A Tale of Two Peaks: Troubleshooting Poor Peak Shape

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January 17, 2019

“Everything was Just Fine and then this Happened!”

“How do I go about Troubleshooting?”

Track events- log book

- Changed column, liner, septum, syringe, etc.
- Injected samples, other method, etc.
- Did maintenance, cut column, inlet flush, etc.

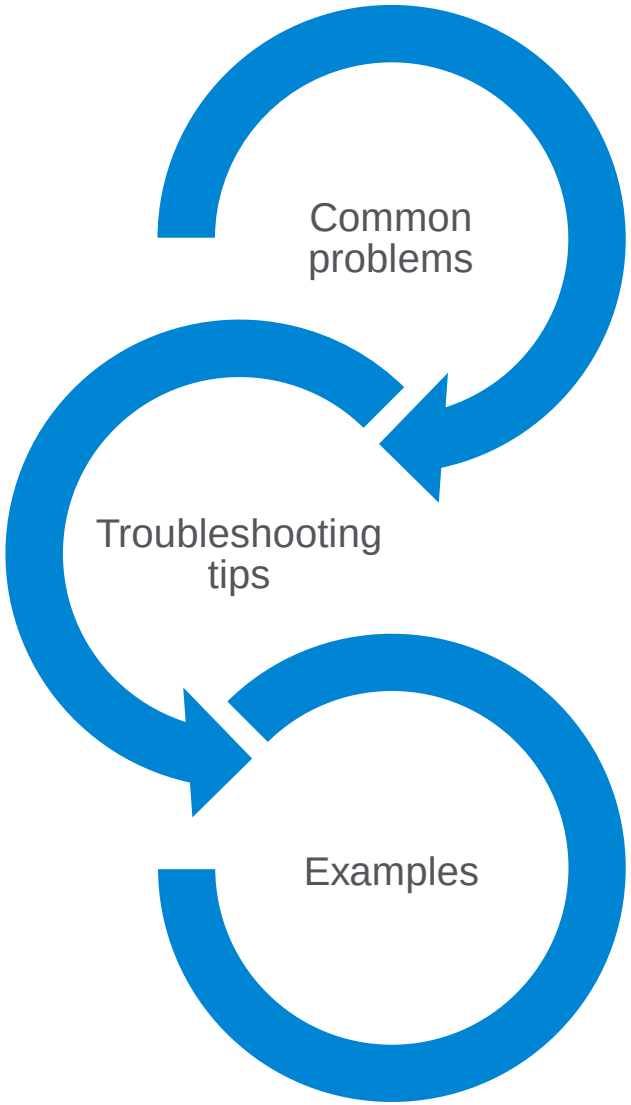
Logic = Something changed (slowly or sudden) = Something is different

Logical Troubleshooting

Troubleshooting starts with isolating the problem

- There are five basic areas from where the problem arises
  - -Injector
  - -Flow
  - -Column
  - -Detector
  - -Electronics
- But of course it can always be some combination
- Knowing what can and cannot cause the symptom is the key

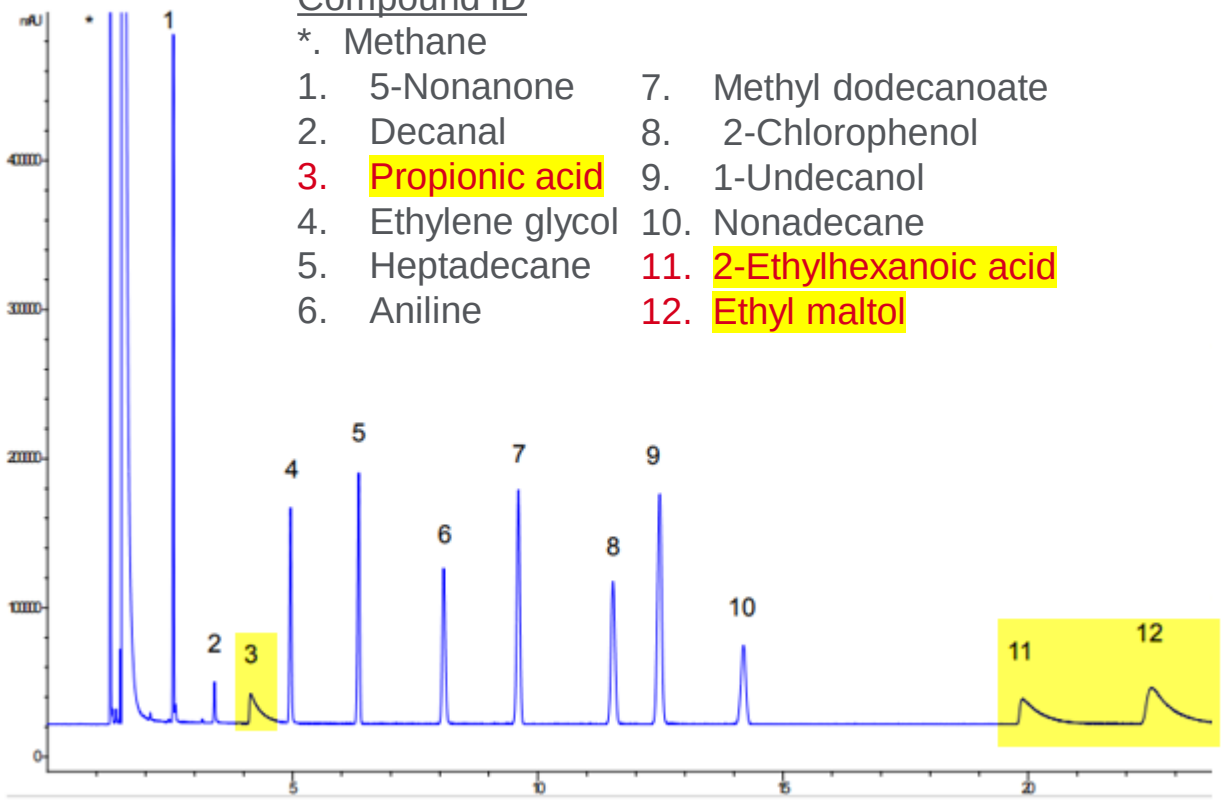
Agenda



Act 1: Common Peak Shape Issues

- **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

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

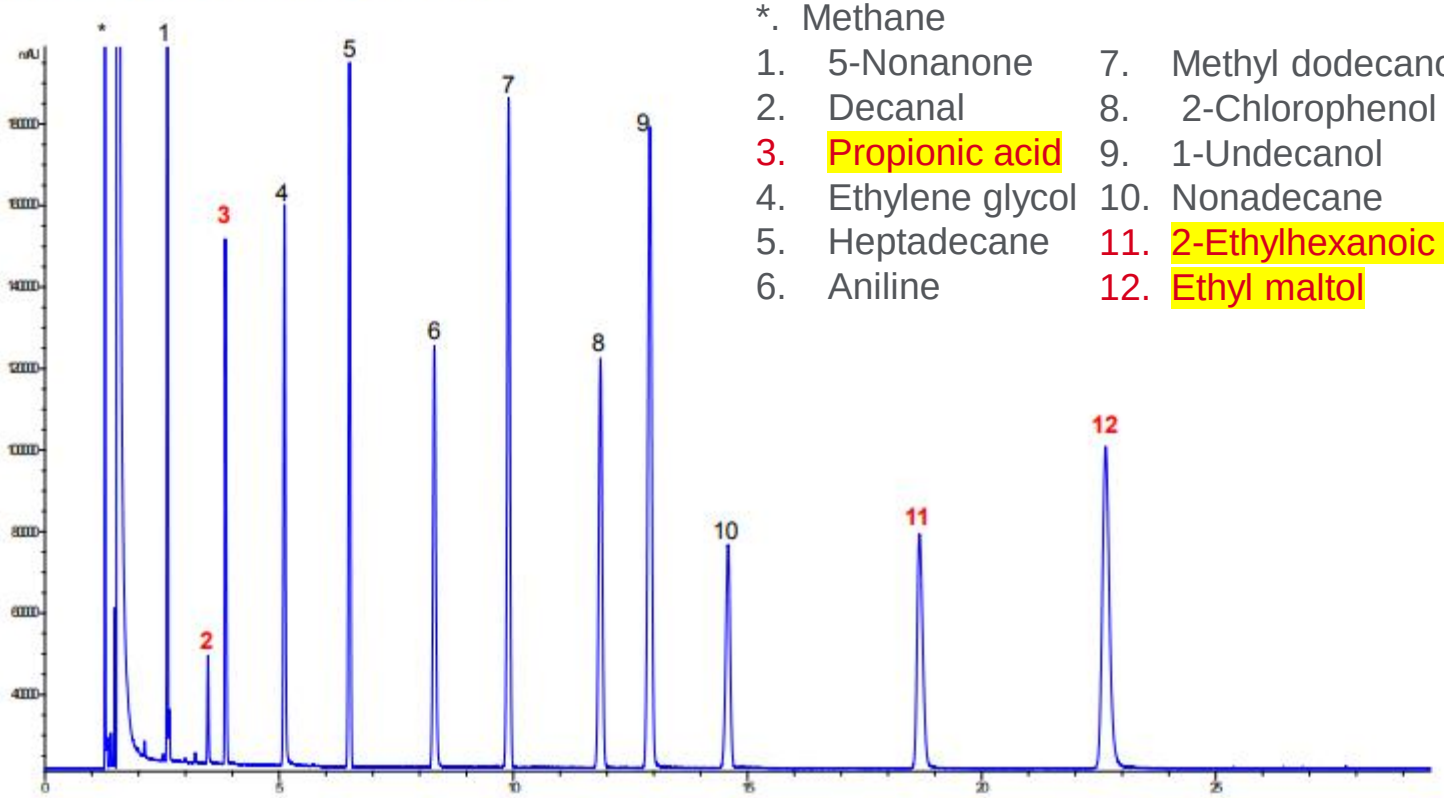
Miscellaneous - overloading of PLOT columns, co-elution, polarity mismatch between phase, solute or solvent, and some compounds always tail

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

Agilent Inert Flow Solution

Modified Agilent J&W DB-WAX UI mix on DB-WAX UI, 122-7032UI

*Every column is tested individually



Compound ID

*. Methane
1. 5-Nonanone
2. Decanal
3. Propionic acid
4. Ethylene glycol
5. Heptadecane
6. Aniline
7. Methyl dodecanoate
8. 2-Chlorophenol
9. 1-Undecanol
10. Nonadecane
11. 2-Ethylhexanoic acid
12. Ethyl maltol

Brochure 5991-6709EN

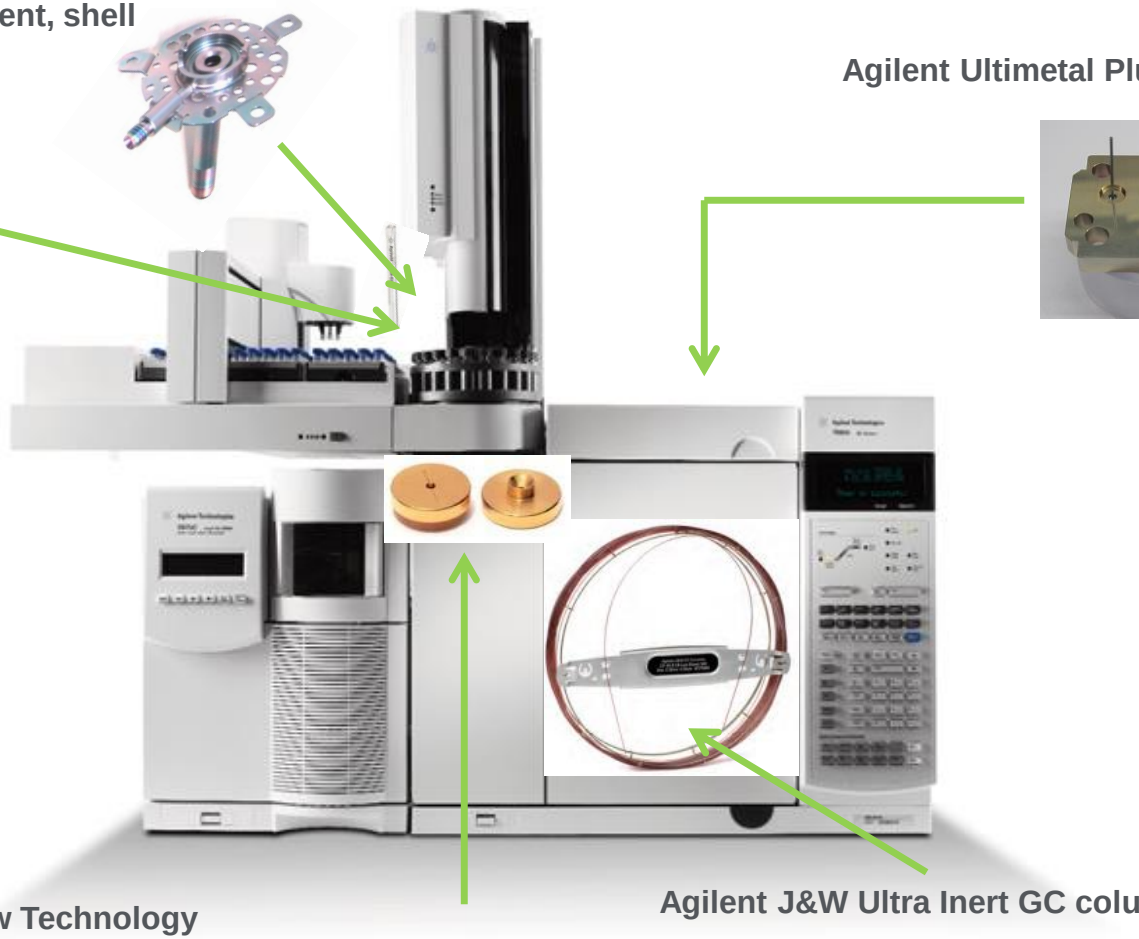
Agilent Inert Flow Solution

Agilent Ultimetal Plus inlet weldment, shell and transfer lines

Agilent ultra inert inlet liner

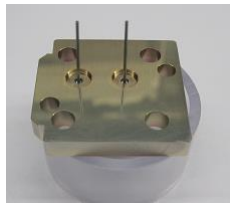
Agilent Ultimetal Plus ferrules

Agilent Ultimetal Capillary Flow Technology Devices, Ultimate union



Agilent Ultra Inert gold seal

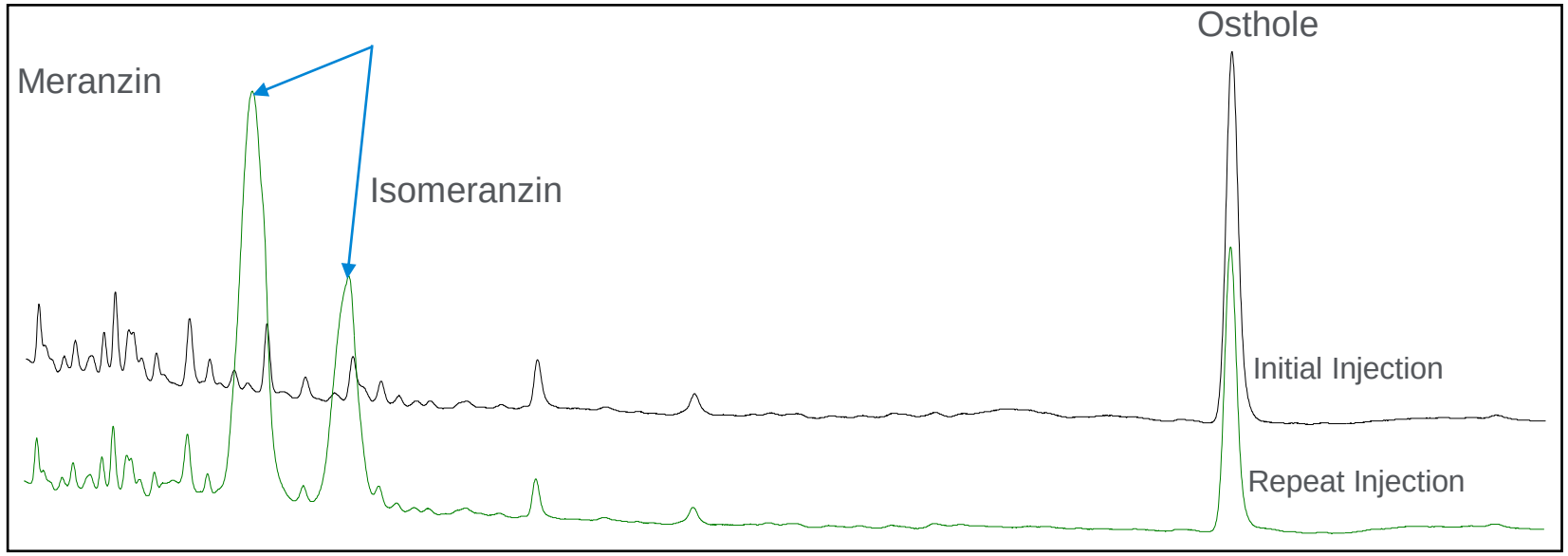
Agilent Ultimetal Plus– TCD, FPD, NPD/FID jets



Agilent J&W Ultra Inert GC column

5990-8532EN brochure

Bonus or Ghost Peaks



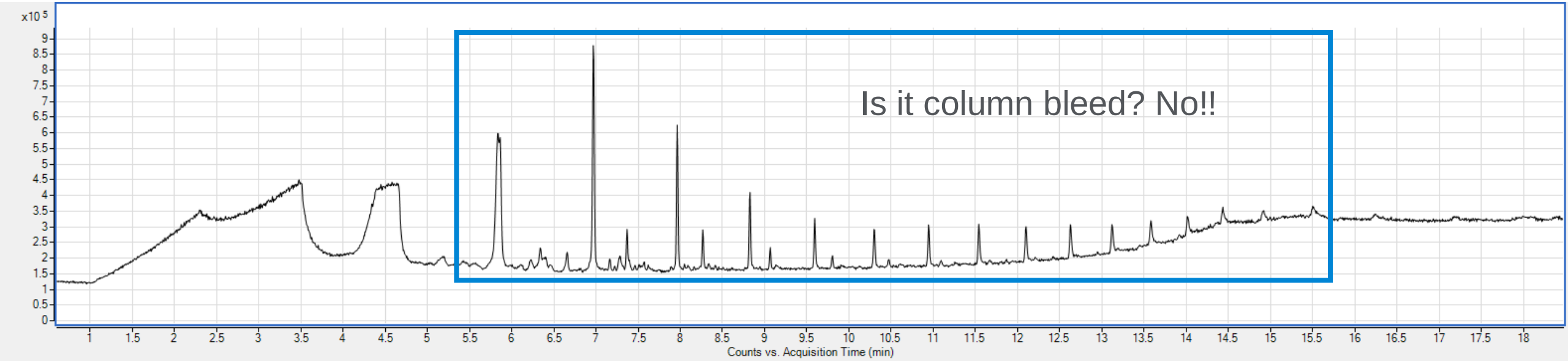
5991-9078EN

Contamination in injector, column or flow (carrier gas)

- Carry-over 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!

