Advanced Analytical Technologies for Analyzing Environmental Matrixes Contaminated with Petroleum Hydrocarbons

PAH Analyzers

GC-Q and GC-QQQ

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Analytical Needs for Oil Spill Contaminants

- **Sample extraction from environmental matrices, such as seafood**
- **Poly-aromatic Hydrocarbons (PAH) in seafood, sediment, water**
- **Volatile and Semi-volatile Organic Compounds (VOC, SVOC)**
- **Petroleum hydrocarbon (PHC) fingerprinting and source identification**

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

QuEChERS

- **Q**uick, **E**asy, **Ch**eap, **E**ffective, **R**obust and **S**afe
- **Developed by the US FDA and EU Food Regulatory Agencies**
- **Procedure was validated in 2003, “toddler stage”**
- **Extraction and analysis of pesticides in food product**

QuEChERS

- Majority of current method for pesticides in food use SPE
- SPE requires multiple methods for specific classes of compounds
- A single QuEChERS method can extract 250+ pesticides
- Amenable to GC/MS and LC/QQQ analysis

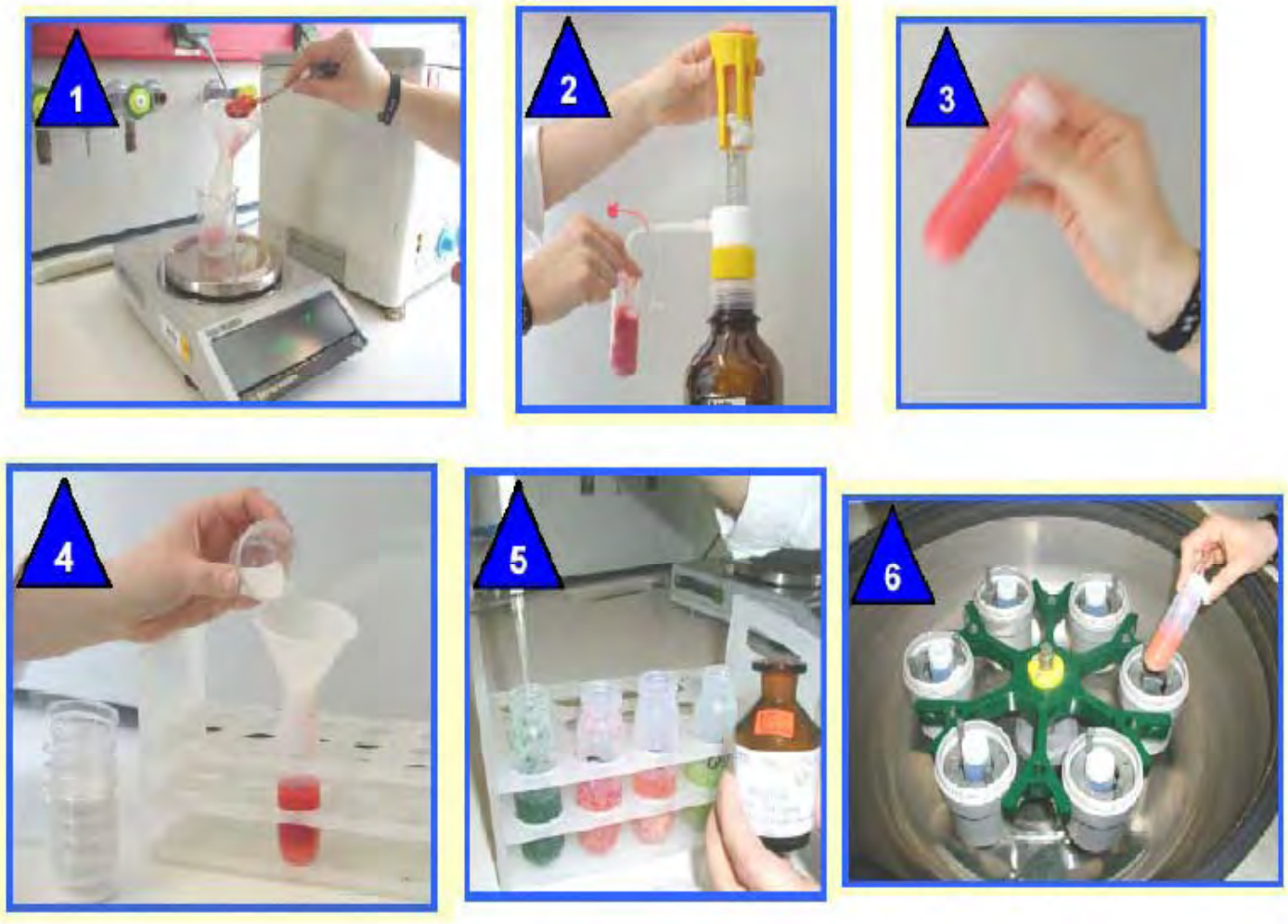
Why is QuEChERS?

- ***Reduced solvent, reduced labor, increased lab productivity***

Standard SPE Methods	QuEChERS Method
Sample Processing: 120 min.	30 min. 25% of the time
Solvent usage: 60-90 ml	Solvent usage: 10-15 ml
Chlorinated Solvents: 20-30 ml	Chlorinated Solvents: None

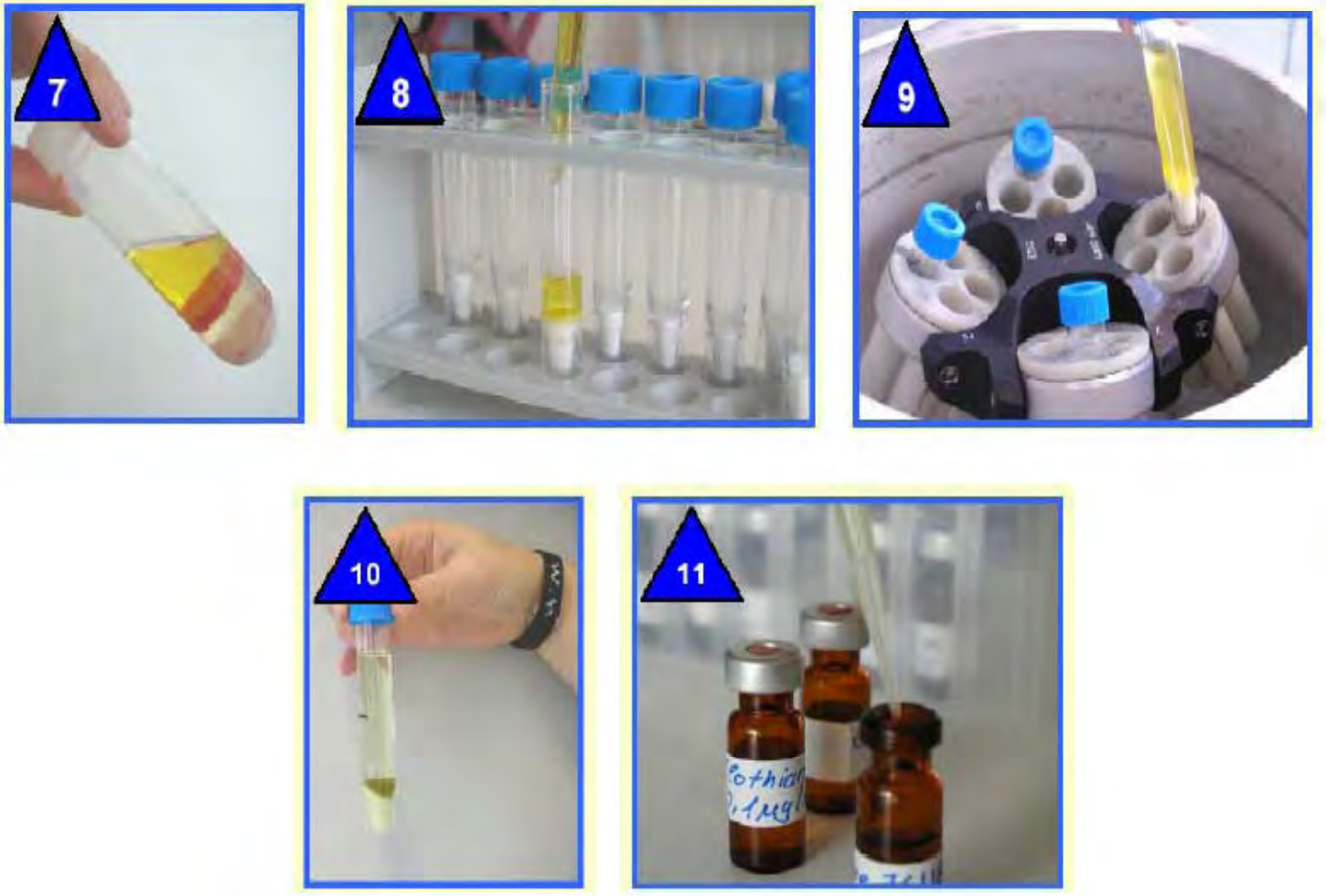
First Step: QuEChERS Extraction

Pictorial Representation of the QuEChERS Steps



Second Step: Dispersive-SPE

Pictorial Representation of the QuEChERS Steps



Advantages of Dispersive SPE Over Standard SPE

Dispersive SPE =	
No SPE Apparatus	No Flow Control
No SPE Cartridges	No Elution Solvent
No Vacuum	No Dilution of Extract
No pretreatment	No Solvent Evaporation
No Channeling	Less Sorbent
No Drying Out	Less Time
No Collection	Less Cost

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Why QuEChERS?

When Compared to SPE and GPC:

- 25-50%+ time savings
- Reduced solvent usage: 10-15 mL/sample
- No chlorinated solvents required
- Extract multiple families of compounds with one extraction method
- Does not require advanced sample preparation experience

QuEChERS Seafood Extraction Method:

Step1:Extraction

Finfish, shellfish

Weigh 3 g into 50 mL tube,
add 2 ceramic homogenizers

Add 12 mL of water, vortex 30 sec

Add 15 mL of ACN (1% AA), vortex 1 min

Vertically shake for
1 min, centrifuge at 4000 rpm for 5 min

Aspirate and transfer 8 mL of extract
To dispersive SPE (fatty sample)

Step2: d-SPE (dispersive-SPE)

Transfer 8 mL of extract from Step 1
To d-SPE (fatty sample)

Vortex 1 min

Centrifuge 400 rpm, 5 min

Aspirate extract , filter through 0.45 um
Nylon filter, transfer to GC or HPLC Vial

Step 3: Analyze
GC/MS or GC/QQQ
LC/UV/FLD or LC/QQQ
(require dilution with water 1:4 or 1:5
prior to LC)

No solvent exchange required for GC/MS analysis

The Challenge with Trace Analysis Today

The EU now mandates that no pesticides be present in baby food above 1 ppb

The Japanese Positive List has similar limits for over 400 specified pesticides

How much PAH is in the shrimp?

- **Old problems…**

  Identification and quantitation at 1 ppb

- **New problems….**

  It's the baby food and crude oil!

Matrix Problems Are Many!

- Interferences obscure peaks and hinder identification
- Interferences ruin calibration and quantitation
- Rising baselines
- Shifting retention times
- Contaminated columns
- Contaminated detectors

GC/MS Requirements are Changing

Technology	2007-2012 Growth Rate
Single quad	5.0%
Ion trap	4.7%
Time-of-flight	7.5%
Triple quad	20.9%

SDI Global Assessment Report, 10th Edition, Sept. 2008

Why?

- Lower required MDLs
- Challenges of more complex matricies
- Methods can be much cheaper to operate

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.

