

It's Peak Season for Great Peak Shape

Tips and tricks on troubleshooting in GC chromatography

Mark Sinnott
Alexander Ucci
6 August, 2020



“Everything Was Just Fine.. and Then This Happened!”

“How do I troubleshoot?”

Track your actions/log book:

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

It's Peak Season for Great Peak Shapes

DE.2851967593



Logic
=
**Something changed
(slowly or suddenly)**
=
Something is different

Logical Troubleshooting

Troubleshooting starts with isolating the problem.

- There are five basic areas from where problems can arise:

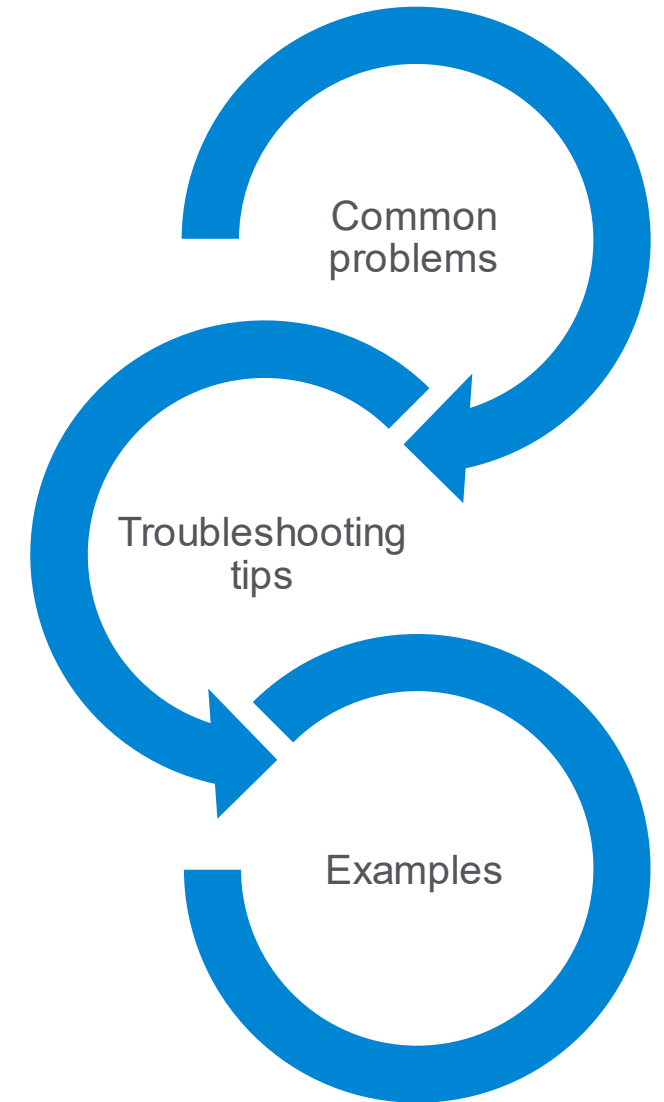
- Injector
- Flow
- Column
- Detector
- Electronics

Or...

- A combination of these

Knowing what can and cannot cause the symptom is the key, and most importantly **DON'T PANIC!**

