 25:75 DCM/Hexane

Evaporate to 0.25 mL (not to dryness!)

SPE Cleanup for GC/MS(MS) Confirmation

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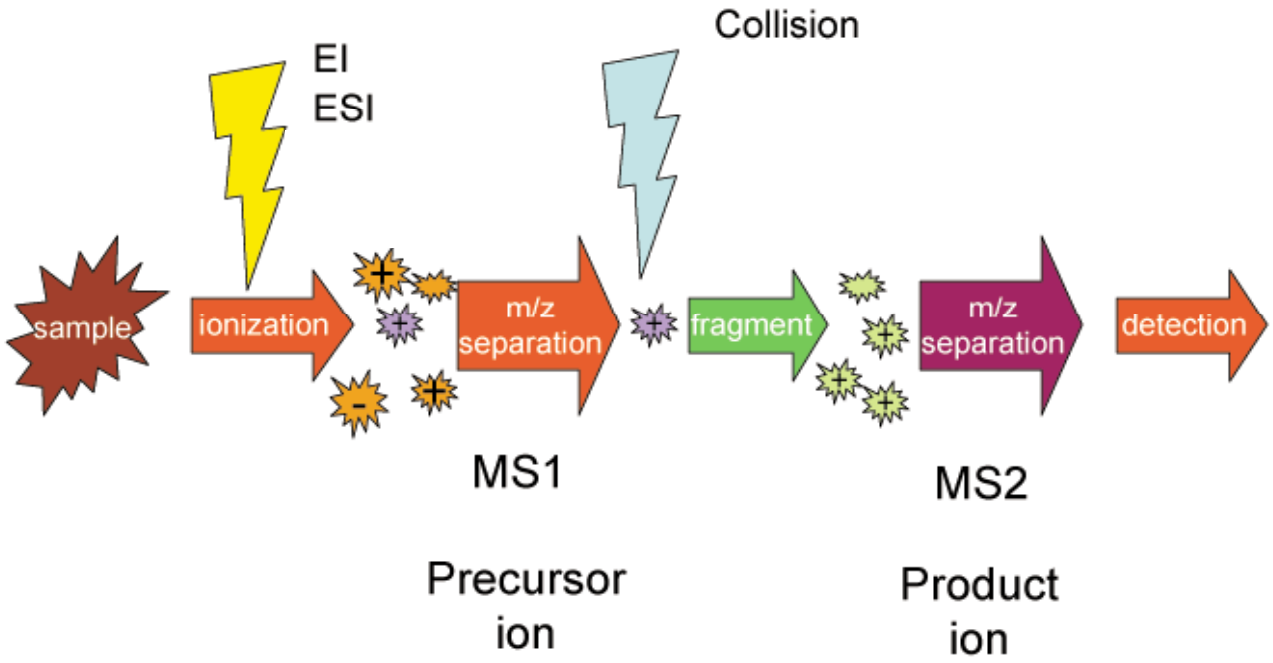
- If all PAH must be recovered at > 75%, then use tandem cleanup (Oasis HLB and Sep-Pak Silica)
- If high recovery of 1 and 2 ring PAH are not crucial, then only Sep-Pak Silica cleanup is required
  - Evaporate 1 mL of QuEChERs extract
    - there will be evaporative loss of naphthalene, acenaphthylene and acenaphthene
  - Take up residue in 0.5 mL hexane
    - Perform SPE on Certified Sep-Pak Silica cartridge

