Advanced Analytical Technologies for Analyzing Environmental Matrixes Contaminated with Petroleum Hydrocarbons

QuEChERS with GC-Q and GC-QQQ PAH Analyzers

Fred Feyerherm
Application Chemist
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References

”Extraction, Cleanup, and Gas Chromatography/Mass Spectrometry Analysis of Sediments and Tissues for Organic Contaminants”, Sloan, C.A., Brown, D.W., Pearce, R.W., Boyer, R.H., Bolton, J.L., Burrows, D.G., Herman, D.P., and Krahn, M.M.U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-59, 47 pp., 2004

“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

“The Analysis of Poly Aromatic Hydrocarbons in Biota and Sediment Extracts Using GC-MS/MS with the Agilent 7000A GC-QQQ System” Chris Sandy, Agilent Technologies UK, 44 pp, Oct 2009

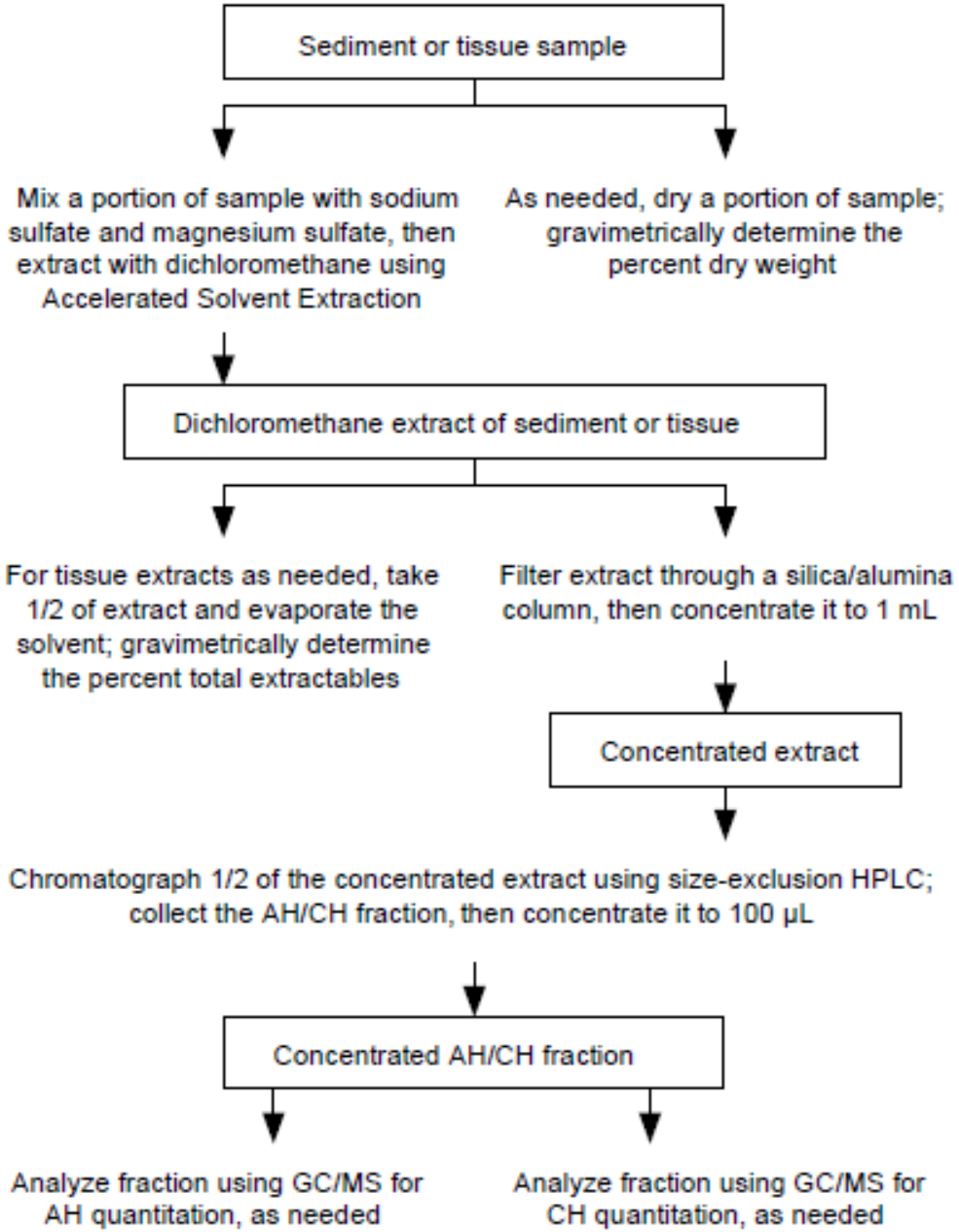
“GC/MS Analysis of European Union (EU) Priority Polycyclic Aromatic Hydrocarbons (PAHs) using an Agilent J&W DB-EUPAH GC Column with a Column Performance Comparison”, Doris Smith and Ken Lynam, Agilent Technologies, USA, 6 pp, pub 5990-4883EN, Oct 2009.

“Analysis of polycyclic aromatic hydrocarbons in fish: evaluation of a quick, easy, cheap, effective, rugged, and safe extraction method”, Ramalhosa M.J. et al, Journal of Separation Science, 2009, 32, 3529-3538

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**

NOAA Sample Preparation Procedure



Alternative Procedure: QuEChERS

QuEChERS: **Qu**ick, **E**asy, **Ch**eap, **E**ffective, **R**obust and **S**afe

- **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**

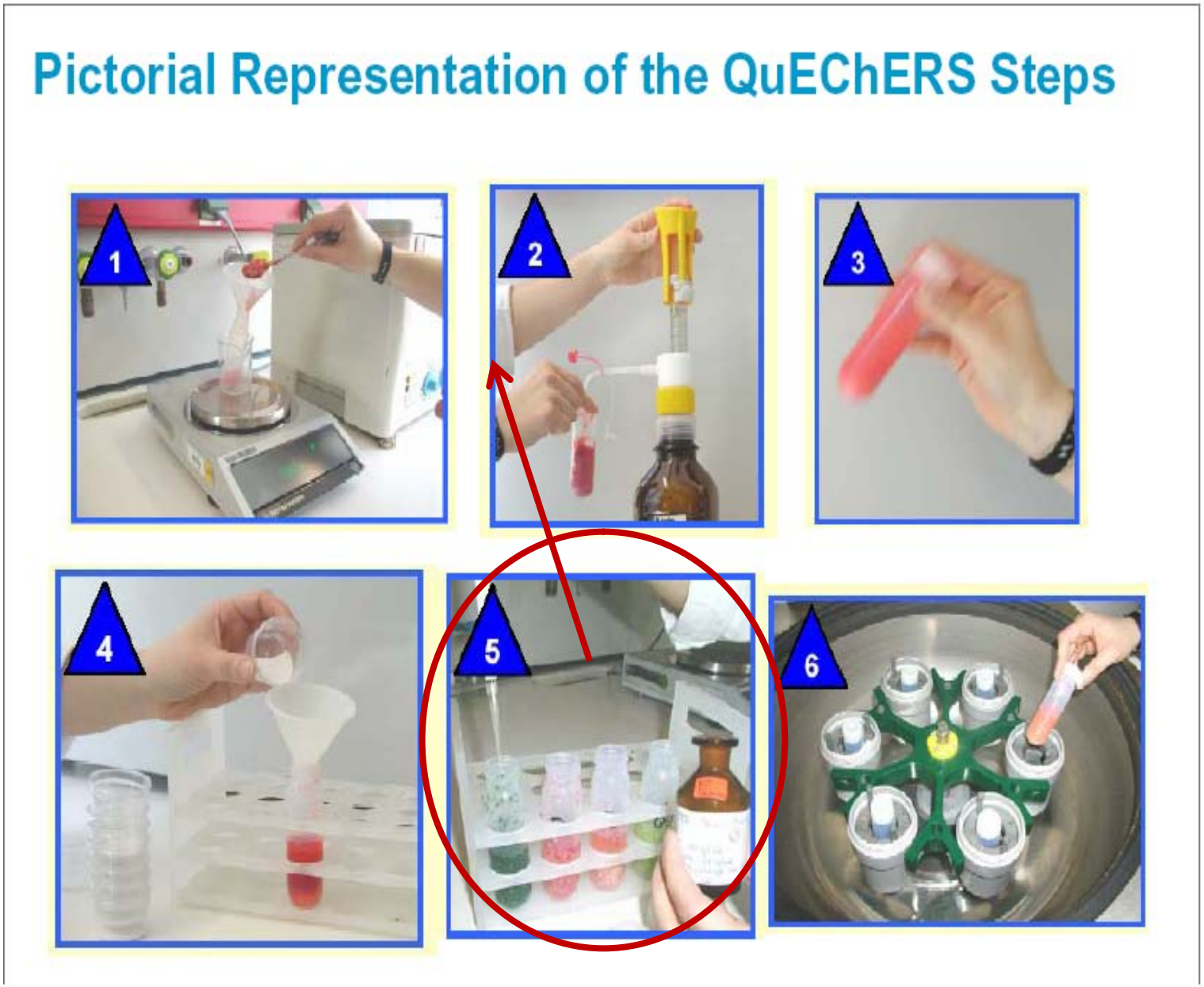
  - **Why: Because of its "NAME"**
    - > Takes 10 minutes versus overnight for the NOAA method
      - Less time, Less solvent, Less glassware, Less cost, Less solvent disposal, Less subject to error, No chlorinated solvent

