Advanced Analytical Technologies for Analyzing Environmental Matrixes Contaminated with Petroleum Hydrocarbons

Part II
PAH Analyzers
GC-Q and GC-QQQ

Mike Szelewska
Application Chemist
July, 08, 2010

“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**
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 with collaborators.
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

7693A Tower and Tray

5 mL/min bleeder
9 cm x 0.12 id

Aux 3.0 psig

MMI Inlet

23 psig RTLocked

7890A GC 240V

1 mL/min CF

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

7000B EI QQQ

or

5975C EI MSD

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Principle Of Backflushing

**During GC Run**

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

**After GC Run**

- **S/SL Inlet**: 1 psi
- **Split Vent**:
- **Aux EPC**: 60 psi
- **Capillary Flow Device**:
- **Detector(s)**

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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.
Matrix, 42 min elution

Sample, with backflushing

4 min. BF | 20 min. Timesavings

Blank after backflush

Scale 20x more sensitive than above
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

**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

--- Improved reliability and speed

### Internal Std
- 1 Naphthalene-d8
- 5 Biphenyl
- 6,2,6-dimethylnaphthalene
- 16 Anthracene
- 17 1-methylphenanthrene
- 21 Benz[a]anthracene
- 26 Benzo[k]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
- 2 Naphthalene
- 12 Fluorene
- 13 Dibenzothiophene
- 18 Fluoranthene
- 20 Pyrene
- 21 Benz[a]anthracene
- 22 Triphenylene
- 23 Chrysene
- 24 Benzo[a]pyrene
- 25 Benzo[b]fluoranthene
- 31 Dibenz[a,c]anthracene
- 32 Dibenz[a,h]anthracene
- 33 Indeno[1,2,3-cd]pyrene
- 34 Benzo[ghi]perylene

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### Internal Std
- 1: Naphthalene-d8
- 2: Naphthalene
- 3: 1-methylnaphthalene
- 4: 2-Methylnaphthalene
- 5: Biphenyl
- 6: 2,6-dimethylnaphthalene
- 7: HMB
- 8: Acenaphthylene
- 9: Acenaphthene-d10
- 10: Acenaphthene
- 11: 2,3,5-trimethylnaphthalene

### Target Compounds
- 12: Fluorene
- 13: Dibenzothiophene
- 14: Dibenzothiophene
- 15: Phenanthrene
- 16: Anthracene
- 17: 1-methylphenanthrene
- 18: Fluoranthene
- 19: 1-methylphenanthrene-d10
- 20: Pyrene
- 21: Benz[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

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### r^2 values for 7 level cal curves, GC-QQQQ and GC-Q