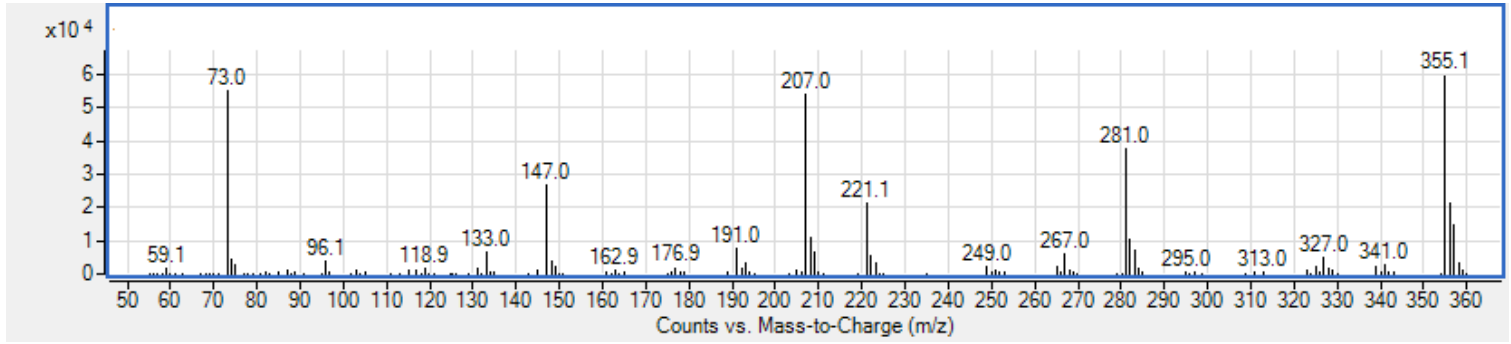
What are these Repeating Peaks?



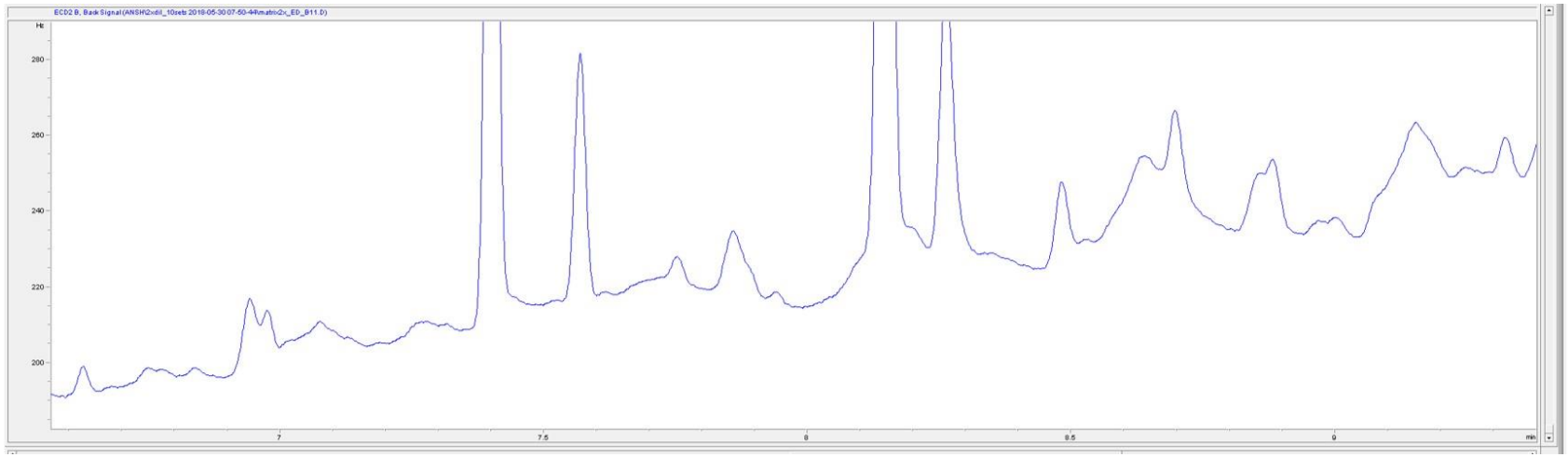
Common ions for siloxane molecules:
73
147
207
281
355

Septa contamination in wash vials or inlet liners can be diagnosed by looking for siloxane polymers in your total ion chromatogram. Each peak in the chromatogram corresponds to a cyclized (ring structure) siloxane molecule. These molecules fragment with very similar patterns.

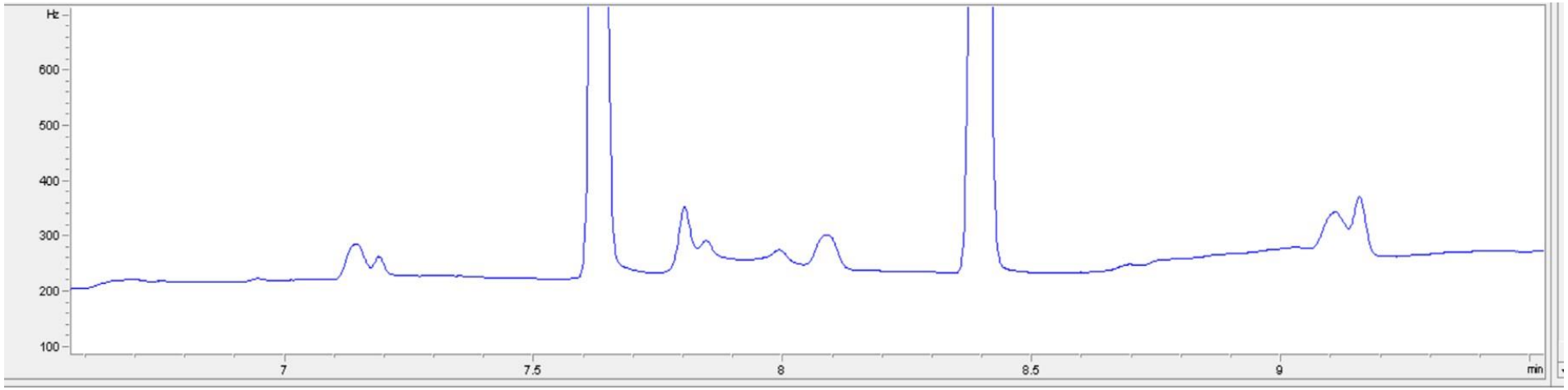
Example spectrum:



Does your Baseline look like this? Do you See Extra Peaks?

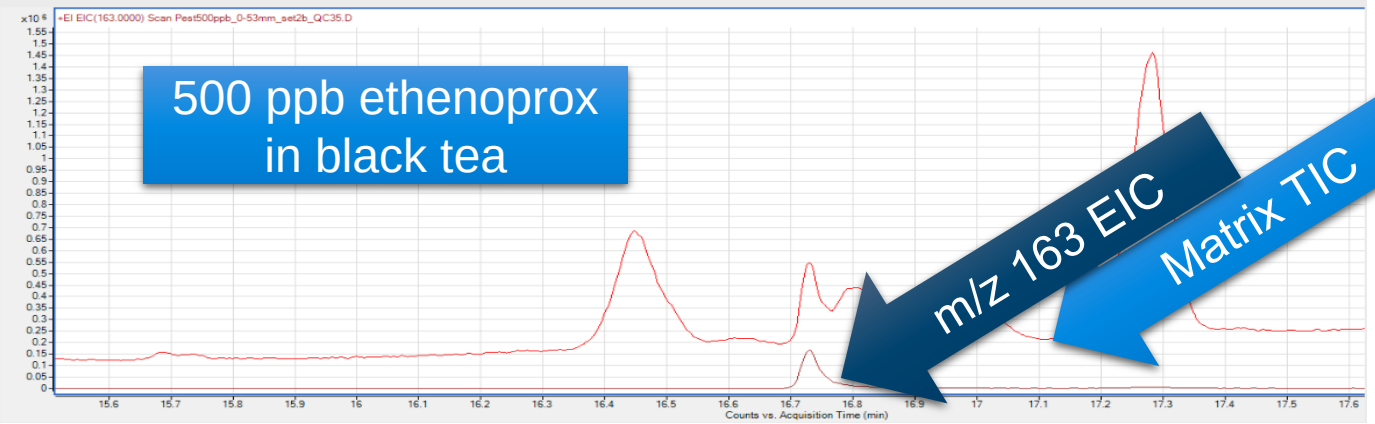


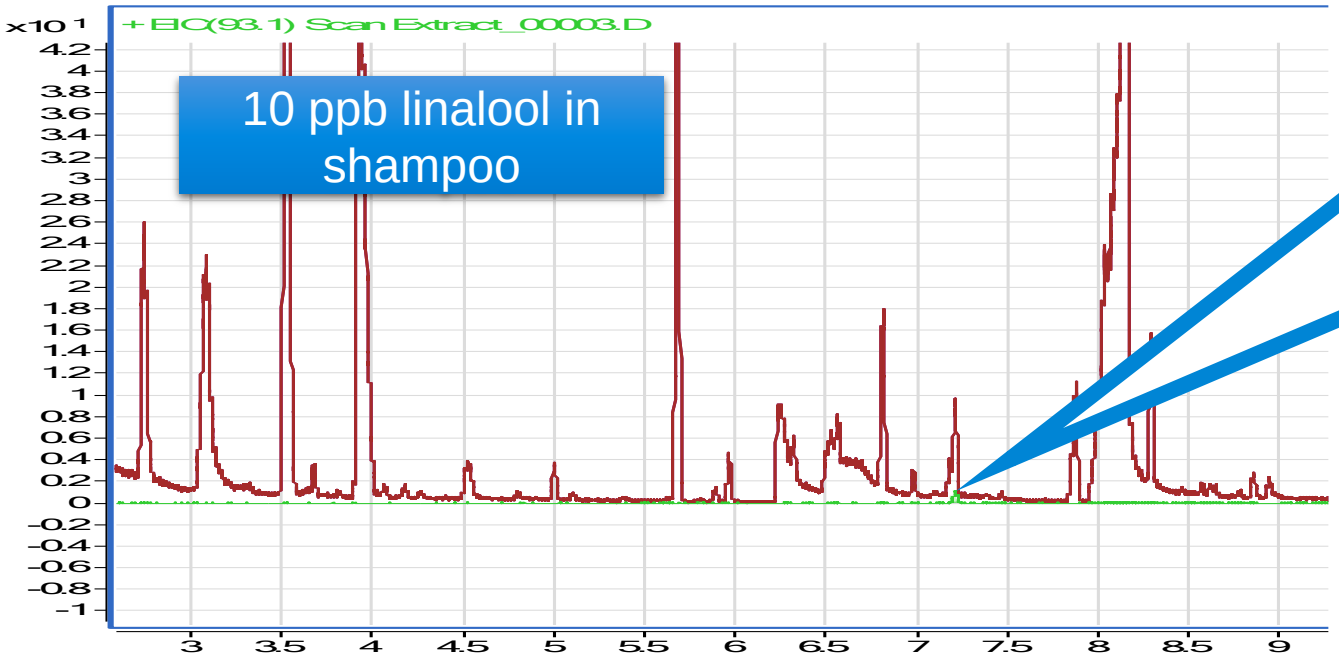
When it should look like …..

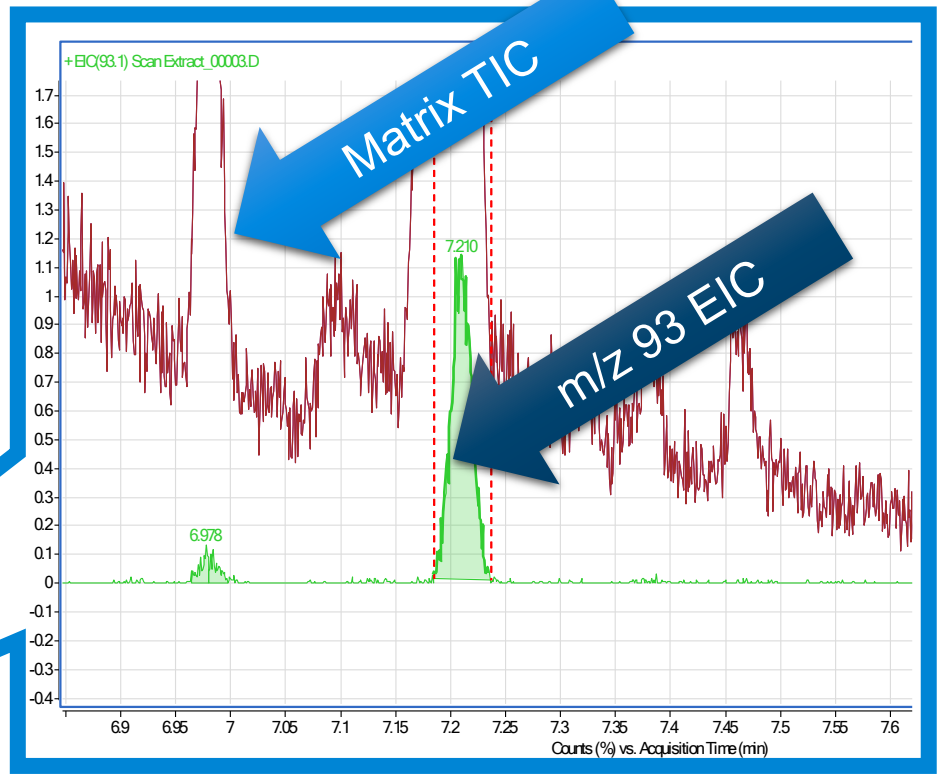


The Matrix

If your target ions are buried beneath matrix peaks, it might be time to trim the column or do sample clean-up

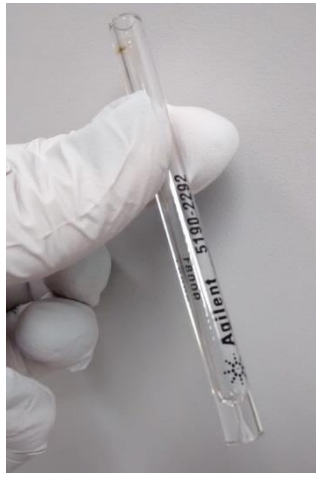




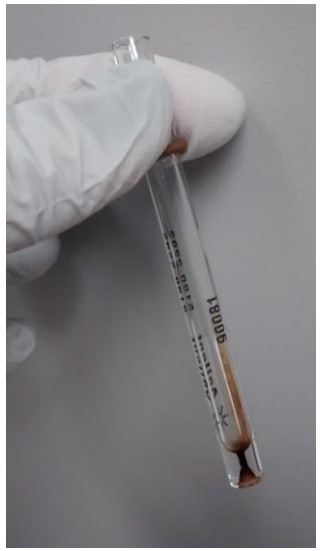


…(or improve your sample cleanup)