  - So let's take a look at QuEChERS

QuEChERS Procedure:

Chop then

First Step – Extraction/Partitioning



1) Weigh sample
2) Add Ceramic Homogenizers
2) Add standards
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 at1500 rpm

Centrifuge at 4000 rpm for 5 min

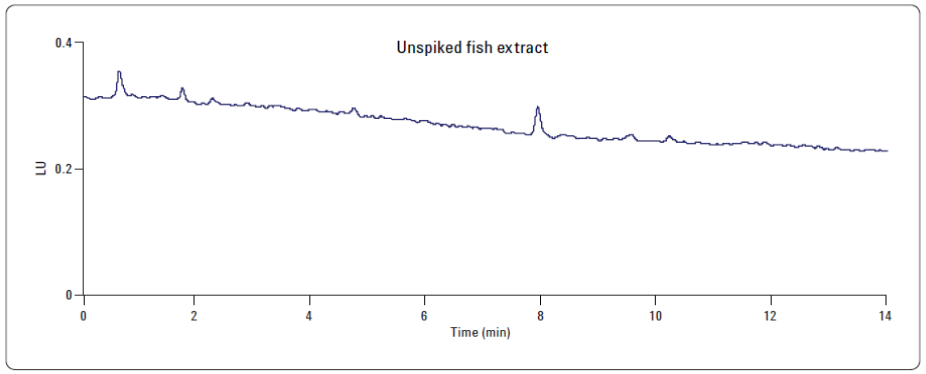
Transfer 1 mL of upper ACN layer to
SampliQ AOAC Fatty dispersive SPE 2 ml tube (Agilent part # 5982-5122)
Or 8 mL to SampliQ AOAC Fatty dispersive SPE 15 mL tube (Agilent part # 5982-5158)

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

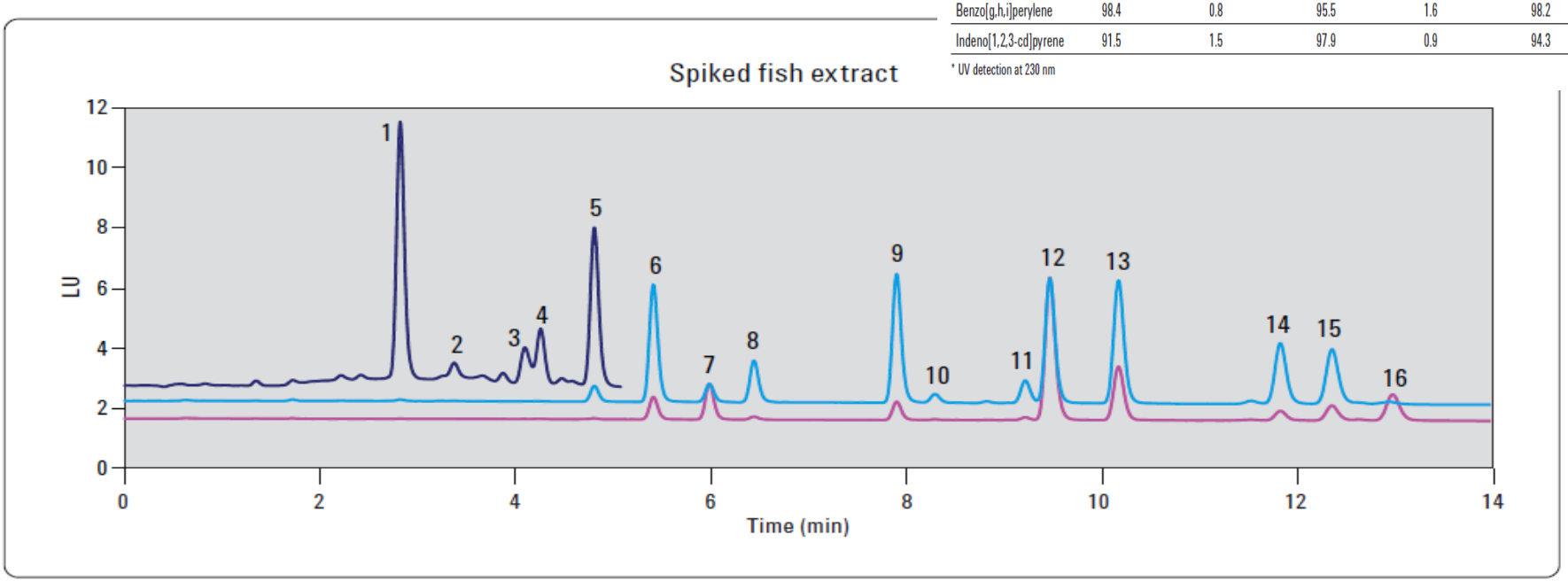
PAH Analysis by LC/FLD



Recoveries and RSDs for the Sixteen Polycyclic Aromatic Hydrocarbons in Fish Sample (n = 6)

PAH	Level of spiking (ng/g) (n = 6)					
	1		2		3	
	%Recovery	%RSD	%Recovery	%RSD	%Recovery	%RSD
Naphthalene	94.7	1.4	97.9	1.1	93.8	1.4
*Acenaphthylene	87.8	1.7	96.3	1.2	85.6	0.8
Acenaphthene	92.1	1.5	93.0	1.8	96.7	0.8
Fluorene	98.1	1.5	89.9	1.0	97.2	0.9
Phenanthrene	90.6	0.9	93.8	0.8	83.1	1.7
Anthracene	96.7	1.0	87.6	0.8	92.1	0.6
Fluoranthene	83.4	1.3	93.9	1.5	95.9	1.2
Pyrene	93.5	1.8	86.1	1.3	95.0	1.4
1,2-Benzanthracene	94.5	1.3	89.6	1.6	94.9	1.0
Chrysene	101.0	1.4	97.8	1.7	87.2	1.6
Benzo[e]pyrene	88.8	1.5	85.2	1.9	95.0	1.4
Benz[e]acenaphthylene	95.5	0.7	92.7	0.7	89.2	0.9
Benzo[k]fluoranthene	93.5	0.8	94.6	0.9	98.9	0.8
Dibenzo[a,h]anthracene	88.2	0.9	97.3	1.1	97.1	0.6
Benzo[g,h,i]perylene	98.4	0.8	95.5	1.6	98.2	0.7
Indeno[1,2,3-cd]pyrene	91.5	1.5	97.9	0.9	94.3	0.7

* UV detection at 230 nm



Overlay HPLC – FLD chromatograms of the spiked fish sample containing: 1. Nap 2. Acy 3. Ace 4. Flu 5. Phe 6. Ant 7. Fln 8. Pyr 9. BaA 10. Chr 11. BeP 12. BeA 13. BkF 14. DahA 15. BghiP 16. InP. The spiking level for this sample was level 1 The blue portion of the chromatogram used the following excitation/emission wavelengths: 260-nm/352-nm; the red portion 260-nm/420-nm; the light blue portion: 260-nm/440-nm. For acenaphthylene, UV detection at 230-nm was used

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

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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.

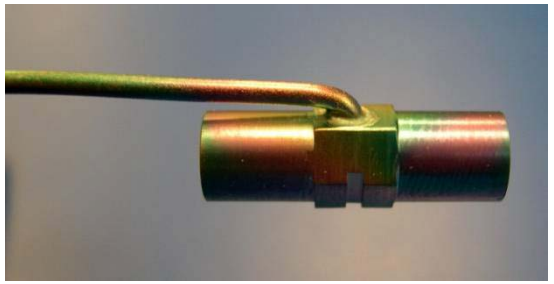
4. **Backflushing** is done via a capillary flow technology purged union connected post column. Cycle time is reduced as column bake-out is eliminated. Source cleaning is reduced.

