Advanced Analytical Technologies for Analyzing Environmental Matrixes Contaminated with Petroleum Hydrocarbons

QuEChERS with GC-Q and GC-QQQ PAH Analyzers

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References


“Protocol for Interpretation and Use of Sensory Testing and Analytical Chemistry Results for Re-Opening Oil-Impacted Areas Closed to Seafood Harvesting”, 2010_0529_NOAA Opening Protocol Final, 8 pp., 2010


1. Compatible with QuEChERS, which is a fast and simple sample prep technique.

2. Capillary Flow Technology based backflush reduces system maintenance needs even with dirty matrices. Method parameters are pre-set.

3. PAH MRM acquisition method (QQQ) has been optimized and preloaded.

4. PAH SIM target and qualifier ions (Q) set in acquisition and data analysis.

5. Analyzer is offered as a turnkey system that has been factory configured and undergone chemical testing prior to shipment.

6. PAH calibration standards and ISTDs are included, reducing start up time.

7. PAH-specific column used for optimized PAH separation.
NOAA Sample Preparation Procedure

1. Sediment or tissue sample
   - Mix a portion of sample with sodium sulfate and magnesium sulfate, then extract with dichloromethane using Accelerated Solvent Extraction.
   - As needed, dry a portion of sample; gravimetrically determine the percent dry weight.

2. Dichloromethane extract of sediment or tissue
   - For tissue extracts as needed, take 1/2 of extract and evaporate the solvent; gravimetrically determine the percent total extractables.
   - Filter extract through a silica/alumina column, then concentrate it to 1 mL.

3. Concentrated extract
   - Chromatograph 1/2 of the concentrated extract using size-exclusion HPLC; collect the AH/CH fraction, then concentrate it to 100 μL.

4. Concentrated AH/CH fraction
   - Analyze fraction using GC/MS for AH quantitation, as needed.
   - Analyze fraction using GC/MS for CH quantitation, as needed.
Alternative Procedure: QuEChERS

QuEChERS: Quick, Easy, Cheap, Effective, Robust and Safe

• Initial purpose/validation was to determine pesticides in fruit and vegetables

• “QuEChERS works so well with pesticides can it work for other compound extracts”

• Advancements in QuEChERS has offered PAH determination in seafood

  – Why: Because of its “NAME”
    > Takes 10 minutes versus overnight for the NOAA method
      ▪ Less time, Less solvent, Less glassware, Less cost, Less solvent disposal, Less subject to error, No chlorinated solvent

  – So let’s take a look at QuEChERS
QuEChERS Procedure:

Chop then Freeze

Grind
First Step – Extraction/Partitioning

1) Weigh sample
2) Add Ceramic Homogenizers
3) Vortex
4) Add ACN (1% AA)
5) Vortex
6) Add salts
7) Shake 1 min
8) Centrifuge
Second Step – Dispersive SPE

1) Choose d-SPE
2) Transfer volume
3) Vortex 1 min
4) Centrifuge
5) Analyze
QuEChERS and d-SPE Sample Preparation Workflow

Weigh 3 g fish sample (+/- 0.1g) in 50 mL centrifuge tube

Add Surrogate/IS solution, and QC spike solution if necessary, Vortex 1 min

Add 12 mL of DI water and 2 ceramic bars to the sample (Agilent part #5982-9313), Vortex 1 min

Add 15 mL of ACN containing 1%HAc, Vortex 1 min

Add Agilent SampliQ QuEChERS AOAC extraction salt packet (Agilent part #5982-5755)

Cap and shake vigorously for 1 min on Geno/Grinder at 1500 rpm

Centrifuge at 4000 rpm for 5 min

Transfer 1 mL of upper ACN layer to
- SampliQ AOAC Fatty dispersive SPE 2 ml tube (Agilent part # 5982-5122)  
- Or 8 mL to SampliQ AOAC Fatty dispersive SPE 15 mL tube (Agilent part # 5982-5158)

Vortex 1 min, Centrifuge at 13000 rpm for 2 min for 2 mL tubes,  
Or at 4000 rpm for 5 min for 15 mL tubes

Transfer 500 µL extract to autosampler vial

Analyze by LC/FLD or GC/MS
PAH Analysis by LC/FLD

Unspiked fish extract

![Graph showing uns spiked fish extract](image)

Spiked fish extract

![Graph showing spiked fish extract](image)