Tandem (MS/MS) Mass-Spectrometry

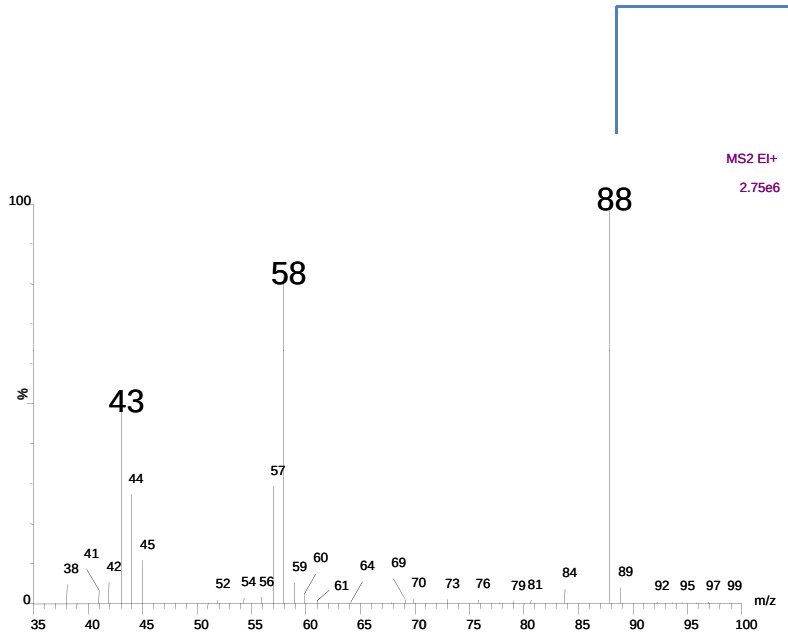


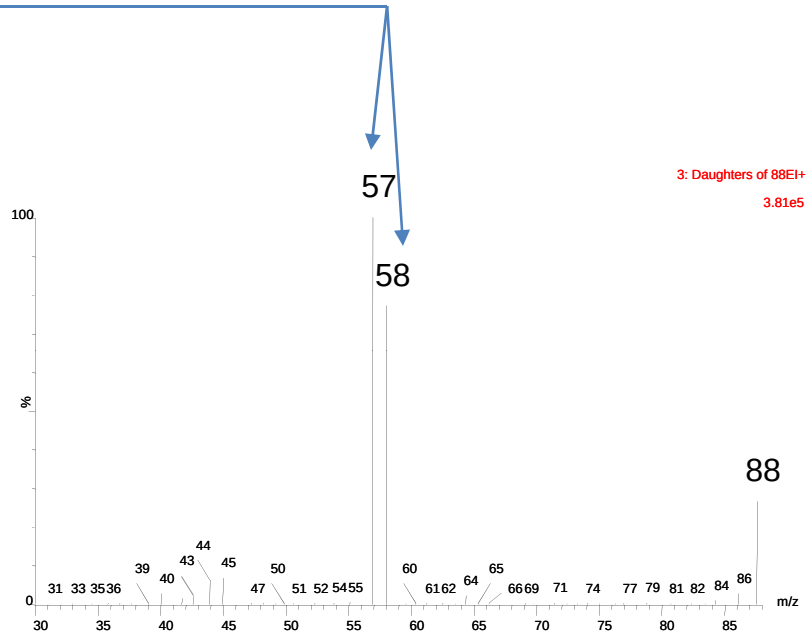
K. Murray, Wikipedia

Selected Reaction Monitoring (SRM) or Multiple Reaction Monitoring (MRM)

- More selective than SIR (SIM)
- Usually more sensitive than SIR

Example: GC-MS(MS)