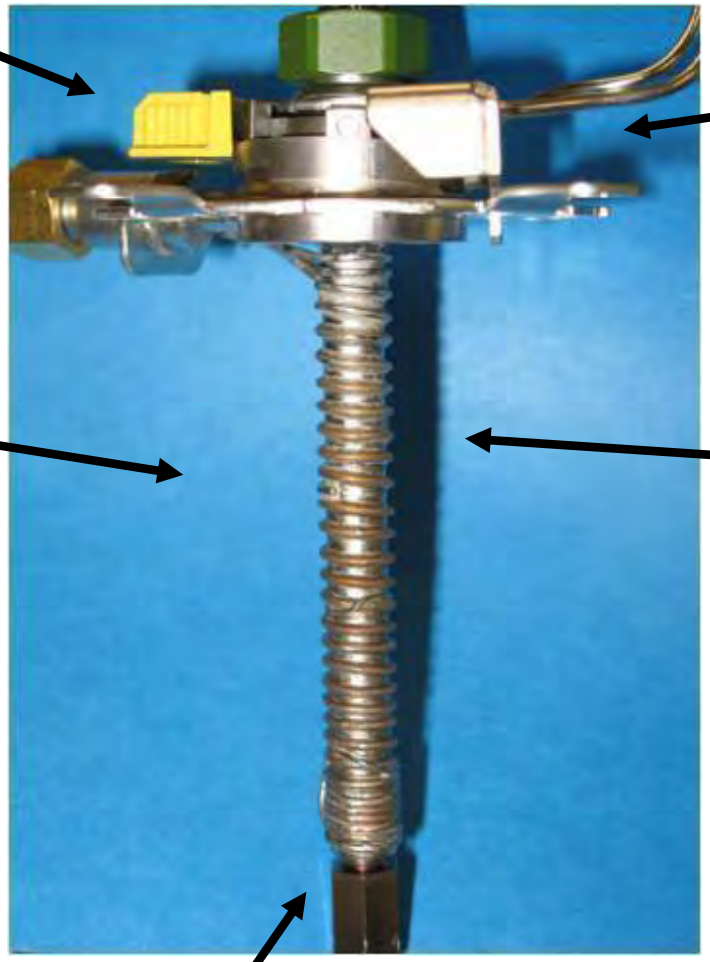
4. **Backflushing** is done via a capillary flow technology purged union connected post column. Cycle time is reduced as column bake-out is eliminated. Source cleaning is reduced.

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 with collaborators

Improving Detection limits: Large Volume Injection

Standard 11mm septa

Turn-top easy liner exchange



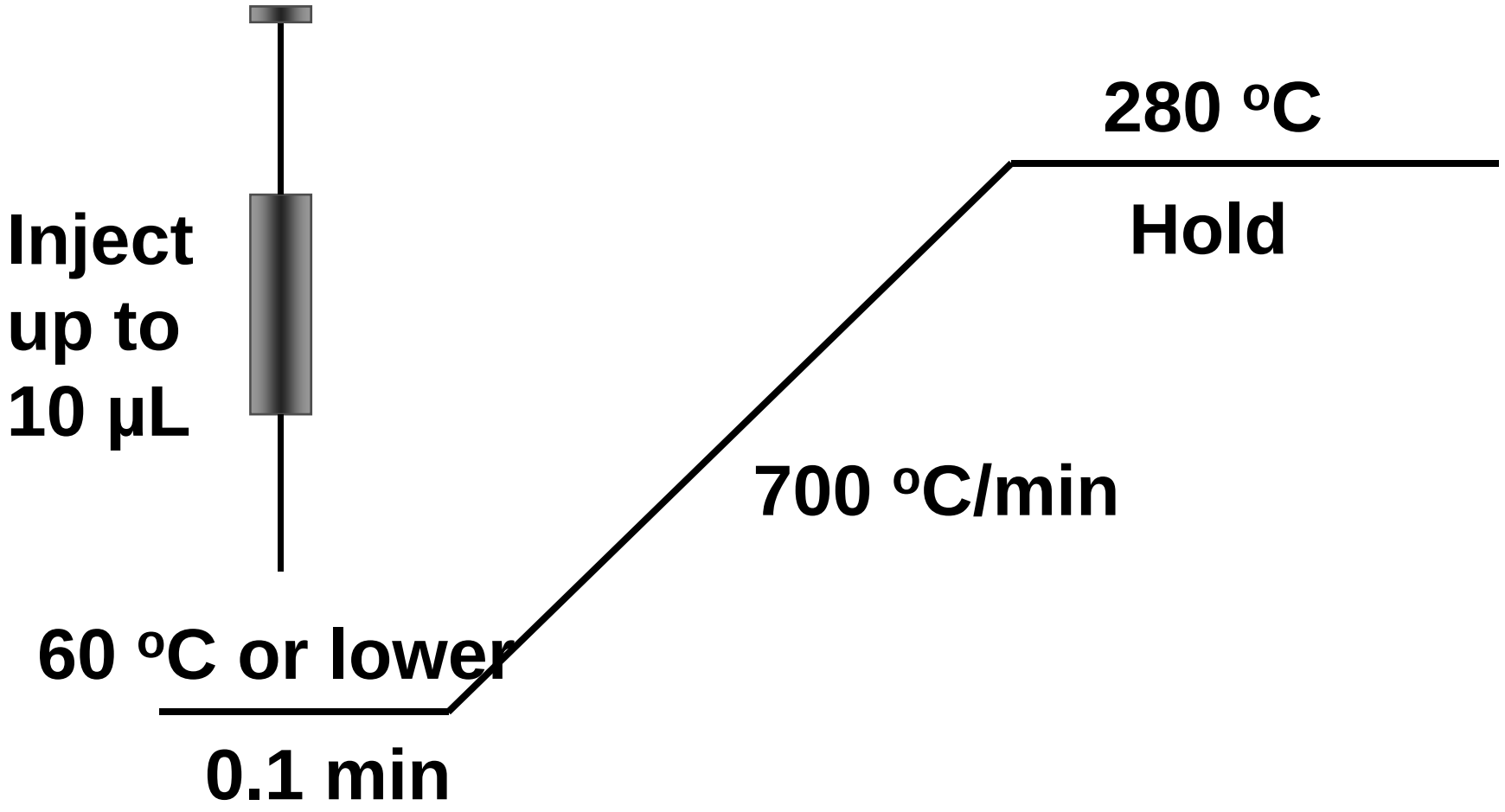
No leaks at liner

Air plus CO2, N2 cryogenic cooling

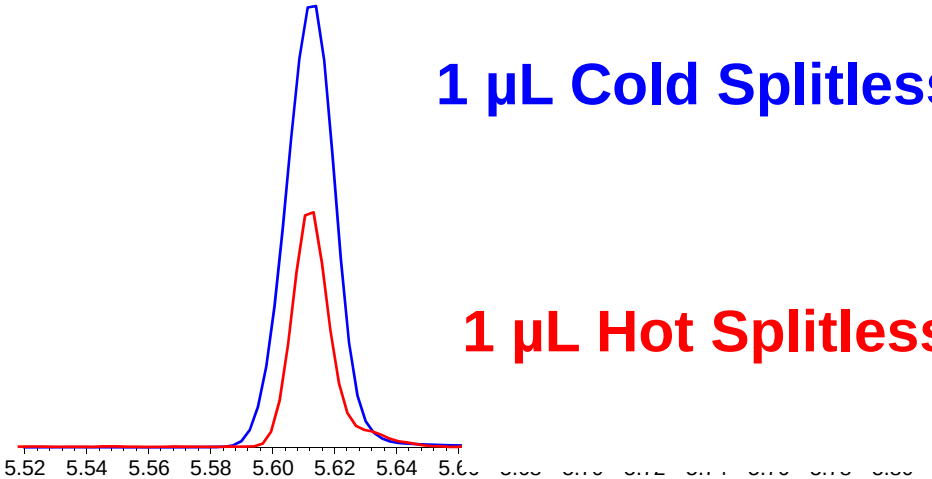
Standard liner dimensions

Standard column nut

Cold Splitless Injection Really Works – Inject up to 10 µL (liner dependent) without Solvent Vent

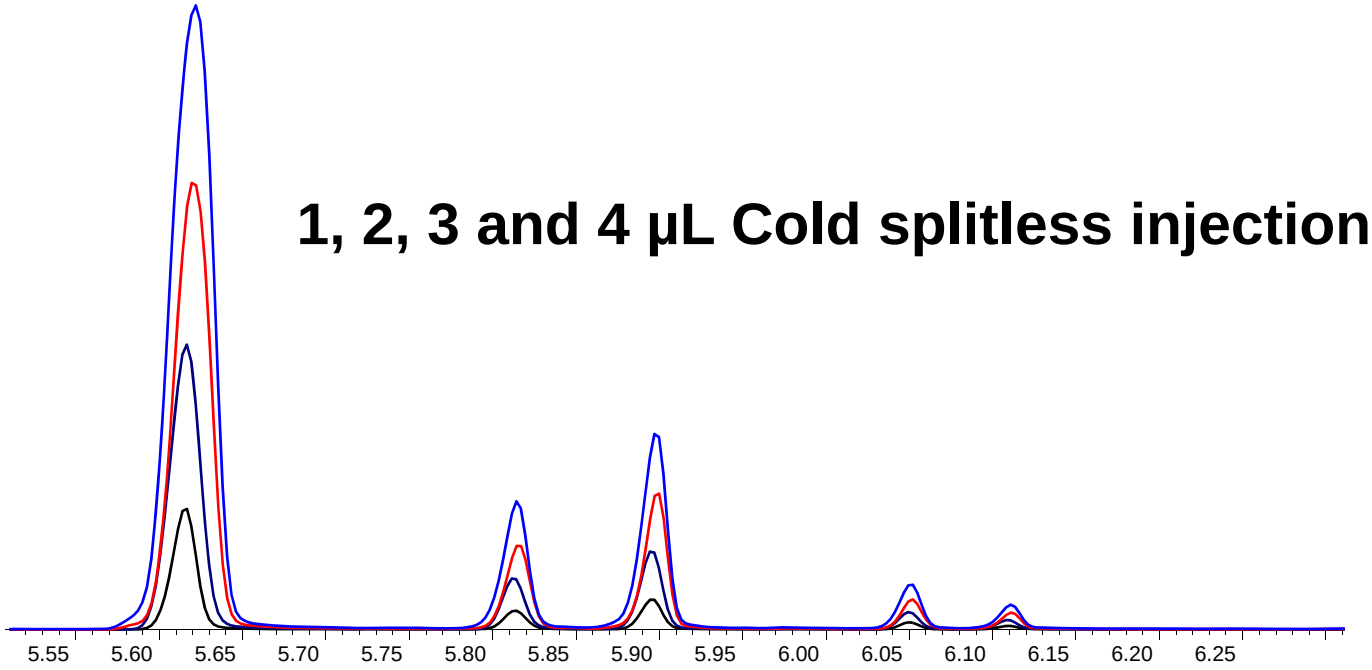


Multimode Injection LVI of Triazine Herbicides



1 µL Cold Splitless injection

1 µL Hot Splitless injection



1, 2, 3 and 4 µL Cold splitless injections (ethyl acetate)