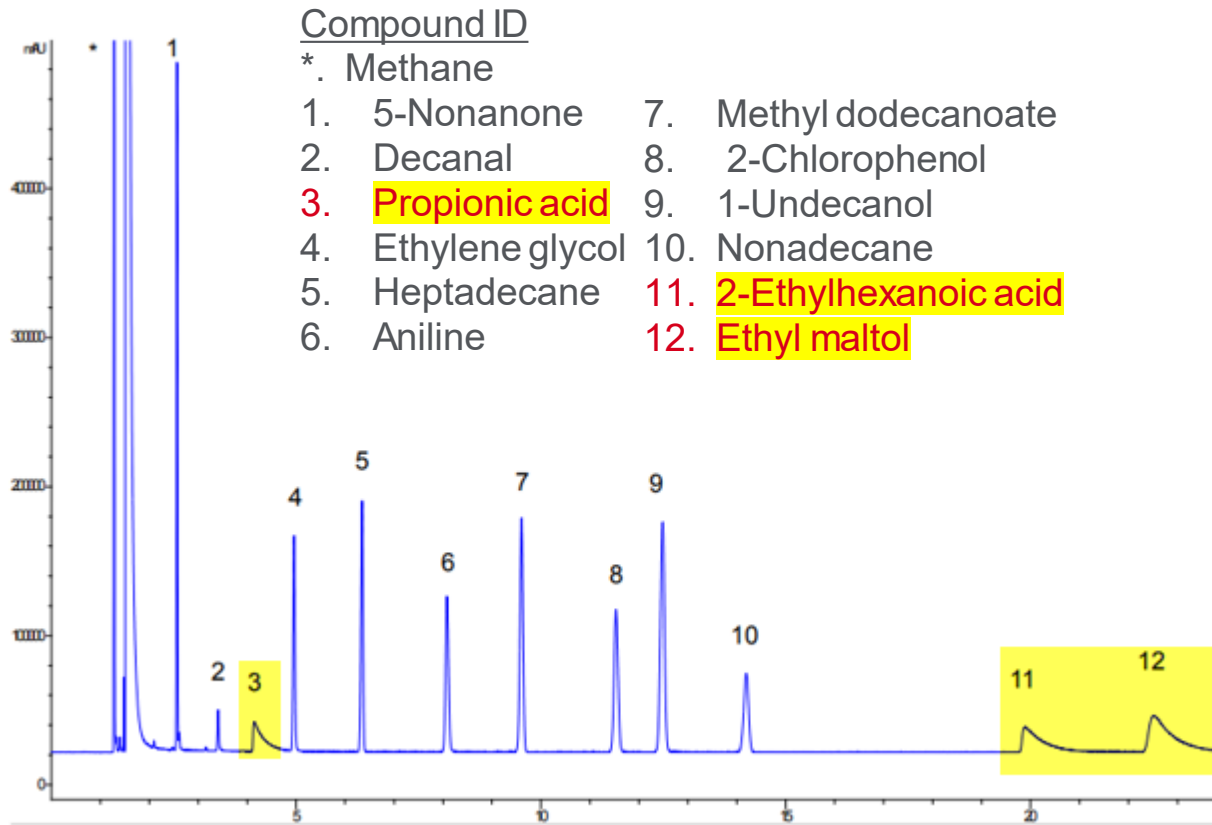
Agenda



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

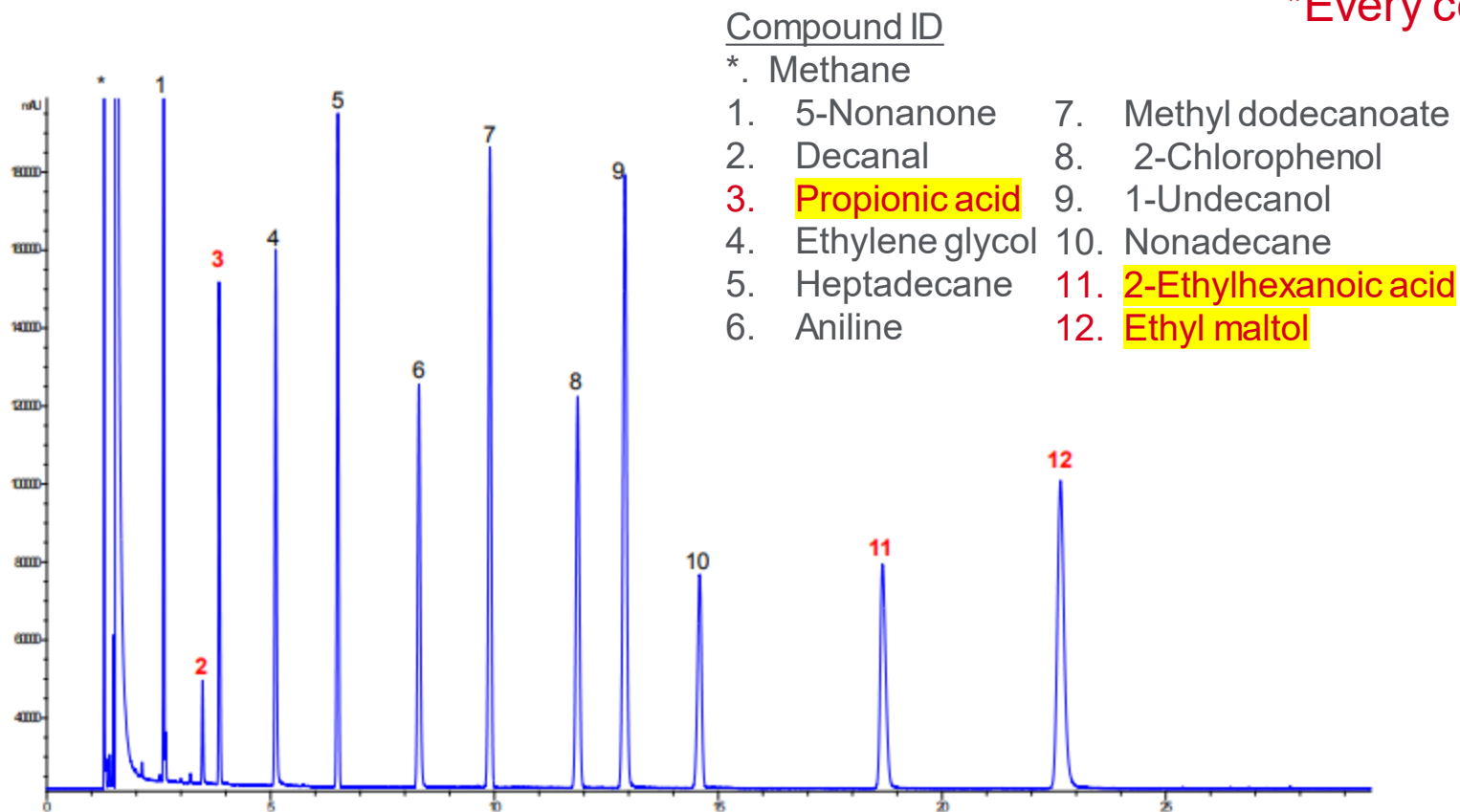
Miscellaneous - overloading of PLOT columns, co-elution, polarity mismatch between phase, solute or solvent, and some compounds always tail