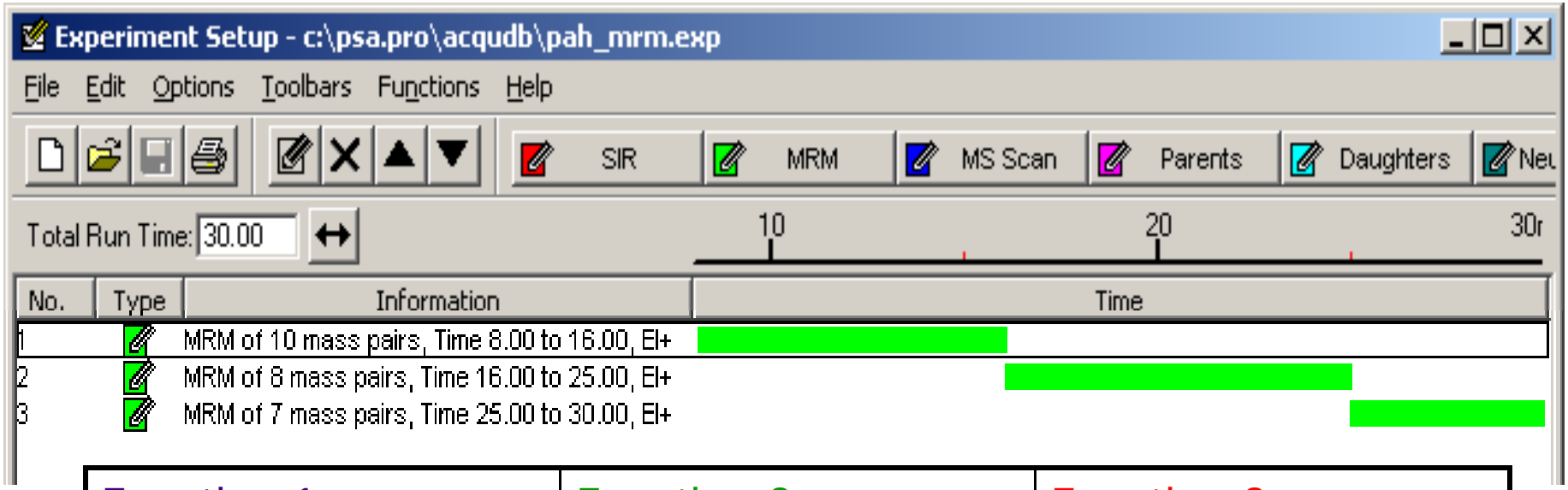


GC-MS: *Full scan spectrum produced from a 1 µg/mL standard 1,4-dioxane.*

GC-MS(MS): *Product ion spectrum produced from a 1 µg/mL standard (argon 5 eV CID)*

MRM: 88 > 57
88 > 58

MS(MS) Functions (Quattro micro GC)



Function 1	Function 2	Function 3
1. Naphthalene	5. Phenanthrene	11. Benzo(b)fluoranthene
2. Acenaphthene	6. Anthracene	12. Benzo(k)fluoranthene
3. Acenaphthylene	7. Fluoranthene	13. Benzo(a)pyrene
4. Fluorene	8. Pyrene	14. Indenopyrene
	9. Benz(a)anthracene	15. Dibenz(a,h)anthracene
ISTD: acenaphthene-d10	10. Chrysene	16. Benzoperylene
	ISTD: phenanthrene-d10	ISTD: perylene-d12

GC/MS Conditions

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System: Waters Quattro micro GC™
Ion Mode: EI+
Ion Energy: 70 eV
Inter Channel Delay: 0.01 sec
Dwell: 0.03 sec

PAH	MRM1	Collision (eV)	MRM2	Collision (eV)
Function 1				
1. Naphthalene	128>128	15	128>102	20
2. Acenaphthene	152>151	20	152>150	25
3. Acenaphthylene	154>153	20	154>152	30
4. Fluorene	166>165	20	166>164	35
ISTD1: Acenaphthene-d10	136>136	15		
Function 2				
5. Phenanthrene	178>151	40	178>152	15
6. Anthracene	178>151	40	178>152	15
7. Fluoranthene	202>202	20	202>200	35
8. Pyrene	202>202	20	202>200	35
9. Benz(a)anthracene	228>226	30	228>228	25
10. Chrysene	228>226	30	228>228	25
ISTD2: Phenanthrene-d10	188>160	40		
Function 3				
11. Benzo[b]fluoranthene	252>250	30	252>252	25
12. Benzo[k]fluoranthene	252>250	30	252>252	25
13 Benzo[a]pyrene	252>250	30	252>252	25
14. Indeno(1,2,3-cd)pyrene	276>274	40	276>276	25
15. Dibenz(a,h)anthracene	278>276	35	278>278	25
16. Benzo[ghi]perylene	276>274	40	276>276	25
ISTD3: perylene-d12	264>260	30		

PAH Analysis
GC/MS(MS)

