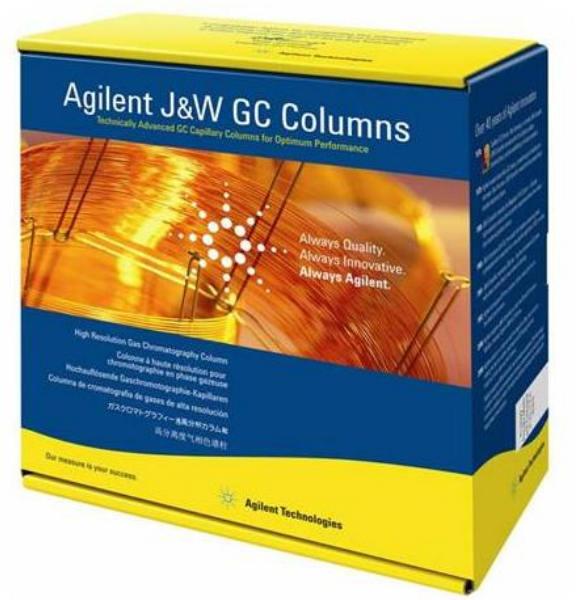
GC Best Practices & Troubleshooting



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.

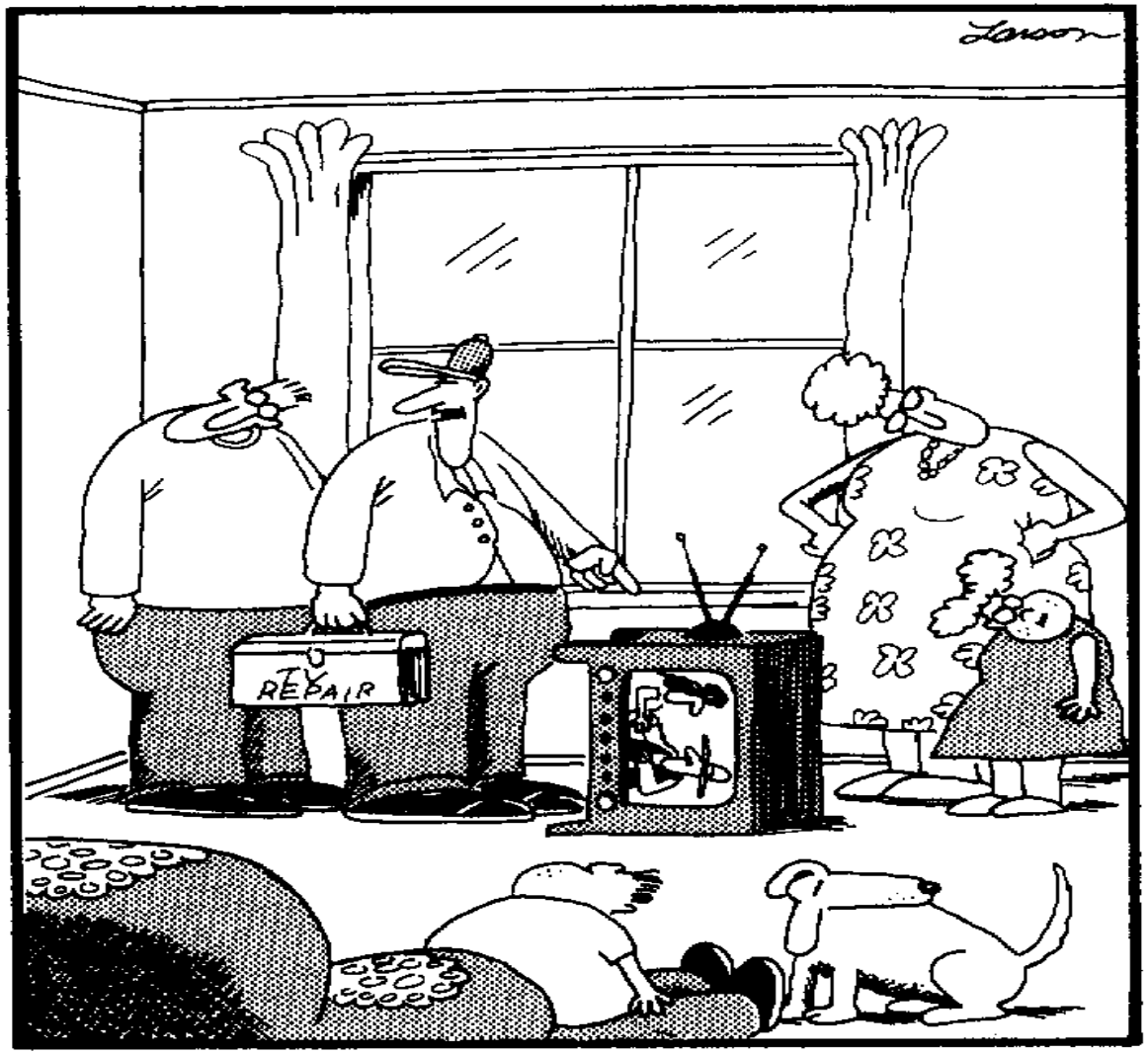
3. Compare before/after chromatograms.

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

4. Make sure it makes sense to do what you're doing…

5. Be careful of distractions

6. Sometimes just a fresh set of eyes is all that is needed.



"Well, here's your problem, Mr. Schueler."

What Can Possibly Go Wrong?

INJECTOR – contamination, flow settings, flow path issues, overload, valve settings, faulty consumables

COLUMN – contamination, flow settings, flow path issues, damage (activity & bleed), breakage

DETECTOR – contamination, flow settings, flow path issues, electronics

Logical Troubleshooting

- **Troubleshooting Starts with Isolating the problem**
  - There are 5 basic areas from where the problem arises

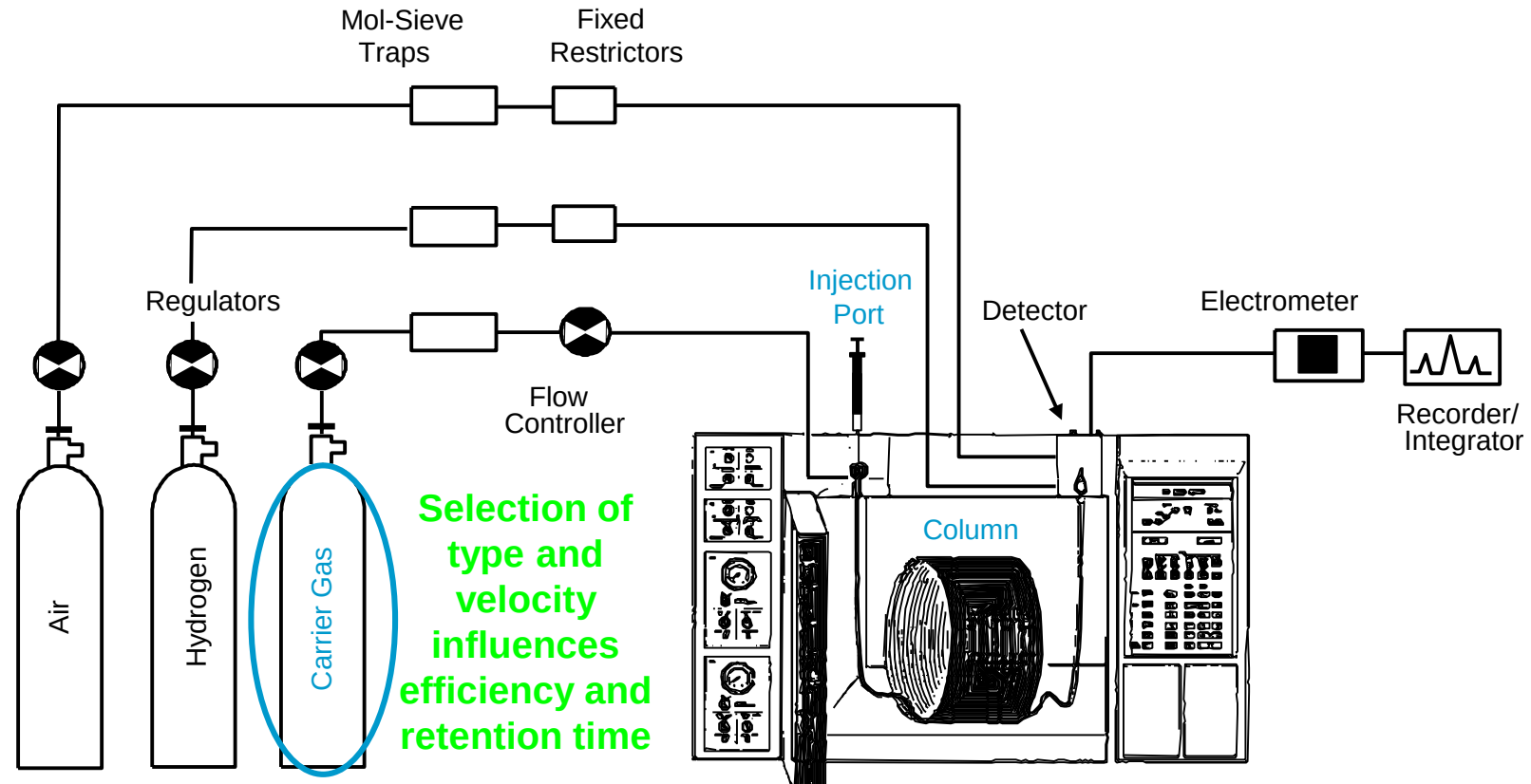
  - FLOW
  - INJECTOR
  - COLUMN
  - DETECTOR
  - ELECTRONICS

  (Temperature)

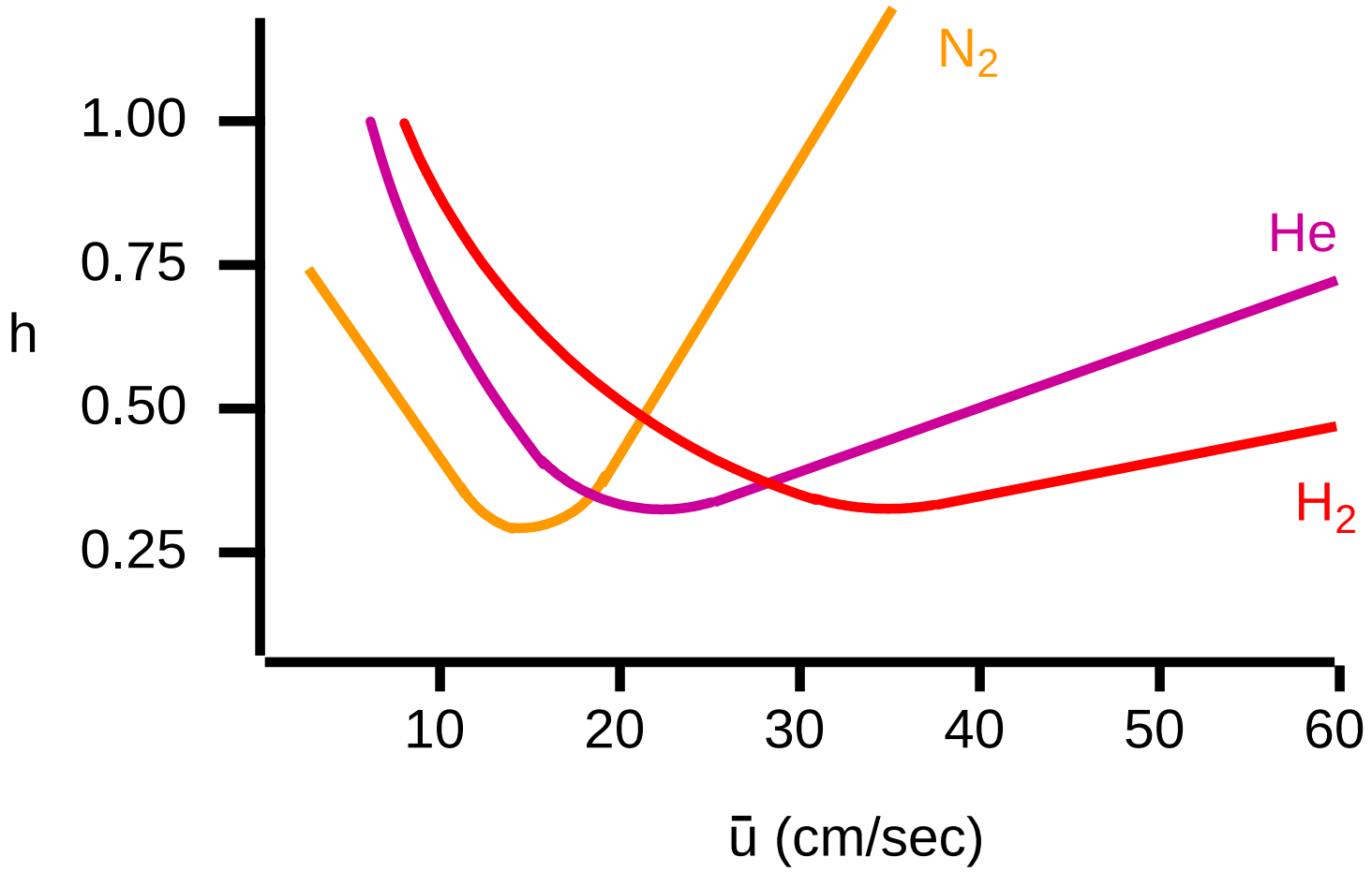
  - But of course it can always be some COMBINATION

- **Knowing what can & <u>can't</u> cause the symptom is the key**

Typical Gas Chromatographic System



van Deemter Curves

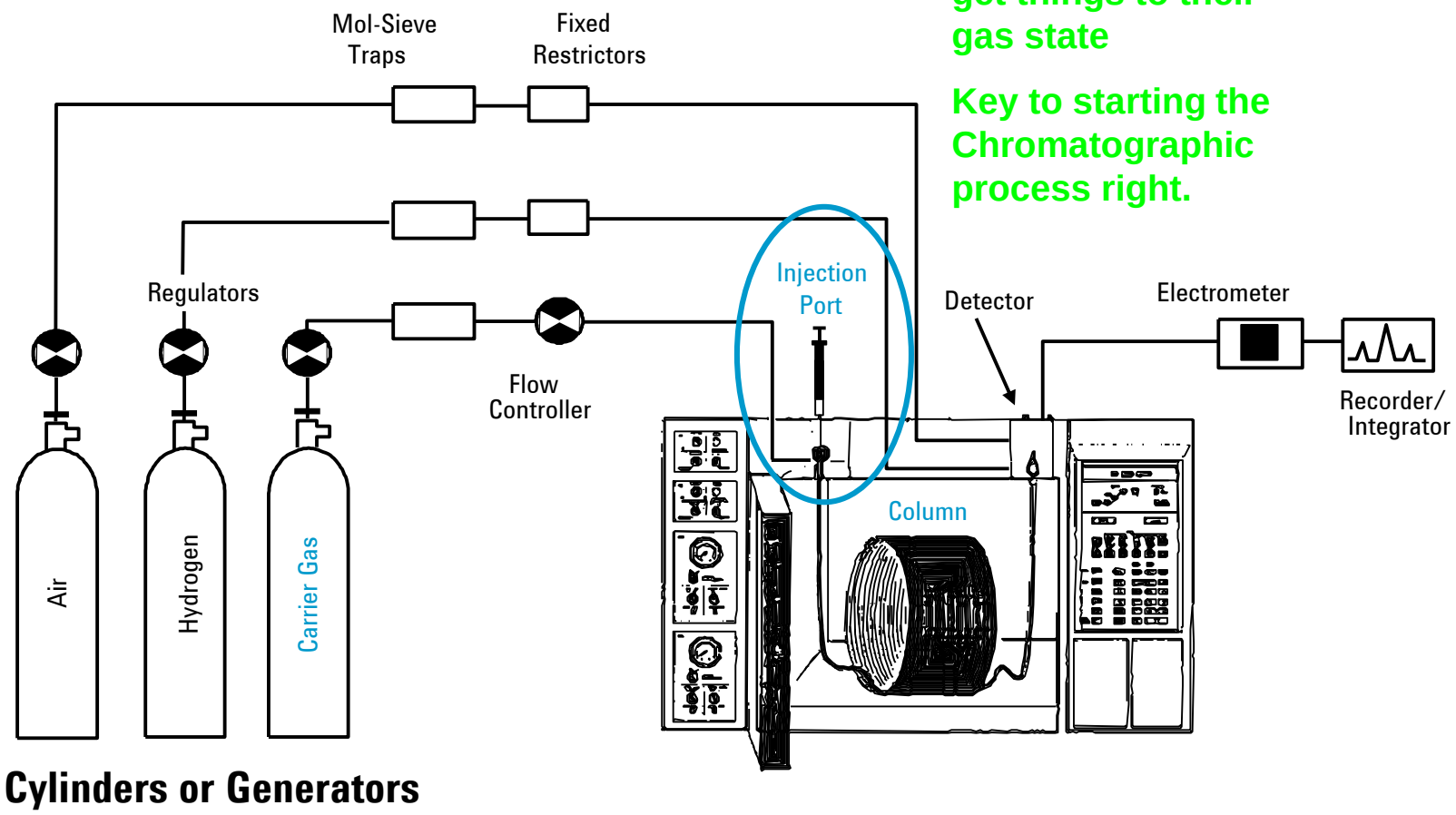


CARRIER GAS

Type	Velocity Range (u_{opt} – OPGV)
Nitrogen	8-16
Helium	20-40
Hydrogen	30-55

Gas Clean Filters

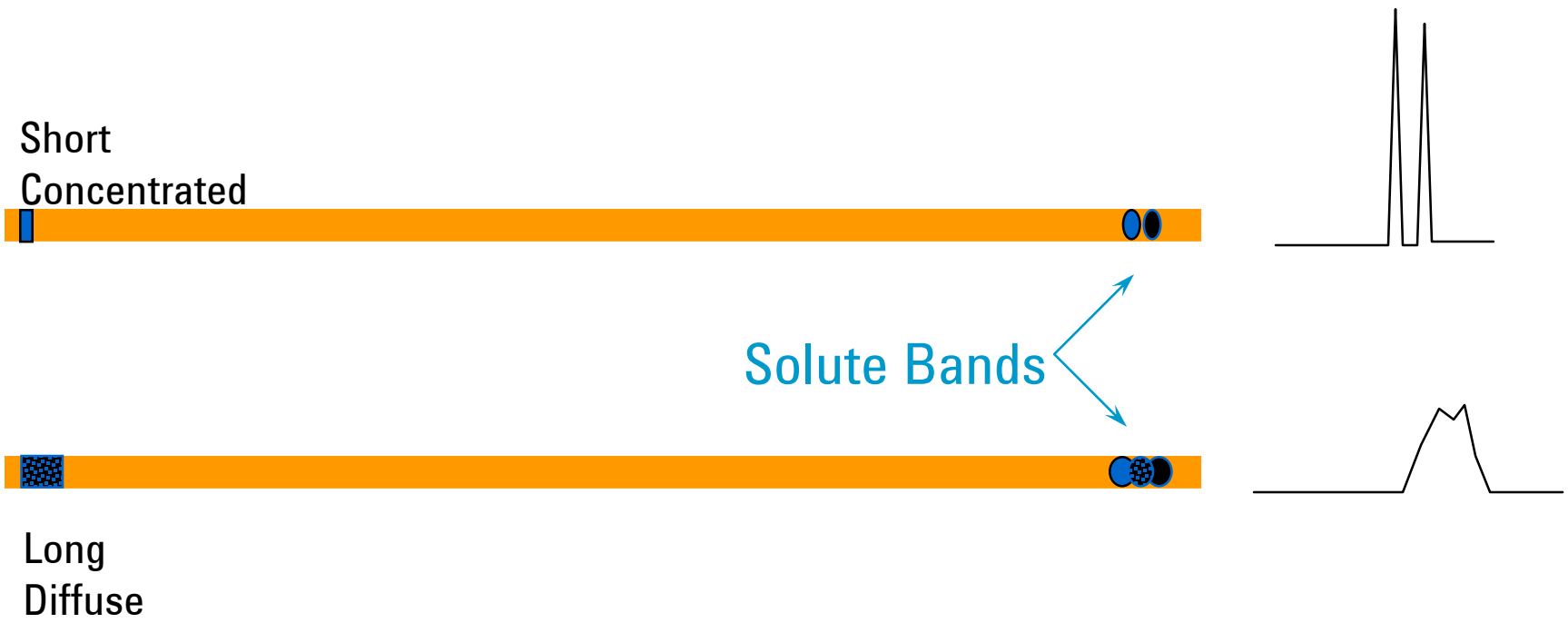
Typical Gas Chromatographic System



Have to be able to get things to their gas state

Key to starting the Chromatographic process right.