PAH Analyzer(s), 7890GC-7000B QQQ and GC-5975C Q

1. Compatible with QuEChERS, which is a fast and simple sample prep technique
2. Capillary Flow Technology based backflush reduces system maintenance needs even with dirty matrices. Method parameters are pre-set.
3. PAH MRM acquisition method (QQQ) has been optimized and preloaded
4. PAH SIM target and qualifier ions (Q) set in acquisition and data analysis
5. Analyzer is offered as a turnkey system that has been factory configured and undergone chemical testing prior to shipment
6. PAH calibration standards and ISTDs are included, reducing start up time
7. PAH-specific column used for optimized PAH separation
PAH Method for Productivity, GC-QQQ and GC-Q

1. Multimode Inlet for versatility. S/SL could be used for hot splitless PAHs but the MMI offers large volume injection if needed. Cold splitless also available when the system is used for thermally labile compounds.

2. PAH specific column, 20m x 0.18mm x 0.14um DB-EUPAH, p/n 121-9627. This offers separations that a DB5-MS does not, but the DB5-MS could be used. Run time is 18 minutes.

3. Retention Time Locking done on the method and column shipped. The system only needs to be relocked on installation.

4. Backflushing is done via a capillary flow technology purged union connected post column. Cycle time is reduced as column bake-out is eliminated. Source cleaning is reduced.

5. SIM target ion (Q) is the most abundant and qualifier ions are the next 3 most abundant. These can be optimized against matrix background using the Ion Optimization program in the latest software release.

6. MRM (QQQ) optimization is ongoing
GC-QQQ (or GC-Q) PAH Analyzer

(1) CF Column  20 m X 0.18 mm id X 0.14 um DB-EUPAH part# 121-9627

(2) CP Restrictor  0.70 m X 0.15 mm id deactivated tubing

7890A GC
240V

5 mL/min bleeder
9 cm x 0.12 id

1 mL/min CF

4 temperature ramps
Run time = 18 min plus
4 minute backflush

23 psig
RTLocked

7693A Tower
and Tray

MMI Inlet

5 mbar

Aux
3.0 psig

Purged
Union

7000B
EI QQQ
or
5975C
EI MSD

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Principle Of Backflushing

During GC Run:
- Split Vent
- S/SL Inlet
- 25 psi
- Capillary Flow Device
- 3.0 psi
- Aux EPC
- Detector(s)

After GC Run:
- Split Vent
- S/SL Inlet
- 1 psi
- Capillary Flow Device
- 60 psi
- Aux EPC
- Detector(s)
Heavy Compounds May Be Left in Head of Column After Each Injection

These heavy materials build up and travel further into the column with each injection.

This buildup of heavy materials causes retention time shifts, peak distortion, higher bleed, and loss of sensitivity.
Backflushing After Each Injection

Backflushing removes heavy materials after each injection.
Environmental - Gasoil Backflush Example

Matrix, 42 min elution

Sample, with backflushing

4 min. BF

20 min. Timesavings

Scale 20x more sensitive than above

Blank after backflush

7.00 12.00 17.00 22.00 27.00 32.00 37.00
10% Fish Oil In Acetone: Retention Time Shifts Eliminated With Backflushing

10 Runs without Backflushing: Retention times shift ~4-5 sec during 10 runs

10 Runs with Backflushing: RT shift eliminated
PAH Analysis, NOAA 29: GC/MS with Column Backflush

--- Improved reliability and speed

Oven Program
50 °C for 0.8 min
then 70 °C/min to 180 °C for 0 min; then 7 °C/min to 230 °C for 1 min
then 40 °C/min to 280 °C for 1 min; then 25 °C/min to 335 °C for 3 min

Run Time 18.25 min

Mode Pulsed Splitless Temperature: 320 °C

Column DB-EUPAH, 20 m x 180 μm x 0.14 μm
Column Flow constant flow at 1 mL/min (pressure = 25.885 psi)