*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



Brochure 5991-6709EN

Agilent Inert Flow Solution

Agilent Ultimet Plus inlet weldment, shell and transfer lines



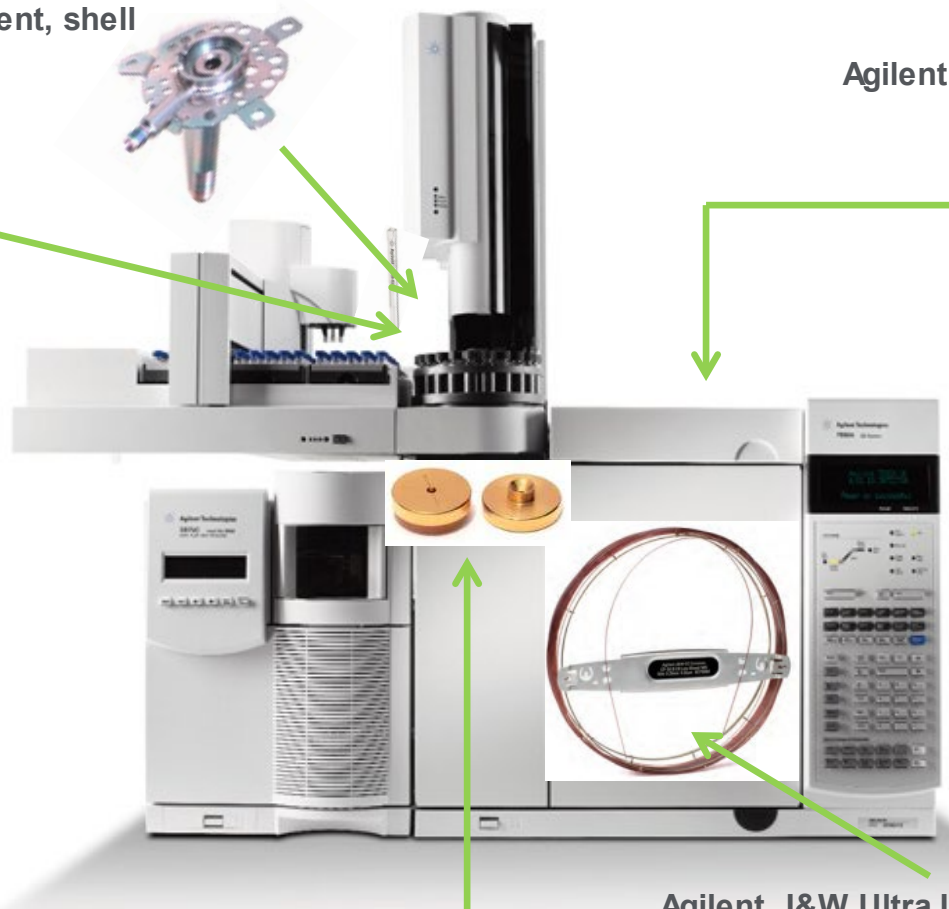
Agilent Ultra Inert inlet liner



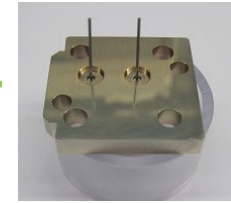
Agilent Ultimet Plus ferrules



Agilent Ultimet Capillary Flow Technology Devices, Ultimate union



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

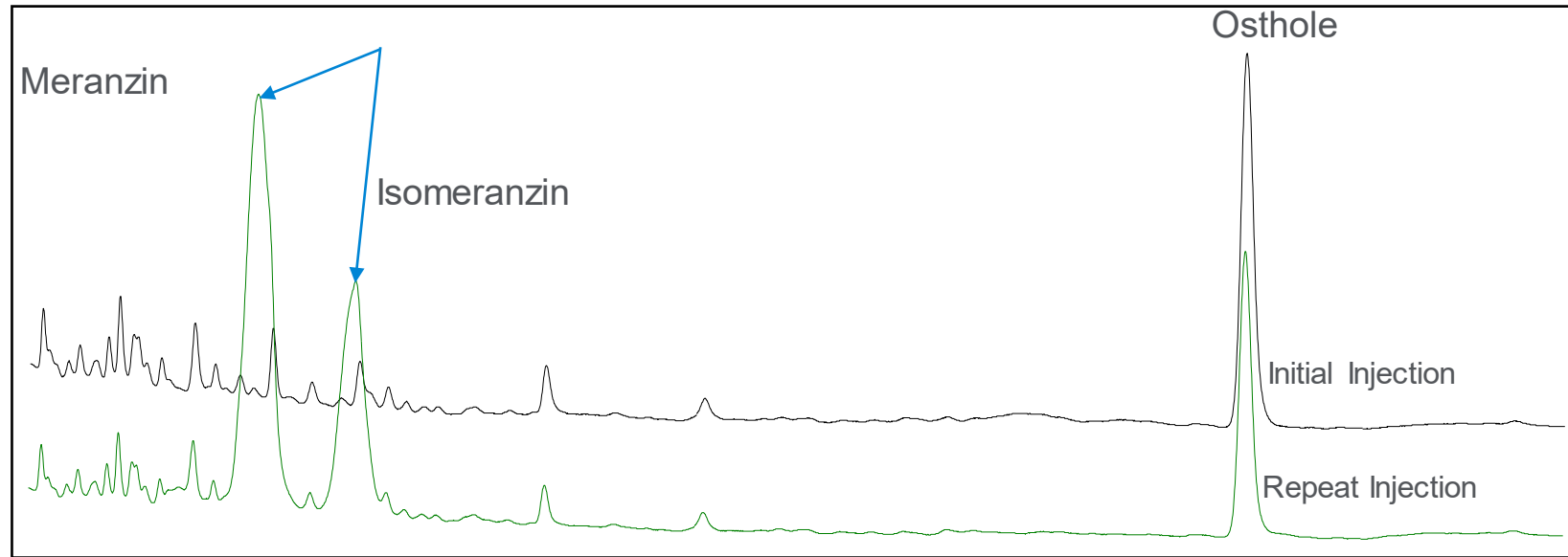


Agilent J&W Ultra Inert GC column

Agilent Ultra Inert gold seal

5990-8532EN brochure

Bonus or Ghost Peaks



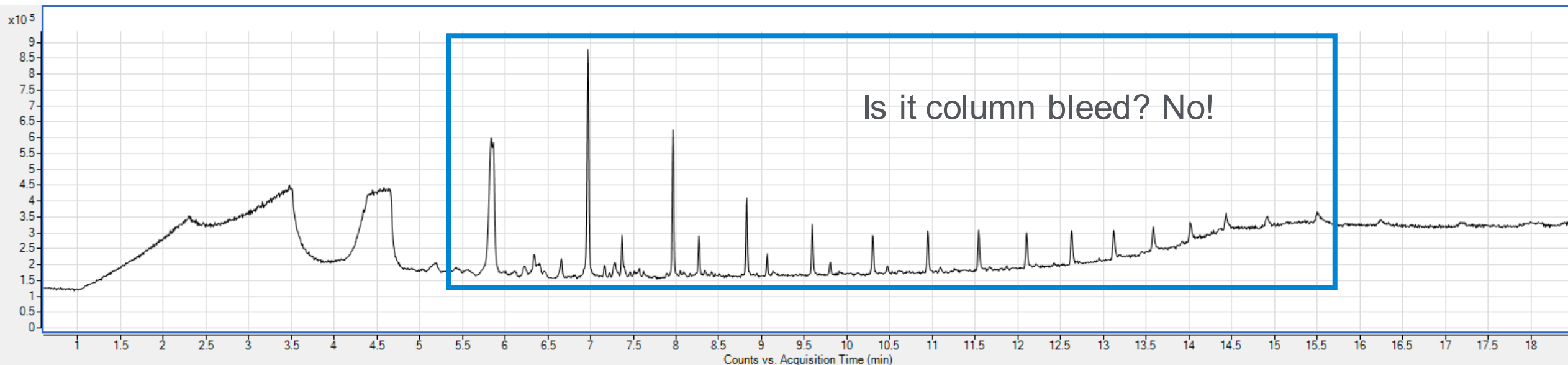
5991-9078EN

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

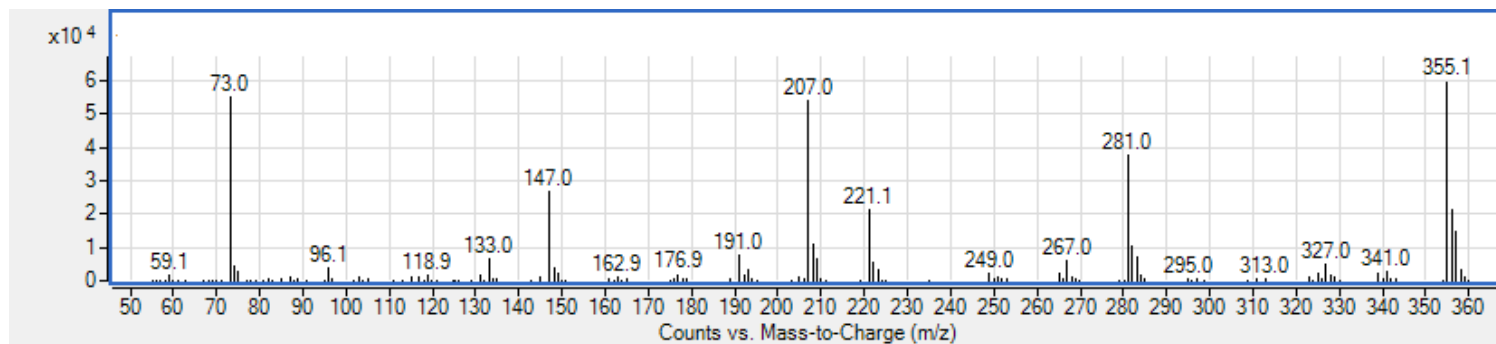
207

281

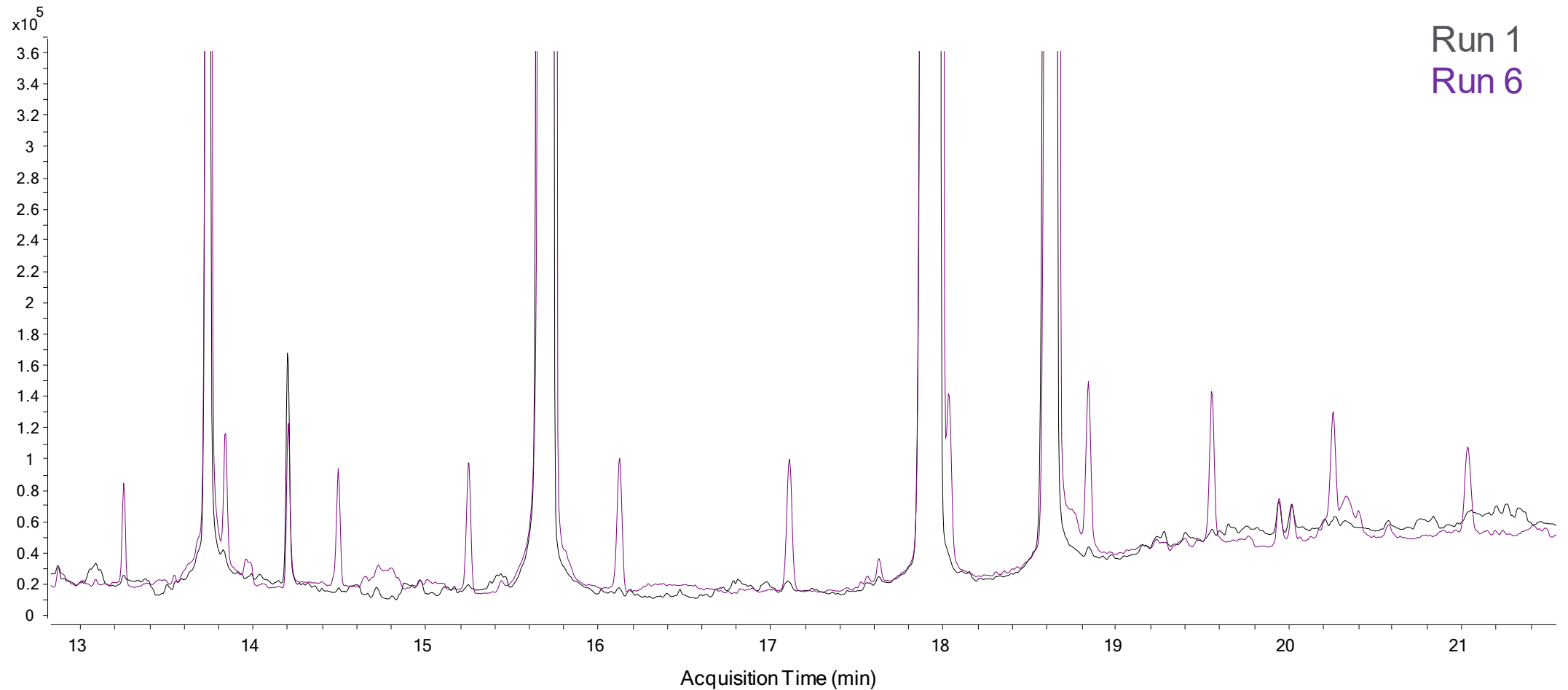
355

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:



