Troubleshooting

Symptoms and Solutions

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Troubleshooting Tips

1. Isolate the problem.

(Blank Runs, Inject Un-retained Compound, Know what it is not)

2. Change only one variable at a time.

3. Compare before/after chromatograms.

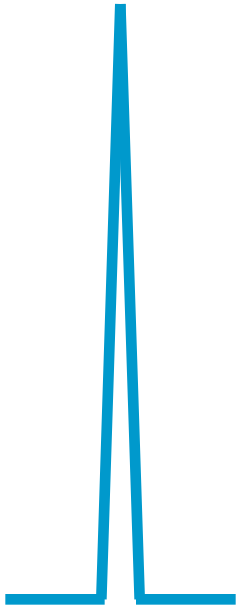
(Peak shape, response, retention, baseline rise, background, look for trends, etc.)

Symptoms and Reasons (Cheat Sheet)

*Solution = Get Rid of the Reason

- Peak Tailing – Flow Path or Activity
- Bonus Peaks – In Sample or Back Flash (Carry Over)
- Split Peaks – Injector Problems, Mixed Solvent, Cold Spots
- Broad Peaks – Injection Problems, Temperature/Flow, Solubility Problems
- No Peaks – Wasn't Introduced, Wasn't Detected
- Response Changes – Activity, Injector Discrimination, Detector Problem
- Peak Fronting – Overload or Solubility Mismatch, Injector Problems
- Shifting Retention – Leaks, Column Aging, Contamination or Damage
- Loss of Resolution – Separation Decreasing, Peak Broadening
- Baseline Disturbances – Column Bleed, Contamination, Electronics
- Noisy or Spiking Baseline – Electronics or Contaminated Detector
- Quantitation Problems – Activity, Injector or Detector Problems

Peak Tailing

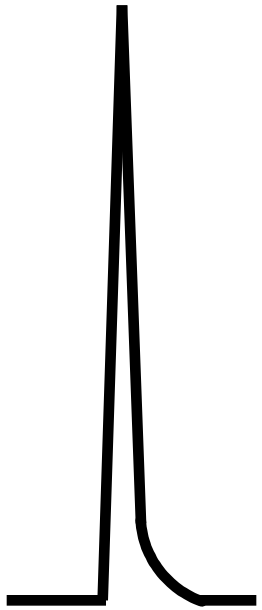


INJECTOR or COLUMN is Active

-Reversible adsorption of active compounds

(-OH, -NH, -SH)

FLOW problem
- dead volume, obstruction, poor installation, or severe column contamination



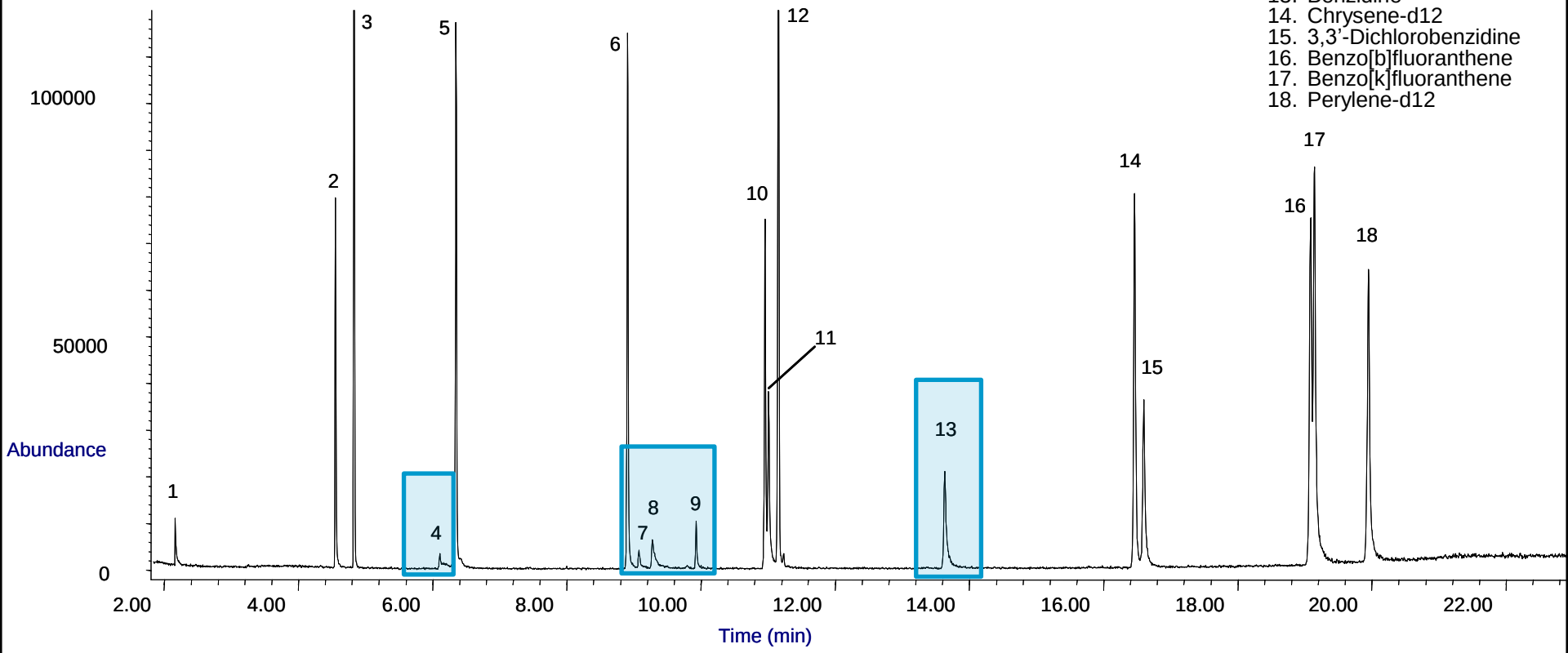
Miscellaneous – temperature issues for late eluters, overloading of PLOT columns, co-elution, polarity mismatch between phase, solute or solvent, and some compounds always tail

*Tip = Inject a light hydrocarbon, should not tail unless flow path problem.

Symptom – Tailing of Active Compounds

Sample:	0.5 ng on column loading with ISTD
Column:	20m 0.18mm 0.18µm
Carrier:	Helium 37cm/sec, Ramped flow; 0.7ml/min (0.1min) to 1.3ml/min (15ml/min^2)
Oven:	35°C (2.5 min) to 80°C (40°C/min), 15°C/min to 200°C, 8°C/min to 275°C (2 min)
Injection:	0.5µl, Splitless, 280°C, purge flow 30ml/min at 0.75 min
MSD:	Transfer Line 290°C, Source 300°C, Quad 180°C

1. n-Nitrosodimethylamine
2. Aniline
3. 1,4-Dichlorobenzene-d4
4. Benzoic Acid
5. Naphthalene-d8
6. Acenaphthene-d10
7. 2,4-Dinitrophenol
8. 4-Nitrophenol
9. 2-Me-4,6-dinitrophenol
10. 4-Aminobiphenyl
11. Pentachlorophenol
12. Phenanthrene-d10
13. Benzidine
14. Chrysene-d12
15. 3,3'-Dichlorobenzidine
16. Benzo[b]fluoranthene
17. Benzo[k]fluoranthene
18. Perylene-d12



Solution – Ultra Inert GC Column

Sample:	0.5 ng on column loading with ISTD
Column:	20m 0.18mm 0.18µm
Carrier:	Helium 37cm/sec, Ramped flow; 0.7ml/min (0.1min) to 1.3ml/min (15ml/min^2)
Oven:	35°C (2.5 min) to 80°C (40°C/min), 15°C/min to 200°C, 8°C/min to 275°C (2 min)
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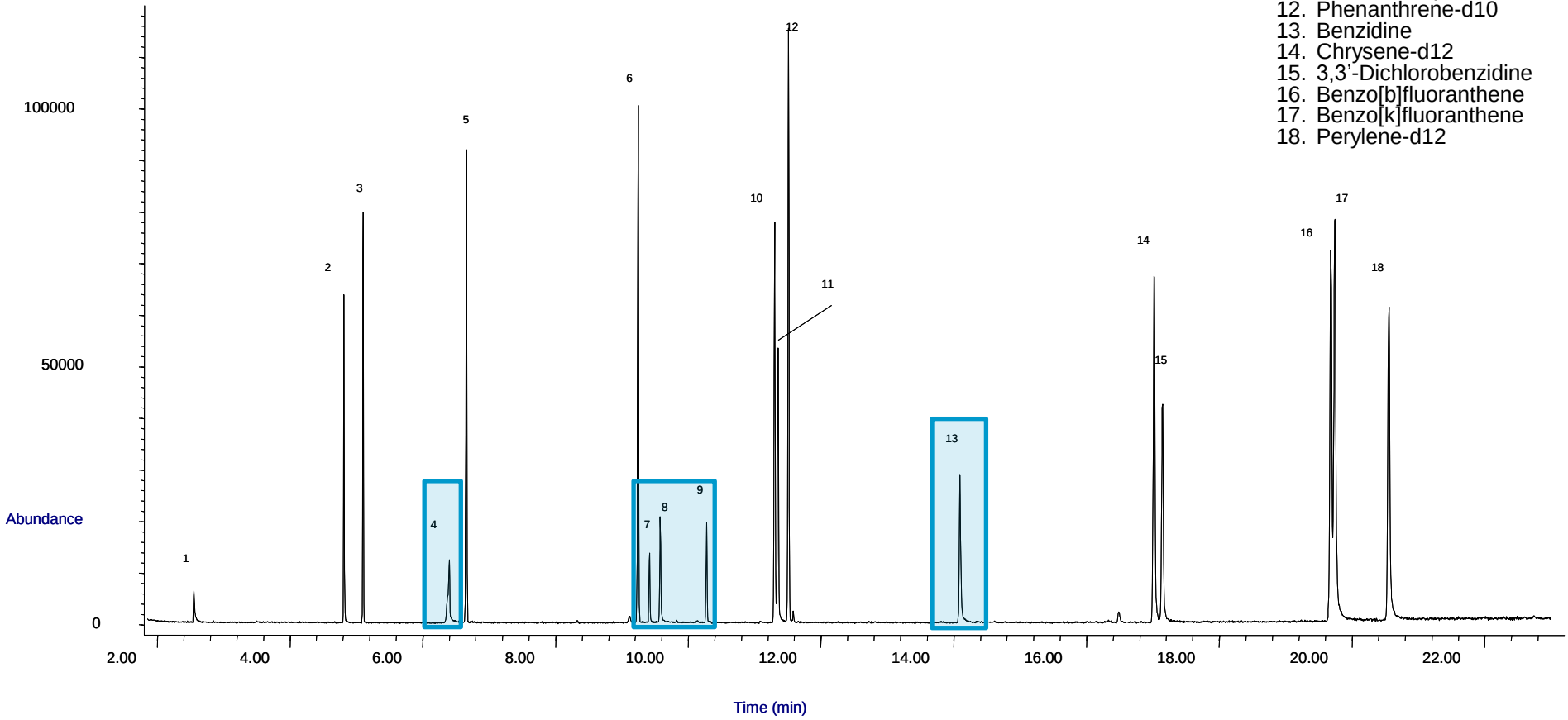
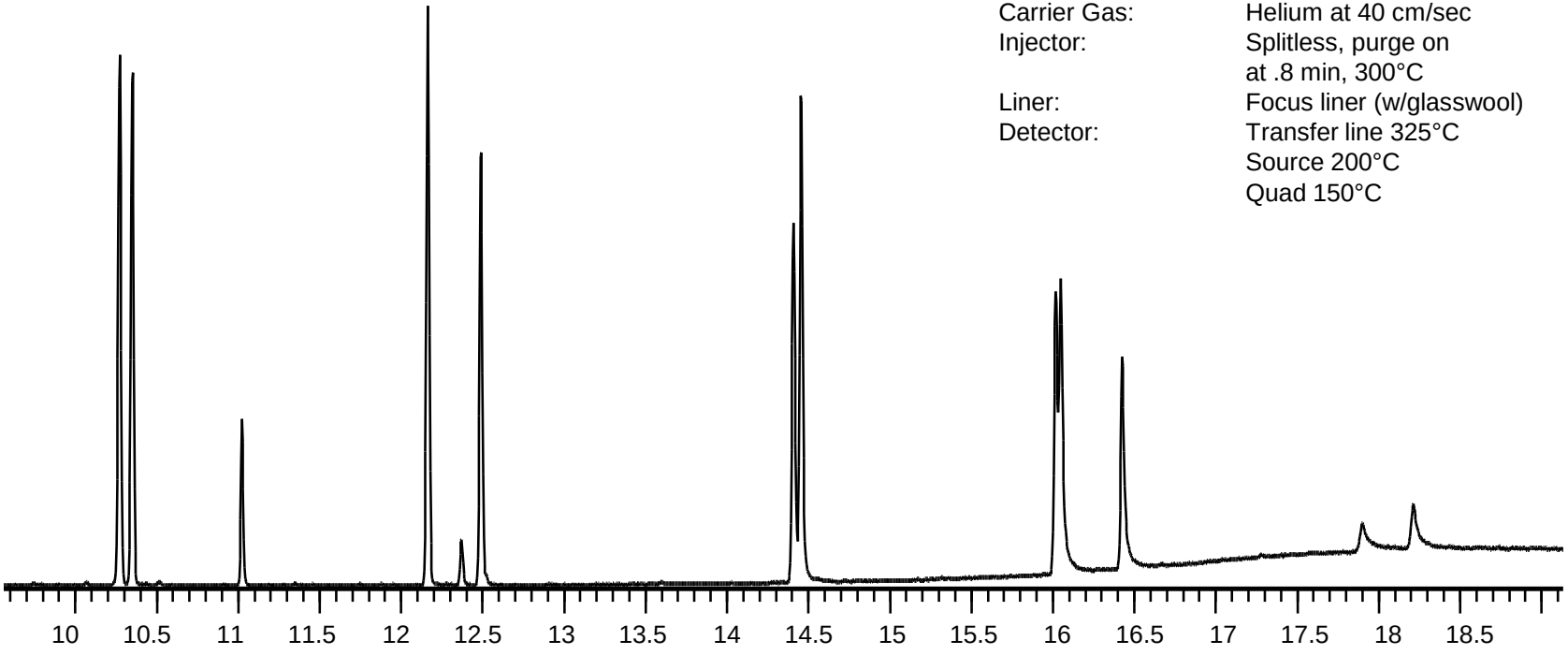
8. 4-Nitrophenol
9. 2-Me-4,6-dinitrophenol
10. 4-Aminobiphenyl
11. Pentachlorophenol
12. Phenanthrene-d10
13. Benzidine
14. Chrysene-d12
15. 3,3'-Dichlorobenzidine
16. Benzo[b]fluoranthene
17. Benzo[k]fluoranthene
18. Perylene-d12



Symptom - Tailing is Worse as Retention Increases

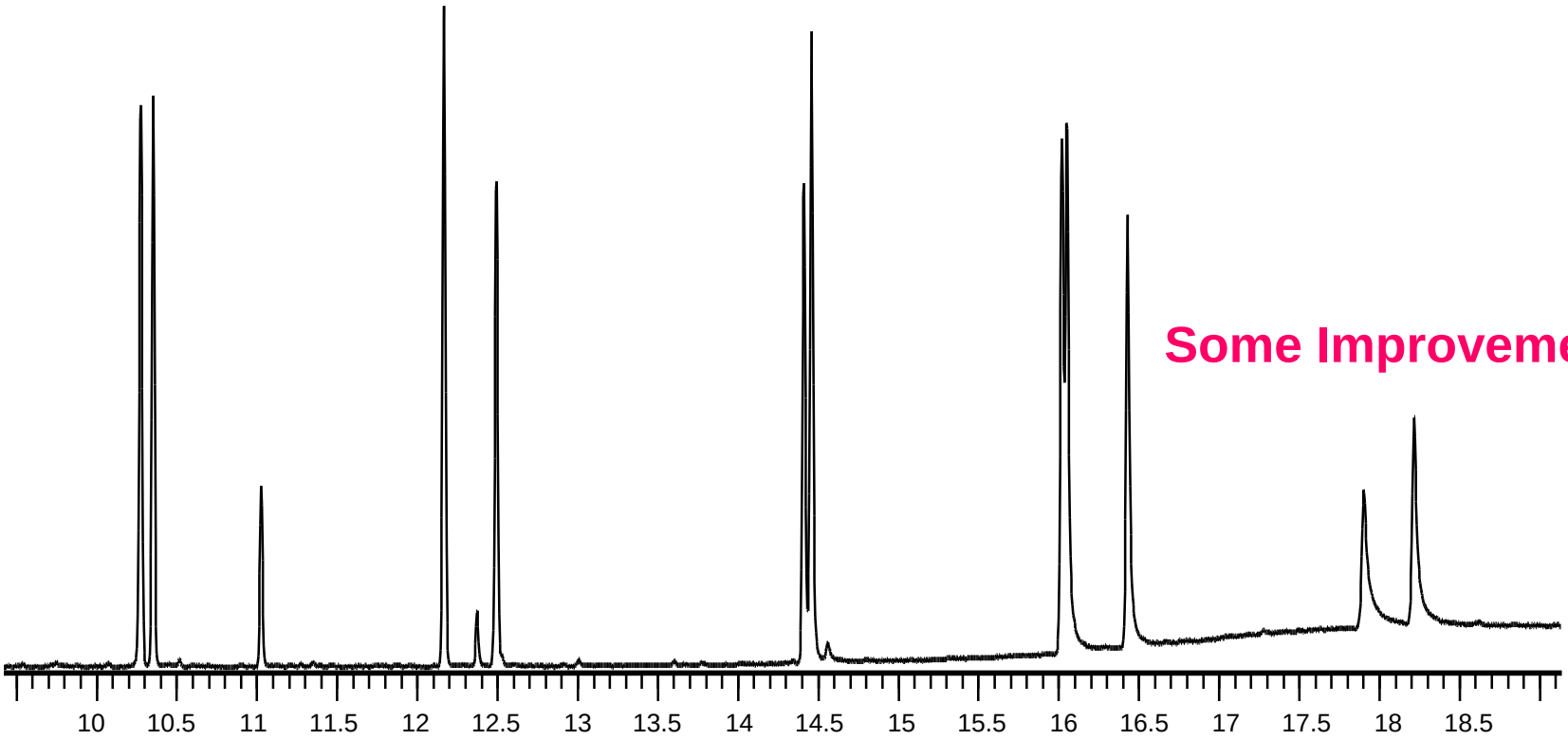
PAHs

Column:	**DB-1ms**
	30 m x 0.25 mm I.D., 0.25 µm
J&W P/N:	**123-1522**
Test Temperature:	80°C for 0.8 min
	80-325°C at 15°/min
	325°C for 2 min
Carrier Gas:	Helium at 40 cm/sec
Injector:	Splitless, purge on
	at .8 min, 300°C
Liner:	Focus liner (w/glasswool)
Detector:	Transfer line 325°C
	Source 200°C
	Quad 150°C



Solution - Increase Injector Temperature

From 300°C to 350°C

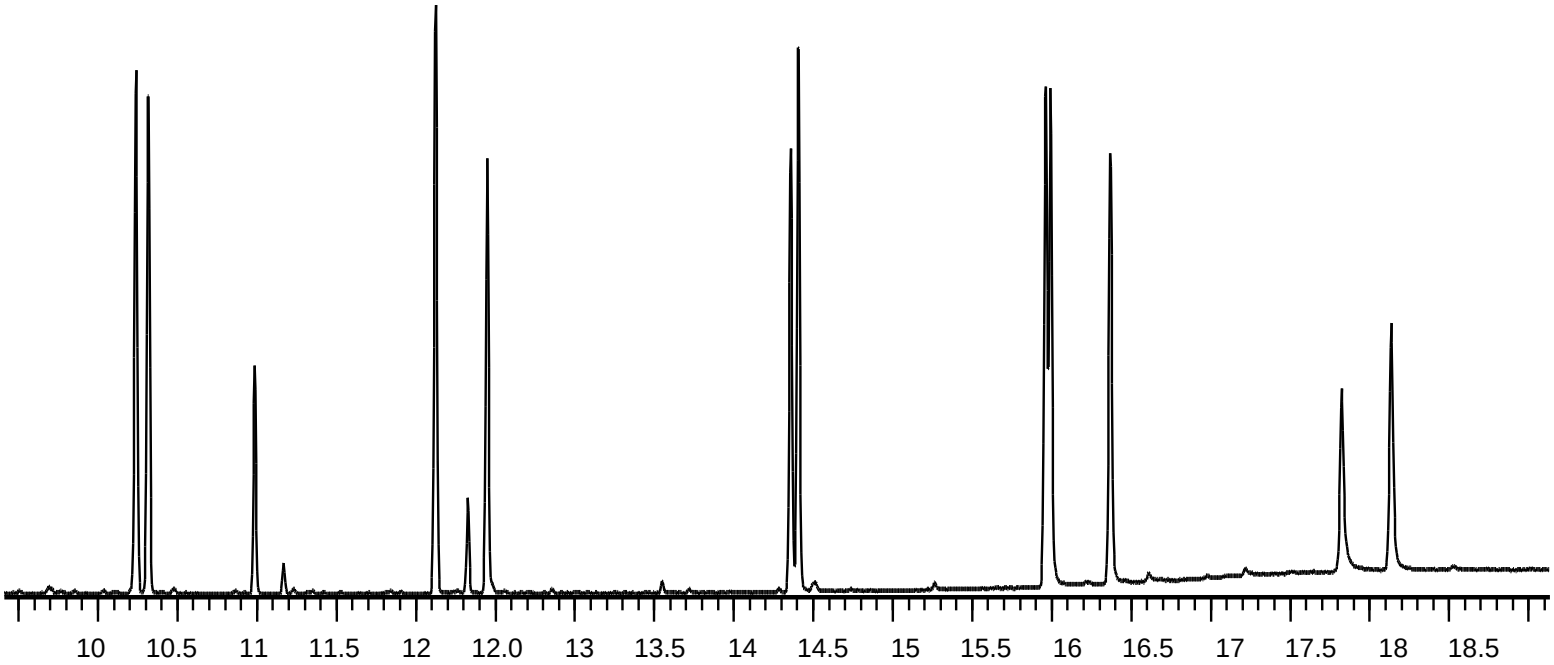


Some Improvement

Oven: 80°C for 0.8 min, 80-325°C at 15°/min, 325°C for 2 min
Carrier Gas: Helium at 40 cm/sec

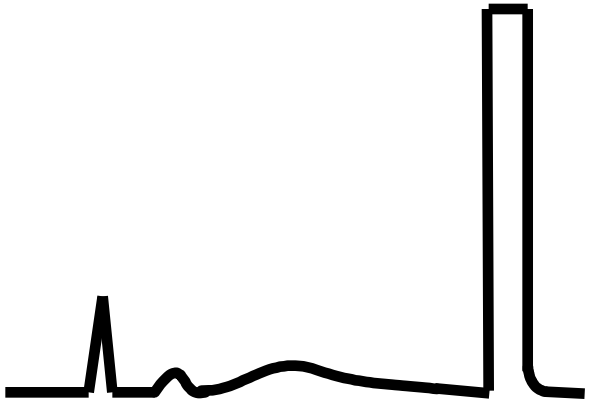
Solution - Increase Detector Temperatures

Transfer line: remains 325°C
Source: from 200°C to 250°C
Quad: from 150°C to 200°C



Oven: 80°C for 0.8 min, 80-325°C at 15°/min, 325°C for 2 min
Carrier Gas: Helium at 40 cm/sec

Bonus Peaks or Ghost Peaks



Contamination in INJECTOR or FLOW (carrier gas)

-Contaminated consumables

-Carryover from a backflash or previous sample

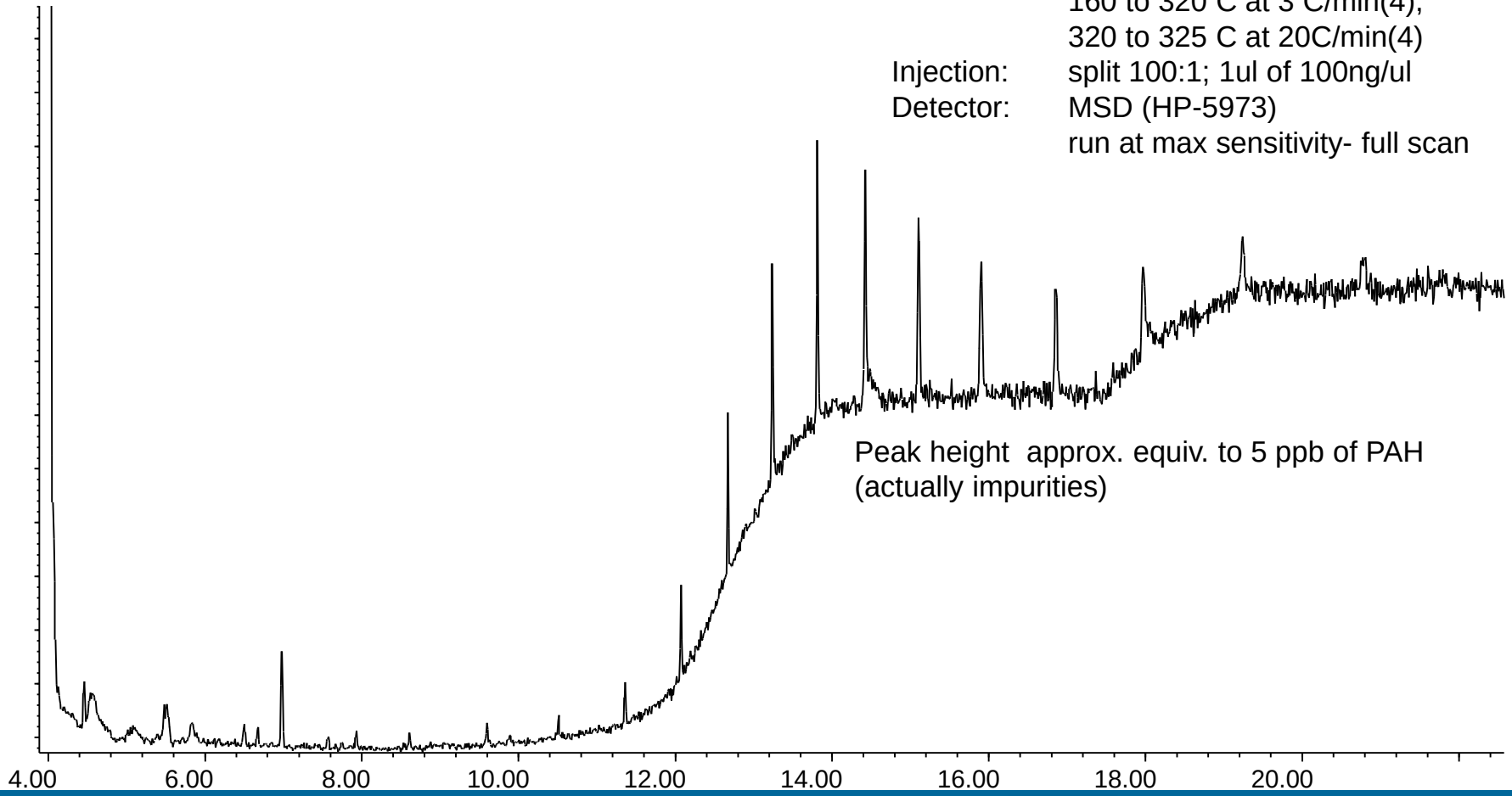
-Bad tank of gas or traps have expired

-Septum bleed

*TIP = Run a blank run…it should be blank!

