Large Volume Injection

Presented By
Tim Anderson
GC Product Manager
Phenomenex

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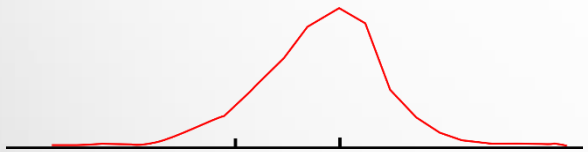
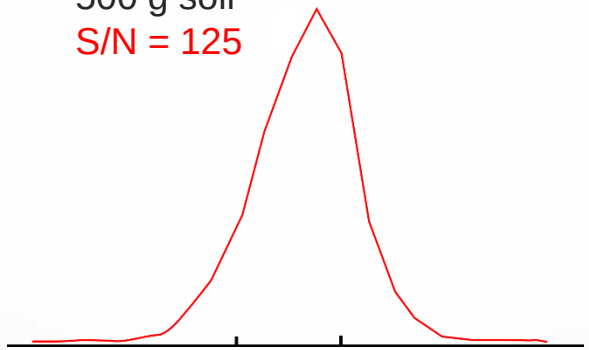
Goal

Develop a method to use the least amount of sample and achieve the highest possible s/n response

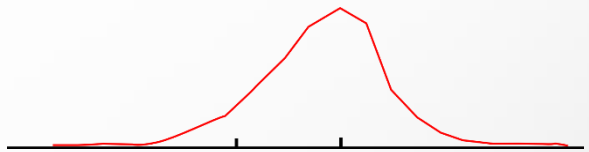
Typical Analysis
1.0 µL Splitless
500 g soil
S/N = 25



Larger Injection
5.0 µL Splitless
500 g soil
S/N = 125



Larger Injection / Smaller Sample
5.0 µL Splitless
100 g soil
S/N = 25



What to Watch Out For!

1.0 µL Dichloromethane @ 250 °C & 14 psi = 336 µL

Liner Volume ≈ 990 µL

3.0 µL = 1008 µL; exceeds liner volume!

- Flashback
- Ghost peaks
- Loss in sensitivity / linearity

Preliminary

Assumptions

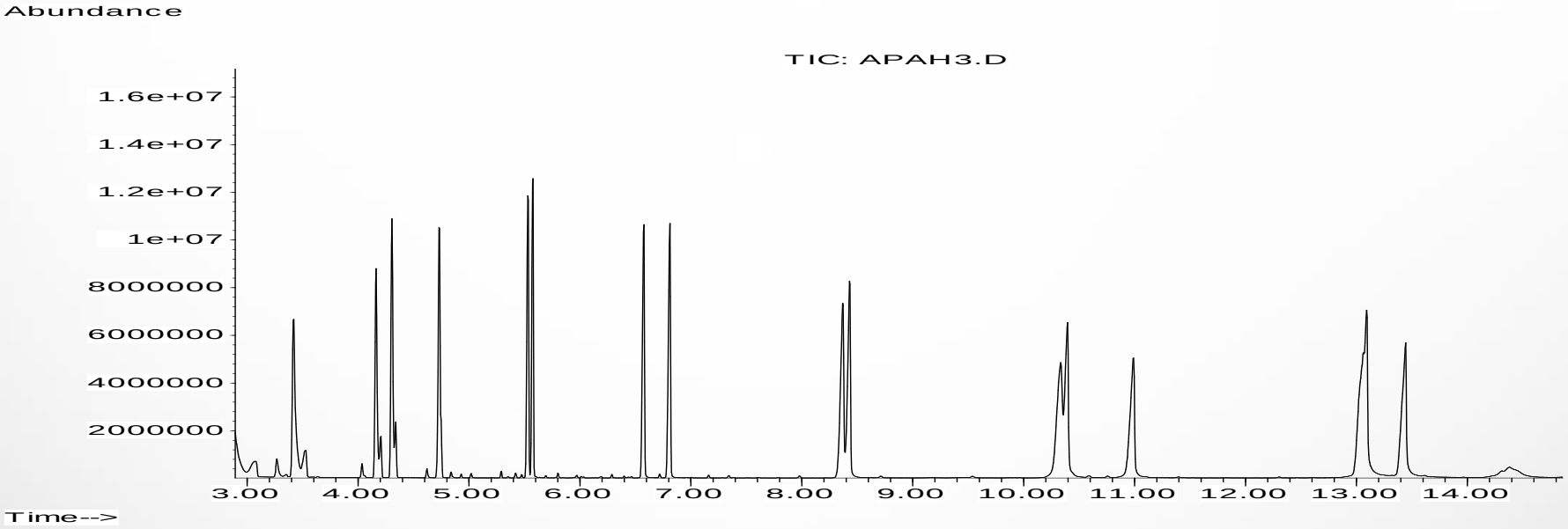
- Pressure Pulsed injection tend to improve analyte responses
- Improves transfer of analytes from the inlet into the column
- Started with Pressure Pulse and tested this assumption during throughout the experiment

Analytes:

- Started with a relatively simple list of Polycyclic Aromatic Hydrocarbons (PAHs)
- Contains relatively wide range of retention times
- Subsequent work includes other compounds

Initial Conditions

Column:	**Zebron ZB-SemiVolatiles 30 m x 0.25 mm x 0.25 µm**
Column Flow:	**1.4 mL/min Helium (constant flow)**
Oven Program:	**100 °C for 0.5 to 260 °C @ 30 °C/min to 295 °C @ 6 °C/min to 325 °C @ 25 °C/min for 2 min**
Inlet Liner:	**Direct Connect Liner with top taper and bottom hole**
Injection:	**Pressure Pulse @ 30 psi for 0.66 min, splitless for 0.60 min**
Detector:	**MSD; 46-450 amu, transfer line = 320 °C**



Liner Experiments

What is the best liner?

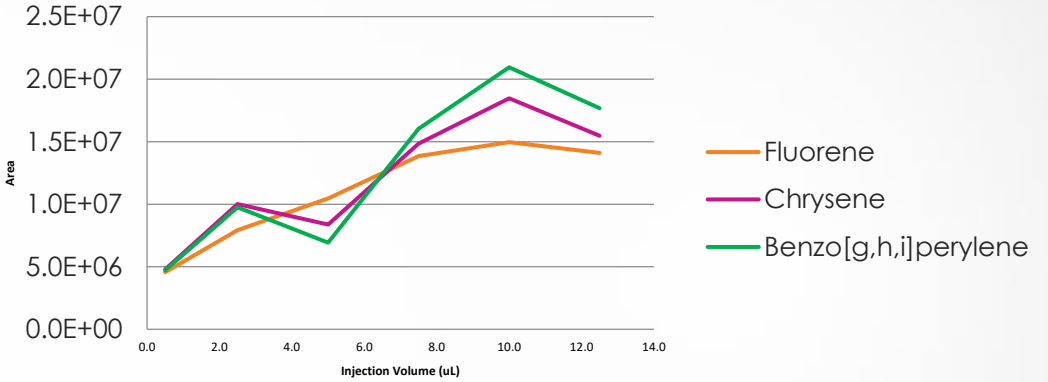
Different styles

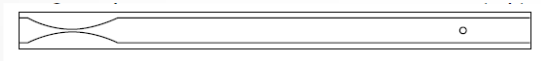
Compared early, mid, and late eluters

Liner Performance

DirectConnect Dual Taper, bottom hole

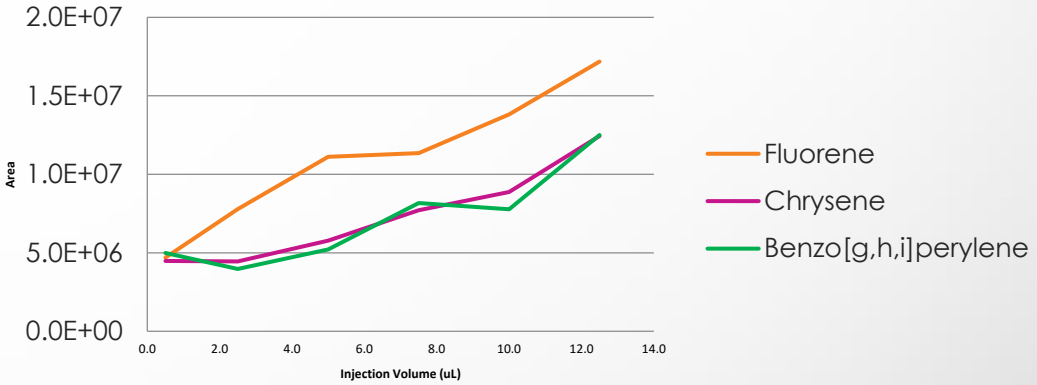
- **Maximum Injection volume = 10 µL**
- **Maximum intensity = 1.67e7**
- **Better response for higher MW**
- **Response decreases after 10 µL!**





DirectConnect Top Hole

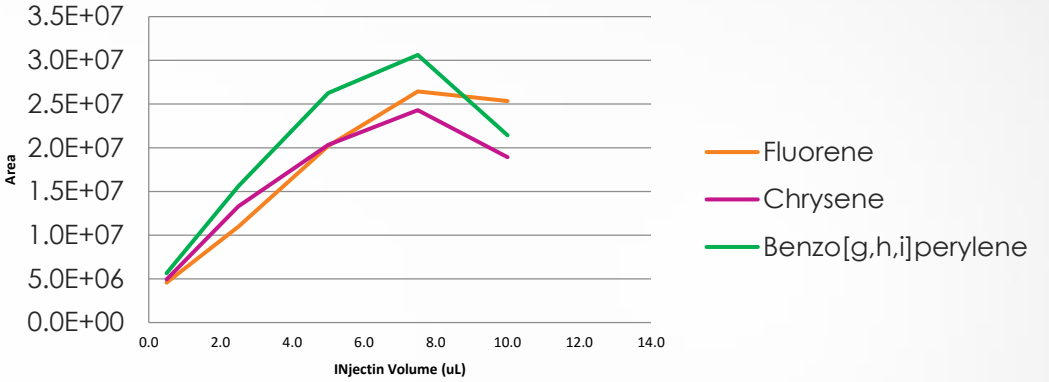
- **Maximum Injection volume = 12.5 µL+**
- **intensity = 1.40e7**
- **Better response for lower MW**