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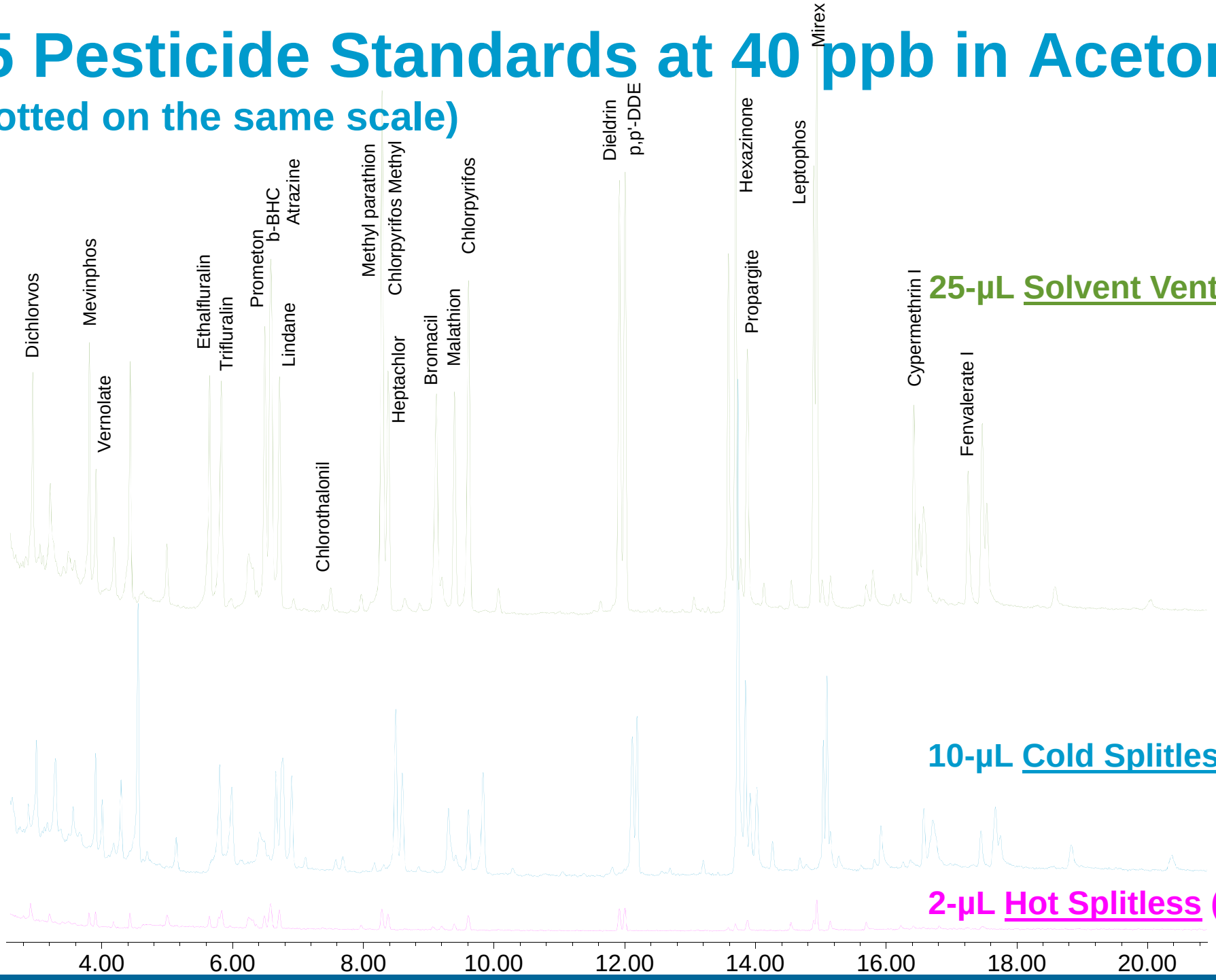
25 Pesticide Standards at 40 ppb in Acetone

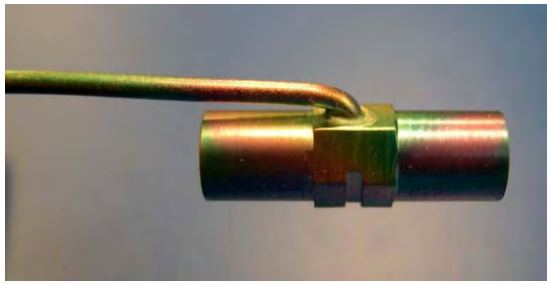
(plotted on the same scale)



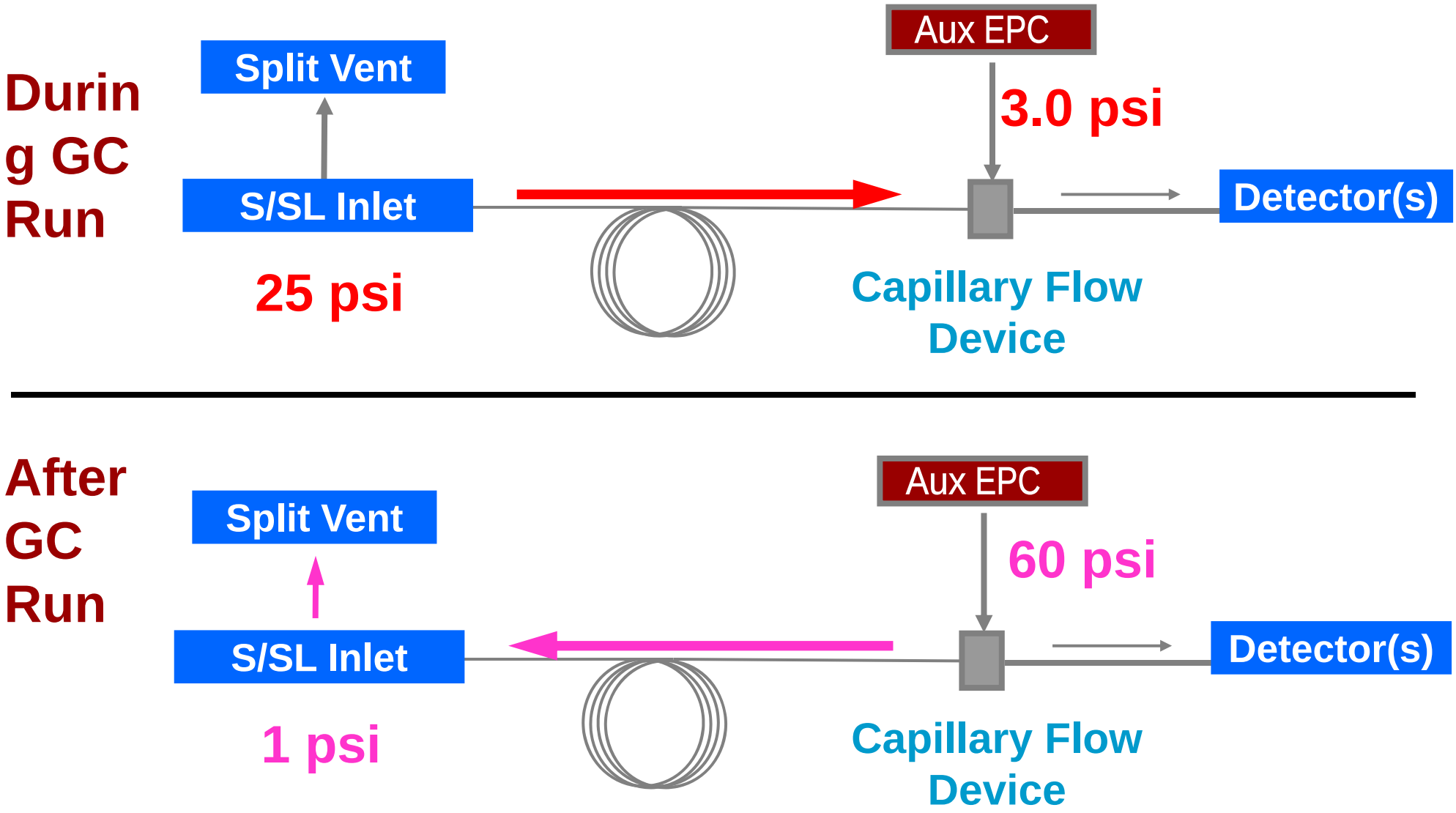
GC-QQQ (or GC-Q) PAH Analyzer

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

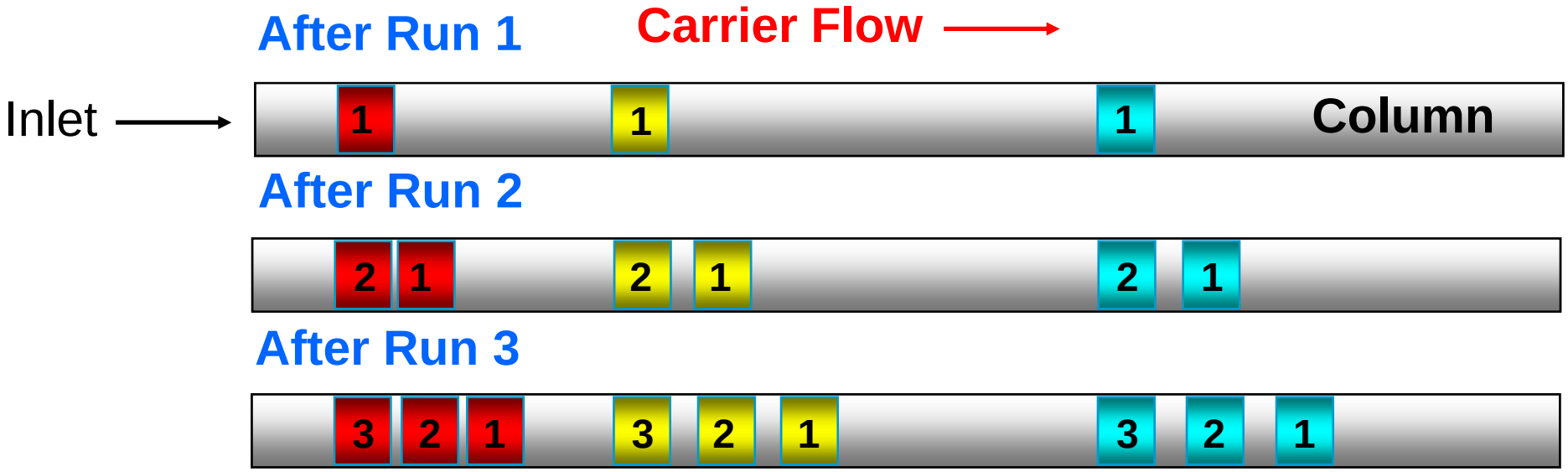
(2) CP Restrictor 0.70 m X 0.15 mm id deactivated tubing



Principle Of Backflushing



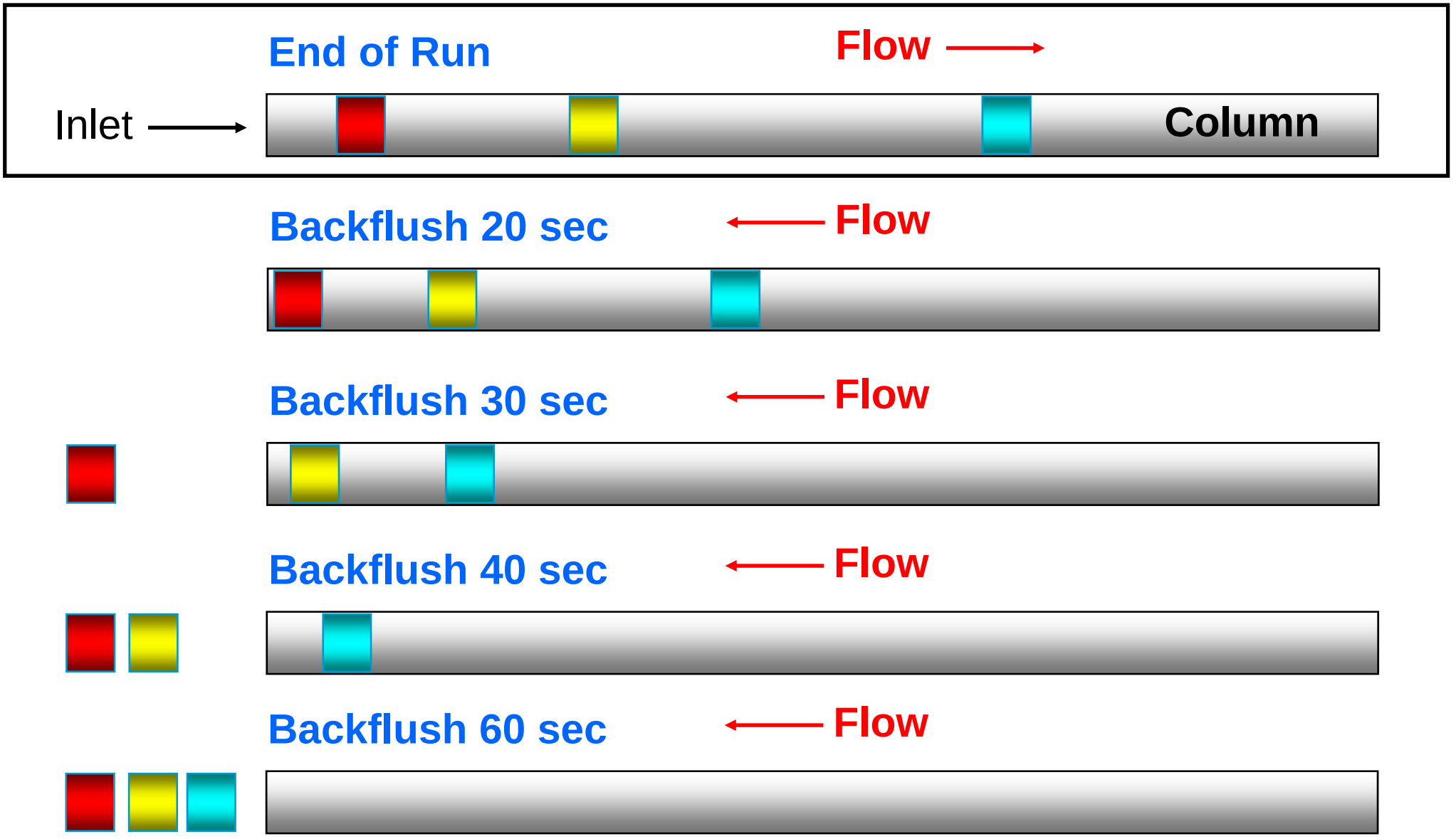
Heavy Compounds May Be Left in Head of Column After Each Injection