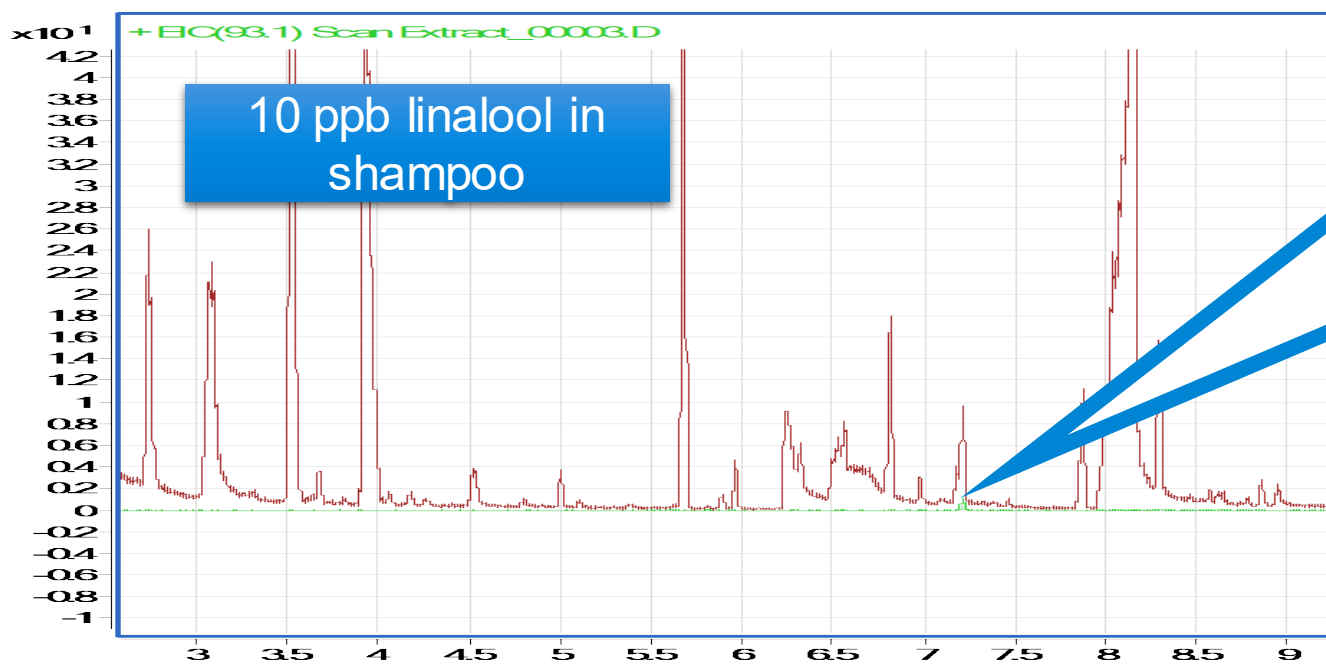
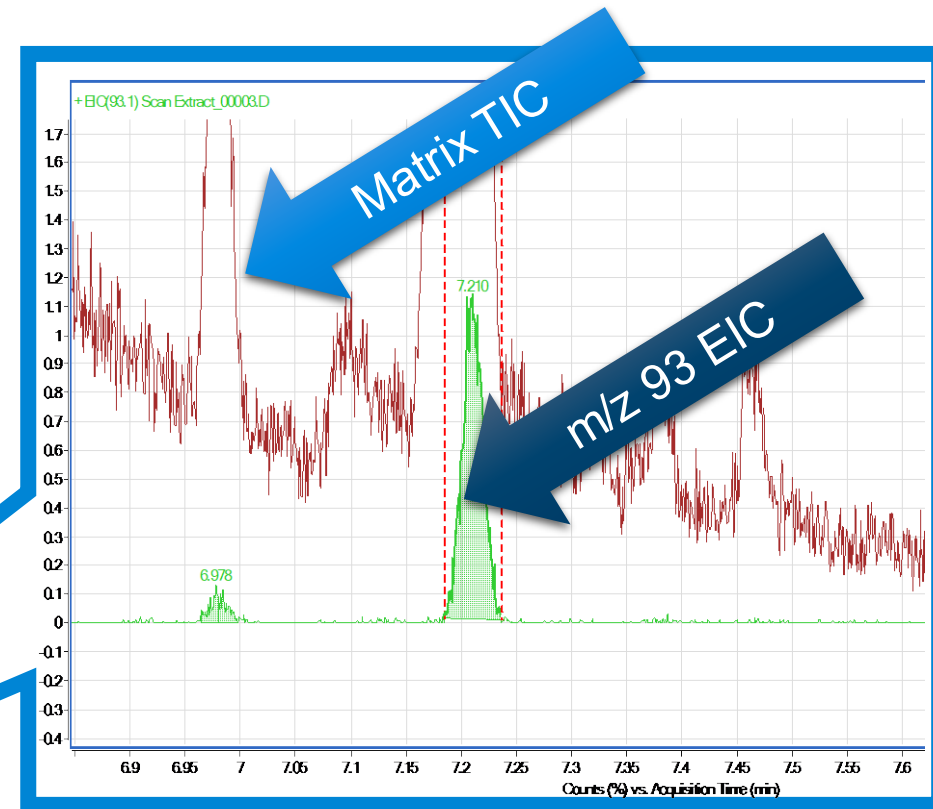
Multiple Injections from the Same Vial: Siloxanes!



Does Your Baseline Look Like This? Do You See Extra Peaks?

The Matrix

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

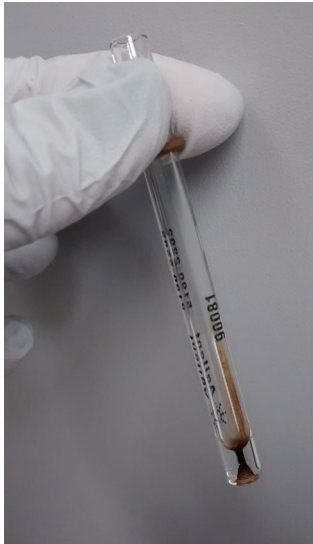


...(or improve your sample cleanup)