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

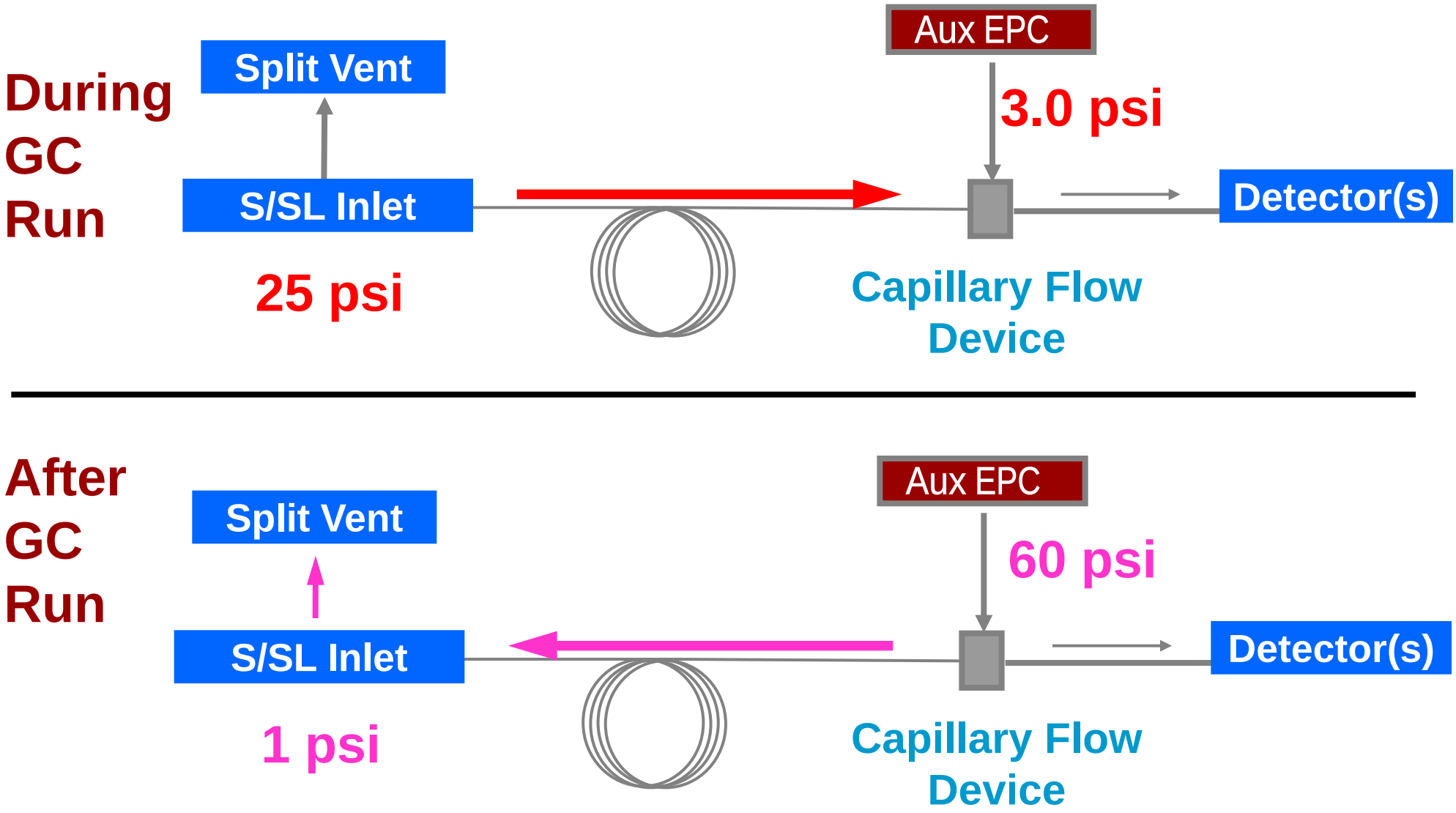
GC-QQQ (or GC-Q) PAH Analyzer

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

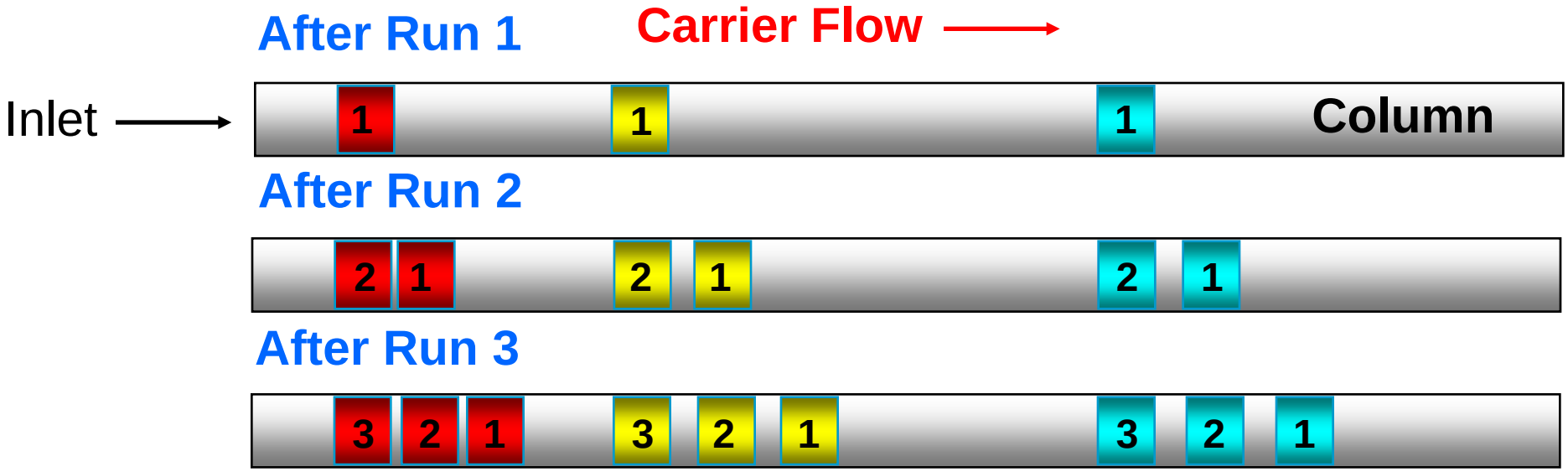
(2) CP Restrictor 0.70 m X 0.15 mm id deactivated tubing



Principle Of Backflushing



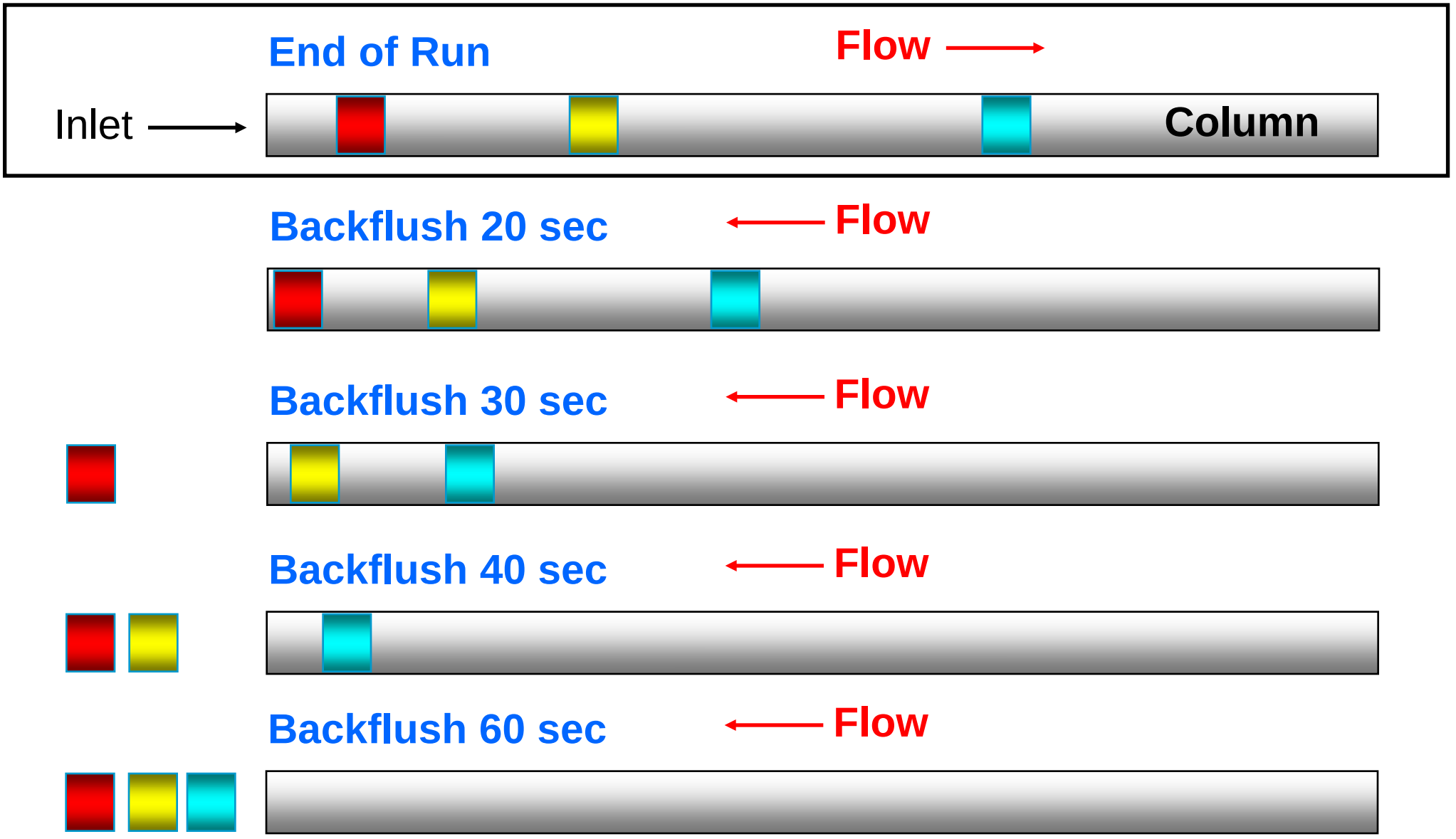
Heavy Compounds May Be Left in Head of Column After Each Injection