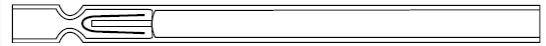


Liner Performance

Single Taper With Wool

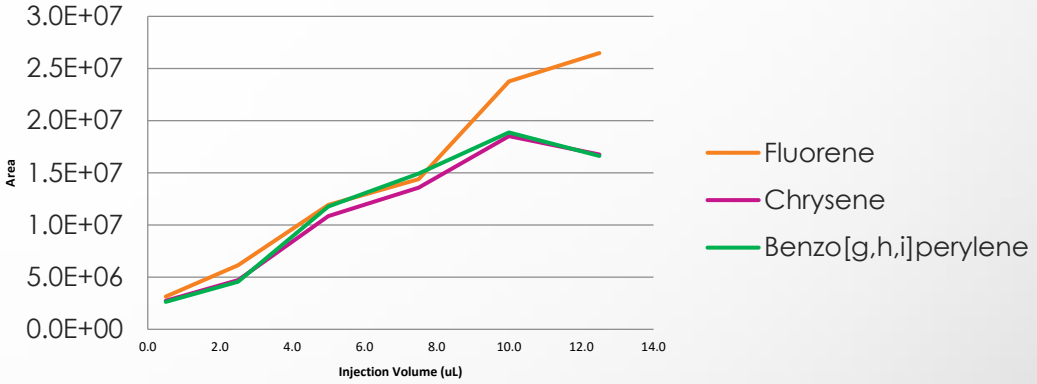
- **Maximum Injection volume = 10 µL**
- **Maximum intensity = 2.07e7**
- **Consistent response, except at larger volumes which benefit lower MW**
- **Response decreases after 10 µL!**





Cup Liner

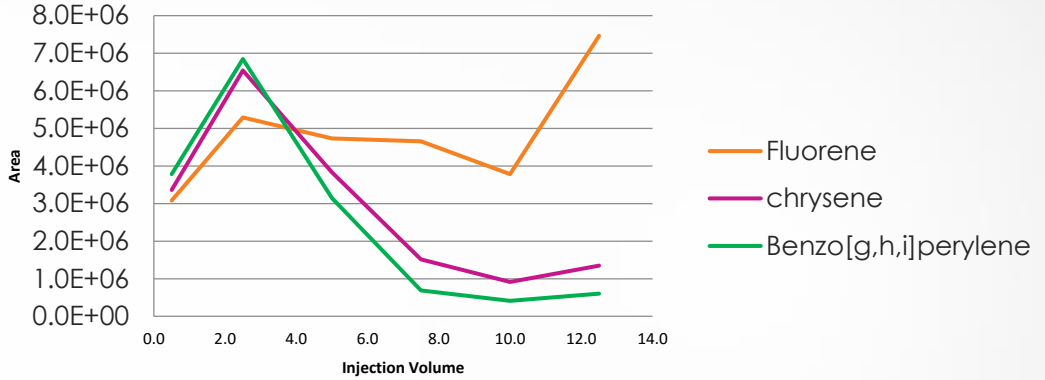
- **Maximum Injection volume = 7.5 µL**
- **Maximum intensity = 2.71e7**
- **Slightly better response for higher MW**
- **Response decreases after 7.5 µL**



Liner Performance

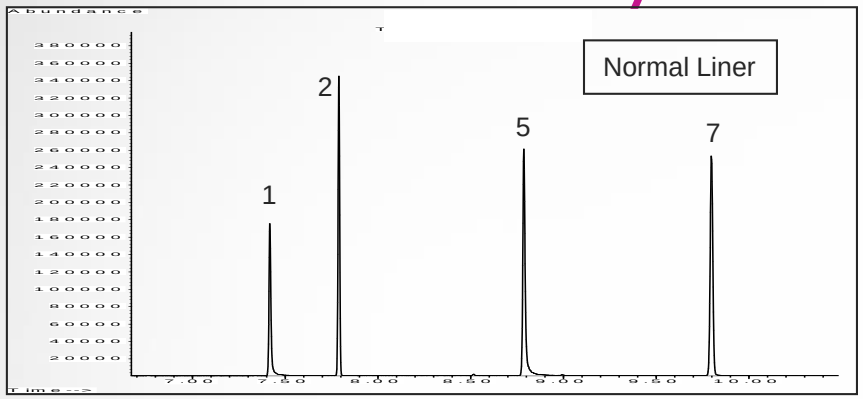
FocusLiner (single taper with wool in middle)

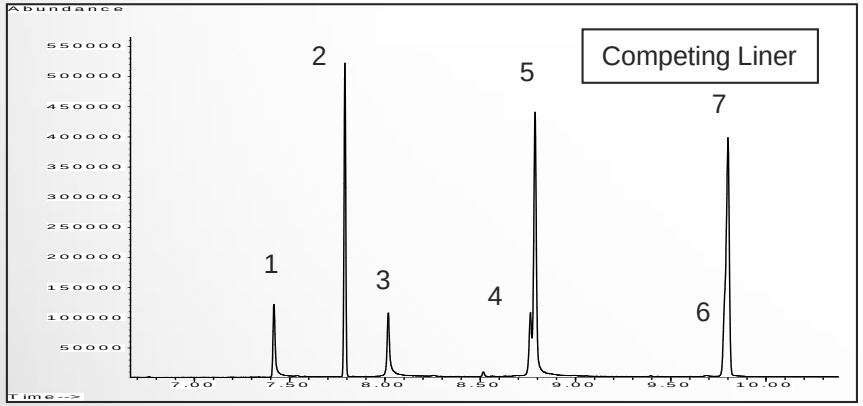
- **Maximum Injection volume = 2.5 µL**
- **Maximum intensity = 6.23e6**
- **Much better response for lower MW**
- **Possible cooling of glass wool**



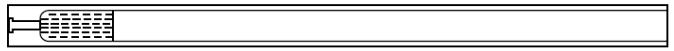
Average Liner Performance

Liner Activity





Splitless, Single Taper Liner with wool



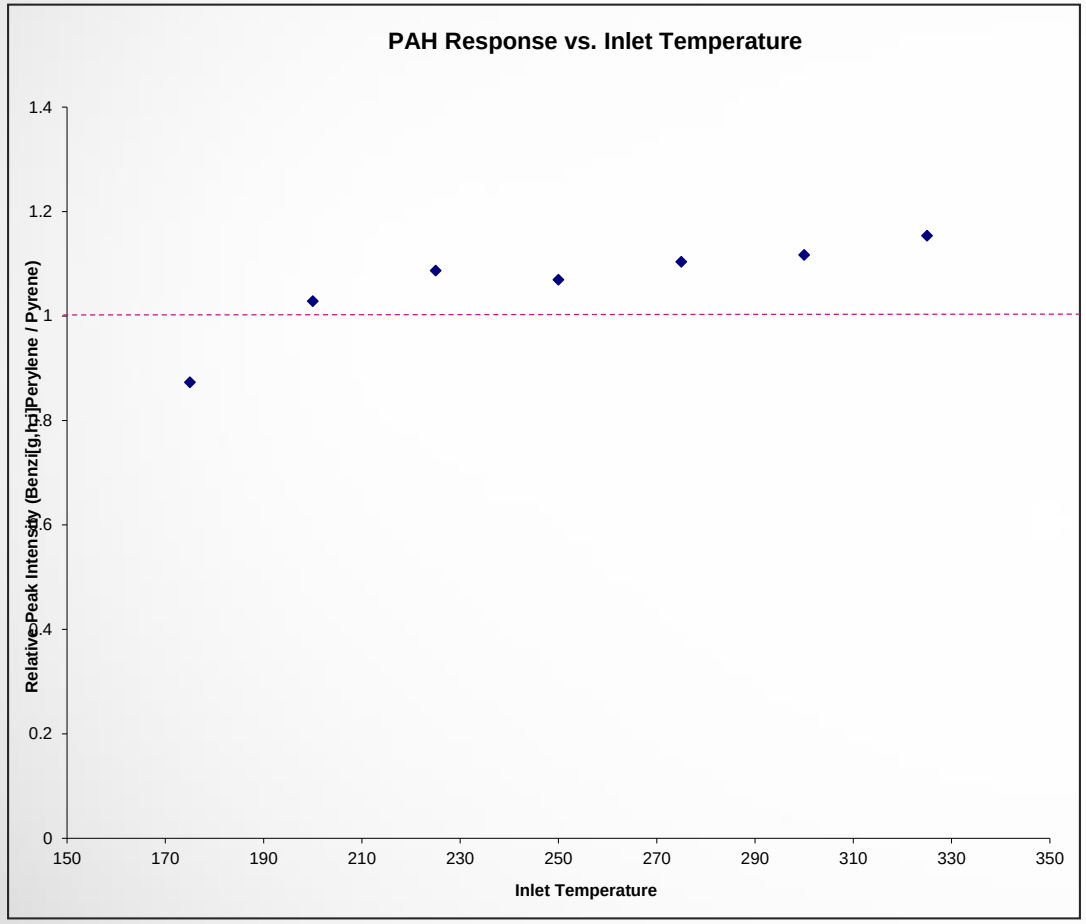
Inlet Temperature = 275 ºC

Analytes

1. Pentachlorophenol
2. DFTPP
3. Hexadecanoic acid
4. Octadecanoic acid
5. Benzidine
6. Hexanedioic acid, bis(2-ethylhexyl)ester
7. DDT

Relative Responses	1-taper	Wool-middle	Wool-bottom	straight	Cup	Wool-bottom 2
PCP	0.44	0.70	0.60	0.43	0.80	0.32
Benzidine	0.87	1.17	1.11	0.90	1.22	1.34
DDT	1.23	1.24	1.19	0.99	1.17	1.59

Injection Temperature



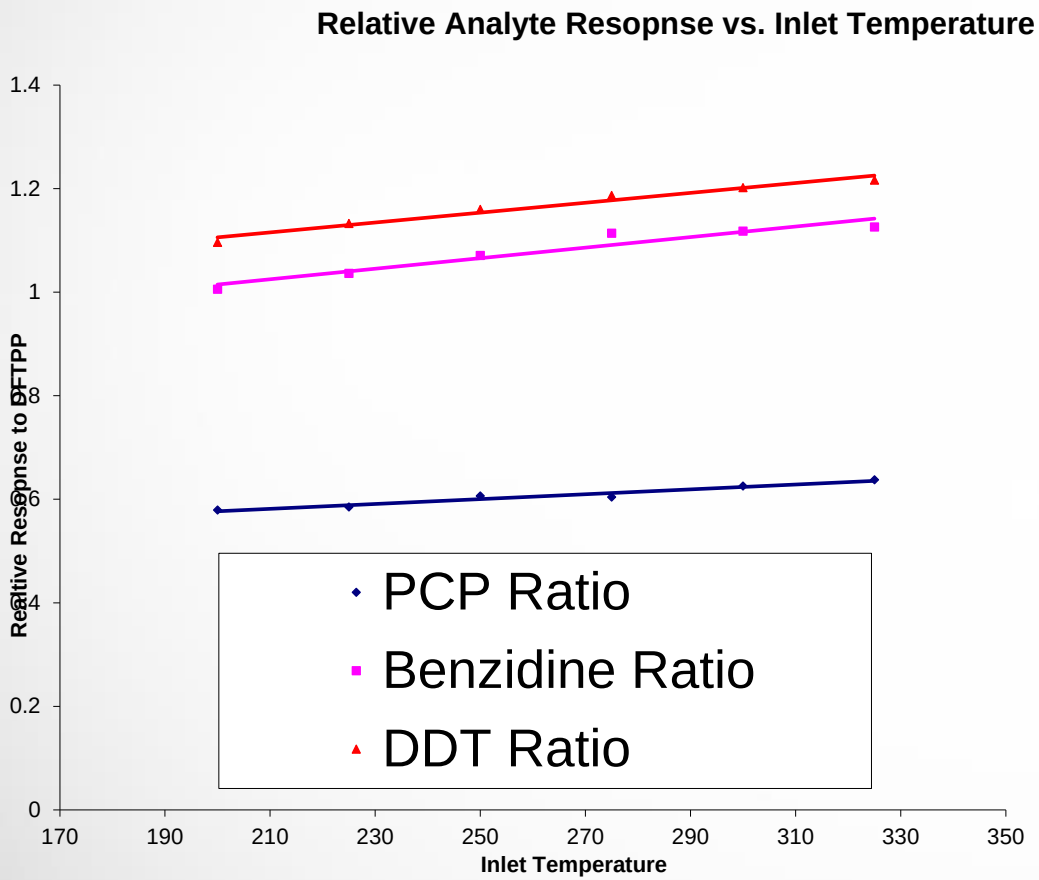
Ratio of over middle PAH

- Benzo(g,h,i)perylene / Pyrene

Inlet Discrimination:

- Reduced response of later eluting compounds due to their lower volatility at a given temperature.
- Higher Inlet Temperatures = Greater Response for Late Eluting Compounds

Active Analytes vs. Temperature



- Kinetics states that reactions occur faster at higher temperatures
- Expected less relative response at higher temperatures
- Liner was new and CLEAN!