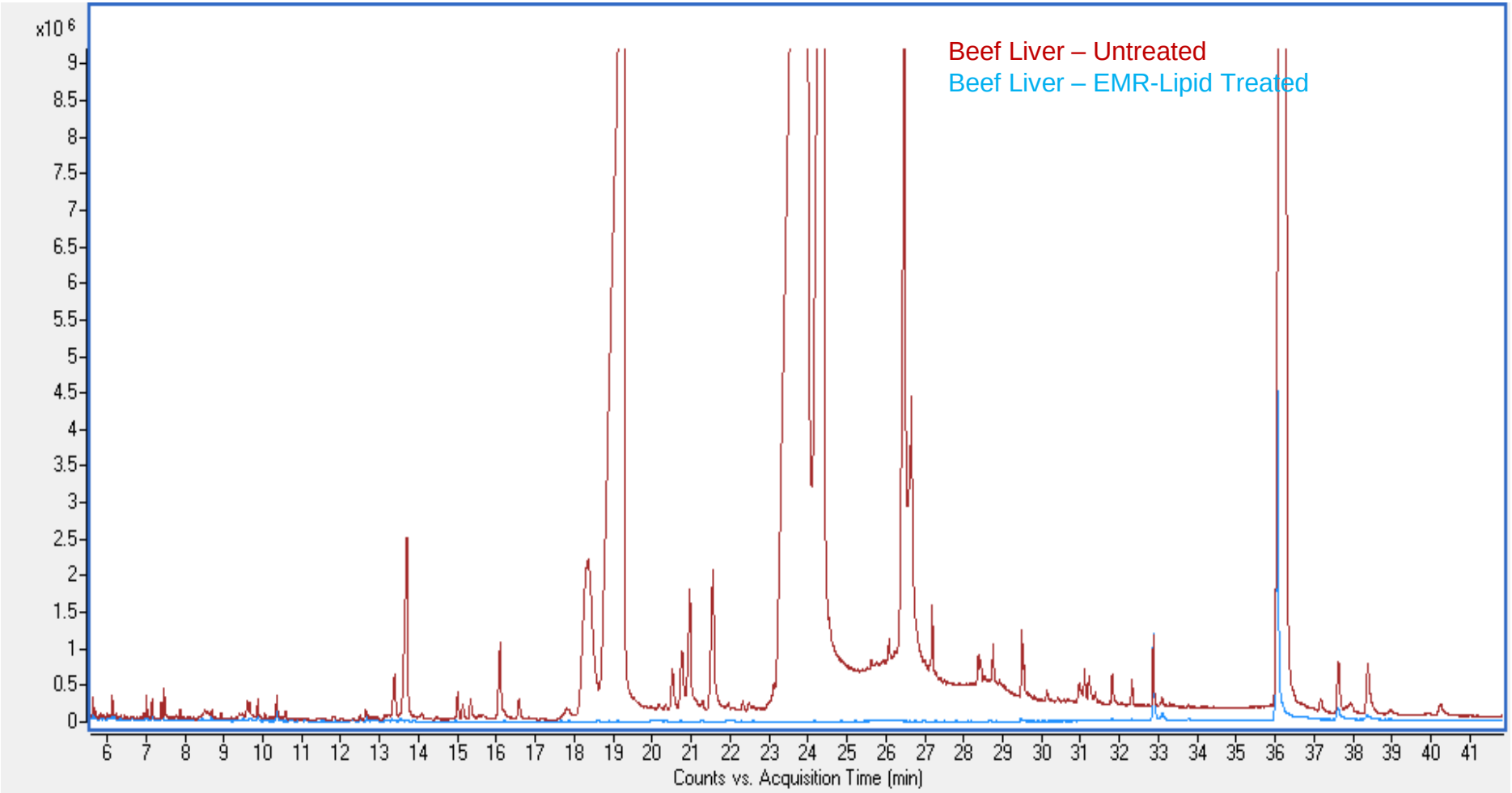
The Importance of Sample Cleanup



50 samples
with clean-up

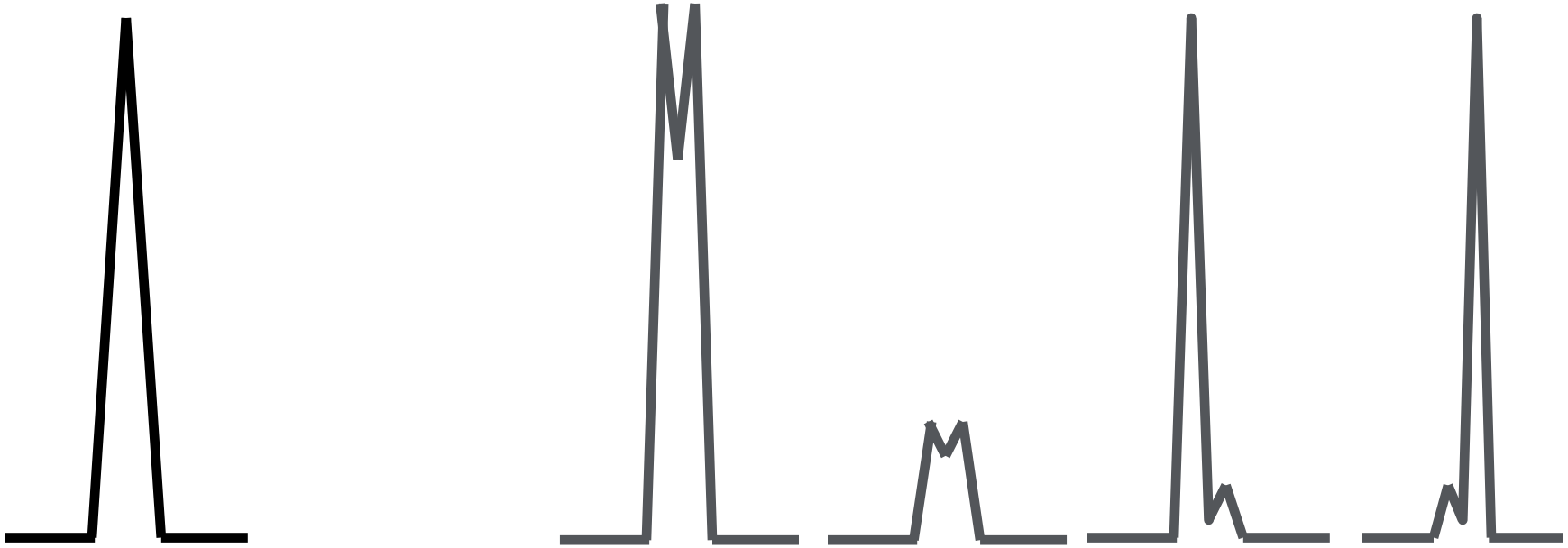


50 samples
without clean-up



For sample clean-up help, please contact us! spp-support@agilent.com

Split Peaks



Injector (poor sample introduction)

- Injecting the sample twice (somehow?)
- Mixed sample solvent (polarity difference)
- Sample in syringe needle (manual inject)

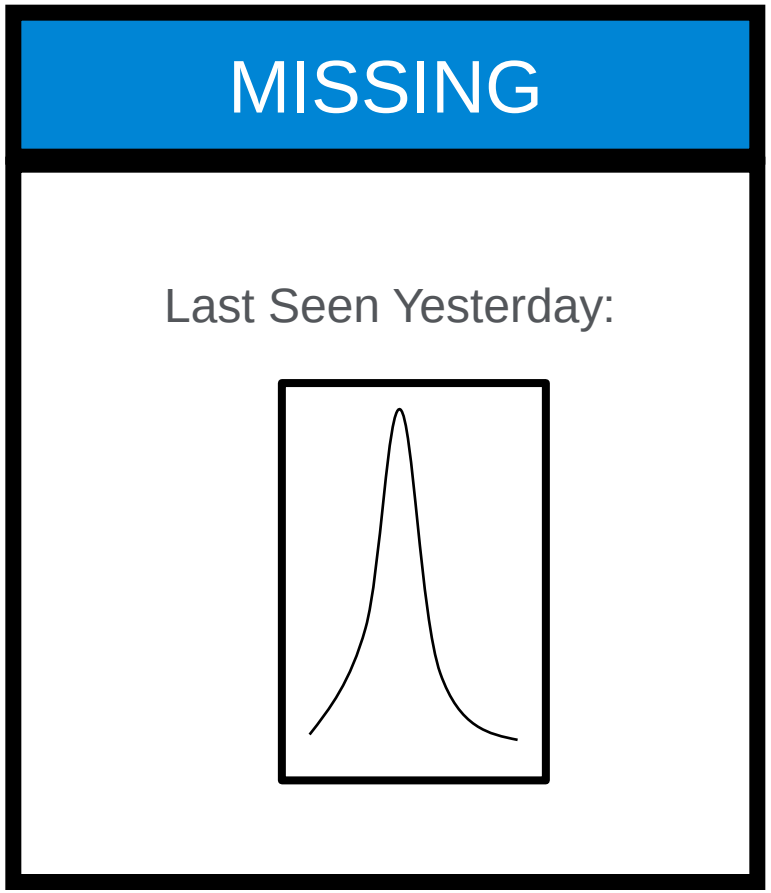
Injector (activity)

- 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

No Peaks



Detector (not on or not operational)

Injector (not working)

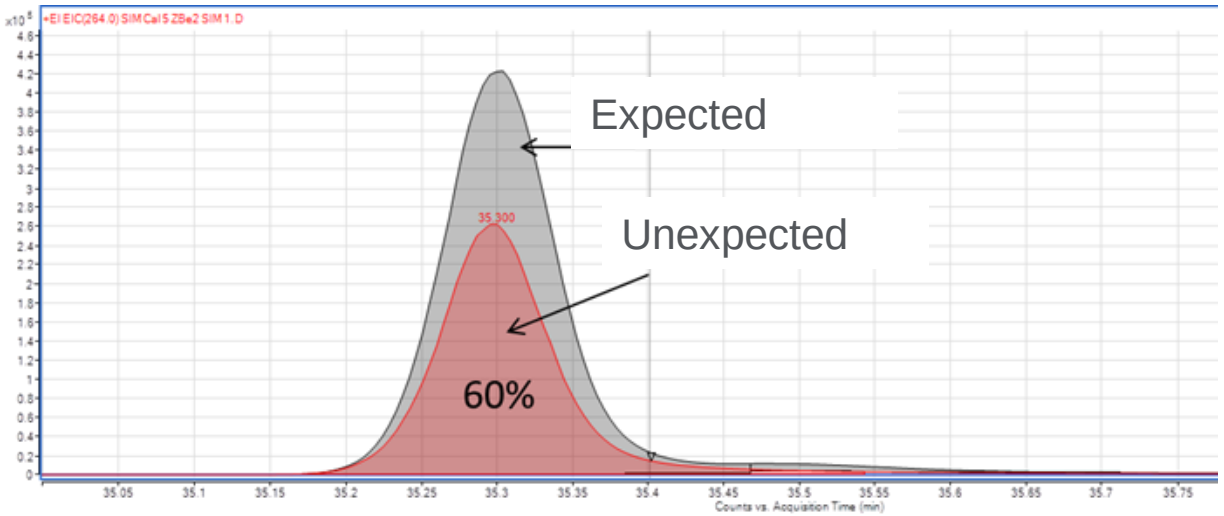
Plugged syringe/plunger not moving

- Wrong injector (or detector)
- Huge leak (older systems)
- No carrier gas flow

Not the column unless…

- broken column or no column

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*

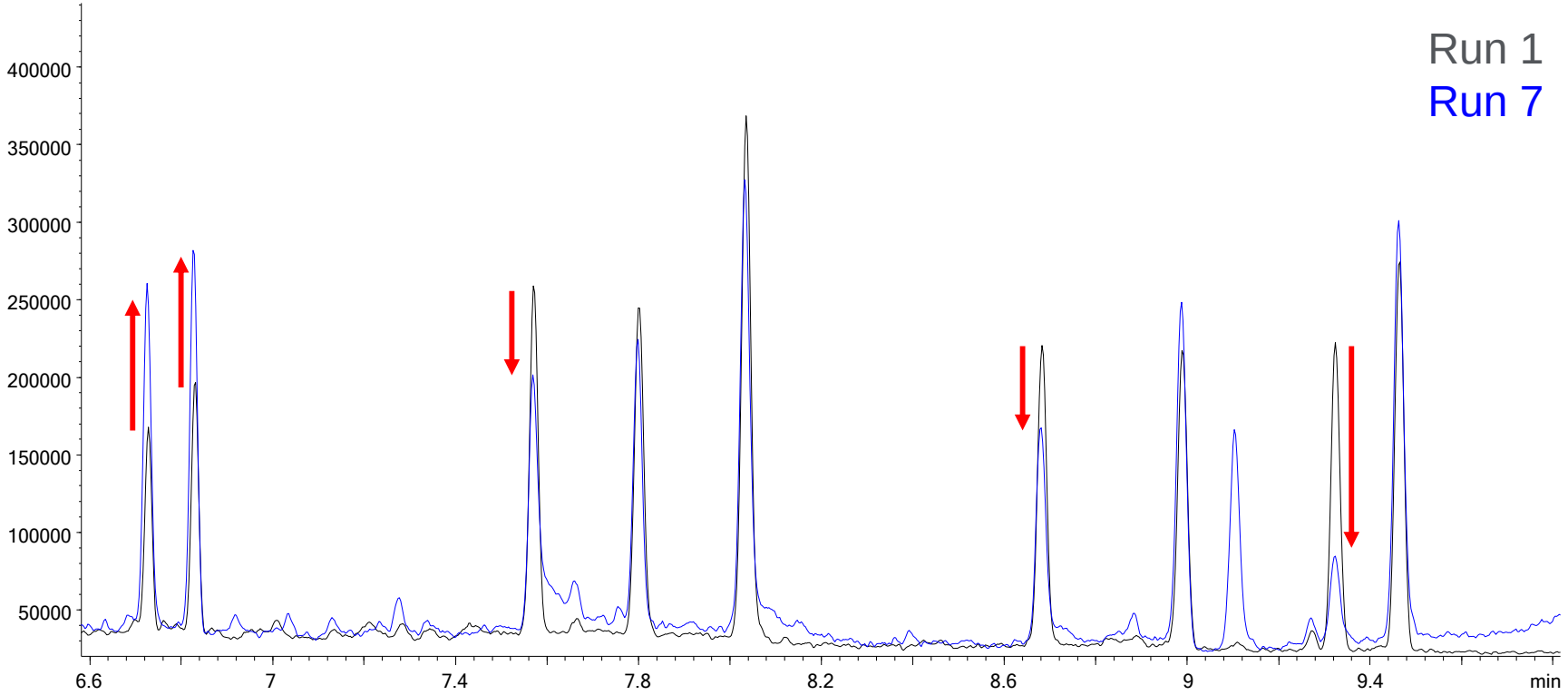
Detector (response problem)

- 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

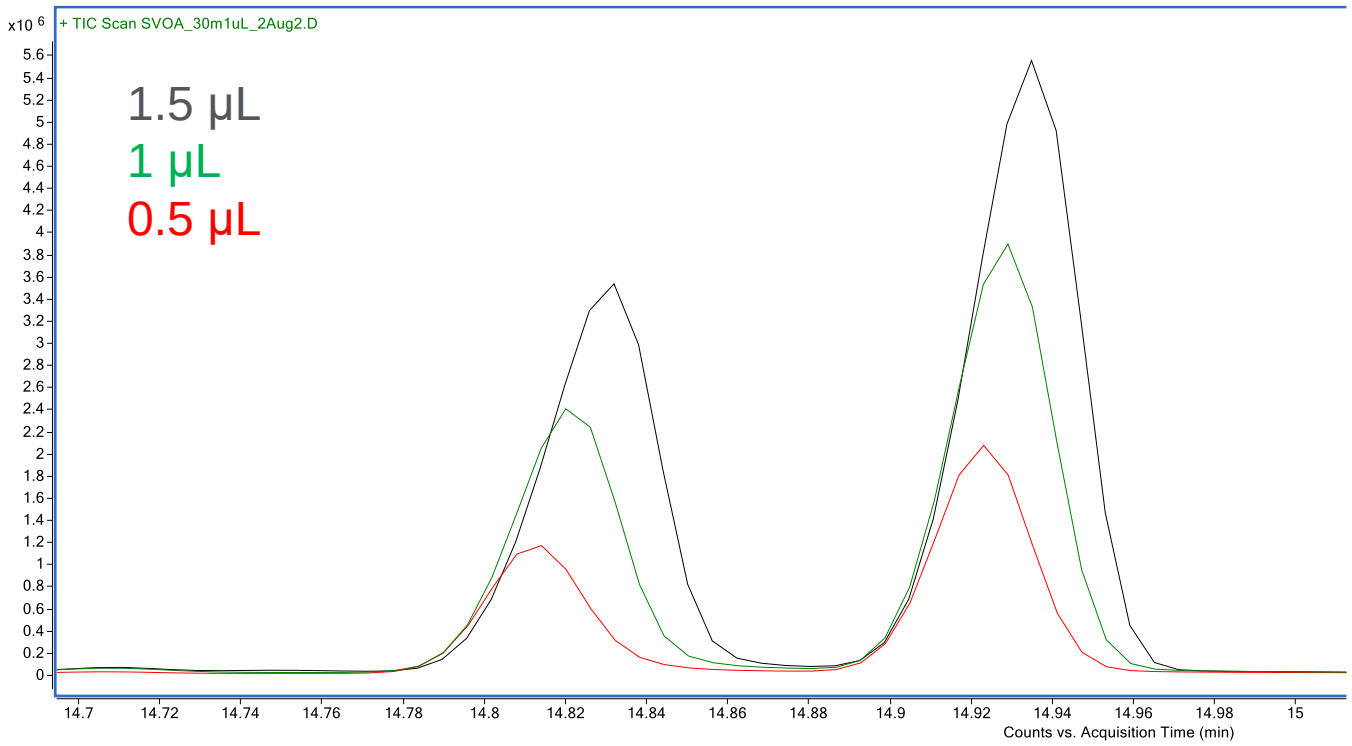
- Irreversible adsorption of active compounds (-OH, -NH, -SH)

Decomposition of sample

- Temperature change – Discrimination
- Evaporation from sample

Peak Fronting

Shark fin-shaped or just slight



Column (contaminated)