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

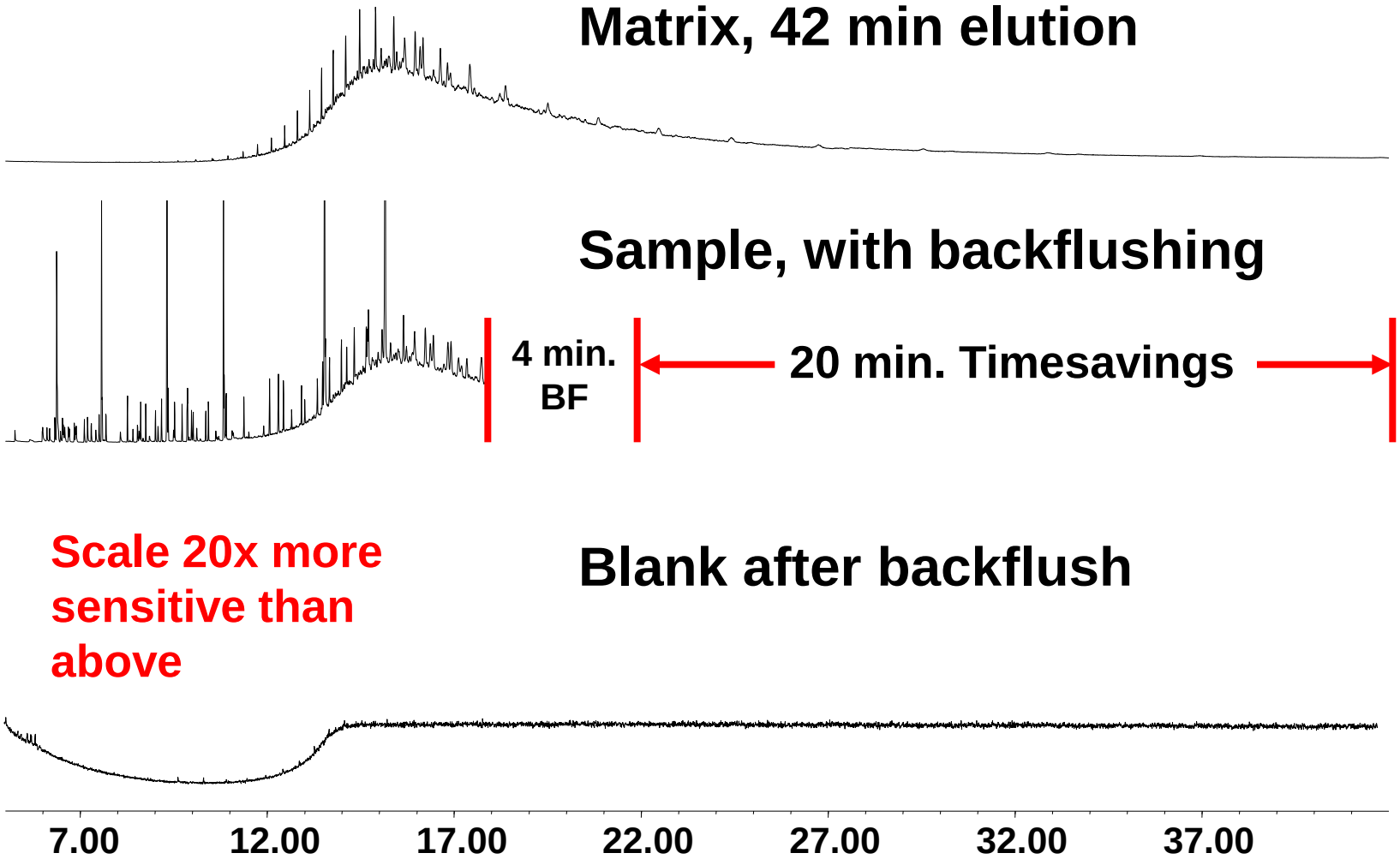
This buildup of heavy materials causes retention time shifts, peak distortion, higher bleed, and loss of sensitivity

Backflushing After Each Injection



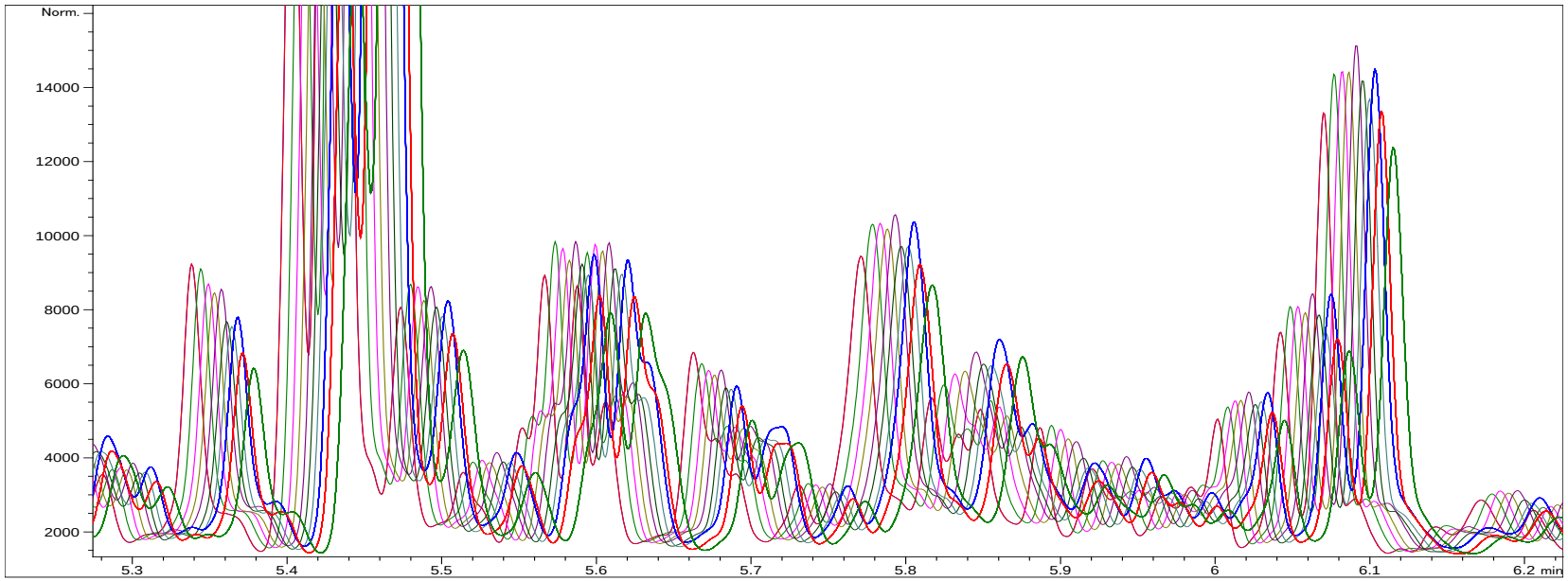
Backflushing removes heavy materials after each injection.

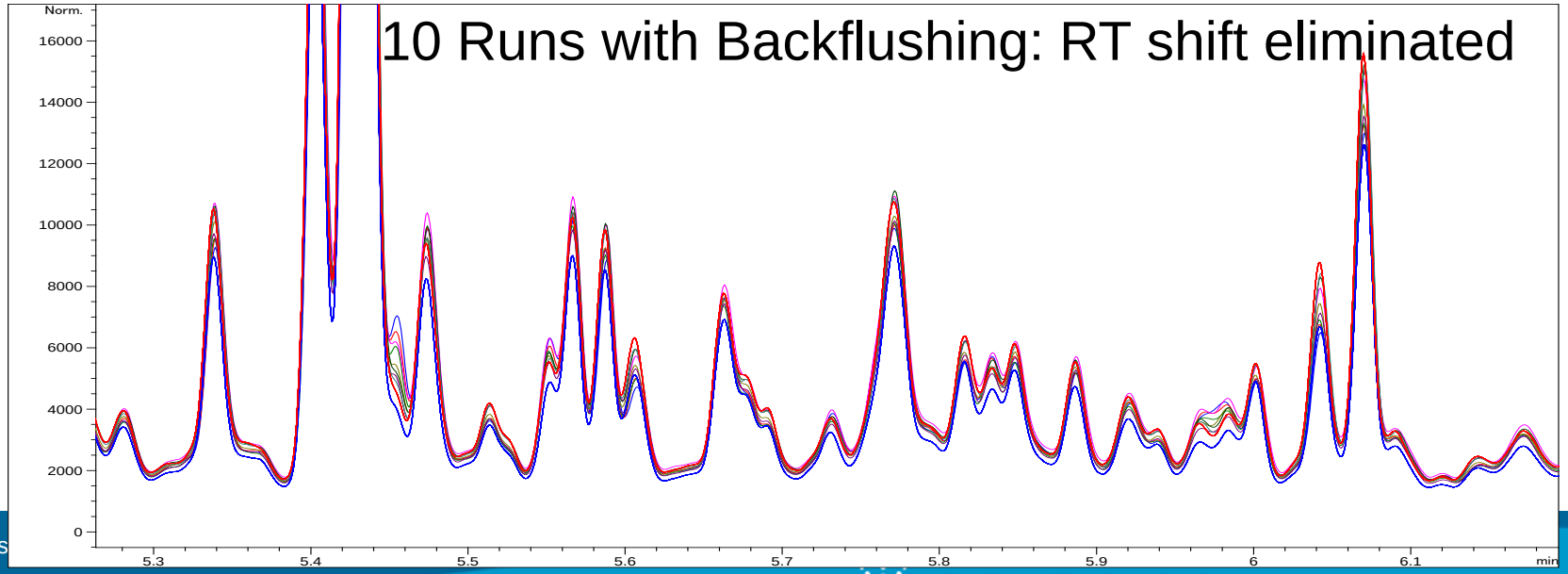
Environmental - Gasoil Backflush Example