Symptom – Bonus Peaks in Blank Run
(MSD spectra says siloxanes)

Column:	HP 5MS 30mx0.25mmx0.25um
Oven:	80 to 160C at 25 C/min, 160 to 320 C at 3 C/min(4), 320 to 325 C at 20C/min(4)
Injection:	split 100:1; 1ul of 100ng/ul
Detector:	MSD (HP-5973) run at max sensitivity- full scan



Peak height approx. equiv. to 5 ppb of PAH (actually impurities)

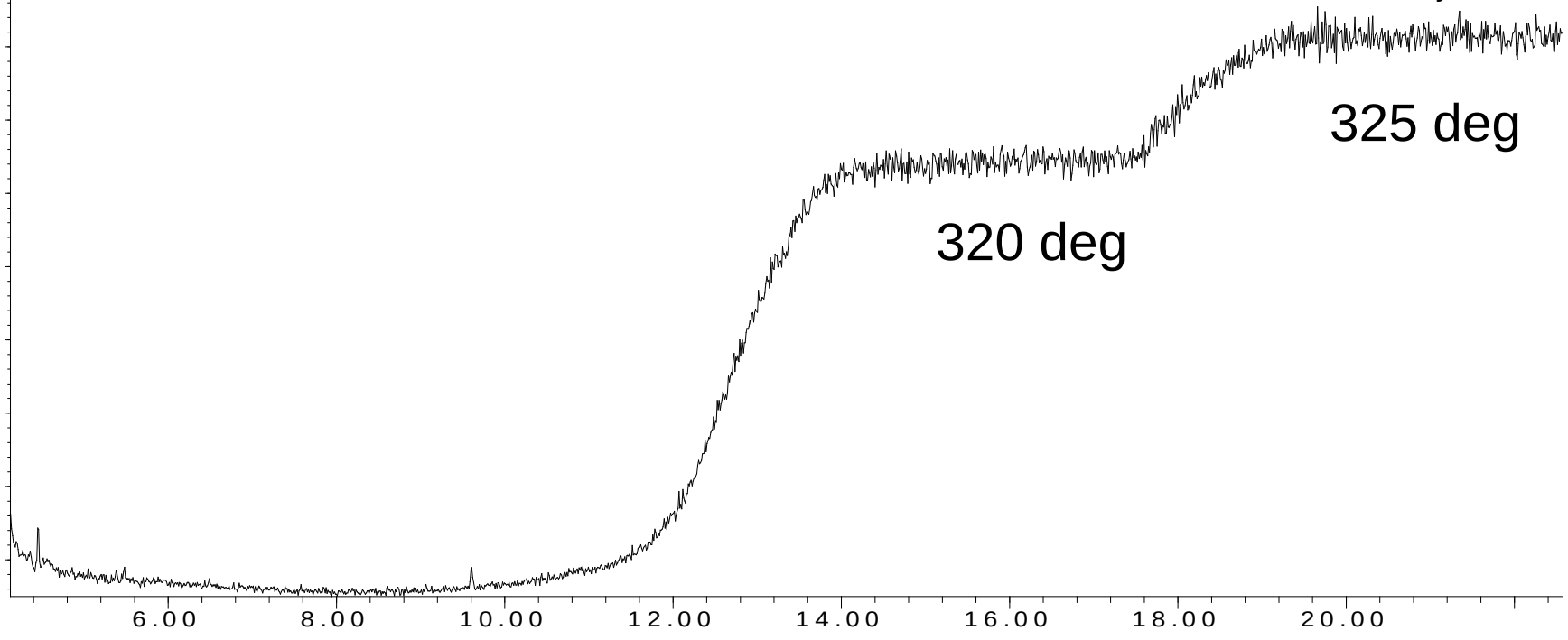
Solution – Change Septum

Septa Bleed vs Column Bleed (MSD)

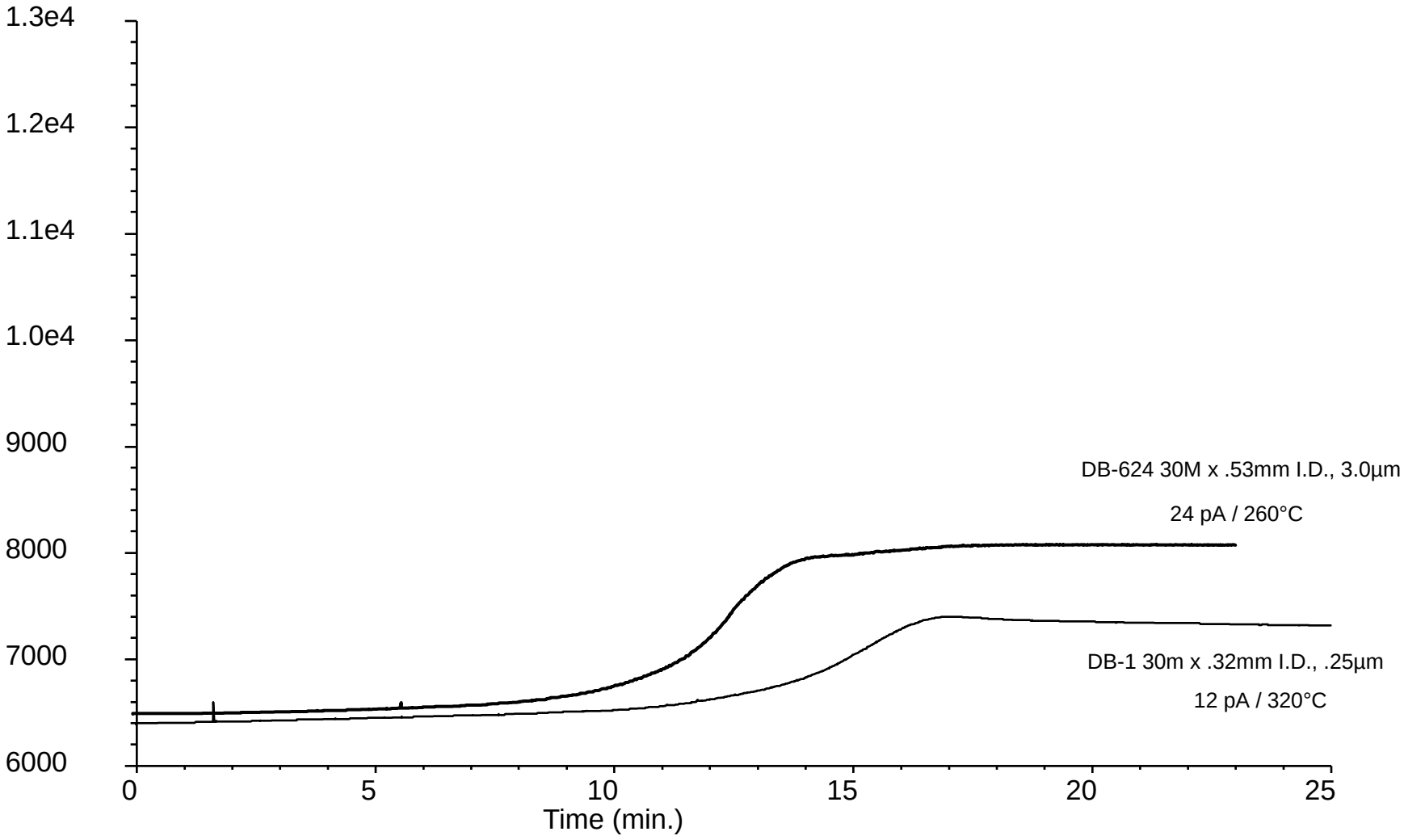
Source of peaks from outside of the column *ELIMINATED*

Columns:	HP 5 MS 30mx0.25mmx0.25um
Oven:	80 to160 C at 25 C/min, 160 to 320 C at 3 C/min(4) 320 to 325 C at 20C/min(4)
Injection:	split 100:1; 1ul of 100ng/ul
Detector:	MSD (HP-5973) run at max sensitivity- full scan

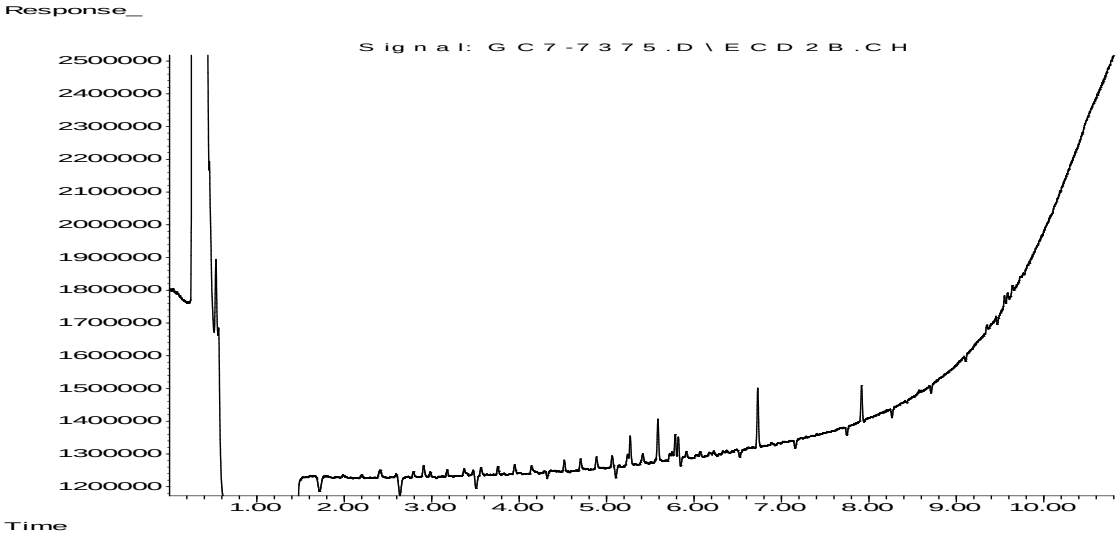
HP Advanced Green Septa
P/N 5183-4594 (11 mm)

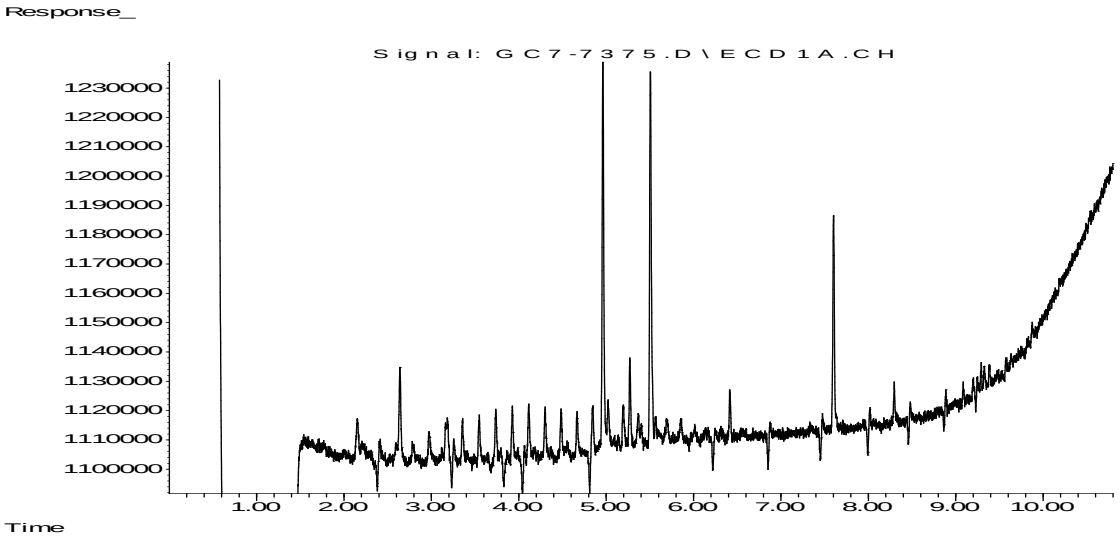


Affect of Column Dimensions/Phase Type on Bleed

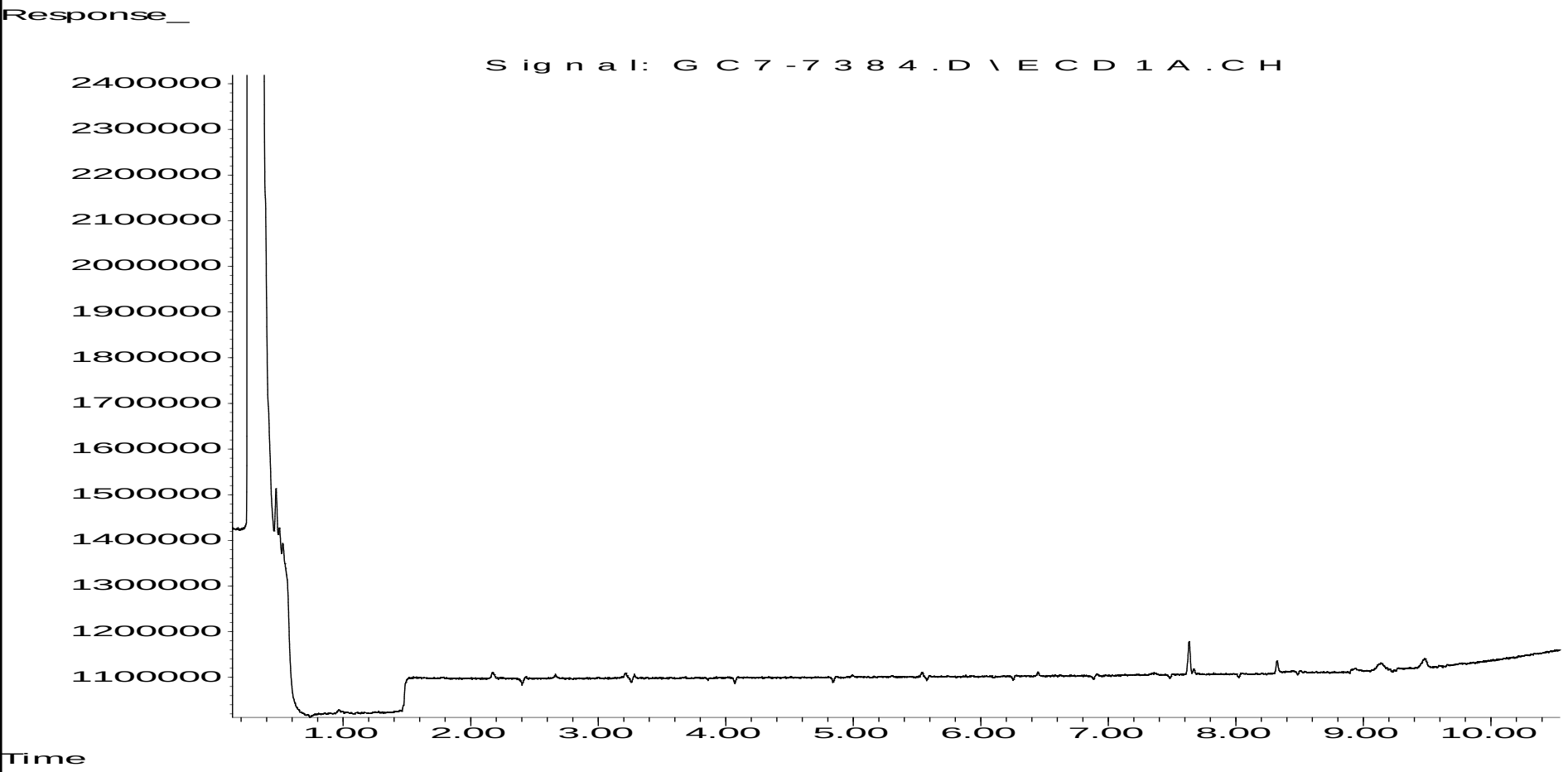


Symptom – Bonus Peaks in Blank Runs
(Contaminated Vial Inserts from Plastic Bag)

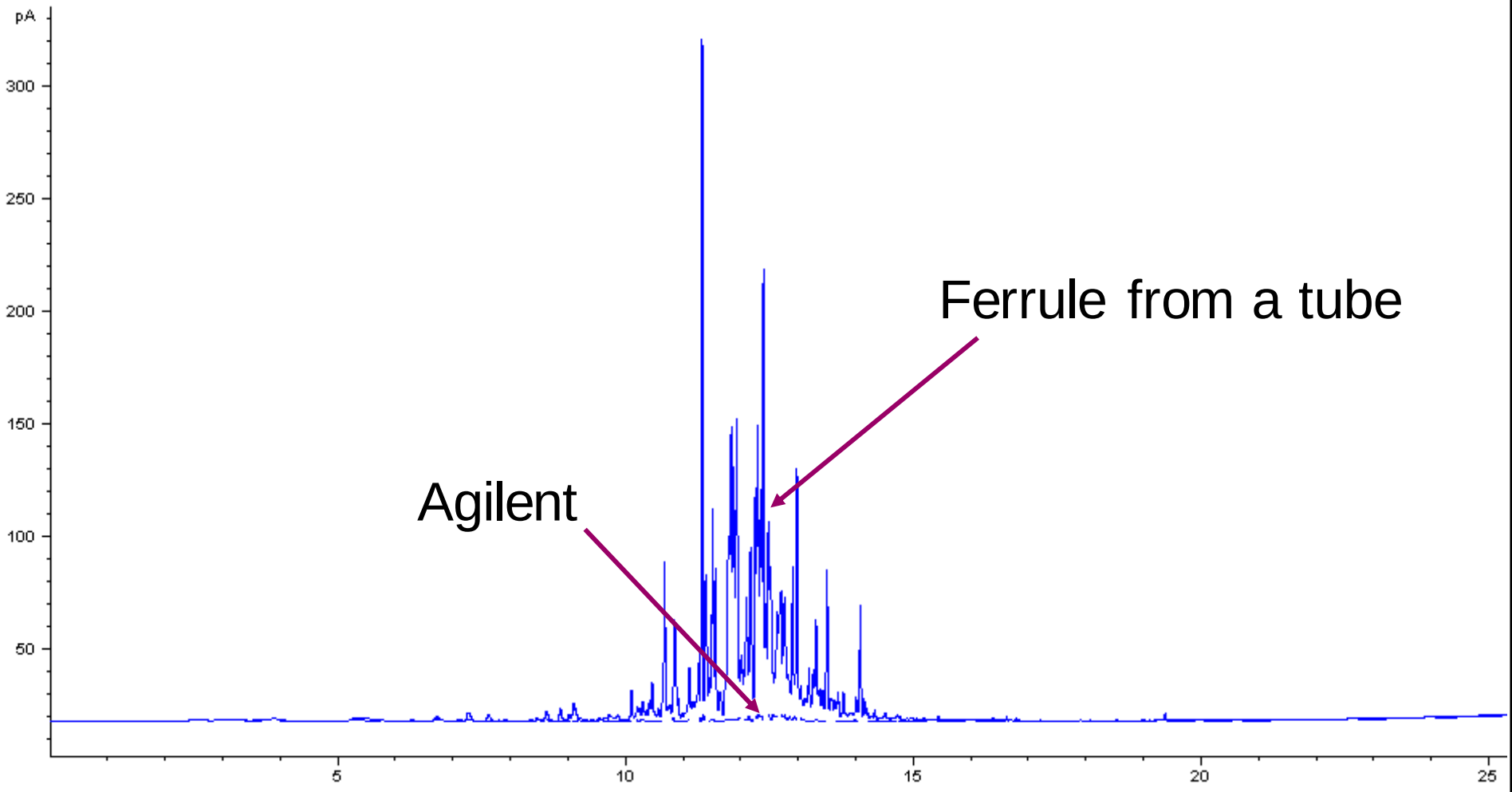




Solution - Rinse Insert with Hexane

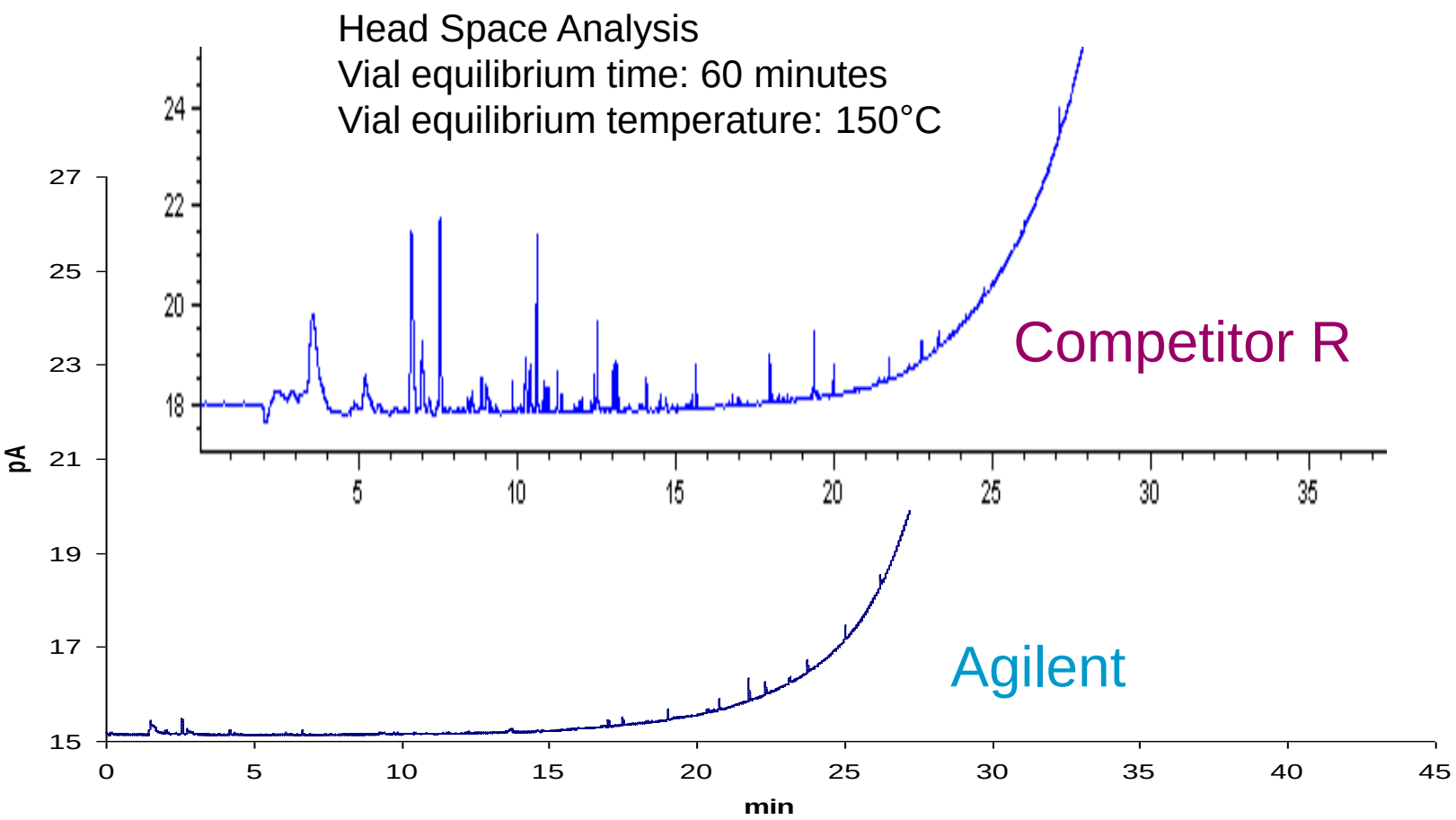


Symptom - Bonus Peaks (Ferrule Contamination)



Solution – Agilent Ferrules in Touchless Packaging

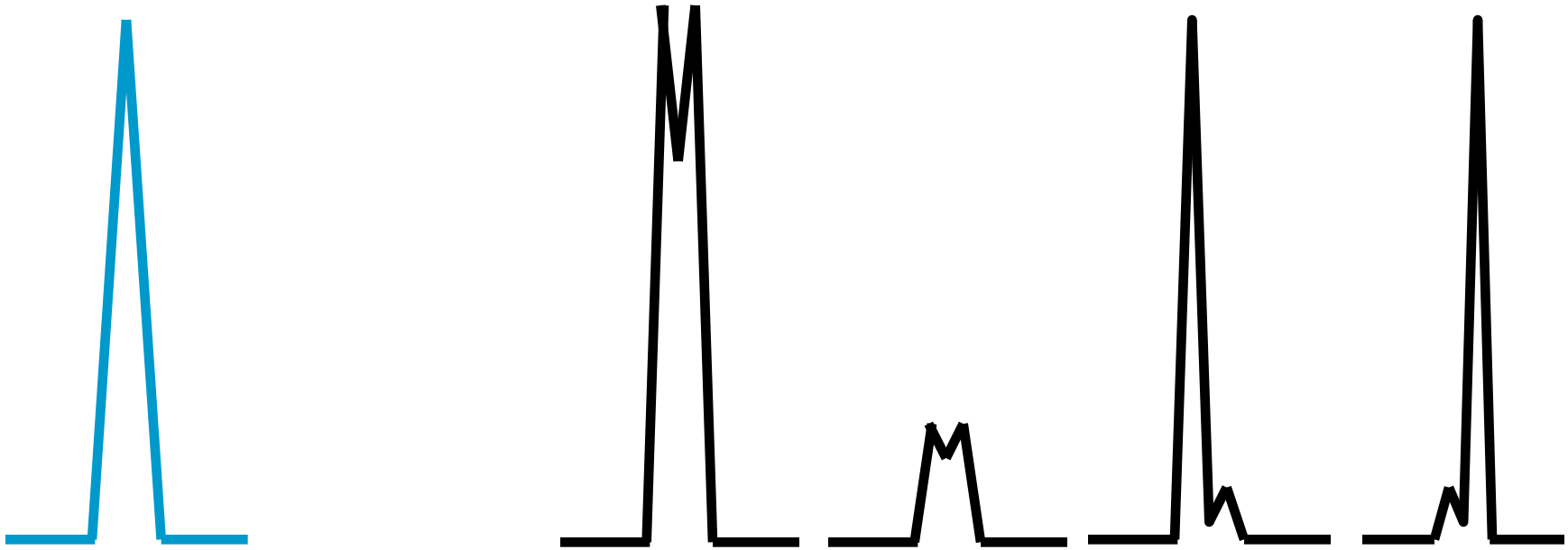
Symptom - Bonus Peaks (O-Ring Contamination)



Solution – Agilent Non-Stick O-Ring in Touchless Packaging

“Touchless” Packaging is the Future

Split Peaks



INJECTOR (poor sample introduction)

-Injecting the sample twice (some how?)