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

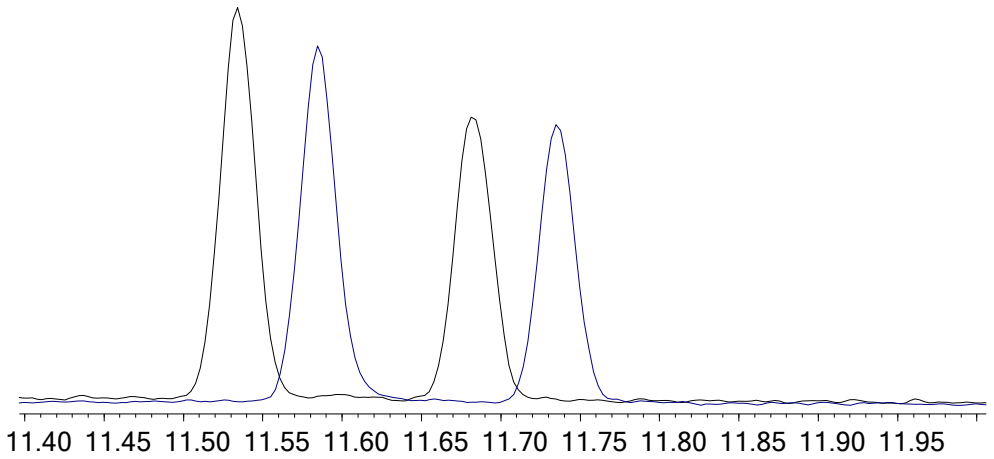
Injector

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

Other

- Co-elution
- Breakdown

Retention Time Shift



Injector

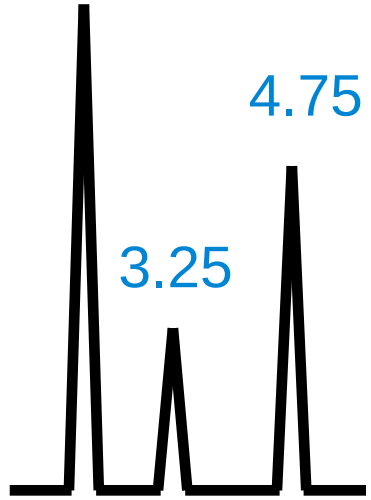
- Leak in the septum
- Change in injection solvent
- Large change in sample concentration

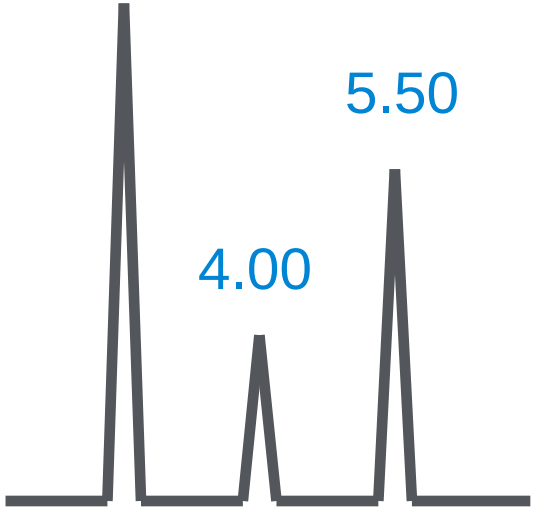
Flow

- Change in gas velocity

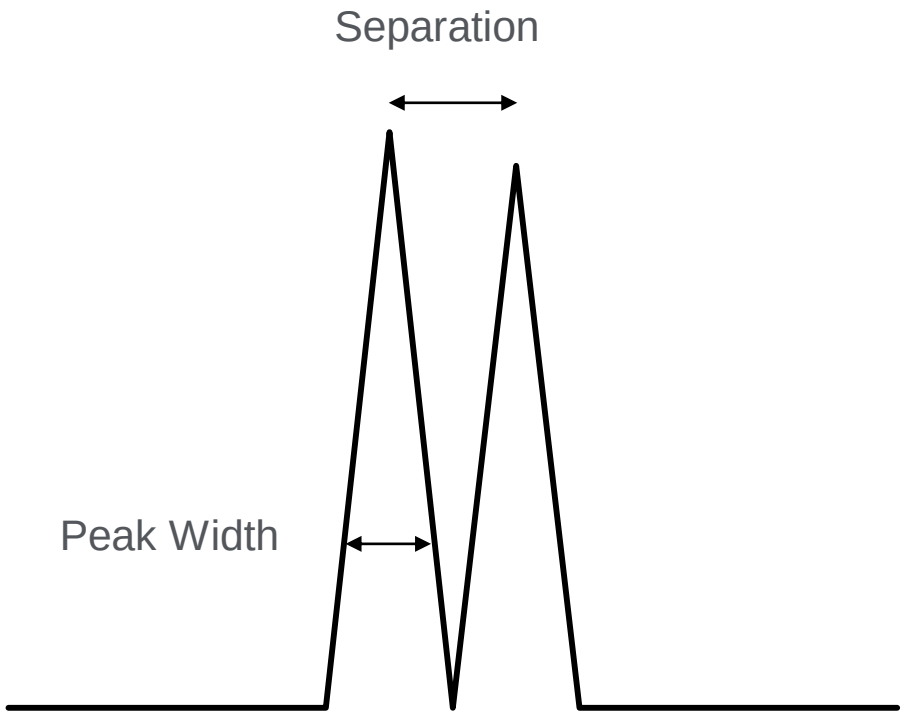
Column

- Contamination
- Damaged stationary phase
- Loss of stationary phase
- Change in temperature





Loss of Resolution



Resolution is a function of separation and peak width

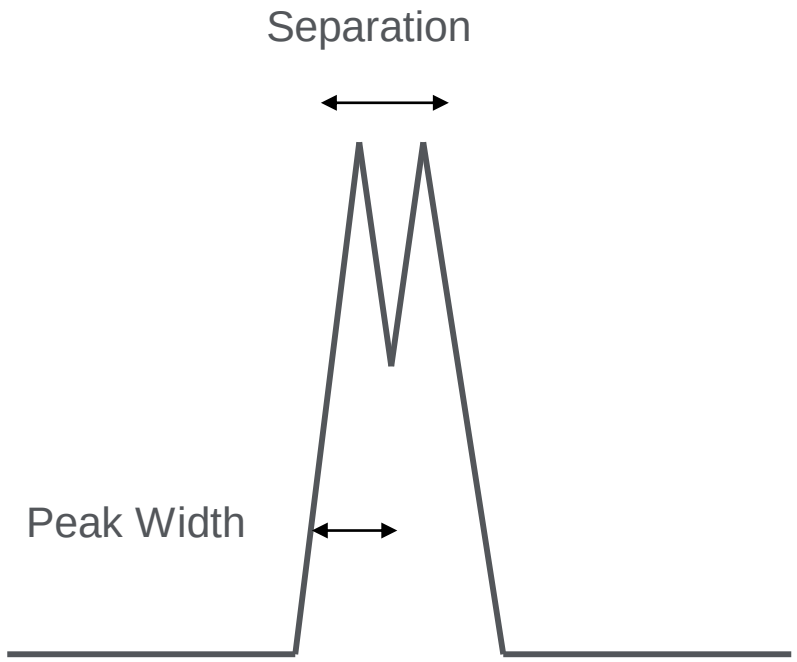
Loss of Resolution - Separation Decrease

Column

- Different column temperature
- Contamination (more phase?)
- Matrix components co-eluting

Flow

- Change in velocity?



Loss of Resolution - Peak Broadening

Flow

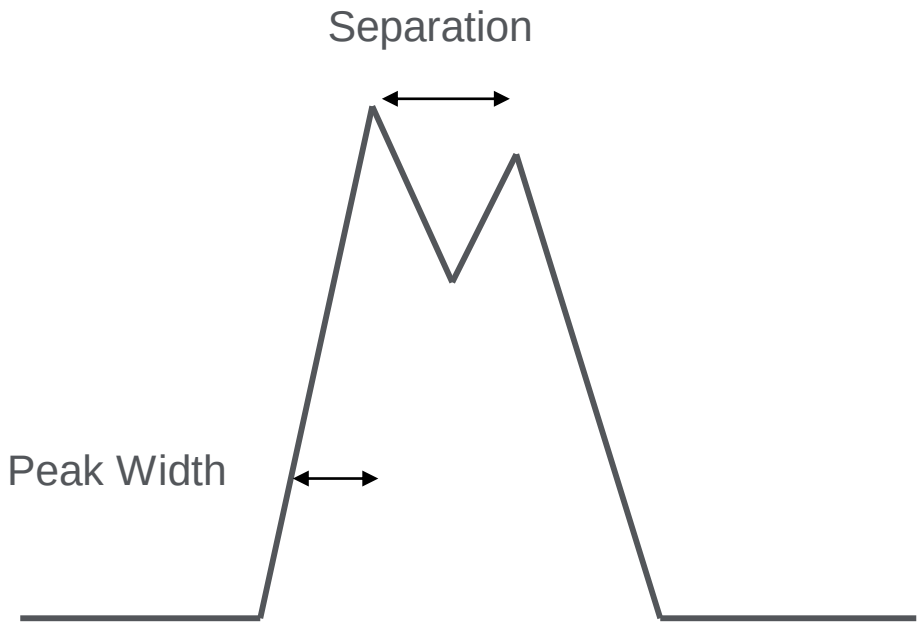
- Make-up gas

Column

- Contamination
- Phase degradation

Injector (efficiency)

- Settings, liner, installation, etc.



Baseline Disturbances

Sudden Changes, Wandering, or Drifting

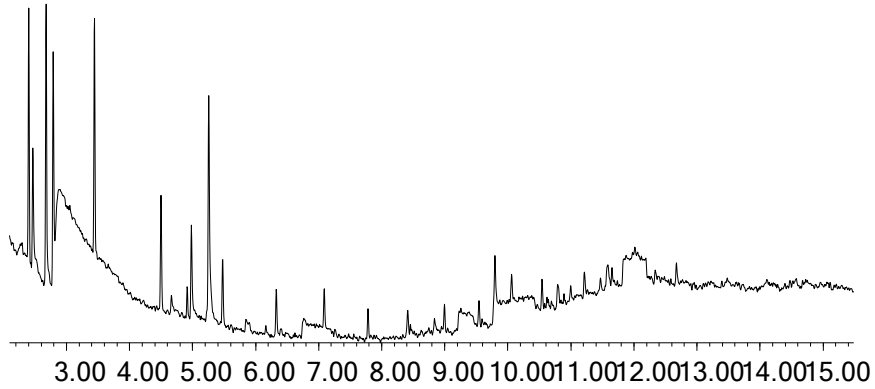
Drifting/Wandering/Weird Disturbances

Column or detector

- Not fully conditioned or stabilized (electronics)
- Contamination

Flow

- Changes in carrier and/or detector gas flows
- Valves switching, leaks



Noisy Baseline

Mild

Severe

Flow

- Contaminated gas
- Incorrect detector settings

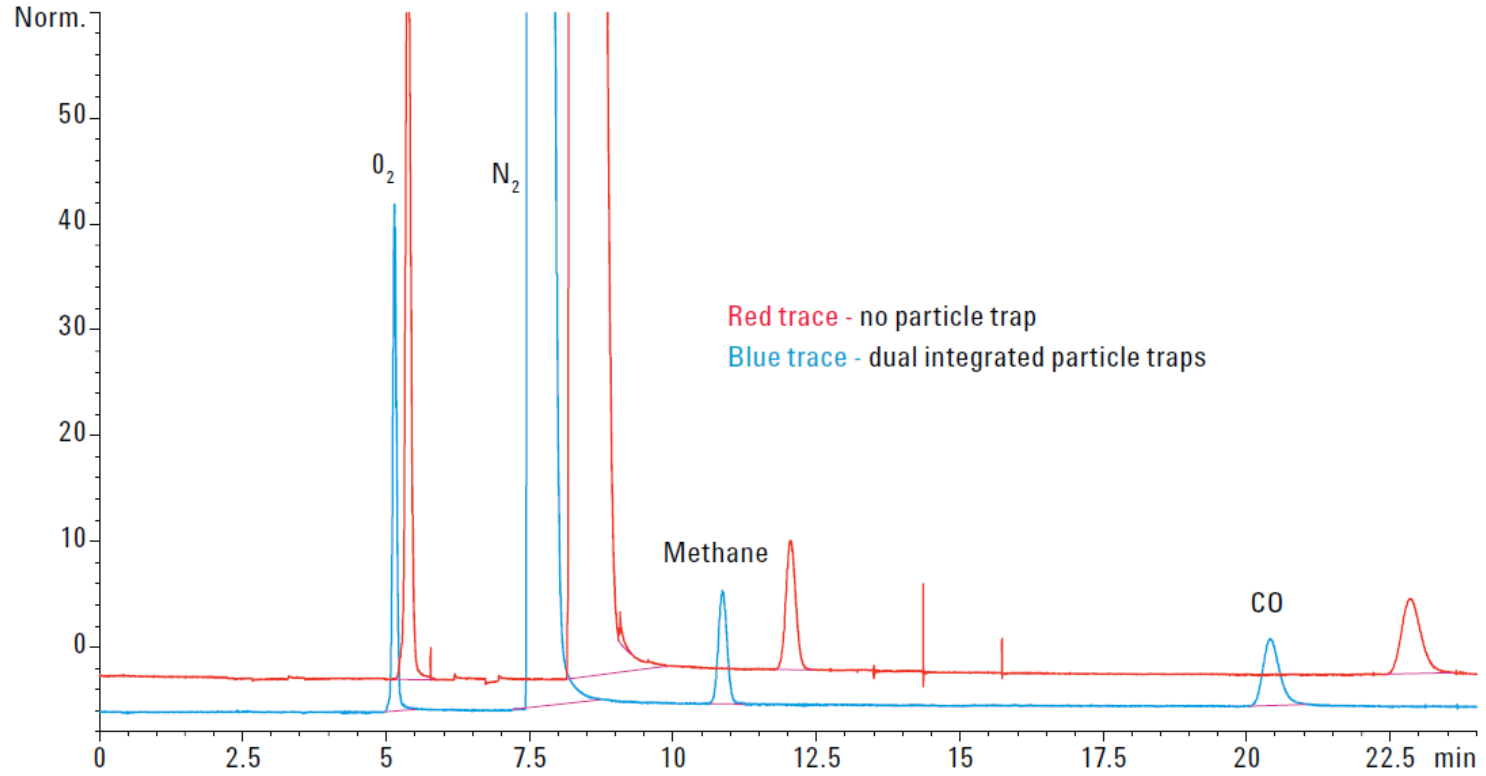
Column

- 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)

Application Note 5991-2975EN

Quantitation Problems

Detector

- Poor stability (electronics) or baseline disturbances (contamination)
- Outside detector's linear range or wrong settings

Activity (adsorption) in injector or column

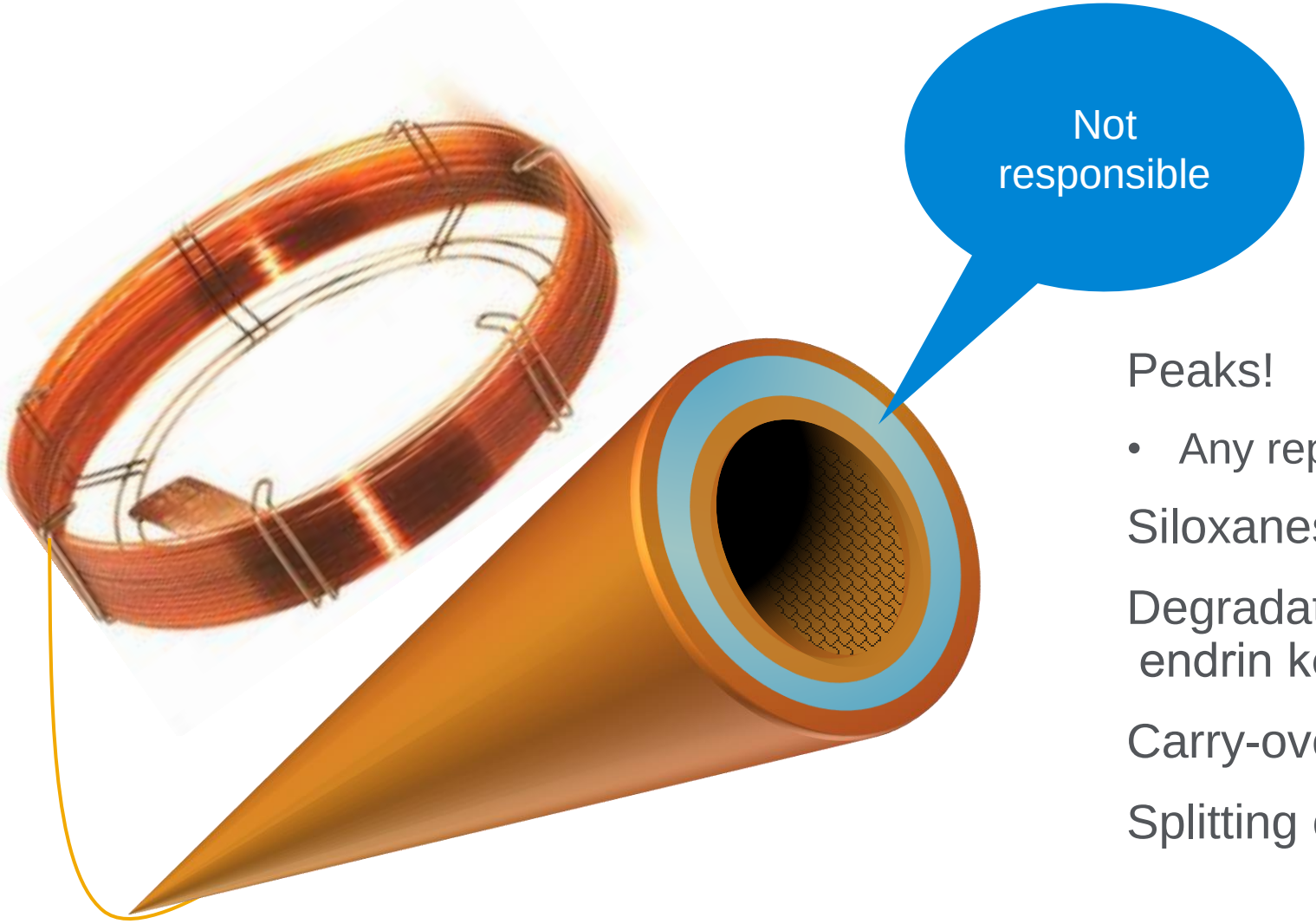
Injector

- Technique, settings, conditions
- Syringe worn

Other

- Co-elution
- Matrix effects
- Sample evaporation – leaky vials
- Sample decomposition

What is not caused by a Column???