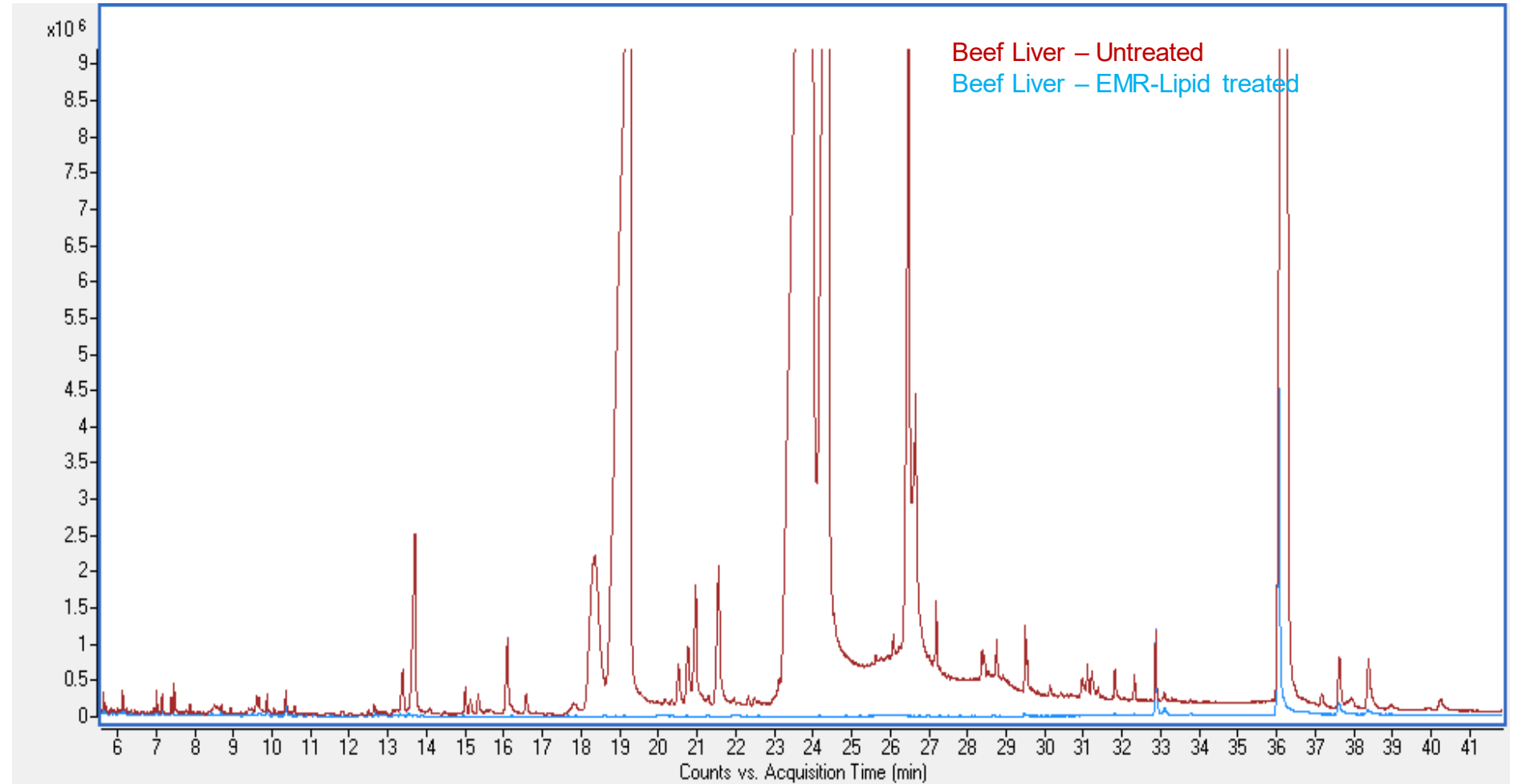
The Importance of Sample Cleanup



50 samples
with clean-up

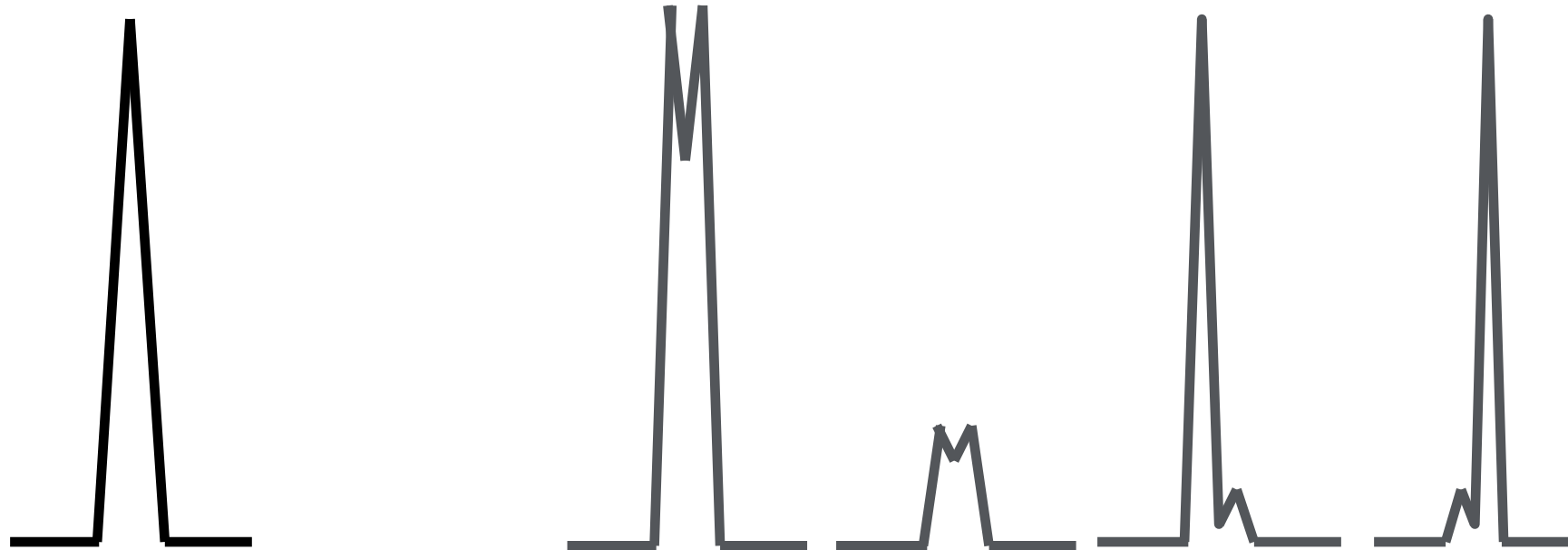


50 samples
without clean-up



For sample cleanup 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)

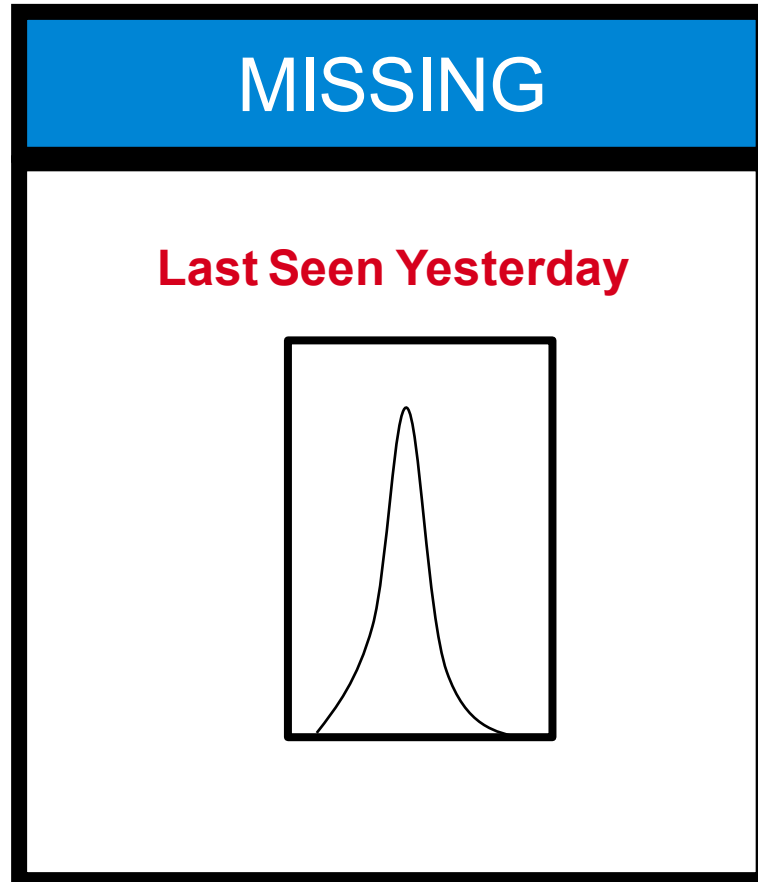
Injector (activity)

- Breakdown (not really a split peak, two peaks)
- Sample degradation in injector

Volatility

- High boilers dropping out on cold spots
- Transfer line temperatures
- Unions or fittings not tracking column temperature

No Peaks



Detector (not on, or not operational)

Injector (not working)

Plugged syringe/plunger not moving

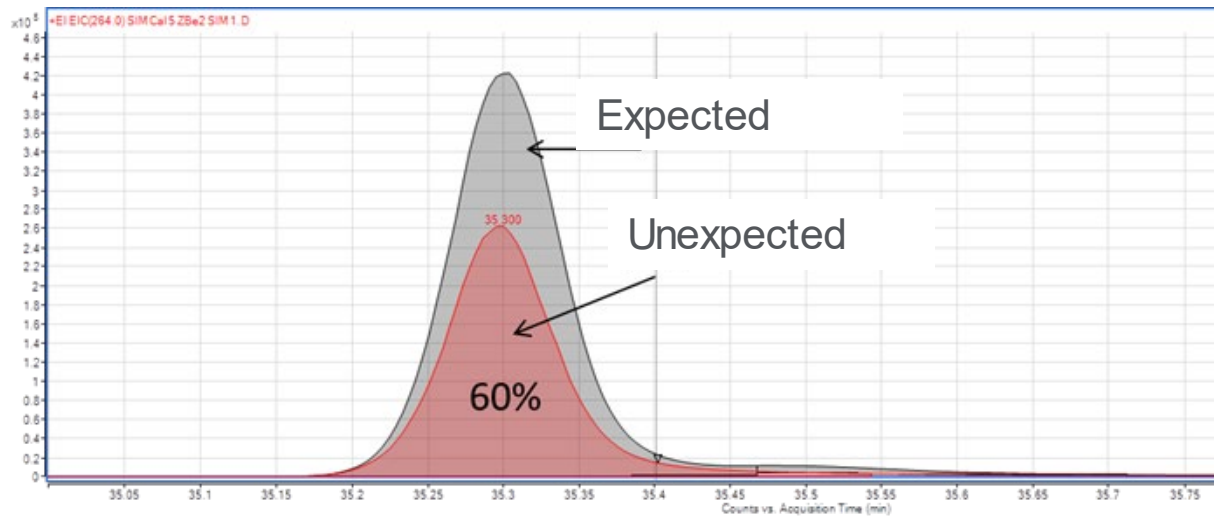
- Wrong injector (or detector)
- Huge leak (older systems)
- No carrier gas flow