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

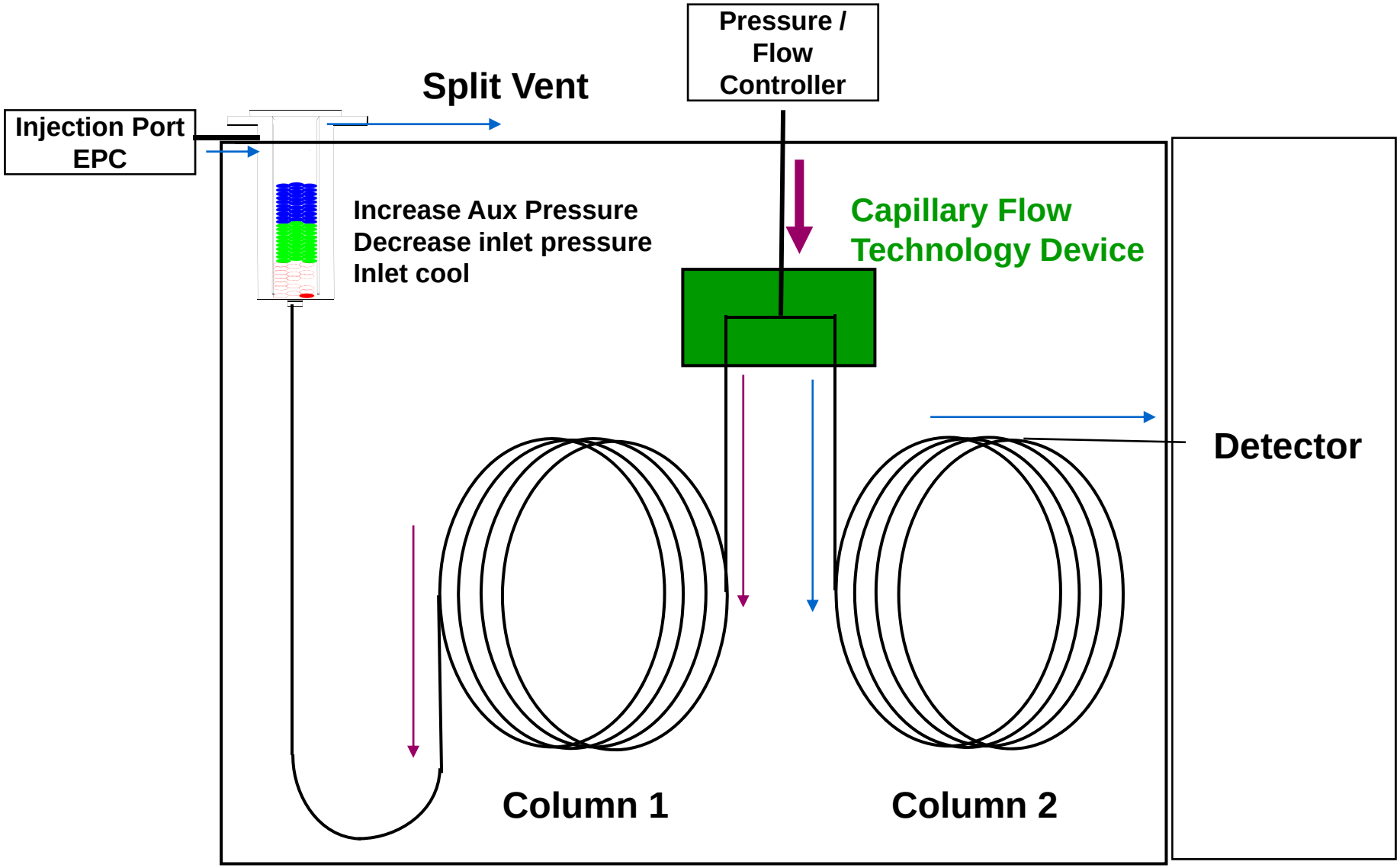
This buildup of heavy materials causes retention time shifts, peak distortion, higher bleed, and loss of sensitivity

Backflushing After Each Injection

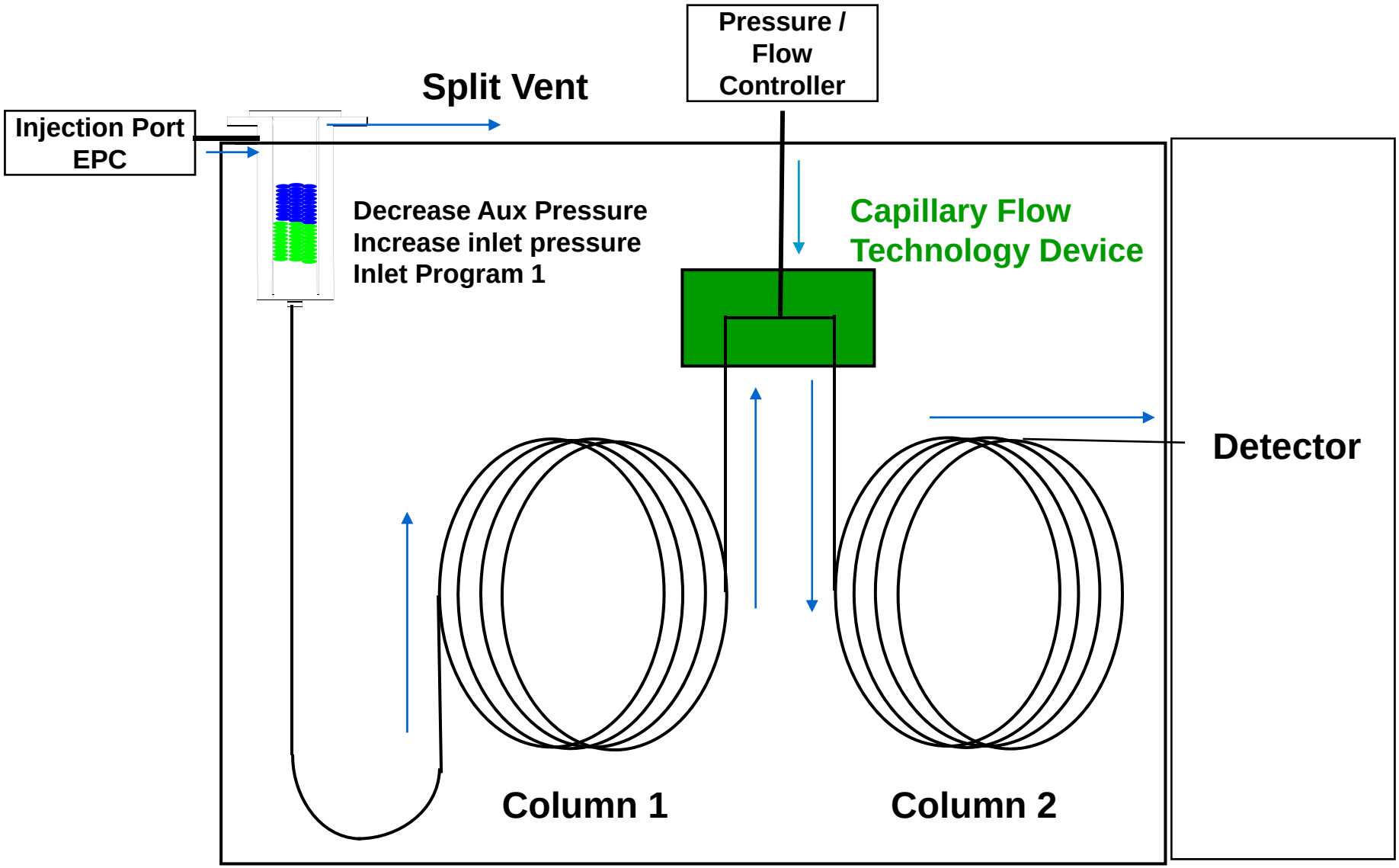


Backflushing removes heavy materials after each injection.

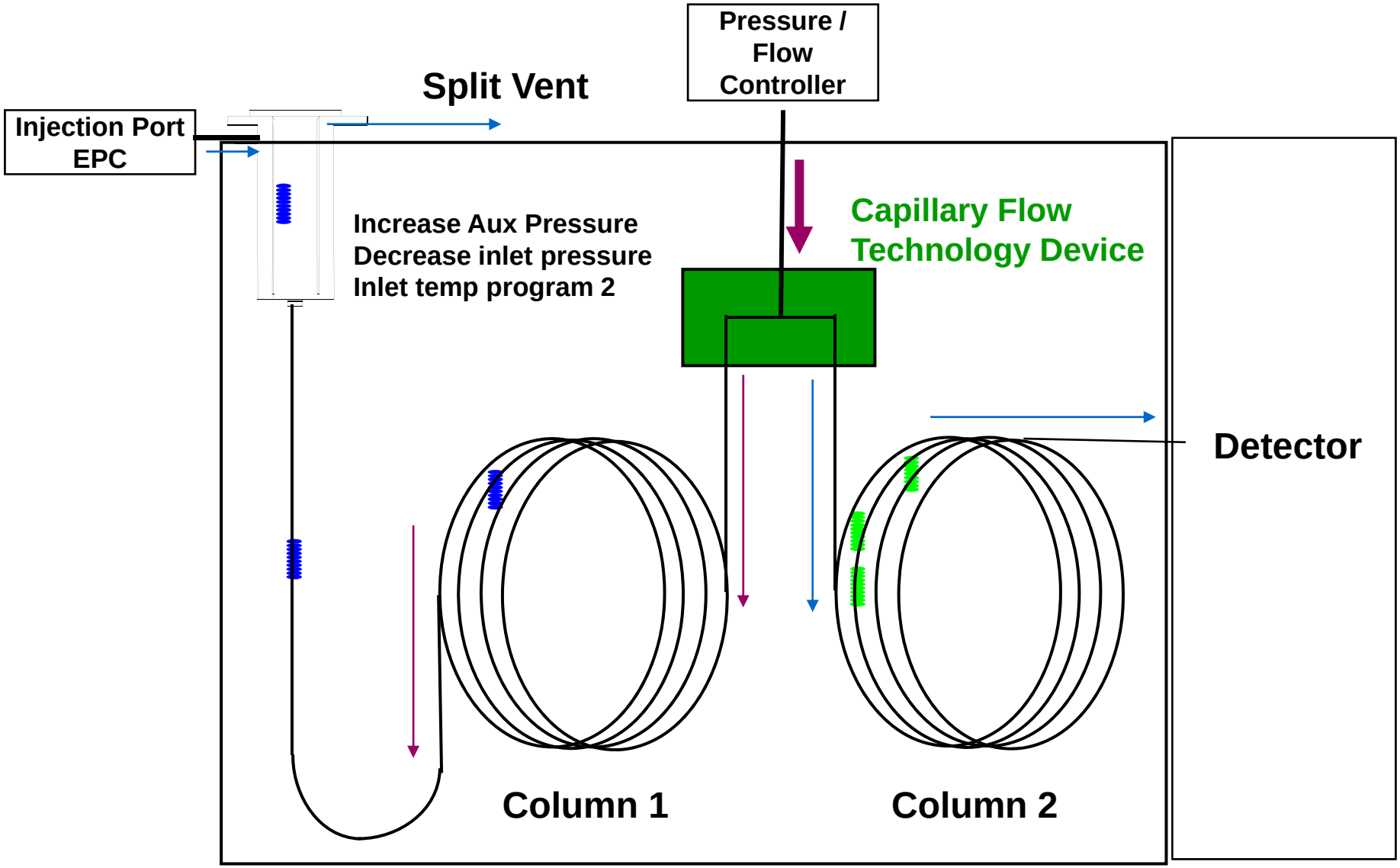
Backflush of Pre-Column to Vent: Inject Mode