10% Fish Oil In Acetone: Retention Time Shifts Eliminated With Backflushing

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





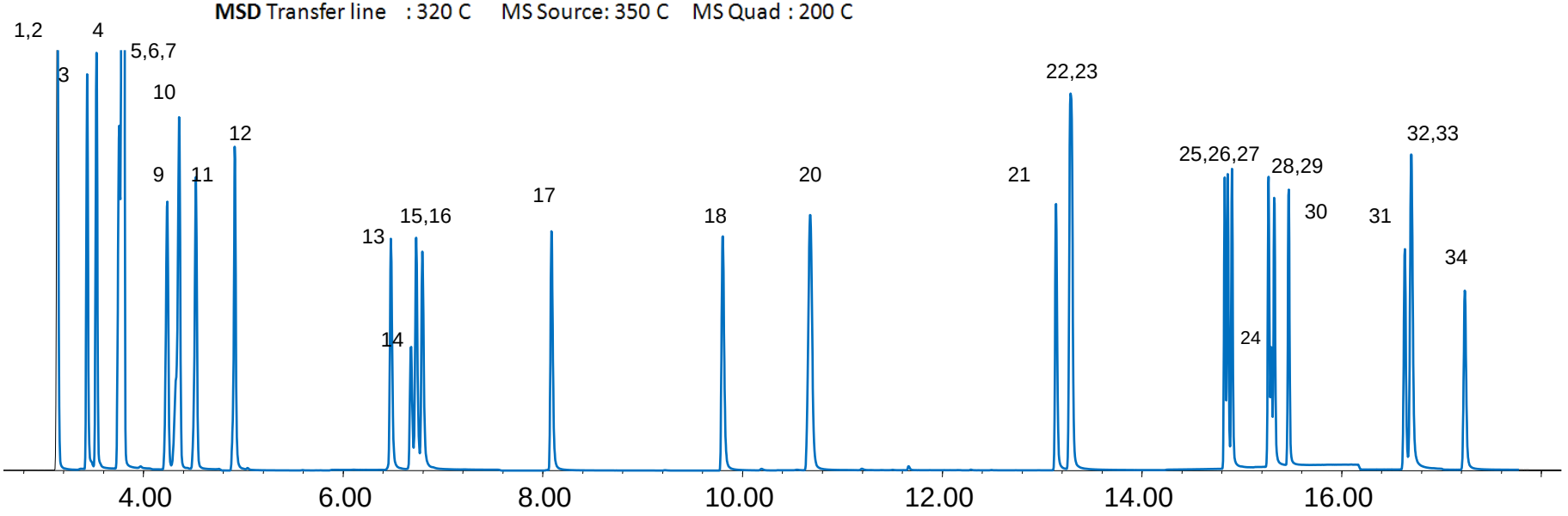
10 Runs with Backflushing: RT shift eliminated

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		
MM Inlet			
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

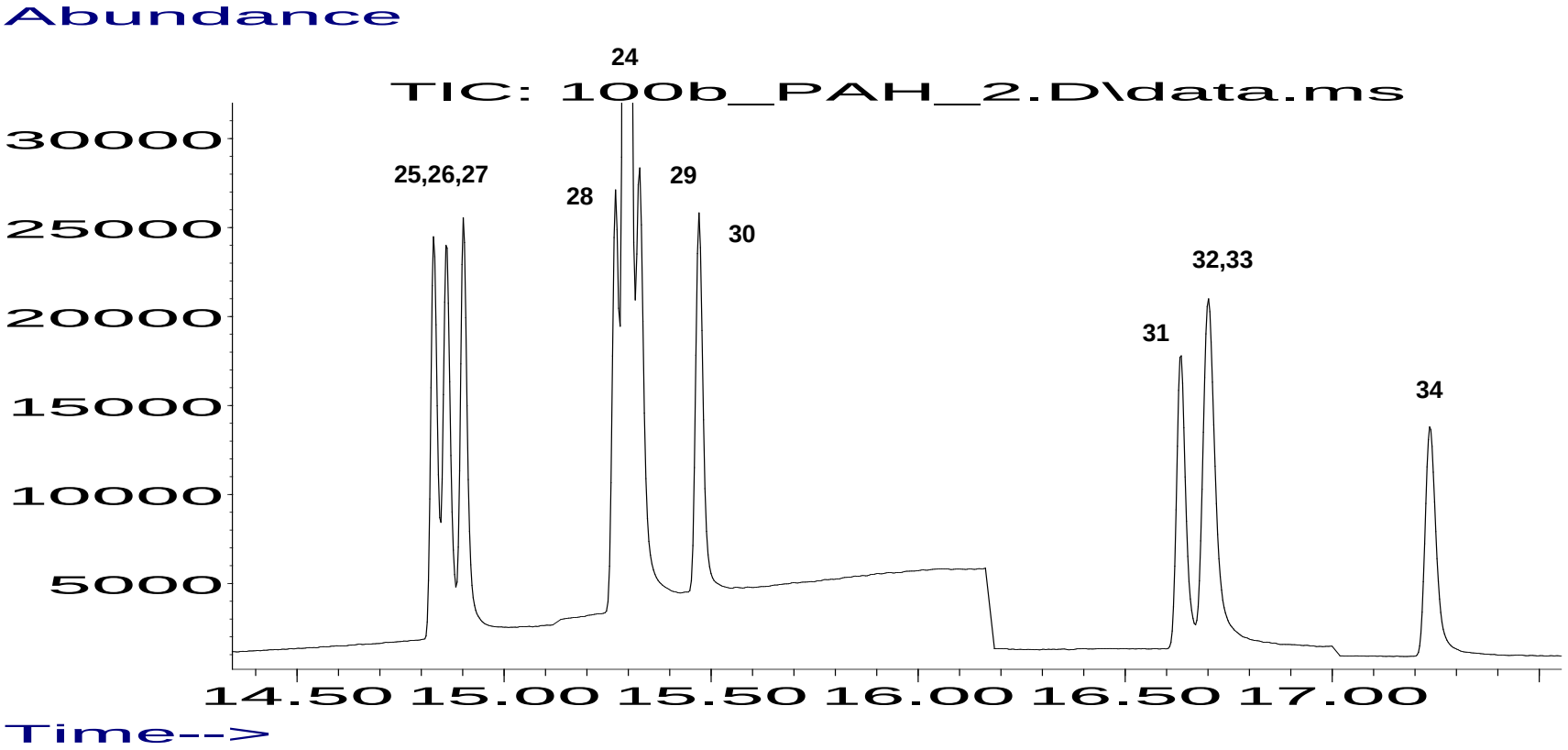
-- Improved reliability and speed



	Internal Std	4	2-Methylnaphthalene	15	Phenanthrene	26	Benzo[k]fluoranthene
1	Naphthalene-d8	5	Biphenyl	16	Anthracene	27	Benzo[j]fluoranthene
9	Acenaphthene-d10	6	2,6-dimethylnaphthalene	17	1-methylphenanthrene	28	Benzo[e]pyrene
14	Phenanthrene-d10	7	HMB	18	Fluoranthene	29	Benzo[a]pyrene
24	Benzo[a]pyrene-d12	8	Acenaphthylene	20	Pyrene	30	Perylene
		10	Acenaphthene	21	Benz[a]anthracene	31	Dibenz[a,c]anthracene
	Target Compounds	11	2,3,5-trimethylnaphtha...	22	Triphenylene	32	Dibenz[a,h]anthracene
2	Naphthalene	12	Fluorene	23	Chrysene	33	Indeno[1,2,3-cd]pyrene
3	1-methylnaphthalene	13	Dibenzothiophene	25	Benzo[b]fluoranthene	34	Benzo[ghi]perylene

mike_szelewski@agilent.com

PAH Analysis: GC/MS SIM Late Eluters



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

	7 levels --->	1 - 1000	1 - 100	1 - 1000
RT		QQQ A	QQQ V	Q
3.14	Napthalene	0.9998	0.9972	0.9997
3.43	1-methylnaphthalene	0.9998	0.9995	0.9998
3.53	2-methylnaphthalene	0.9999	0.9995	0.9996
3.76	Biphenyl	0.9998	0.9902	0.9998
3.78	2,6-dimethylnaphthalene	0.9998	0.9983	0.9999
4.24	Acenapthylene	0.9999	0.9994	0.9998
4.80	Acenapthene	0.9999	0.9999	0.9997
4.97	2,3,5-trimethylnaphthalene	0.9999	0.9998	0.9998
5.35	Fluorene	0.9999	0.9998	0.9998
6.48	Dibenzothiophene	0.9996	0.9989	0.9998
6.73	Phenanthrene	0.9997	0.9992	0.9999
6.79	Anthracene	0.9997	0.9985	0.9999
8.30	1-methylphenanthrene	0.9997	0.9996	0.9998
9.80	Fluoranthene	0.9960	0.9997	0.9998
10.68	Pyrene	0.9970	0.9998	0.9998
13.14	Benzo(a)anthracene	0.9930	0.9990	0.9998
13.29	Chrysene	0.9940	0.9997	0.9999
14.83	Benzo(b)fluoranthrene	0.9997	0.9980	0.9987
14.86	Benzo(k)fluoranthrene	0.9992	0.9983	0.9985
15.27	Benzo(e)pyrene	0.9999	0.9977	0.9987
15.33	Benzo(a)pyrene	0.9998	0.9971	0.9987
15.47	Perylene	0.9996	0.9977	0.9986
16.70	Indeno(1,2,3,-cd)pyrene	0.9997	0.9899	0.9996
16.69	Dibenz(a,h)anthracene	0.9980	0.9895	0.9996
17.23	Benzo(ghi)perylene	0.9888	0.9889	0.9991