Peaks!

- Any reproducible sharp chromatographed peak

Siloxanes

Degradation product peaks: Endrin Aldehyde, endrin ketone, DDE, DDD…

Carry-over of sample compounds

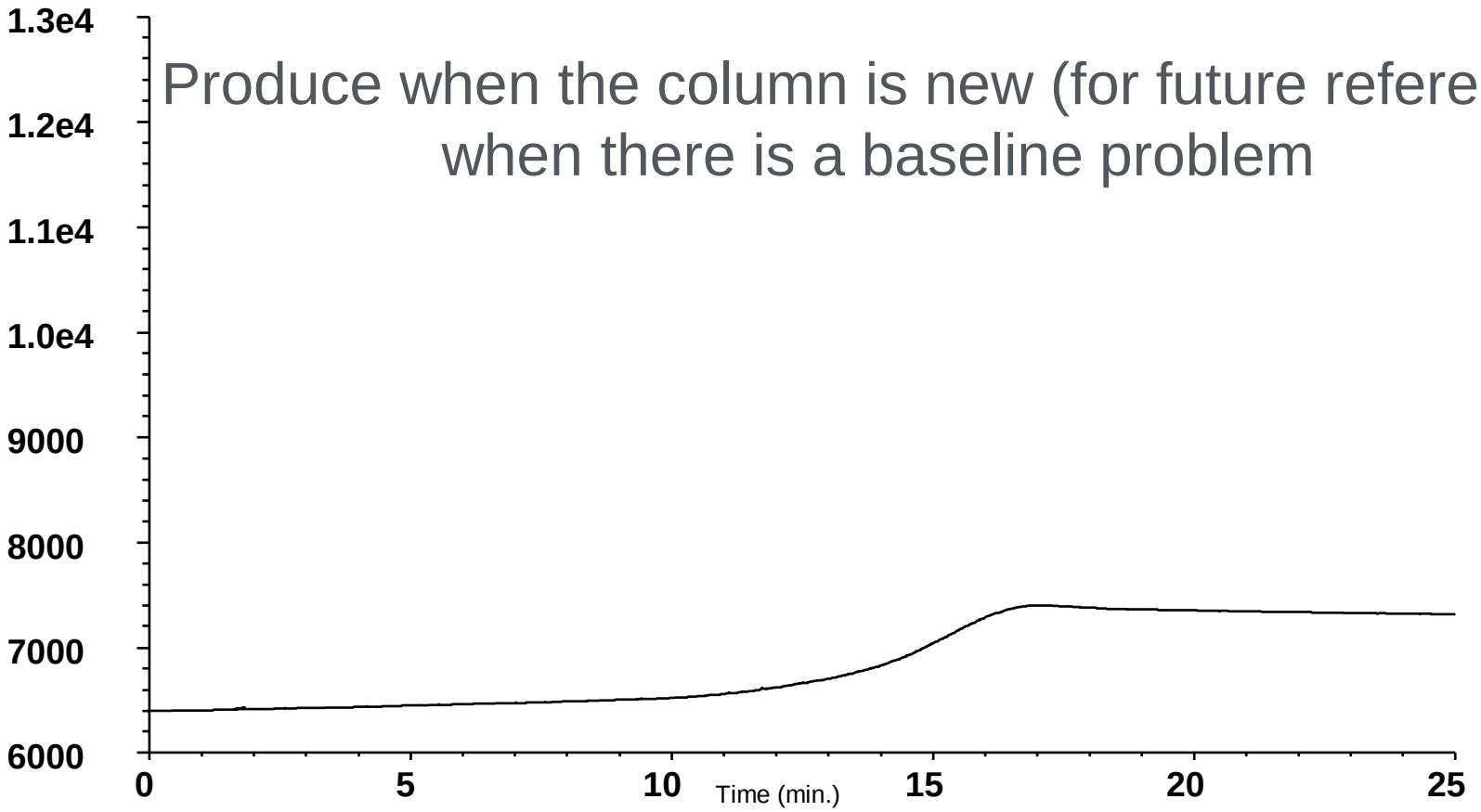
Splitting of peaks

Act 2: Troubleshooting Tools

Bleed profile: *baseline problems*
Inject a nonretained peak: *peak shape problems*
Test mix: *all problems*
Isolate the components: *all problems*

Condensation test: *baseline problems*
Jumper tube test: *baseline problems*

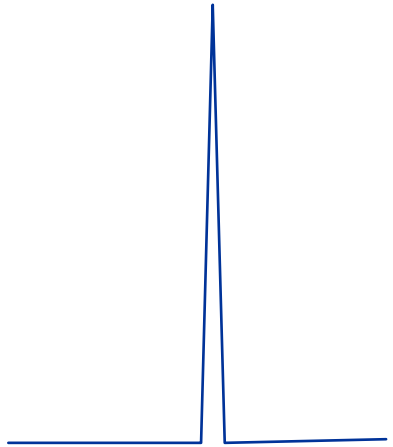
Generating a Bleed Profile



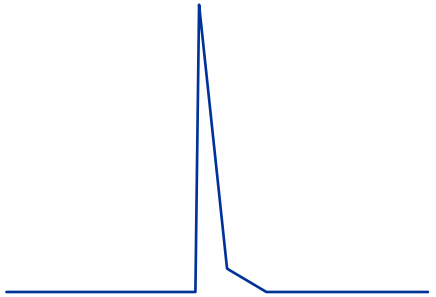
***Agilent J&WDB-1 30 m x .32 mm I.D., .25 µm**
Temperature program // 40°C, hold 1 min // 20°/min to 320 °C, hold 10 min.

Inject a Nonretained Compound to check Flowpath

Used to Check Flowpath



Good Installation



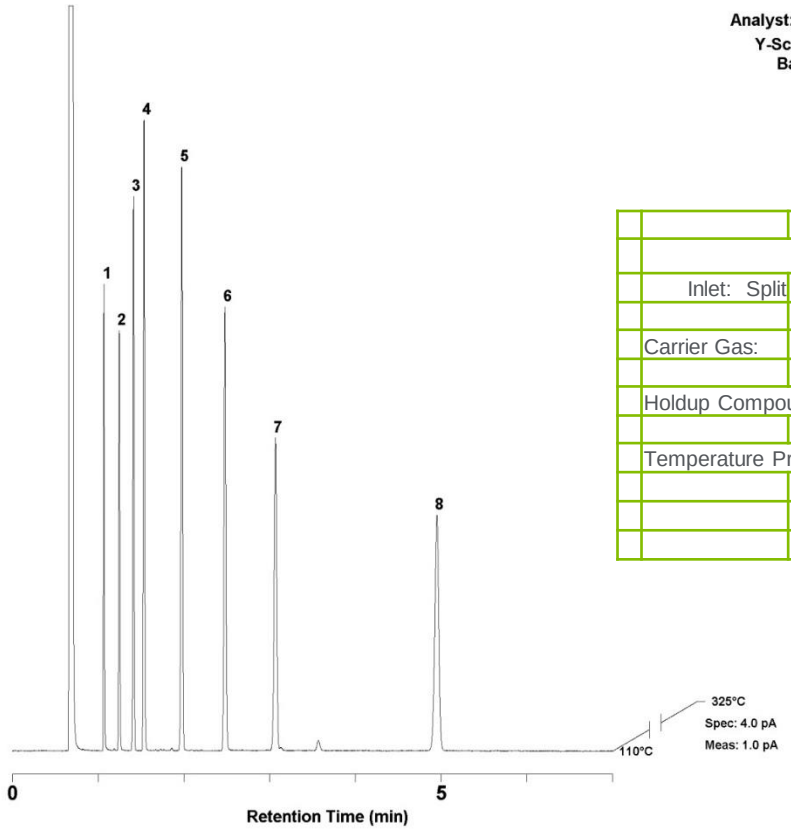
Improper Installation or Injector Leak

Potential problems:

- Injector or septum leak
- Too low of a split ratio
- Liner problem
  - -(broken, leaking, misplaced)
- Column position in injector and detector

Test Mix- Make your Own!

Used to determine how “good” the column is or if the problem is related to the chemical properties of the analytes.

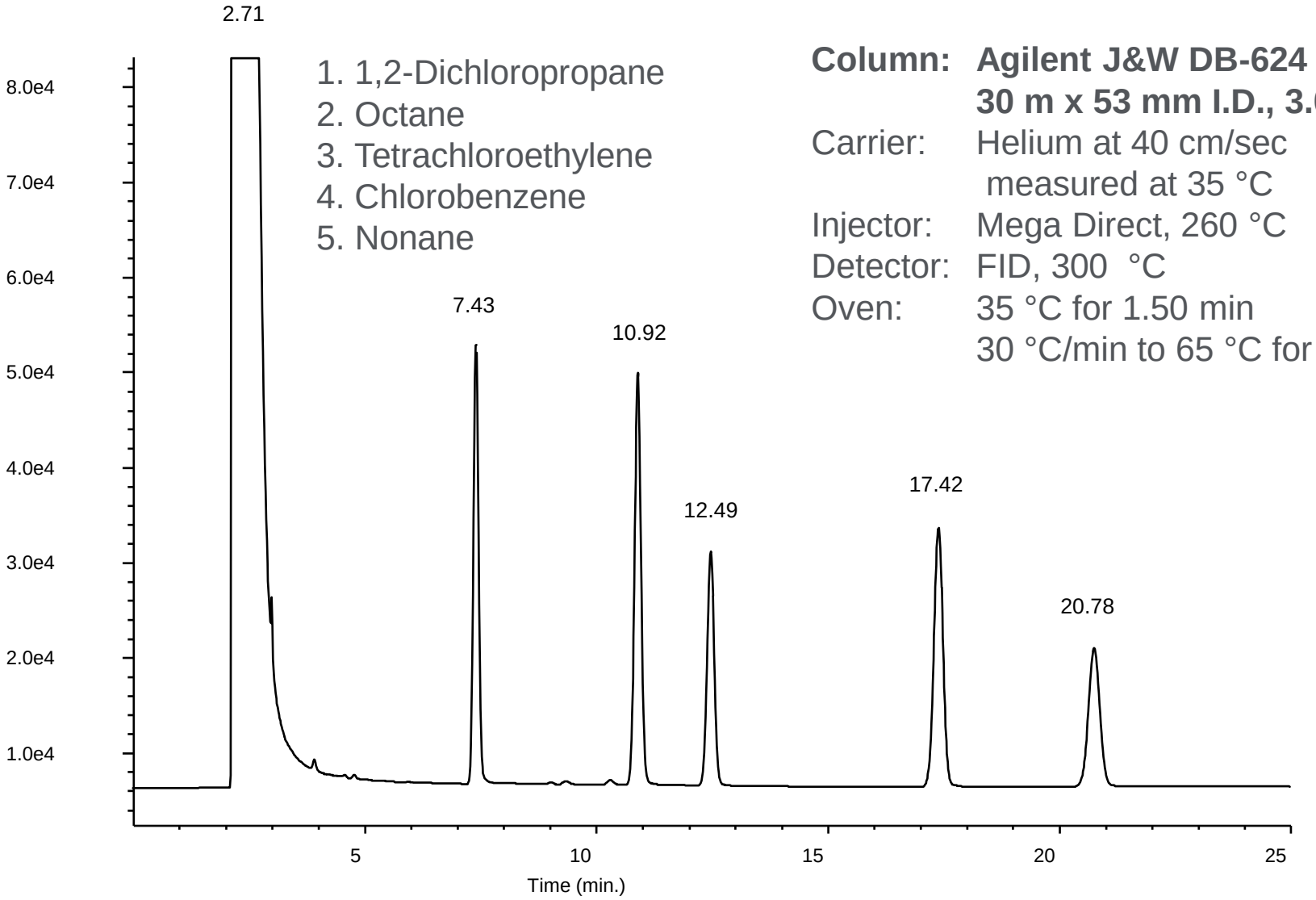


Test Conditions			
Inlet: Split	(250°C)	Detector: FID	(320°C)
Carrier Gas:	Hydrogen	Flow:	37.3 cm/sec (1.8 ml/min)
Holdup Compound:	Methane		(0.671-min)
Temperature Program:	Isothermal at 110°C		

Compounds	Purpose
Hydrocarbons	Efficiency
	Retention
Alcohols	Activity
FAMEs, PAHs	Retention
Acids	Acidic Character
Bases	Basic Character

Agilent J&W DB-624 Column

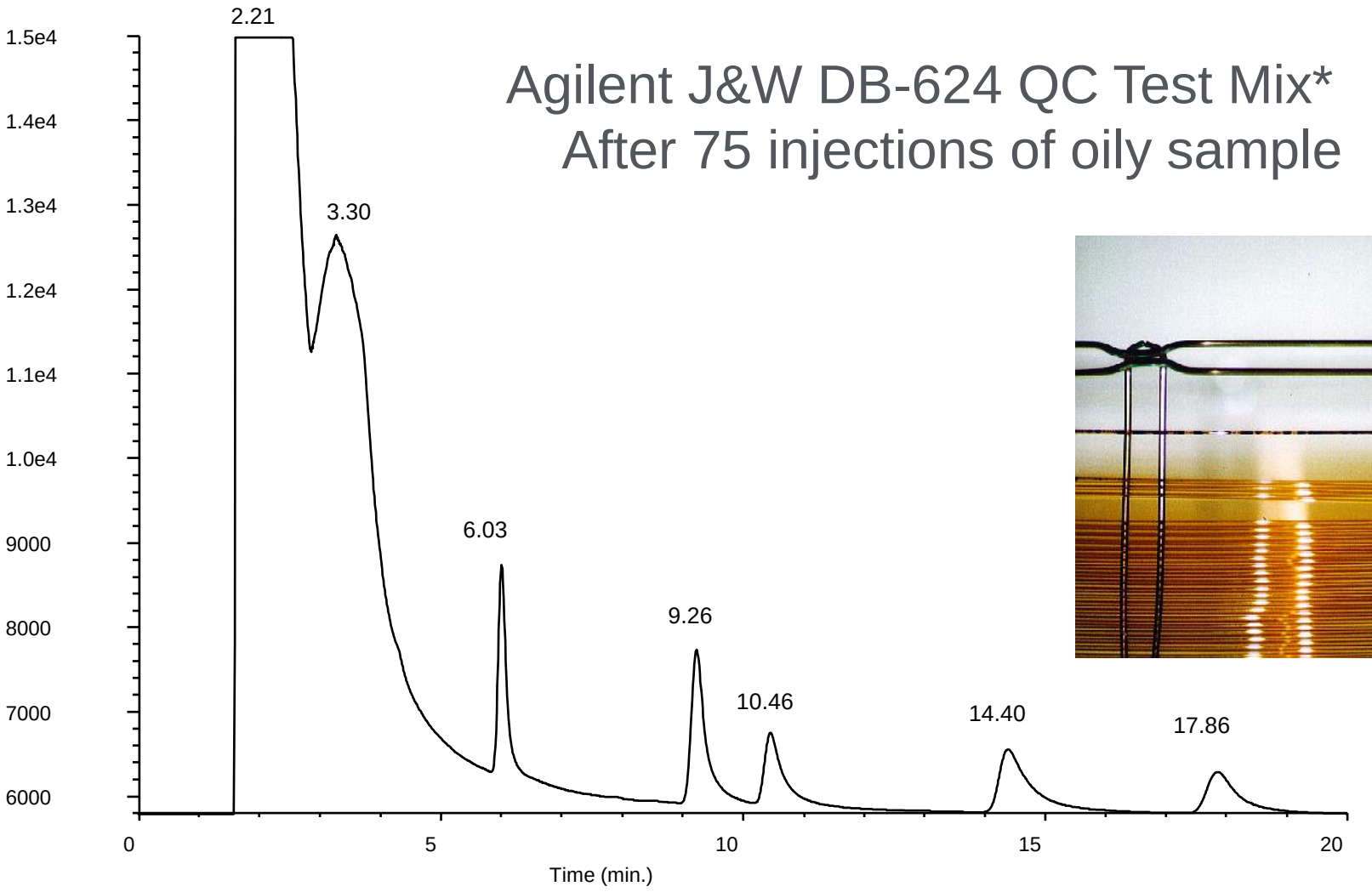
QC Test Mix



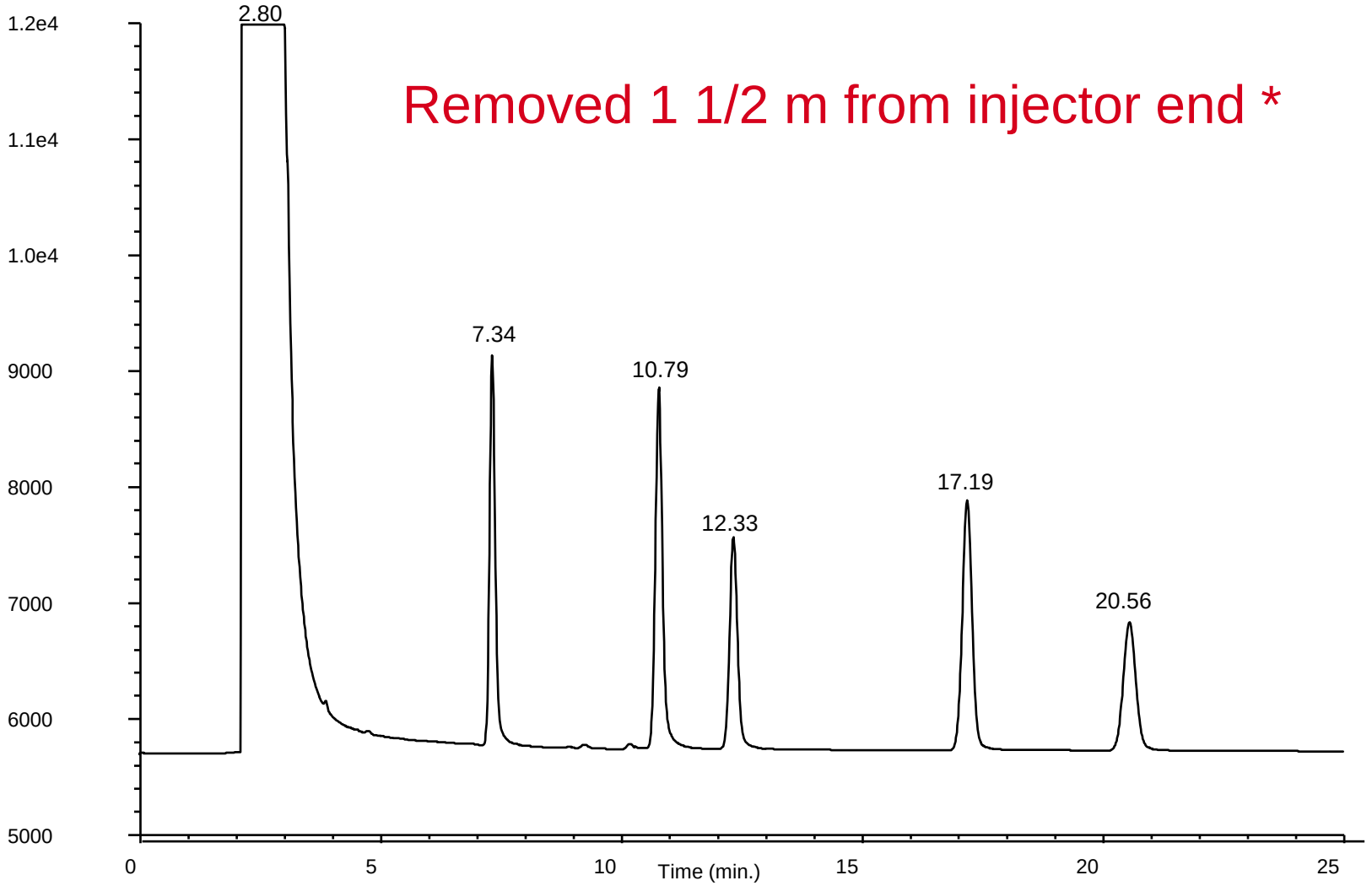
1. 1,2-Dichloropropane
2. Octane
3. Tetrachloroethylene
4. Chlorobenzene
5. Nonane