Influence of Injection Efficiency



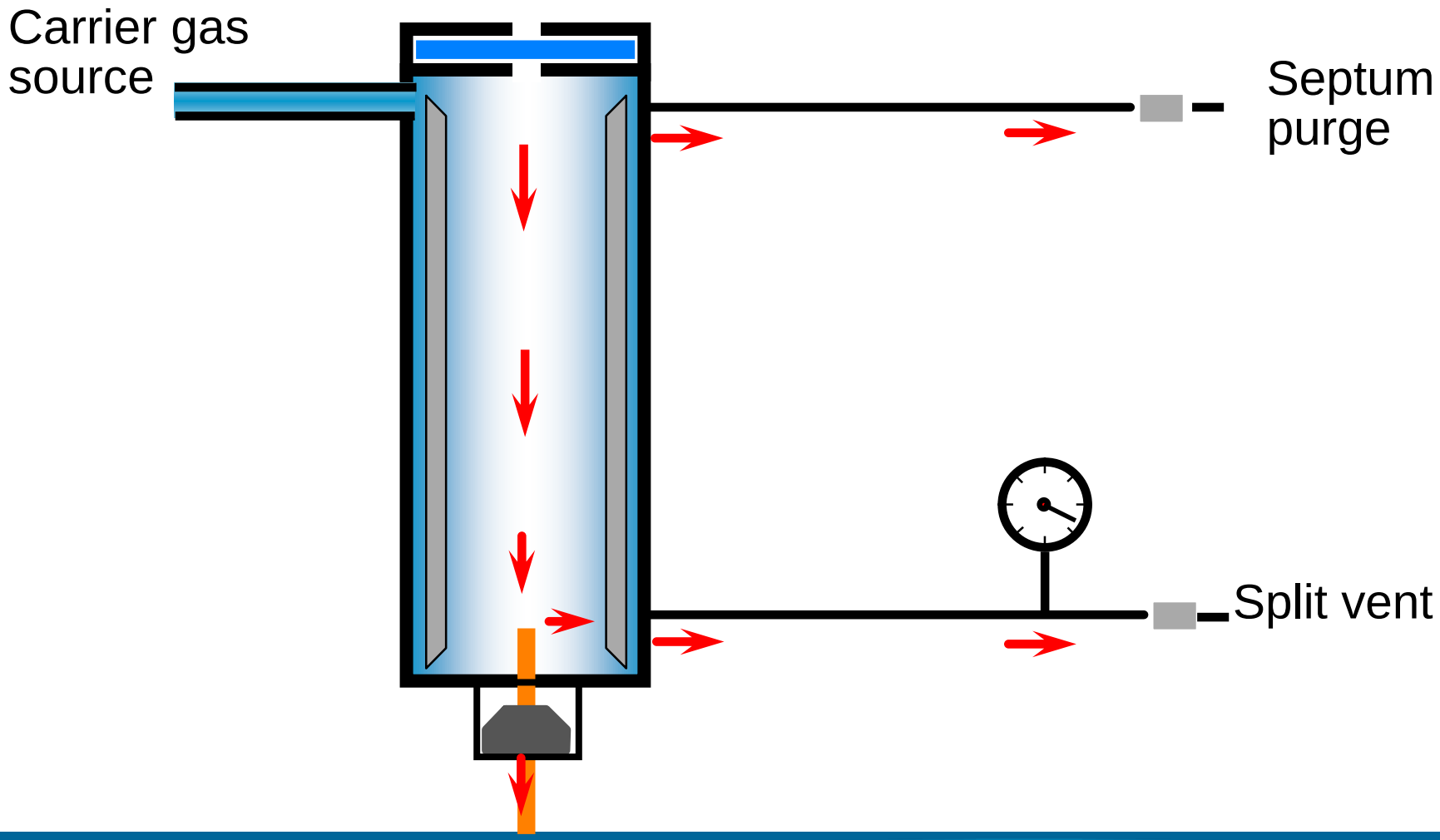
Same column, same chromatographic conditions

Injectors

Split

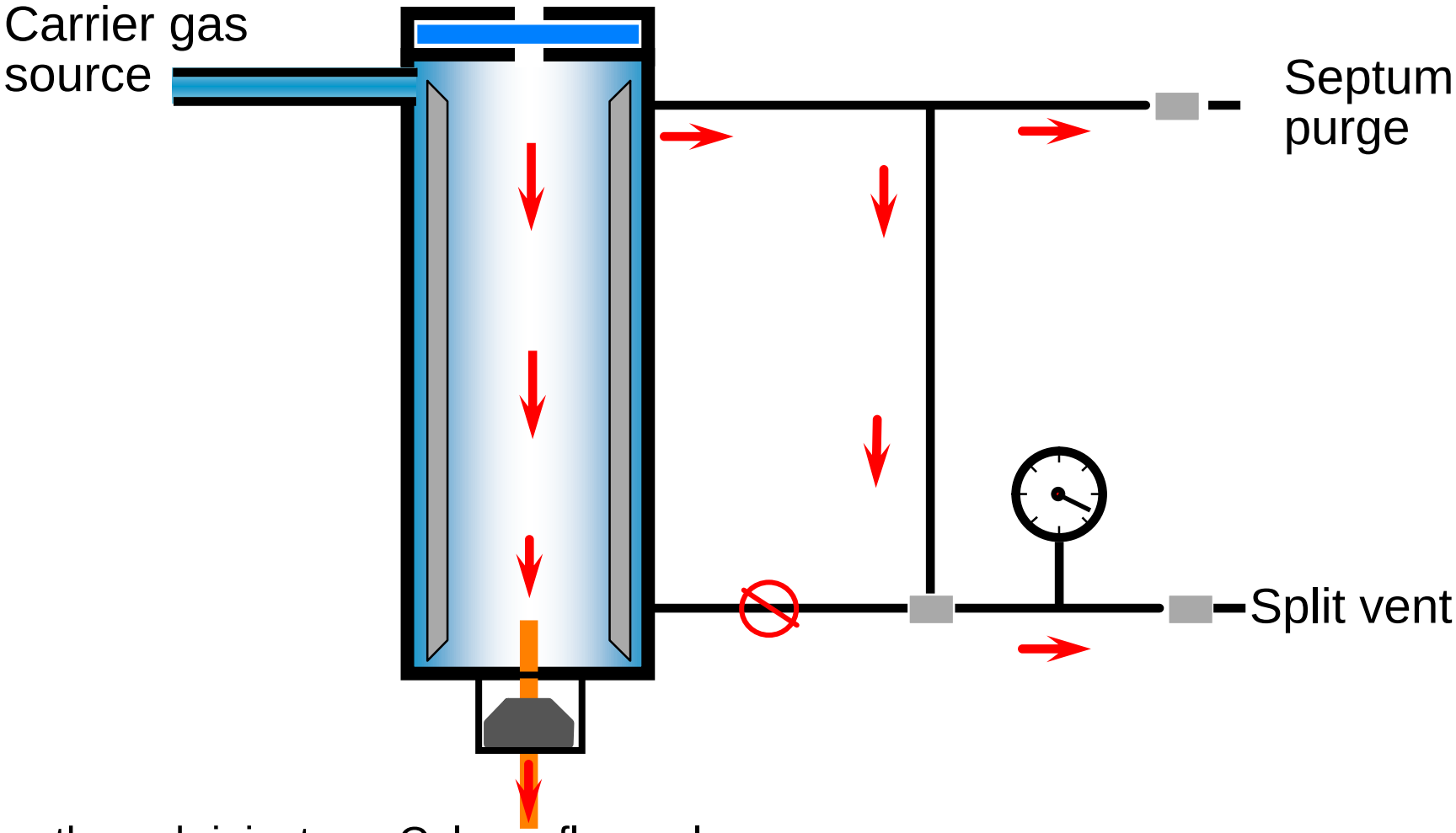
Splitless

Split Injector
Flow Path



Splitless Injector

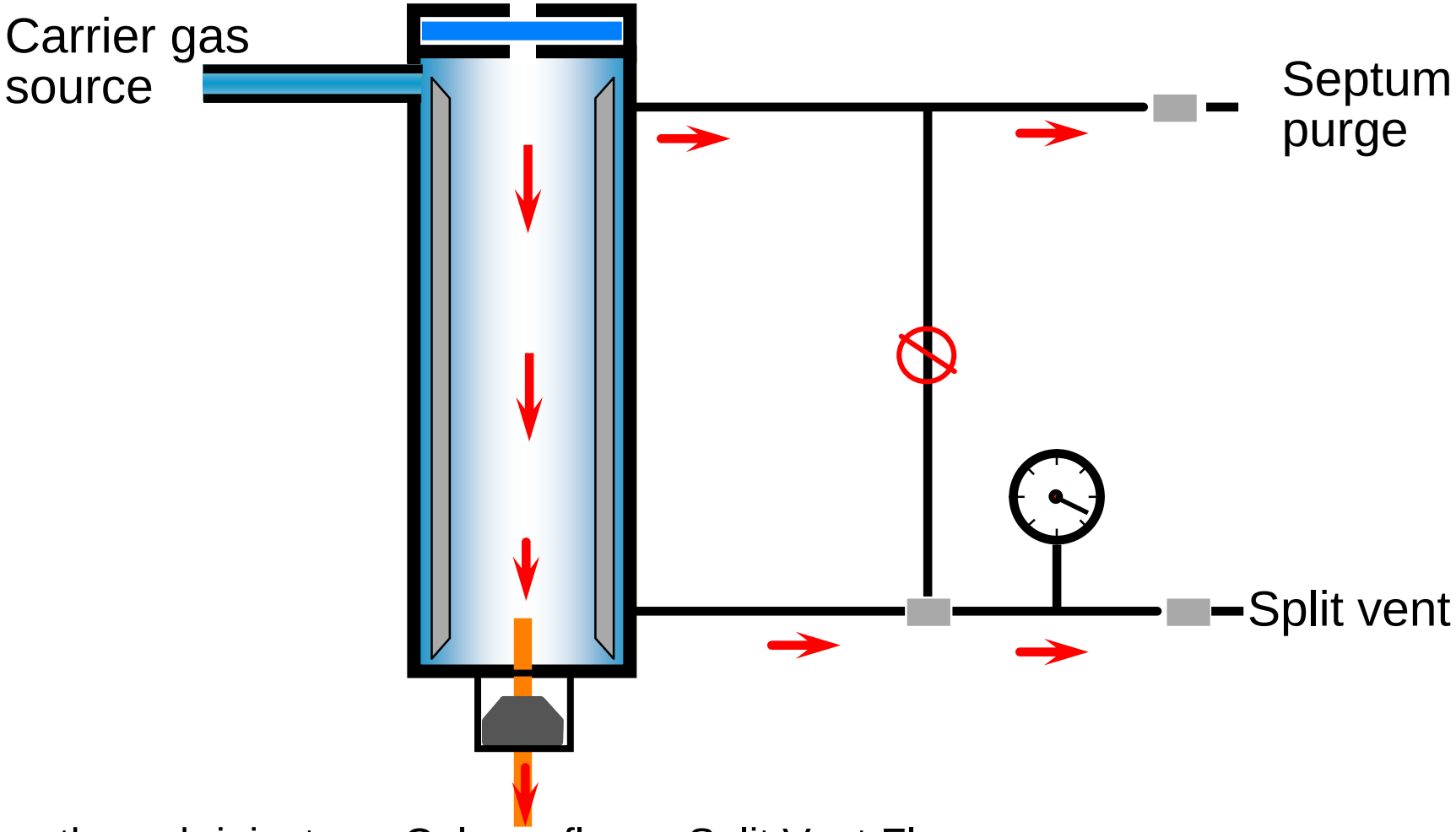
Purge Off At Injection



Flow through injector = Column flow only

Splitless Injector

Purge On After Injection



Flow through injector = Column flow + Split Vent Flow

Split Injector
Major Variables

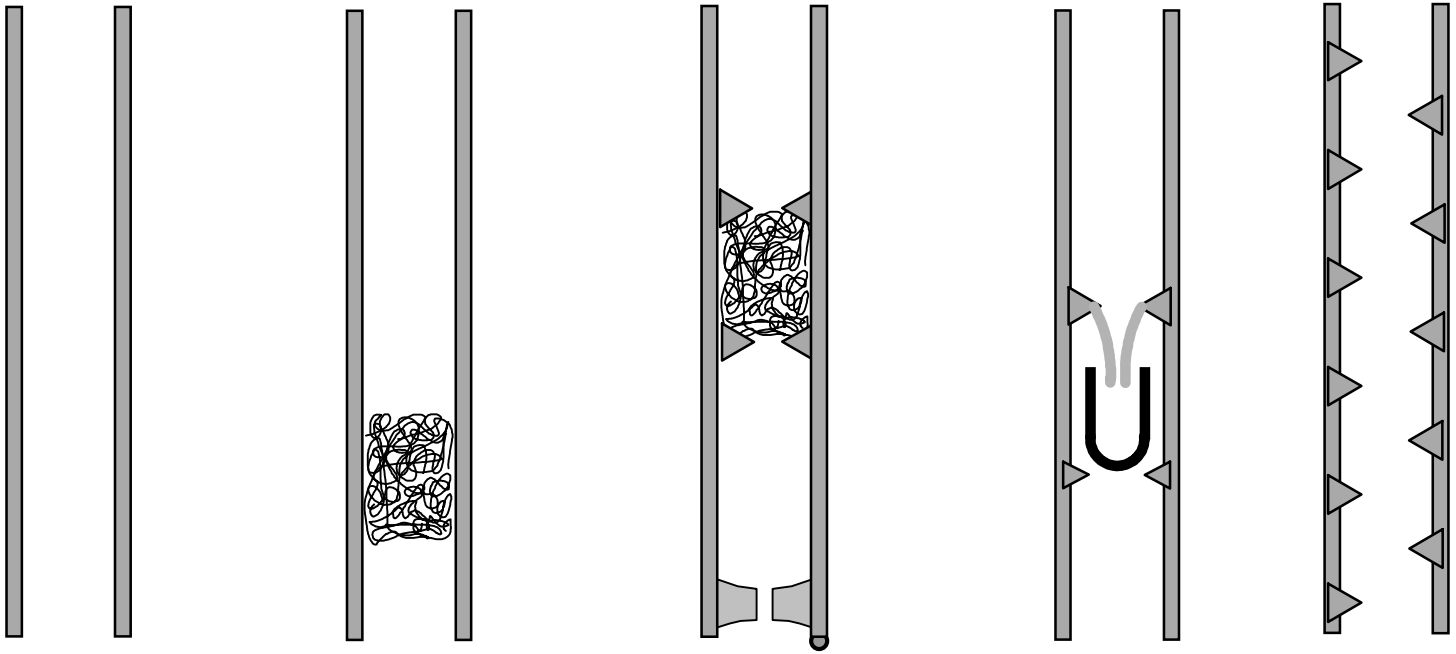
Split ratio - determines amount of sample onto column and efficiency of injection (sensitivity vs peak shape)

Liner - influences efficiency of vaporization/discrimination

Temperature - hot enough to vaporize sample without degradation or causing backflash

Injection volume - typically 1-3uL, increasing it does not have as much of an effect as one might think

Split Liners – What's What?