<table>
<thead>
<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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<tbody>
<tr>
<td>3.14</td>
<td>Napthalene</td>
<td>0.9998</td>
<td>0.9972</td>
<td>0.9997</td>
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<tr>
<td>3.43</td>
<td>1-methylnaphthalene</td>
<td>0.9998</td>
<td>0.9995</td>
<td>0.9998</td>
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<tr>
<td>3.53</td>
<td>2-methylnaphthalene</td>
<td>0.9999</td>
<td>0.9995</td>
<td>0.9996</td>
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<tr>
<td>3.76</td>
<td>Biphenyl</td>
<td>0.9998</td>
<td>0.9902</td>
<td>0.9988</td>
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<tr>
<td>3.78</td>
<td>2,6-dimethylnaphthalene</td>
<td>0.9998</td>
<td>0.9983</td>
<td>0.9999</td>
</tr>
<tr>
<td>4.24</td>
<td>Acenaphtylene</td>
<td>0.9999</td>
<td>0.9994</td>
<td>0.9998</td>
</tr>
<tr>
<td>4.80</td>
<td>Acenaphthene</td>
<td>0.9999</td>
<td>0.9999</td>
<td>0.9997</td>
</tr>
<tr>
<td>4.97</td>
<td>2,3,5-trimethylnaphthalene</td>
<td>0.9999</td>
<td>0.9998</td>
<td>0.9998</td>
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<tr>
<td>5.35</td>
<td>Fluorene</td>
<td>0.9999</td>
<td>0.9998</td>
<td>0.9998</td>
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<tr>
<td>6.48</td>
<td>Dibenzothiophene</td>
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<td>0.9989</td>
<td>0.9998</td>
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<tr>
<td>6.73</td>
<td>Phenanthrene</td>
<td>0.9997</td>
<td>0.9992</td>
<td>0.9999</td>
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<tr>
<td>6.79</td>
<td>Anthracene</td>
<td>0.9997</td>
<td>0.9985</td>
<td>0.9999</td>
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<tr>
<td>8.30</td>
<td>1-methylphenanthrene</td>
<td>0.9997</td>
<td>0.9996</td>
<td>0.9998</td>
</tr>
<tr>
<td>9.80</td>
<td>Fluoranthene</td>
<td>0.9960</td>
<td>0.9997</td>
<td>0.9998</td>
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<tr>
<td>10.68</td>
<td>Pyrene</td>
<td>0.9970</td>
<td>0.9998</td>
<td>0.9998</td>
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<tr>
<td>13.14</td>
<td>Benzo(a)anthracene</td>
<td>0.9930</td>
<td>0.9990</td>
<td>0.9998</td>
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<tr>
<td>13.29</td>
<td>Chrysene</td>
<td>0.9940</td>
<td>0.9997</td>
<td>0.9999</td>
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<tr>
<td>14.83</td>
<td>Benzo(b)fluoranthrene</td>
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<td>0.9980</td>
<td>0.9987</td>
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<tr>
<td>14.86</td>
<td>Benzo(k)fluoranthrene</td>
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<td>0.9985</td>
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<tr>
<td>15.27</td>
<td>Benzo(e)pyrene</td>
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<td>0.9977</td>
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<tr>
<td>15.33</td>
<td>Benzo(a)pyrene</td>
<td>0.9998</td>
<td>0.9971</td>
<td>0.9987</td>
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<tr>
<td>15.47</td>
<td>Perylene</td>
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<td>0.9986</td>
</tr>
<tr>
<td>16.70</td>
<td>Indeno(1,2,3-cd)pyrene</td>
<td>0.9997</td>
<td>0.9899</td>
<td>0.9996</td>
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<tr>
<td>16.69</td>
<td>Dibenz(a,h)anthracene</td>
<td>0.9980</td>
<td>0.9895</td>
<td>0.9996</td>
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<tr>
<td>17.23</td>
<td>Benzo(ghi)perylene</td>
<td>0.9888</td>
<td>0.9889</td>
<td>0.9991</td>
</tr>
</tbody>
</table>

QQQ A and Q calibration stds were in iso-octane solvent.