-Mixed sample solvent (polarity difference)

-Sample in syringe needle (manual inject)

INJECTOR (activity)

-Breakdown (not really a split peak, 2 peaks)

-Sample degradation in injector

VOLATILITY

High boilers dropping out on Cold Spots

-Transfer line temps

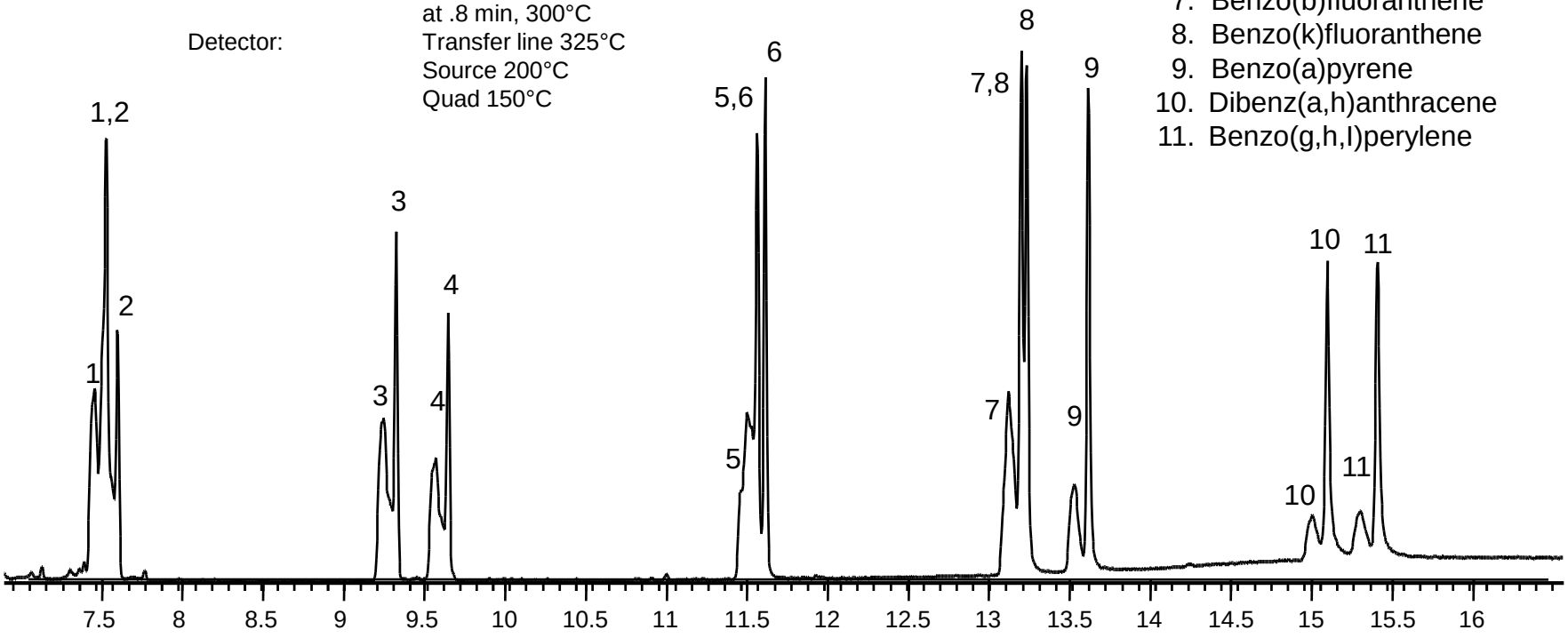
-Unions or fittings not tracking column temp

Symptom – Split Peaks After Installation

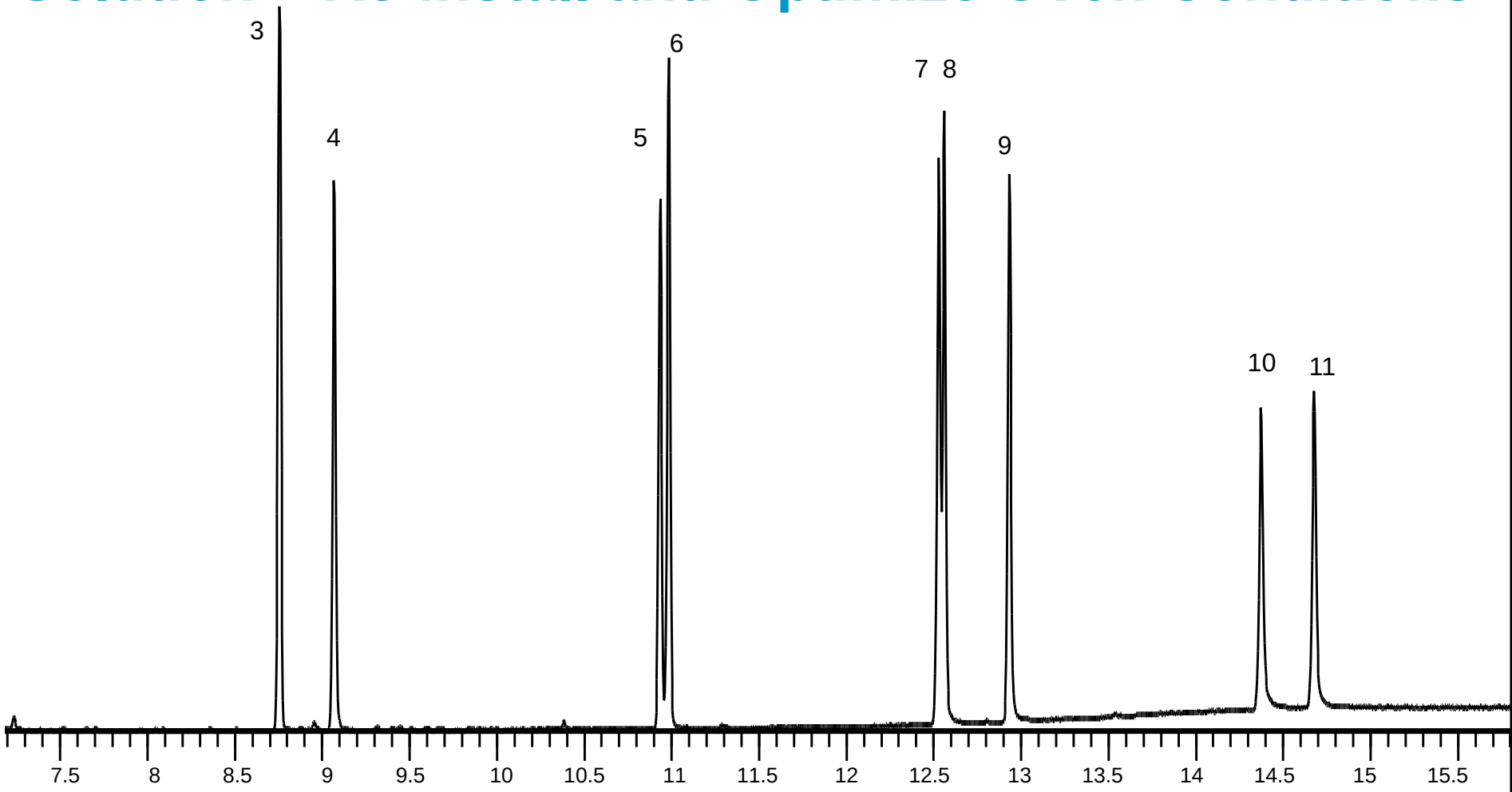
PAH's in Toluene

Column:	**DB-1ms**
	30 m x 0.25 mm I.D., 0.25 µm
J&W P/N:	**123-1522**
Test Temperature:	80°C for 0.8 min
	80-325°C at 15°/min
	325°C for 2 min
Carrier Gas:	Helium at 40 cm/sec
Injector:	Splitless, purge on
	at .8 min, 300°C
Detector:	Transfer line 325°C
	Source 200°C
	Quad 150°C

1. Phenanthrene
2. Anthracene
3. Fluoranthene
4. Pyrene
5. Benzo(a)anthracene
6. Chrysene
7. Benzo(b)fluoranthene
8. Benzo(k)fluoranthene
9. Benzo(a)pyrene
10. Dibenz(a,h)anthracene
11. Benzo(g,h,I)perylene

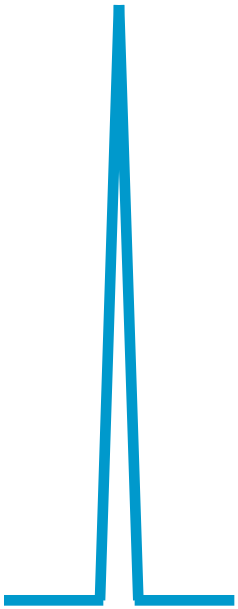


Solution – Re-install and Optimize Oven Conditions



Oven: **100°C for 0.8 min, 100-325°C at 15°/min, 325°C for 2 min**
Carrier Gas: **Helium at 40 cm/sec**

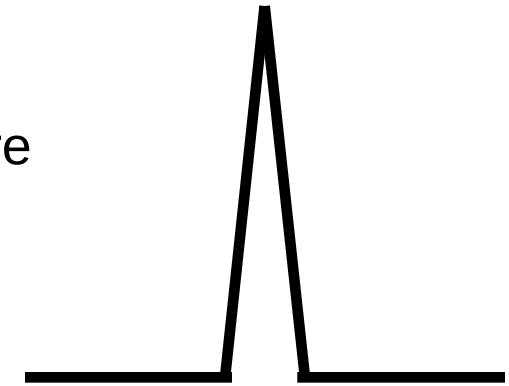
Broad Peaks (peak distortion)



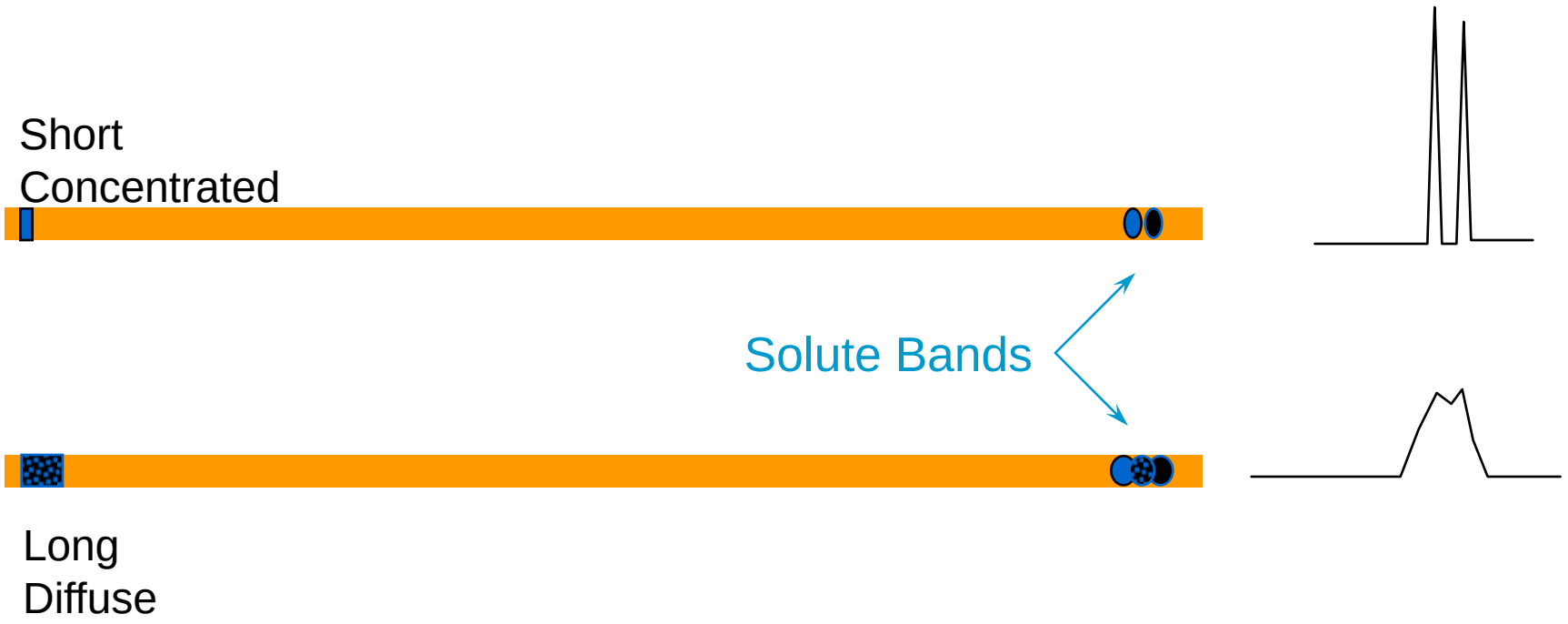
INJECTOR
-Poor Installation
-Change in injection solvent
-Change in settings (temps/flows)
-Poor sample focusing
-Large change in sample concentration
-Breakdown or adsorption

FLOW
-Change in gas velocity
-Constant Flow vs Constant Pressure

COLUMN
-Contamination
-Damaged/old stationary phase
-Breakdown or adsorption
-Reverse Solvent Effect

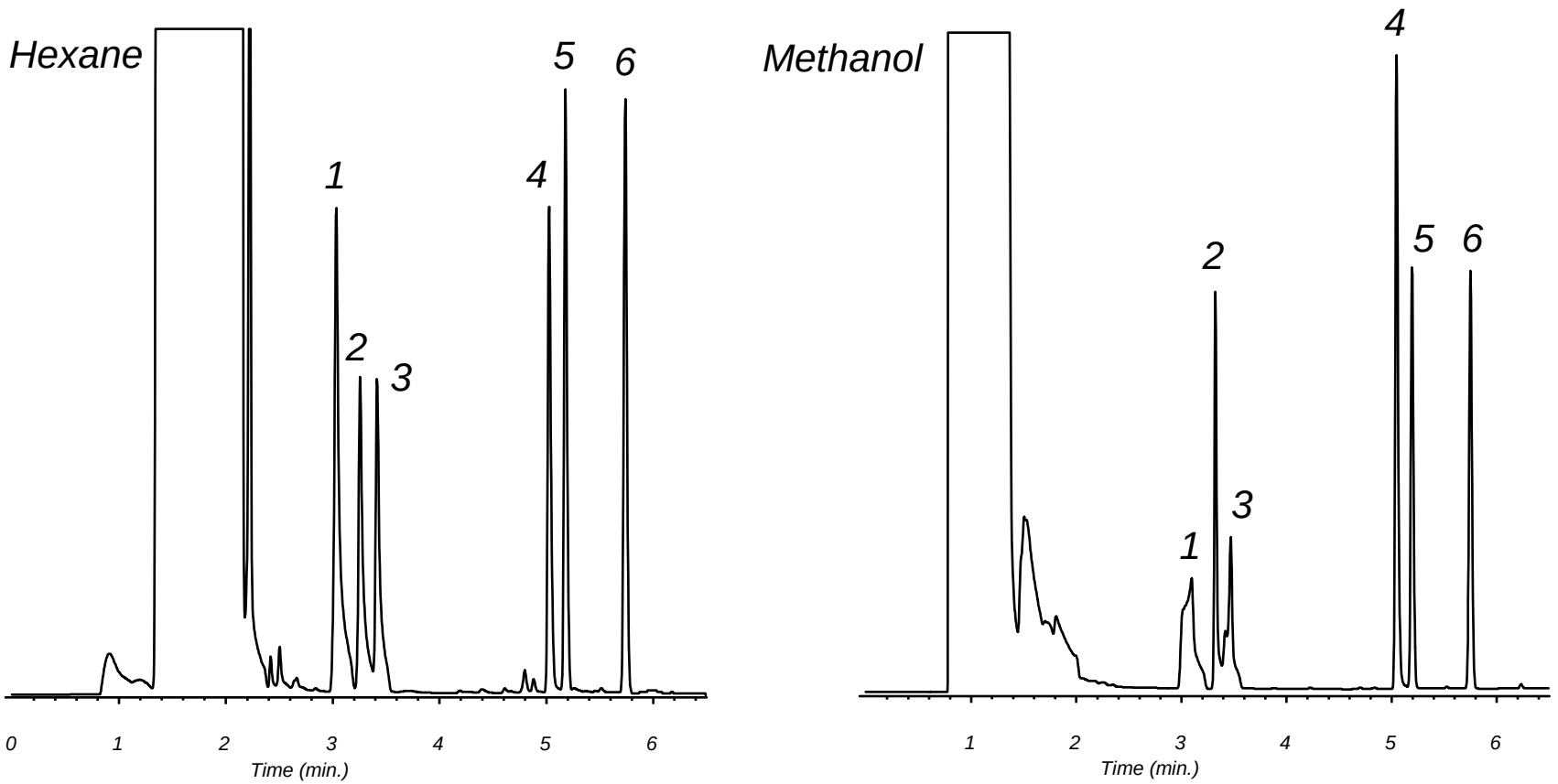


Influence of Injection Efficiency



Same column, same chromatographic conditions

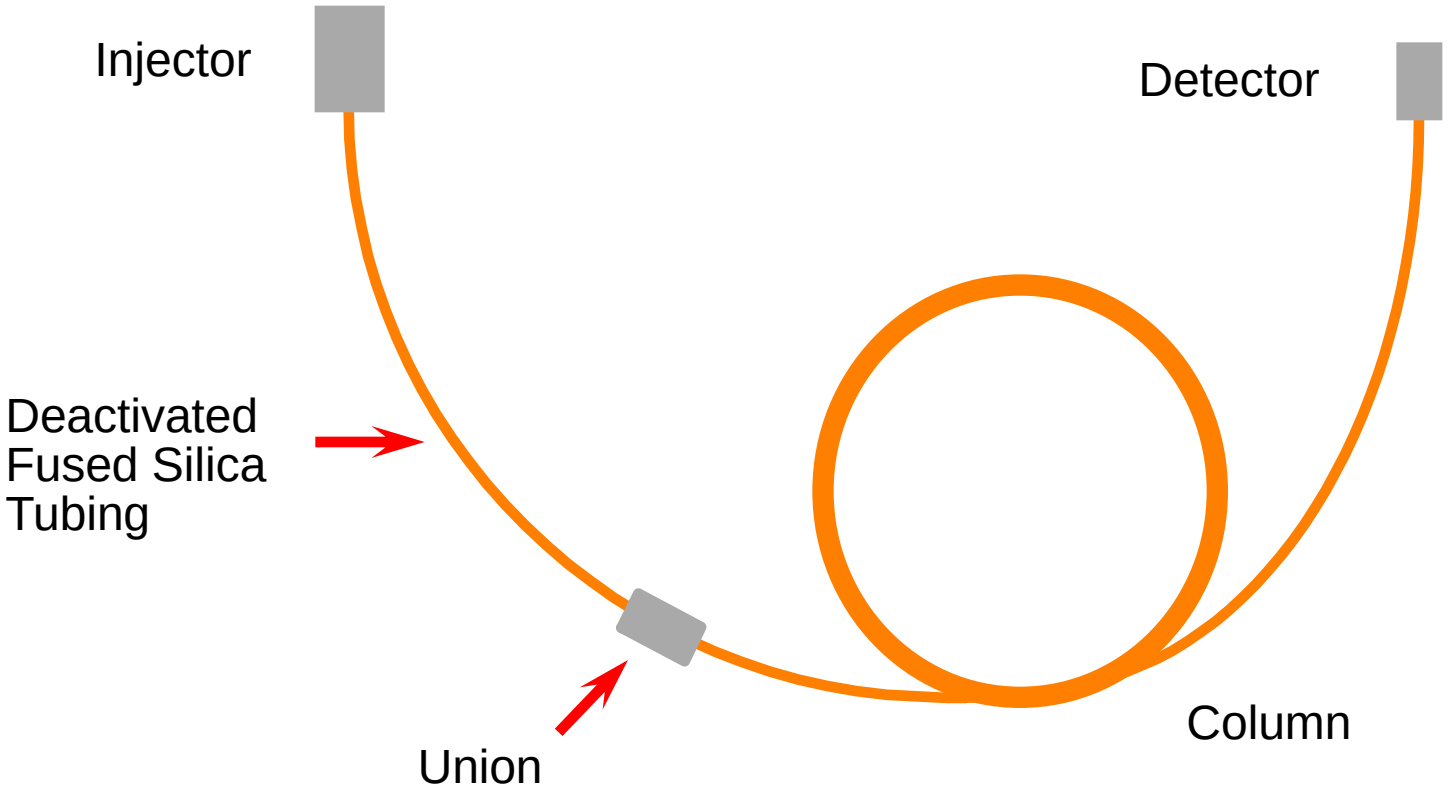
Splitless Injection
Reverse Solvent Effect



DB-1, 15 m x 0.25 mm I.D., 0.25 µm
50°C for 1 min, 50-210°C at 20°/min; Helium at 30 cm/sec
1. 1,3-DCP 2. 3-hexanol 3. butyl acetate 4. 1-heptanol 5. 3-octanone 6. 1,2-dichlorobenzene

Retention Gap

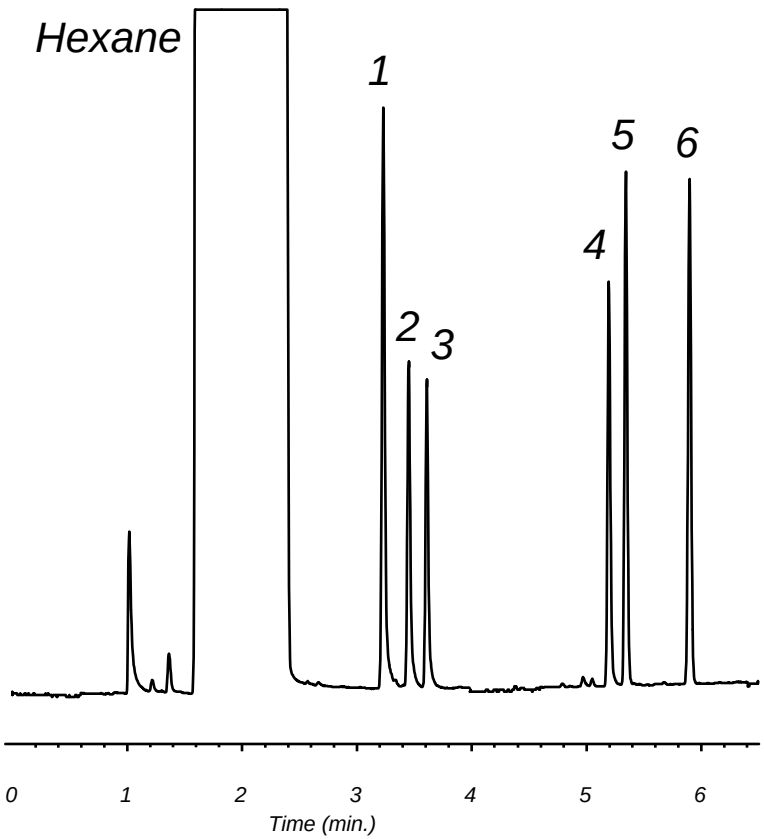
Also Called A Guard Column

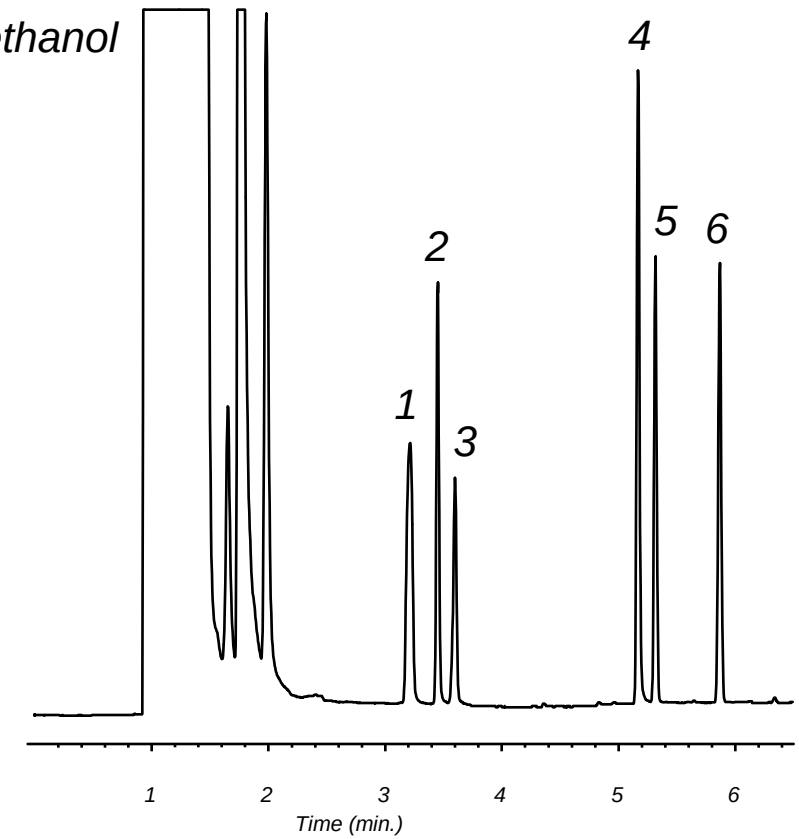


Usually 2-10 meters long and same diameter as the column
(or larger if needed)