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*

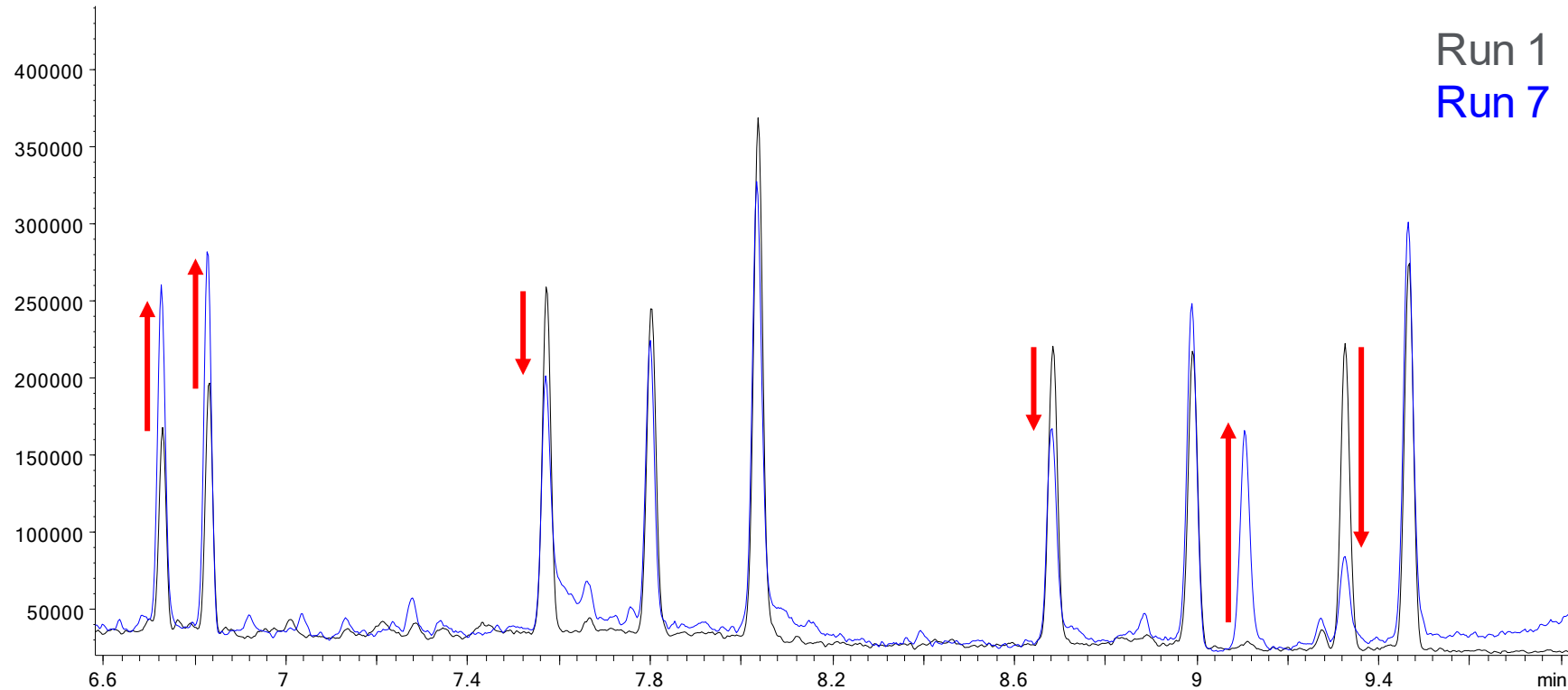
Detector (response problem)

- 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



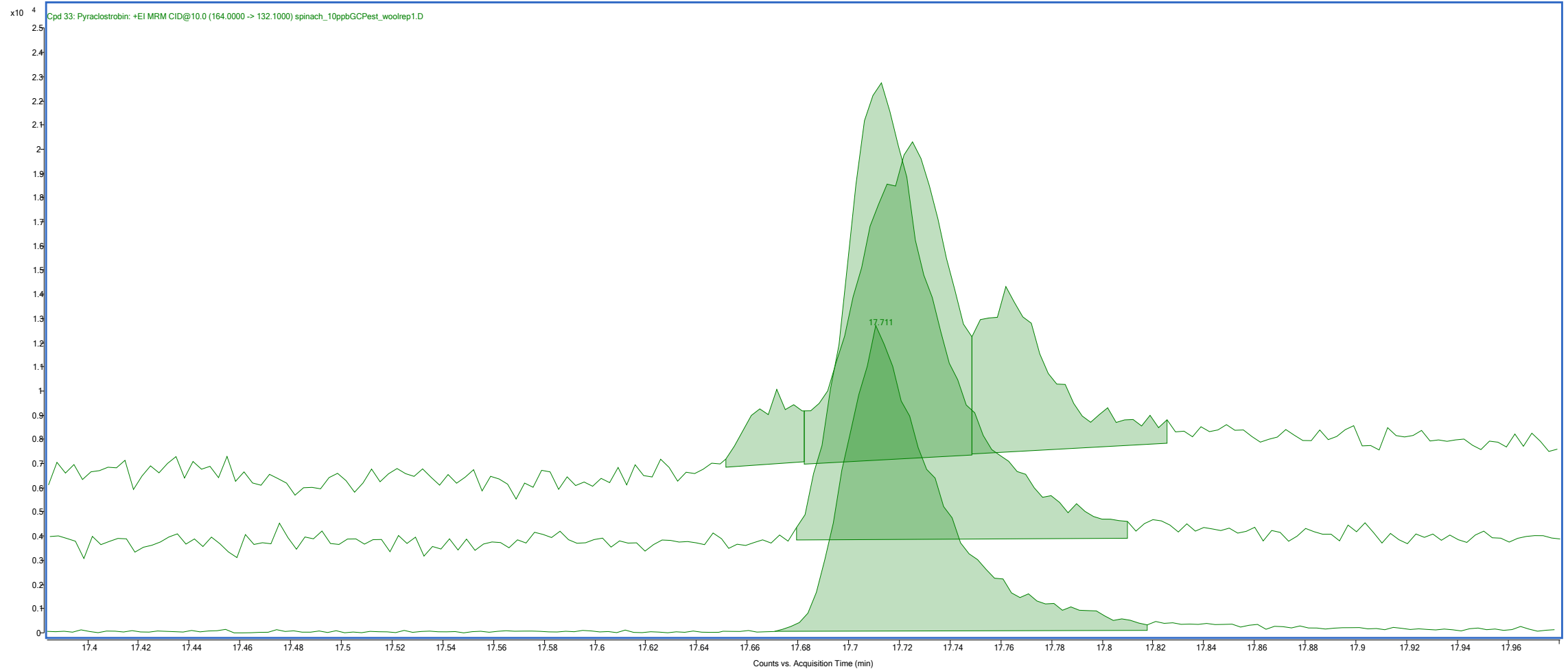
Injector or column is active/contaminated