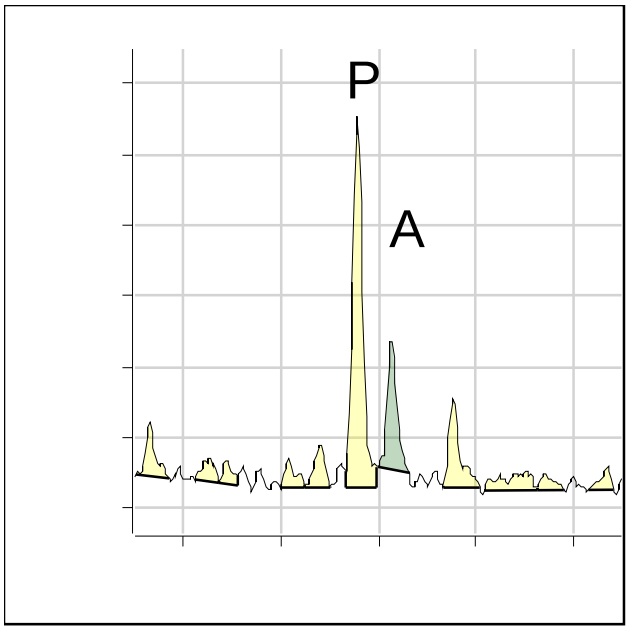
QQQ A and Q calibration stds were in isooctane solvent.

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

Data from Ralph Hindle, Vogon Labs, 7000A

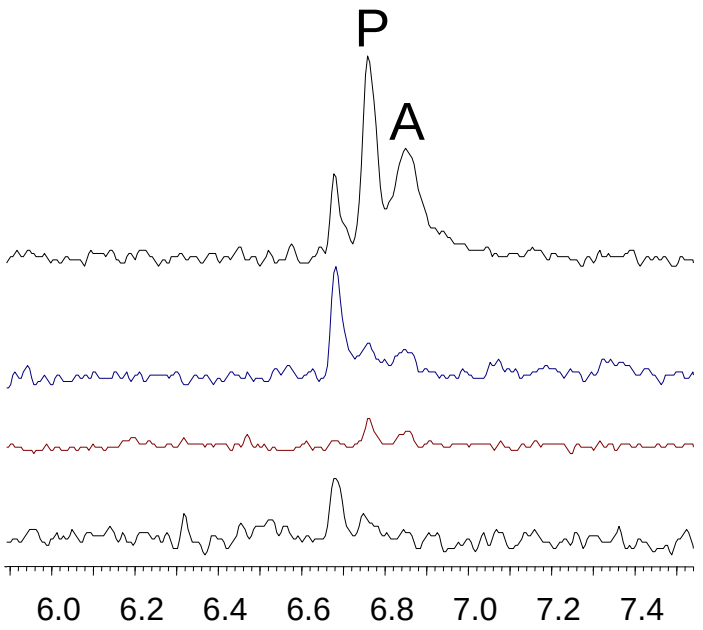
Phenanthrene and Anthracene 1.0 ppb Standard

MRM 7000A QQQ Std made in QuEChERS fish extract



Vogon Labs

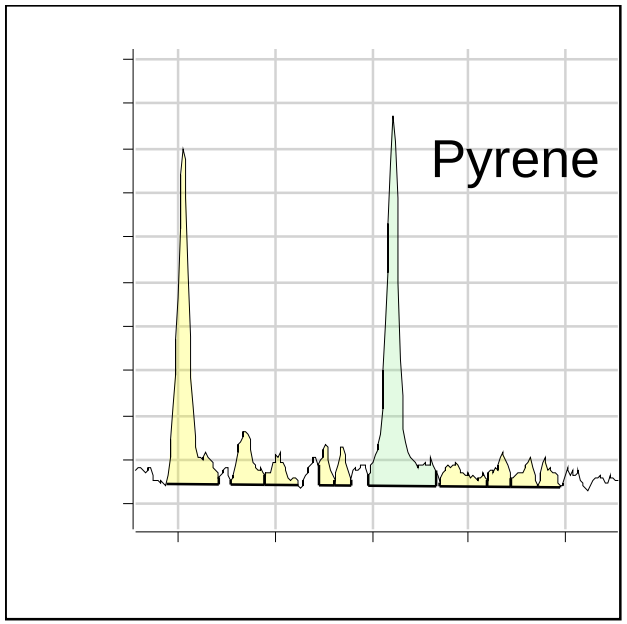
SIM 5975 Q in Isooctane



Agilent LFS

Pyrene 1.0 ppb Standard

MRM 7000A QQQ Std made in QuEChERS fish extract



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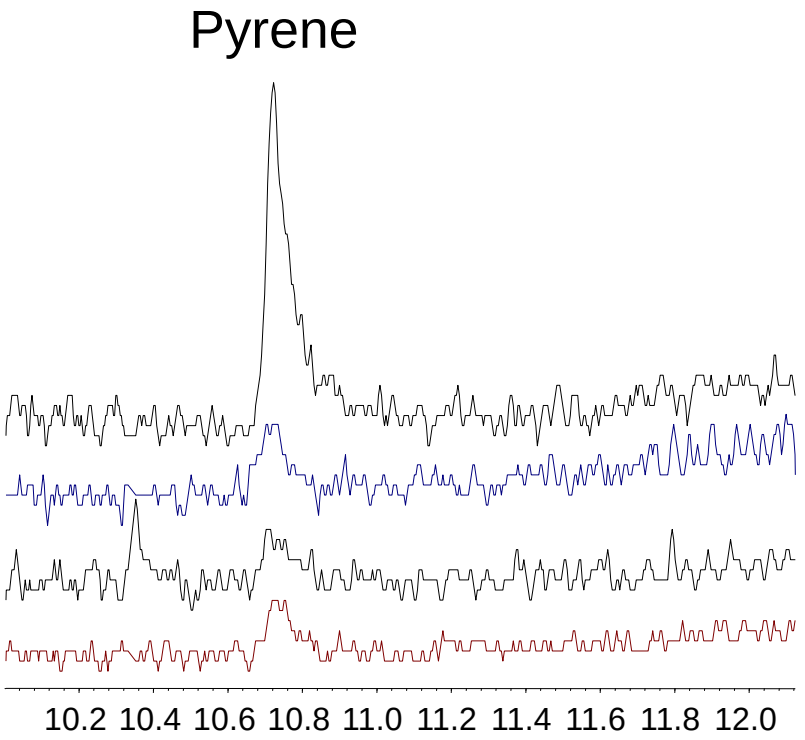


Agilent LFS

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

	25 ppb spike 1	25 ppb spike 2	25 ppb spike 3	Avg % Rec
Acenaphthylene	23.8	25.0	25.7	99
Acenaphthene	23.3	24.8	22.5	94
Fluorene	31.3	30.6	29.2	121
Phenanthrene	24.5	27.1	26.4	104
Anthracene	22.5	23.6	24.3	94
Fluoranthene	25.7	25.9	26.8	105
Pyrene	22.9	22.9	24.1	93
Benz[a]anthracene	29.2	27.9	29.9	116
Chrysene	24.0	23.4	24.3	96
Benzo[b]fluoranthene	22.0	23.1	23.6	92
Benzo[k]fluoranthene	20.7	21.9	22.2	86
Benzo[a]pyrene	27.0	29.5	31.7	117
Dibenz[a,h]anthracene	18.8	19.4	19.9	77
Indeno[1,2,3-cd]pyrene	17.3	17.9	18.7	72
Benzo[ghi]perylene	17.3	18.0	18.7	72