Note: Conditions were for Pulsed Split injection parameters; inlet liner with wool at bottom

Chromatography Optimization

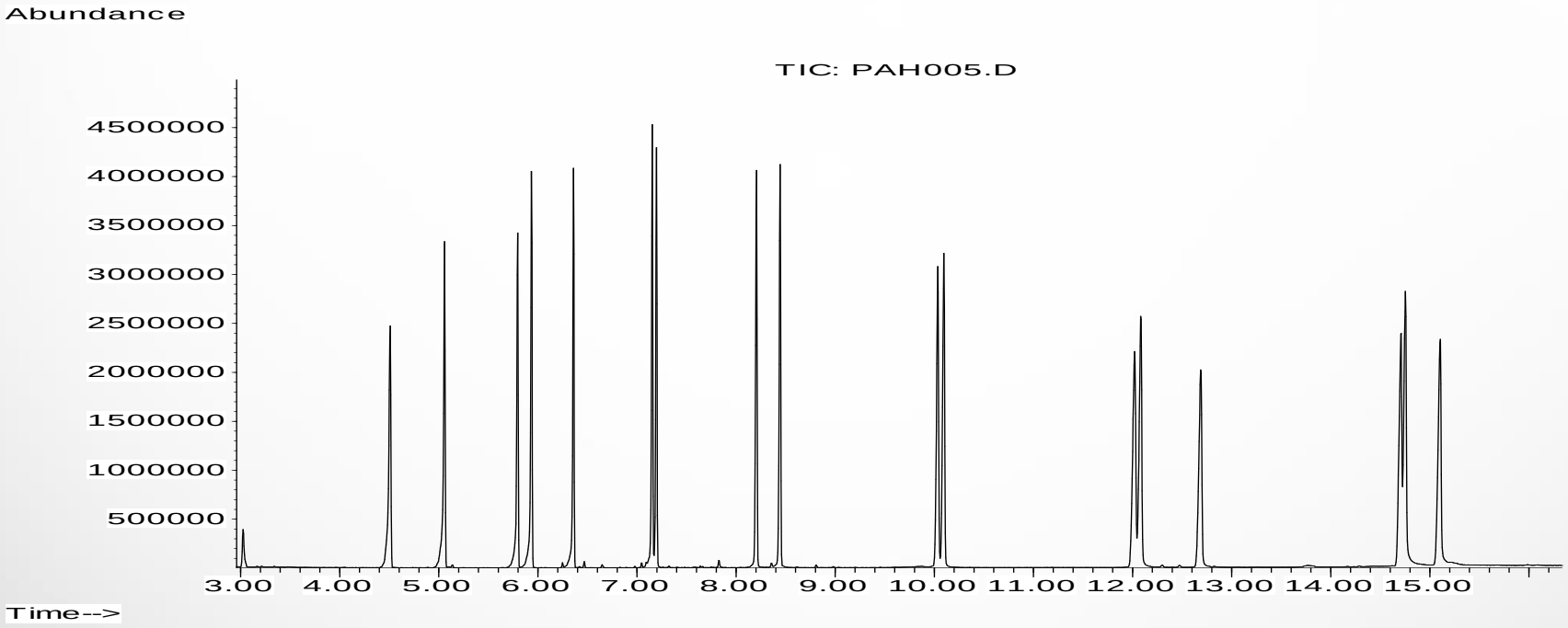
60 °C gave much better initial peak shape

Initial peak shape still not perfect

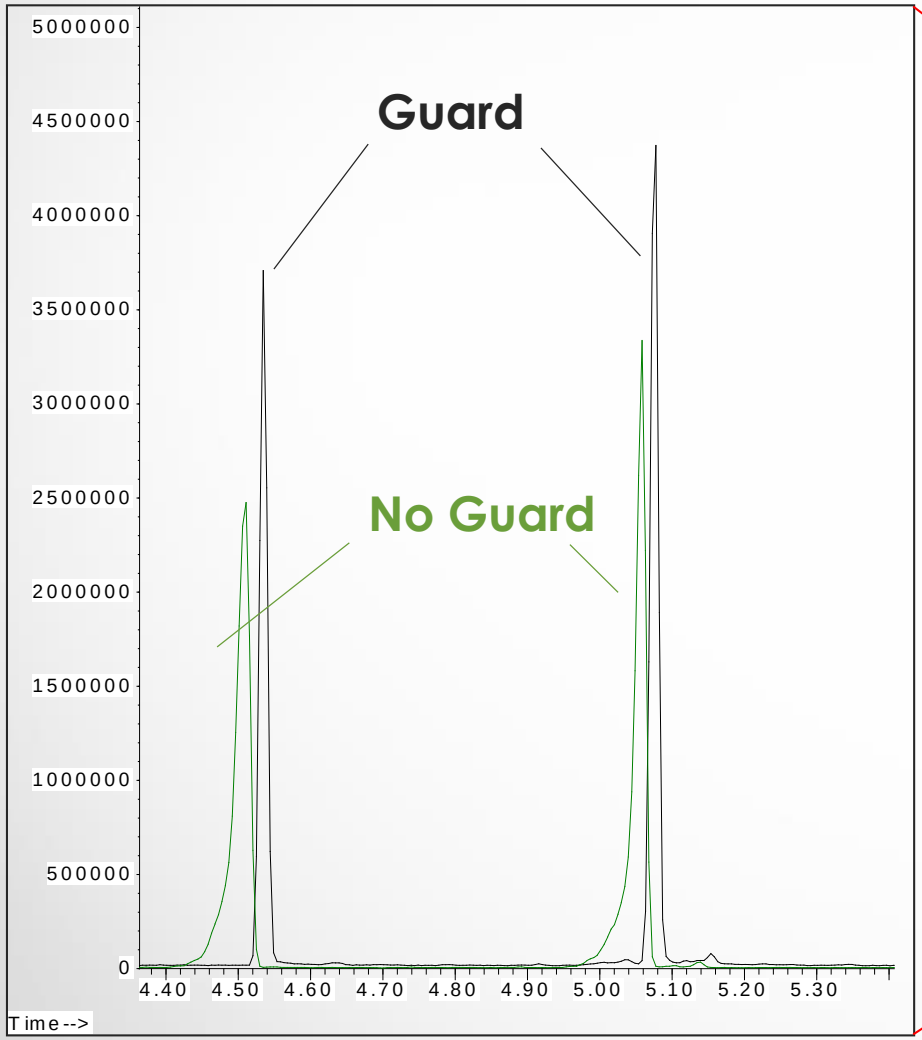
Naphthalene at 4.5 min, last PAH at 15.2 min

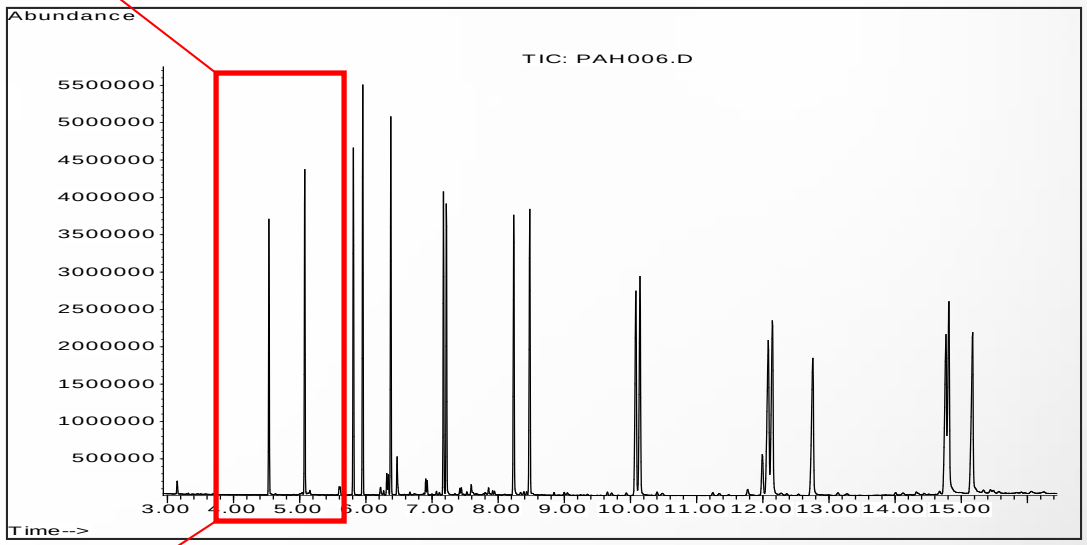
Benzo[b]fluoranthene / Benzo[k]fluoranthene = 6.45% valley height (8270D requirement = 50%)