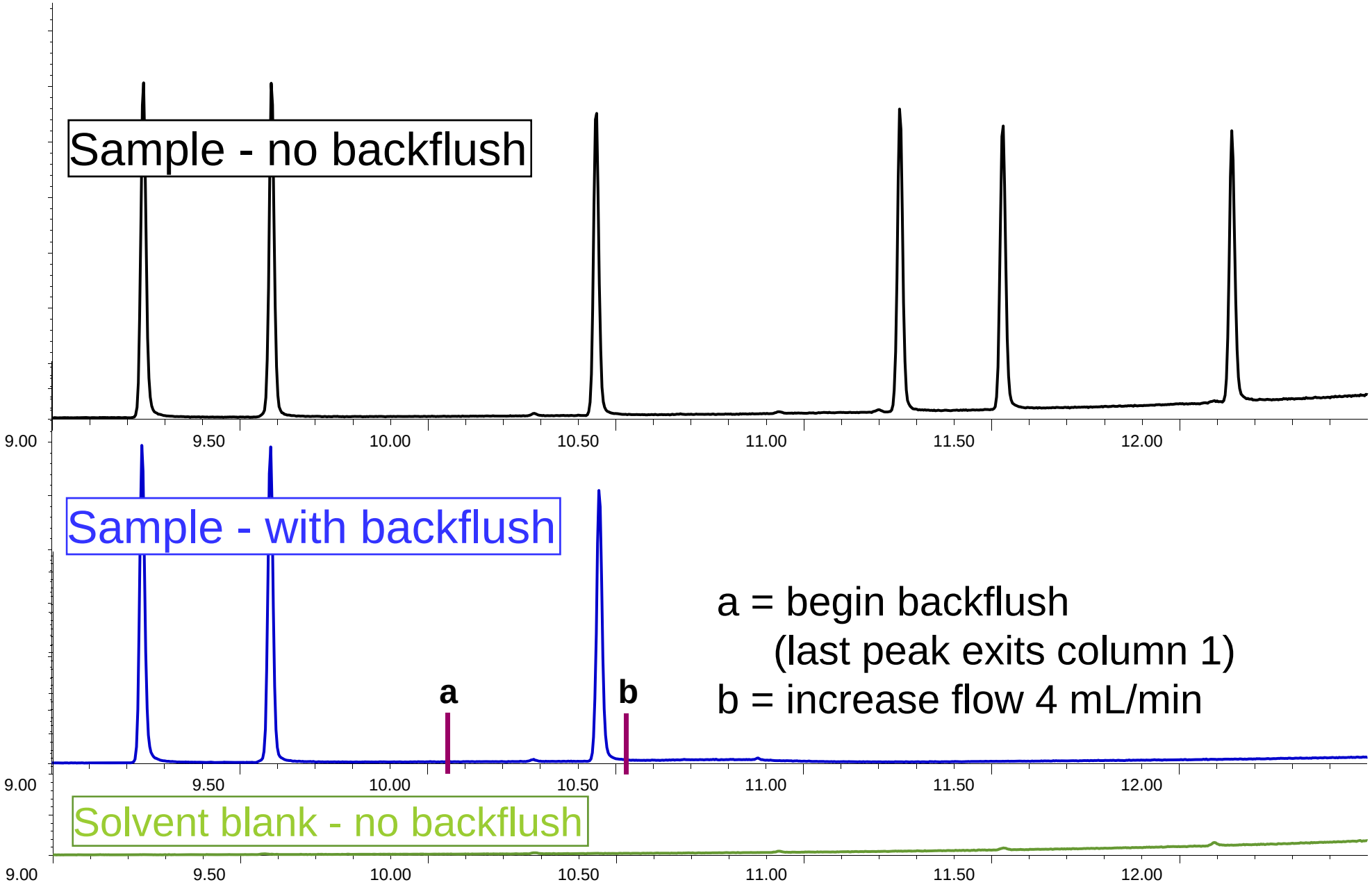
Backflush of Pre-Column to Vent: Transfer Analytes



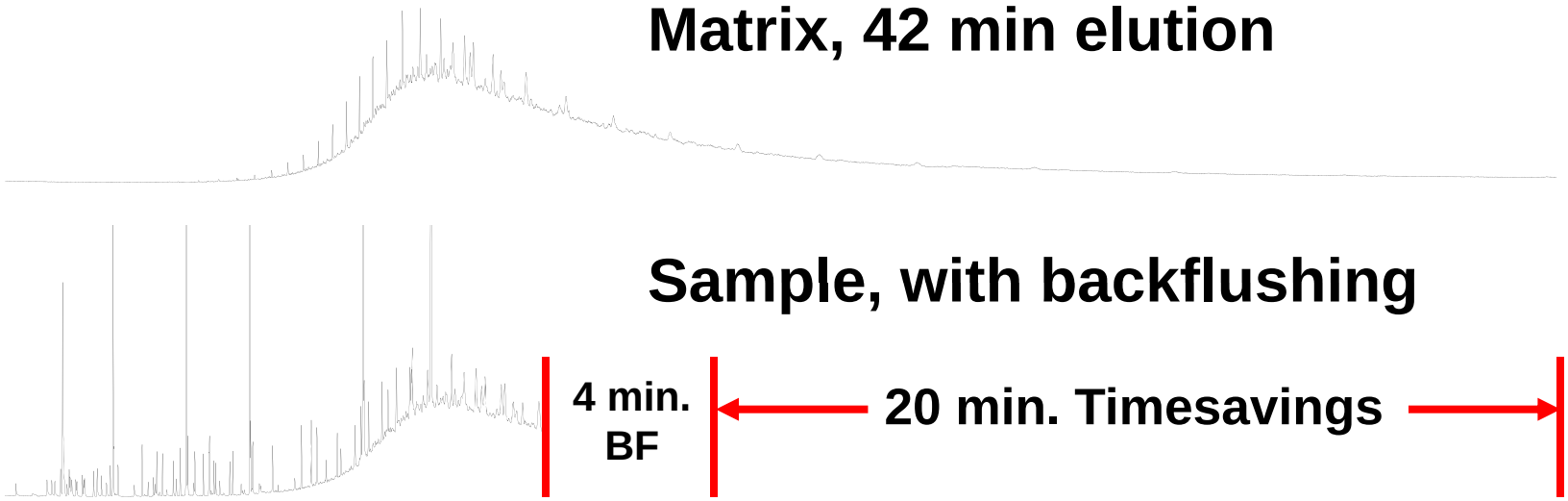
Backflush of Pre-Column to Vent: Backflush Matrix