Column: **Agilent J&W DB-624**
30 m x 53 mm I.D., 3.0 µm
Carrier: Helium at 40 cm/sec
measured at 35 °C
Injector: Mega Direct, 260 °C
Detector: FID, 300 °C
Oven: 35 °C for 1.50 min
30 °C/min to 65 °C for 10 min

Example of Column Contamination



Example of Column Contamination

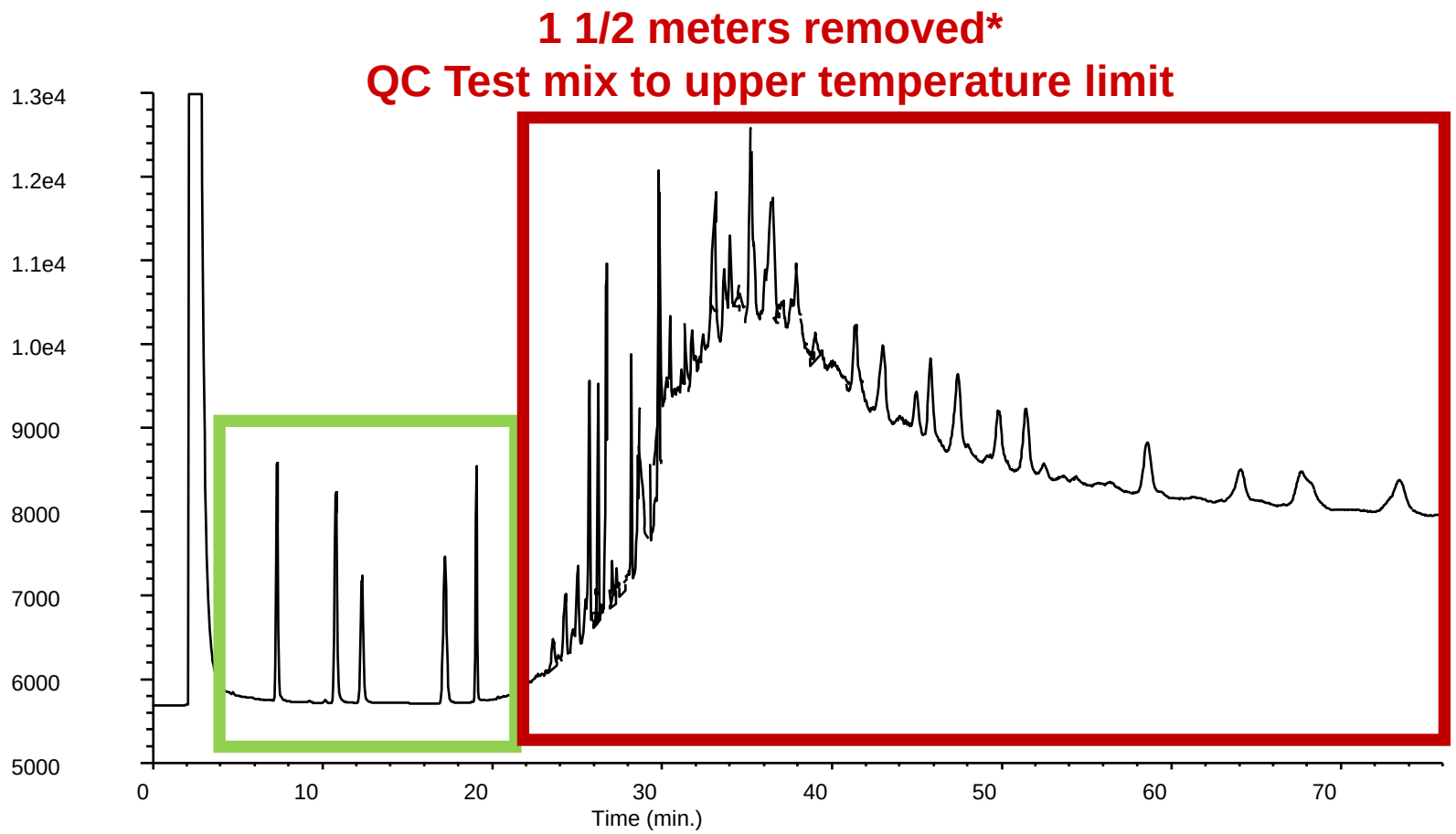


Removed 1 1/2 m from injector end *

*Before column rinse and bake

Temperature program // 35 °C hold 1.50 min // 30° C/min to 65 °C, hold 10 min

Example of Column Contamination



We have more semivolatile contamination!

*Before column bake
Temperature program // 35 °C, hold 1.50 min // 30 °C/min to 65 °C, hold 15 min // 20 °C/min to 260 °C, hold 50 min

Condensation Test

Used* to isolate the cause of:

- Erratic baselines
- Ghost peaks or carry-over

***Use when problems are worse after periods of GC non-use**

Condensation Test

Procedure

- Leave GC at 40-50 °C for > 8 hours
- Blank run
- Repeat a blank run immediately after the first blank run is complete
- Compare the two blank runs

Condensation Test

Results

First blank run is worse

- Contaminants (from injector, lines, traps or carrier gas) carried into the column
- Blank runs the same: *contaminants are not strongly focused on the front of the column*

Jumper Tube Test

Purpose

- Helps to locate the source of contamination or noise
- Isolates GC components

Jumper Tube Test

Isolate the detector

- Remove column from the detector
- Cap detector and turn on
- Blank run

Jumper Tube Test

Isolation of Detector - Results

Detector OK

Detector is the problem

Jumper Tube Test

Isolate the Injector

- Connect the injector and detector
  - 1-2 meters deactivated fused silica tubing
- Turn on carrier gas
- Blank run

Jumper Tube Test

Isolate the Injector - Results

Injector OK

Injector, lines or carrier gas contaminated

Jumper Tube Test

Isolate the Column

- Re-install the column
- Set up as before
- Blank run

Jumper Tube Test

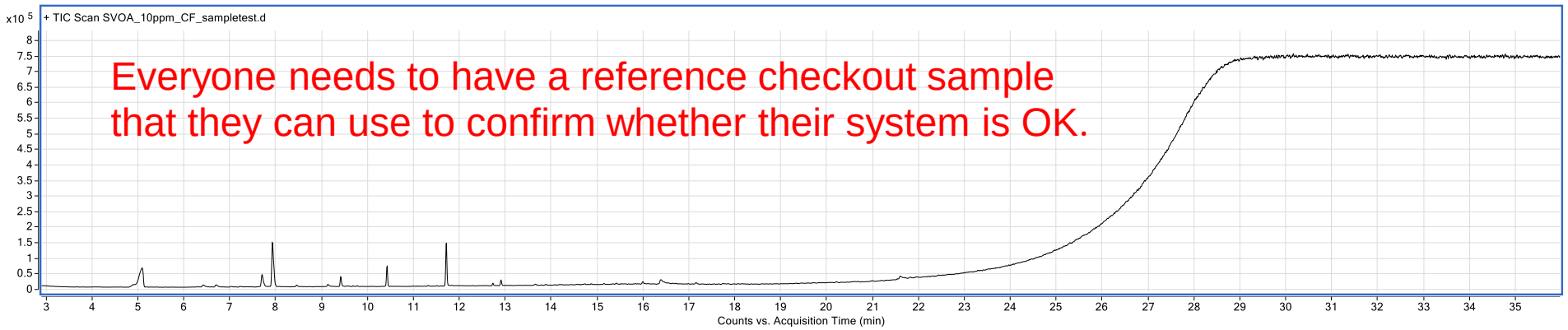
Isolate the Column - Results

- Problem returns: it's the column
- Problem gone: previous leak, solid debris, or installation problem

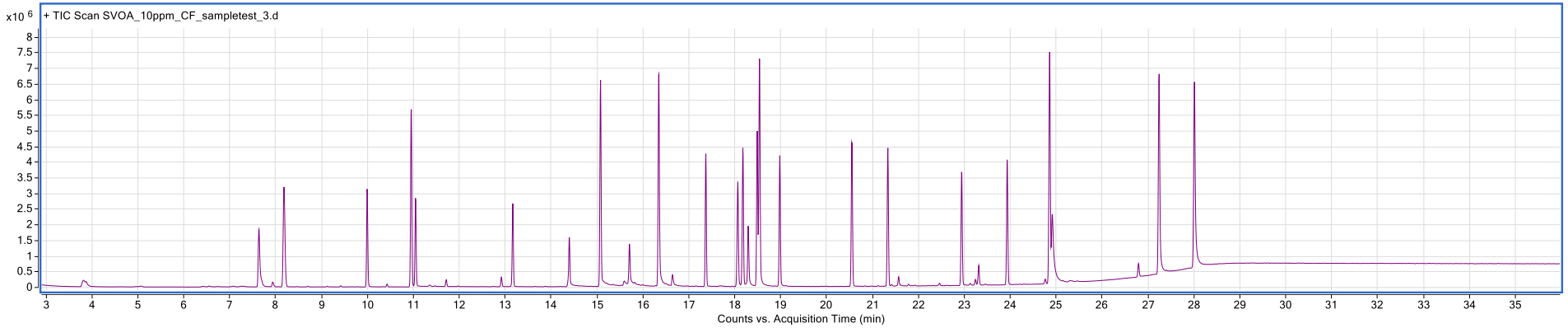
Act 3: Troubleshooting Example

Problem: No Peaks with Semivolatiles Checkout Mixture

What my TIC looked like:



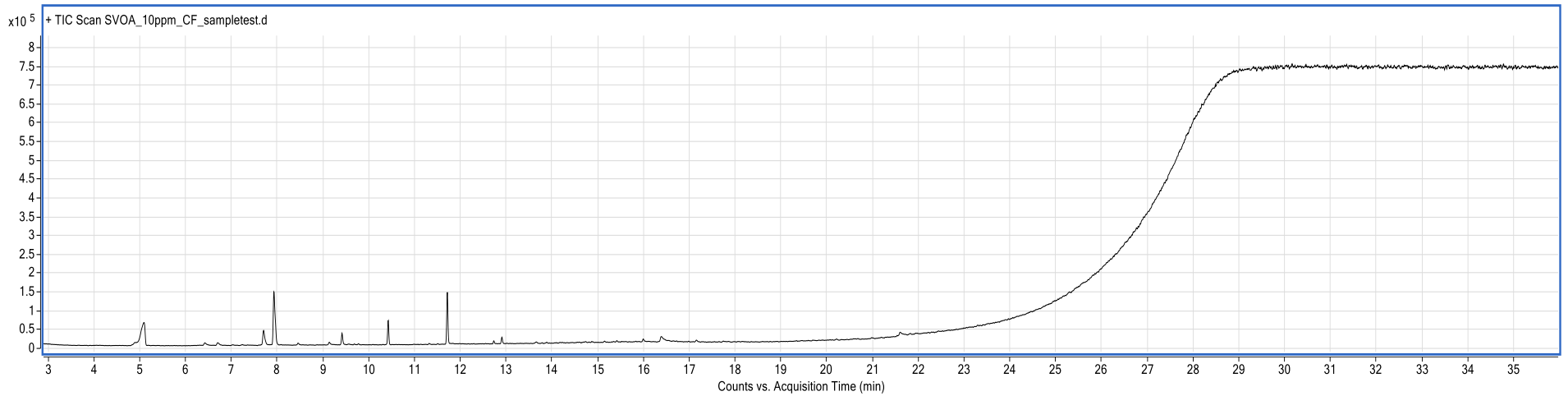
What my TIC should look like:



Problem: No Peaks with Semivolatiles Checkout Mixture

What could cause this?

- The wrong vial was injected
- The sample has degraded
- The inlet is leaking
- The column is damaged



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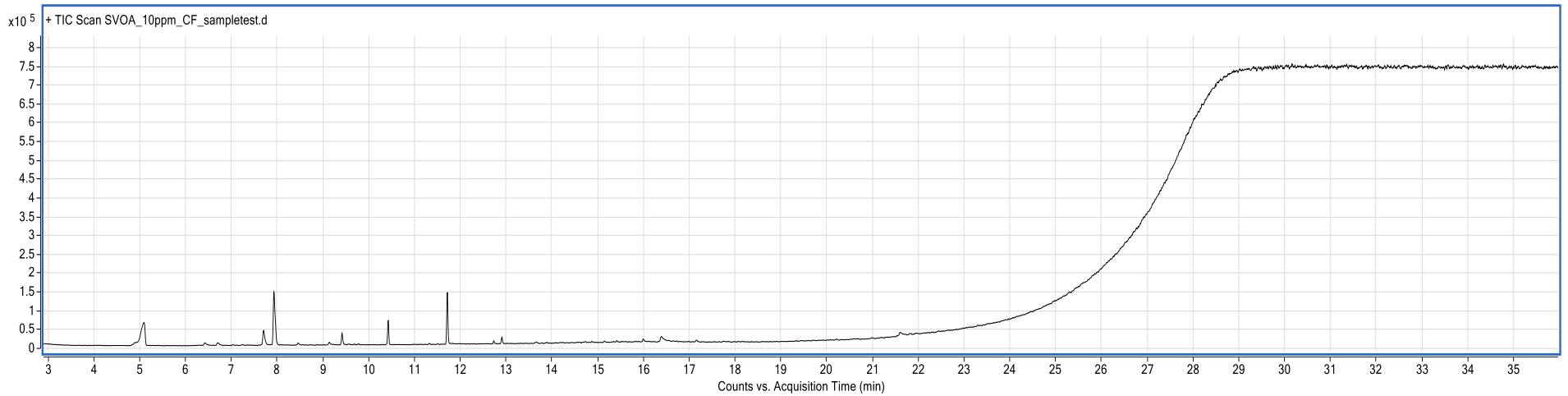

Problem: No Peaks with Semivolatiles Checkout Mixture

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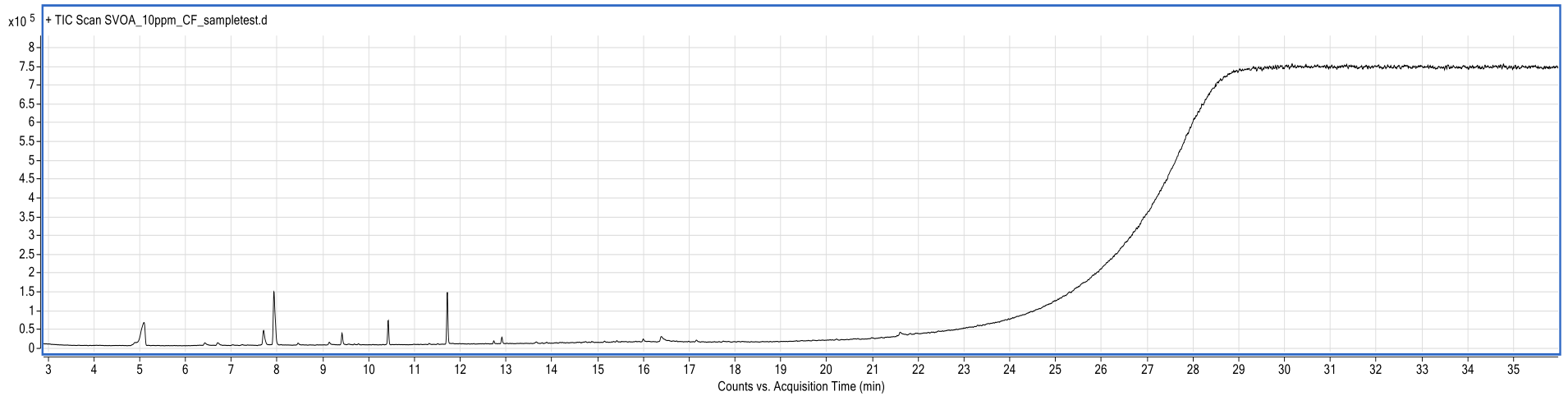