Indeno[1,2,3-cd]pyrene / Dibenz[a,h]anthracene = 15.8%

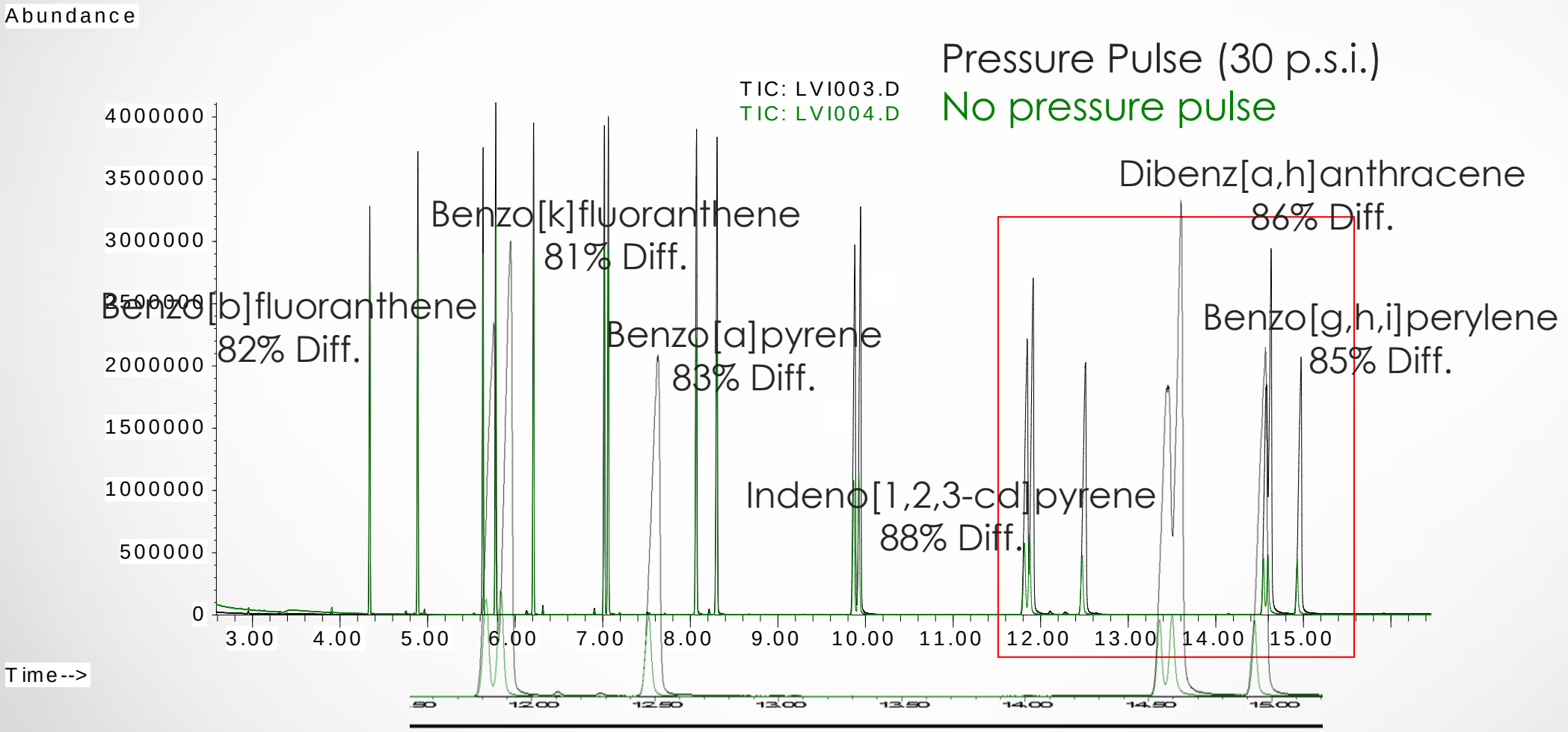


Guard Columns





Confirmation of Pressure Pulse



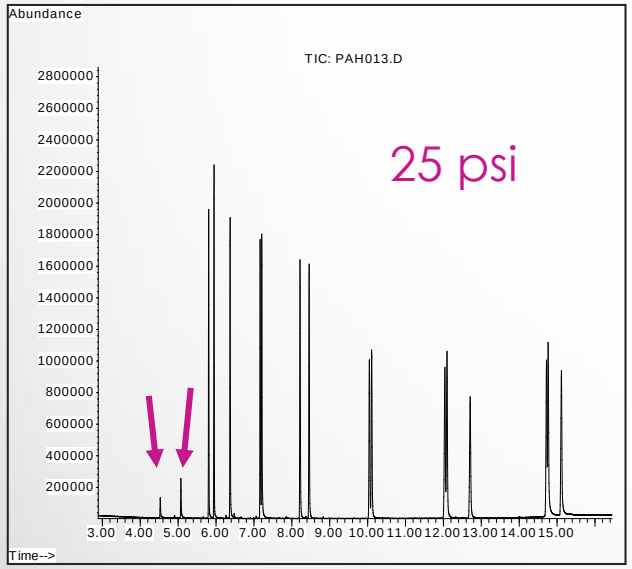
Optimization of Pressure Pulse

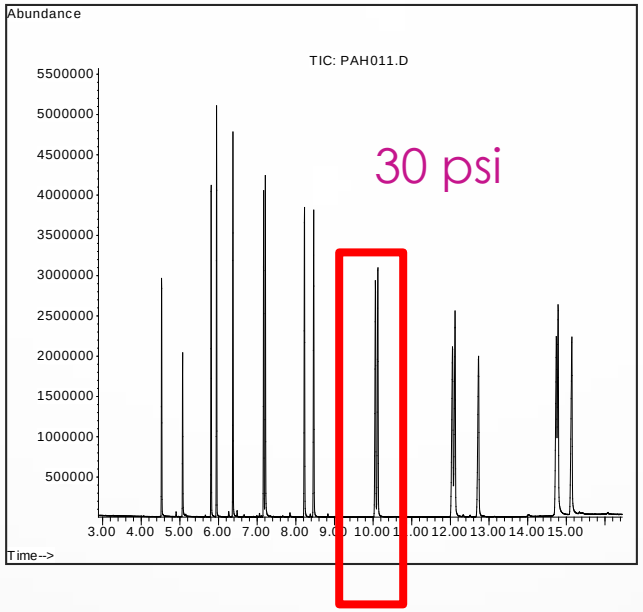
Higher pressures decreased initial responses

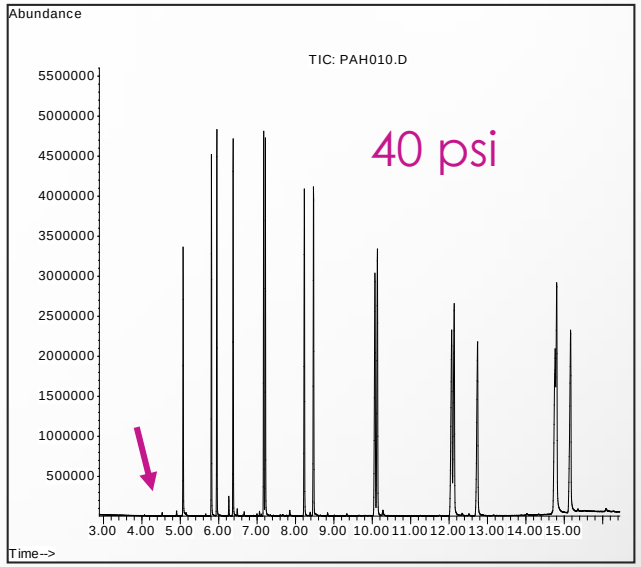
Lower pressures decreased all signals – less transfer on-column

Optimum was found to be 30 psi

Decreasing pressure time at 40 psi did NOT bring back naphthalene

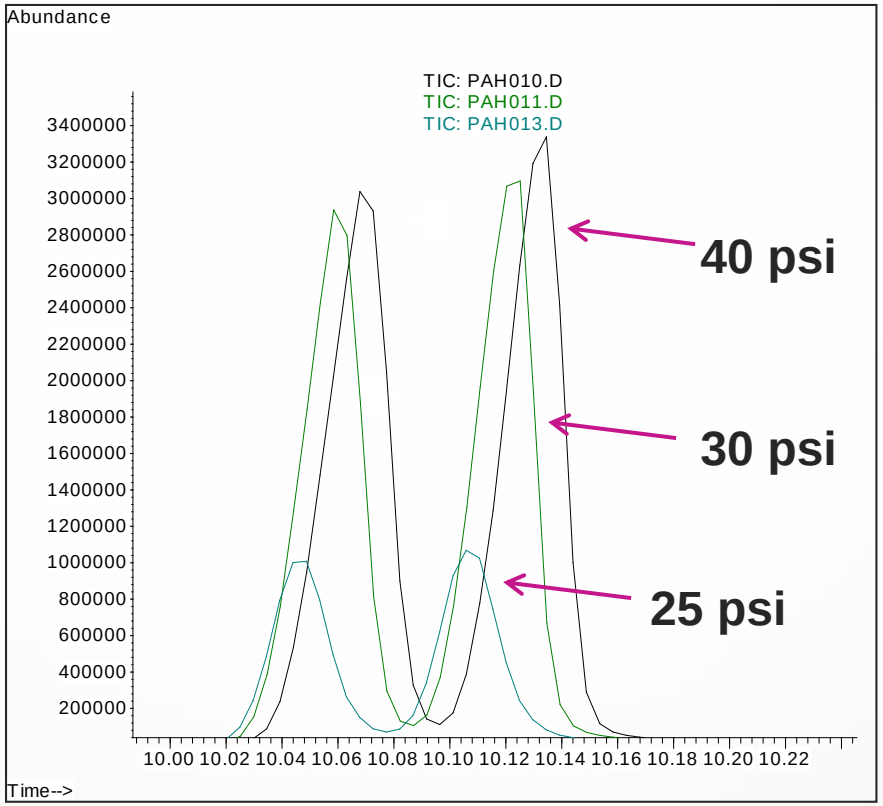






Optimization of Pressure Pulse

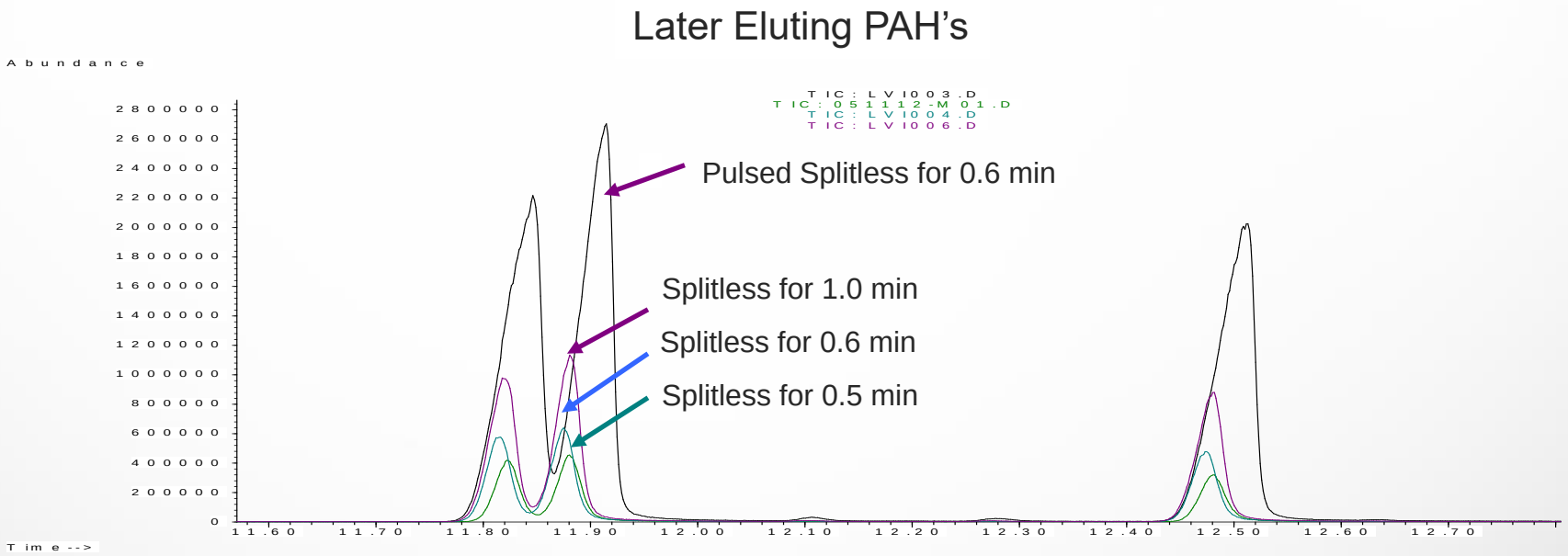
Zoom in on Benz[a]anthracene and Chyrsene