MSD Transfer line 320 C MS Source: 350 C MS Quad : 200 C

**Internal Std**

1. Naphthalene-d8
2. 2-Methylnaphthalene
3. 1-Methylnaphthalene
4. 2-Methylnaphthalene
5. Biphenyl
6. 2,6-dimethylnaphthalene
7. HMB
8. Acenaphthylene
9. Phenanthrene-d10
10. Acenaphthene
11. 2,3,5-trimethylnaphthalene
12. Fluorene
13. Dibenzothiophene
14. Phenanthrene-d10
15. Phenanthrene
16. Anthracene
17. 1-methylphenanthrene
18. Fluoranthene
19. Acenaphthene
20. Pyrene
21. Benzo[a]anthracene
22. Triphenylene
23. Chrysene
24. Benzo[a]pyrene
25. Benzo[b]fluoranthene
26. Benzo[k]fluoranthene
27. Benzo[j]fluoranthene
28. Benzo[e]pyrene
29. Benzo[a]pyrene
30. Perylene
31. Dibenz[a,c]anthracene
32. Dibenz[a,h]anthracene
33. Indeno[1,2,3-cd]pyrene
34. Benzo[ghi]perylene

**Target Compounds**

1. Naphthalene
2. 1-Methylnaphthalene
3. 2-Methylnaphthalene
4. Biphenyl
5. 2,6-dimethylnaphthalene
6. Phenanthrene
7. HMB
8. Acenaphthylene
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25. Benzo[a]pyrene
26. Perylene
27. Dibenz[a,c]anthracene
28. Dibenz[a,h]anthracene
29. Indeno[1,2,3-cd]pyrene
30. Benzo[ghi]perylene
PAH Analysis: GC/MS SIM Late Eluters

**Internal Std**
1. Naphthalene-d8
2. Antipyrene-d10
3. Phenanthrene-d10
4. Benzo[a]pyrene-d12
5. Biphenyl
6. 2,6-dimethylnaphthalene
7. HMB
8. Acenaphthylene
9. Acenaphthene-d10
10. Acenaphthene
11. 2,3,5-trimethylnaphthalene
12. Fluorene
13. Dibenzothiophene
14. Phenanthrene-d10
15. Phenanthrene
16. Anthracene
17. 1-methylphenanthrene
18. Fluroanthene
19. 2,3,5-trimethylnaphthalene
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22. Triphenylene
23. Chrysene
24. Benzo[k]fluoranthene
25. Benzo[b]fluoranthene
26. Benzo[k]fluoranthene
27. Benzo[j]fluoranthene
28. Benzo[e]pyrene
29. Benzo[a]pyrene
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**Target Compounds**
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10. Acenaphthene
11. 2,3,5-trimethylnaphthalene
12. Fluorene
13. Dibenzothiophene
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26. Benzo[k]fluoranthene
27. Benzo[j]fluoranthene
28. Benzo[e]pyrene
29. Benzo[a]pyrene
30. Perylene
31. Dibenzo[a,c]anthracene
32. Dibenzo[a,h]anthracene
33. Indeno[1,2,3-cd]pyrene
34. Benzo[ghi]perylene
**r^2 values for 7 level cal curves, GC-QQQQ and GC-Q**

<table>
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<tr>
<th>RT</th>
<th>7 levels ---&gt;</th>
<th>1 - 1000</th>
<th>1 - 100</th>
<th>1 - 1000</th>
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<td>0.9996</td>
<td>0.9998</td>
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<td>15.47</td>
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QQQ A and Q calibration stds were in iso-octane solvent.

QQQ V calibration stds were in QuEChERS extract of fish at 1g/mL

Data from Ralph Hindle, Vogon Labs, 7000A
Phenanthrene and Anthracene 1.0 ppb Standard

MRM 7000A QQQ
Std made in QuEChERS fish extract

SIM 5975 Q in Isooctane

Vogon Labs

Agilent LFS
Pyrene 1.0 ppb Standard

MRM 7000A QQQ
Std made in QuEChERS fish extract

SIM 5975 Q in Isooctane

Pyrene

Vogon Labs

Agilent LFS

mike_szelewski@agilent.com
Recovery Values for PAHs, Spiked into Mussel Tissue at 125 ppb and Extracted Using QuEChERS + Dispersive SPE with no Additional Cleanup nor Concentration

<table>
<thead>
<tr>
<th>PAH</th>
<th>25 ppb spike 1</th>
<th>25 ppb spike 2</th>
<th>25 ppb spike 3</th>
<th>Avg % Rec</th>
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<td>Acenaphthylene</td>
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<td>25.0</td>
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<td>Acenaphthene</td>
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<td>24.8</td>
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<td>Fluorene</td>
<td>31.3</td>
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<tr>
<td>Phenanthrene</td>
<td>24.5</td>
<td>27.1</td>
<td>26.4</td>
<td>104</td>
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<tr>
<td>Anthracene</td>
<td>22.5</td>
<td>23.6</td>
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<td>94</td>
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<tr>
<td>Fluoranthene</td>
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<tr>
<td>Pyrene</td>
<td>22.9</td>
<td>22.9</td>
<td>24.1</td>
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<td>Benz[a]anthracene</td>
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<td>Chrysene</td>
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<td>96</td>
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<tr>
<td>Benzo[b]fluoranthene</td>
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<td>Benzo[k]fluoranthene</td>
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<td>Indeno[1,2,3-cd]pyrene</td>
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<td>17.3</td>
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</table>

Extracts measured by both GC-QQQ MRM and GC-Q SIM. Recovery values were the same.