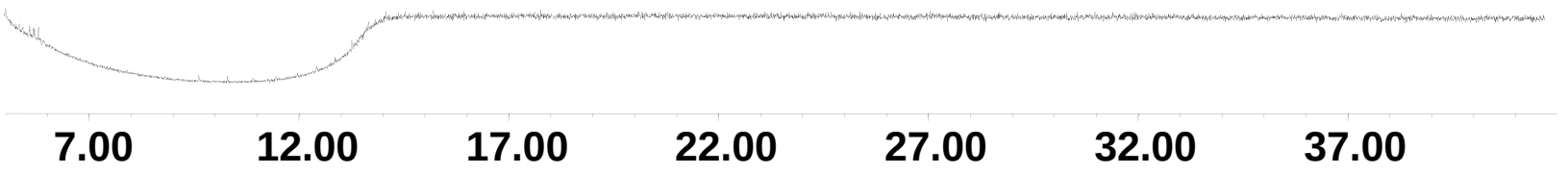
Backflush of Pre-Column to Vent



Environmental - Gasoil Backflush Example

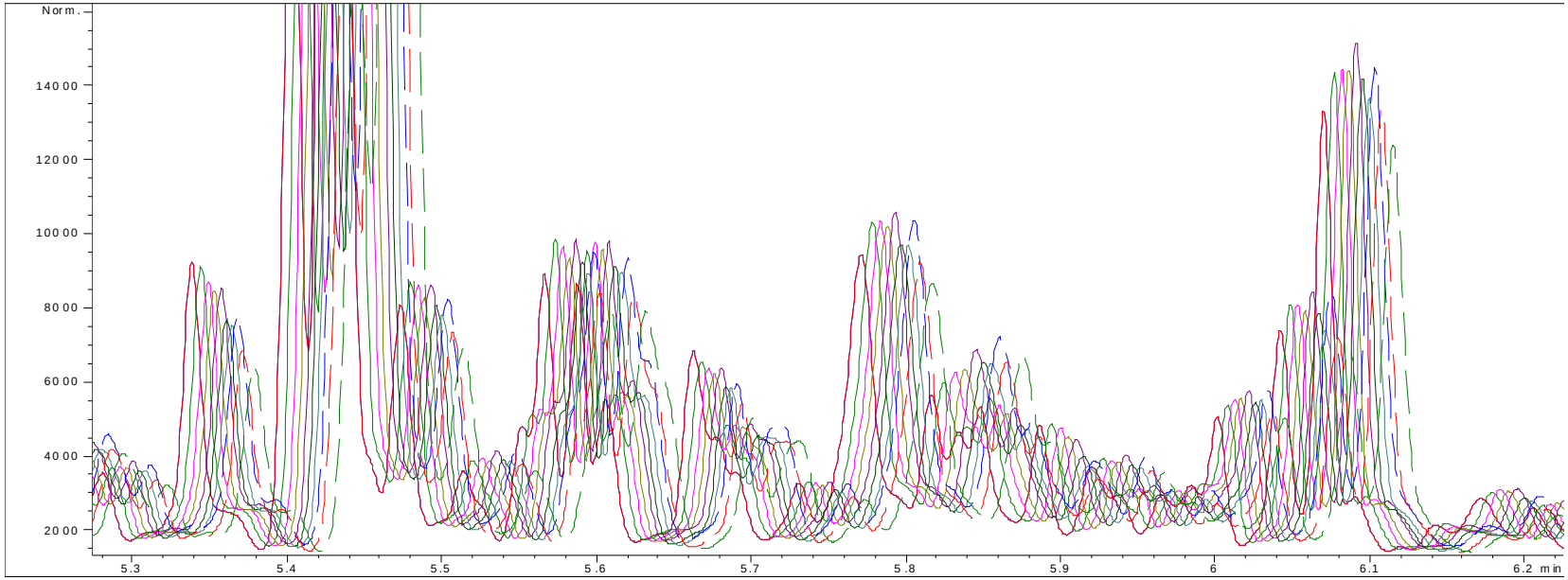


Scale 20x more sensitive than above

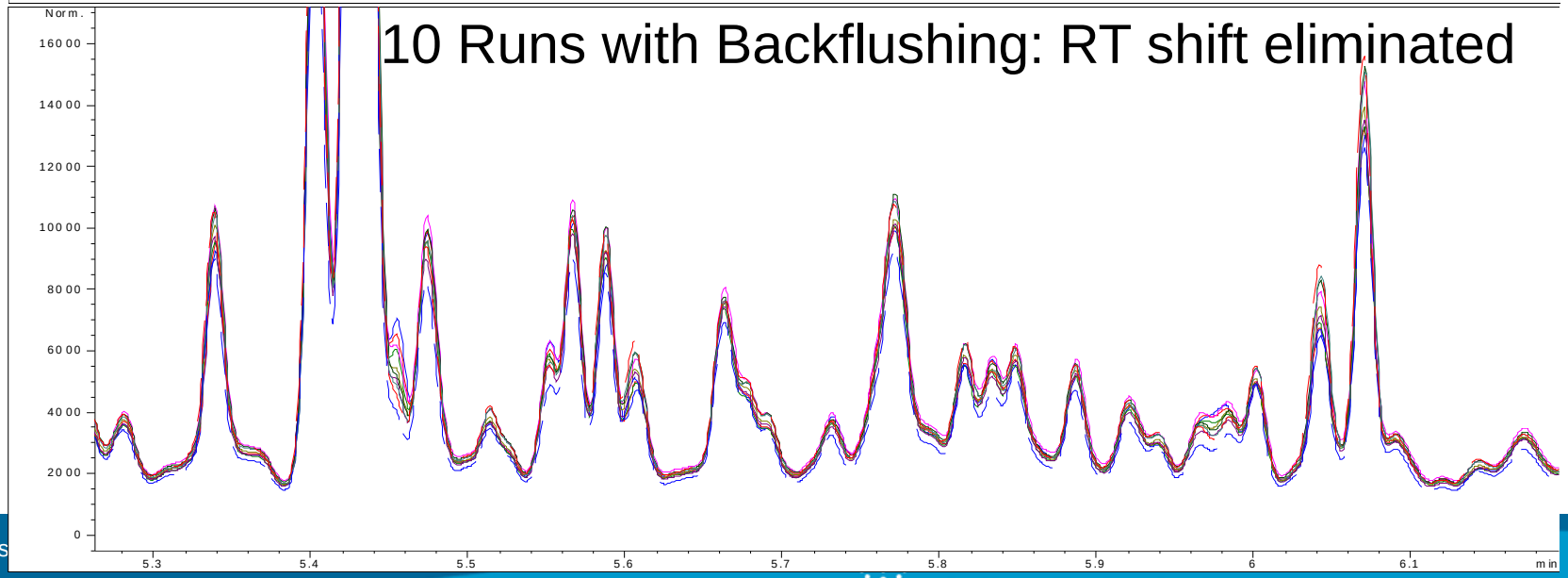


10% Fish Oil In Acetone: Retention Time Shifts Eliminated With Backflushing

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

MM Inlet

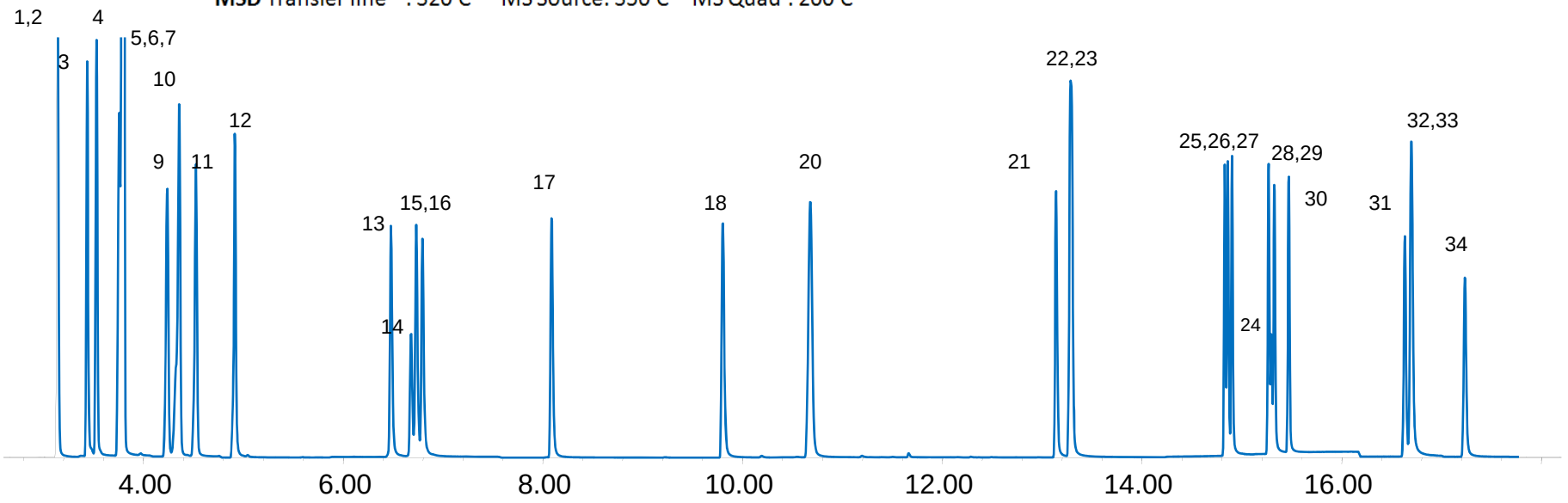
Mode Pulsed Splitless Temperature: 320 °C

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

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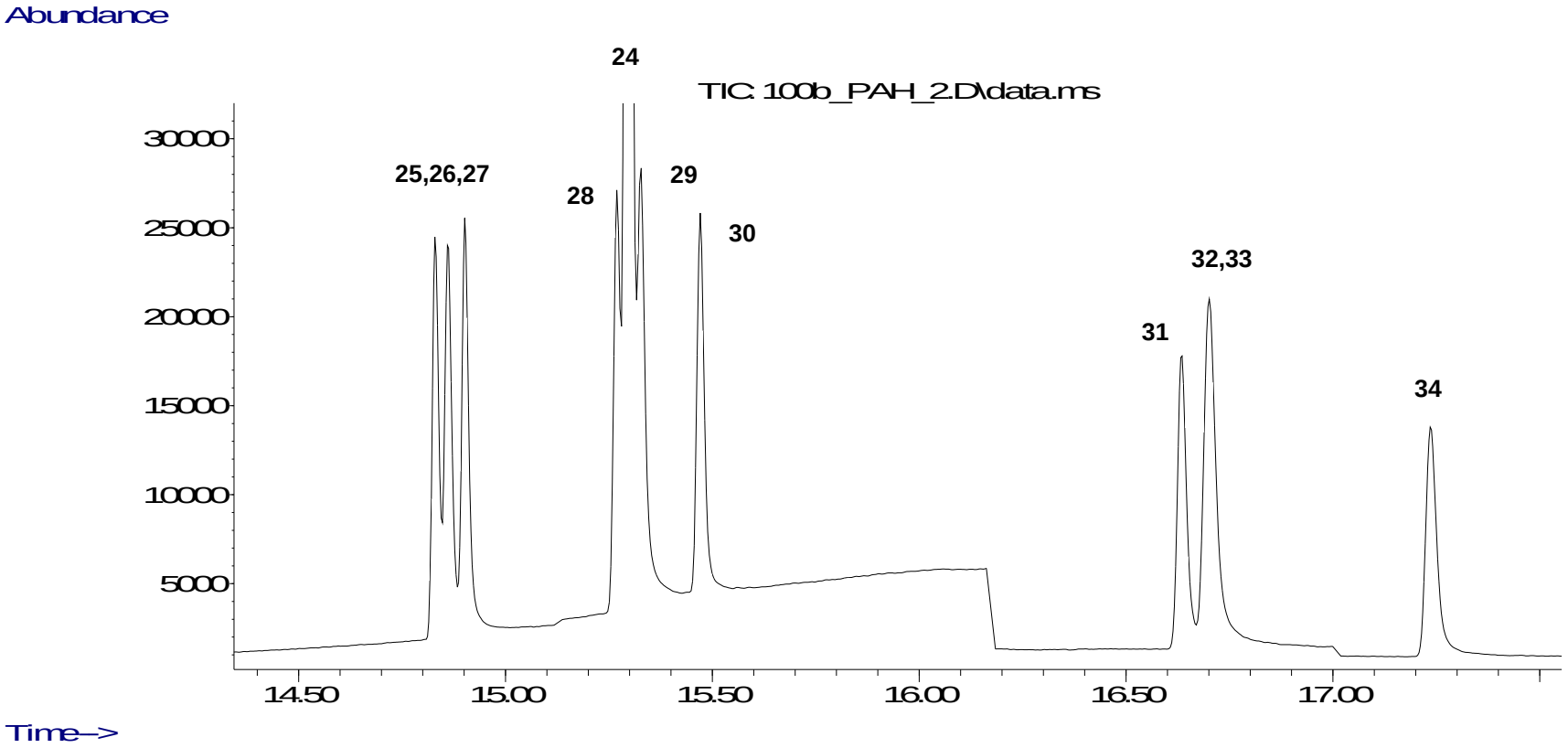
PAH Analysis: GC/MS SIM Late Eluters



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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

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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

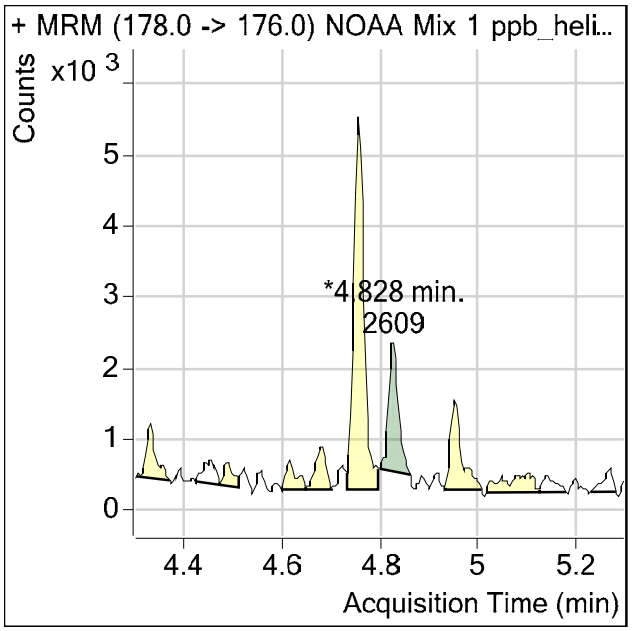
QQQ A and Q calibration stds were in isooctane solvent.

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

Data from Ralph Hindle, Vogon Labs, 7000A

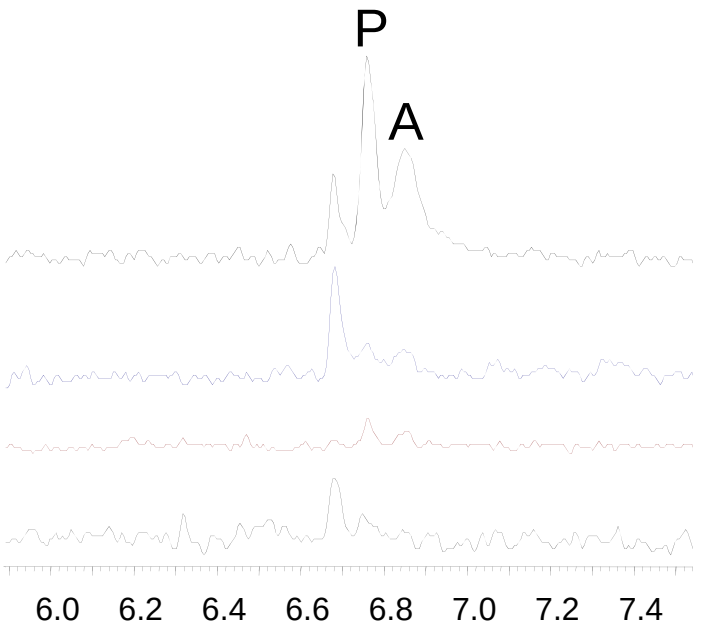
Phenanthrene and Anthracene 1.0 ppb Standard