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

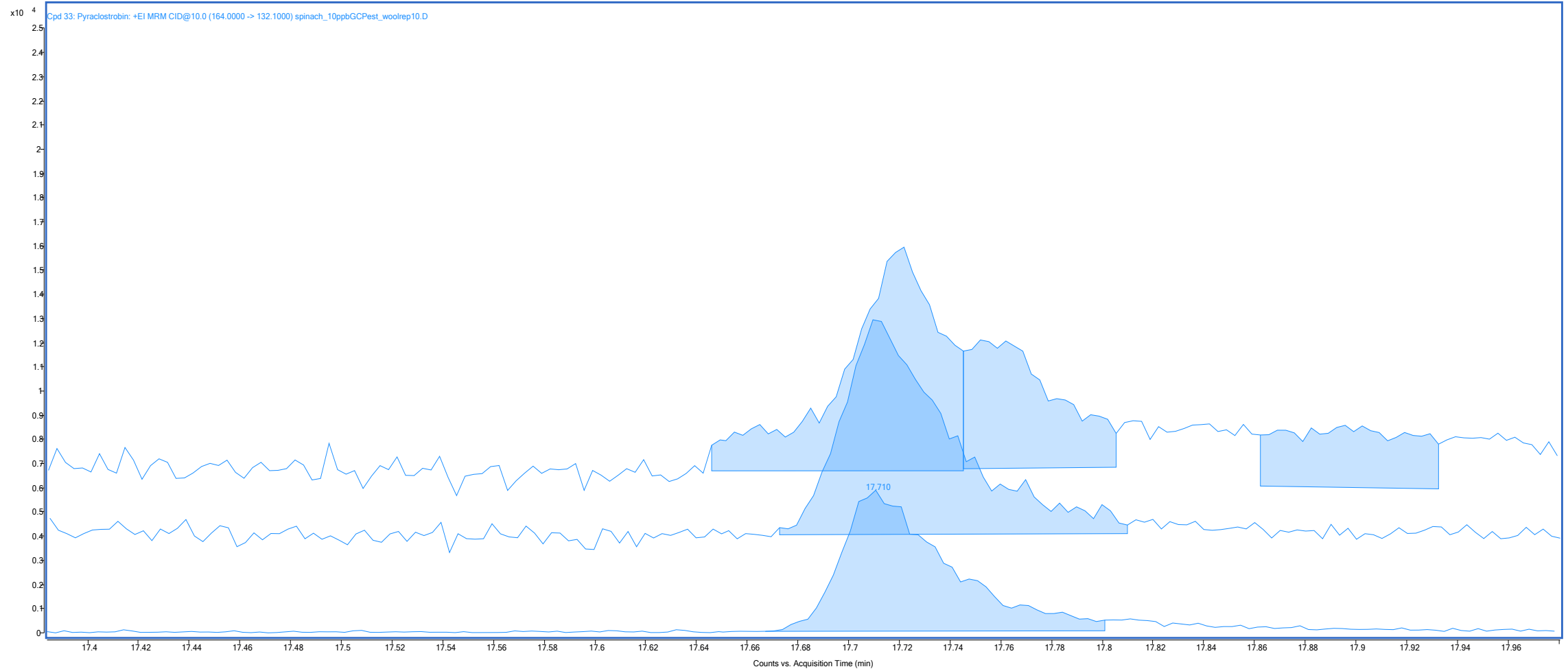
Decomposition of sample

- Temperature change – Discrimination
- Evaporation from sample

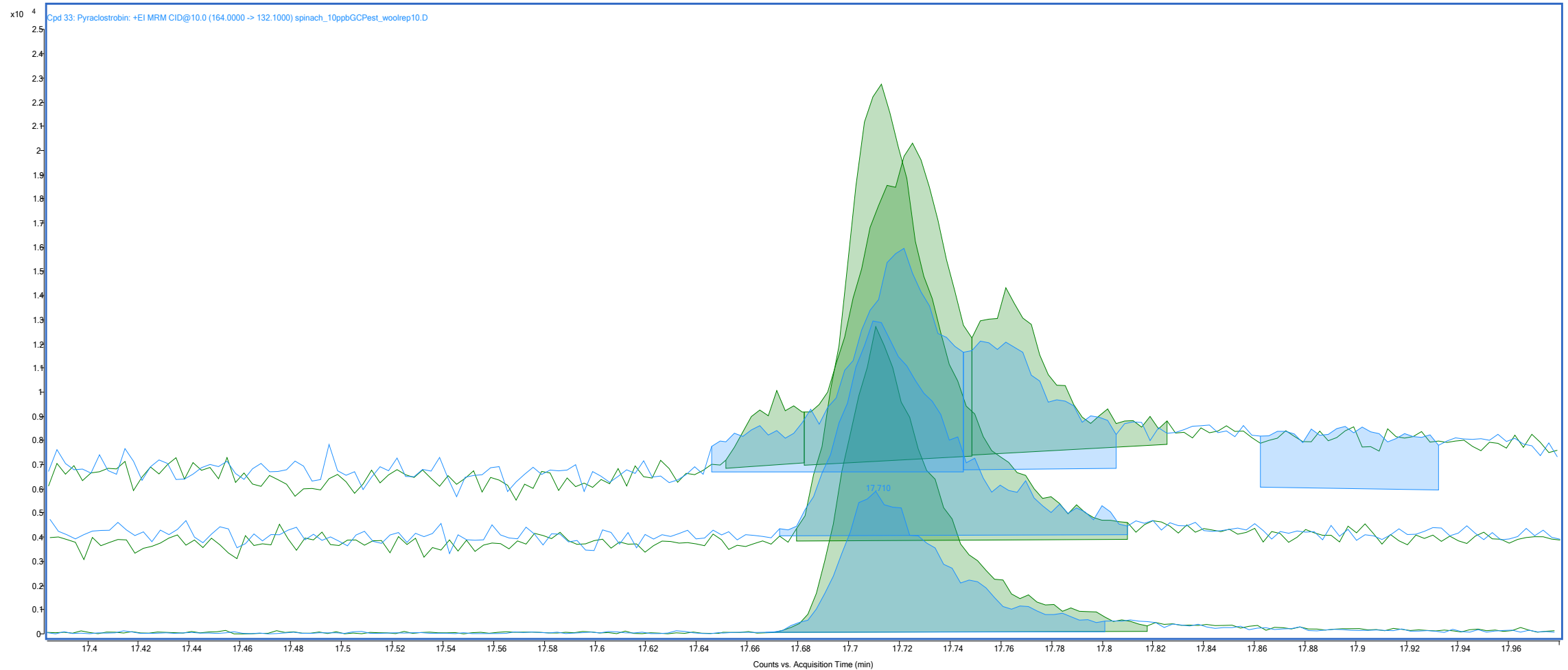
Change in Response: Pyraclostrobin in Spinach on Run 1



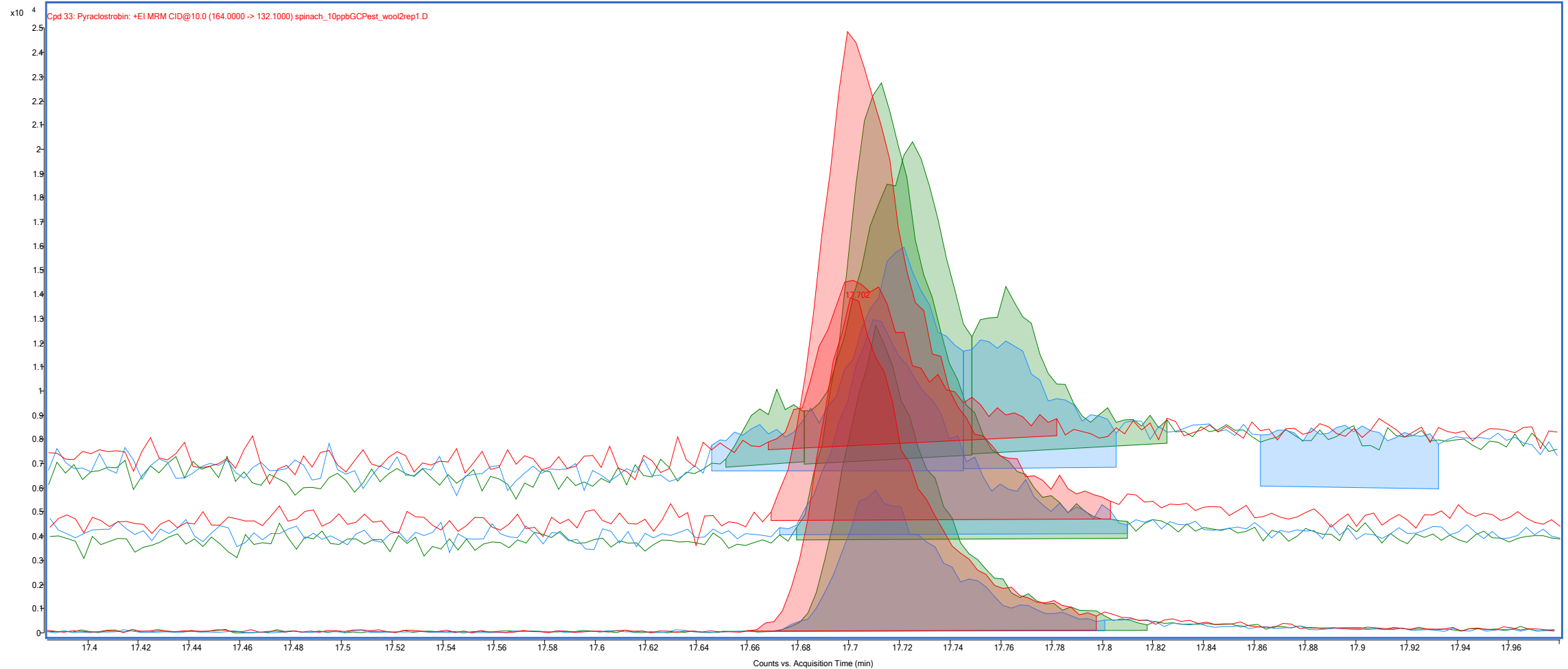
Change in Response: Pyraclostrobin in Spinach on Run 65