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

Concentration in 3 g mussel tissue = 125 ppb

mike_szelewski@agilent.com

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

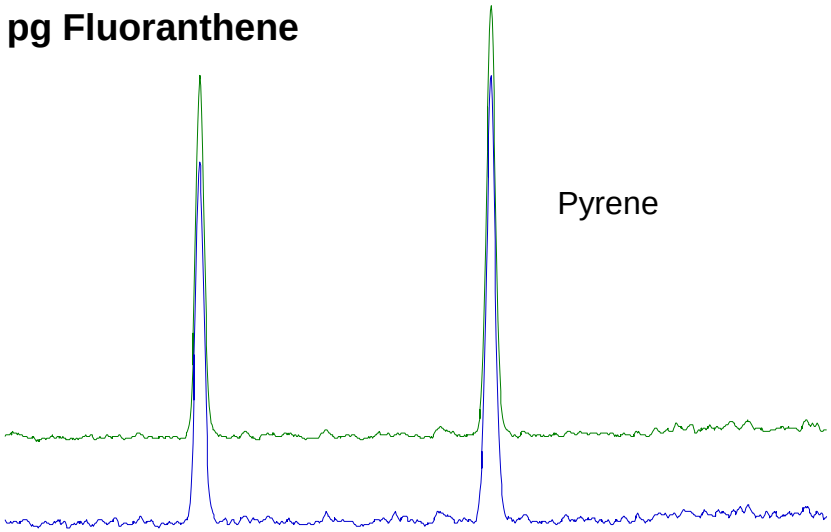
	7000B		5975C		7000B		5975C
	MRM		SIM		MRM		SIM
	Std		Std		Spike		Spike
	1 ppb		1ppb		25 ppb		25 ppb
Naphthalene	36		23		---		---
Fluorene	8.0		7.2		112		92
Phenanthrene	6.7		8.8		121		69
Anthracene	6.8		5.7		100		60
Fluoranthene	8.0		5.3		88		43
Pyrene	6.3		4.6		105		39
Benz[a]anthracene	22		5.0		130		128
Chrysene	21		5.1		130		121
Benzo[a]pyrene	15		10		60		11

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.

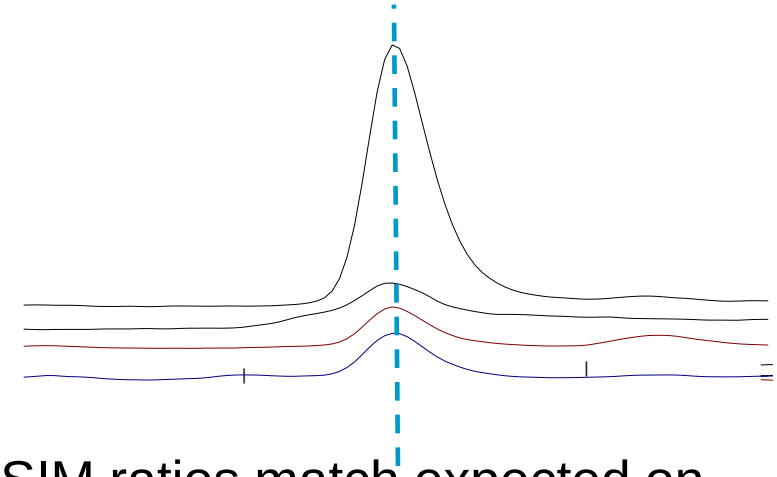
Both sets of EICs are from a QuEChERS extract concentrated 10x in ACN. Background is still low.

15 pg Fluoranthene



MRM ratios match expected on QQQ

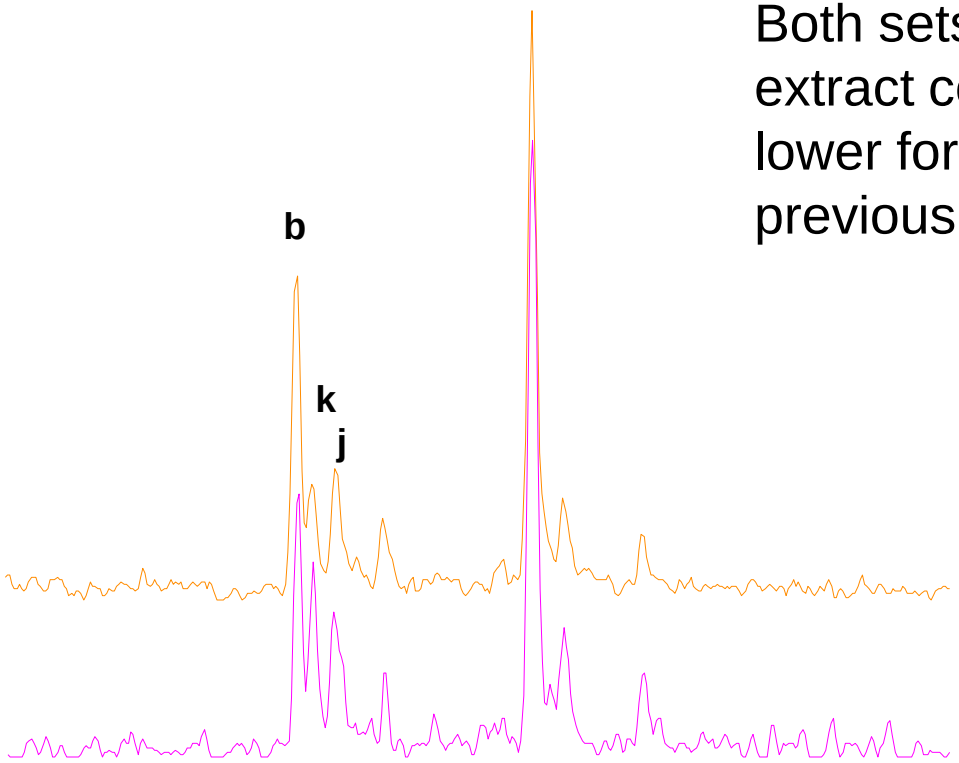
15 pg Fluoranthene



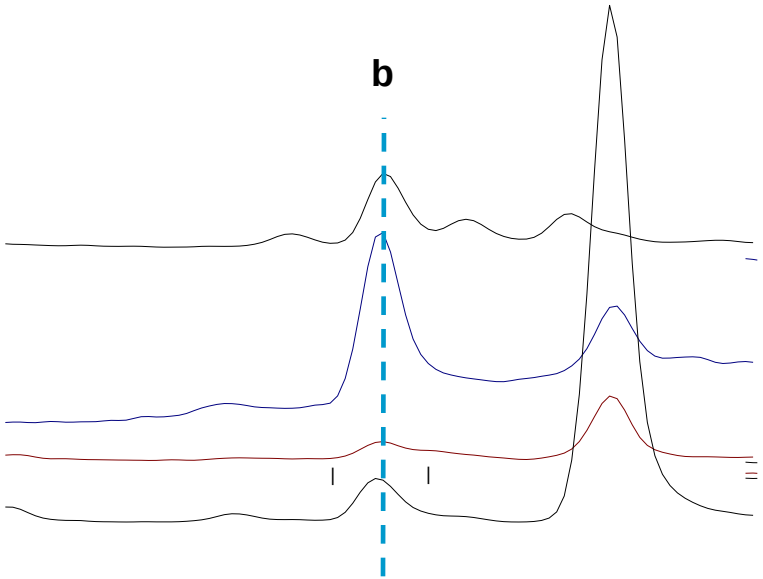
SIM ratios match expected on GC-Q. RTs align.

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.

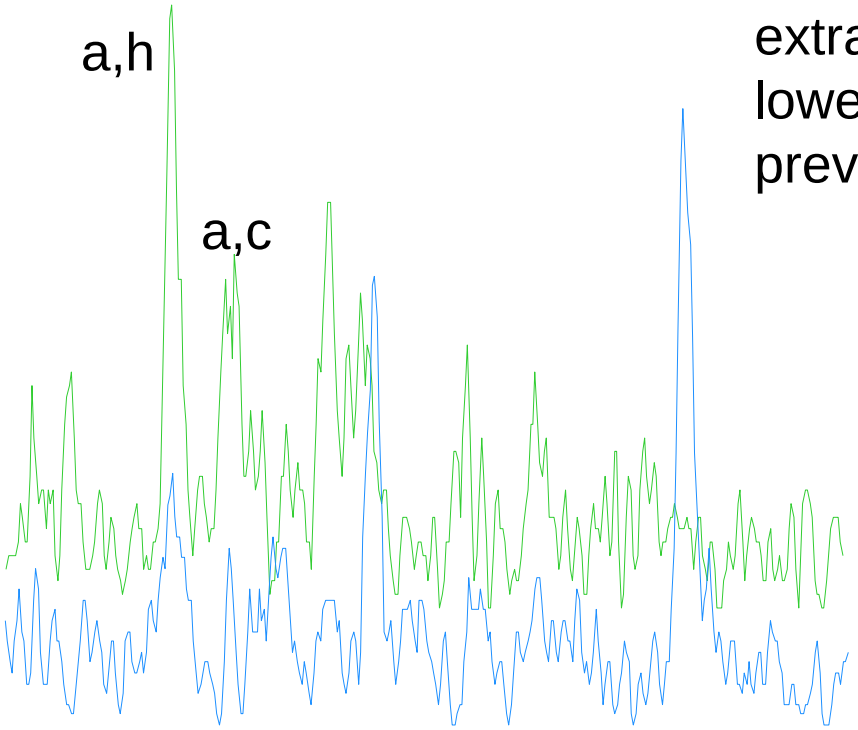


MRM ratios match expected on QQQ

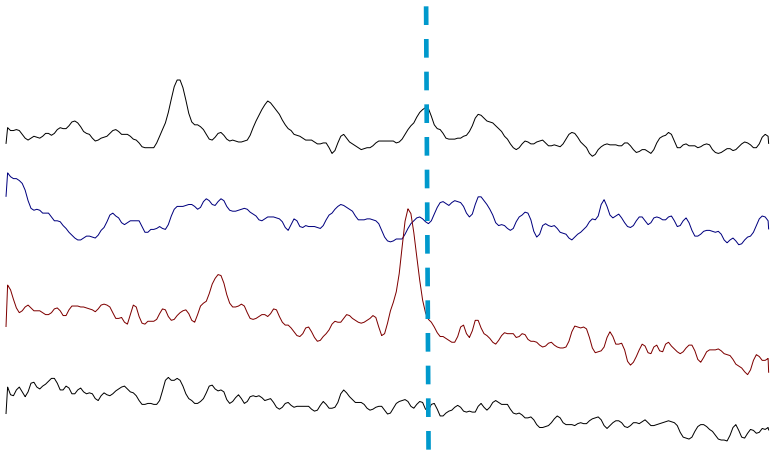
SIM ratios do not match expected on GC-Q. RTs do not align

Instead of concentrating 10x, you could use a 10 uL solvent vent injection with the MMI

Same 10x Concentrated QuEChERS Extract from previous slide. Dibenz(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.