Fixing the Reverse Solvent Effect

3 m x 0.25 mm I.D. Retention Gap





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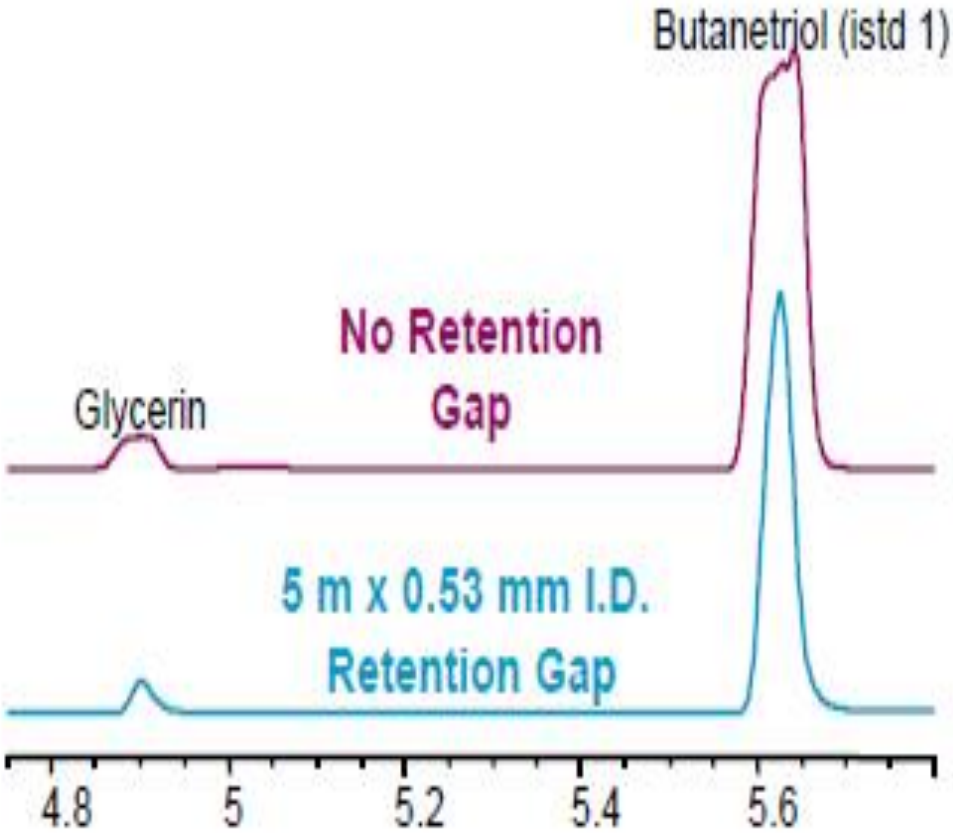
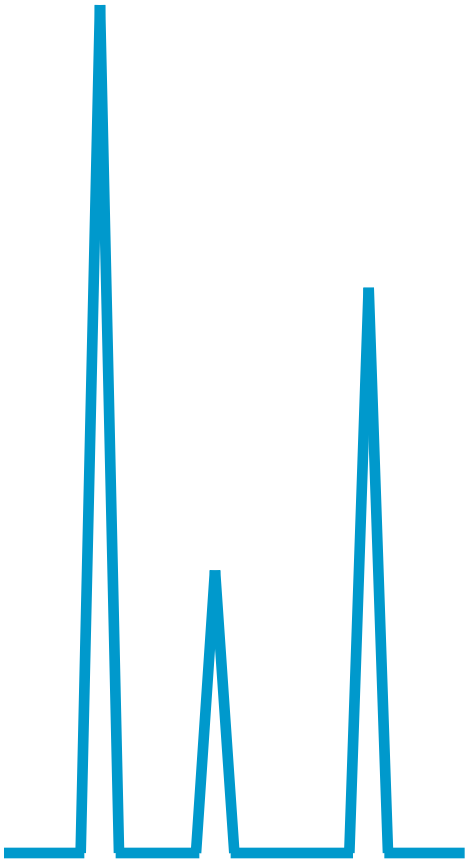
Ultimate Union and Retention Gap



No Peaks



DETECTOR (not on or not operational)

INJECTOR (not working)

-Plugged syringe/plunger not moving

-Wrong injector (or detector)

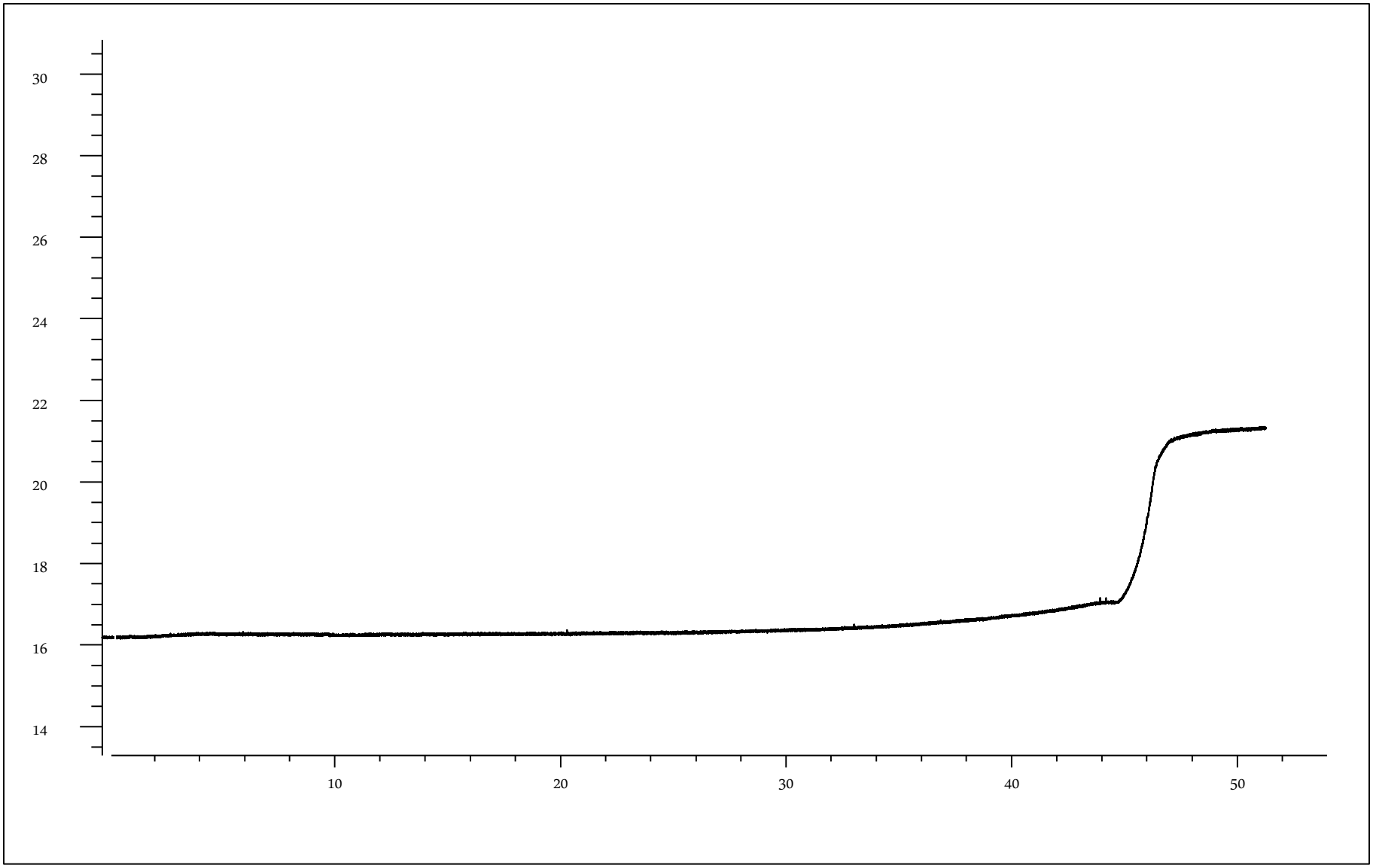
-Huge leak/no carrier gas flow (older systems)

NOT the COLUMN Unless…

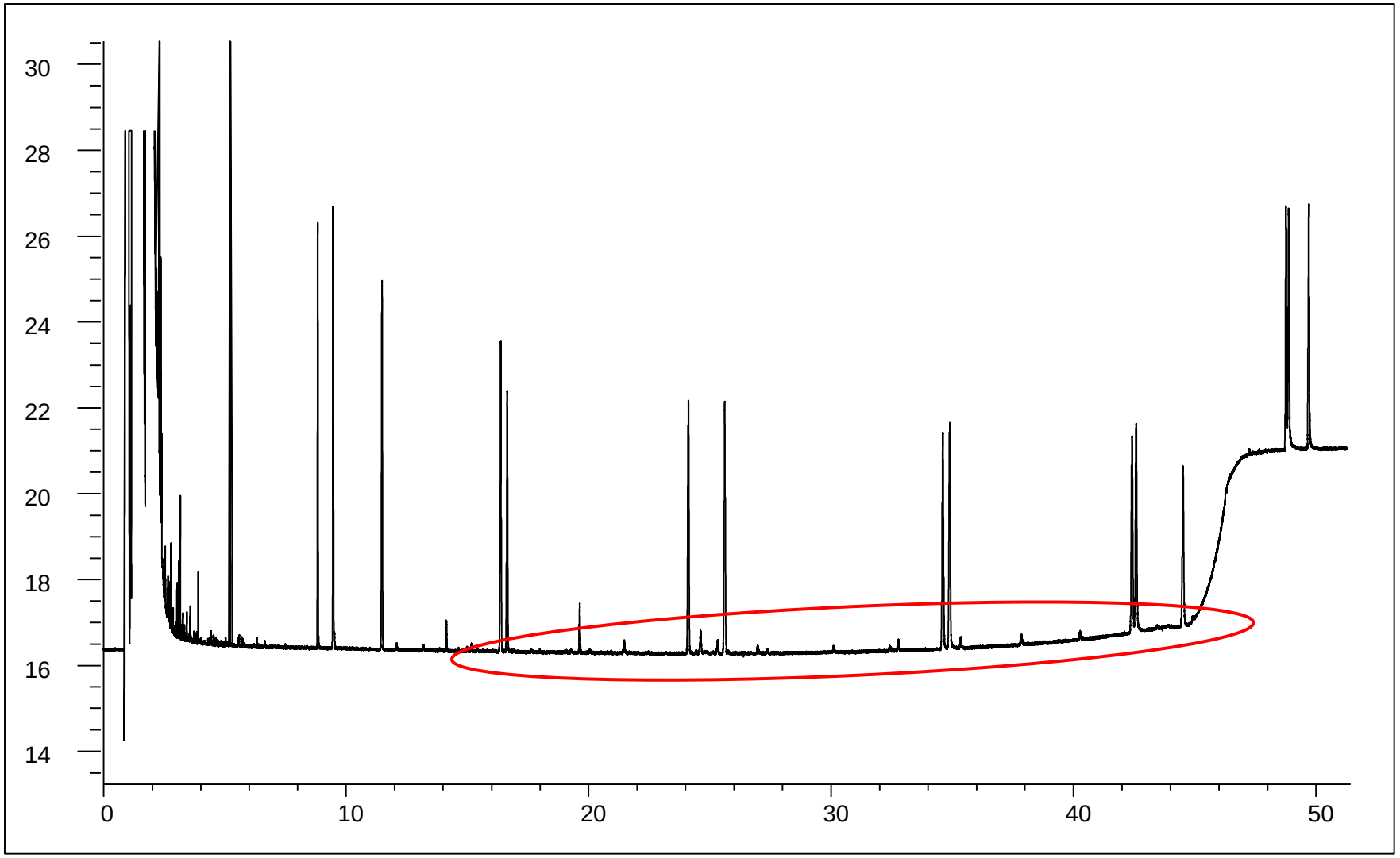
-Broken column

-No column

Symptom – No Peaks

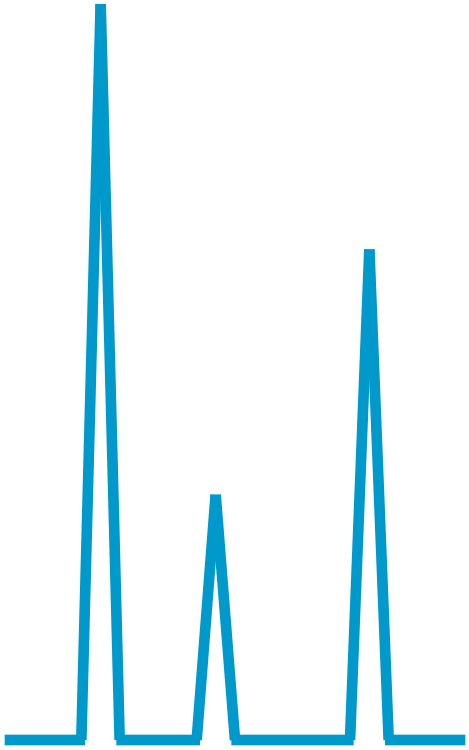


Solution - Unplugged Syringe



Peak Response

All Change in Size



INJECTOR

-Leaky syringe

-Split ratio set incorrectly

-Wrong purge activation time

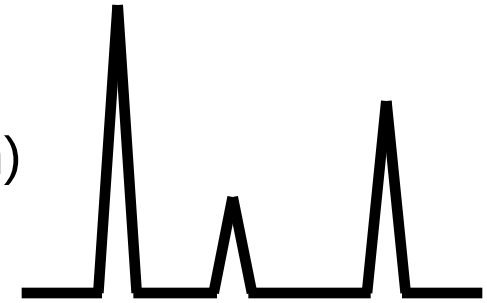
-Septum purge flow too high

-Injector temperature too low*

DETECTOR (response problem)

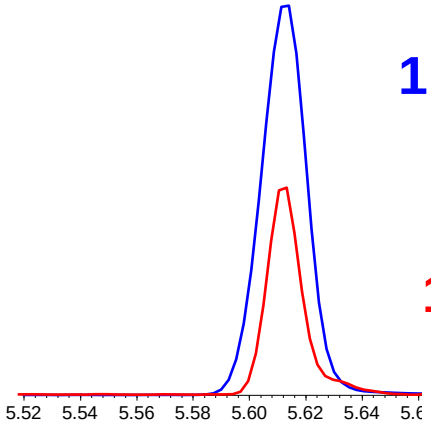
-Settings or flows changed

-Electronics failing



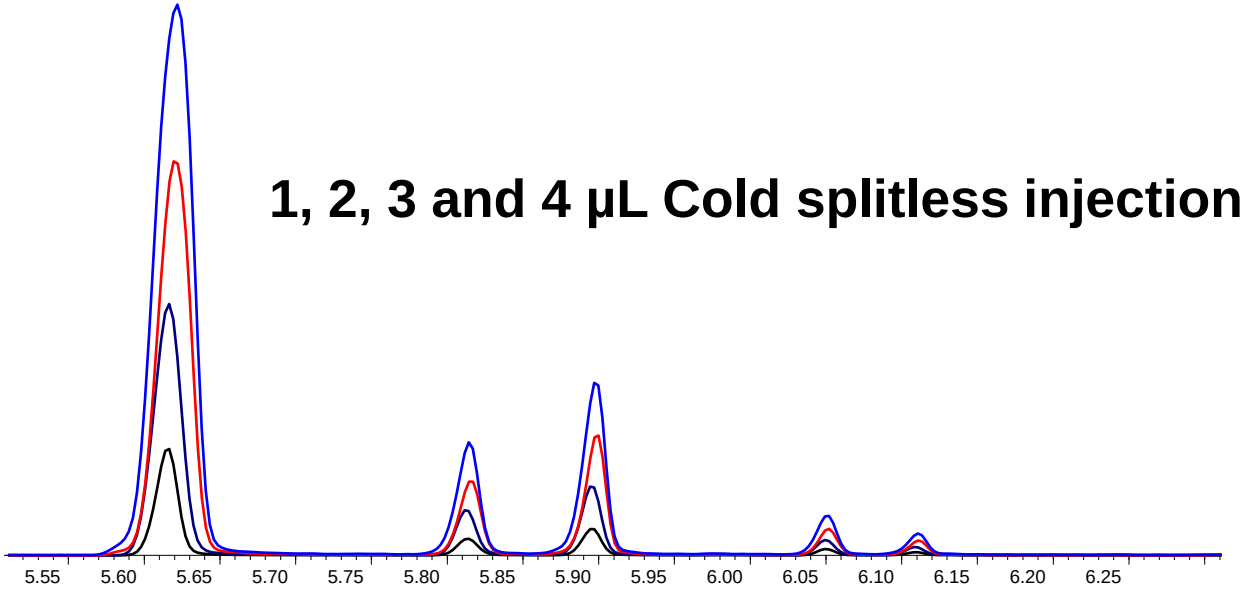
*Tip = Ask is it all of them or some of them, if all then injector or detector

Response Changes with Inlet Conditions
Multimode Injection LVI of Triazine Herbicides



1µL Cold Splitless injection

1µL Hot Splitless injection



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

Compare cold to hot splitless S/N using the new Multimode Inlet on a 7890-5975 GC/MSD system

Most “active” compound gives largest improvement

	RT	Mass	Ratio Cold S/N : Hot S/N
Fluorene	11.152	166	1.36
Aldrin	15.205	66	1.33
		263	1.33
Mevinphos	9.338	127	1.33
Pentachlorophenol	12.989	266	2.19
Endrin	17.600	263	1.48
p,p'-DDT	18.600	235	1.51

Response Changes with Inlet Conditions

MMI , N-nitrosodimethylamine, earliest eluter at 3.83 min

1 Hot Splitless

2 Pulsed Hot Splitless

3 Pulsed Hot Splitless with SPC

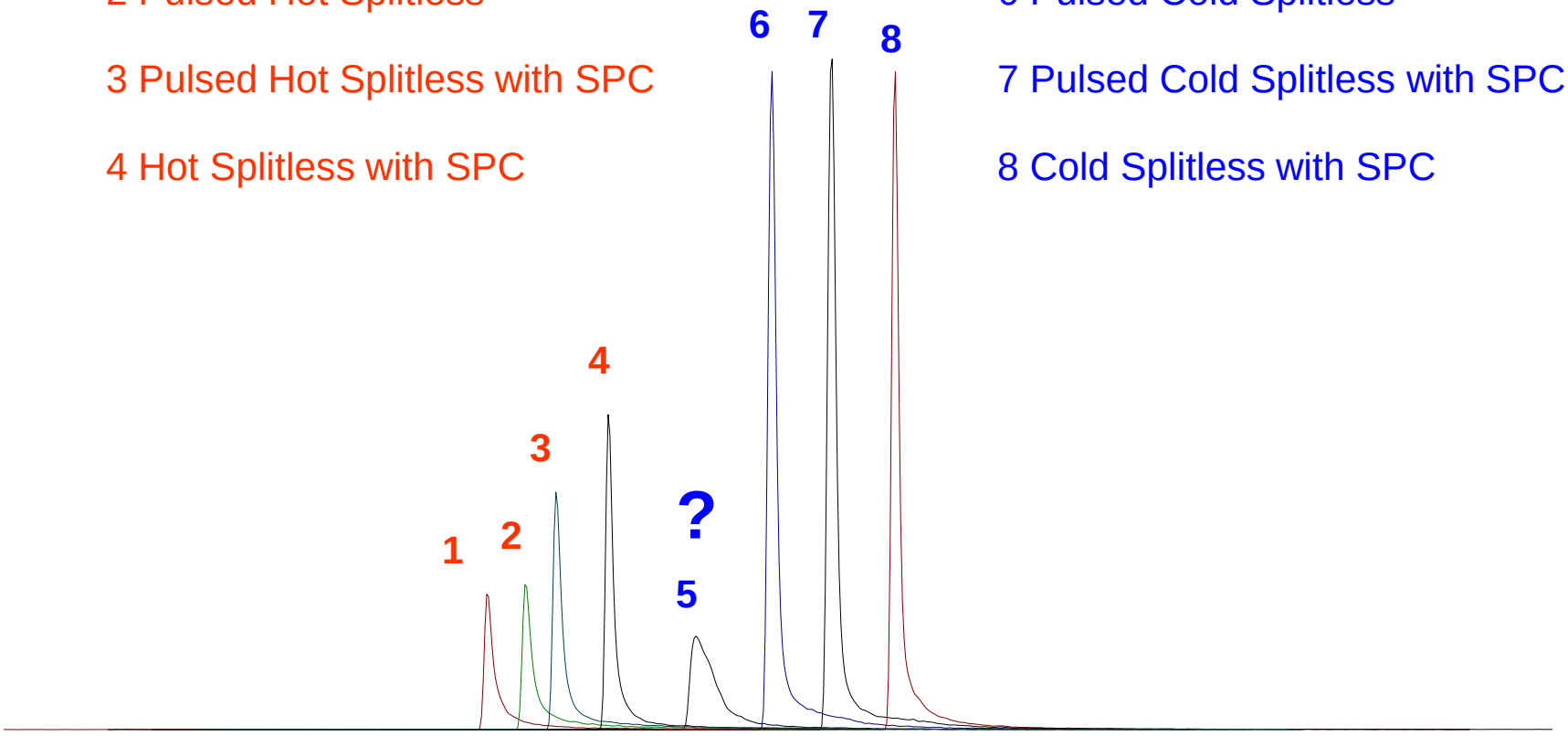
4 Hot Splitless with SPC

5 Cold Splitless

6 Pulsed Cold Splitless

7 Pulsed Cold Splitless with SPC

8 Cold Splitless with SPC



Data intentionally offset on y-axis

Reponse Changes with Inlet Conditions

MMI , Pentachlorophenol, 10.8 min

1 Hot Splitless

2 Pulsed Hot Splitless

3 Pulsed Hot Splitless & SPC

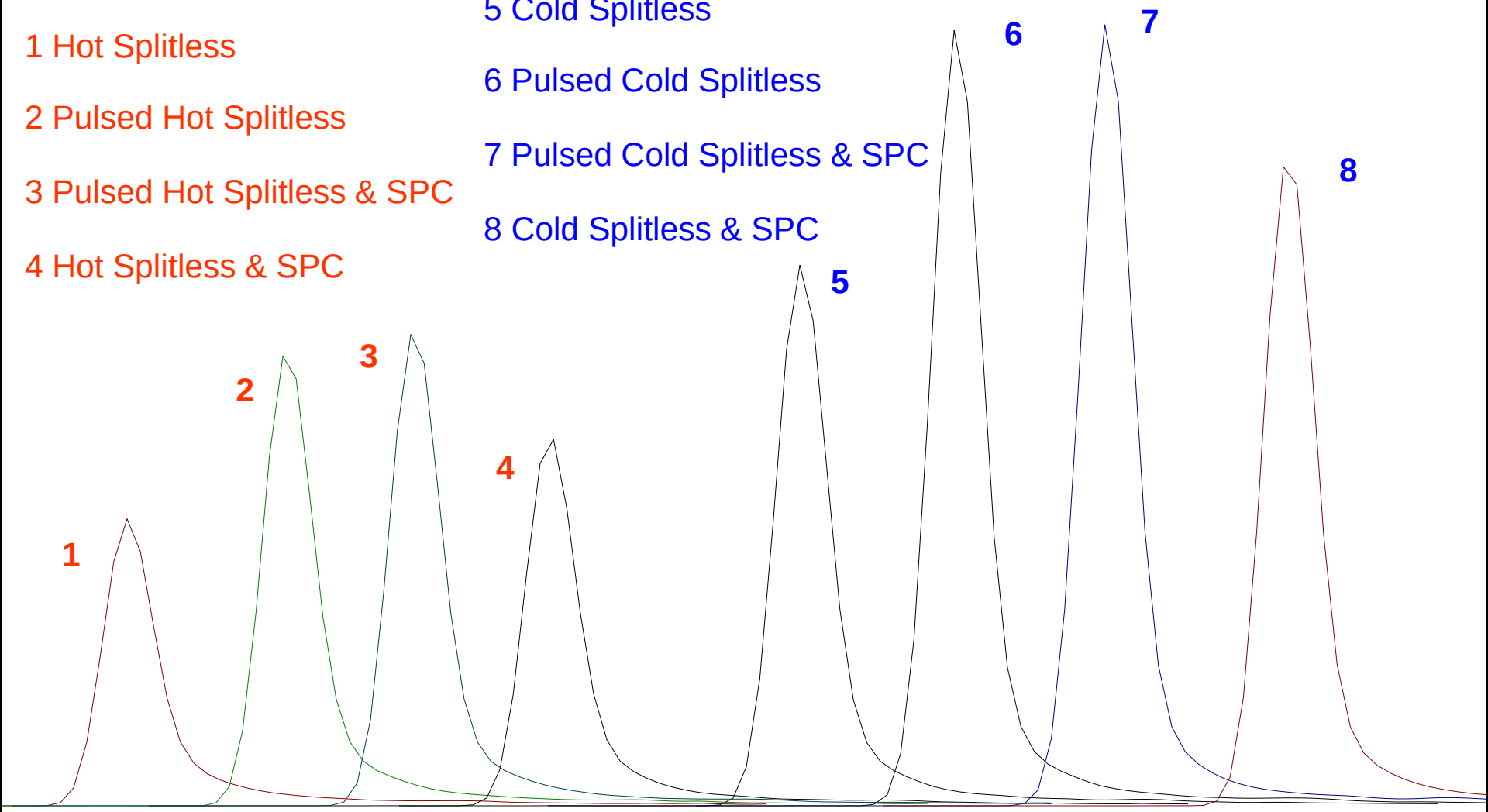
4 Hot Splitless & SPC

5 Cold Splitless

6 Pulsed Cold Splitless

7 Pulsed Cold Splitless & SPC

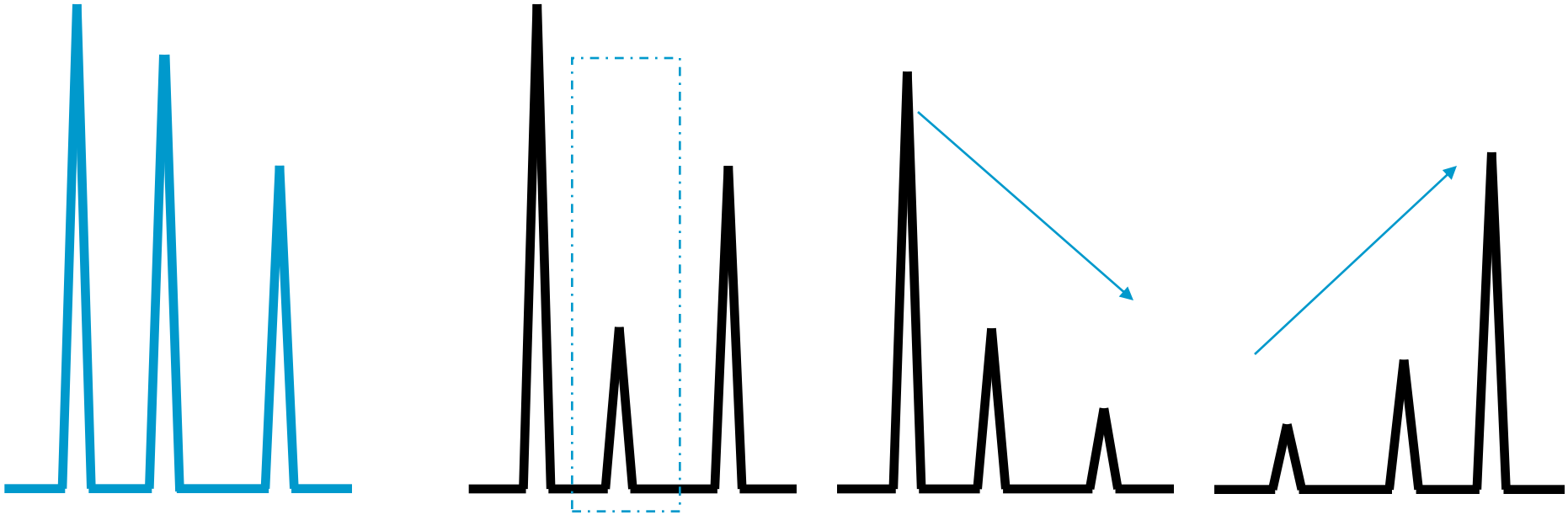
8 Cold Splitless & SPC



Data intentionally offset on y-axis

Peak Response

Some Change in Size



INJECTOR or COLUMN is active/contaminated

-Irreversible adsorption of active compounds (-OH, -NH, -SH)