Concentration in 3 g mussel tissue = 125 ppb
Signal to Noise (pk-pk) for NOAA PAHs (5/29/2010 list)  
GC-QQQ and GC-Q  
1 ppb Standard and 125 ppb Spike in mussels

<table>
<thead>
<tr>
<th></th>
<th>7000B</th>
<th>5975C</th>
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<td></td>
<td>MRM</td>
<td>SIM</td>
<td>MRM</td>
<td>SIM</td>
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<tr>
<td>Std 1 ppb</td>
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<td>Std 1 ppb</td>
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<td>7.2</td>
<td>112</td>
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<tr>
<td>Phenanthrene</td>
<td>6.7</td>
<td>8.8</td>
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<td>Anthracene</td>
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<td>4.6</td>
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<td>39</td>
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<td>15</td>
<td>10</td>
<td>60</td>
<td>11</td>
</tr>
</tbody>
</table>

Sensitivity for standards is similar in the 2 systems but better in the QQQ when matrix is present. Spiked mussel tissue extracted with QuEChERS + dispersive SPE.
What if my QuEChERS extract does not have enough sensitivity? Concentrate the extract 10-fold.

Both sets of EICs are from a QuEChERS extract concentrated 10x in ACN. Background is still low.

15 pg Fluoranthene

MRM ratios match expected on QQQ

SIM ratios match expected on GC-Q. RTs align.

Instead of concentrating 10x, you could use a 10 uL solvent vent injection with the MMI
Same 10x Concentrated QuEChERS Extract from previous slide. Benzo[b,k,j]fluoranthenes at ~1-6 pg.

Both sets of EICs are from a QuEChERS extract concentrated 10x in ACN. S/N is lower for these ions compared to previous slide.

MRM ratios match expected on QQQ

SIM ratios do not match expected on GC-Q. RTs do not align

Instead of concentrating 10x, you could use a 10 uL solvent vent injection with the MMI
Same 10x Concentrated QuEChERS Extract from previous slide. Dibenzo(a,h) & (a,c) anthracene at ~ 0.2 pg

Both sets of EICs are from a QuEChERS extract concentrated 10x in ACN. S/N is lower for these ions compared to previous slide.

MRM ratios do not match expected on QQQ, but s/n is better than Q

SIM data useful if you squint.

Instead of concentrating 10x, you could use a 10 uL solvent vent injection with the MMI
Sole, Clam & Scallop Samples – Spiked with ISTDs at 67 ppb and Extracted using Agilent QuEChERS

Internal Standards

1. Naphthalene-d8
2. Hexamethylbenzene
3. Acehaphthene-d10
4. Phenanthrene-d10
5. Benzo[a]pyrene-d12

Data from Arkansas DOH on 7000B QQQ-A.
Jeffrey Moran and John Blevins

 mike_szelewski@agilent.com
Background in Scallop Extract vs. Blank Spiked at 67 ppb Before Extraction

**PAHs**
1. Fluoranthene
2. Retene
3. Pyrene
4. Benz[a]anthracene
5. Chrysene + Triphenylene

Data from Arkansas DOH on 7000B QQQQ-A. Jeffrey Moran and John Blevins
Summary

- QuEChERS: offers a simple sample preparation approach to the extraction and analysis of PAHs in finfish and shellfish
- The simplicity and quickness associated with QuEChERS sample preparation allows multitudes of samples to be processes per day versus weeks
- A preconfigured analyzer can help your lab start running PAHs with higher productivity
- Backflushing will reduce cycle time and instrument maintenance for samples with matrix
- Signal-to-noise is about the same on a 5975C-Q using SIM compared to a 7000B-QQQ using MRM for clean samples
- The 7000B-QQQ analyzer can reach lower detection limits for PAHs, with greater confidence, than the 5975C-Q for QuEChERS extracts of seafood