7000A QQQ in QuEChERS fish extract



Vogon Labs

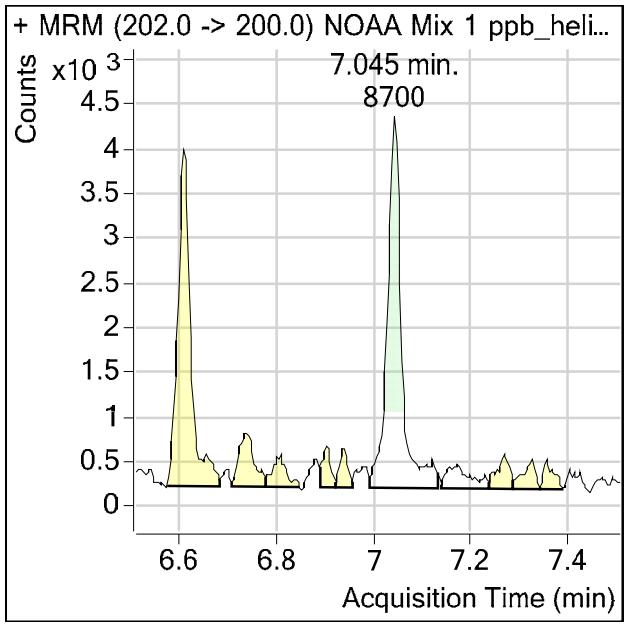
5975 Q in Isooctane



Agilent LFS

Pyrene 1.0 ppb Standard

7000A QQQ in QuEChERS fish extract



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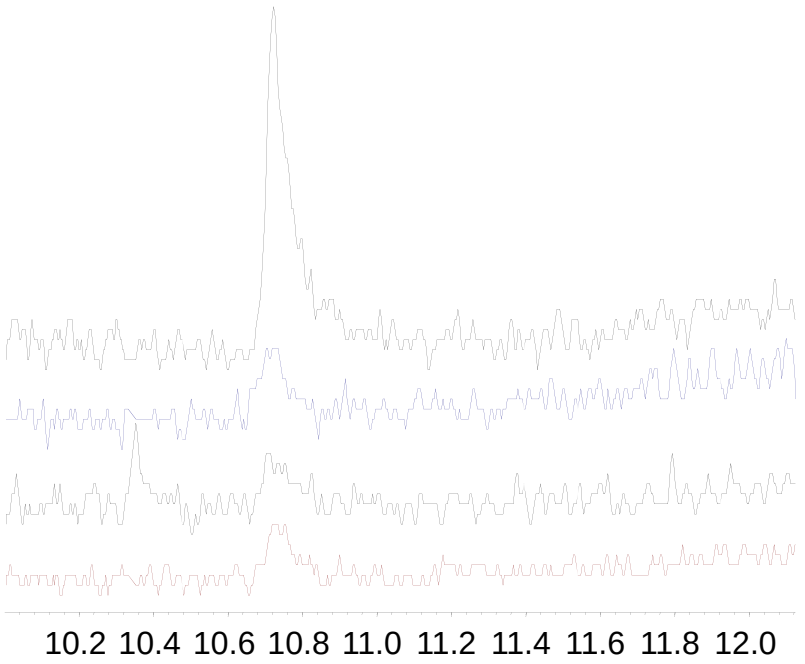


Agilent LFS

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

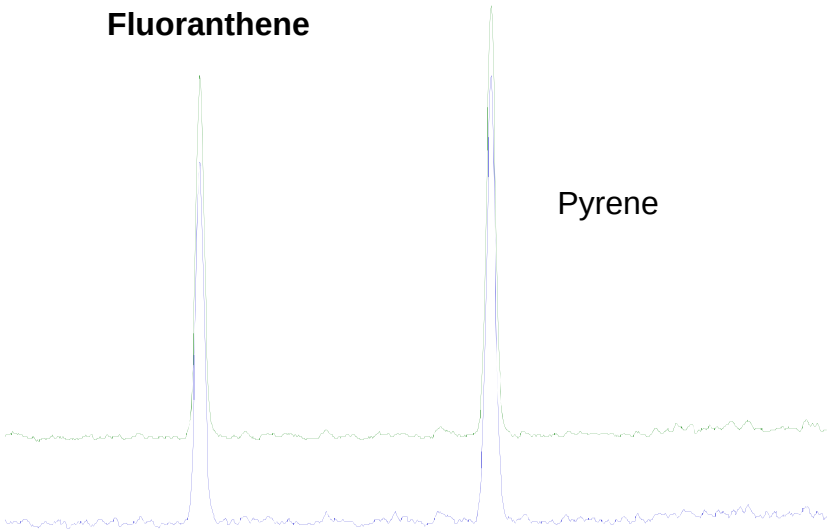
Signal to Noise (pk-pk) for NOAA PAHs (5/29/2010 list)
GC-QQQ and GC-Q
1 ppb Standard and 125 ppb Spike in mussels

	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

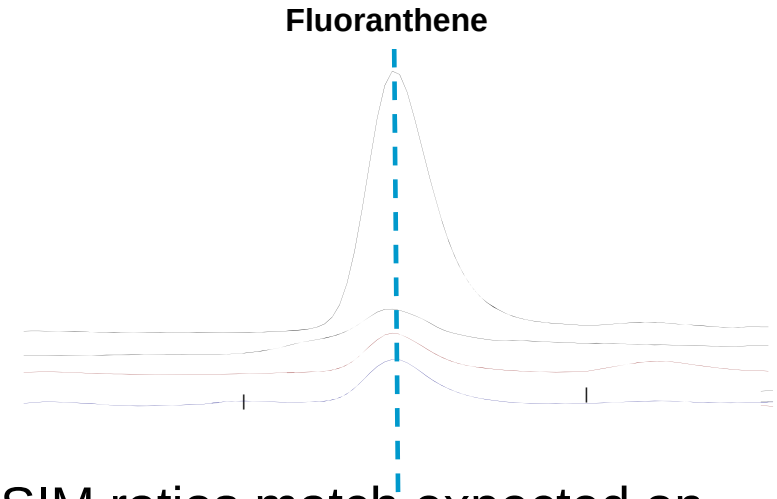
Sensitivity for standards is similar in the 2 systems but better in the QQQ when matrix is present. Spiked mussel tissue extracted with QuEChERS + dispersive SPE.

What if my QuEChERS extract does not have enough sensitivity ? Fluoranthene at ~ 15 pg is Okay.

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



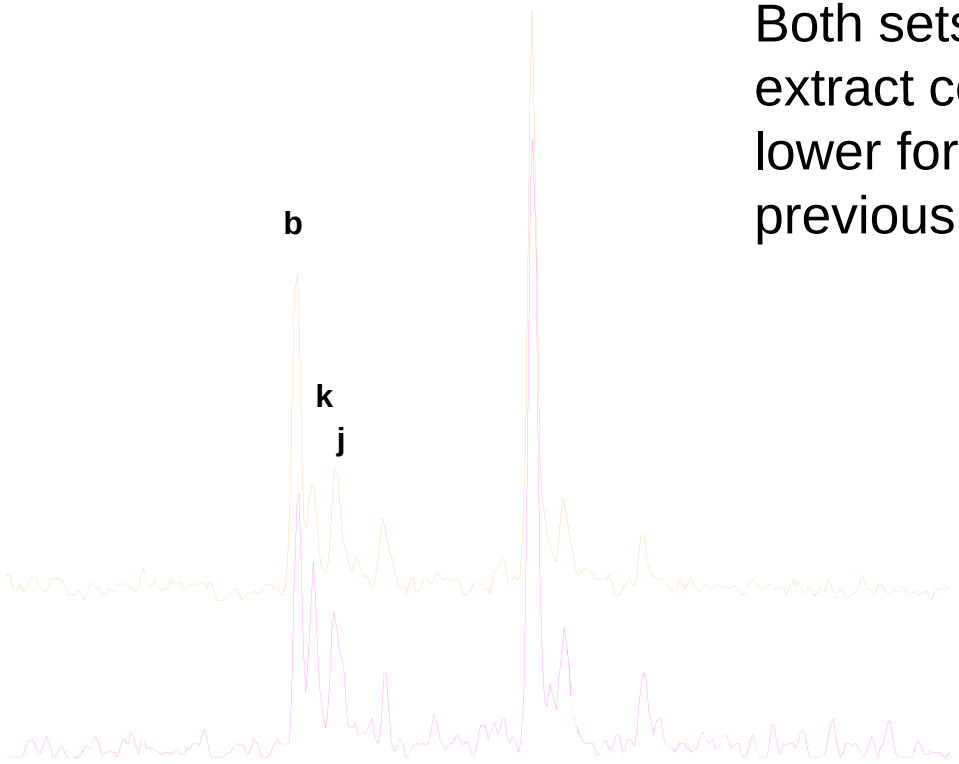
MRM ratios match expected on QQQ



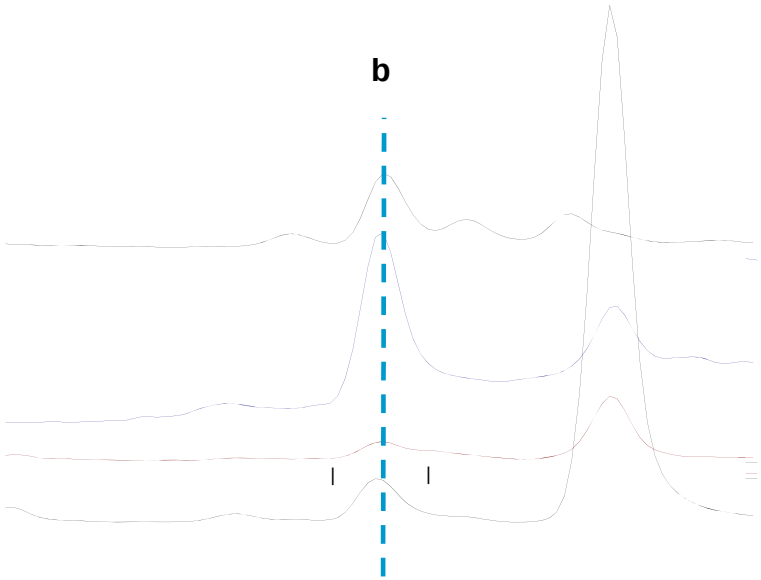
SIM ratios match expected on GC-Q. RTs align.

These also shows how a 10 uL solvent vent injection, of a non-concentrated extract, might appear using an MMI.

What if my QuEChERS extract does not have enough sensitivity ? 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.



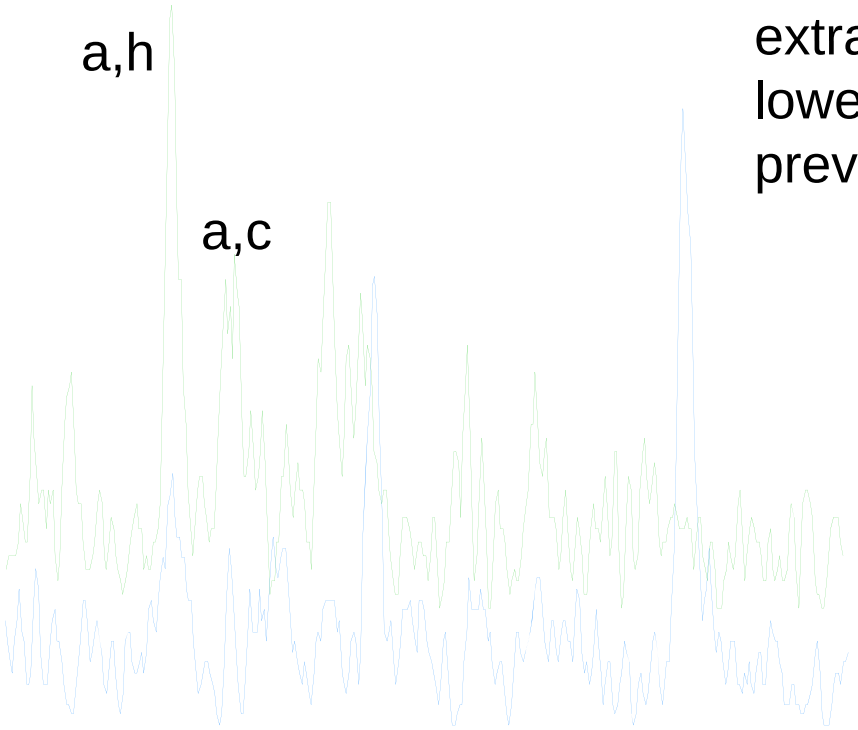
MRM ratios match expected on QQQ

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

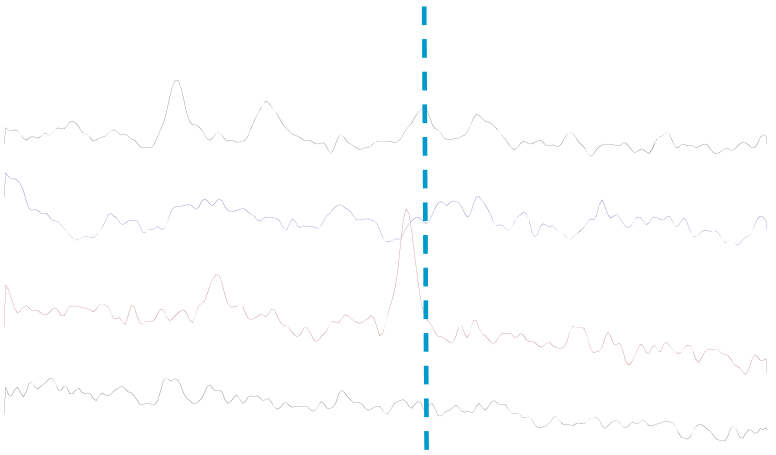
These also shows how a 10 uL solvent vent injection, of a non-concentrated extract, might appear using an MMI.