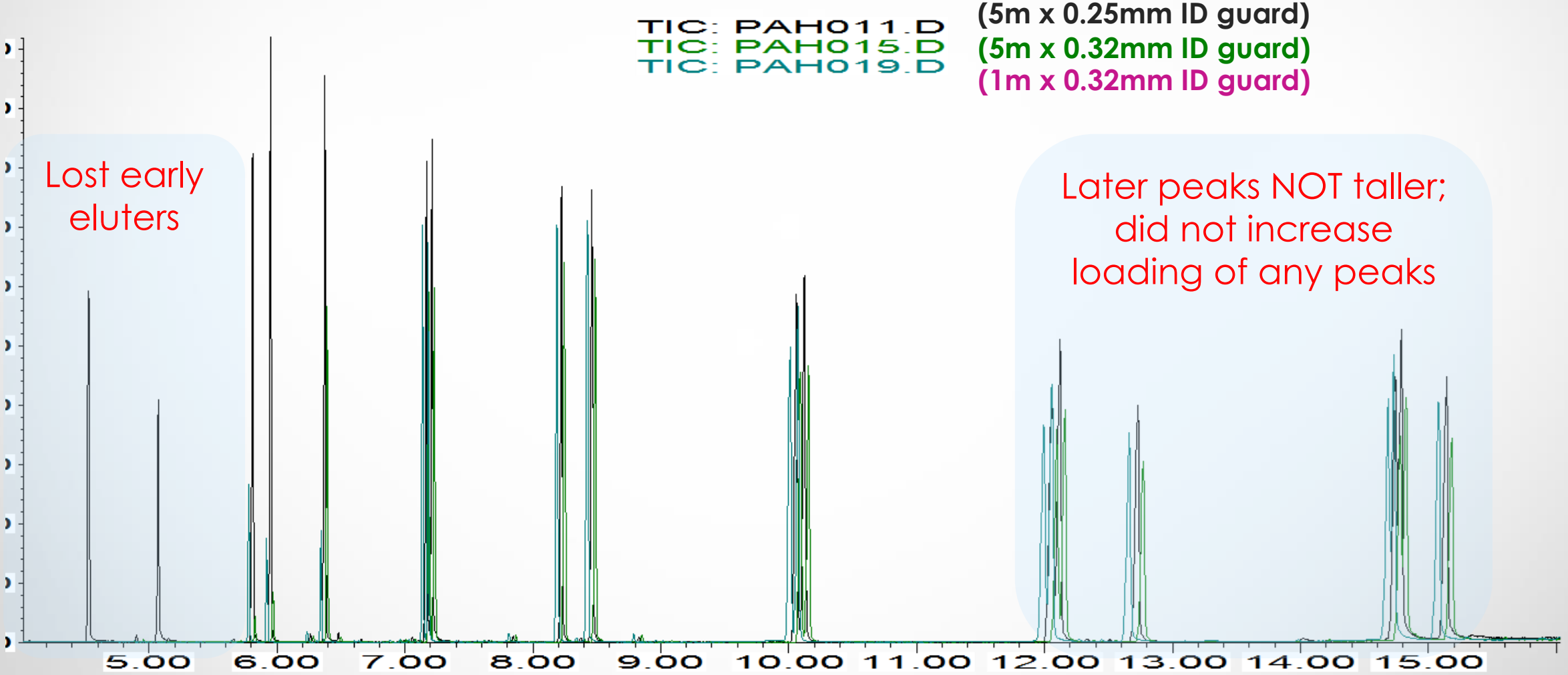
Non-Pulsed Consideration

Expected that standard splitless injection might allow for longer splitless hold times to improve response

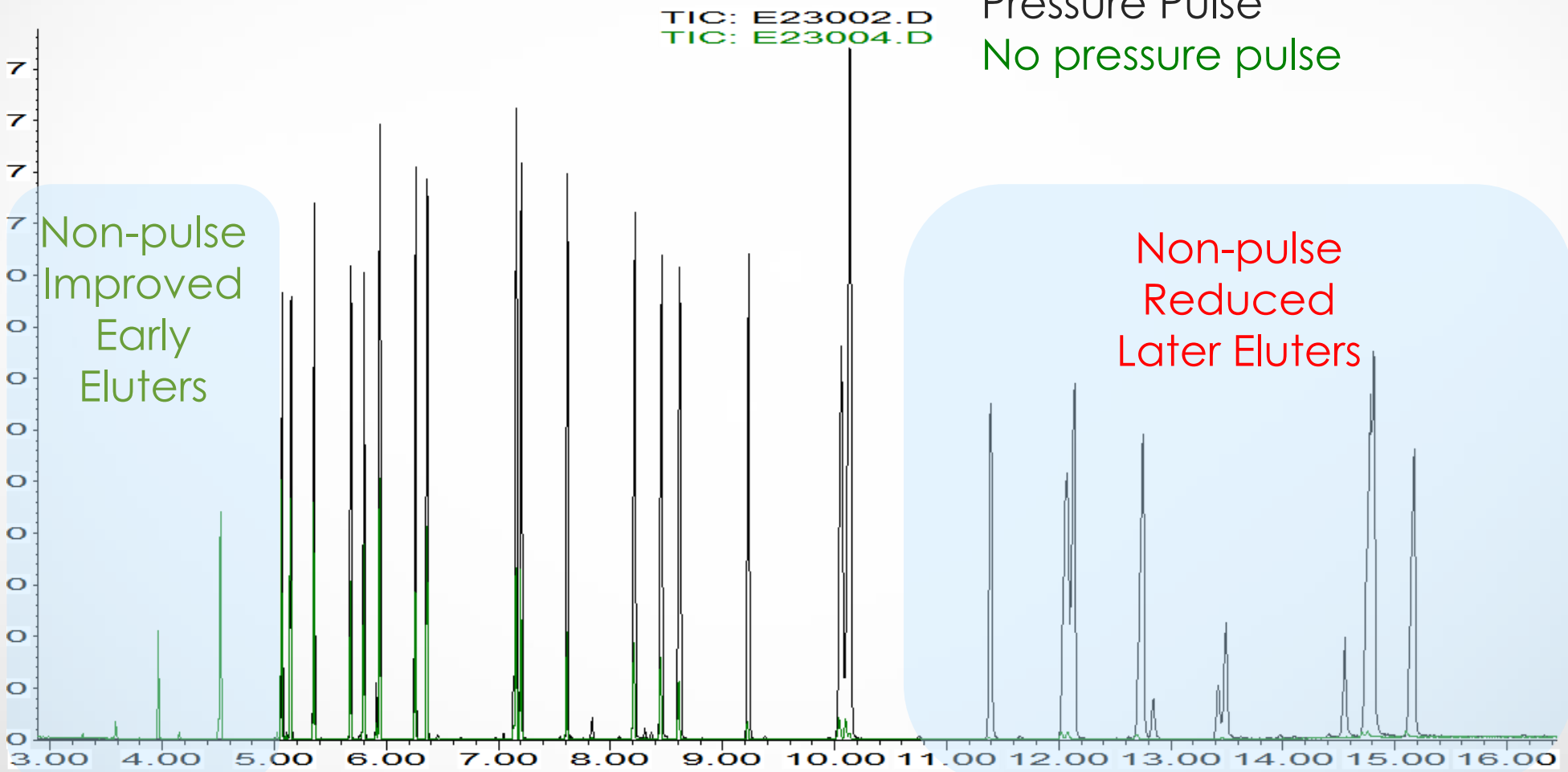
Longer splitless hold times gave better responses, but not comparable to the pulsed splitless injection