-Decomposition of sample

OTHER

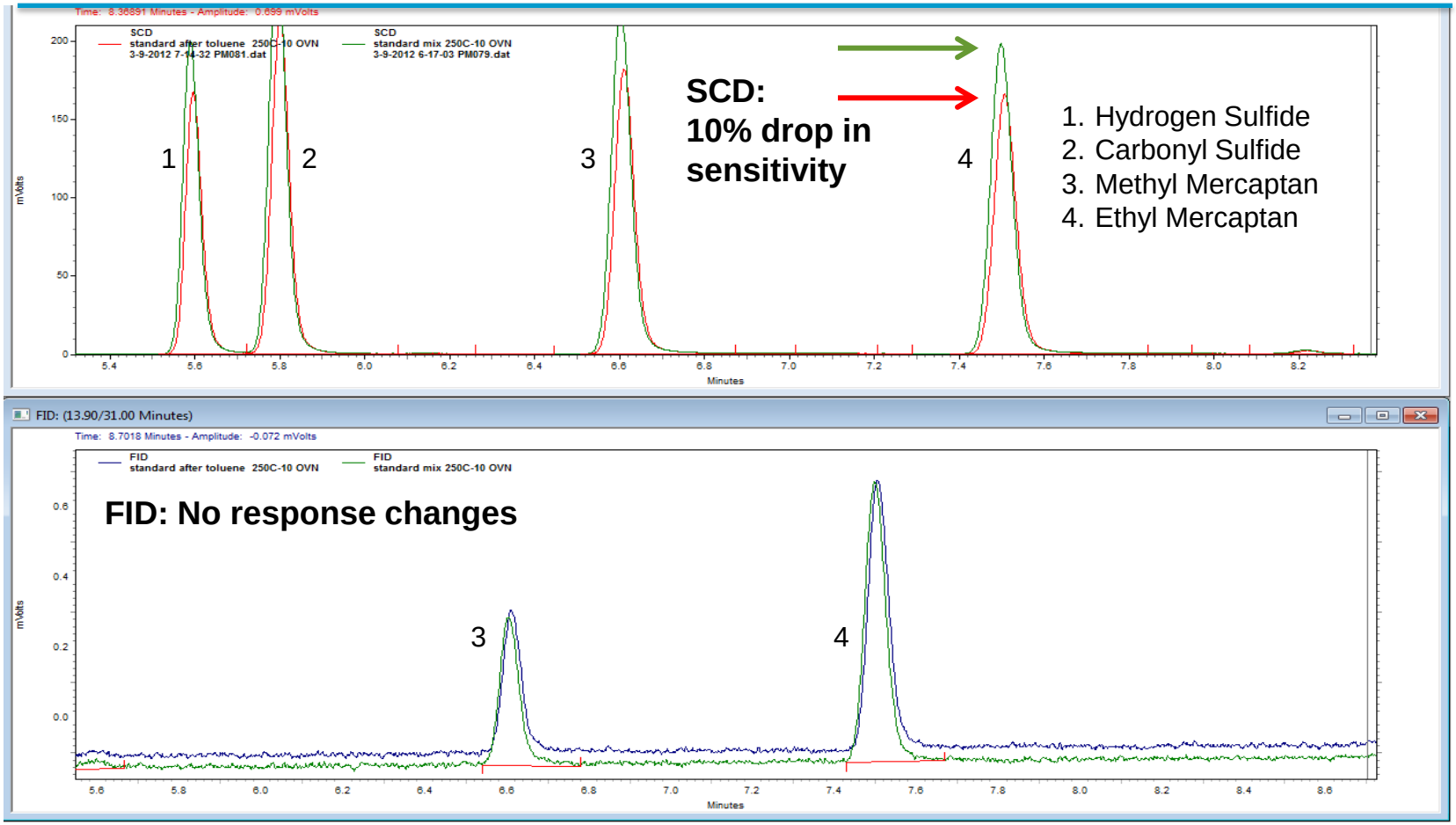
-Temperature Change – Discrimination

-Evaporation from sample

*Tip = If only some change, then ask which ones? If active compounds then activity. If tracks volatility then cold spots or inlet discrimination.

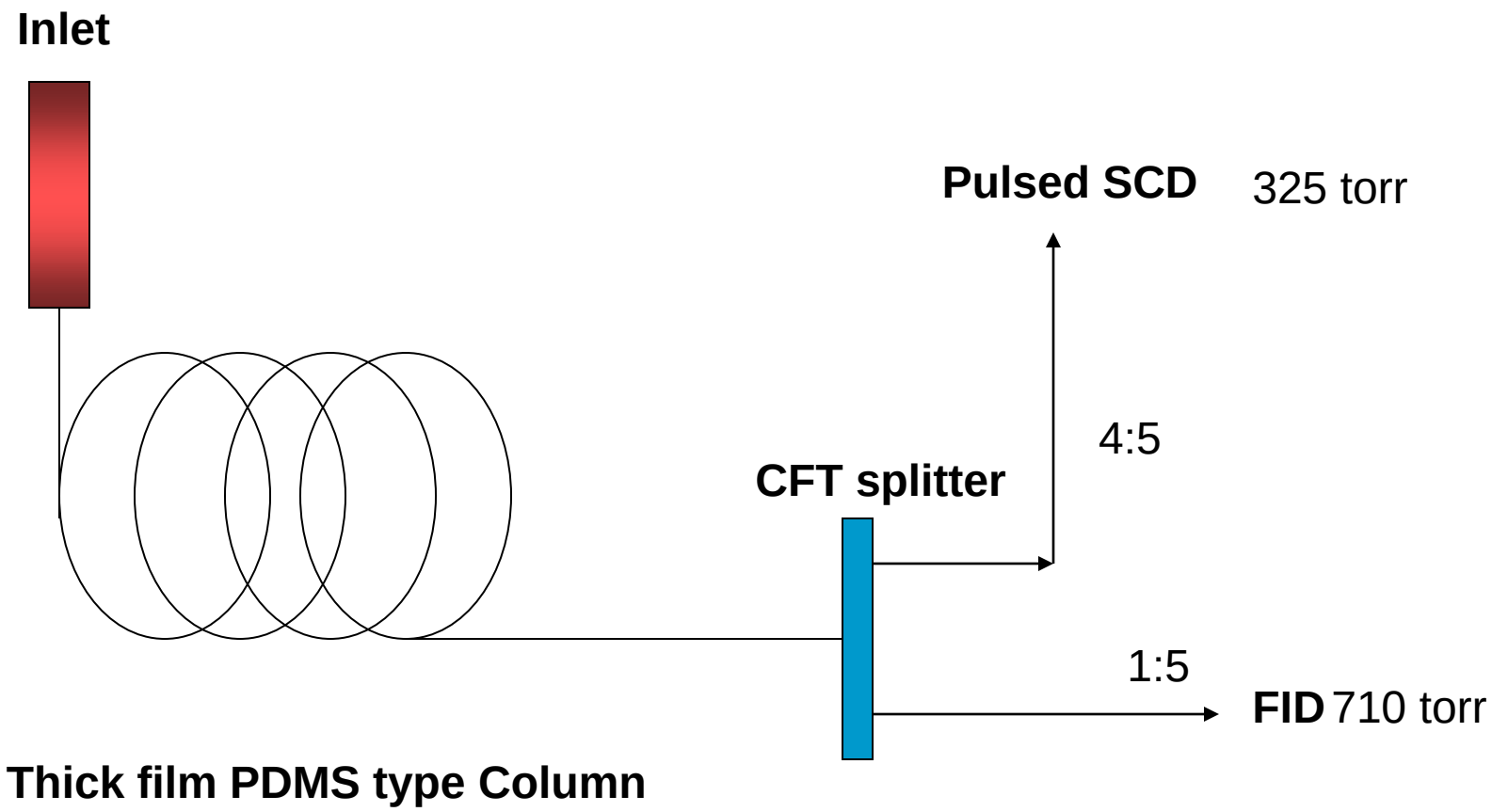
Symptom – Loss of Response of Active Compounds on SCD but not FID

Overlay of before (green) and after 2 x 2 uL neat toluene Injection (red)



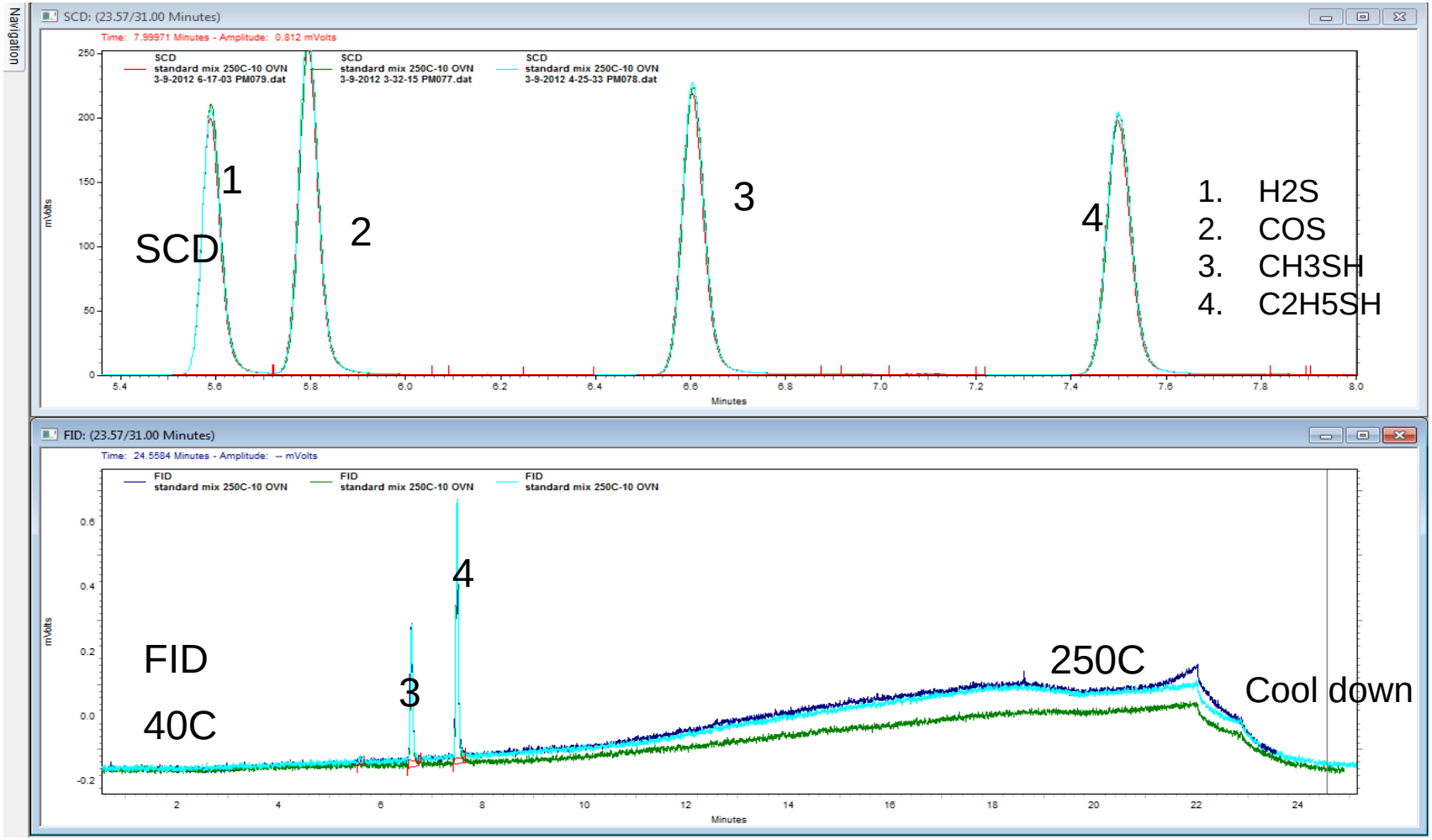
Data courtesy of Jim Luong, Ronda Gras, Myron Hawryluk of Dow Chemical Canada

Configuration to test SCD Quenching Issue



Data courtesy of Jim Luong, Ronda Gras, Myron Hawryluk of Dow Chemical Canada

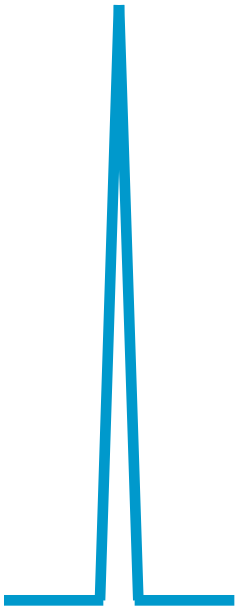
Solution - New DB-Sulfur SCD column
No Column Bleed Contamination of SCD



Data courtesy of Jim Luong, Ronda Gras, Myron Hawryluk of Dow Chemical Canada

Peak Fronting

Shark Fin Shaped or Just Slight



COLUMN (contaminated)

-Overload (More pronounced with large solute and phase polarity differences)

INJECTOR

-Poor efficiency (flow/temp)

-Column installation

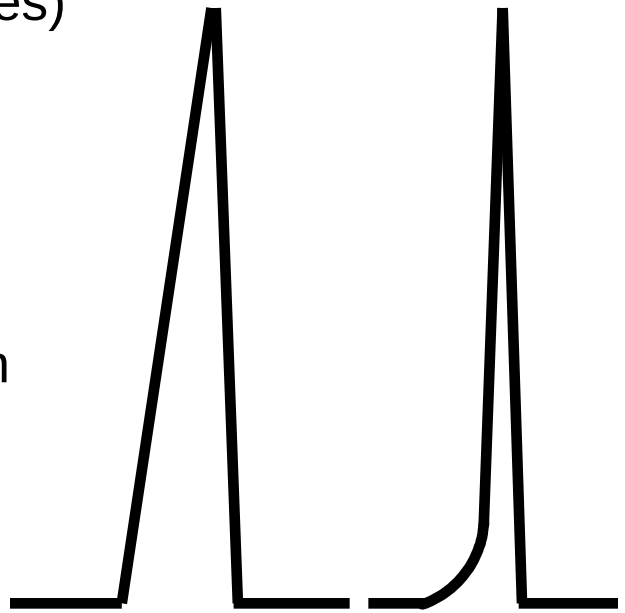
-Compound very soluble in injection solvent (need retention gap)

-Mixed sample solvent

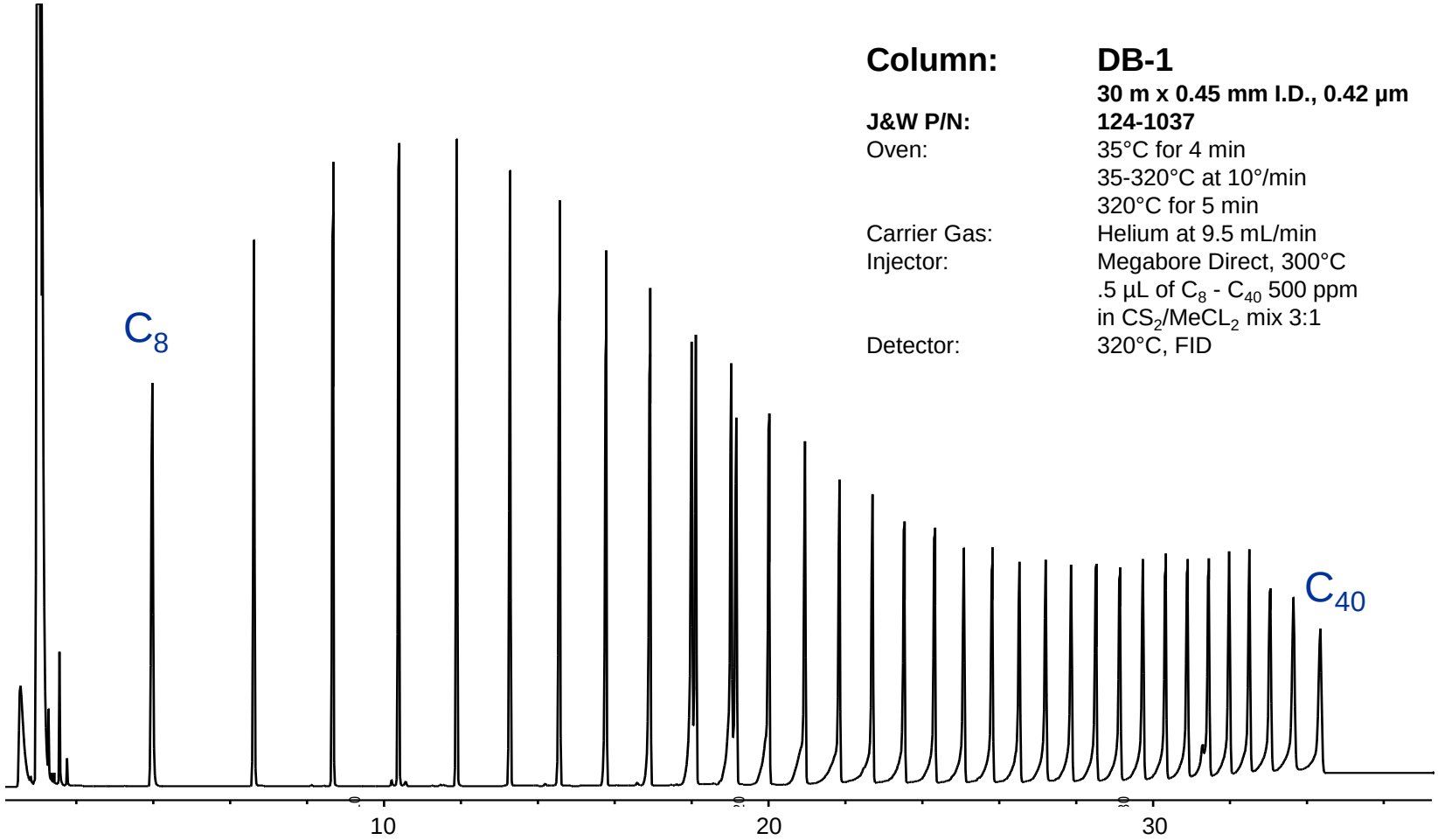
OTHER

-Co-elution

-Breakdown

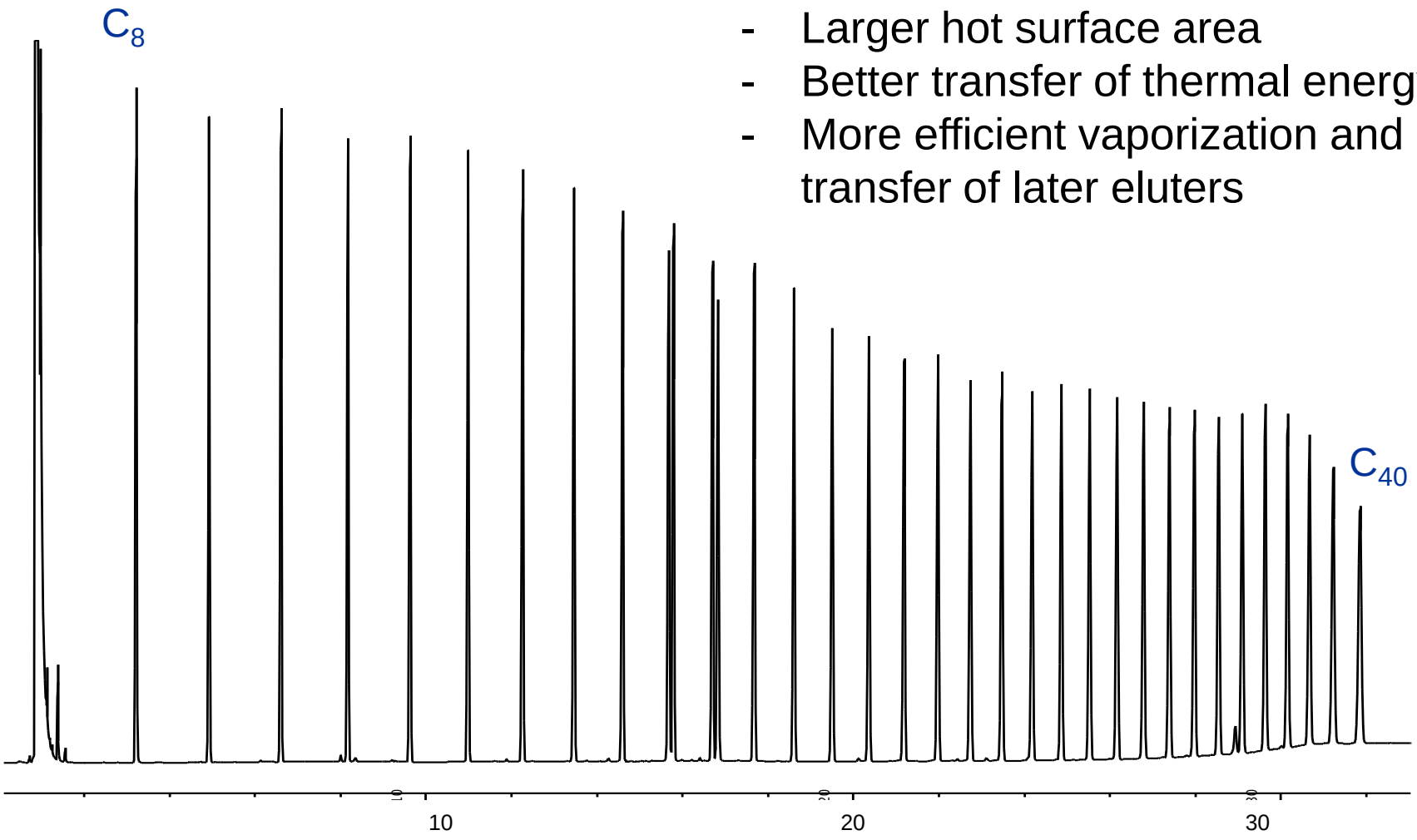


Symptom – Fronting/Discrimination of Later Eluters



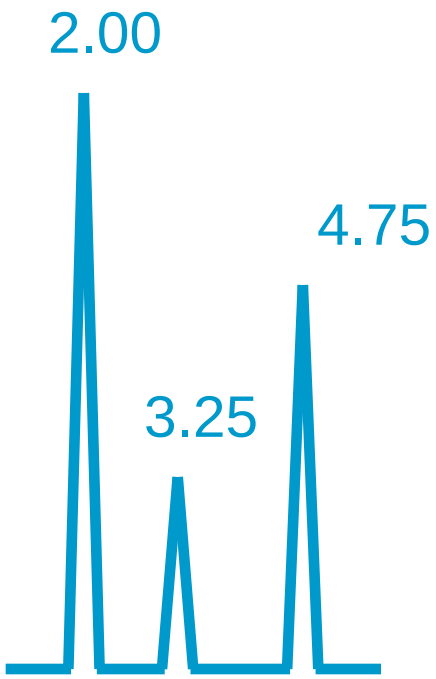
Column:	**DB-1**
	30 m x 0.45 mm I.D., 0.42 µm
J&W P/N:	**124-1037**
Oven:	35°C for 4 min 35-320°C at 10°/min 320°C for 5 min
Carrier Gas:	Helium at 9.5 mL/min
Injector:	Megabore Direct, 300°C .5 µL of C_8 - C_{40} 500 ppm in CS_2/$MeCL_2$ mix 3:1
Detector:	320°C, FID