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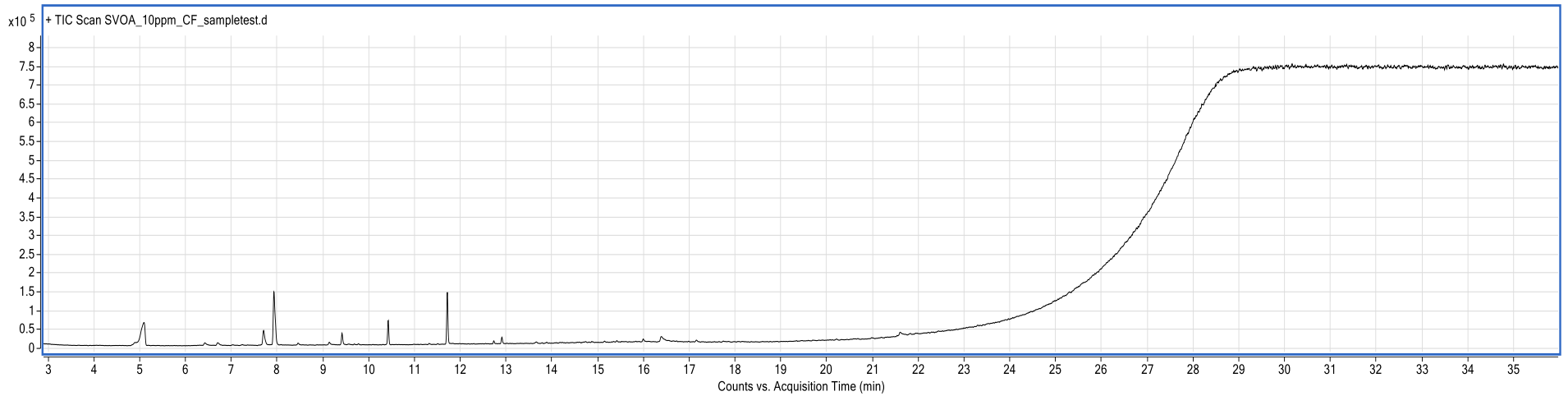
- The column is damaged



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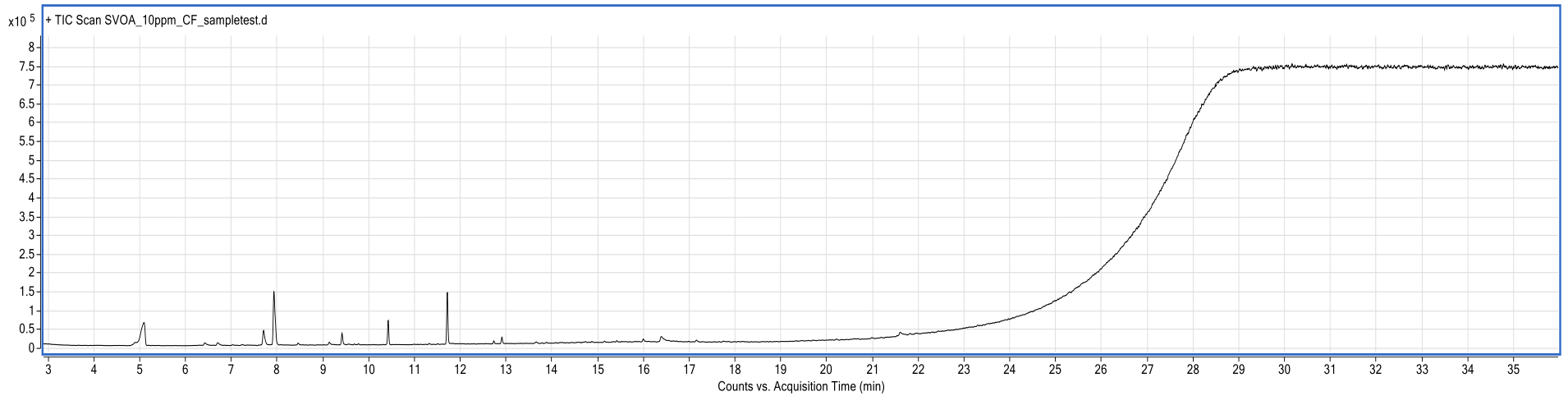
- The column is damaged: **Well, I guess I need to replace my column**



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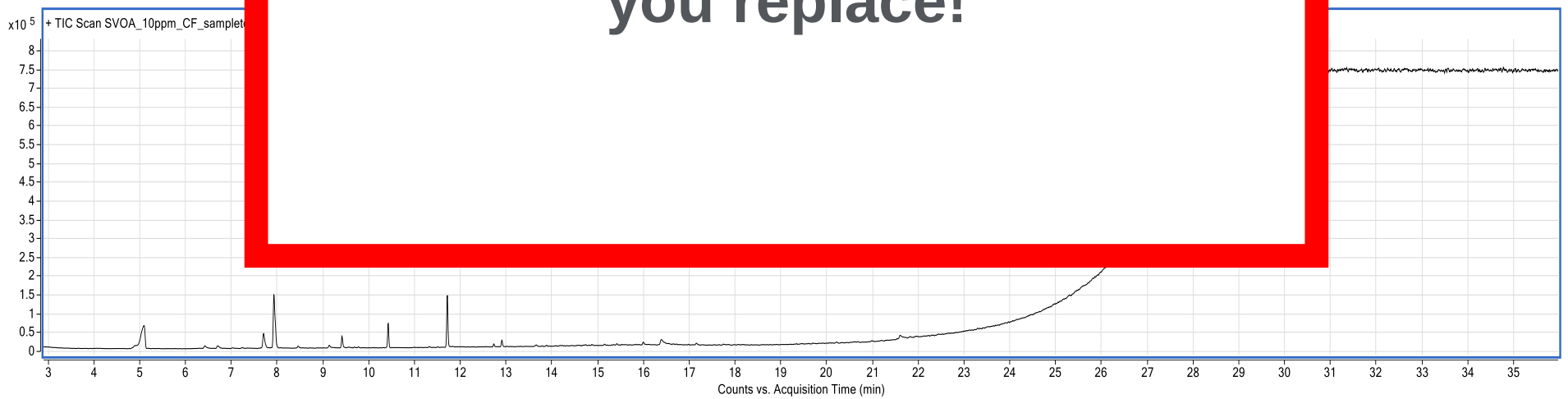
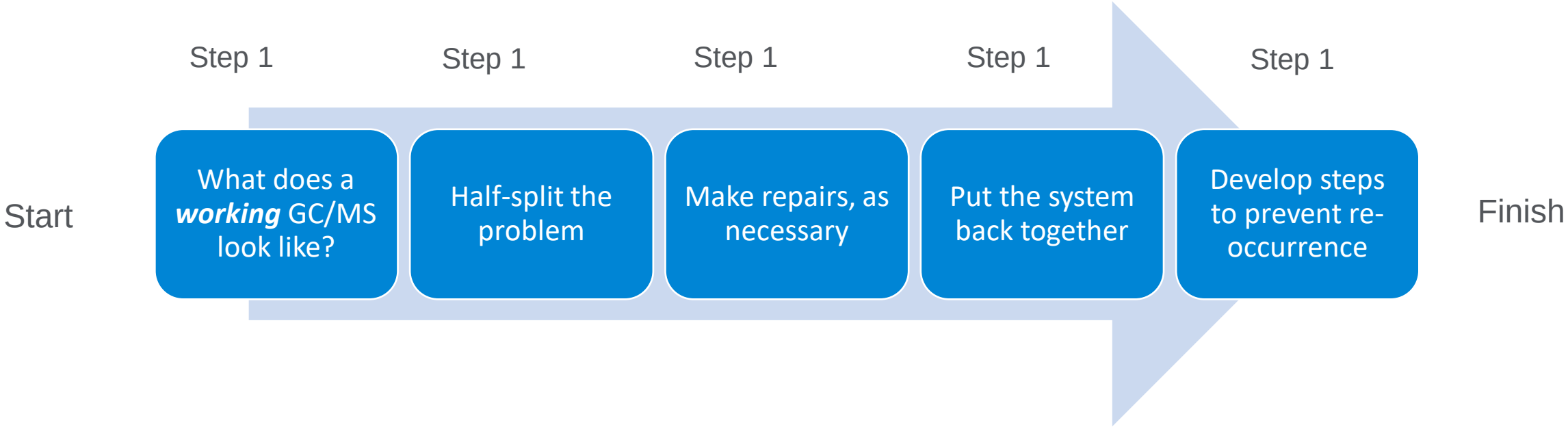
- The inlet is leak **normal**
- The column is d

WAIT

Test (a few more things) before you replace!



Follow a Logical Troubleshooting Procedure!

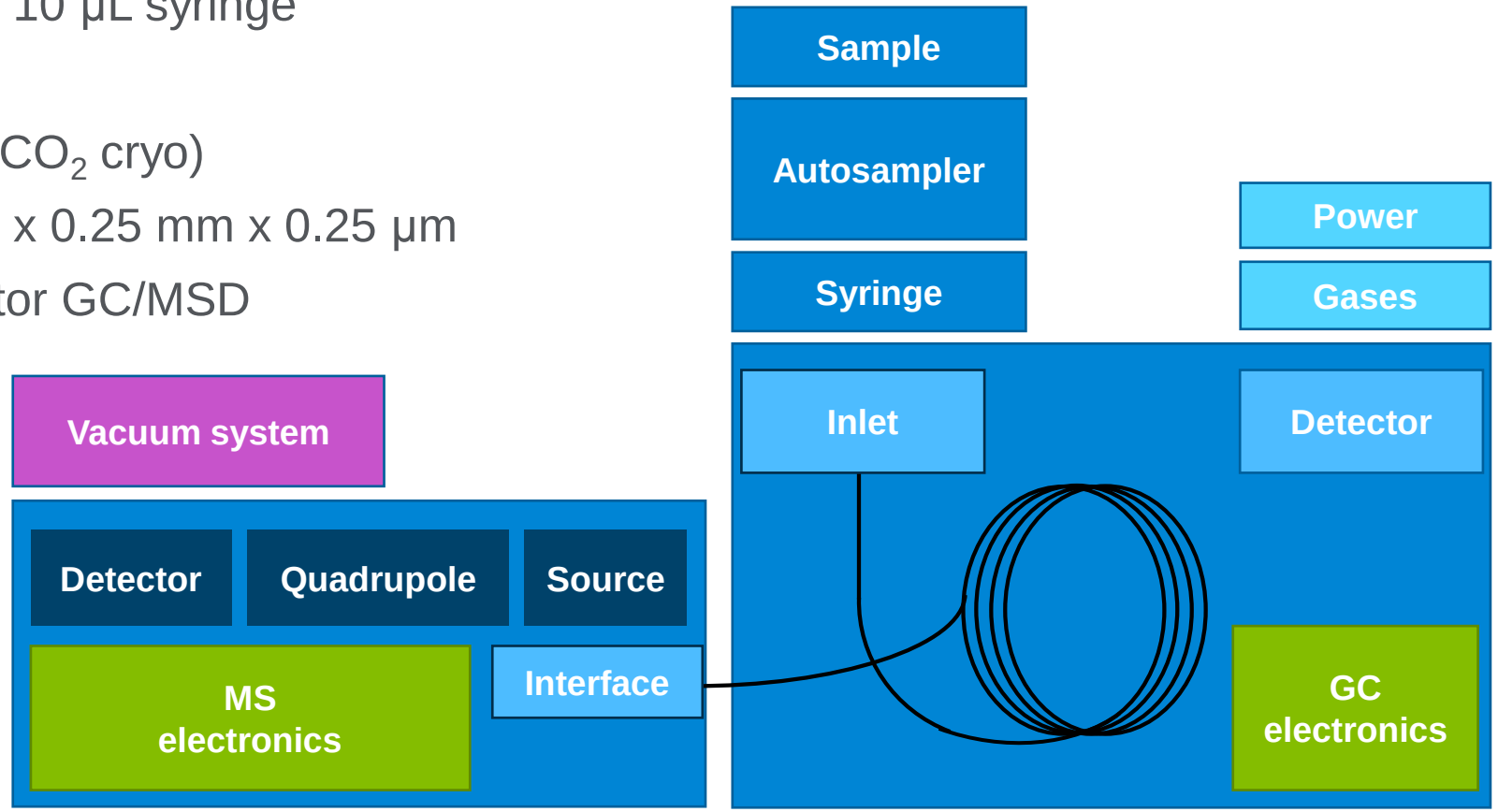


Troubleshooting Step 1: What is the “Working System”?

What does a ***working*** GC/MS look like? | Half-split the problem | Make repairs, as necessary | Put the system back together | Develop steps to prevent re-occurrence

What are the components of the GC/MS system?

- Agilent 7693A autosampler + 10 µL syringe
- Agilent 7890B GC
- Agilent MultiMode Inlet (with CO_2 cryo)
- Agilent J&W HP-5ms UI 30m x 0.25 mm x 0.25 µm
- Agilent 5977A Series Extractor GC/MSD



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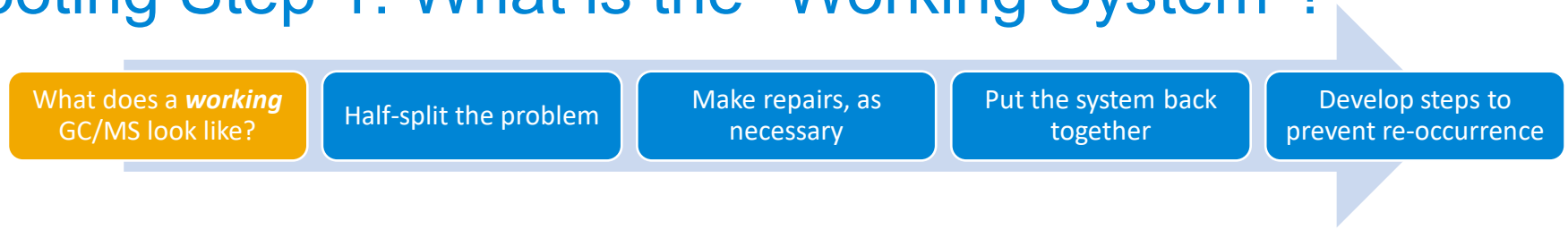


Compare your current data to known good data, when possible.

- How does your background compare to normal?
- Does the problem occur for every run, every analyte, every method?
  Only affects certain samples/analytes?
- Are the peaks smaller or larger than normal?
- Is the peak shape gaussian, or are the peaks splitting, tailing, or saturated?

Troubleshooting Step 1: What is the “Working System”?

Compare your current data to known good data, when possible.

- How does your background compare to normal?
  Background looked a LOT bigger than peaks in that TIC

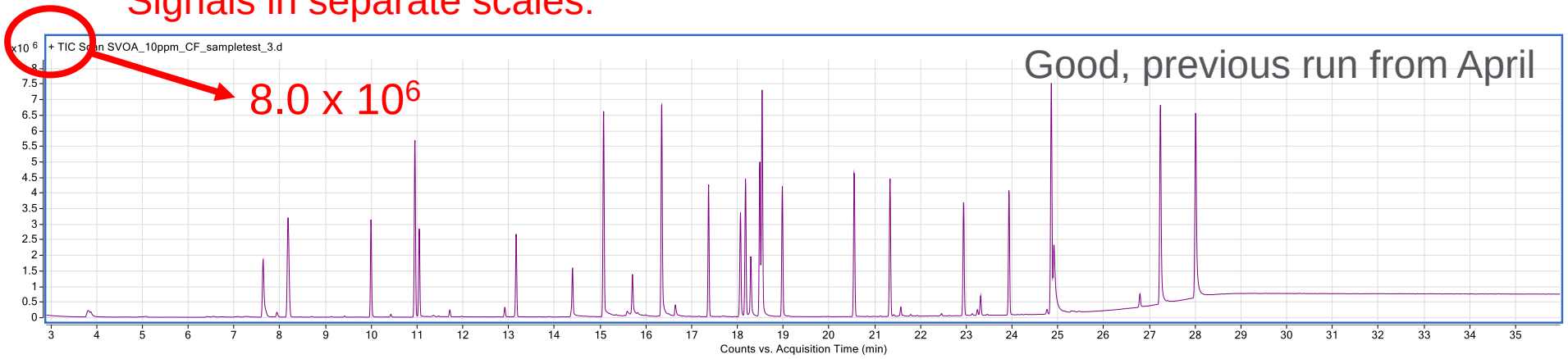
- Does the problem occur for every run, every analyte, every method?
  Only affects certain samples/analytes?
  Occurring on all checkout sample runs attempted

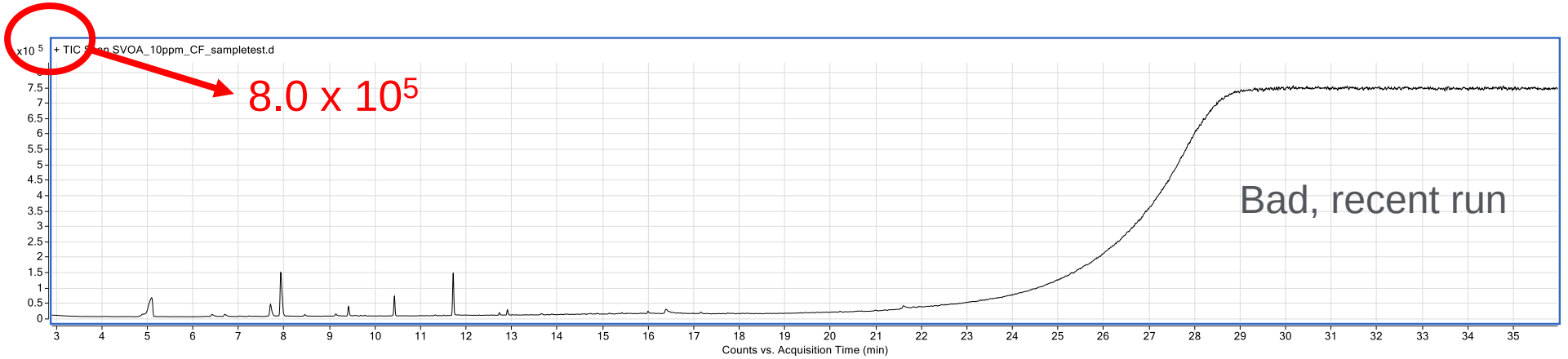
- Are the peaks smaller or larger than normal?
  Definitely smaller

- Is the peak shape gaussian, or are the peaks splitting, tailing, or saturated?
  Let’s find out

Troubleshooting Step 1: What is the “Working System”?