Larger ID Guard: 0.32 mm ID



Pulsed vs. Non-pulsed 0.32 mm ID Guard



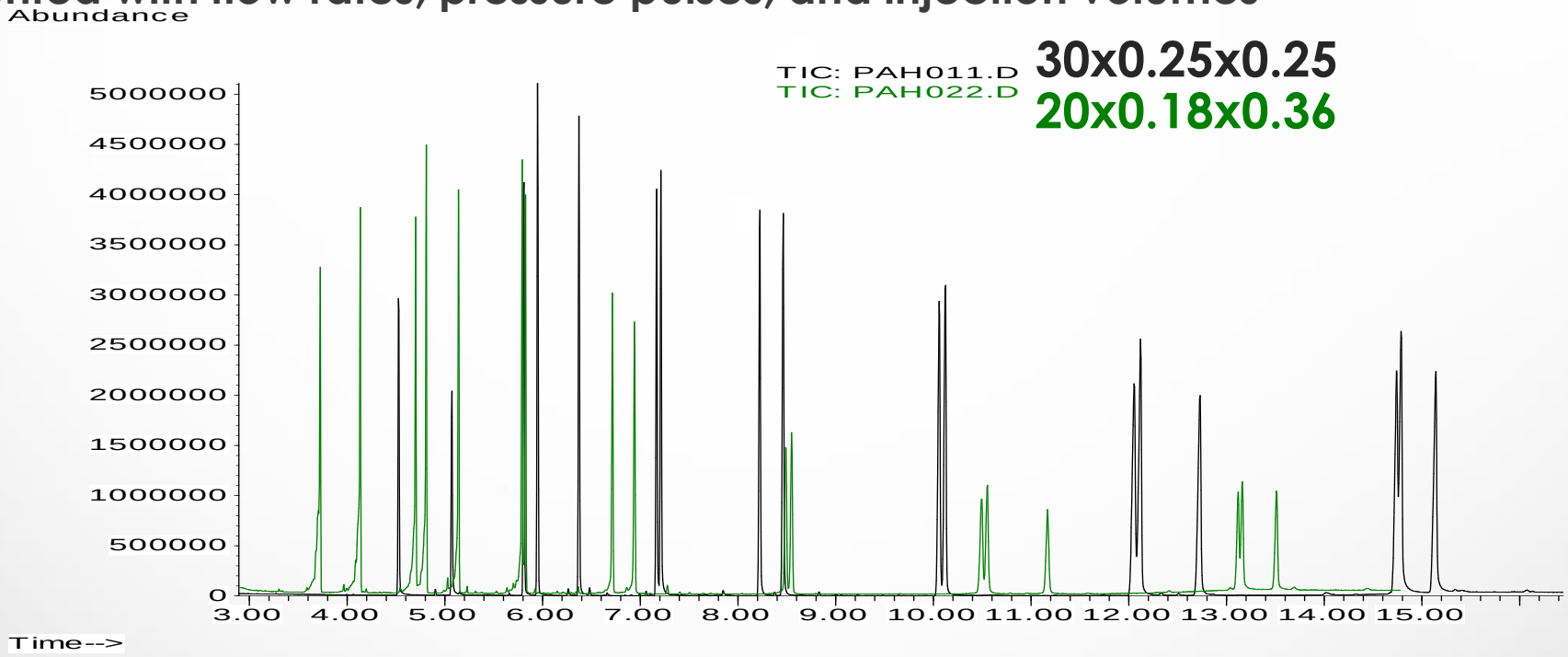
Smaller ID Column

Hoped that improved efficiency would give higher response

Retention times were earlier with last PAH before 14 minutes

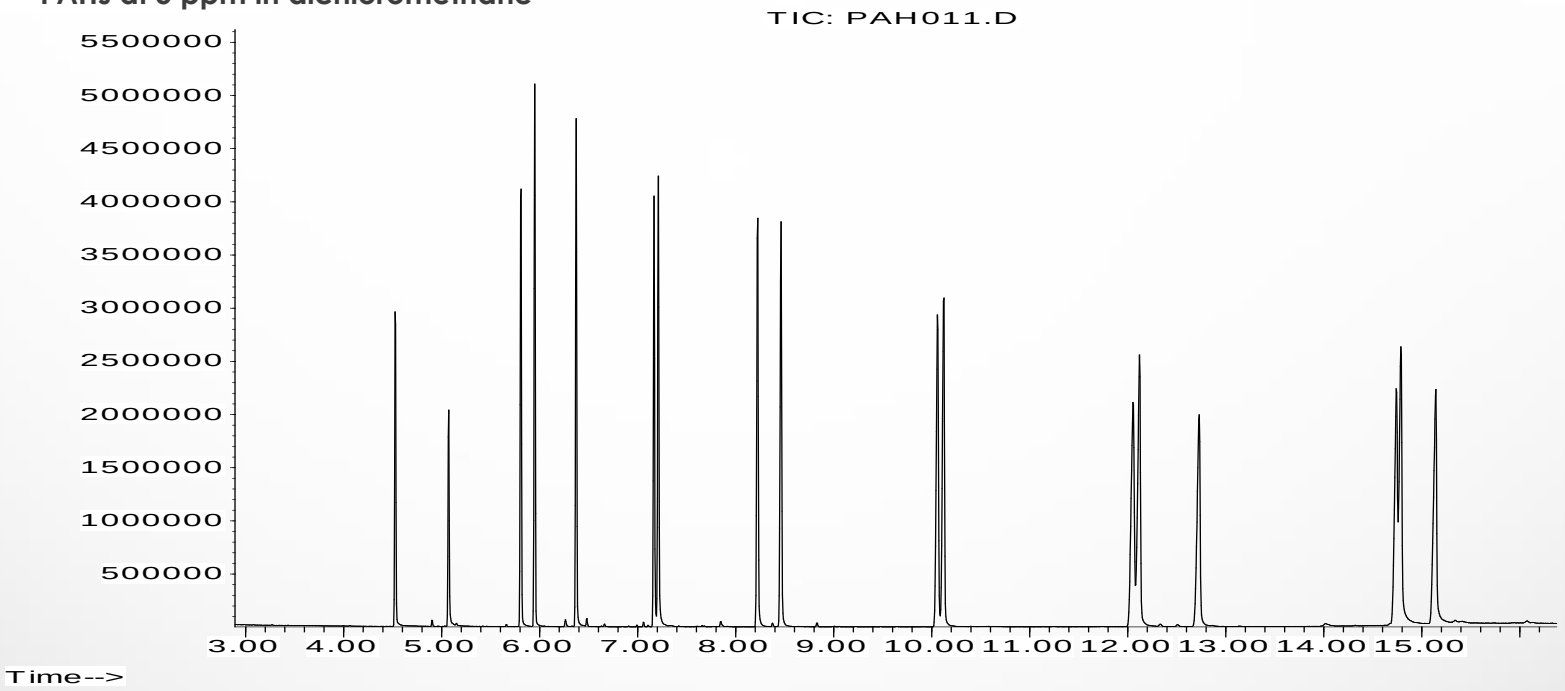
All responses were lower, especially late eluting PAHs

Experimented with flow rates, pressure pulses, and injection volumes

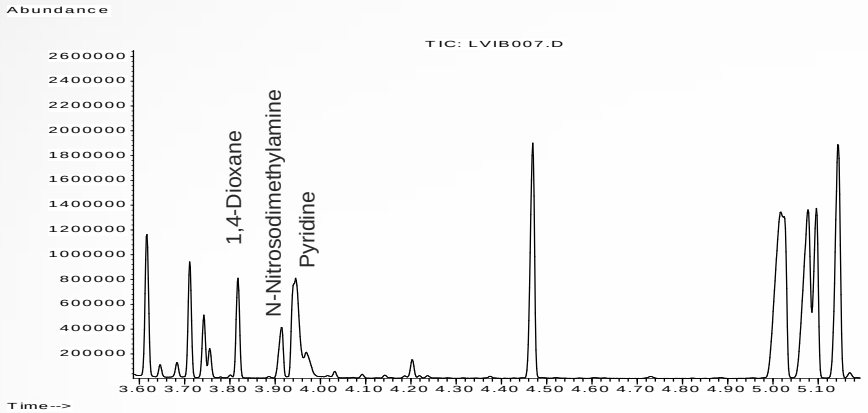


Final Conditions for PAHs

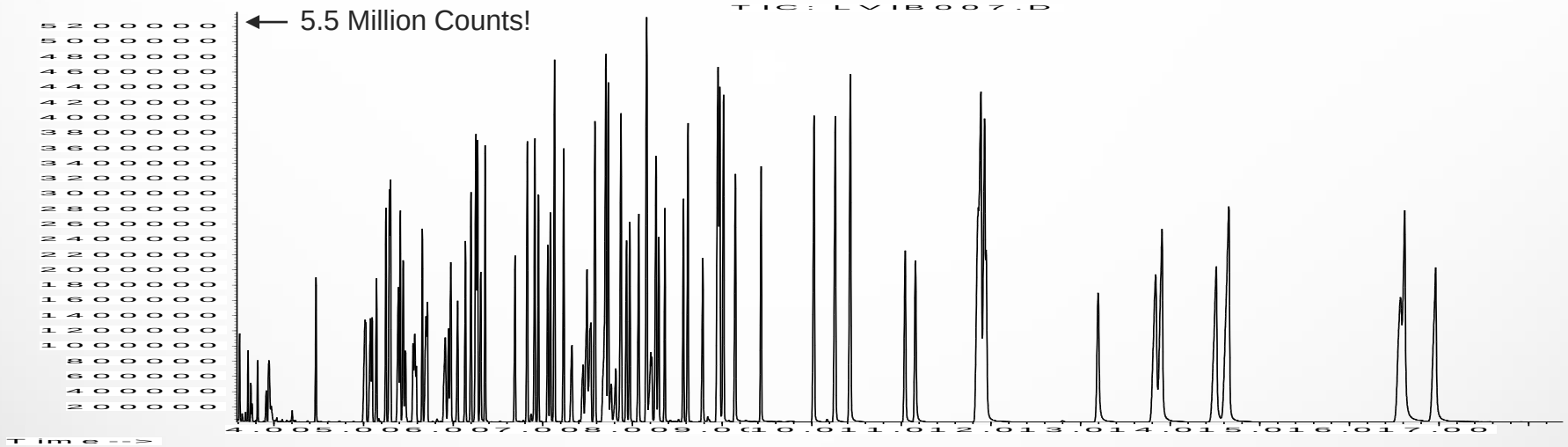
Column Dimension:	**Zebron ZB-SemiVolatiles 30 m + 5 m Guard x 0.25 mm ID x 0.25 µm**
Column Flow:	**1.4 mL/min Helium (constant flow)**
Oven Program:	**60 °C for 0.75 to 260 °C @ 30 °C/min to 295 °C @ 6 °C/min to 325 °C @ 25 °C/min for 2 min**
Inlet Liner:	**Single Taper with wool at bottom**
Injection:	**7.5 µL Pulsed Splitless, Pressure Pulse @ 30 psi for 0.66 min, splitless for 0.60 min**
Detector:	**MSD; 46-450amu, transfer line = 320 °C**
Analytes:	**PAHs at 5 ppm in dichloromethane**



Method Development for Semivolatiles

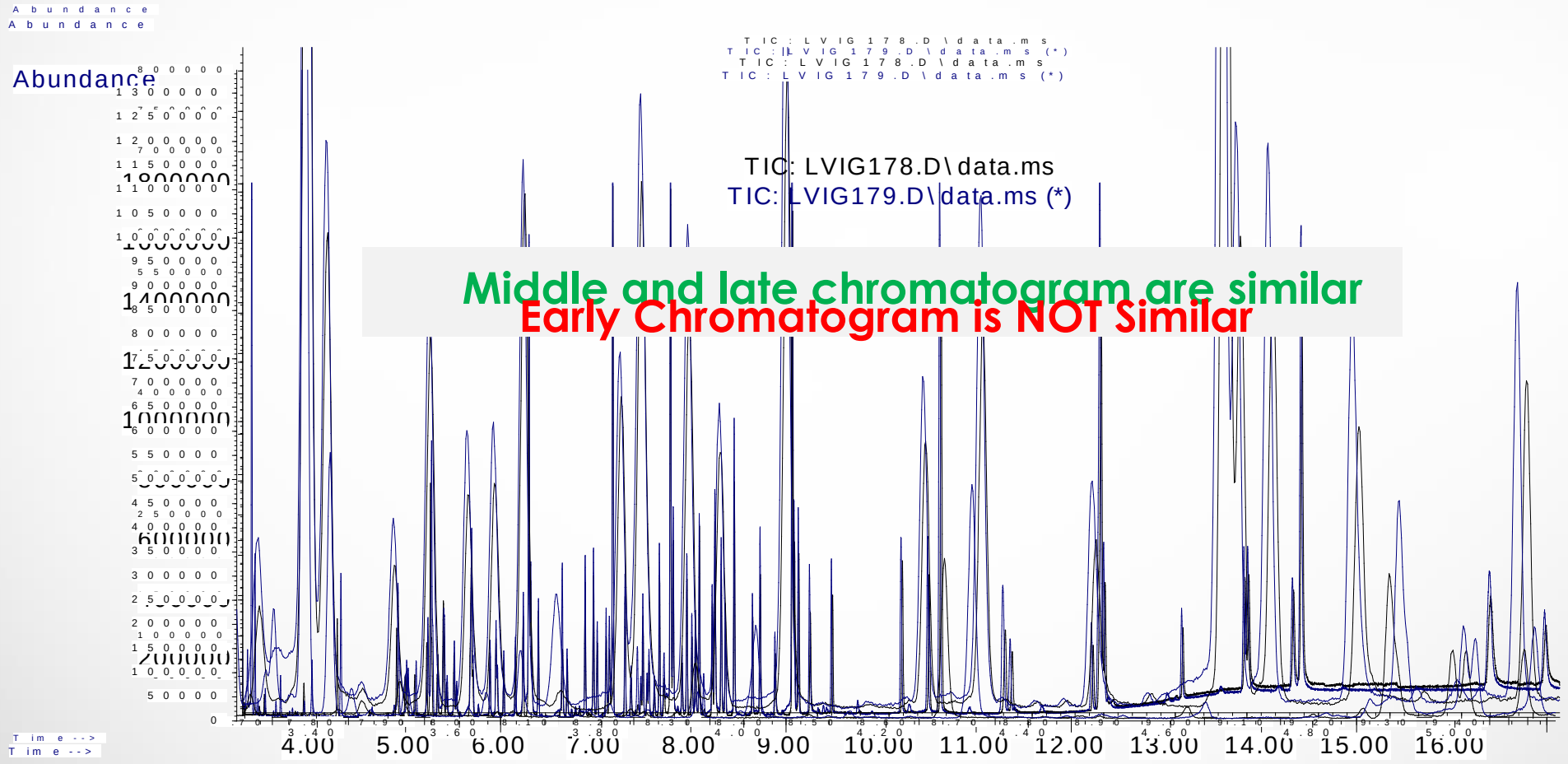


- Used PAH conditions as a starting point
- 17 min run
- Lowered initial temperature to 40 °C for 2 minutes
- 5 ppm Calibration Point with 1,4-Dioxane
- Separation of Benzo[b]/[k]fluoranthene
- Not the best separation of later PAHs