Straight tube

Straight tube with glass wool

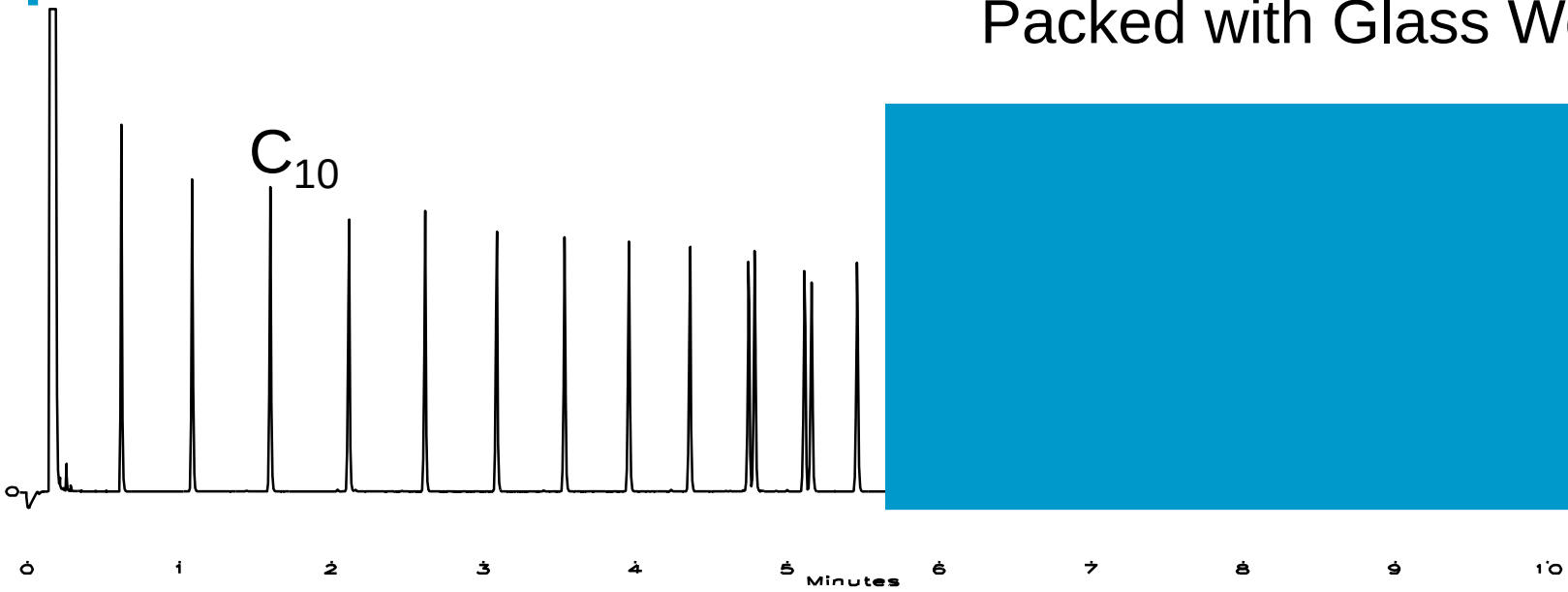
Fixed glass wool

Inverted cup

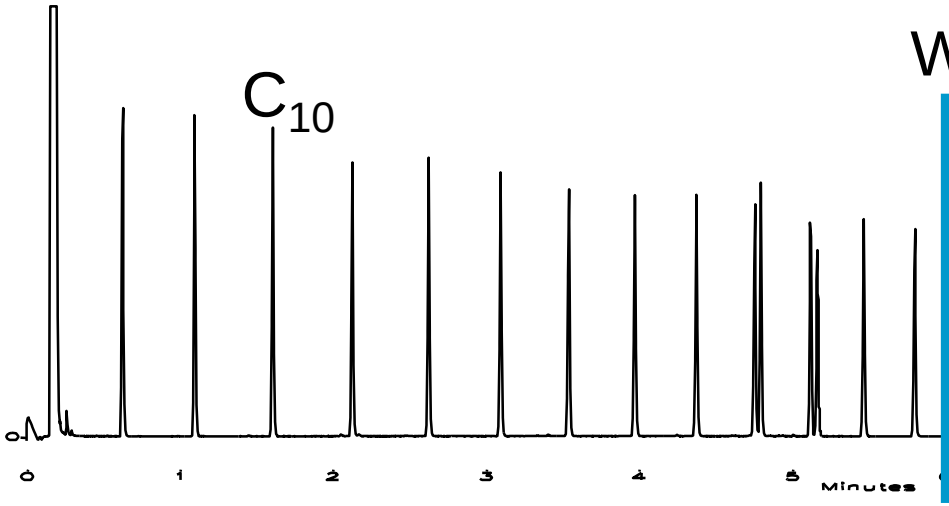
Baffle

Split Liner

Packed with Glass Wool

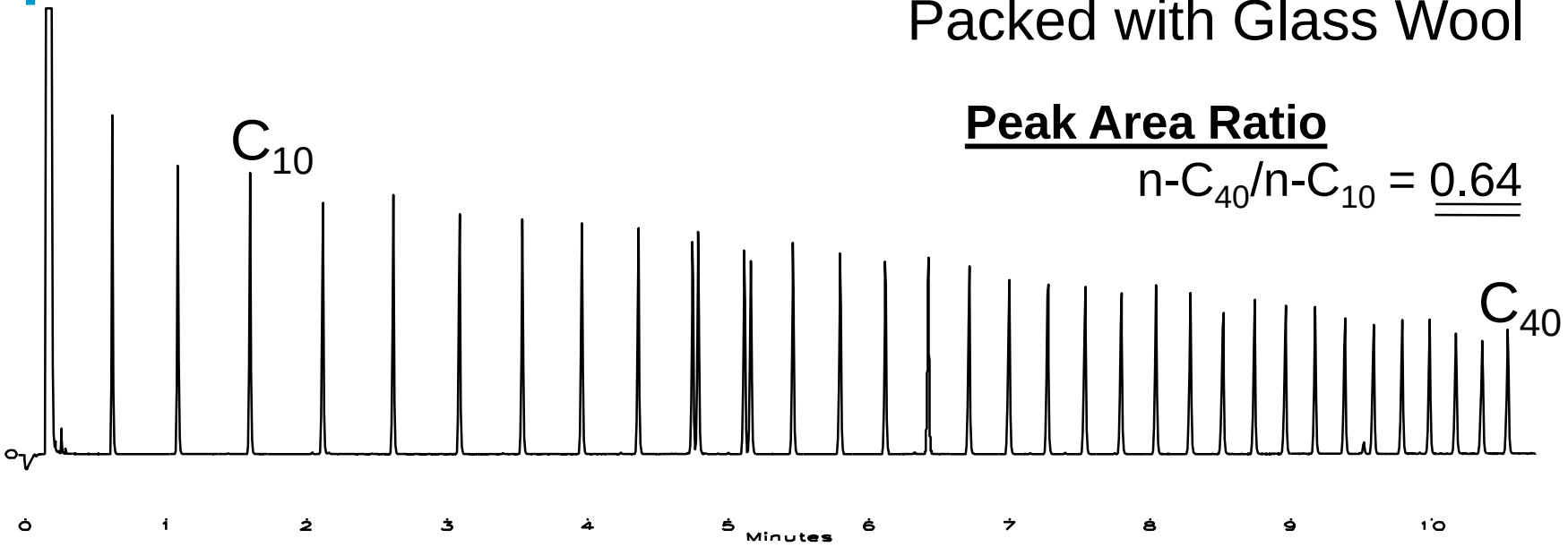


Without Glass Wool Packing



Split Liner

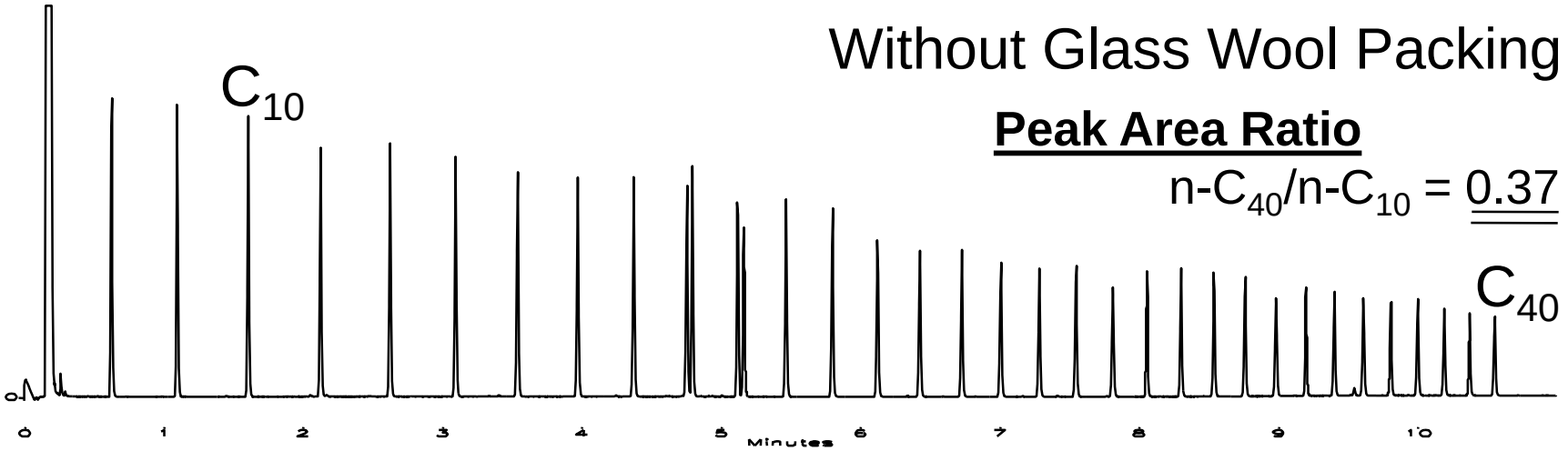
Packed with Glass Wool



Peak Area Ratio

$n\text{-}C_{40}/n\text{-}C_{10}$ = 0.64

Without Glass Wool Packing



Peak Area Ratio

$n\text{-}C_{40}/n\text{-}C_{10}$ = 0.37

Splitless Injector
Overview

Most of the sample is introduced into the column

Used for low concentration samples

Wider peaks are obtained than for split injections

Splitless Injector
Major Variables

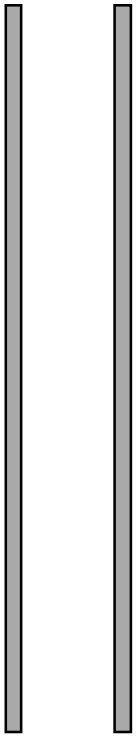
Purge activation time - determines amount of sample onto column and efficiency of injection (sensitivity vs peak shape)

Liner - preventing backflash more critical than vaporization properties (double tapered type recommended)

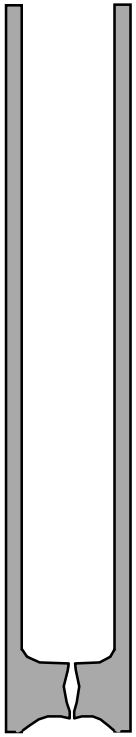
Injection volume - typically 1uL or less (backflash)

Temperature – long residence times allow for lower temps

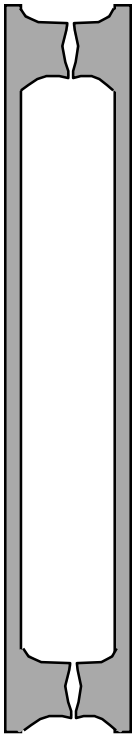
Splitless Injector
Liners



Straight tube

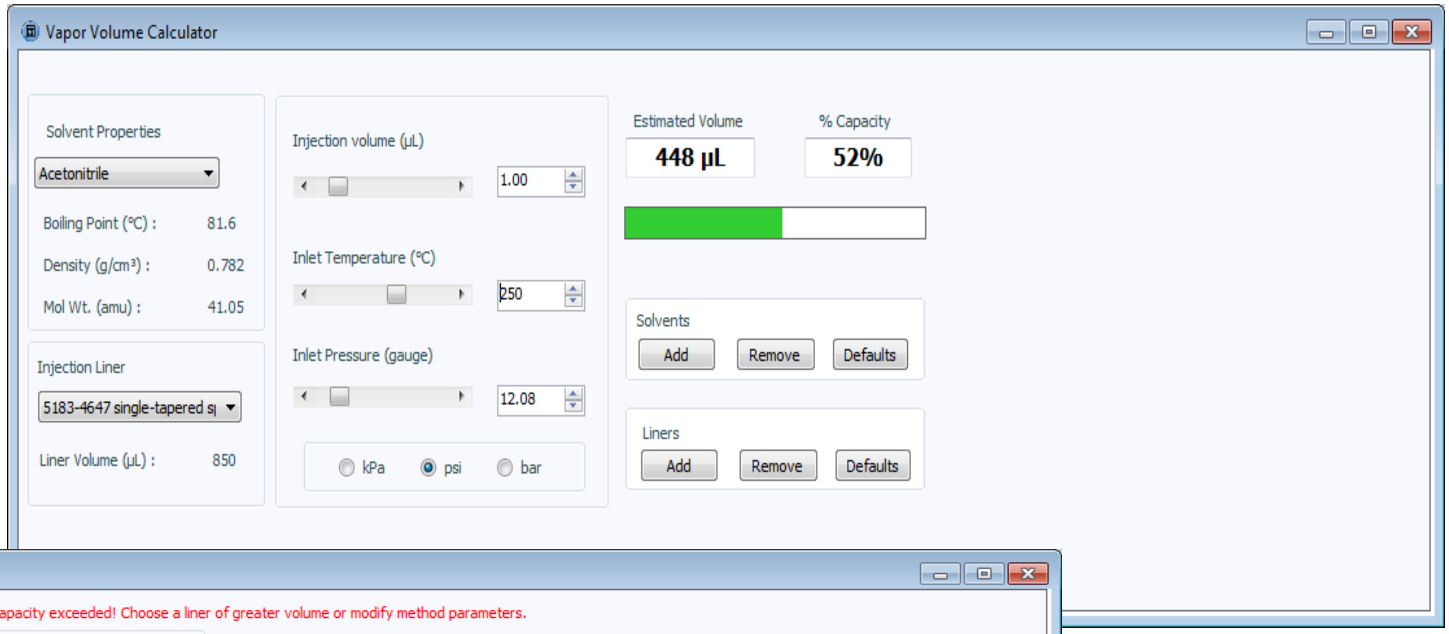


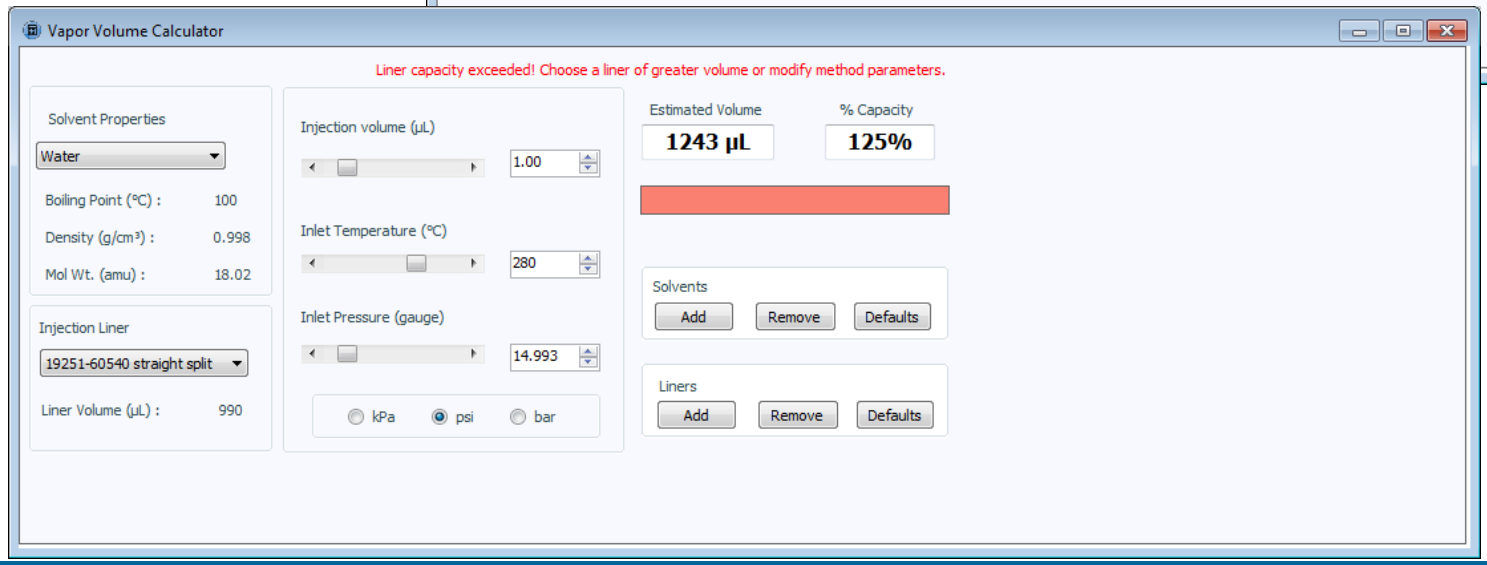
Bottom restriction



Dual restriction

Solvent Vapor Volume Calculator

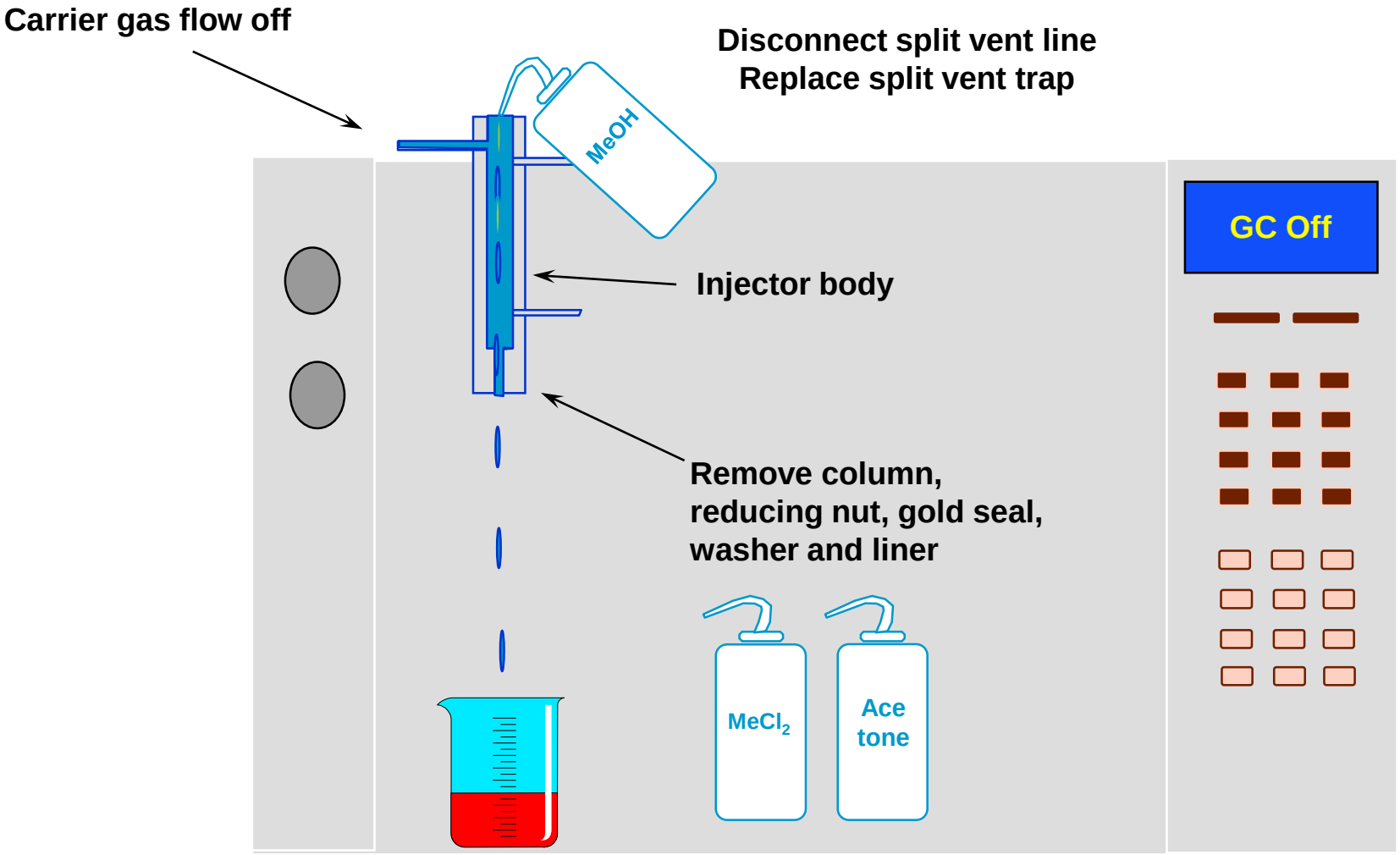




Agilent Technologies

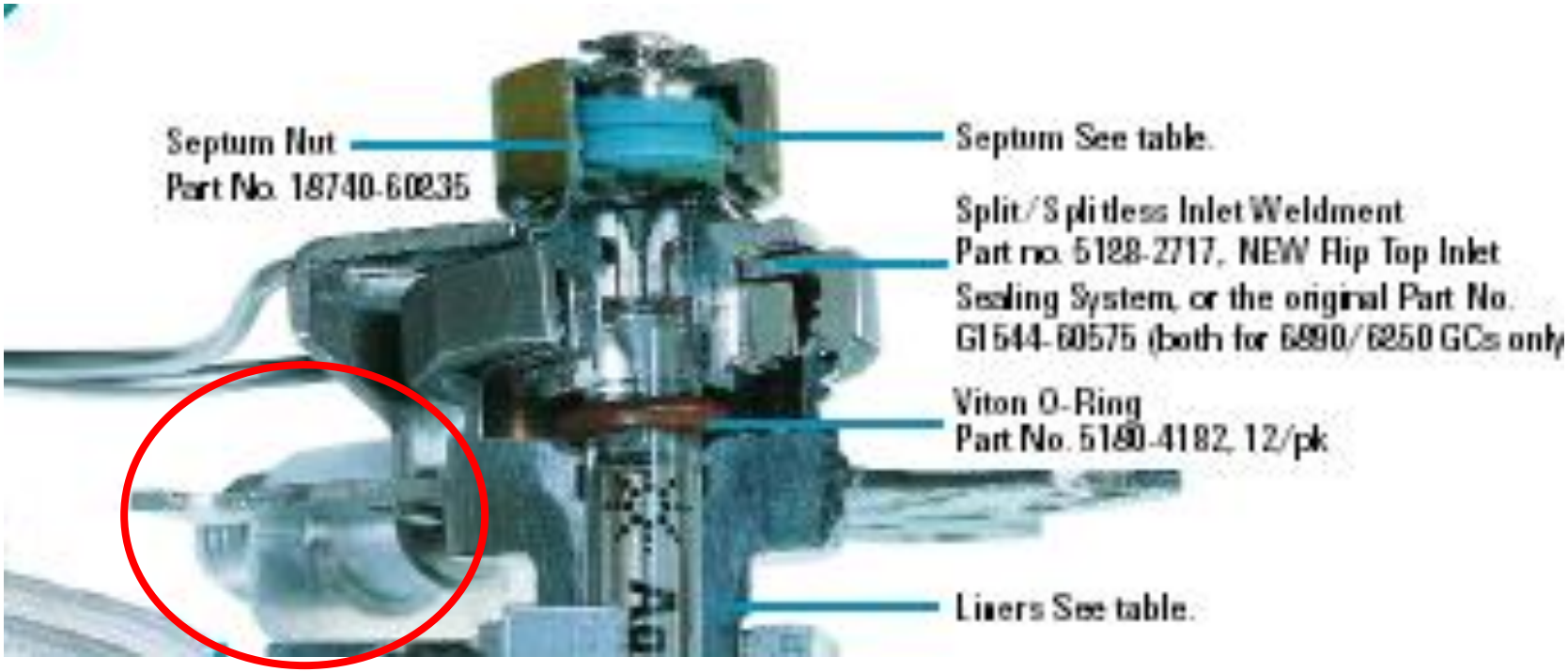
Backflash (carry-over) can give false positives!