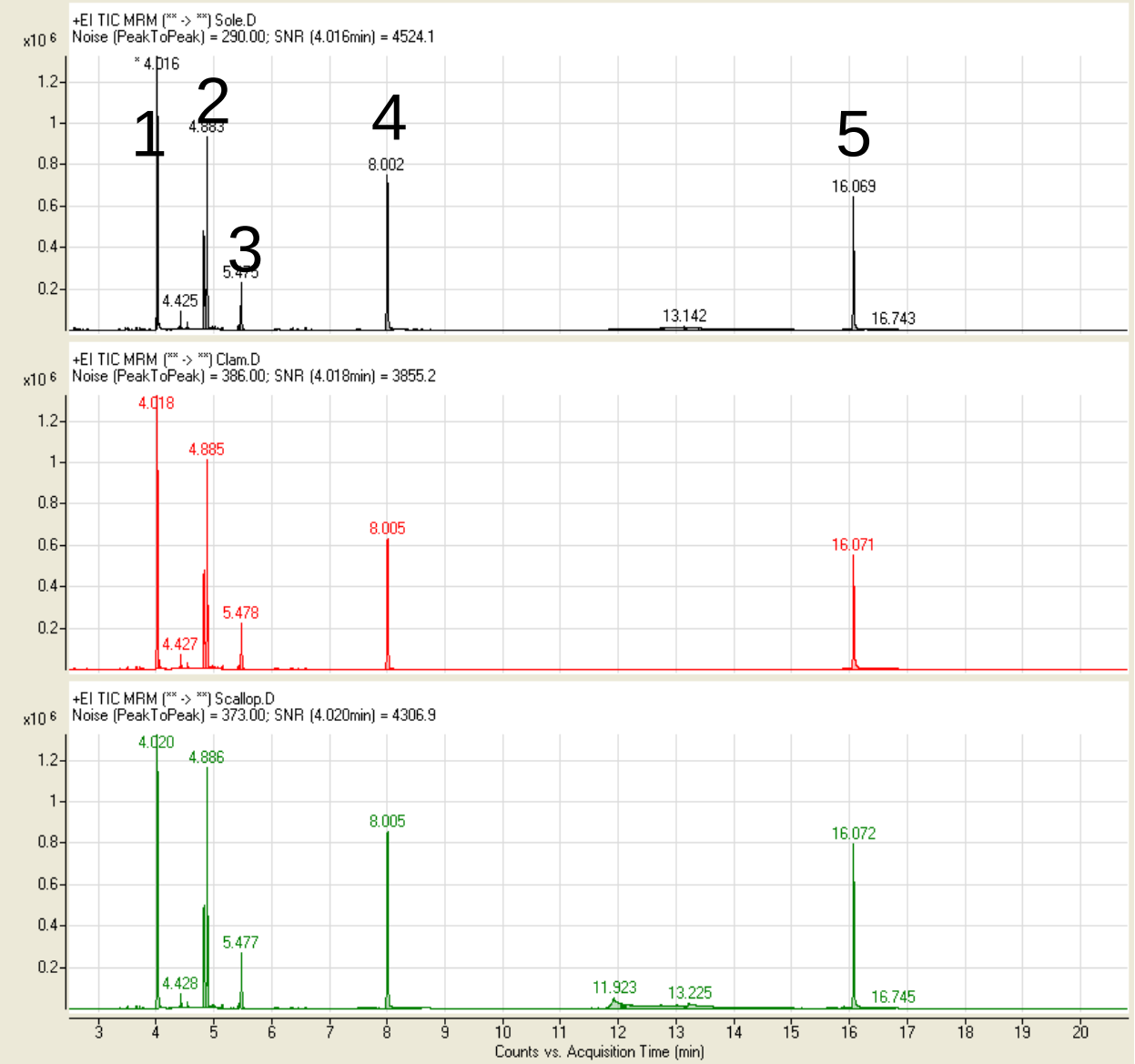


MRM ratios do not match expected on QQQ, but s/n is better than Q

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



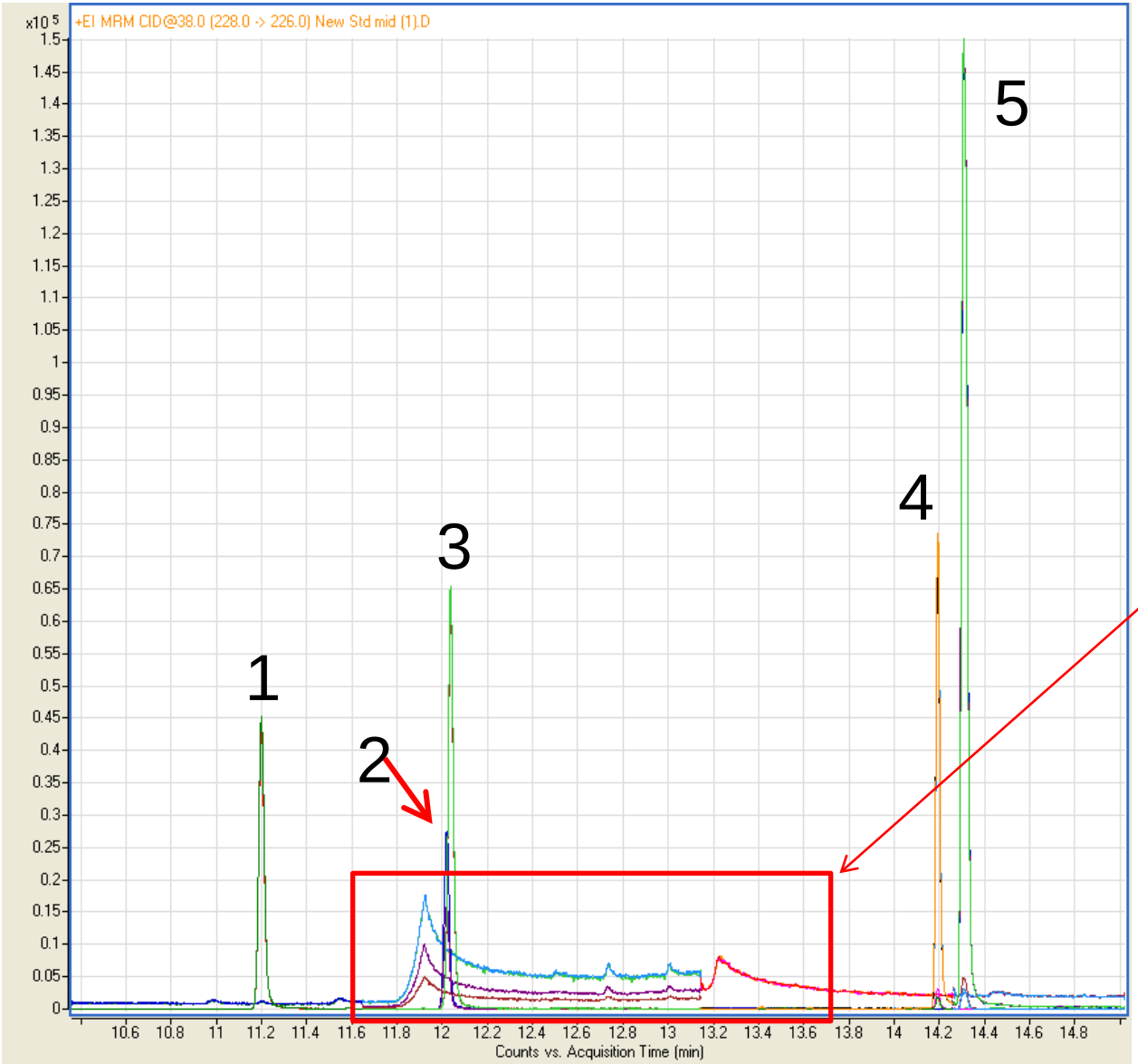
Internal Standards

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

Low level background

Data from Arkansas DOH on 7000B QQQ-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