Determination of PAH in Seafood: Optimized Sample Preparation Procedures for LC-Fluorescence Screening and GC-MS(MS) Confirmation

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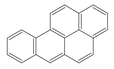
INTRODUCTION

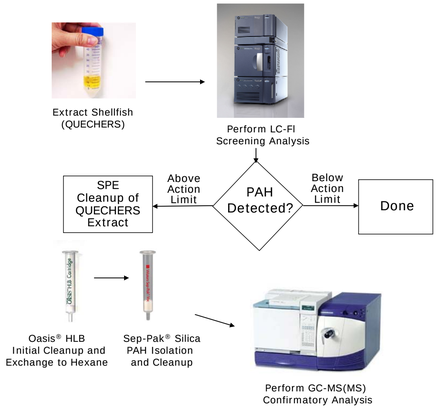
Overview

PAH (polycyclic aromatic hydrocarbons) are toxic compounds common in nature and are constituents of coal and petroleum. Many of these compounds (for example, benzo (a)pyrene, structure below) are carcinogenic. The recent major oil spill in the Gulf of Mexico has focused attention on the problem of PAH contamination and also on the challenges related to PAH analysis of food and environmental samples. In this presentation we will discuss an optimized sample preparation protocol for determination of PAH in shellfish samples. The initial sample extraction is performed using a dispersive (QUECHERS) method by which a sample is extracted with acetonitrile in the presence of an excess of buffer salts. This technique provides a convenient extract well suited for LC analysis with fluorescence detection (LC-FI); no further workup is required. Although LG-FL is a very sensitive technique, any PAH detected by LC-FL may require confirmation by a mass-spectral based analysis. Moreover, the QUECHERS extract is not ideal for GC-MS analysis; for optimum instrumental performance, cleanup and solvent exchange are recommended. A simple, straightforward SPE strategy will be demonstrated for effective PAH confirmation analysis by GC-MS (MS) in oyster and related samples prepared using the QUECHERS approach. Thus, the same sample extract can be used for rapid LC-FI screening and for GC-MS confirmation (after cleanup and solvent exchange).



benzo(a)pyrene

Sample Preparation and Analysis Strategy



SAMPLE PREPARATION

QUECHERS Extraction

Weigh 15 g of homogenized tissue into a 50 mL centrifuge tube. For recovery and QC samples, spike with PAH mixture.

Add 5 mL water to finfish and shrimp samples to aid mixing (oysters do not require added water). Spiked samples are thoroughly mixed and allowed to sit at room temperature for an hour.

To each centrifuge tube add the contents of a DisQuE™ dispersive extraction tube (6 g magnesium sulfate + 1.5 g sodium acetate, pn 186004571) and 15 mL of acetonitrile.

The centrifuge tube is shaken vigorously for one minute.

Cleanup for LC-Fluorescence Analysis

No cleanup is required for the LC analysis!
After centrifuging, a portion of the clear supernatant layer is transferred to an autosampler vial for direct injection to the LC (1 µg/g and 10 µg/g spiked samples are diluted 1:10 and 1:100 respectively prior to injection).

Cleanup for GC-MS(MS) Analysis

Take 1 mL of the supernatant (ACN layer) from the DisQue Extraction and dilute to 3 mL with water.

Add internal standard(s).

Condition Oasis HLB cartridge (3 cc, 60 mg) with 1 mL acetonitrile, 1 mL 25:75 ACN/water (Cartridge 1).

Load diluted extract.

Wash with 1 mL 50:50 ACN/water and dry cartridge under vacuum for five minutes.

Condition Certified Sep-Pak Silica cartridge (500 mg, 3 cc) with 2 mL hexane (cartridge 2).

Attach cartridge 1 atop cartridge 2 with adaptor (tandem).

The tandem cartridges are washed with 2 mL hexane (discard) and eluted with 3 mL 25:75 DCM/Hexane.

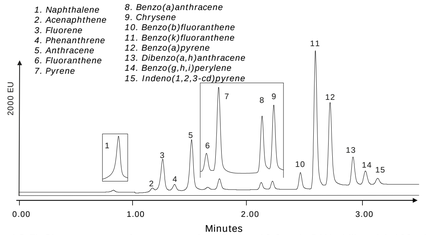
The eluent is evaporated to 0.25 mL (not to dryness!).