Cleaning the Split/Splitless Injector



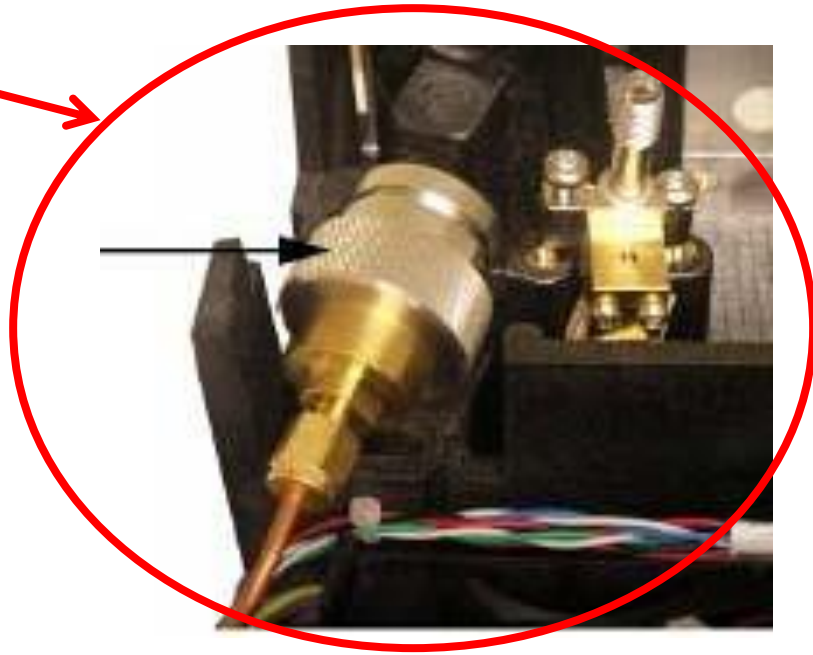
Finding the Split Vent Trap

Follow the split vent line back to the EPC



Finding the Split Vent Trap

Remove cover at Split Vent

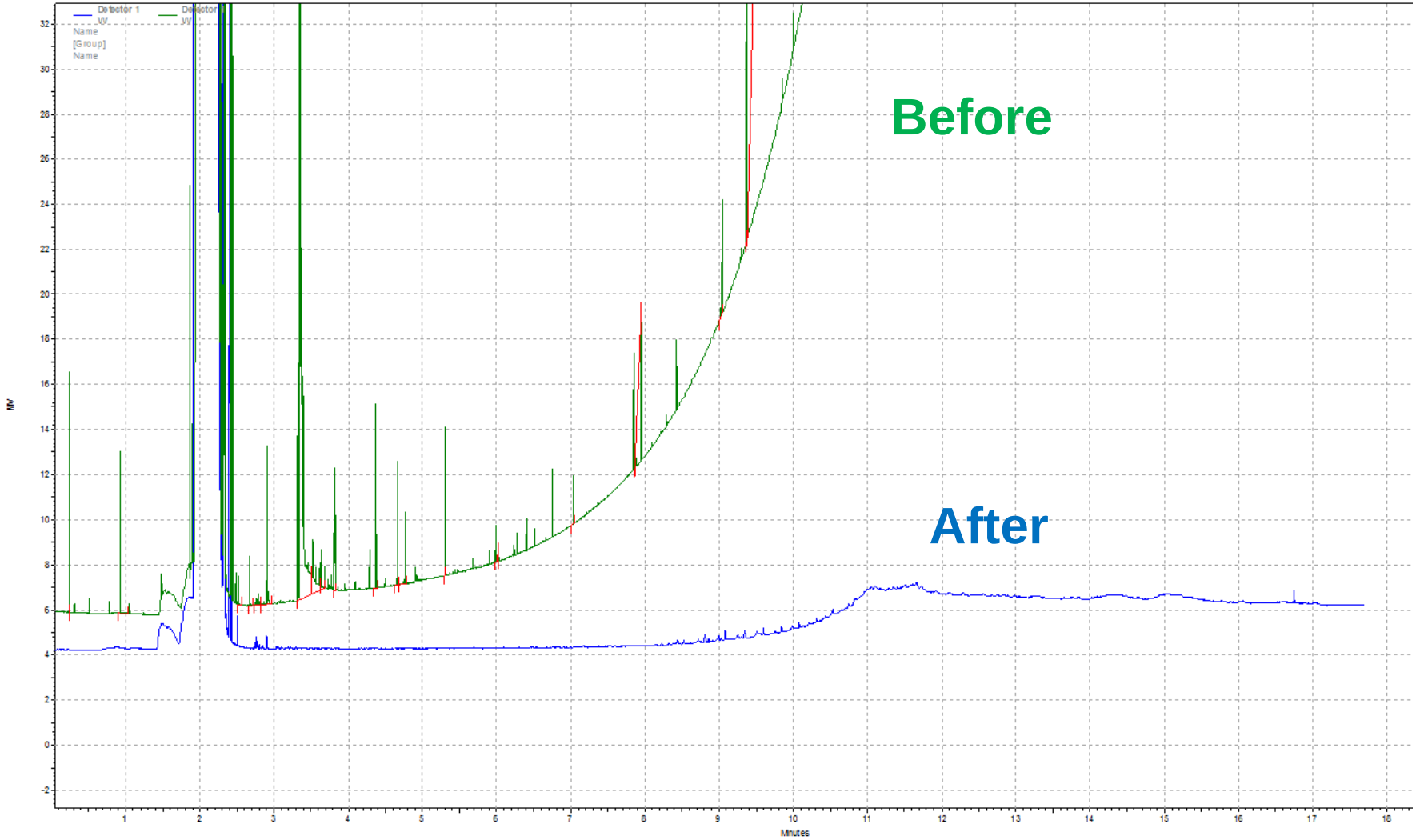


Replacing the Split Vent Trap

Finger Tight Knurled Nut

G1544-80530

Split Vent Trap Changed (Column Bleed?!?)



Split/Splitless Pulsed Injection

Pressure Pulse contains sample expansion and transfers analytes to the column faster.

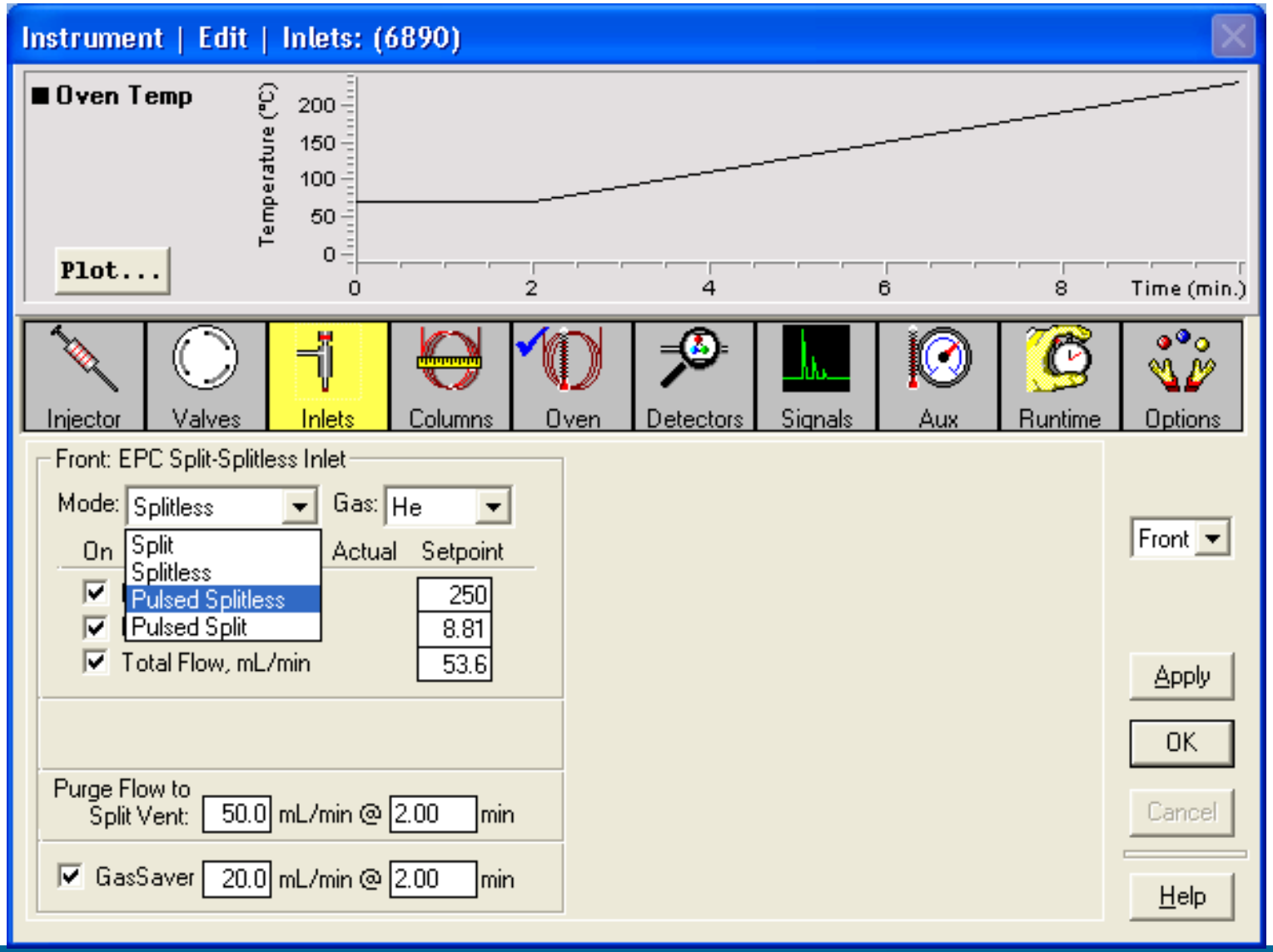
Pulsed Split

- the most volatile components and solvent effected most
- faster sample transfer not as critical since it's already fast

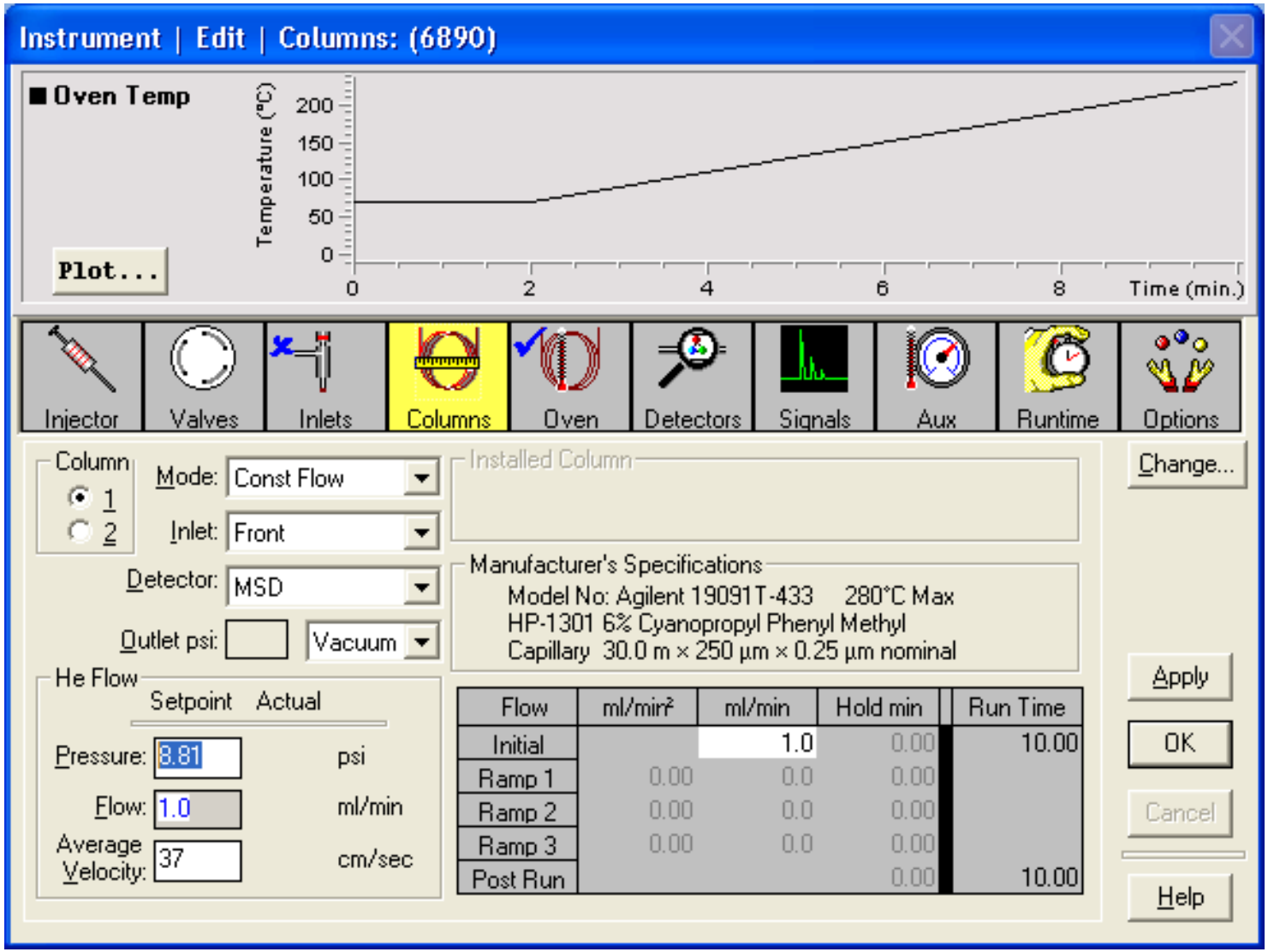
Pulsed Splitless

- sample containment more critical than in split injection
- much sharper peaks than in traditional splitless injection

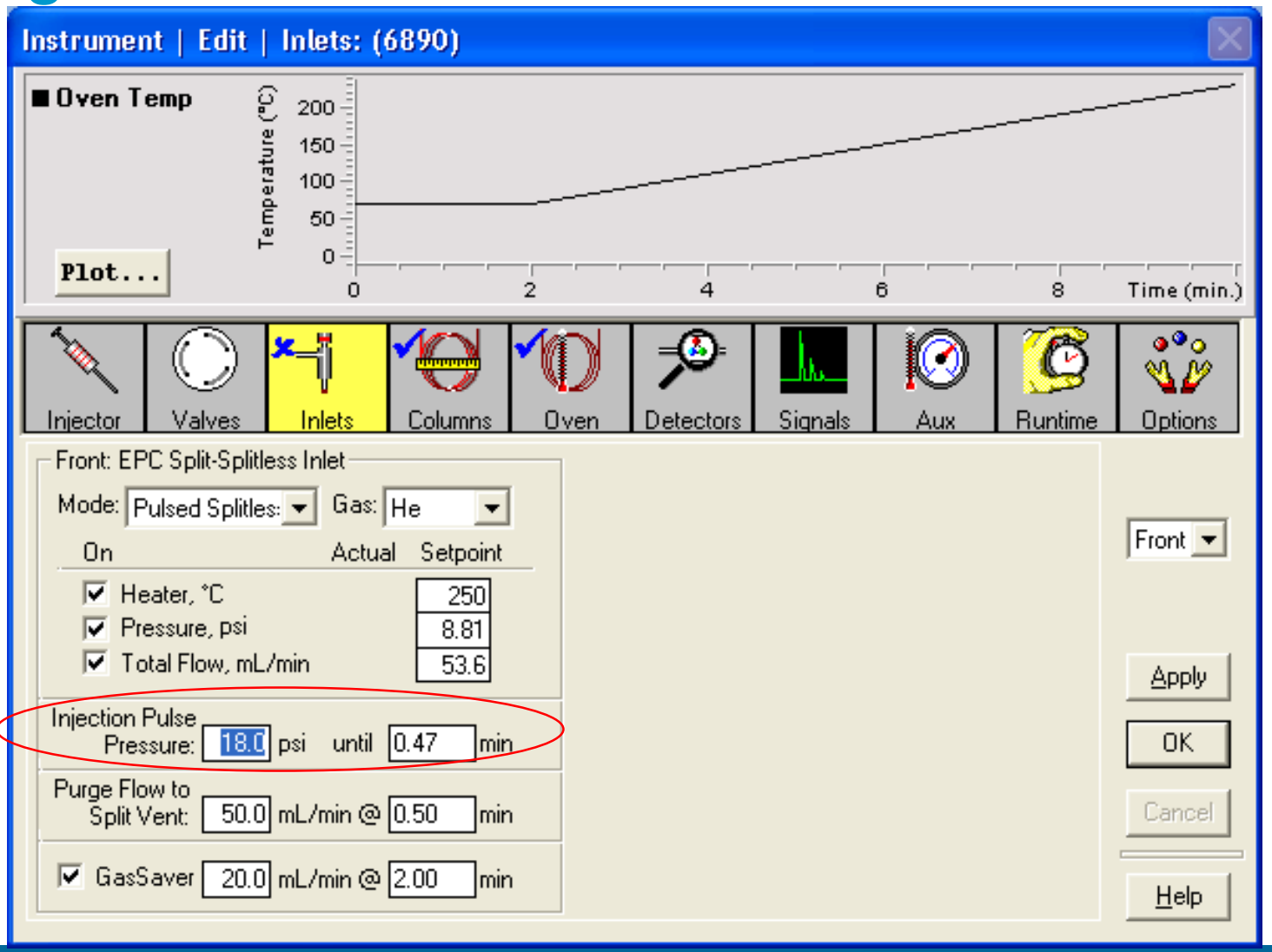
Select Pulsed Splitless Mode in Inlets



Check the Splitless Pressure



Double or Triple the Pressure for ~1 sec less than the Purge Activation Time



The BIGGEST Problem in GC is…

There are more things that DON'T go through a GC than DO!

….therefore, don't inject anything and you'll never have problems.

OK, inject, but realize that everything just got dirty…deal with it!

Where Does it Get Dirty?

Here

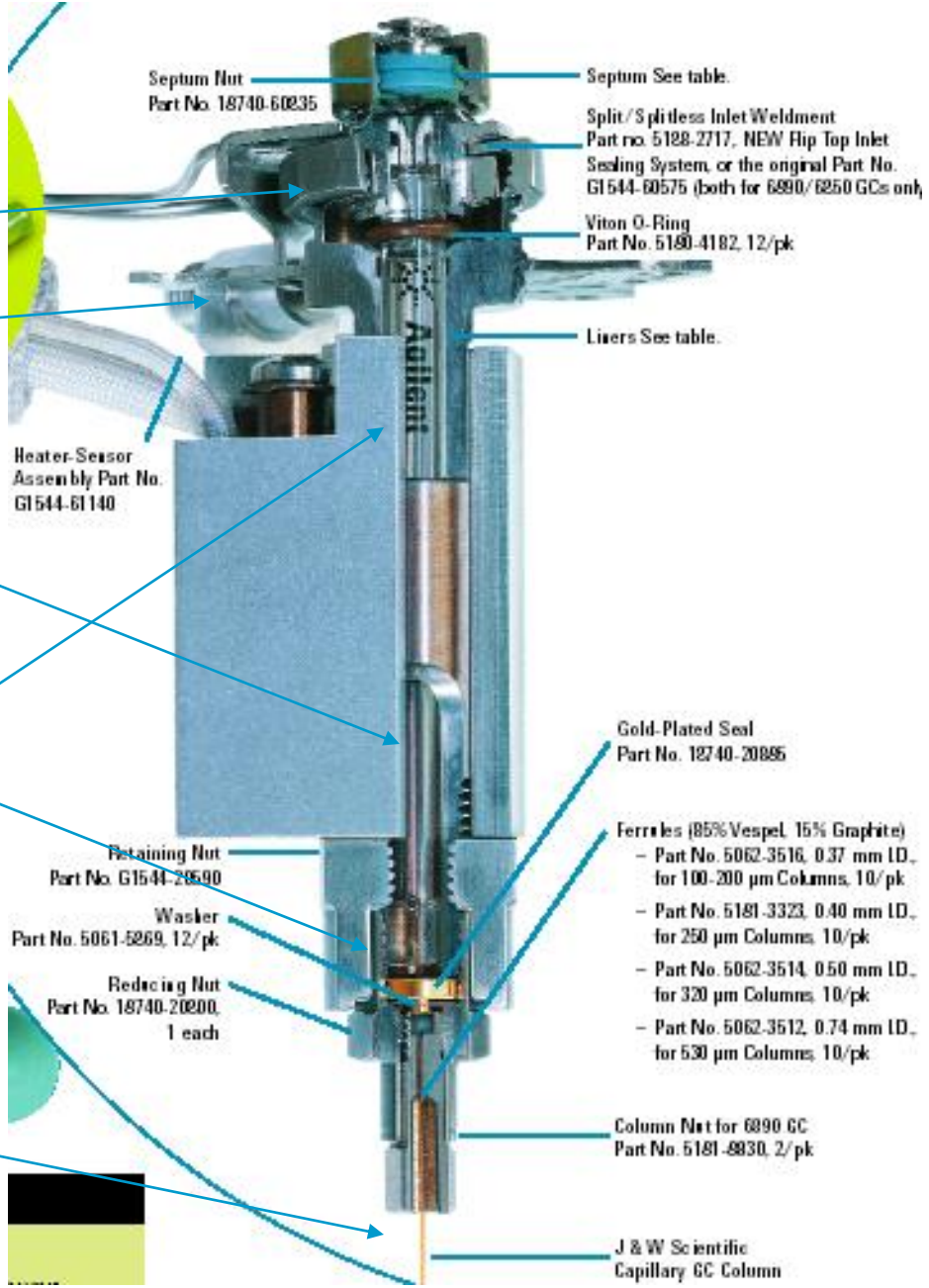
Here

Here

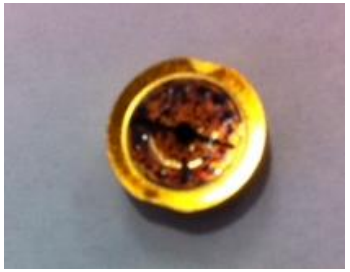
Here

Here

Here



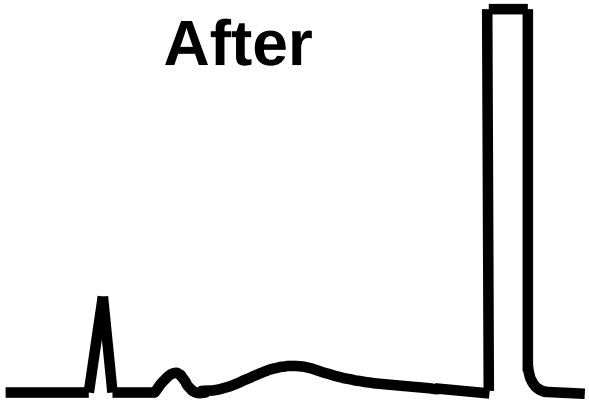
What Are You Doing!?