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

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

7000A QQQ in QuEChERS fish extract

5975 Q in Isooctane

Vogon Labs

Agilent LFS

Mike Szelewski @ Agilent.com

Agilent Technologies

April 2010
Pyrene 1.0 ppb Standard

7000A QQQ in QuEChERS fish extract

5975 Q in Isooctane

Vogon Labs

Agilent LFS
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></th>
<th>25 ppb spike 1</th>
<th>25 ppb spike 2</th>
<th>25 ppb spike 3</th>
<th>Avg % Rec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acenaphthylene</td>
<td>23.8</td>
<td>25.0</td>
<td>25.7</td>
<td>99</td>
</tr>
<tr>
<td>Acenanthrene</td>
<td>23.3</td>
<td>24.8</td>
<td>22.5</td>
<td>94</td>
</tr>
<tr>
<td>Fluorene</td>
<td>31.3</td>
<td>30.6</td>
<td>29.2</td>
<td>121</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>24.5</td>
<td>27.1</td>
<td>26.4</td>
<td>104</td>
</tr>
<tr>
<td>Anthracene</td>
<td>22.5</td>
<td>23.6</td>
<td>24.3</td>
<td>94</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>25.7</td>
<td>25.9</td>
<td>26.8</td>
<td>105</td>
</tr>
<tr>
<td>Pyrene</td>
<td>22.9</td>
<td>22.9</td>
<td>24.1</td>
<td>93</td>
</tr>
<tr>
<td>Benz[a]anthracene</td>
<td>29.2</td>
<td>27.9</td>
<td>29.9</td>
<td>116</td>
</tr>
<tr>
<td>Chrysene</td>
<td>24.0</td>
<td>23.4</td>
<td>24.3</td>
<td>96</td>
</tr>
<tr>
<td>Benzo[b]fluoranthene</td>
<td>22.0</td>
<td>23.1</td>
<td>23.6</td>
<td>92</td>
</tr>
<tr>
<td>Benzo[k]fluoranthene</td>
<td>20.7</td>
<td>21.9</td>
<td>22.2</td>
<td>86</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>27.0</td>
<td>29.5</td>
<td>31.7</td>
<td>117</td>
</tr>
<tr>
<td>Dibenz[a,h]anthracene</td>
<td>18.8</td>
<td>19.4</td>
<td>19.9</td>
<td>77</td>
</tr>
<tr>
<td>Indeno[1,2,3-cd]pyrene</td>
<td>17.3</td>
<td>17.9</td>
<td>18.7</td>
<td>72</td>
</tr>
<tr>
<td>Benzo[ghi]perylene</td>
<td>17.3</td>
<td>18.0</td>
<td>18.7</td>
<td>72</td>
</tr>
</tbody>
</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>
<th>7000B</th>
<th>5975C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Naphthalene</strong></td>
<td>36</td>
<td>23</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td><strong>Fluorene</strong></td>
<td>8.0</td>
<td>7.2</td>
<td>112</td>
<td>92</td>
</tr>
<tr>
<td><strong>Phenanthrene</strong></td>
<td>6.7</td>
<td>8.8</td>
<td>121</td>
<td>69</td>
</tr>
<tr>
<td><strong>Anthracene</strong></td>
<td>6.8</td>
<td>5.7</td>
<td>100</td>
<td>60</td>
</tr>
<tr>
<td><strong>Fluoranthene</strong></td>
<td>8.0</td>
<td>5.3</td>
<td>88</td>
<td>43</td>
</tr>
<tr>
<td><strong>Pyrene</strong></td>
<td>6.3</td>
<td>4.6</td>
<td>105</td>
<td>39</td>
</tr>
<tr>
<td><strong>Benz[a]anthracene</strong></td>
<td>22</td>
<td>5.0</td>
<td>130</td>
<td>128</td>
</tr>
<tr>
<td><strong>Chrysene</strong></td>
<td>21</td>
<td>5.1</td>
<td>130</td>
<td>121</td>
</tr>
<tr>
<td><strong>Benzo[a]pyrene</strong></td>
<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.
Fluoranthene at ~ 15 pg. QuEChERS extract concentrated 10x in ACN.

MRM ratios match expected on QQQ

SIM ratios do match expected on Q. RTs align
Benzo[b,j,k]fluoranthenes at ~1-6 pg. QuEChERS extract concentrated 10x in ACN.

MRM ratios match expected on QQQ

SIM ratios do not match expected on Q. RTs don’t align
Dibenz(a,h) & (a,c) anthracene at ~ 0.2 pg. QuEChERS extract concentrated 10x in ACN.

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

SIM data useful if you squint.
125 ppb EPA PAHs extracted from Swai fish using QuEChERS
DB-5ms 20m 0.18mm 0.18μm
GC/MS SIM TIC

Abundance

TIC 060310b-9.D DATASIMMS
1. Napthalene
2. Acenaphthylene
3. Acenaphthene
4. Fluorene
5. Phenanthrene
6. Anthracene
7. Fluoranthene
8. Pyrene
9. Benz[a]anthracene
10. Chrysene
11. Benzo[b]fluoranthene
12. Benzo[k]fluoranthene
13. Erucylamide
14. Benz[a]pyrene
15. Cholesterol
16. Indeno[1,2,3-c,d]pyrene
17. Dibenz[a,h]anthracene
18. Benzo[g,h,i]perylene

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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

Low level background

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

mike_szelewski@agilent.com
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 processed 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