Perform GC-MS(MS) analysis.

Note: if high recovery of naphthalene and other 2-ring PAH is not important, the QUECHERS extract can be evaporated, the residue taken up in hexane and only the Sep-Pak Silica SPE performed.

LC-RESULTS

LC-Fluorescence Analysis



LC-FL Chromatogram of oyster sample spiked at 10.0 µg/g PAHs (diluted 1:100 with acetonitrile following extraction). Insets show zoomed peaks for naphthalene (1), fluoranthene (6), pyrene (7), benzo(a)anthracene (8) and chrysene (9). Note, acenaphthylene is not detected by fluorescence.

LC Conditions

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: Milli-Q® water
Mobile Phase B: Methanol, Fisher Optima Grade
Mobile Phase C: Acetonitrile, Fisher Optima Grade
Flow Rate: 2.0 ml/min

Gradient Profile

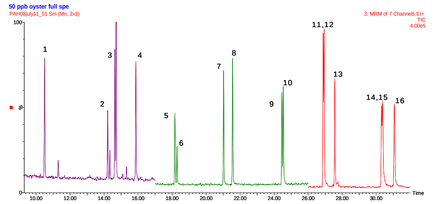
Time (min)	Flow Rate (mL/min)	% A	% B	%C	Curve
0.00	2.0	30	70	0	
2.25	2.0	0	70	30	6
3.50	2.0	0	0	100	6
3.60	2.0	30	70	0	6

Reproducibility and Recovery Data for Oysters	Ave RT N=9		10.0 µg/g N=3		1.0 µg/g N=3		50.0 ng/g N=3	
Compound	RT	%RSD	% Rec	%RSD	% Rec	%RSD	% Rec	%RSD
Naphthalene	0.83	0.1	102	7.8	149	9.8	104	6
Acenaphthene	1.17	0.18	99	5.8	145	13.2	130	2.7
Fluorene	1.27	0.19	100	5.9	143	12.1	100	1.9
Phenanthrene	1.37	0.18	103	9.7	143	16.4	108	3.8
Anthracene	1.52	0.16	80	1.4	116	10.6	67	11.7
Fluoranthene	1.66	0.14	100	15.7	142	17.7	103	5.5
Pyrene	1.76	0.13	107	15.3	149	16.9	108	5.3
Benzo(a)anthracene	2.12	0.09	94	8.6	136	9.4	78	10.1
Chrysene	2.22	0.08	94	5.5	139	9.2	83	8
Benzo(b)fluoranthene	2.46	0.06	94	5.3	137	10.9	83	4.1
Benzo(k)fluoranthene	2.59	0.05	94	6.1	140	9.6	84	4.8
Benzo(a)pyrene	2.72	0.04	86	5.2	125	11.2	75	7.5
Dibenzo(A,h)anthracene	2.92	0.04	78	5.4	124	7.8	81	6
Benzo(g,h,i)perylene	3.03	0.04	72	8.6	114	16.9	78	8.6
Indeno(1,2,3-cd)pyrene	3.14	0.03	86	4.8	126	9	82	5.8

Recovery and Reproducibility Data for Spiked Oysters (similar results were obtained for finfish and shrimp)

GC-MS(MS)-RESULTS

GC-MS(MS) Analysis



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

GC Conditions

System: A-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

MS(MS) Conditions / MRM Transitions

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		

- Using internal standard calculation, correlation (r^2) was 0.995 or better for all PAH (5 point matrix matched curve range 5 to 100 ng/g).
- SPE Recovery was better than 85% for all PAH measured in oyster matrix spiked at the 50 ng/g level.

CONCLUSIONS

LC-Fluorescence Analysis

- Dispersive sample preparation provides a fast and effective method for extracting PAH from different seafood matrices.
- This extraction technique provides a convenient extract well suited for LC analysis with no further workup required.
- Accurate results can be achieved with less sample preparation and in a shorter time compared with other sample preparation techniques.
- LC-FL analysis allows laboratories to quickly screen for PAH in seafood, providing results in a timely and economical manner to ensure product safety.

CONCLUSIONS

GC-MS(MS) Analysis

- 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 confirmation analysis with LOQ below 50 ng/g.
- For GC-MS analysis, the QUECHERS acetonitrile extraction protocol provides equivalent performance compared with ethyl acetate or methylene chloride extraction of seafood.

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