Bonus Peaks or Ghost Peaks

Before

After



Contamination in INJECTOR or FLOW (carrier gas)

-Contaminated consumables

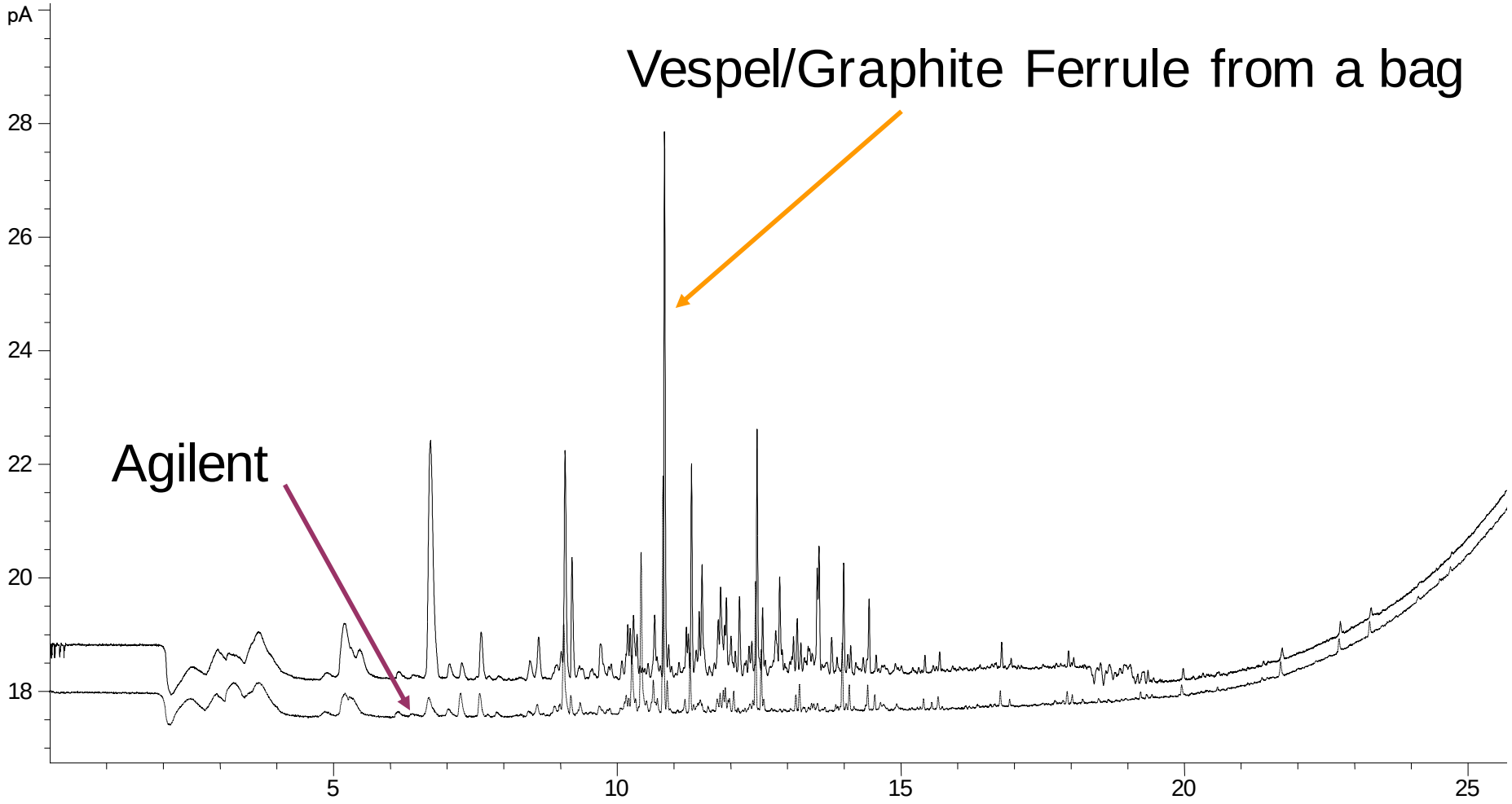
-Carryover from a backflash or previous sample

-Bad tank of gas or traps have expired

-Septum bleed

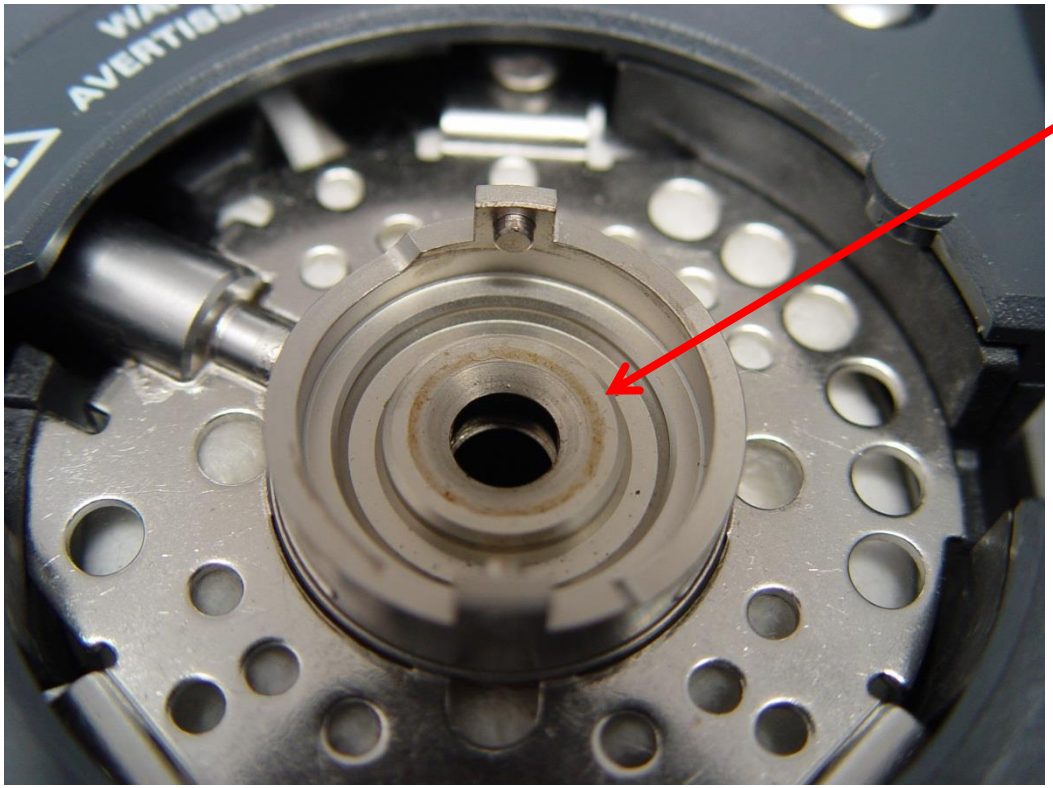
*TIP = Run a blank run…it should be blank!

Bonus Peaks - Ferrule Contamination



More “Off-Brand” O-Ring Issues

Controlled Substances Analysis, H2 Carrier



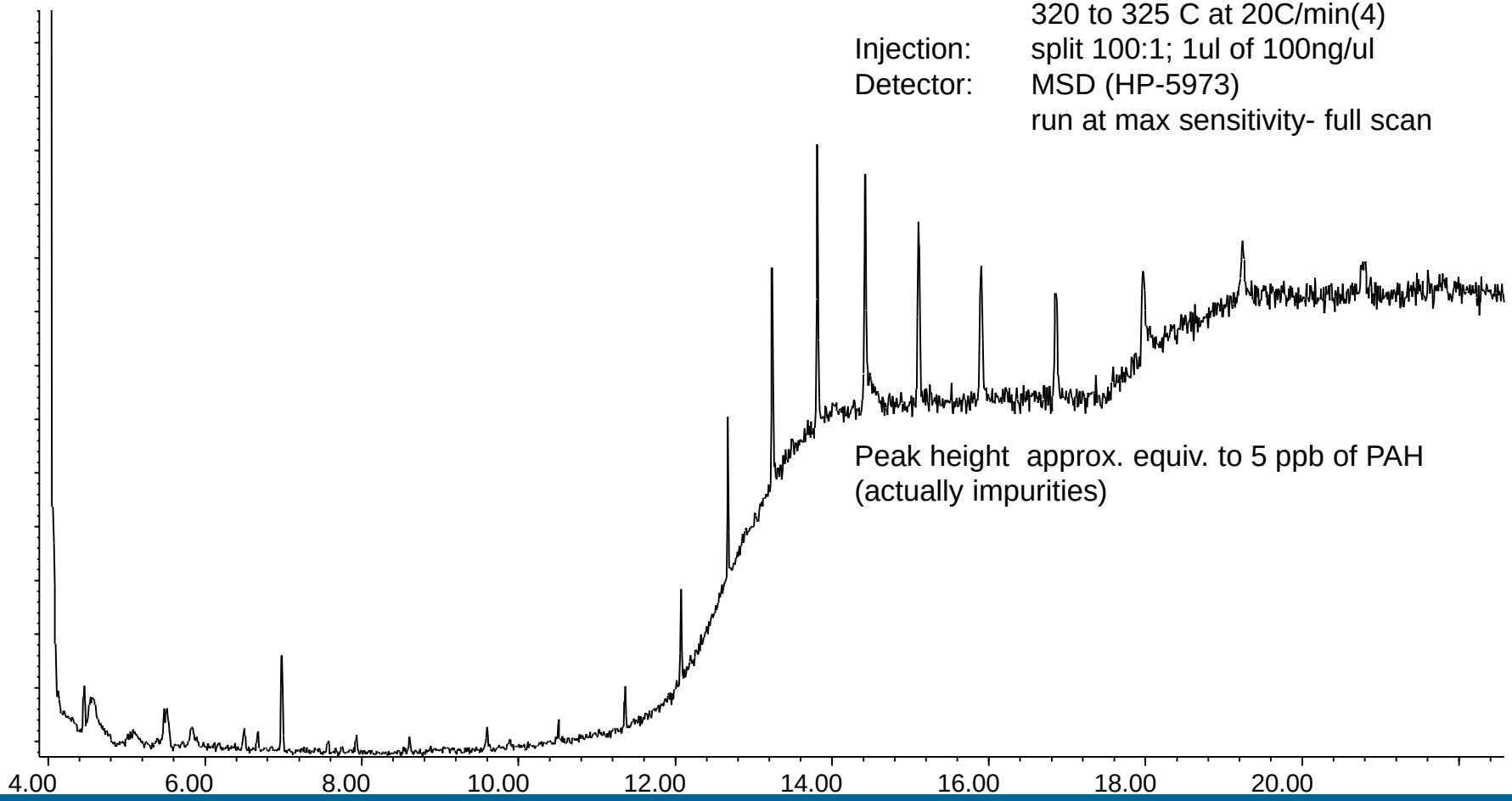
Residue on top of inlet weldment

Problem Resolution:
Agilent Non-Stick Liner O-Ring
p/n 5188-5365, 10PK

Septa Bleed vs Column Bleed (MSD)

Source of peaks from outside of the column

Columns:	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



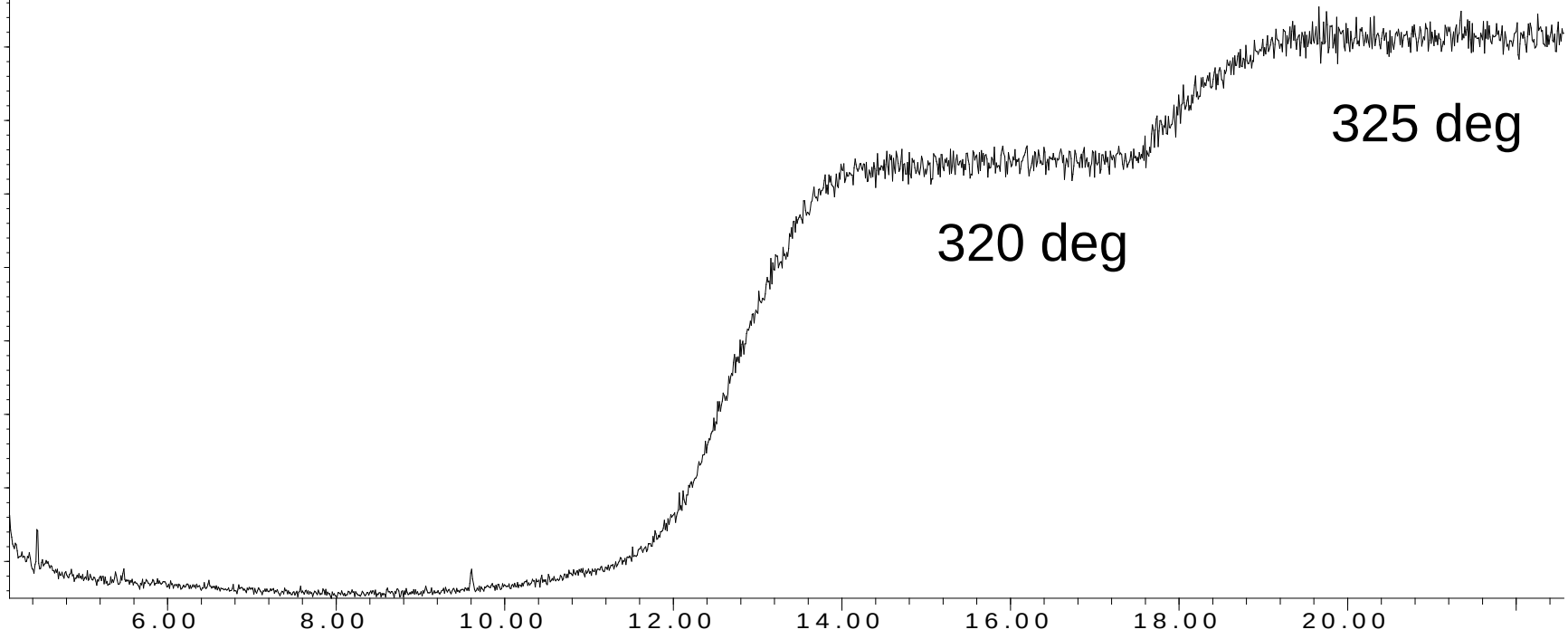
Peak height approx. equiv. to 5 ppb of PAH (actually impurities)

Septa Bleed vs Column Bleed (MSD)

Source of peaks from outside of the column *ELIMINATED*

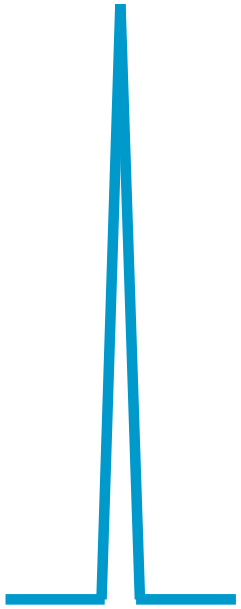
Columns:	HP 5 MS 30mx0.25mmx0.25um
Oven:	80 to160 C at 25 C/min, 160 to 320 C at 3 C/min(4) 320 to 325 C at 20C/min(4)
Injection:	split 100:1; 1ul of 100ng/ul
Detector:	MSD (HP-5973) run at max sensitivity- full scan

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



Peak Tailing

Before



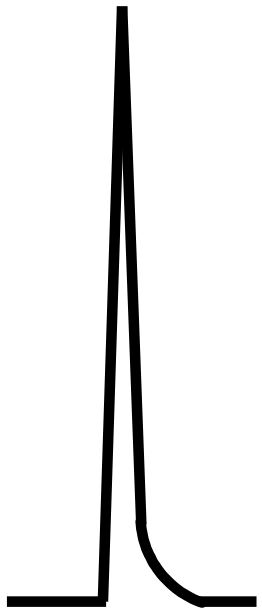
INJECTOR or COLUMN is Active

-Reversible adsorption of active compounds

(-OH, -NH, -SH)

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

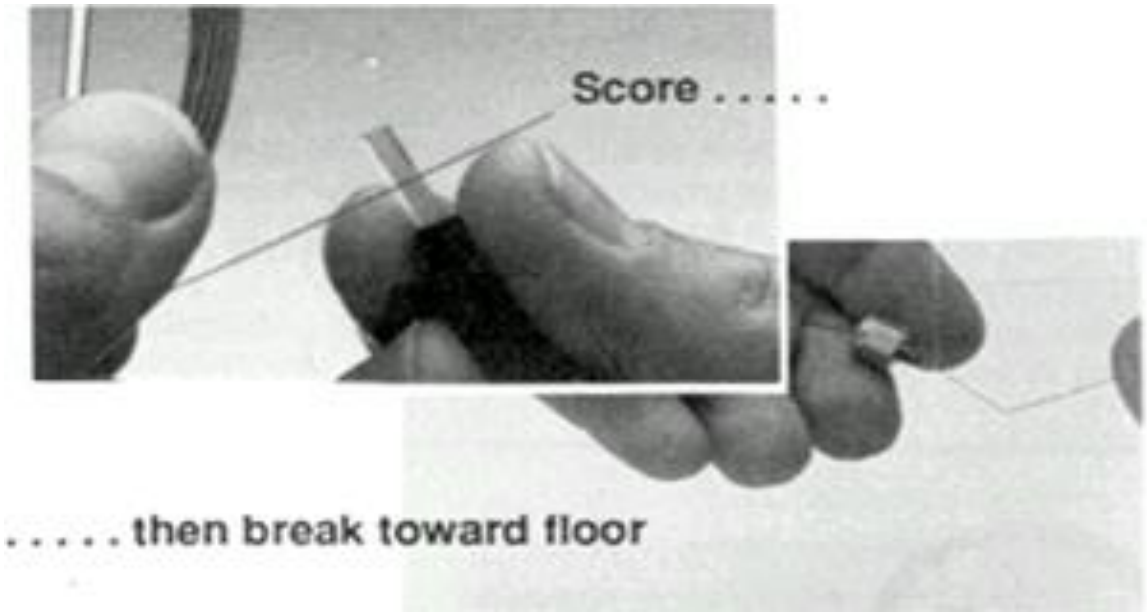
After

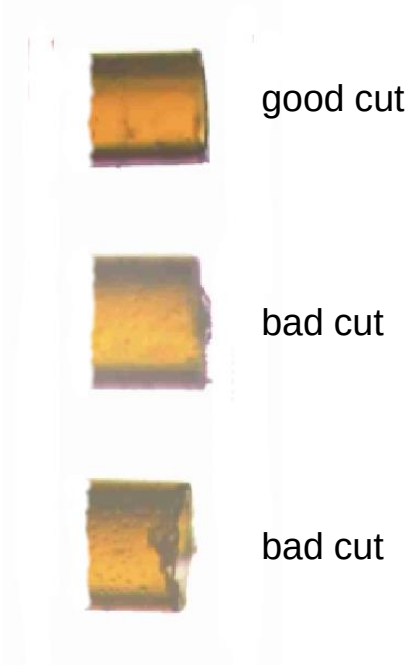


Miscellaneous – temperature issues for late eluters, overloading of PLOT columns, co-elution, polarity mismatch between phase, solute or solvent, and some compounds always tail