Solution - Large Glass Wool Plug Added to Liner



- Larger hot surface area
- Better transfer of thermal energy
- More efficient vaporization and transfer of later eluters

Retention Time Shift



INJECTOR

-Change in injection solvent

-Large change in sample concentration

FLOW

-Leak in the septum

-Change in gas velocity

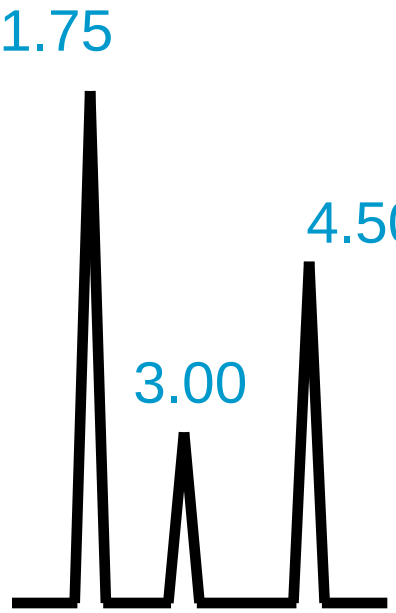
COLUMN

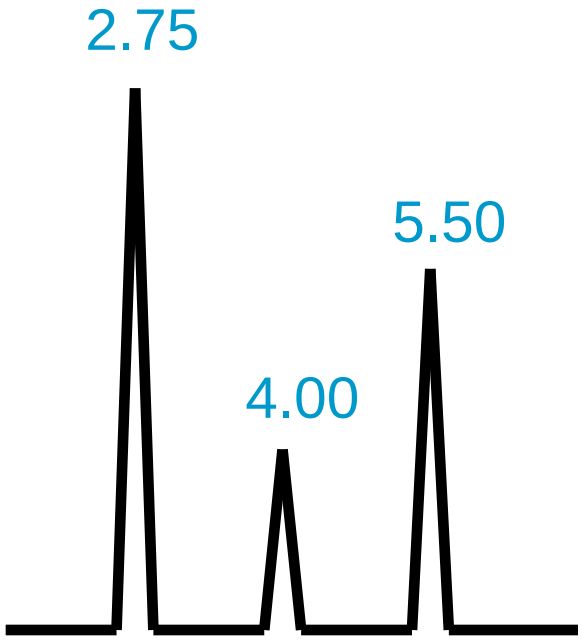
-Contamination

-Damaged stationary phase

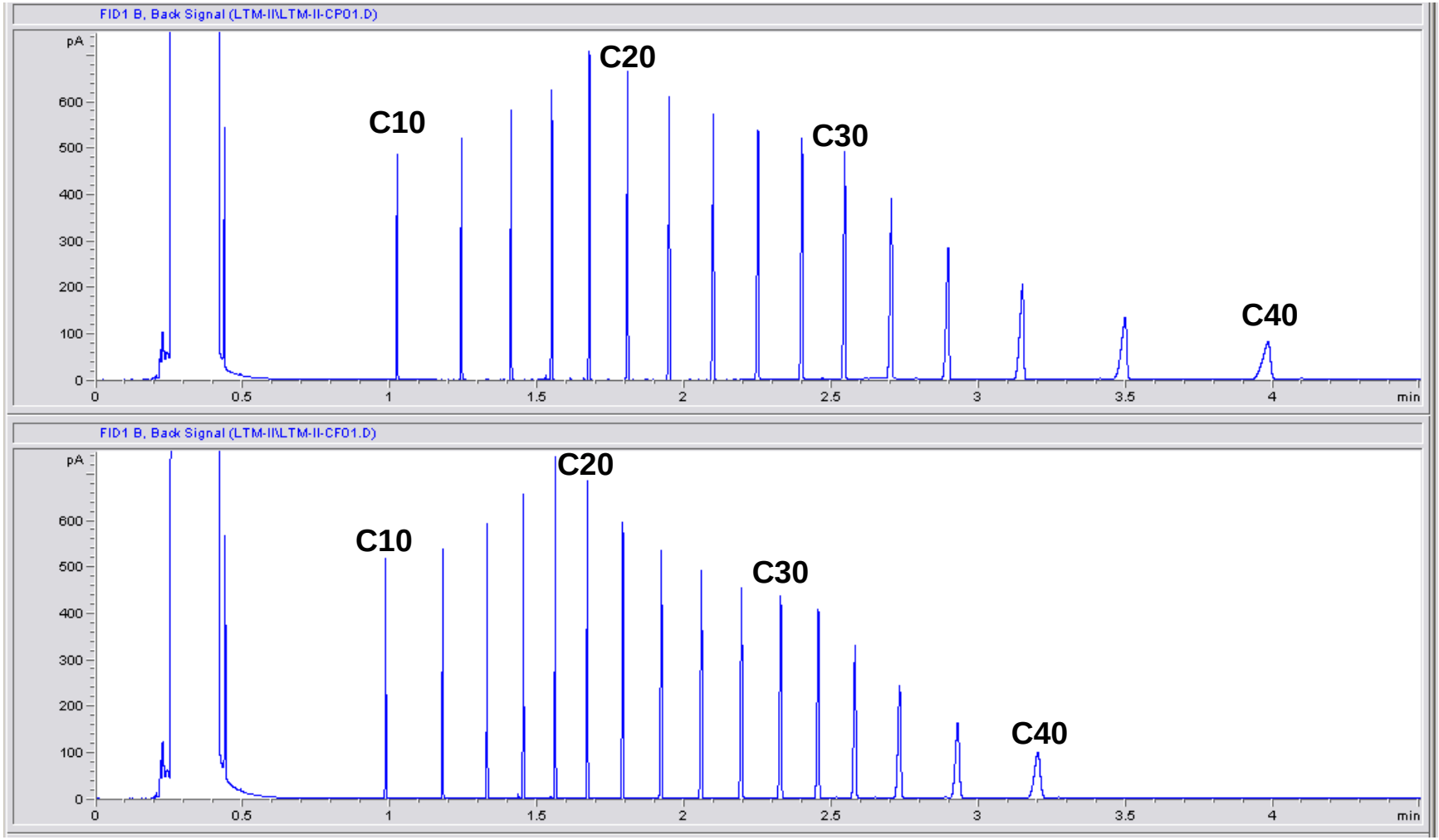
-Loss of stationary phase

-Change in temperature



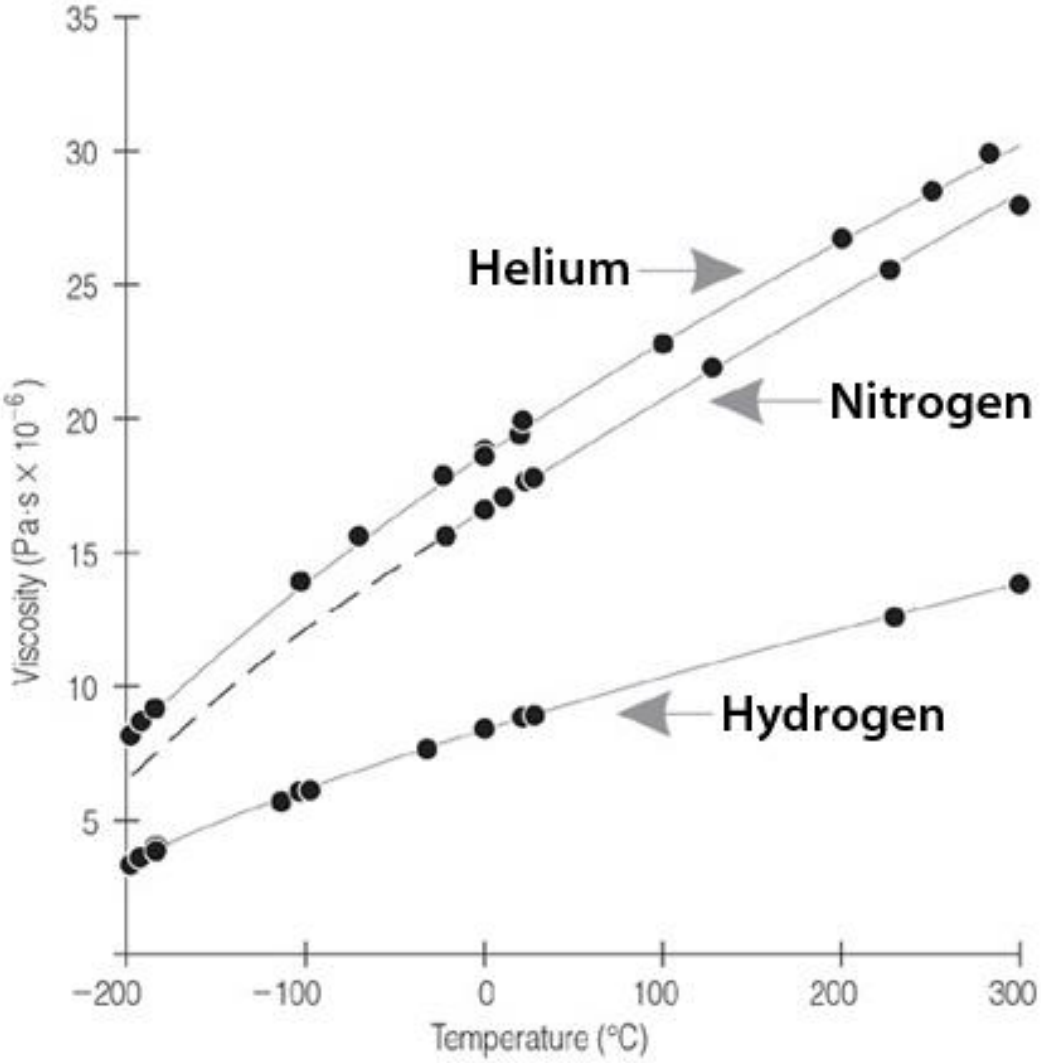


Constant Pressure vs Constant Flow Retention



Under constant pressure conditions, flow decreases as temperature increases. (viscosity of a gas increases as temperature increases)

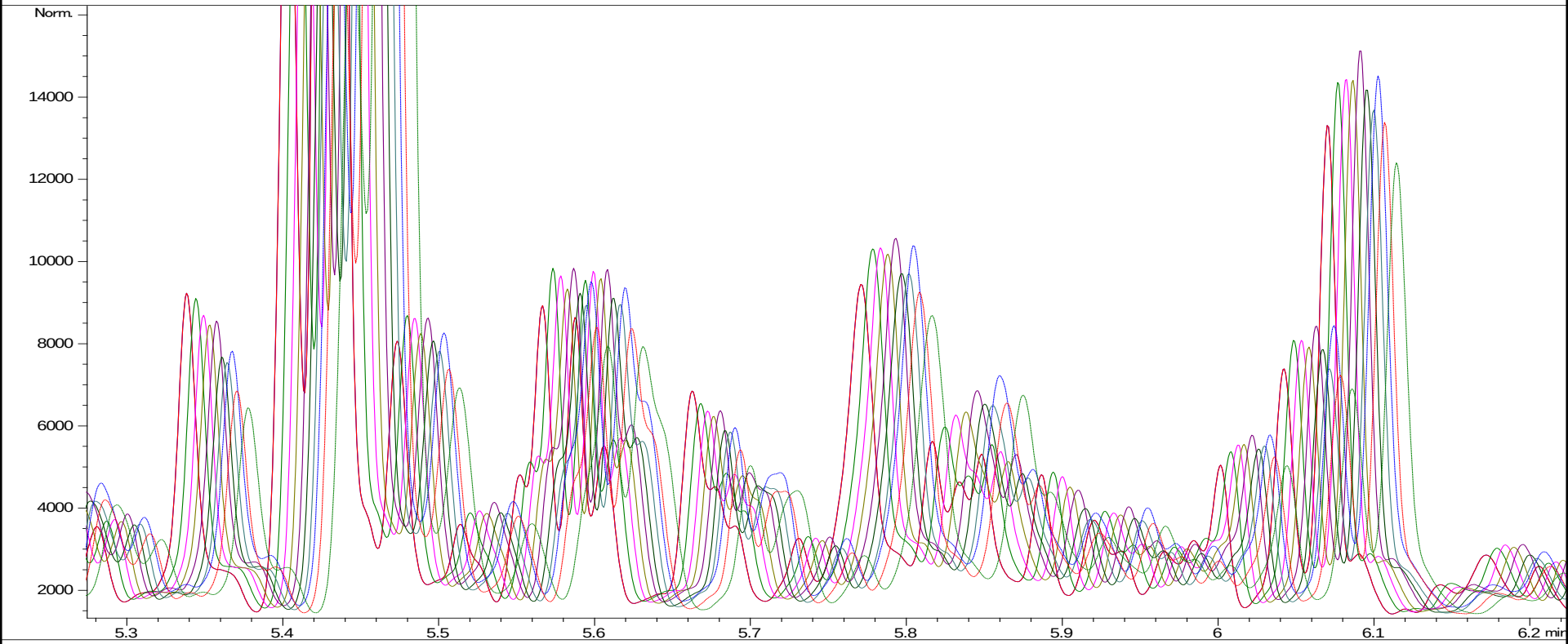
Gas Viscosity vs Temperature



J.V. Hinshaw, Column Connections, LCGC Asia Pacific, 12(2), 1100 (2009).

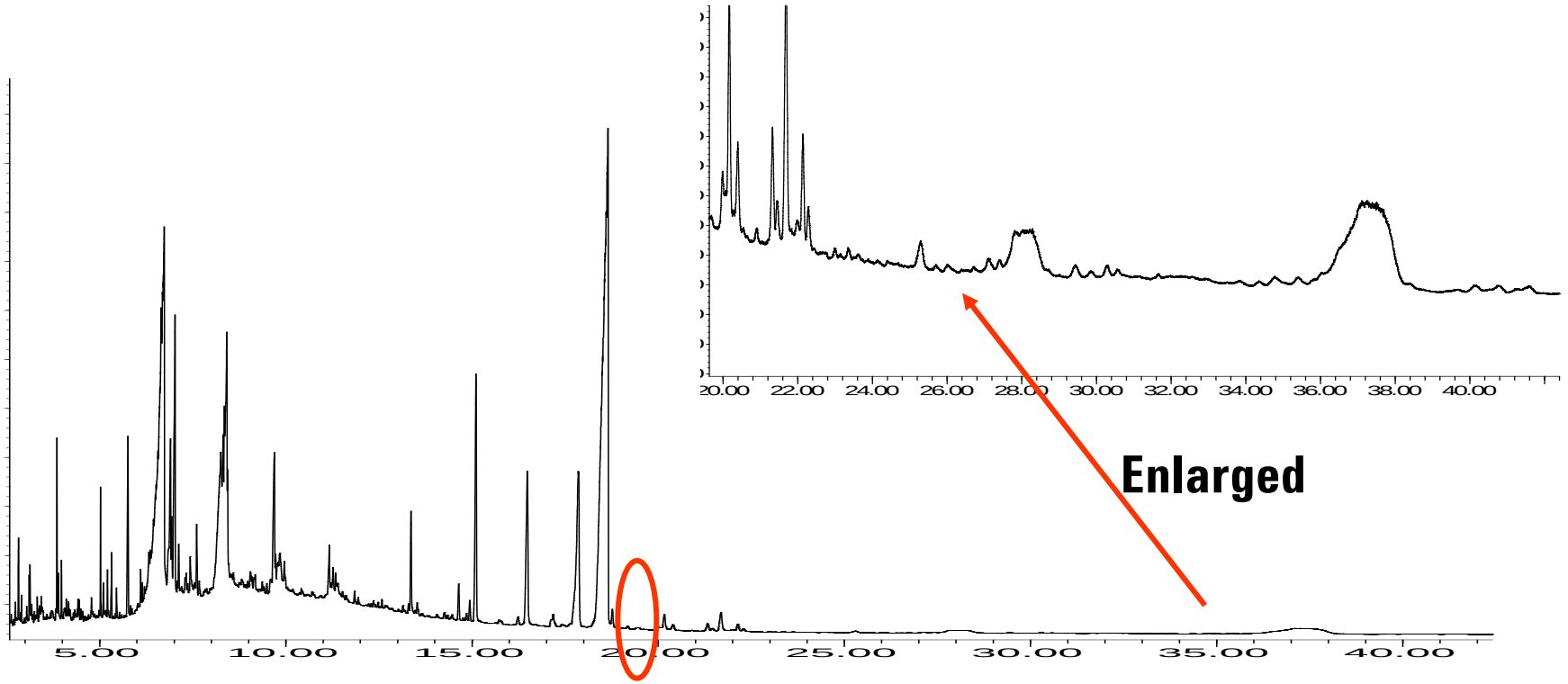
Symptom - Retention Times Shift 4-5 sec

Fish Oil Sample - Overlay of first 10 runs

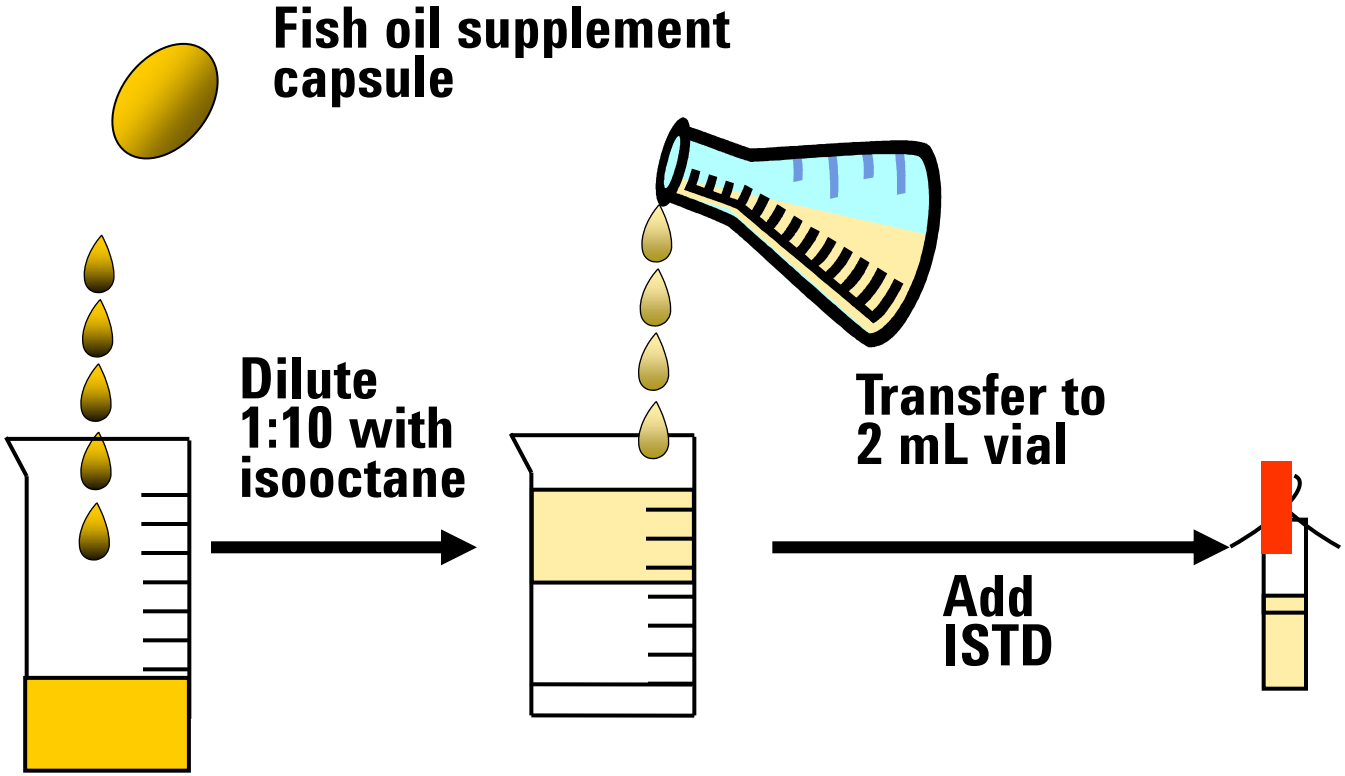


GC-FID Chromatogram of Fish Oil

1-µL splitless injection of 10% fish oil
GC oven was held at 290 °C for 30 min at the end of the run.

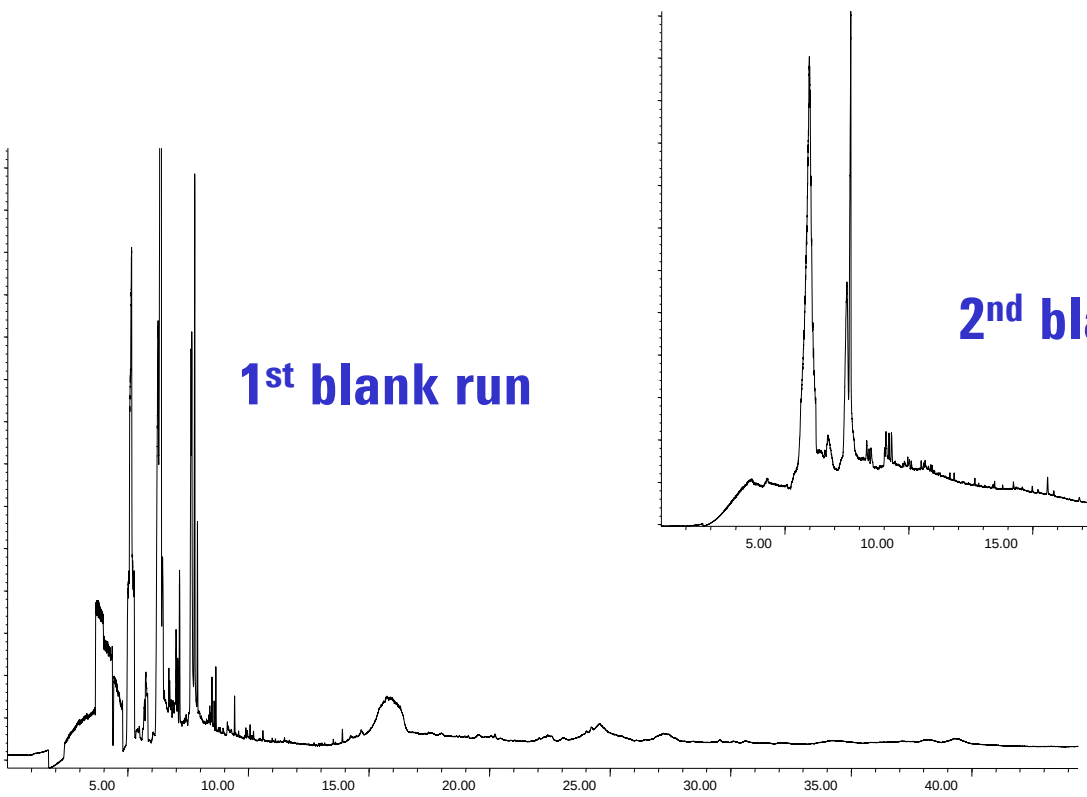


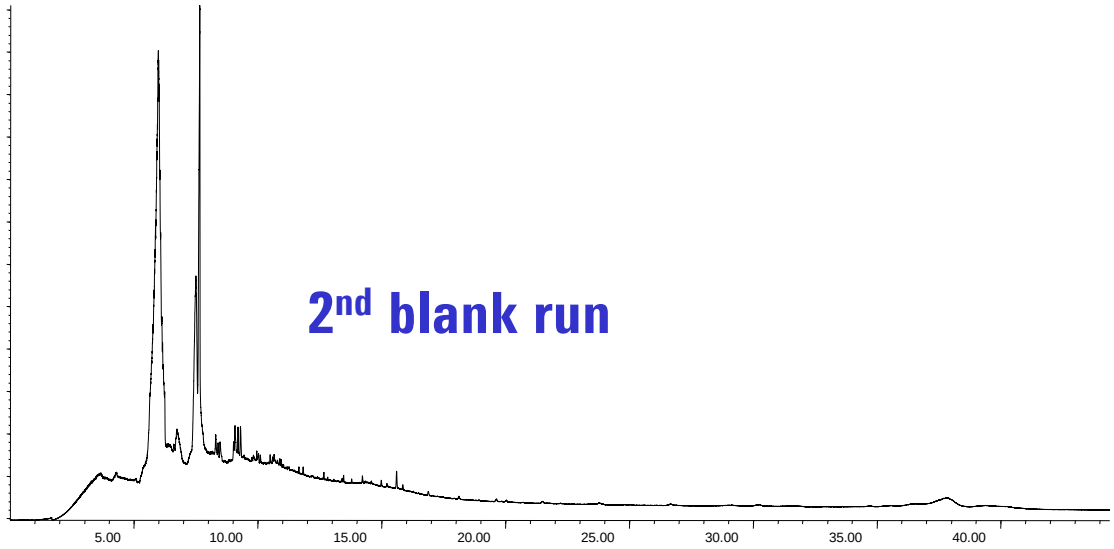
Fish Oil Sample Preparation is “Dilute and Shoot”