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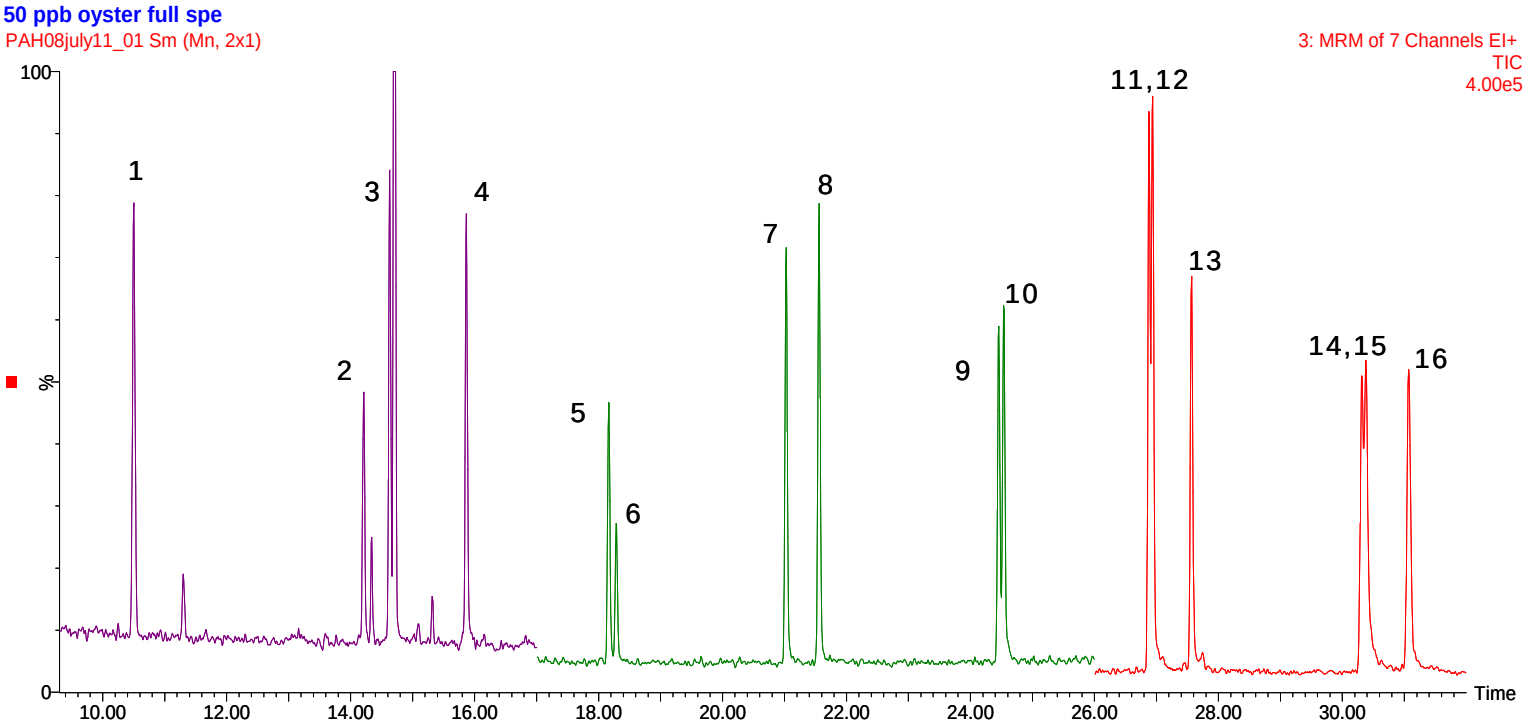
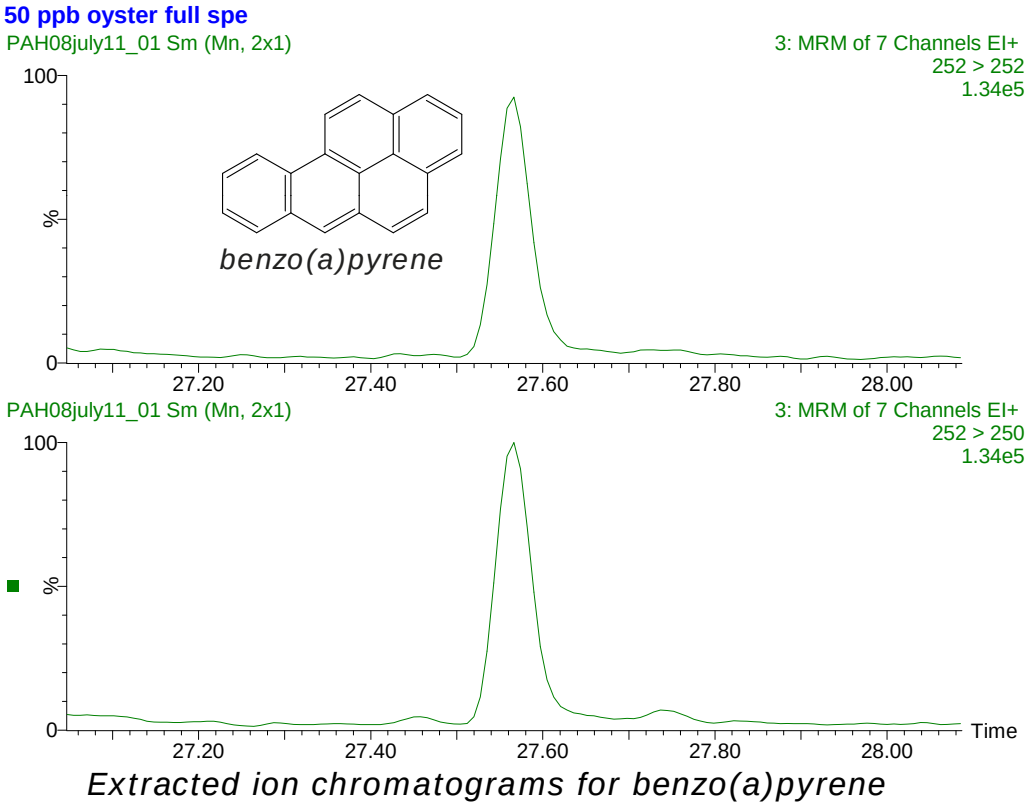
GC: Agilent 6890
Column: Rxi®-5Sil, 30 meter x 0.25 mm (ID), 0.25 µm (df)
Injection Volume: 1.0 µL
Injection Mode: Splitless (purge time 0.75 min)
Carrier Gas: Helium
Flow Rate: 0.8 ml/min (constant flow)
Temperature Program: 50°C initial, hold 1 min, then 10°C/min to 310°C, hold 10 min