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

Flow Path Matters



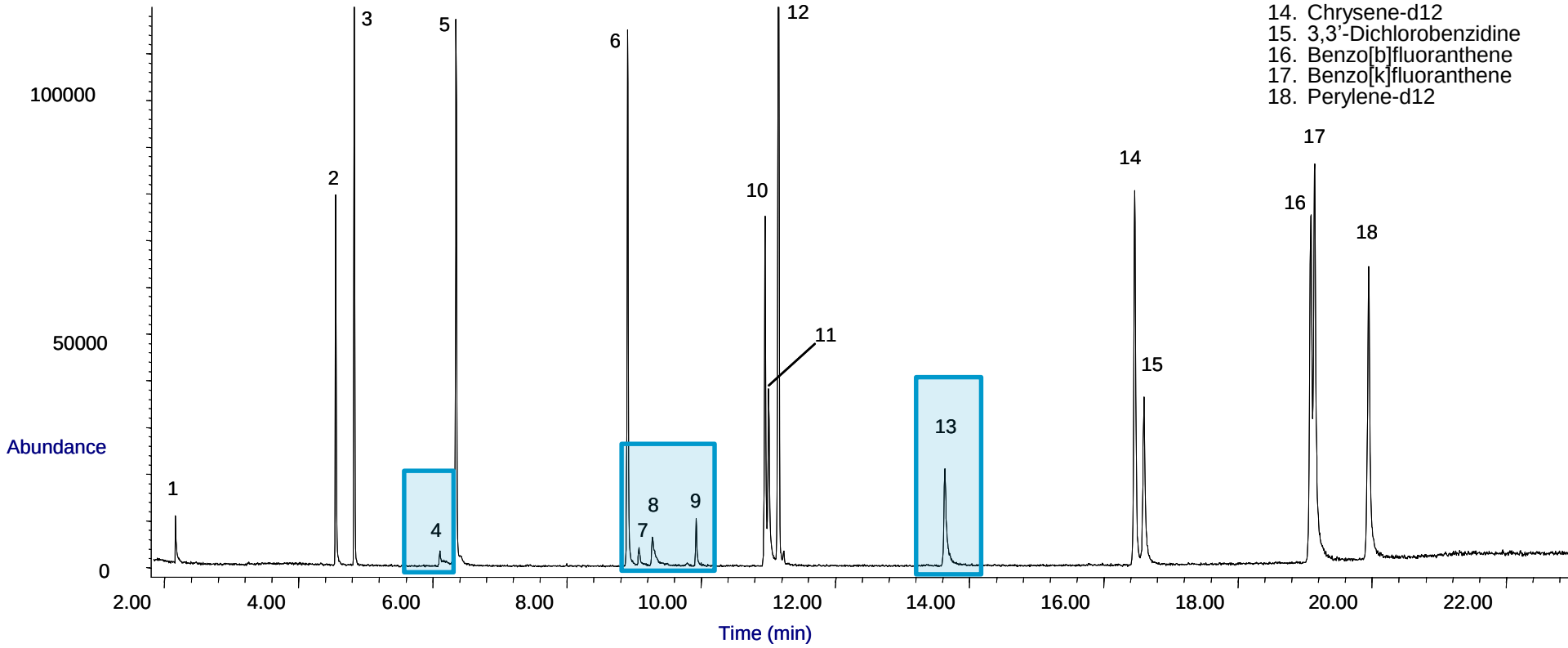


A flat, 90° square cut will be optimal for all connections

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

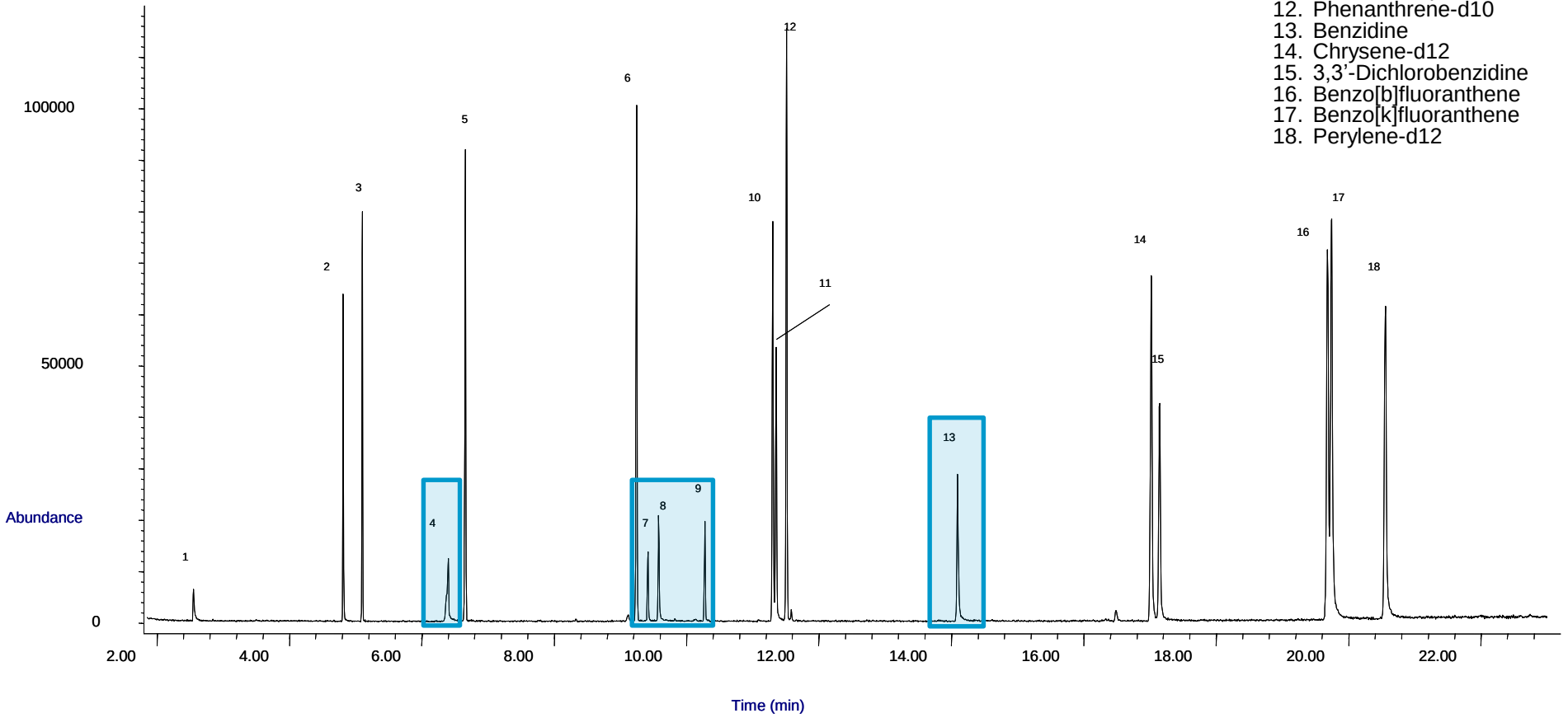
1. n-Nitrosodimethylamine
2. Aniline
3. 1,4-Dichlorobenzene-d4
4. Benzoic Acid
5. Naphthalene-d8
6. Acenaphthene-d10
7. 2,4-Dinitrophenol
8. 4-Nitrophenol
9. 2-Me-4,6-dinitrophenol
10. 4-Aminobiphenyl
11. Pentachlorophenol
12. Phenanthrene-d10
13. Benzidine
14. Chrysene-d12
15. 3,3'-Dichlorobenzidine
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)
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



Improved Performance

Improving the Entire Flowpath

New

Ultra Inert Inlet Liner

UltiMetal – TCD, FPD, NPD/FID Jets

UltiMetal Inlet Weldment, Shell and Transfer Lines

Ultra Inert Gold Seal

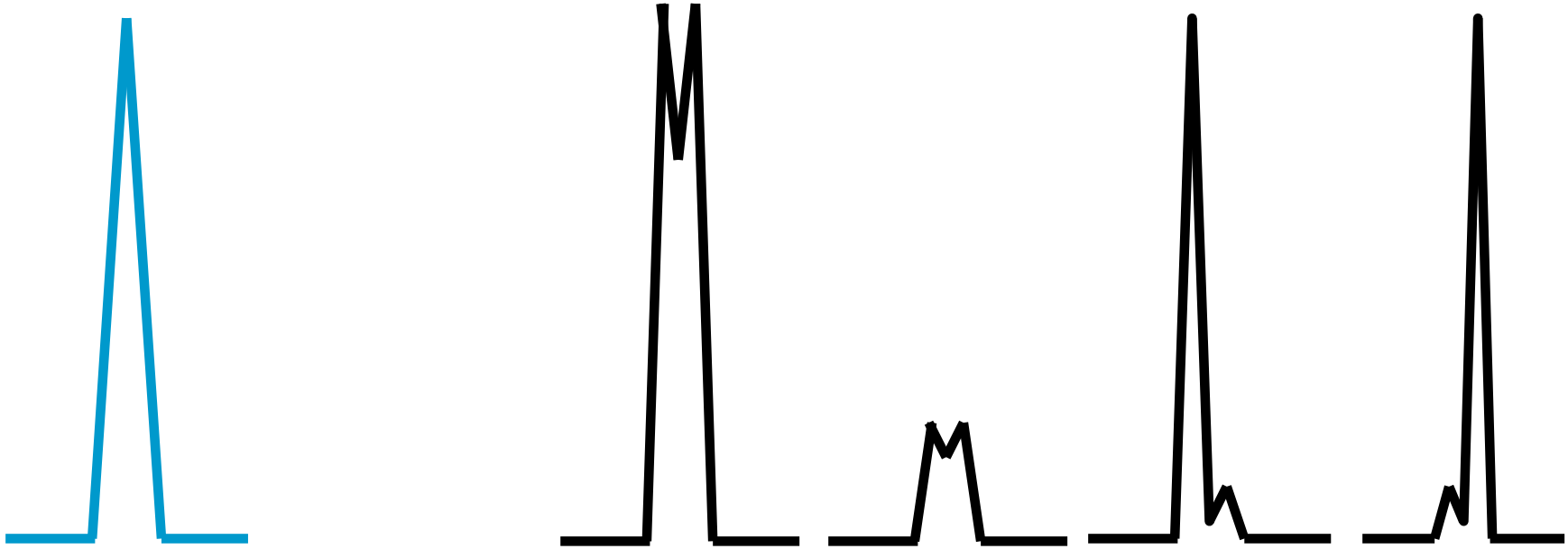
UltiMetal Capillary Flow Technology Devices, Ultimate Union

New UltiMetal FlexiMetal Ferrules

Ultra Inert GC Column

…now from a single supplier

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

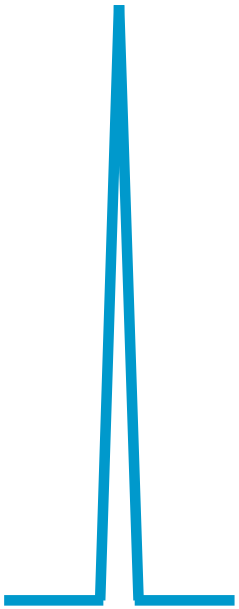
VOLATILITY

High boilers dropping out on Cold Spots

-Transfer line temps

-Unions or fittings not tracking column temp

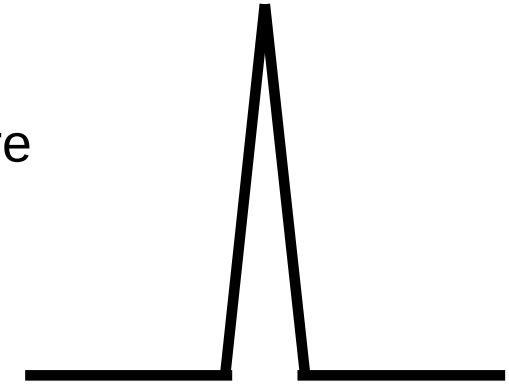
Broad Peaks



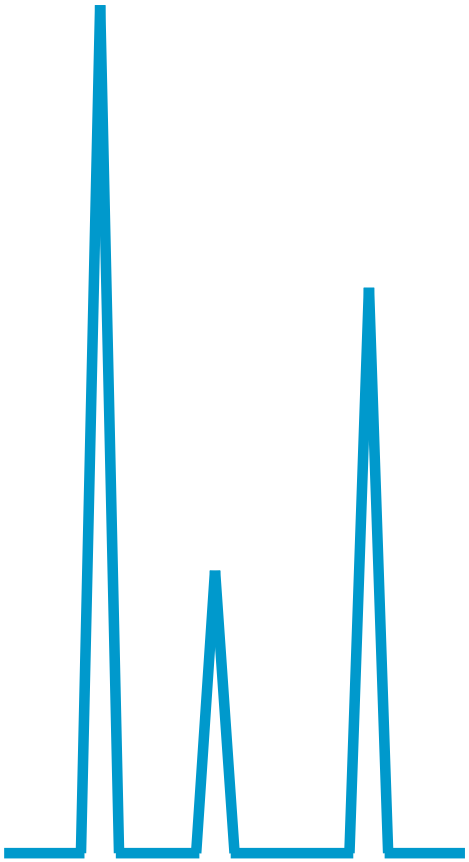
INJECTOR
-Poor Installation
-Change in settings (temps/flows)
-Poor sample focusing
-Large change in sample concentration

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

COLUMN
-Contamination
-Damaged/old stationary phase
-Reverse Solvent Effect



No Peaks



DETECTOR (not on or not operational)

INJECTOR (not working)

-Plugged syringe/plunger not moving

-Wrong injector (or detector)

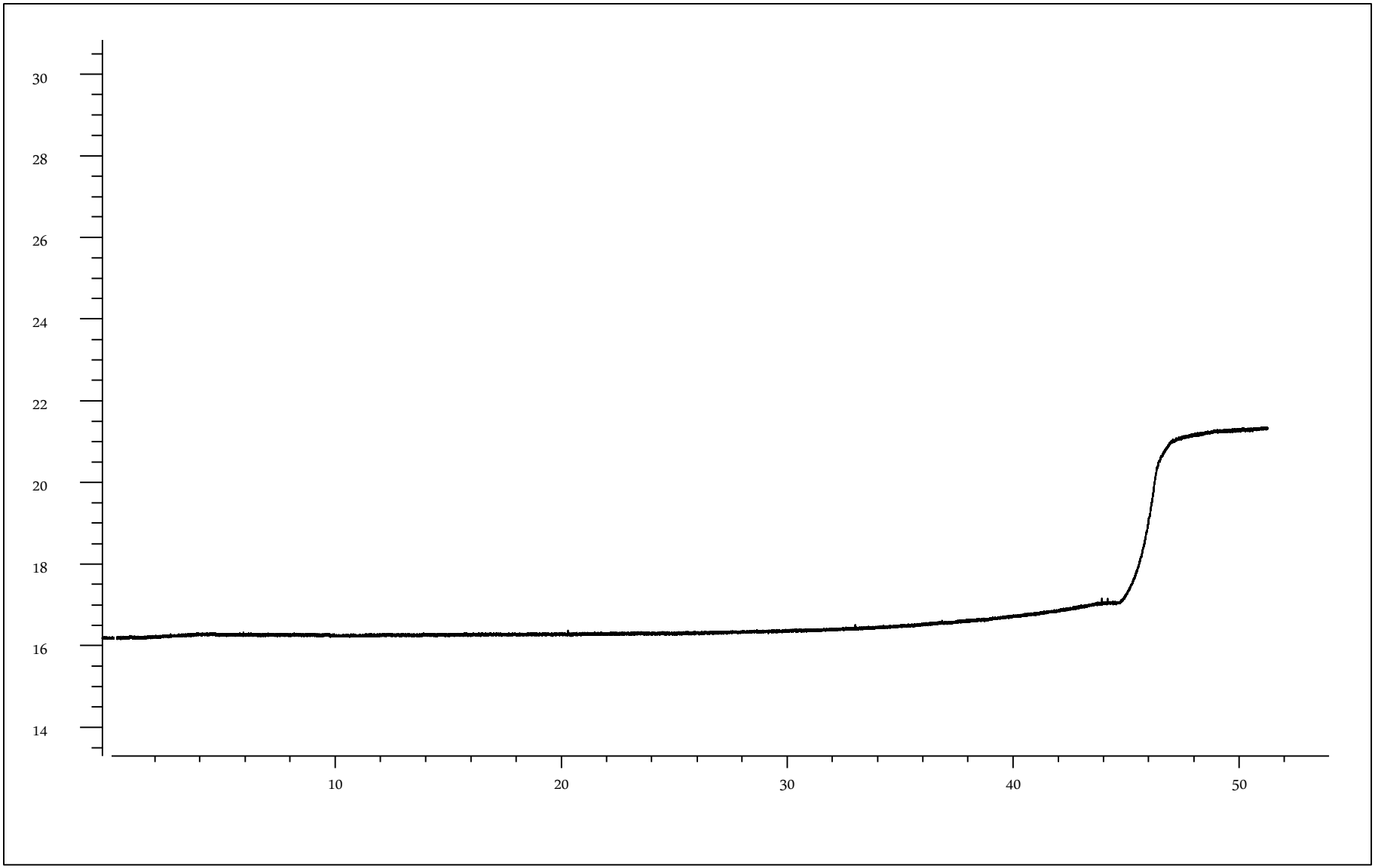
-Huge leak/no carrier gas flow (older systems)

NOT the COLUMN Unless…

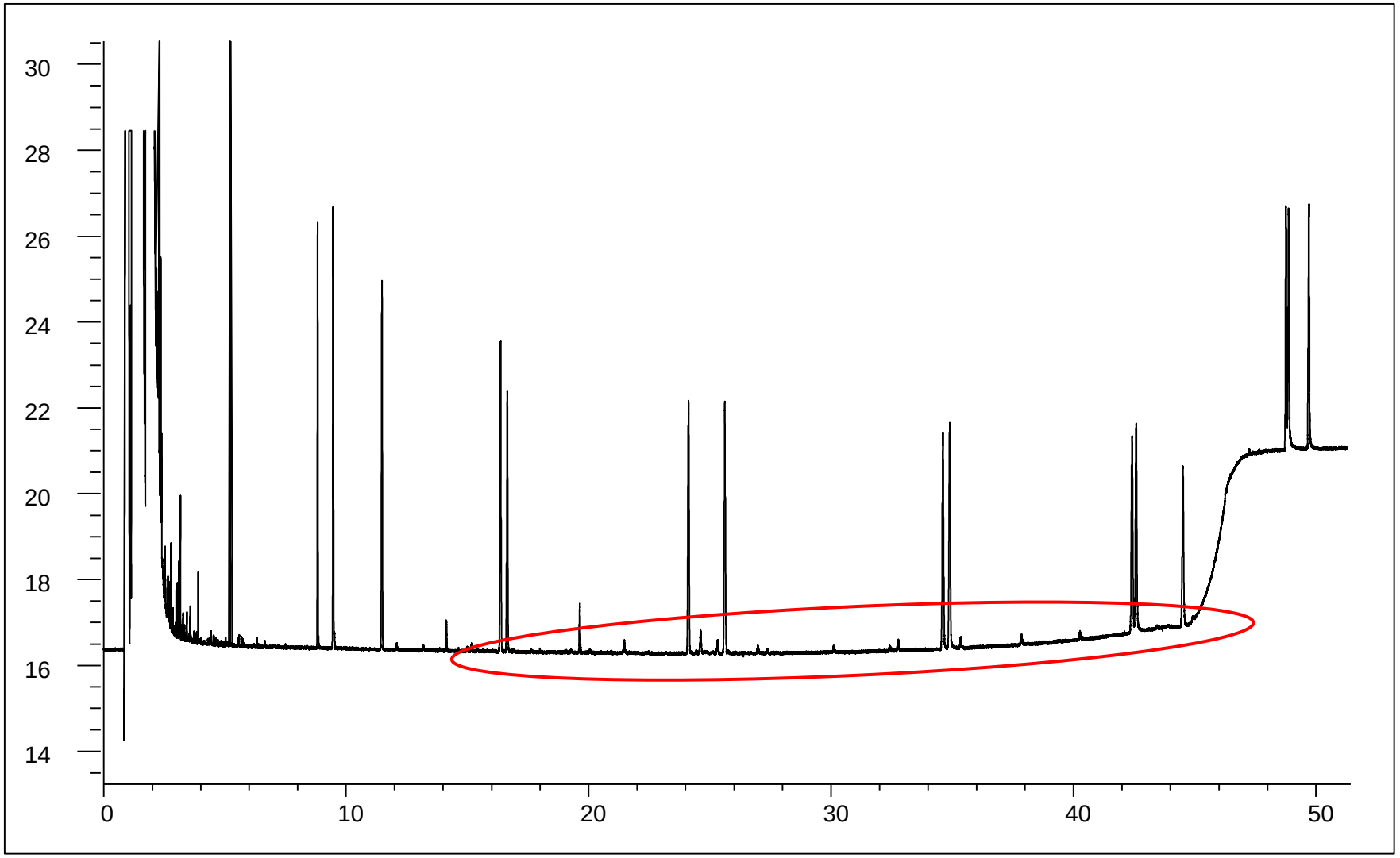
-Broken column

-No column

Symptom – No Peaks

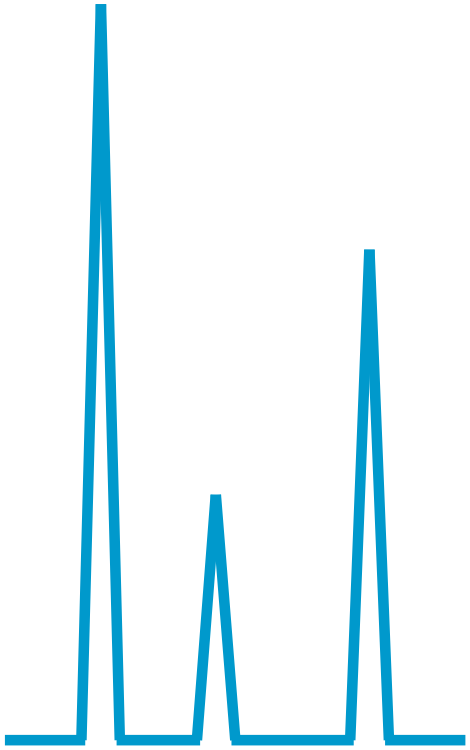


Solution - Unplugged Syringe



Peak Response

All Change in Size



INJECTOR

-Leaky syringe

-Split ratio set incorrectly

-Wrong purge activation time

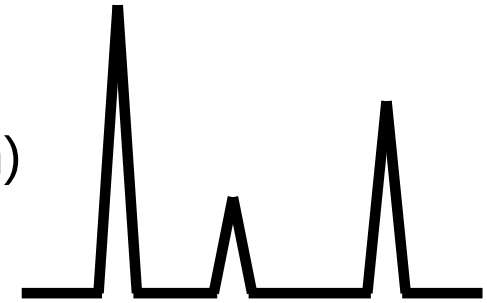
-Septum purge flow too high

-Injector temperature too low*

DETECTOR (response problem)