Compare your current data to known good data.
Now, the data is much clearer, and the background is not significantly higher.
Signals in separate scales:





Troubleshooting Step 1: What is the “Working System”?

Compare your current data to known good data.
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Signals with linked Y axis:

Troubleshooting Step 2: Break Apart (Half-Split) the Problem

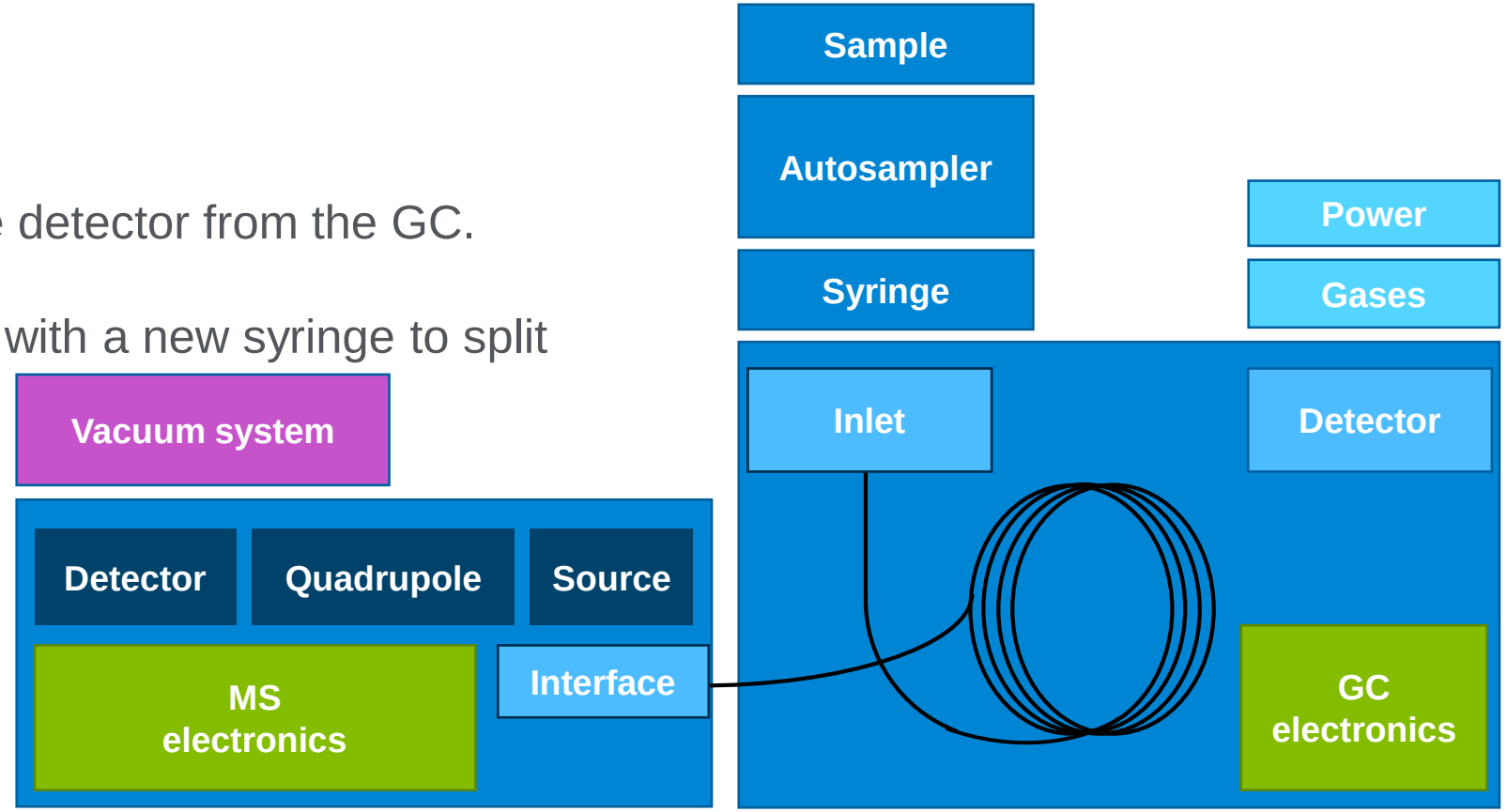
What does a ***working*** GC/MS look like? → Half-split the problem → Make repairs, as necessary → Put the system back together → Develop steps to prevent re-occurrence

Think of a set of tests that will break the system into smaller pieces.

1. Try a new sample.

2. Tune the MS to half-split the detector from the GC.

3. Perform a manual injection with a new syringe to split autosampler and inlet/column.



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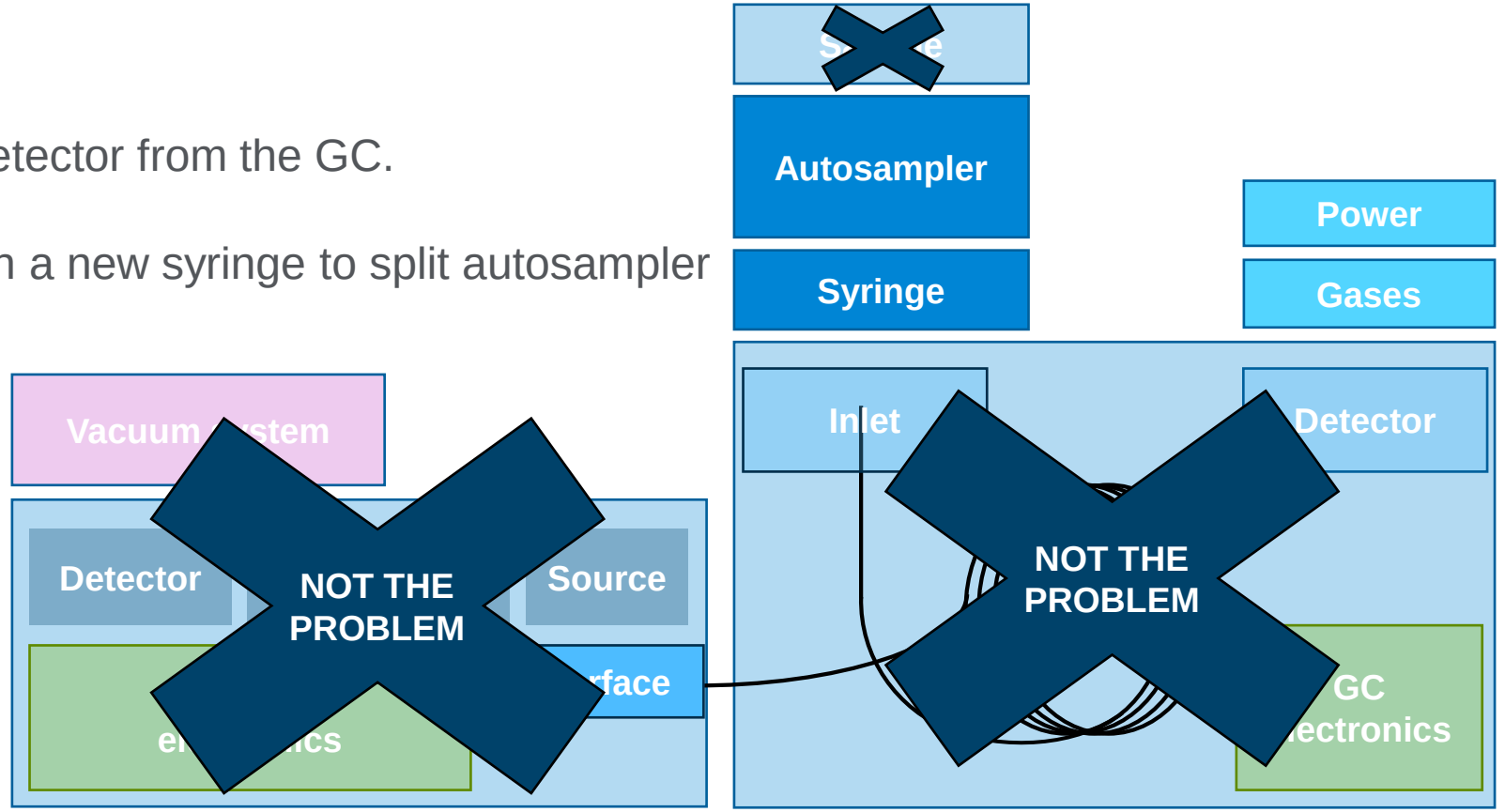

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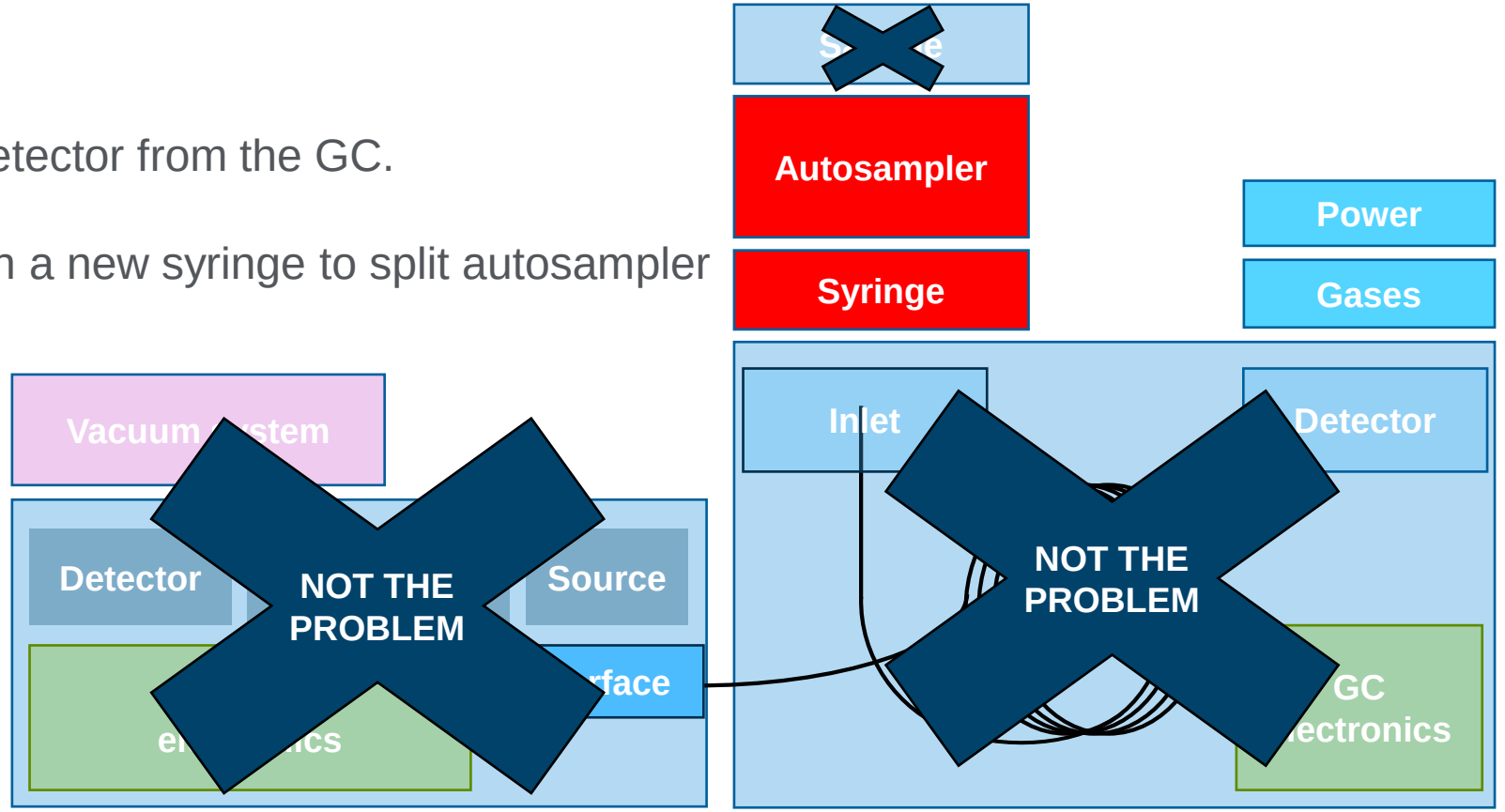


Troubleshooting Step 2: Narrow Focus of the Problem

What does a ***working*** GC/MS look like? | Half-split the problem | Make repairs, as necessary | Put the system back together | Develop steps to prevent re-occurrence

Let's focus on the autosampler and syringe:

Autosampler

Syringe

While sample was new, what is the solvent? Dichloromethane

What kind of syringe? Agilent 10 µl syringe, 23-26s/42/cone (G4513-80204)

Does the autosampler work? Autosampler turns and moves plunger up and down

Does the syringe pull up liquid? No, it doesn't

We may have found the problem!

Troubleshooting Step 3: Make the Repair

What does a ***working*** GC/MS look like? | Half-split the problem | Make repairs, as necessary | Put the system back together | Develop steps to prevent re-occurrence

Replace the syringe with a 10 µL PTFE tipped plunger syringe (G4513-80203) – a much easier repair than venting and changing the column.

PTFE tipped syringes are more chemically resistant and offer a reduced chance of carry over and longer syringe lifetime.

Proper syringe maintenance must still be performed. Clean and refill syringe wash vials frequently.

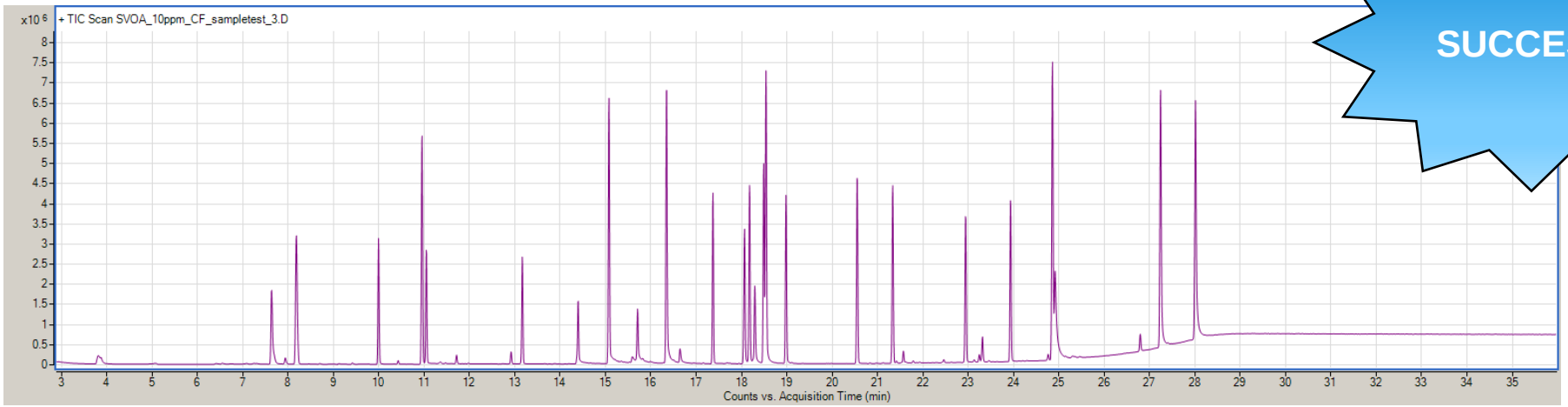
Beware highly concentrated samples and samples with particulates (organic material, salts, etc.)

Syringe

Troubleshooting Step 4: Put the System Back Together

What does a ***working*** GC/MS look like? | Half-split the problem | Make repairs, as necessary | Put the system back together | Develop steps to prevent re-occurrence

What happened with a new syringe?



SUCCESS!

Have a Good Troubleshooting Story?- Let Us Know!

Please call or email us today to share a troubleshooting success story or if you need help troubleshooting!

Troubleshooting Tips

1. Isolate the problem

   (blank run, inject un-retained compound, jumper tube test)

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

Remember

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

Multiple cause and effect

Do not change too many variables at once

Contact Agilent Chemistries and Supplies Technical Support

1-800-227-9770 Option 3, Option 3:

Option 1 for GC/GC/MS Columns and Supplies

Option 2 for LC/LC/MS Columns and Supplies

Option 3 for Sample Preparation, Filtration and QuEChERS

Option 4 for Spectroscopy Supplies

Available in the USA 8-5 all time zones

gc-column-support@agilent.com

lc-column-support@agilent.com

spp-support@agilent.com

spectro-supplies-support@agilent.com