What if my QuEChERS extract does not have enough sensitivity ? 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.



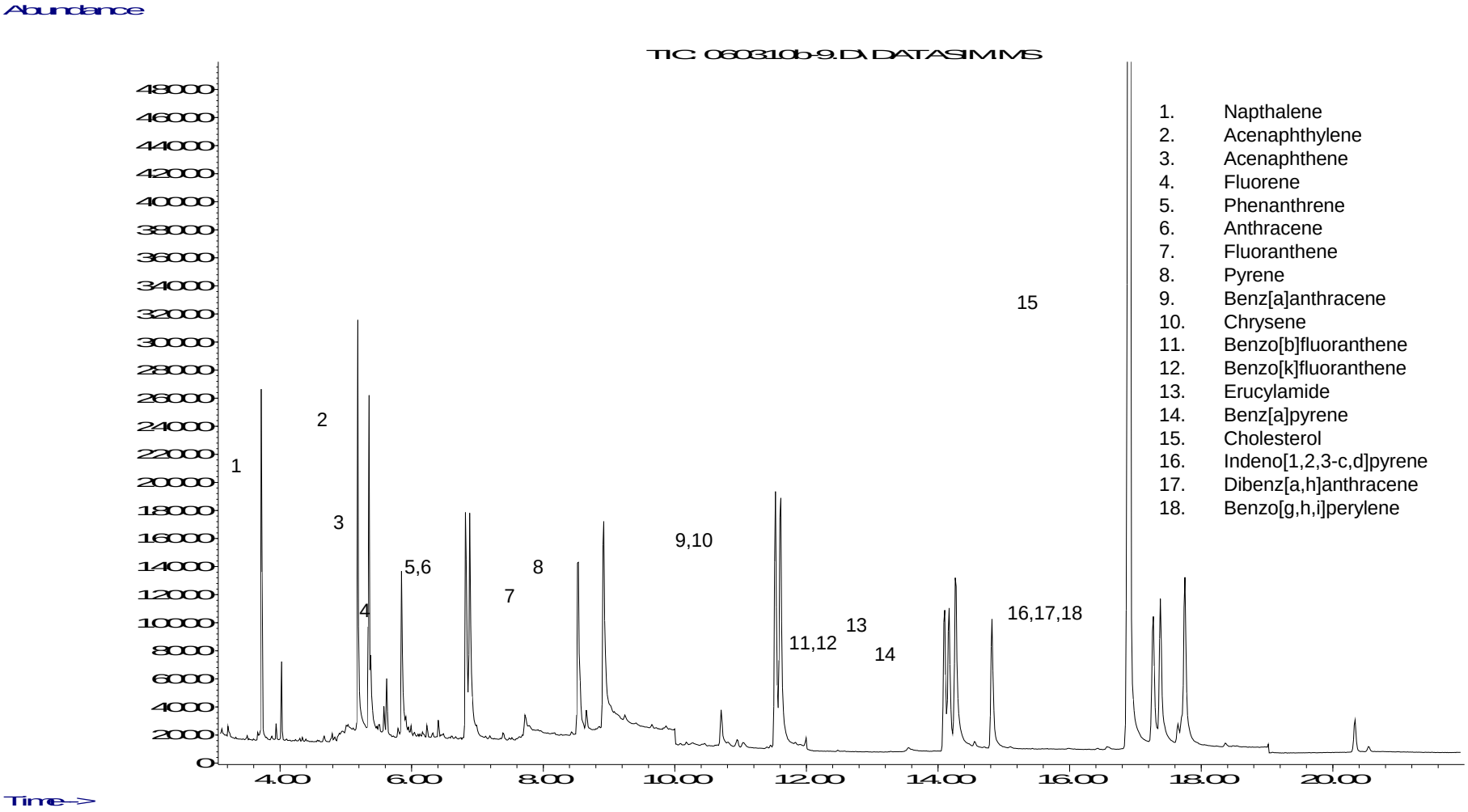
MRM ratios do not match expected on QQQ, but s/n is better than Q



SIM data useful if you squint.

These also shows how a 10 uL solvent vent injection, of a non-concentrated extract, might appear using an MMI.

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



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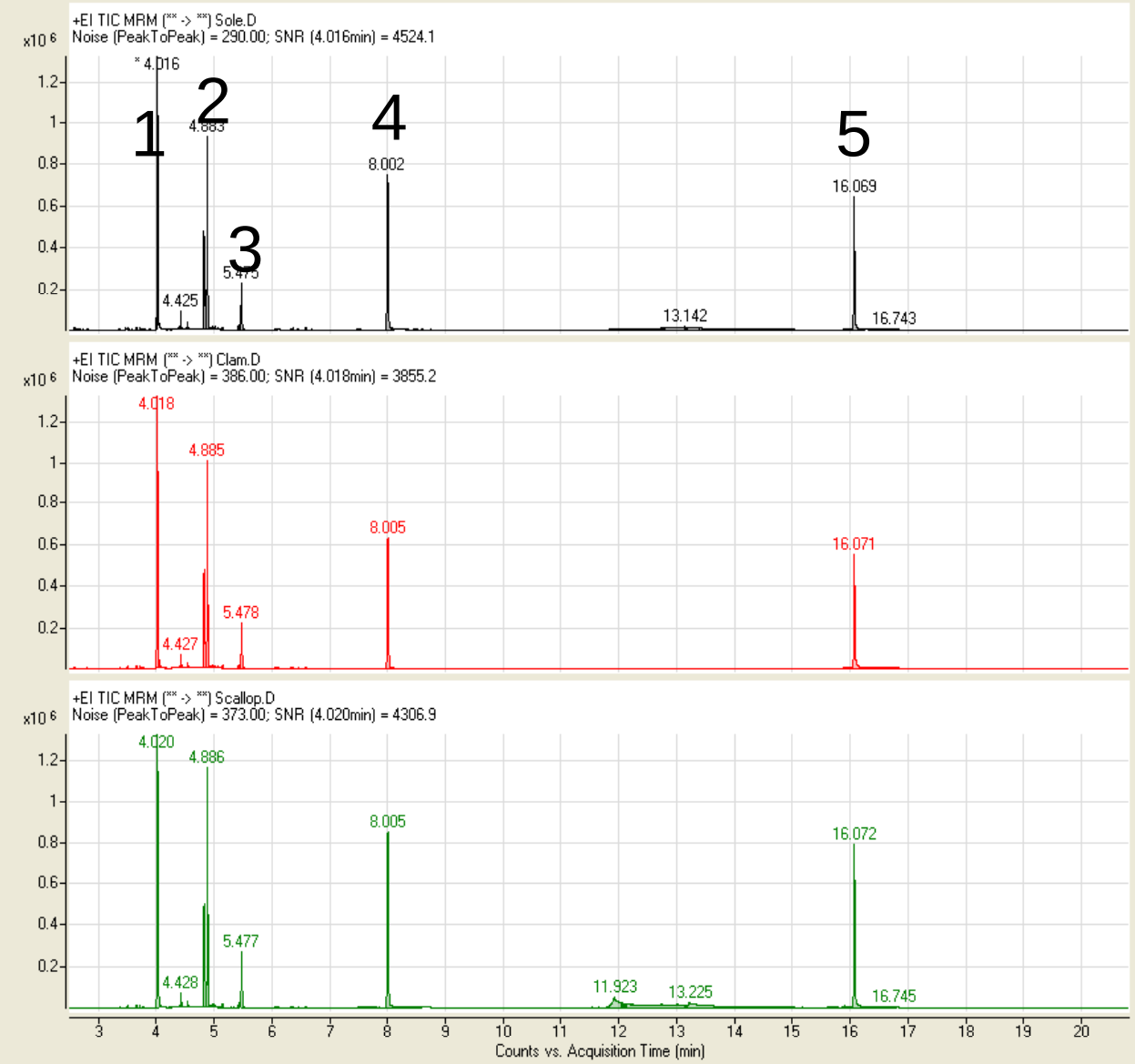
Sole, Clam & Scallop Samples – Spiked with ISTDs at 67 ppb and Extracted using Agilent QuEChERS



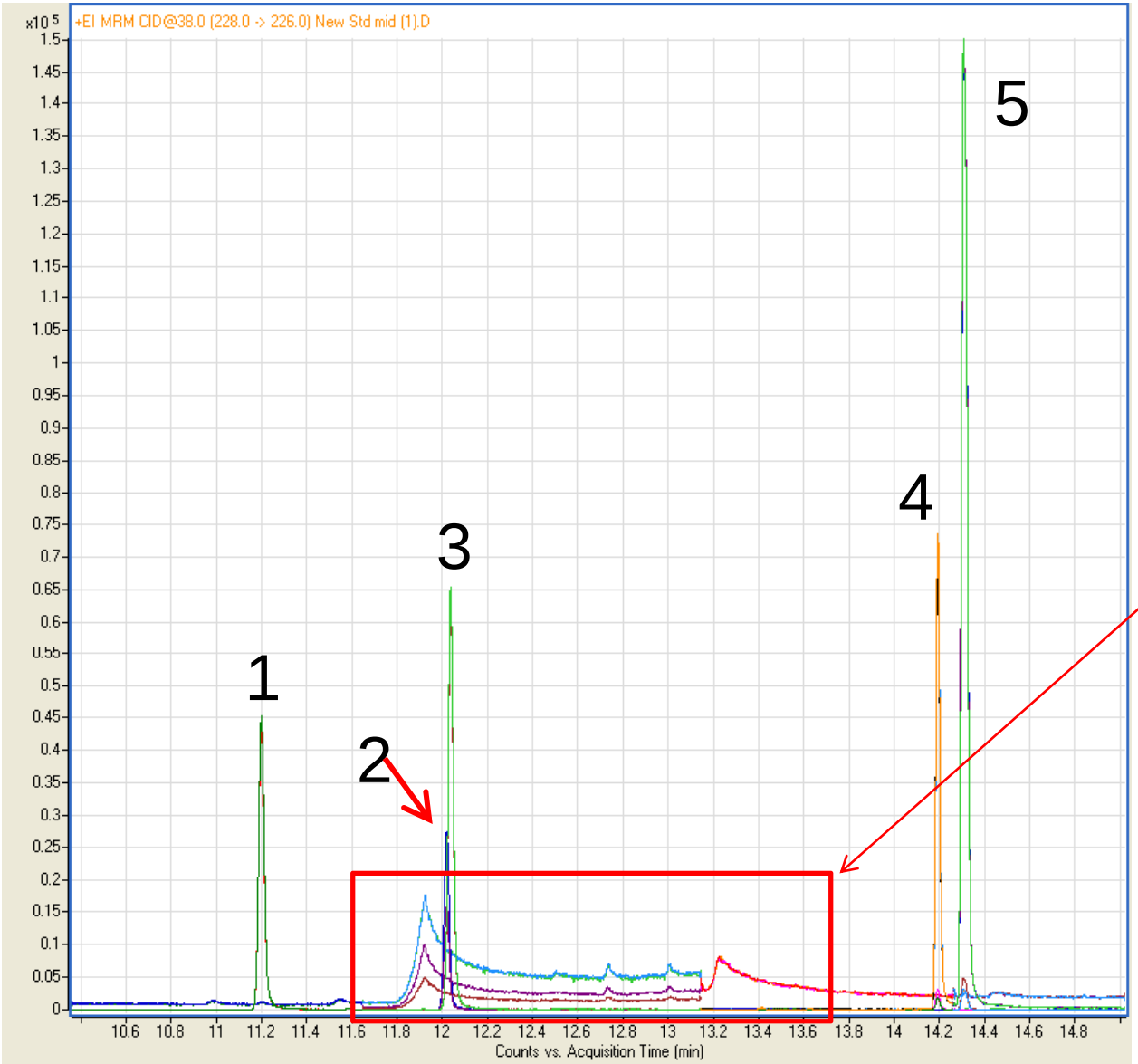
Internal Standards

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-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

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