-Settings or flows changed

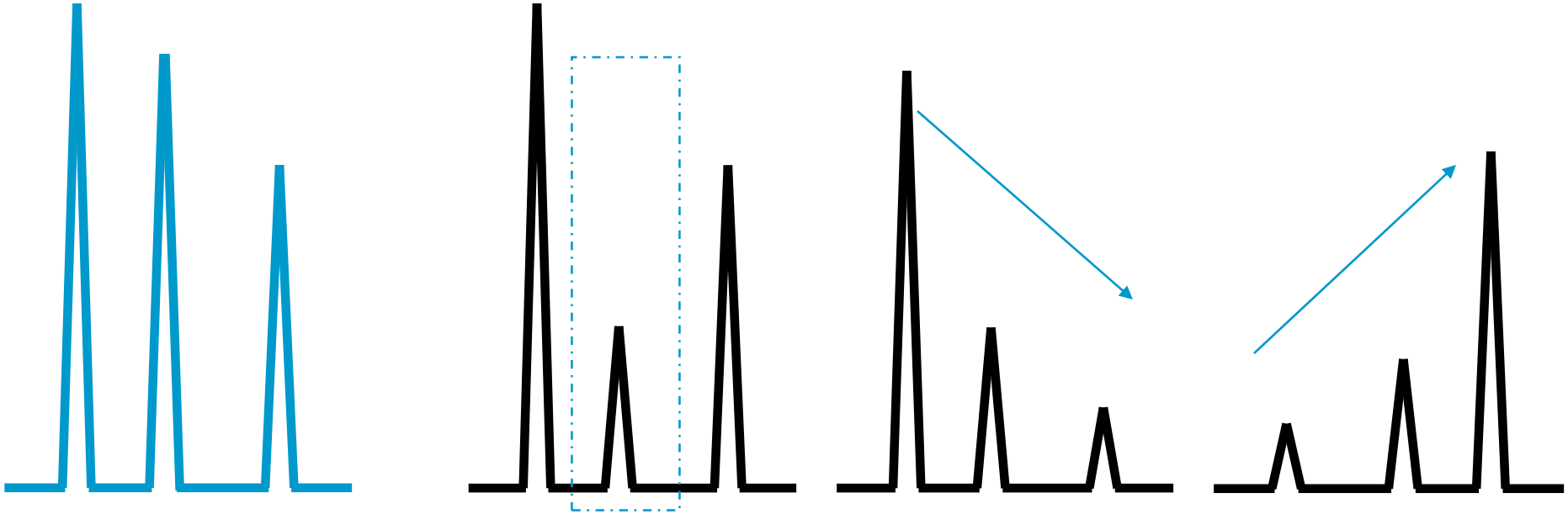
-Electronics failing



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

Peak Response

Some Change in Size



INJECTOR or COLUMN is active/contaminated

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

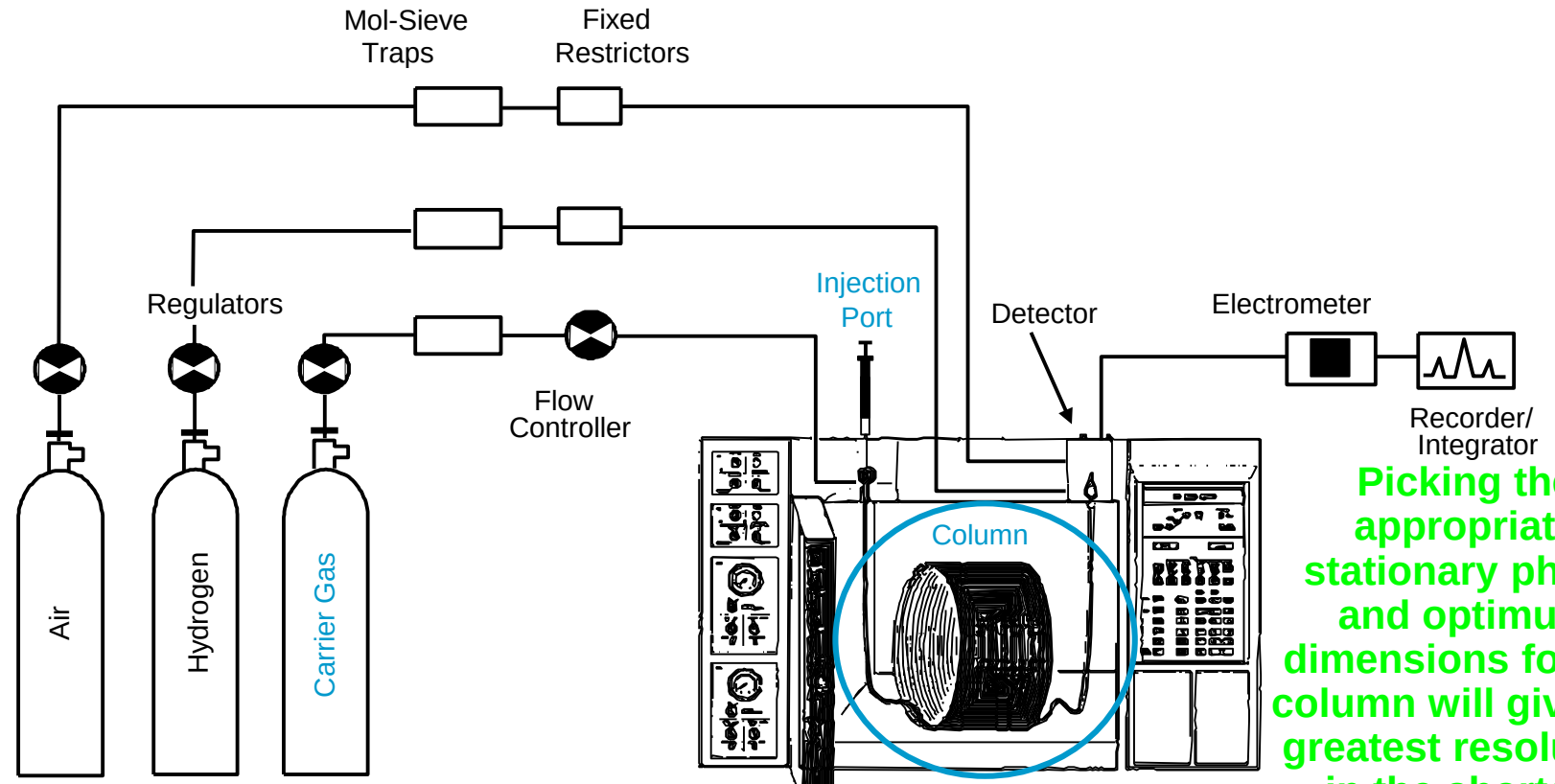
-Decomposition of sample

-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.

Typical Gas Chromatographic System

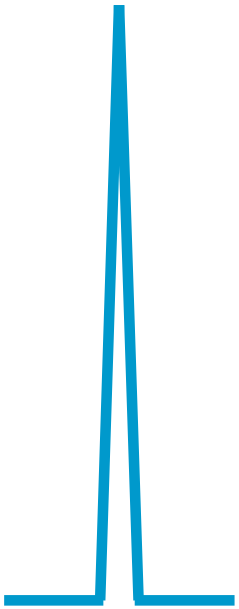


Cylinders or Generators

Picking the appropriate stationary phase and optimum dimensions for the column will give the greatest resolution in the shortest analysis time.

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

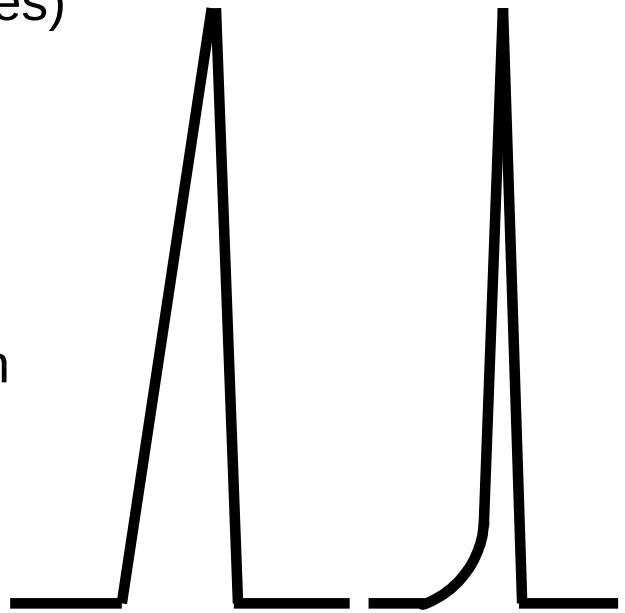
-Compound very soluble in injection solvent (need retention gap)

-Mixed sample solvent

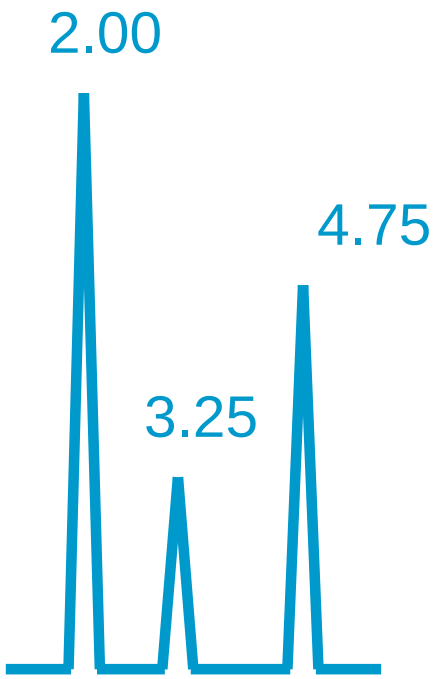
OTHER

-Co-elution

-Breakdown



Retention Time Shift



INJECTOR

-Change in injection solvent

-Large change in sample concentration

FLOW

-Leak in the septum

-Change in gas velocity

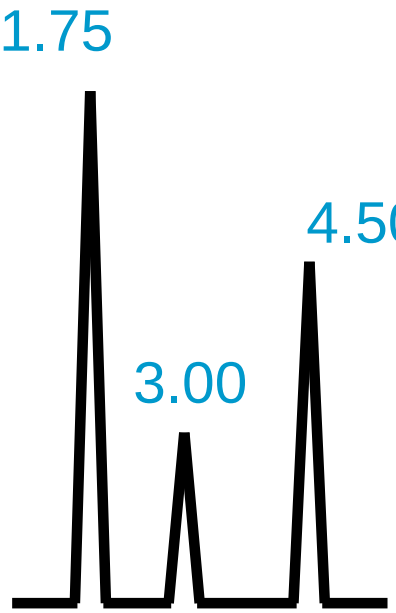
COLUMN

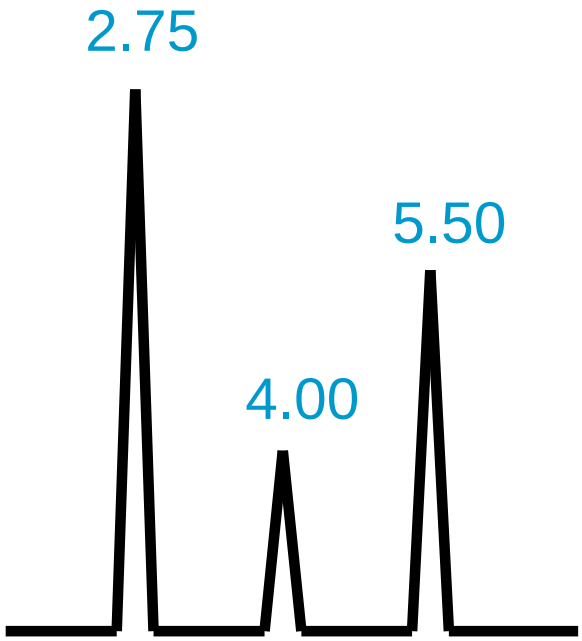
-Contamination

-Damaged stationary phase

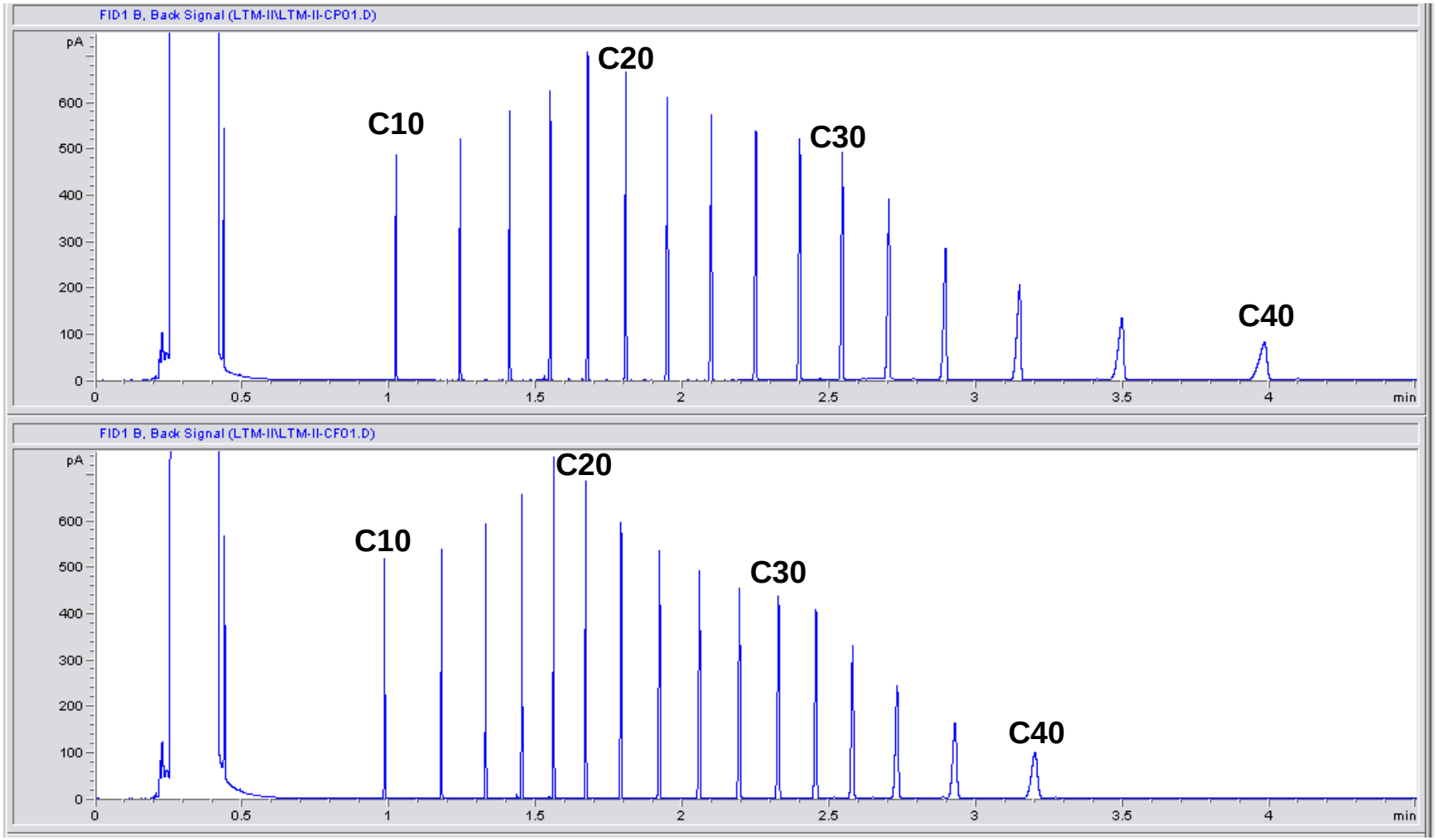
-Loss of stationary phase

-Change in temperature



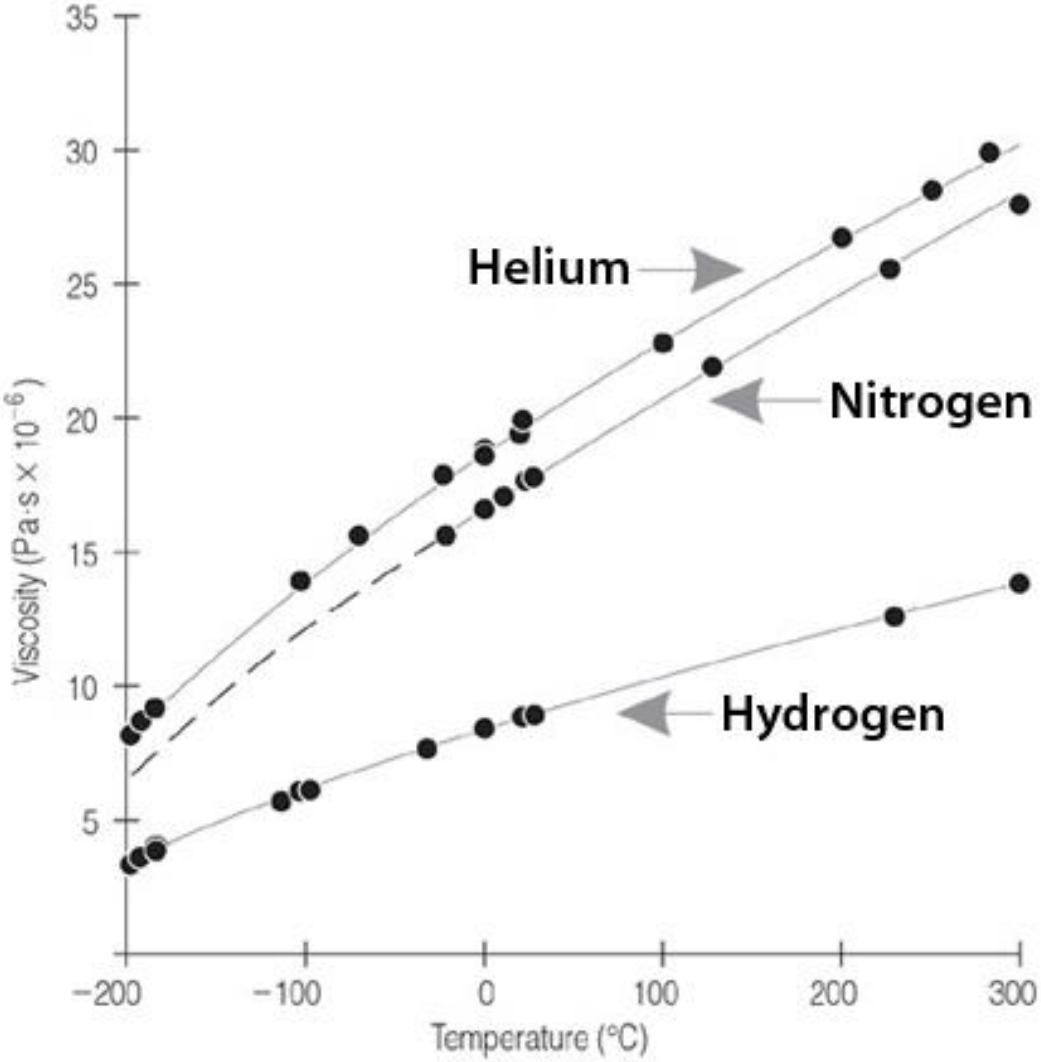


Constant Pressure vs Constant Flow Retention



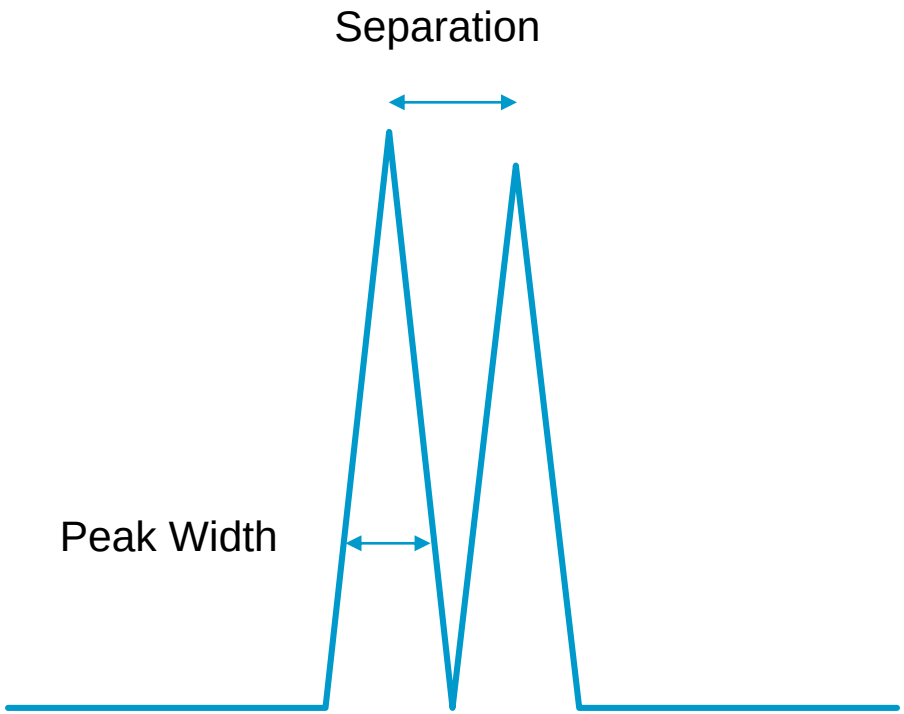
Under constant pressure conditions, flow decreases as temperature increases. (viscosity of a gas increases as temperature increases)