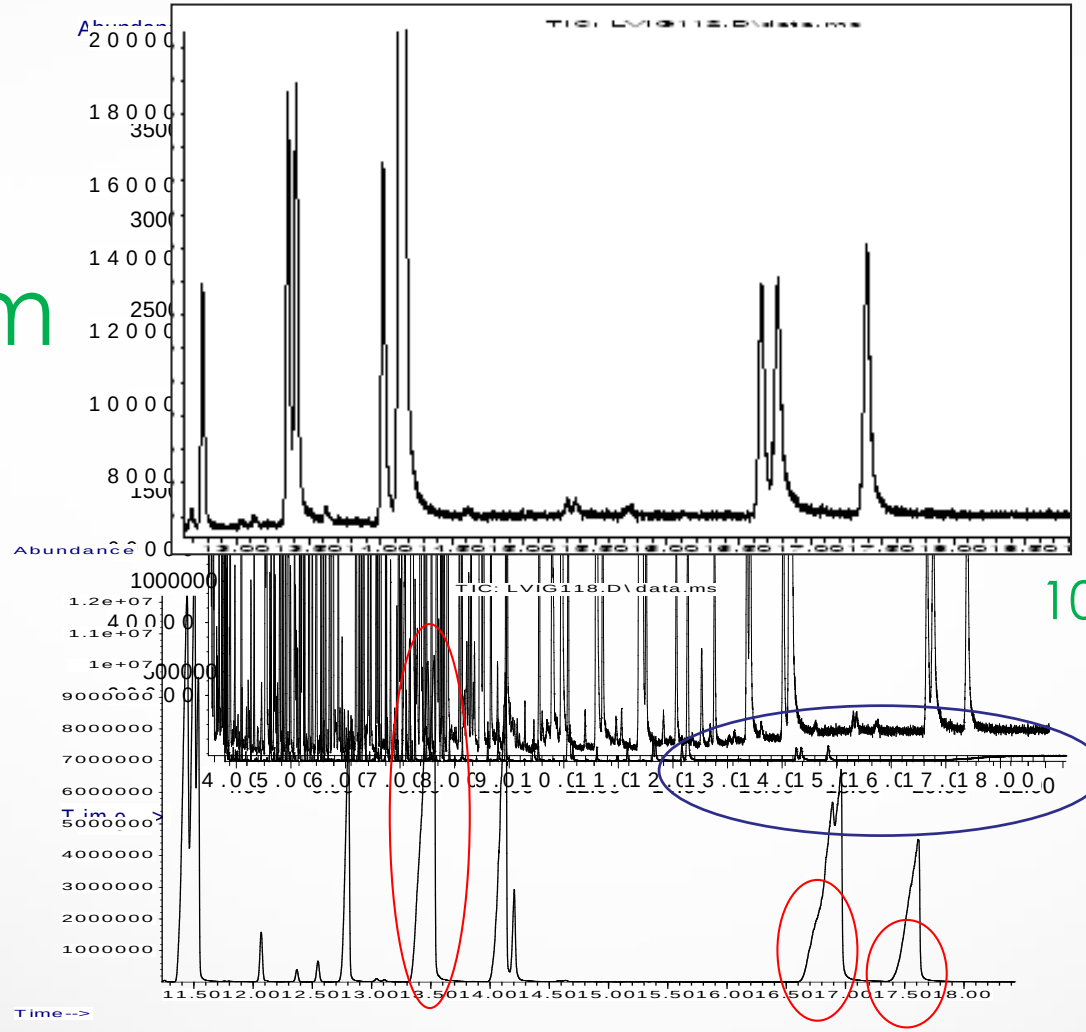
Injection Volume Effects

Overlay of 7.5 µL (file179) and **5.0 µL (file178)**



0.1ppm Calibration Curve

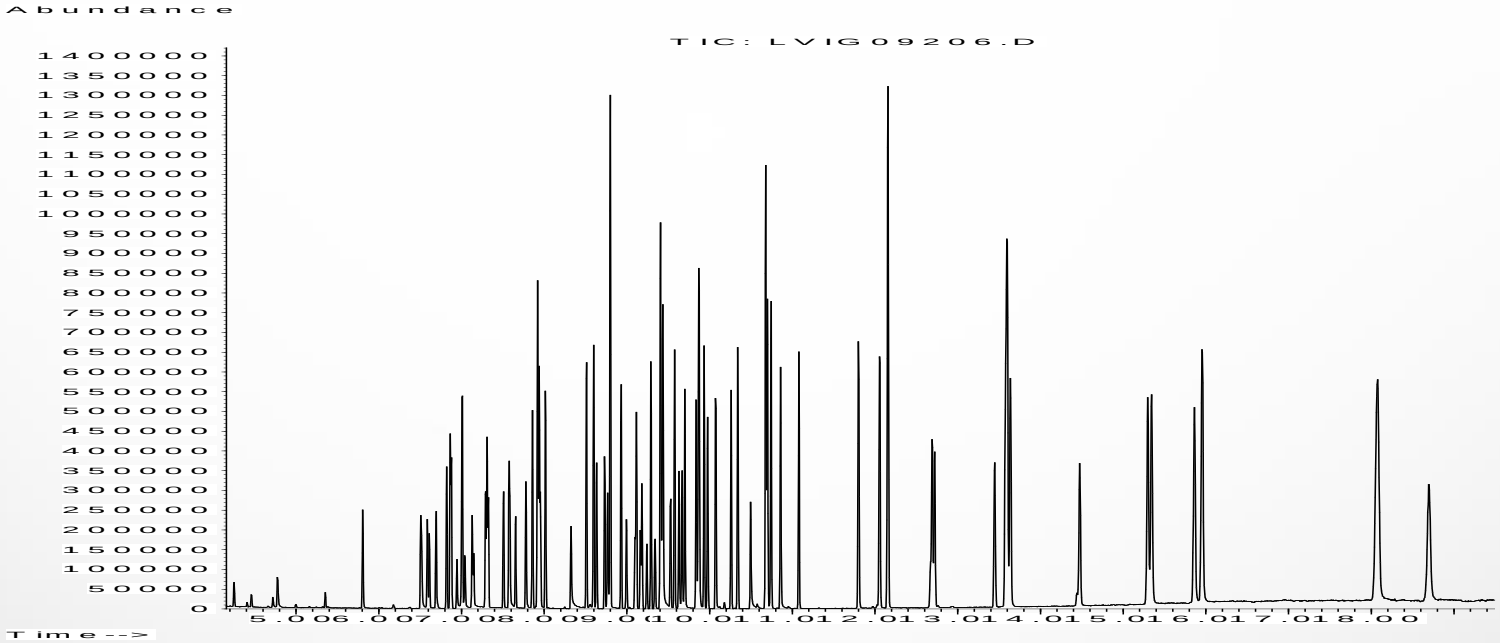
zoom



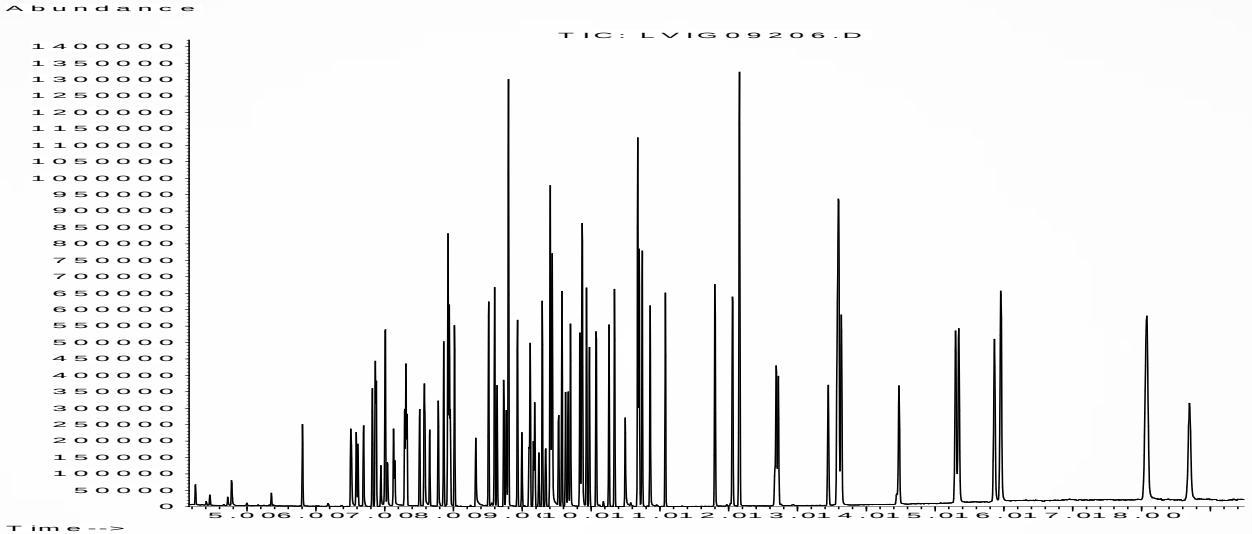
10.0 ppm injection
Later Peaks
(overloading)

LVI: Semivolatiles

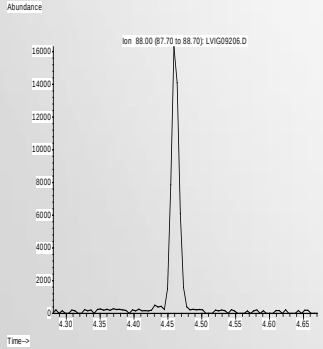
Column:	**Zebron ZB-SemiVolatiles 30 m x 0.25 mm x 0.50 µm with 5 m Guardian**
Liner:	**Single Taper with Wool at the bottom**
Injection:	**5.0 µL pulsed splitless at 30 psi for 0.66 min; 260 °C**
Column Flow:	**1.4 mL/min Helium (constant flow)**
Oven Program:	**45 °C for 3.0 min to 280 °C @ 30 °C/min to 325 °C @ 9 °C/min for 5 min**
Calibration Curve:	**0.1 – 10.0ppm; IS & Surrogate at 1.0ppm**