Two Blank Runs

GC oven temperature held at 320 °C for 30 minutes at end of each run

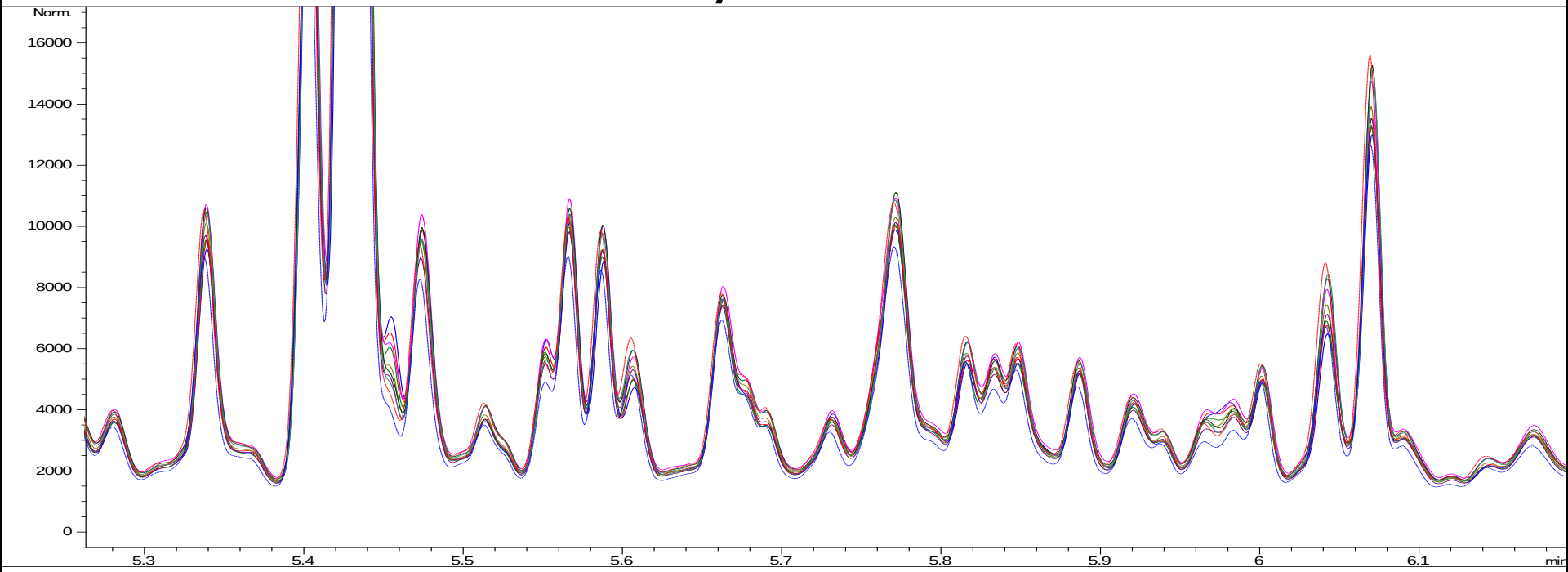




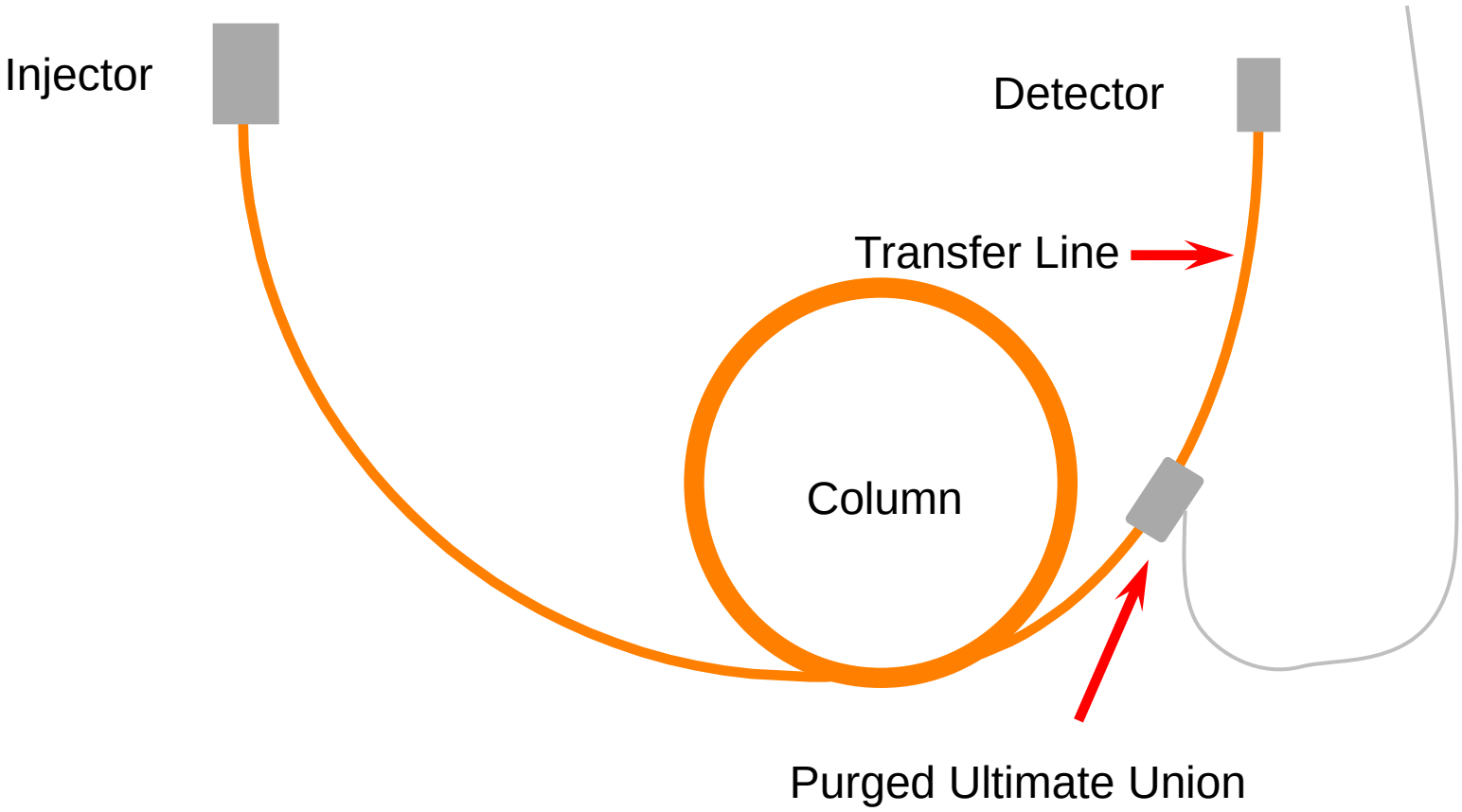
Solution - Get High M.W. Fish Oil Off the Column After Every Run = No More Shifting Retention

Retention Time Precision with Backflushing

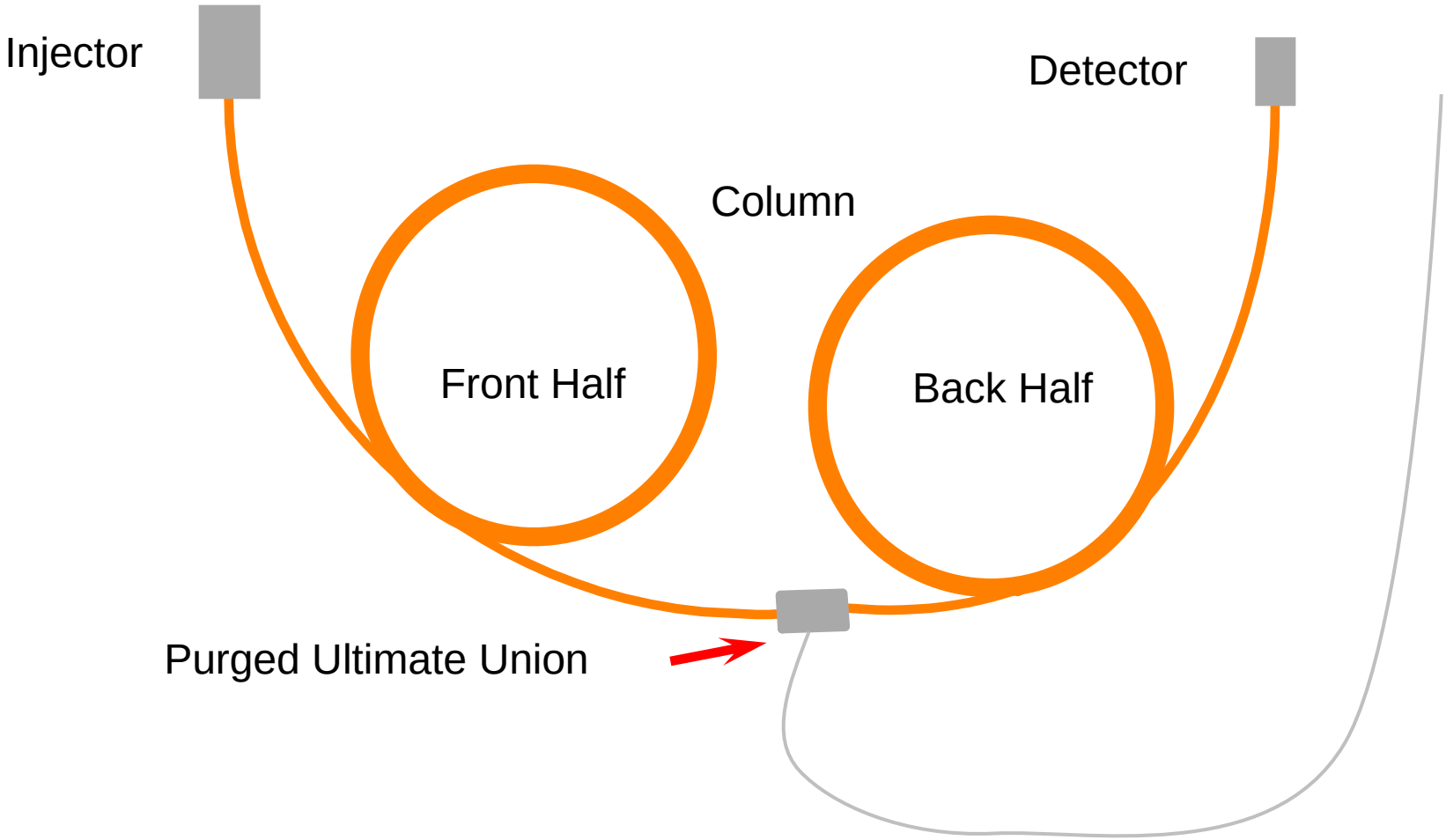
Overlay of 10 Runs



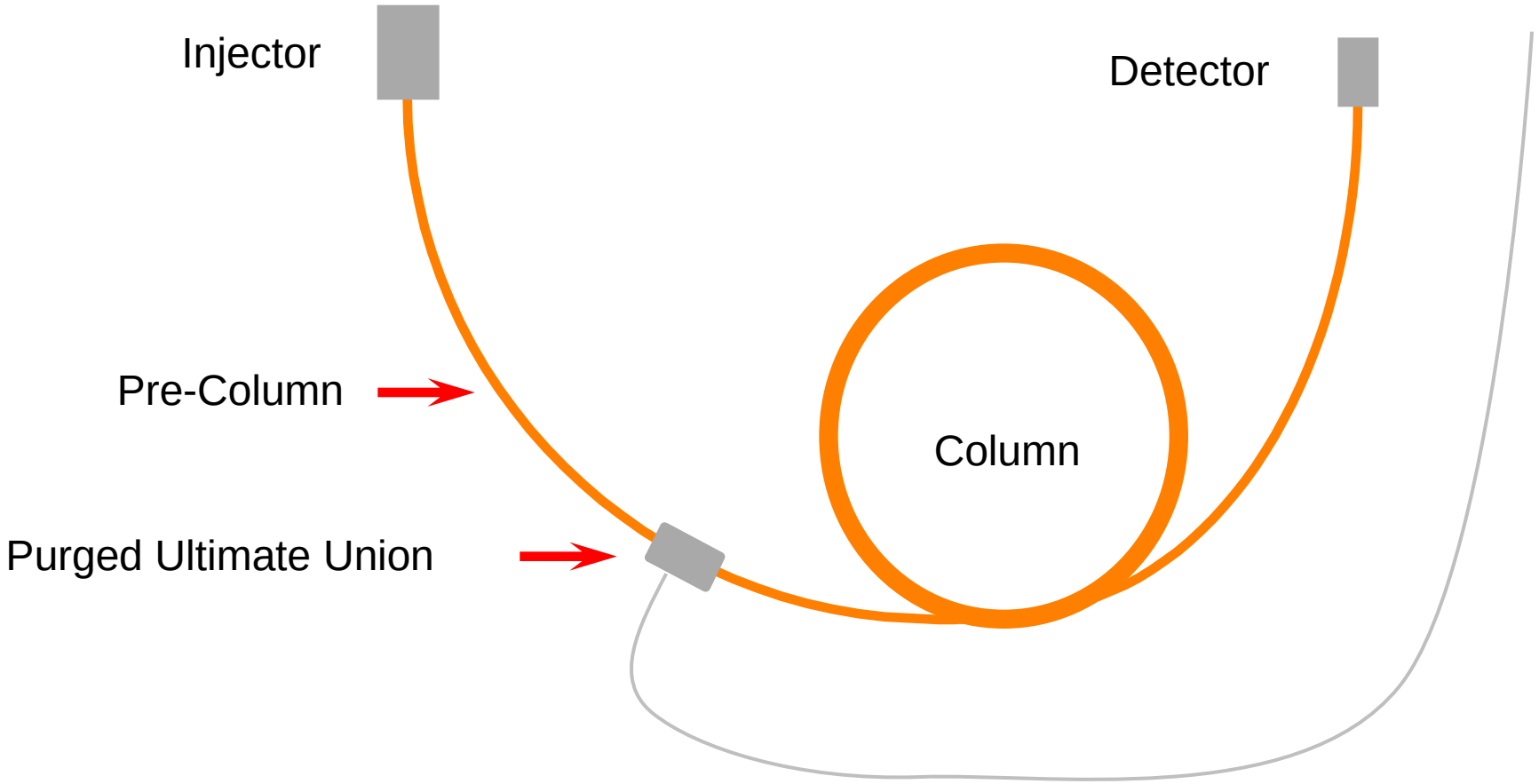
Post-Column Backflush



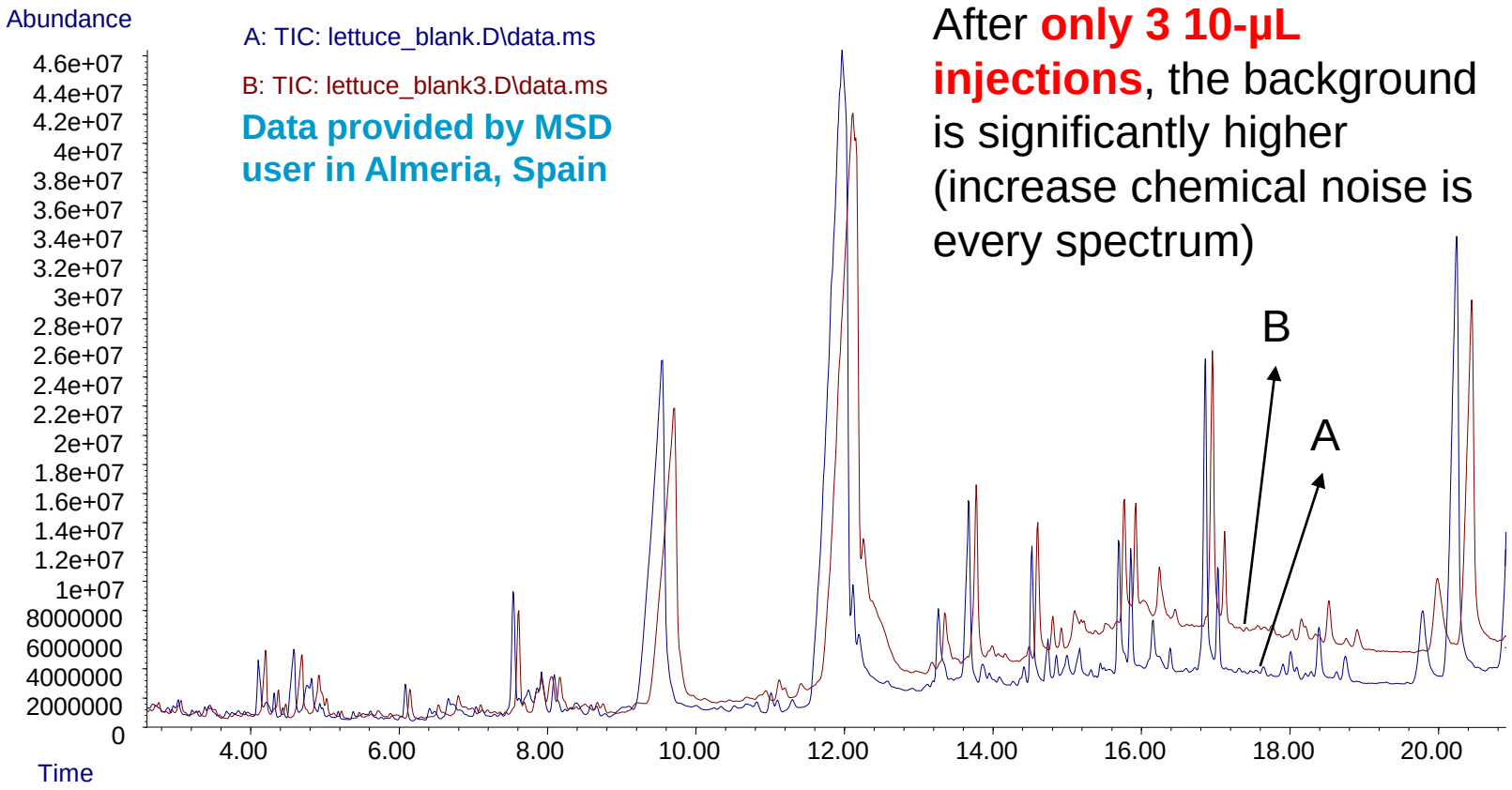
Mid-Column Backflush



Pre-Column Backflush

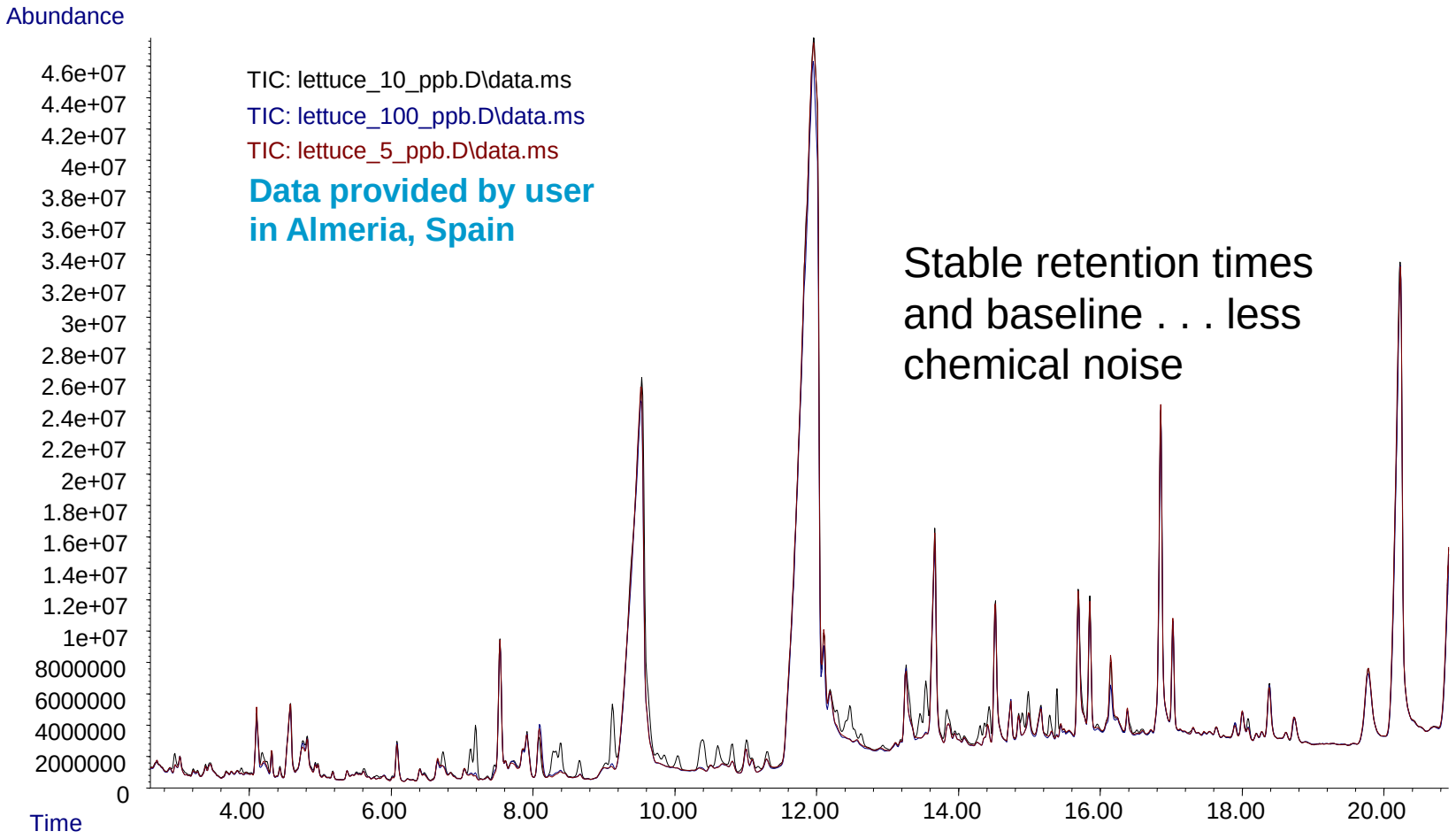


Without Backflush: A Serious Problem



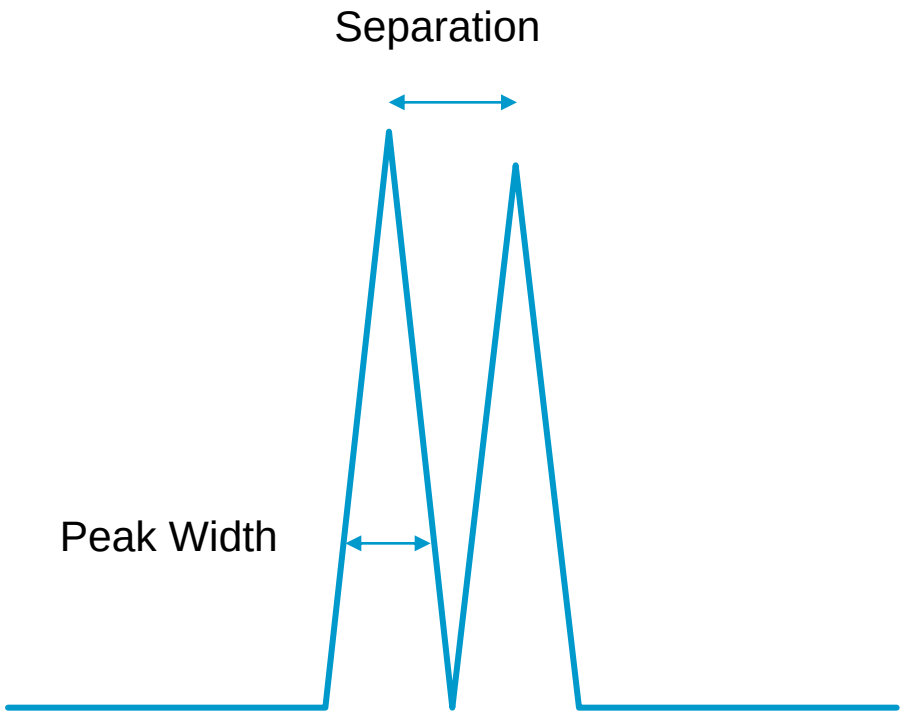
Overlay of two chromatograms of a blank extract injected BEFORE (A) and AFTER (B) three injections without backflush

With Backflush: No Increased Background (Less Spectral Noise) and Consistent Retention Times



Overlay of three chromatograms of lettuce extract run with 2 min of back flush

Loss of Resolution



Resolution is a function of separation and peak width

Loss of Resolution - Separation Decrease

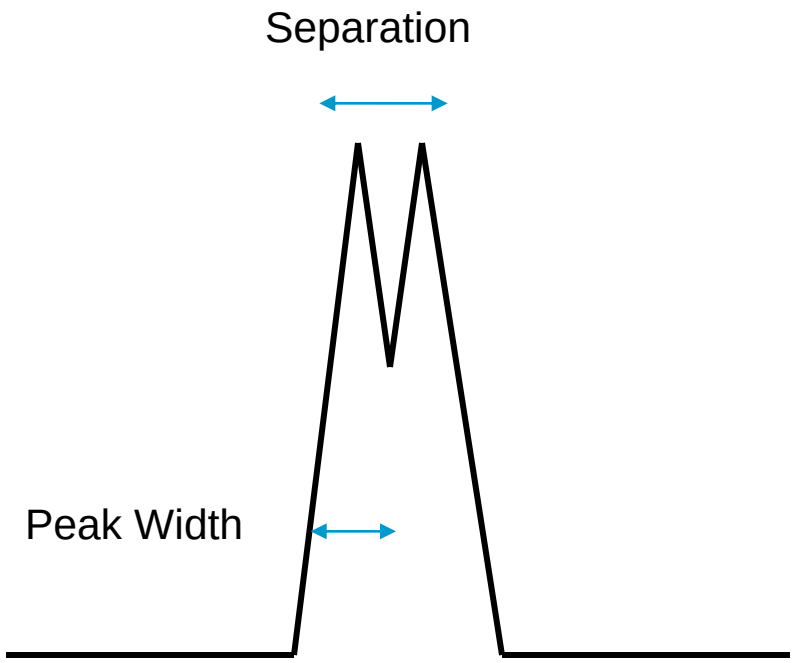
COLUMN

-Different column temperature

-Contamination (more phase?)