Gas Viscosity vs Temperature



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

Loss of Resolution



Resolution is a function of separation and peak width

Loss of Resolution - Separation Decrease

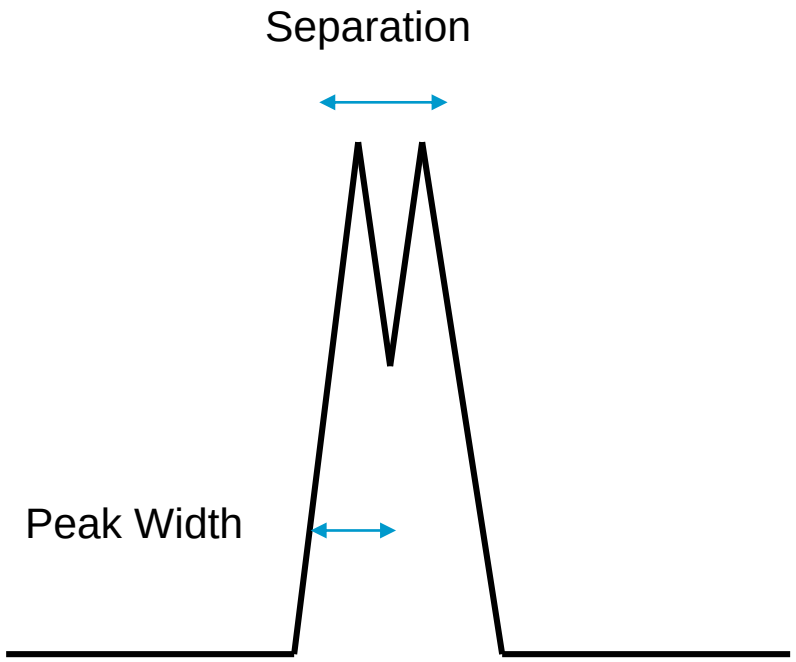
COLUMN

-Different column temperature

-Contamination (more phase?)

-Matrix components co-eluting

-Different column phase?



Loss of Resolution - Peak Broadening

FLOW

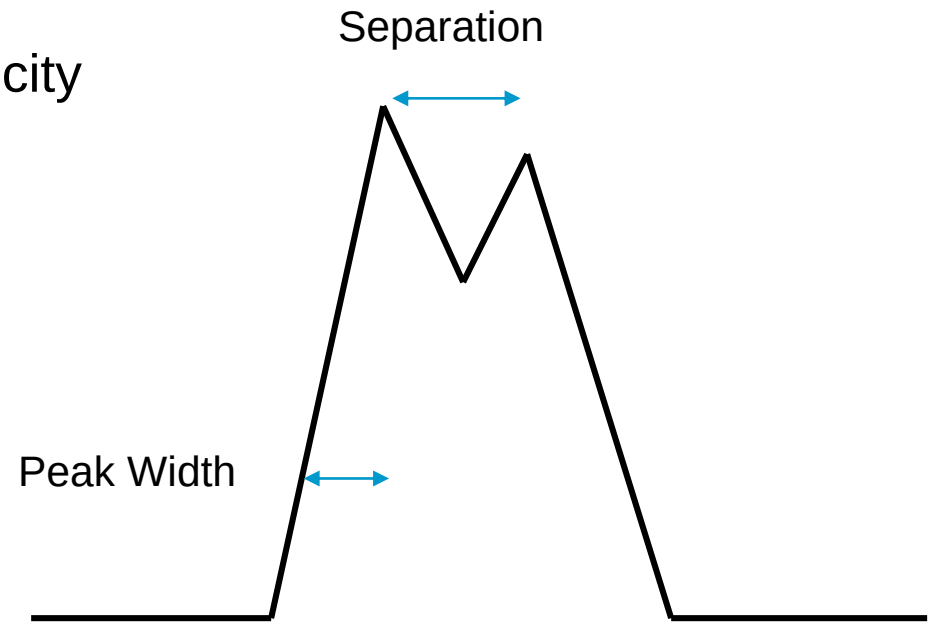
-Change in carrier gas velocity

-Make-up gas

COLUMN

-Contamination

-Phase degradation



INJECTOR (efficiency)

-Settings, Liner, Installation, etc.

Typical Problems of Optimized Methods becoming Unoptimized…and the Reason Why.

- Peak Tailing – Flow Path or Activity
- Bonus Peaks – In Sample or Back Flash (Carry Over)
- Split Peaks – Injector Problems, Mixed Solvent
- 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

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

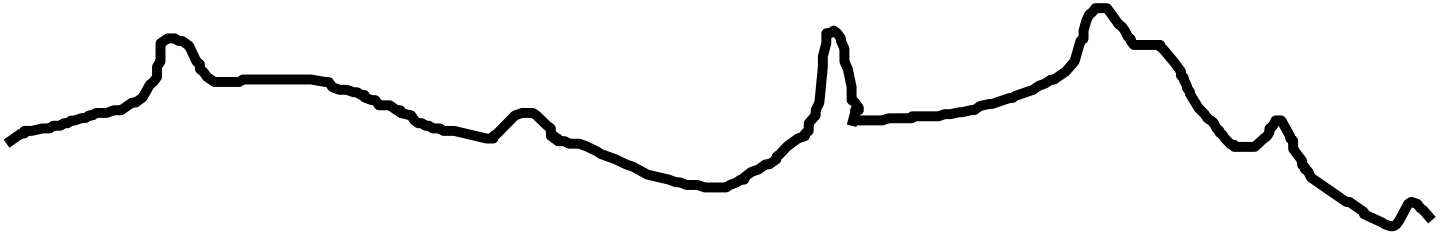
-Sample evaporation – leaky vials

-Sample decomposition

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

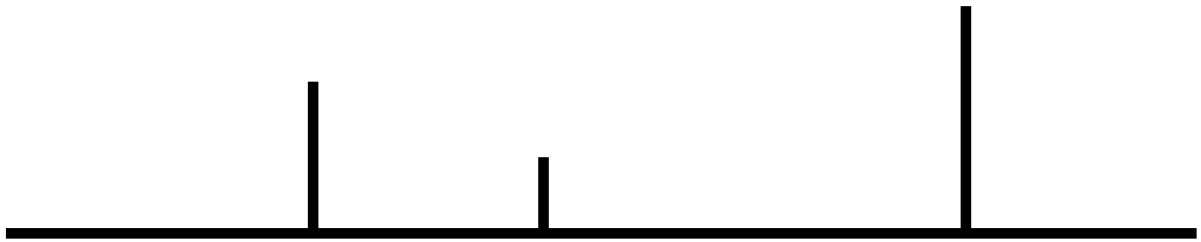
-In detector flame (poor installation)

DETECTOR

-Air leak - ECD, TCD

-Electronics malfunction

Spiking Baseline



DETECTOR

-Particles entering the detector

-Random: poor connection

-Regular: nearby "cycling" equipment (electronics)

Remember

Complete system = Carrier Gas + Injector + Column + Detector + Data System

Multiple cause and effect

Do not change too many variables at once

Self-Tightening Column Nuts

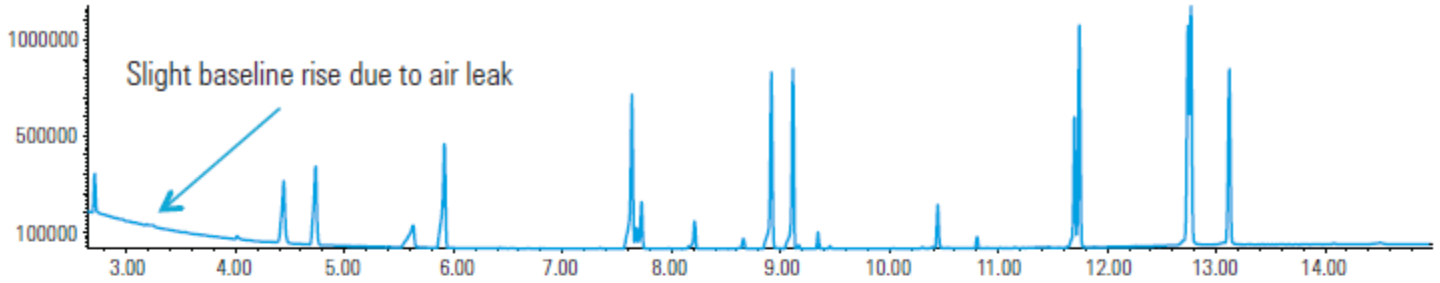
Innovative spring-driven piston continuously presses against ferrule

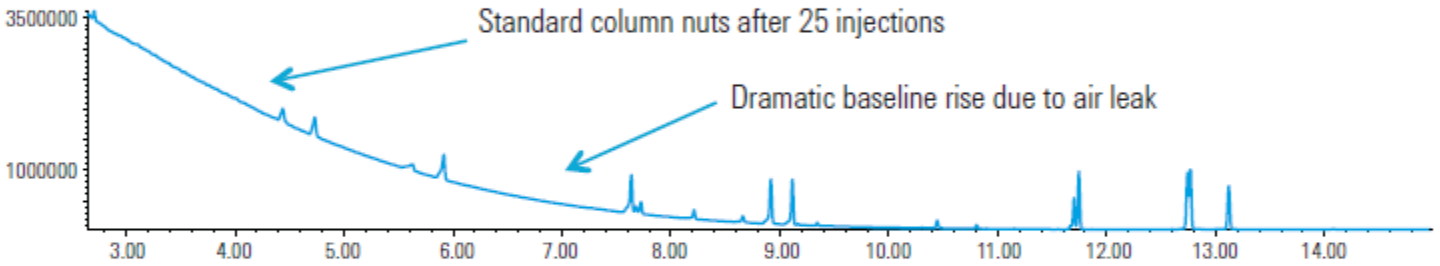
- **Less wasted time:** No retightening needed after repeated thermal cycles
- **Ease of use:** Finger-tight, consistent connections *without tools*
- **Leak Free = Lower column bleed:** Longer column life

Video at **agilent.com/chem/STnutvideo**

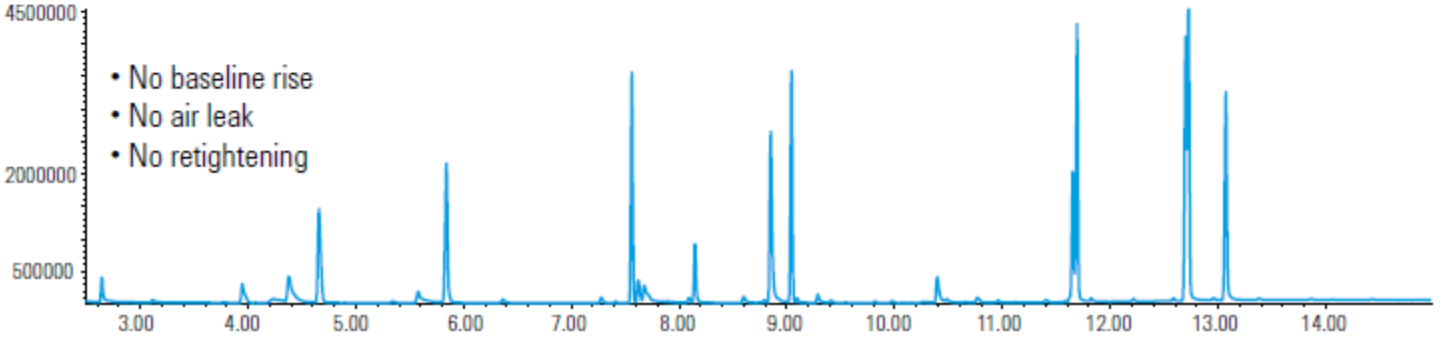
Self-Tightening Column Nuts

Standard column nuts new fitting

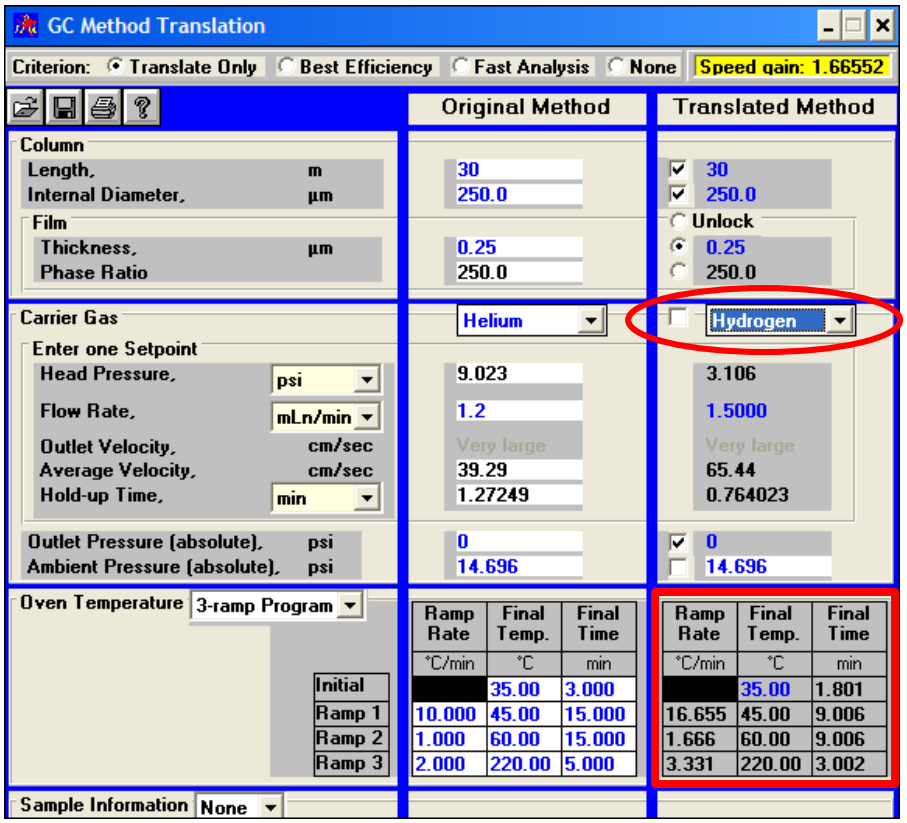


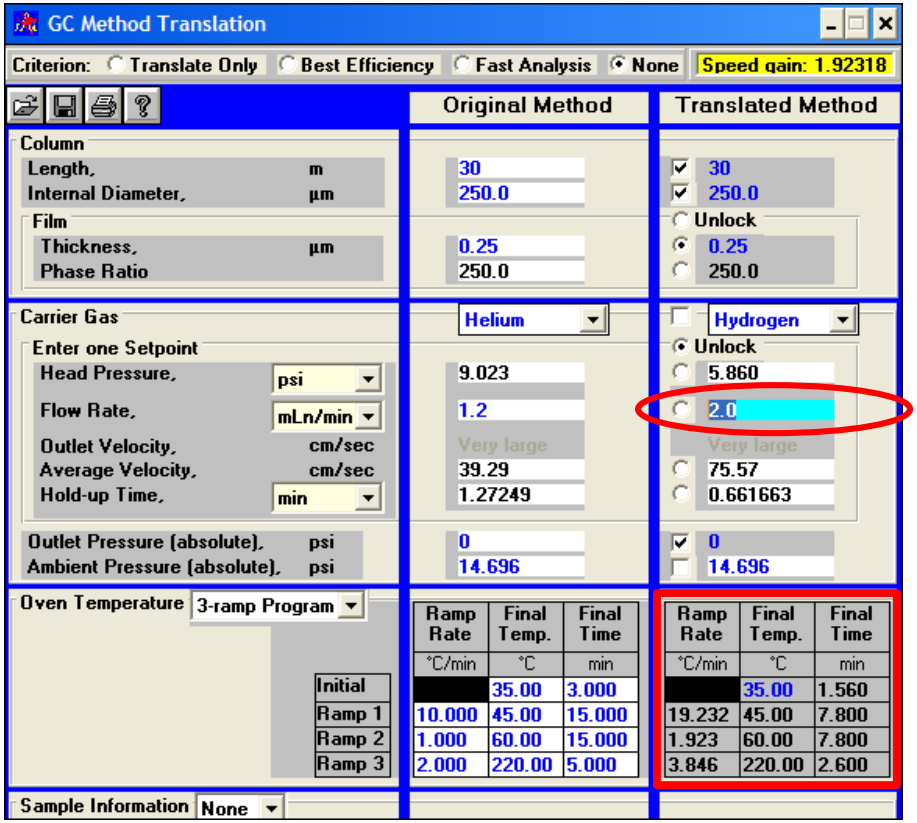


Agilent Self Tightening Column Nuts after 400 injections



Changes in Column Dimensions, Gas Type or Velocity Require Changes in Temp Program Rates





Method Translation Software to the Rescue!

Troubleshooting Resources

Online Troubleshooting and Maintenance Videos

http://www.chem.agilent.com/en-US/Technical-Support/Instruments-Systems/Gas-Chromatography/Pages/troubleshootingvideos.aspx

GC Troubleshooting Guide

http://www.chem.agilent.com/en-US/Products-Services/Instruments-Systems/Gas-Chromatography/pages/gp6770.aspx

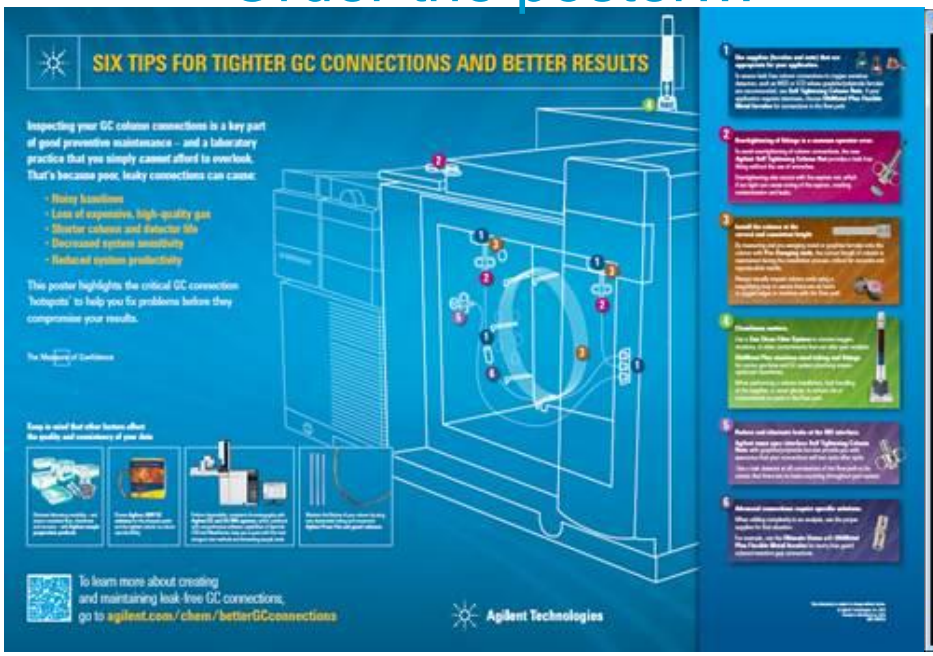
Method Translation Software

http://www.chem.agilent.com/en-US/Technical-Support/Instruments-Systems/Gas-Chromatography/utilities/Pages/gcmethodtranslation.aspx

Agilent Better GC Connections

www.agilent.com/chem/betterGCconnections

Order the poster…



View the video…

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1-800-227-9770, #3

1 (214) 883-2260 (Eric)

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gc-column-support@agilent.com

Eric.pavlich@agilent.com