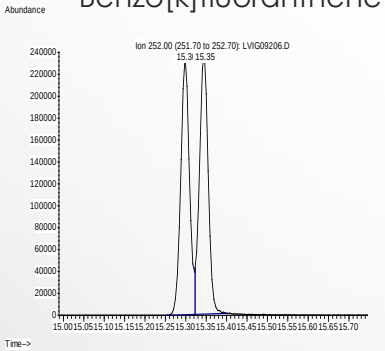
Key Performance Characteristics



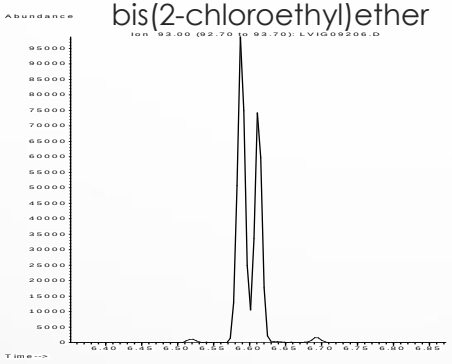
1,4-Dioxane



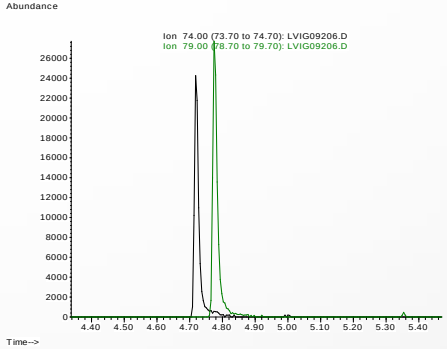
Mass 252:
Benzo[b]fluoranthene &
Benzo[k]fluoranthene



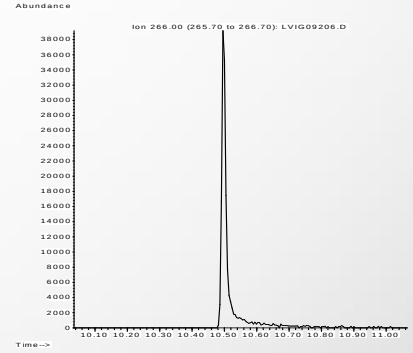
Mass 93:
Aniline &
bis(2-chloroethyl)ether



N-Nitrosoodimethylamine
Pyridine

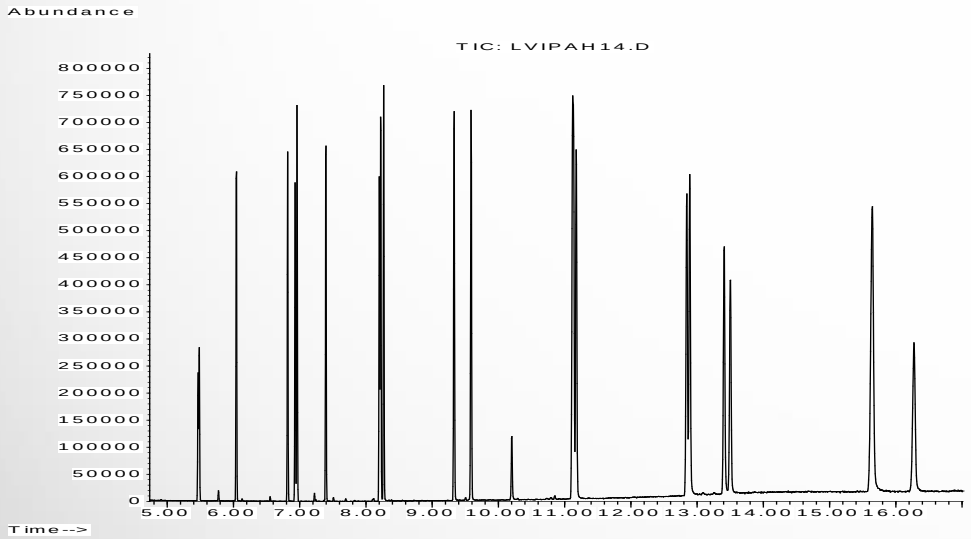


Pentachlorophenol

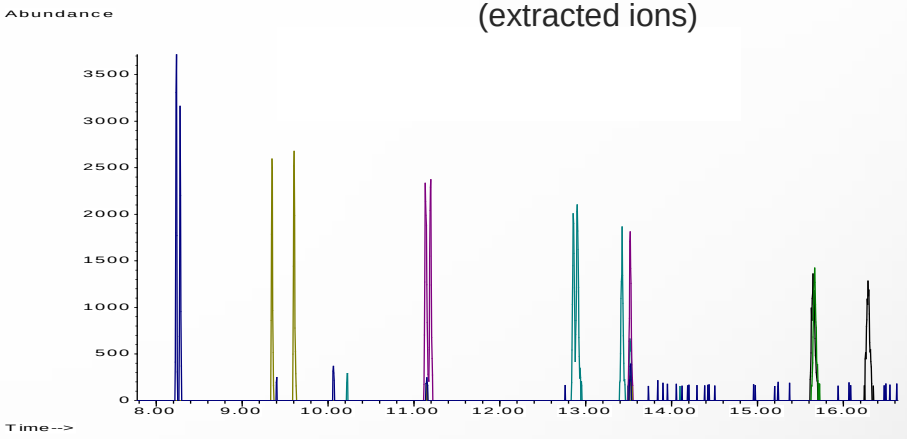


LVI: PAHs

Column:	**Zebron ZB-SemiVolatiles 30 m x 0.25 mm x 0.50 µm with 5 m Guardian**
Liner:	**Single Taper with Wool at the bottom**
Injection:	**5.0 µL pulsed splitless at 30 psi for 0.66 min; 300ºC**
Column Flow:	**1.4 mL/min Helium (constant flow)**
Oven Program:	**60 ºC for 1.0 min to 280 ºC @ 30 ºC/min to 325 ºC @ 9 ºC/min for 5 min**
Calibration Curve:	**0.01 – 1.0 ppm; IS and Surr @ 1.0 ppm**



Calibration Standard of Later Eluting PAHs @ 10 ppb (extracted ions)



This is SCAN data, not SIM!

Choice of Column

Semivolatiles are challenging compounds

Contains Bases, Neutrals, and Acids

Need very inert column for Bases, Neutrals, AND Acids

EPA Method Requirements

DFTPP Tune:

- Decafluorotriphenylphospine, Pentachlorophenol, DDT, Benzidine

Instrument must pass the DFTPP Tune before running samples

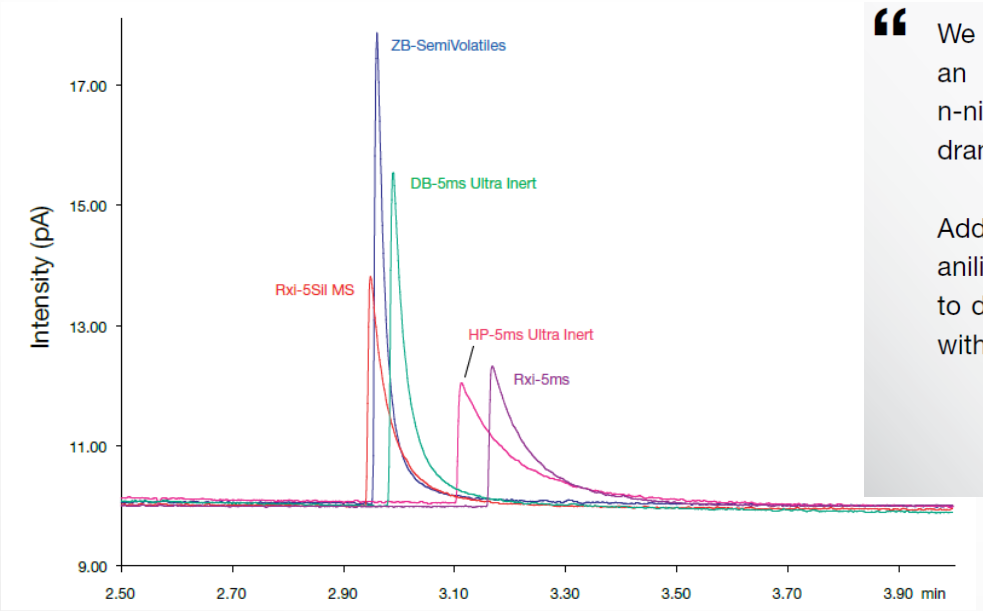
- DFTPP (tests MS response)
  - MS must meet ion ratio criteria
- Pentachlorophenol (test acidic response)
  - Peak skew $\leq$ 2.0
- Benzidine (test basic response)
  - Peak skew $\leq$ 2.0
- DDT (tests other activity)
  - Breakdown $\leq$ 20%

Why is Pyridine Important?

"Pyridine may perform poorly … Therefore, if pyridine is to be determined in addition to other target analytes, it may be necessary to perform separate analyses." – EPA Method 8270D

Labs don't want to run additional analyses = it costs them more money!

Pyridine is also an indicator of column activity for other compounds.



"We made the switch to the ZB-SemiVolatiles column for an increase in performance for separating pyridine and n-nitrosodimethylamine. The improved peak shape has dramatically decreased the %RSD in our calibration curve.

Additionally, we have seen an increase of peak separation for aniline and bis(2-chloroethyl) ether. This has allowed for us to decrease run times while seeing excellent peak resolution without sacrificing quality, something I strive for as an analyst."

Senior Organic Chemist
Phoenix Environmental Laboratories, Inc.

ZB-SemiVolatiles Test Mixes

Traditional Test Mix

- Efficiency
- Polarity
- Bleed
- Activity

Method Specific Test Mix

- Better measure of activity
- This is the DFTPP Tuning Standard
- Also includes Pyridine, more sensitive base than benzidine

The ZB-SemiVolatiles Advantage

Better peak shapes for active compounds

Better calibration RSD values

Makes the 8270D Tuning requirements easy to pass

Resists contamination = longer lifetime

Better quantitation across all concentrations

- Low concentrations: Stronger response
- All concentrations: Better peak shape for easier and more consistent integration

Summary

Single Taper Liner with Wool at Bottom provides the best results for expansion capacity and directing sample on to the column.

Pressure pulse of 30 psi for 0.66 min for controlling expansion volume

Guard column of same ID as analytical column helps the peak shape of initial compounds extend column lifetime

Constant flow of 1.4 mL/min gave the best separation and improved run times

60°C Initial temperature to fully focus early eluters such as naphthalene

The ZB-SemiVolatiles 30 m x 0.25 mm x 0.25 µm with a 5m integrated Guard best separation for crtical PAH pairs in the shortest time (<16 min)

Thank You

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