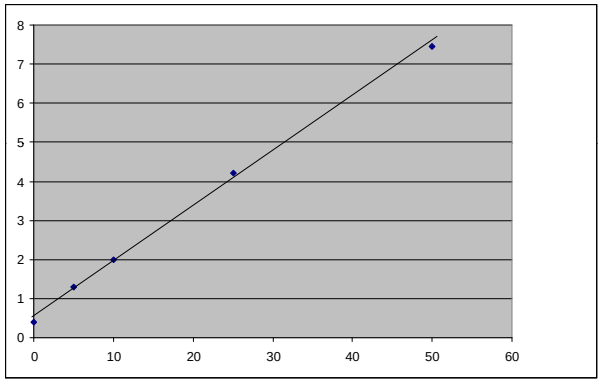


GC-MS(MS) Chromatograms (Functions 1,2,3) of oyster sample spiked at 50 ng/g (Internal standards were omitted for clarity)

Benzo(a)Pyrene in Oyster



Extracted ion chromatograms for benzo(a)pyrene



Matrix-matched calibration curve
(5-50 ng/g)
r^2 = 0.9989

FDA Concern Level 143 ng/g (oyster), 35 ng/g (finfish)

Conclusions

- The dispersive sample preparation (QuEChERS) used for LC-FL provides an extract that can be readily utilized for GC-MS confirmation.
- A straightforward SPE protocol is demonstrated for sample cleanup and solvent exchange to provide optimum GC performance.
- The SPE and GC-MS(MS) approach provides effective confirmation analysis.
- For GC-MS analysis, the QuEChERs acetonitrile extraction protocol provides equivalent performance compared with extraction with methylene chloride (DCM)
  - Extract is suitable for LC-Fl analysis with no cleanup and GC-MS analysis with cleanup; DCM extract is not suitable for LC-Fl
  - GC-MS cleanup for QuEChERs extract is similar to cleanup of DCM extract