-Matrix components co-eluting

-Different column phase?



Loss of Resolution - Peak Broadening

FLOW

-Change in carrier gas velocity

-Make-up gas

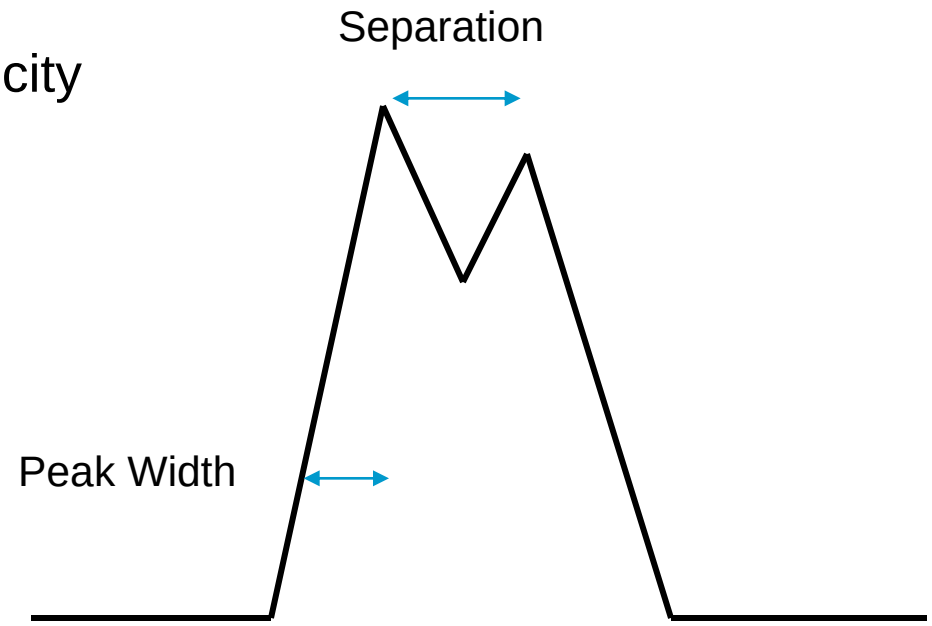
COLUMN

-Contamination

-Phase degradation

INJECTOR (efficiency)

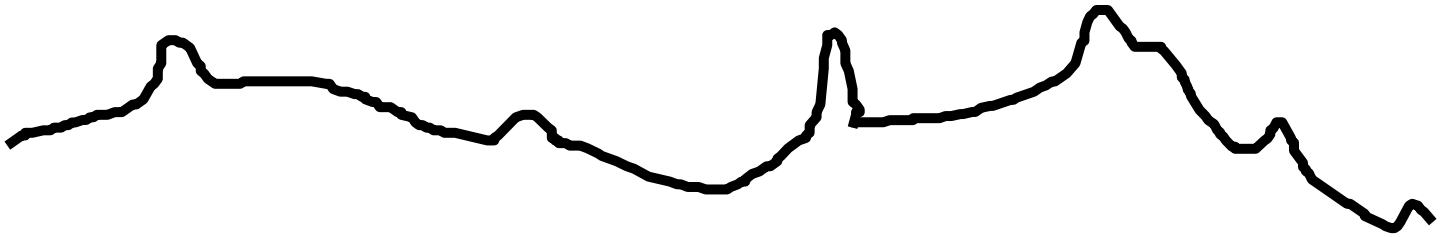
-Settings, Liner, Installation, etc.



Baseline Disturbances

Sudden Changes, Wandering, or Drifting

WANDER



COLUMN or DETECTOR

-Not fully conditioned or stabilized (electronics)

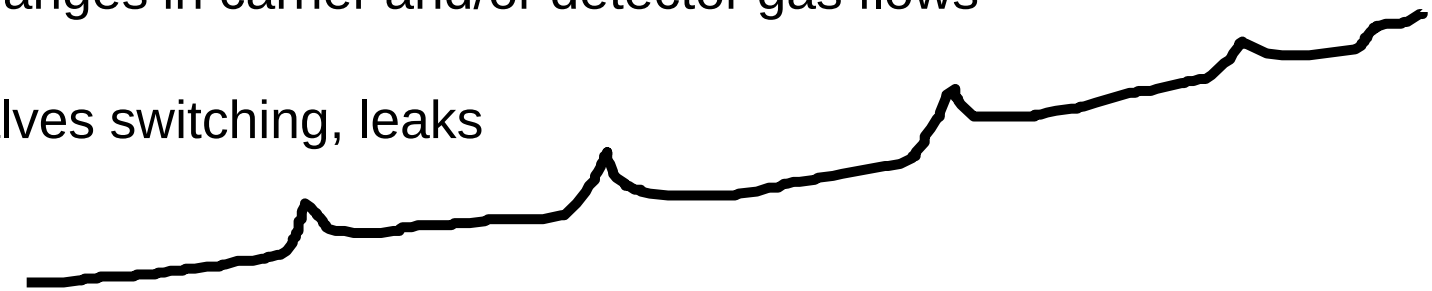
-Contamination

FLOW

-Changes in carrier and/or detector gas flows

-Valves switching, leaks

DRIFT



Noisy Baseline

MILD

SEVERE

FLOW

-Contaminated gas

-Incorrect detector settings

COLUMN

-Bleed if at high temperature

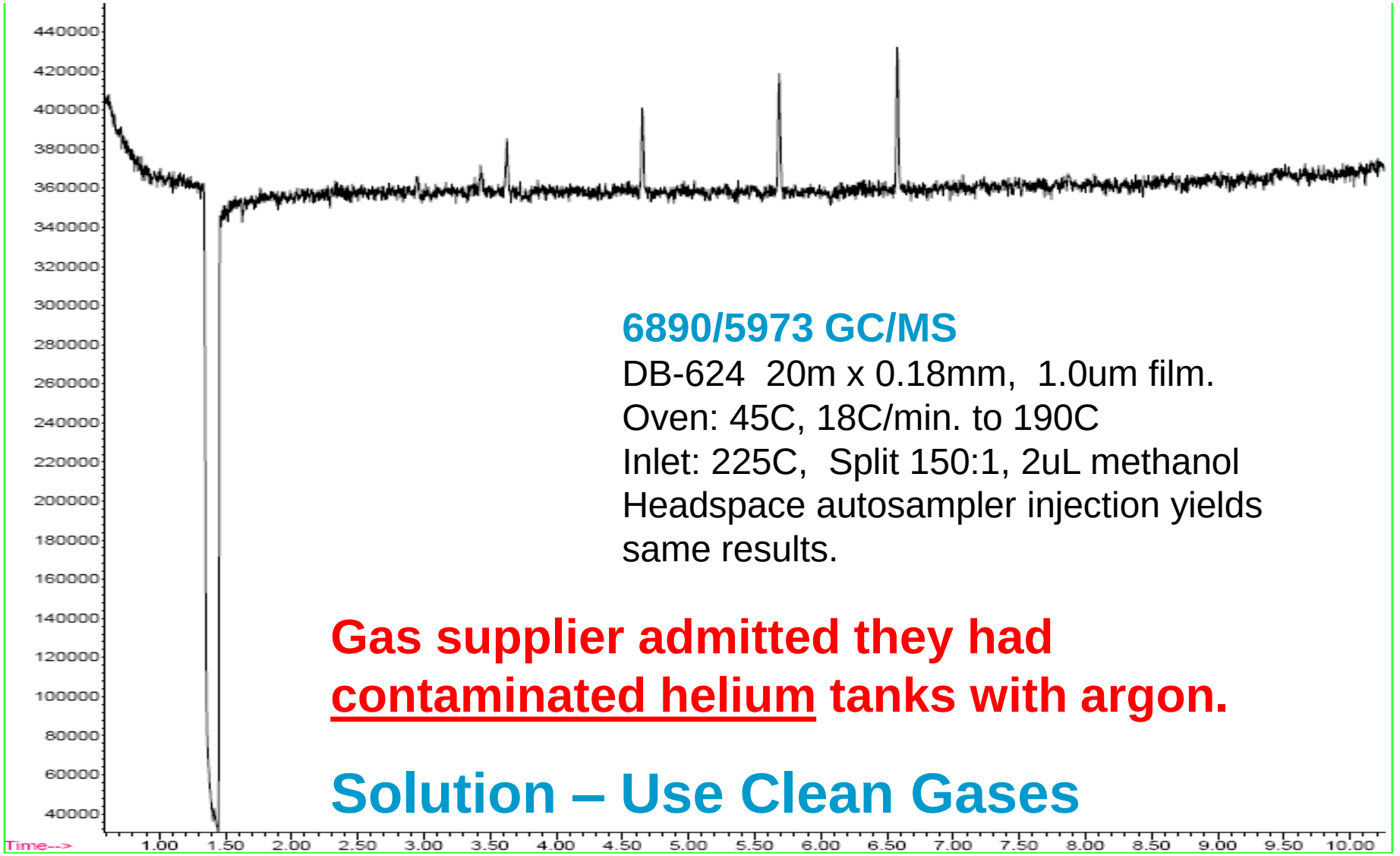
-In detector flame (poor installation)

DETECTOR

-Air leak - ECD, TCD

-Electronics malfunction

Symptom - Noisy Baseline Even at Low Temps



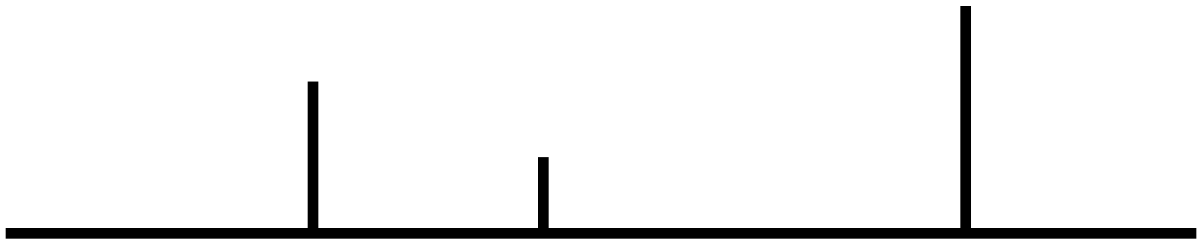
6890/5973 GC/MS

DB-624 20m x 0.18mm, 1.0um film.
Oven: 45C, 18C/min. to 190C
Inlet: 225C, Split 150:1, 2uL methanol
Headspace autosampler injection yields same results.

Gas supplier admitted they had contaminated helium tanks with argon.

Solution – Use Clean Gases

Spiking Baseline



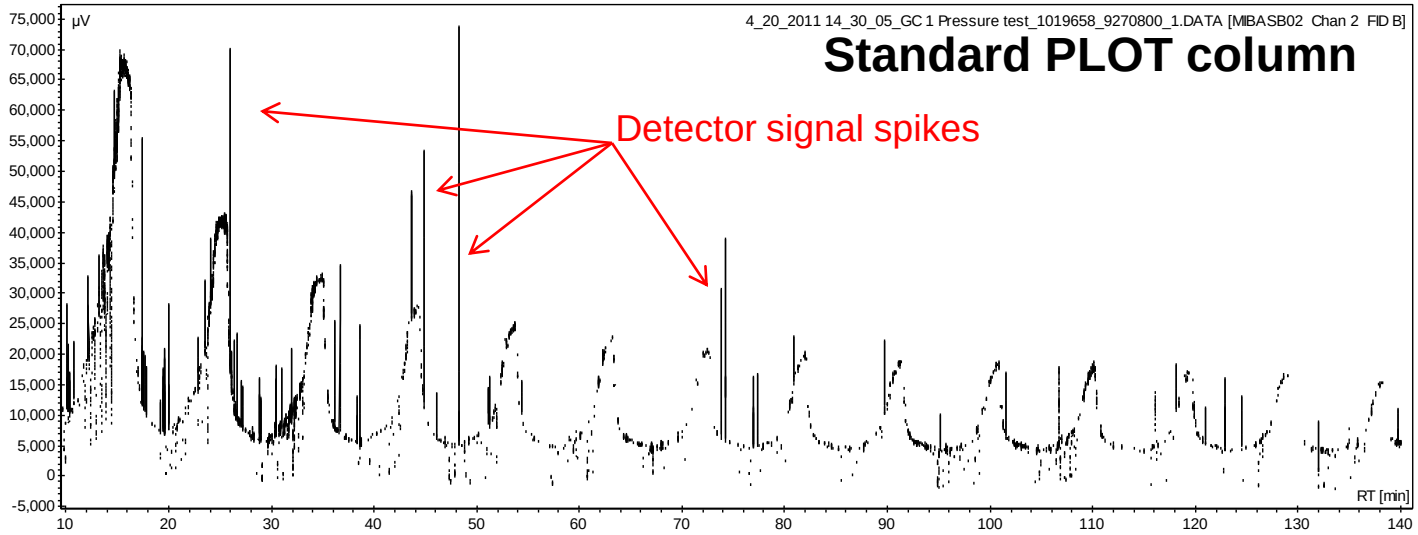
DETECTOR

-Particles entering the detector

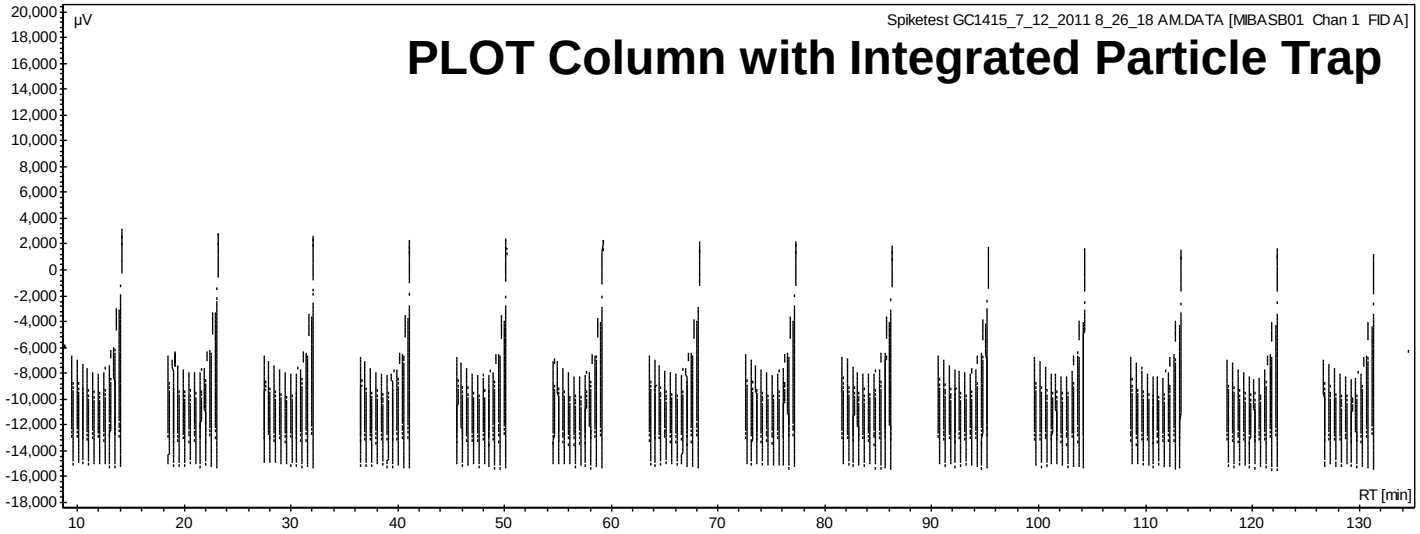
-Random: poor connection

-Regular: nearby "cycling" equipment (electronics)

Symptom – Spiking Baseline with PLOT Columns



- Temperature: 150°C + 20°C/min → 250°C;15 times
- Pressure 3x optimum
- Each run switch off/on carrier gas 10 times

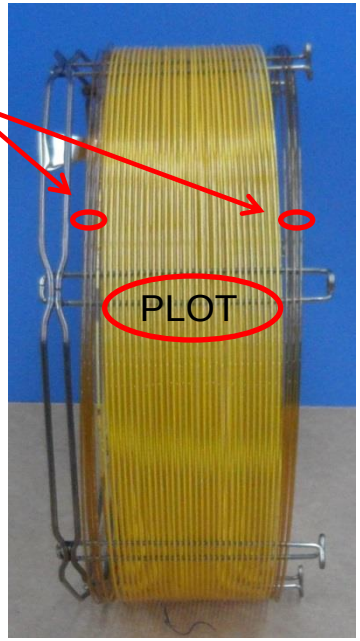


The unusual "chromatogram" shows the detector signal profile of the temp and pressure cycling

Solution – Integrated Particle Traps

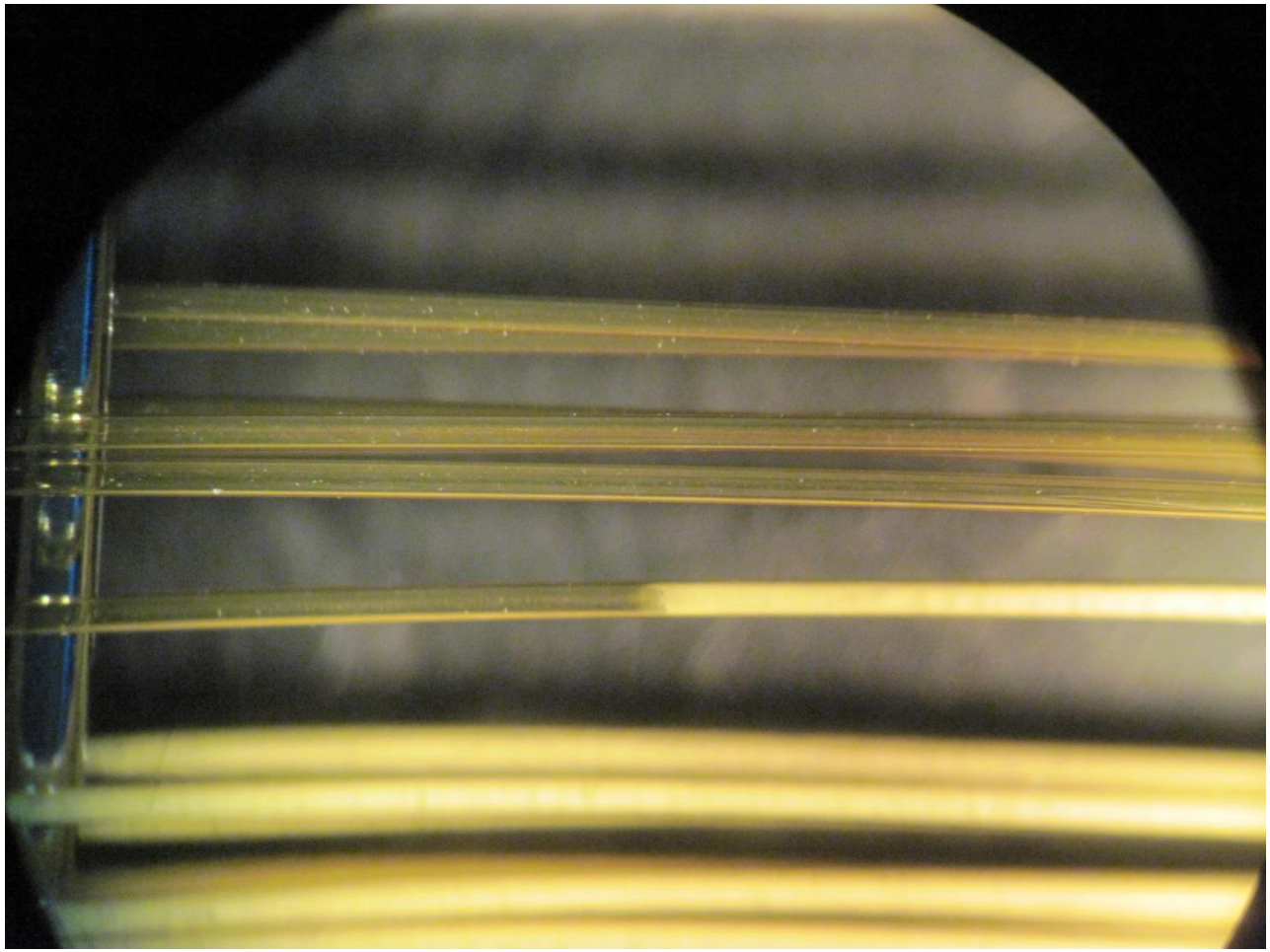
NEW Integrated Particle Trap PLOT Column

2.5 meter integrated particle traps on both ends virtually eliminates the classical particle shedding problem of porous polymer PLOT columns

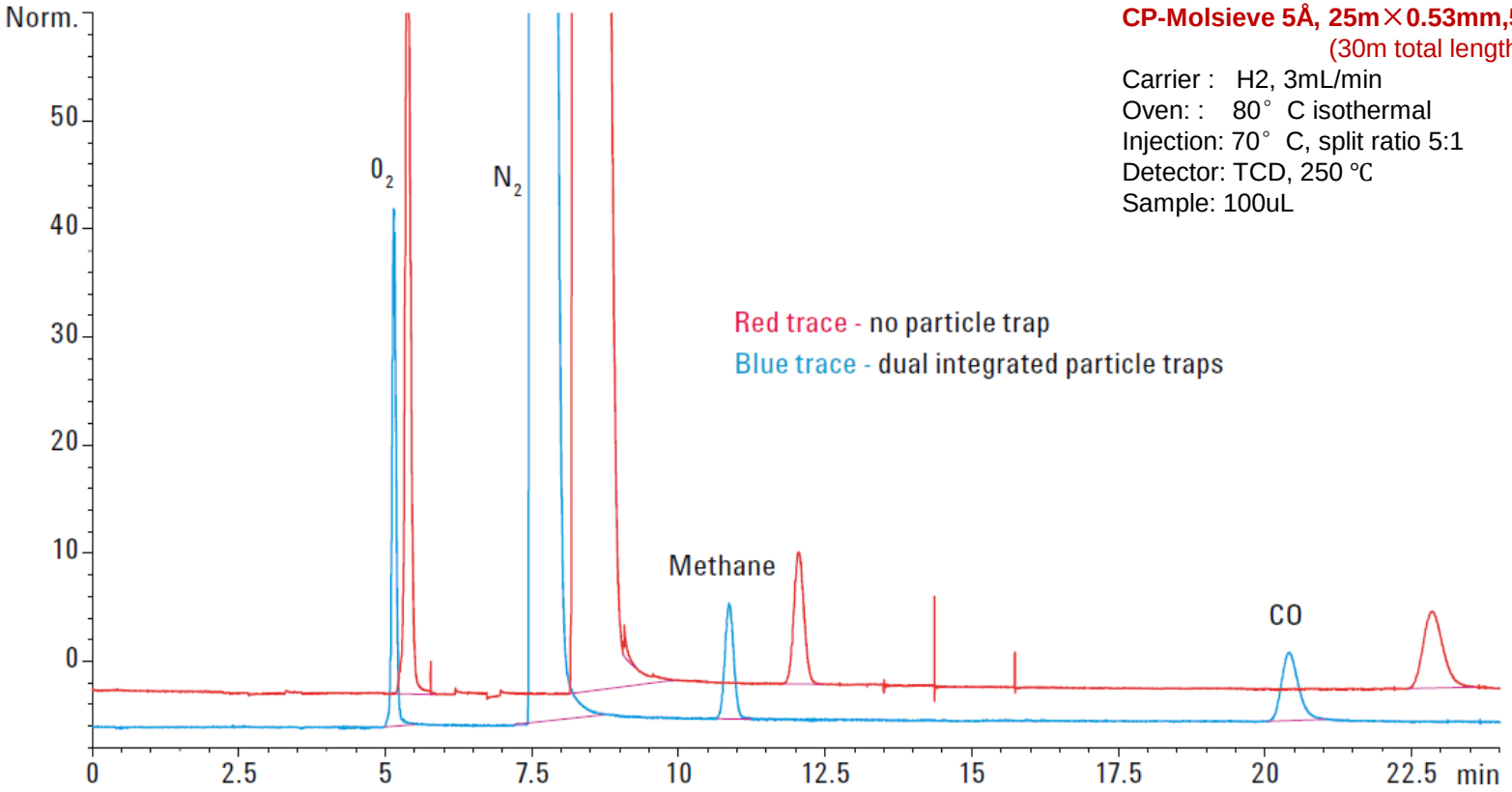


- No unions and fittings
- Compatible with capillary GC, GC/MS and valve switching GC systems, including Capillary Flow Technology (CFT)
- Very similar selectivity, plates and peak shape performance to existing Agilent porous polymer PLOT columns
- Another Agilent first

The Column and Integrated Particle Trap



No spikes at Fixed Gases Analysis on CP-Molsieve 5Å PLOT PT column



CP-Molsieve 5Å, 25m×0.53mm,50um
(30m total length)
Carrier : H2, 3mL/min
Oven: : 80° C isothermal
Injection: 70° C, split ratio 5:1
Detector: TCD, 250 ℃
Sample: 100uL

CP-Molsieve 5Å showing spikes when particle traps are removed (Red trace).
No spikes with manufacturer integrated particle trap (Blue Trace).

Quantitation Problems

DETECTOR

-Poor stability (electronics) or Baseline disturbances (contamination)

-Outside detector's linear range or wrong settings

Activity (adsorption) in INJECTOR or COLUMN

INJECTOR

-Technique, settings, conditions

-Syringe worn

OTHER

-Co-elution

-Matrix effects

-Sample evaporation – leaky vials

-Sample decomposition

Troubleshooting Tips

1. Isolate the problem.

(Blank Runs, Inject Un-retained Compound, Know what it is not)

2. Change only one variable at a time.

3. Compare before/after chromatograms.

(Peak shape, response, retention, baseline rise, background, look for trends, etc.)

4. Utilize Technical Support.

TECHNICAL SUPPORT

1-800-227-9770, #3

1-972-699-6423 (Daron)

1-866-912-6701 (toll free)

E-mail:
Daron_Decker@Agilent.com