Change in Response: Pyraclostrobin in Spinach on Run 1 vs Run 65

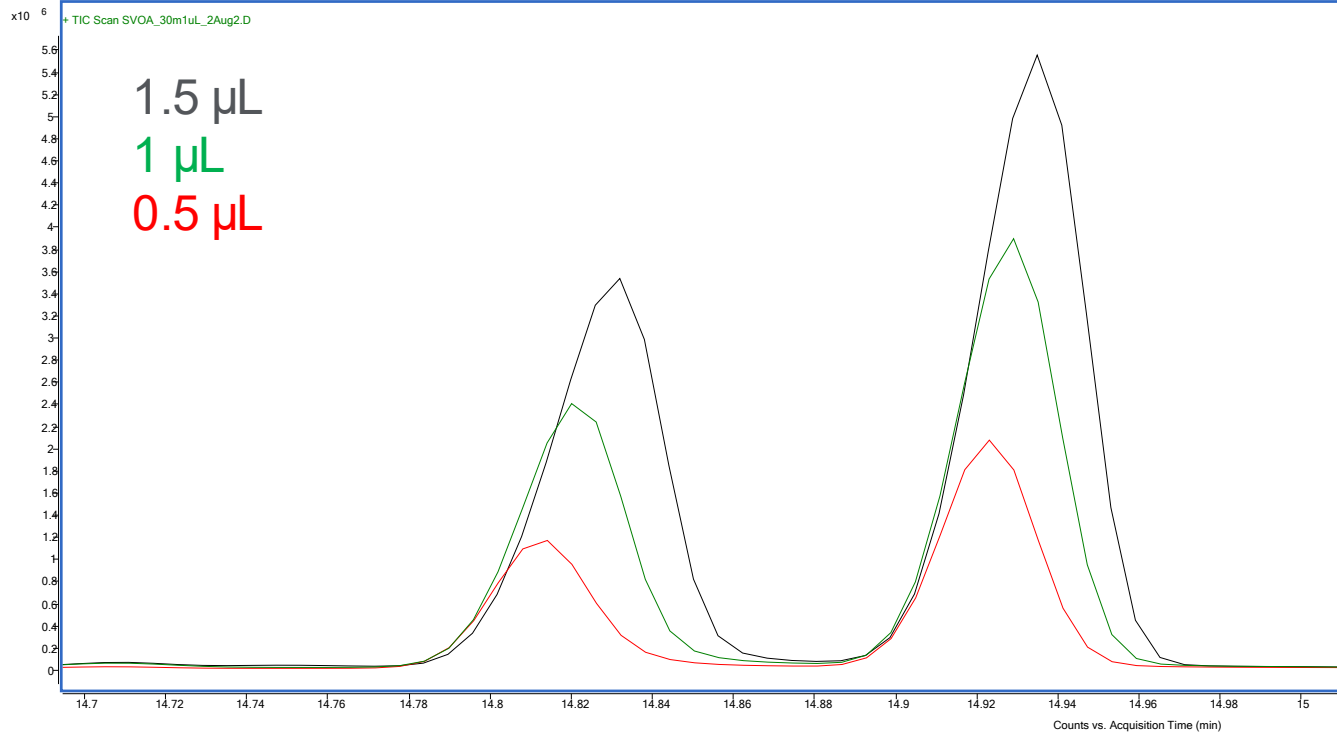


Change in Response: Pyraclostrobin in Spinach with New Liner



Peak Fronting

Shark fin-shaped or just slight



Column (contaminated)

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

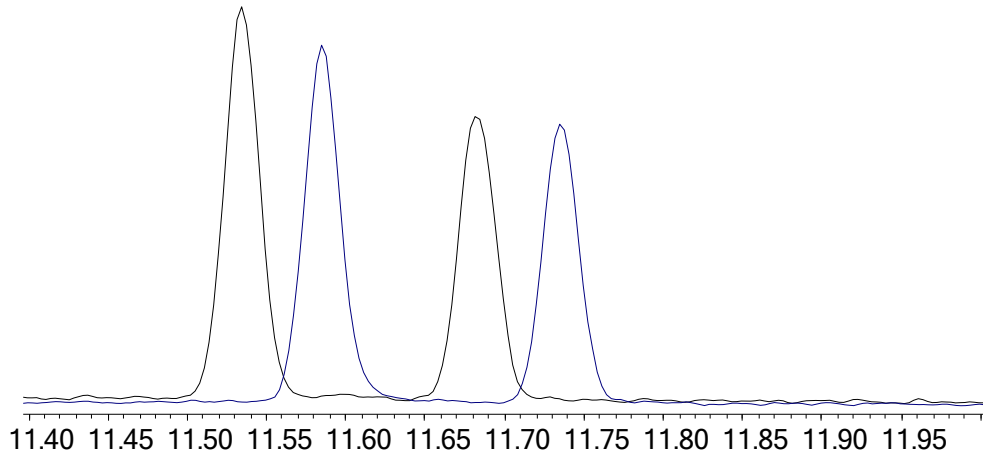
Injector

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

Other

- Co-elution
- Breakdown

Retention Time Shift



Injector

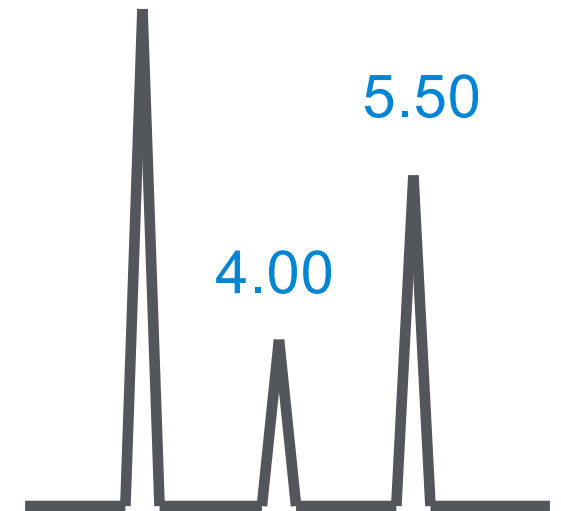
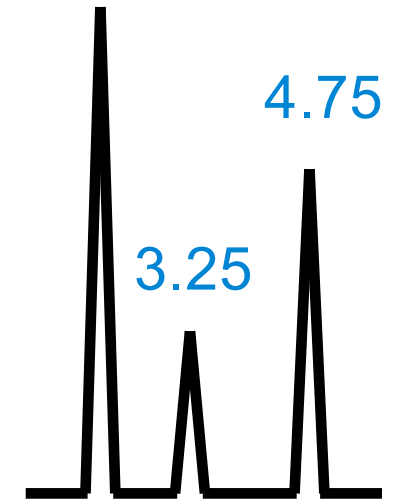
- Leak in the septum
- Change in injection solvent
- Large change in sample concentration

Flow

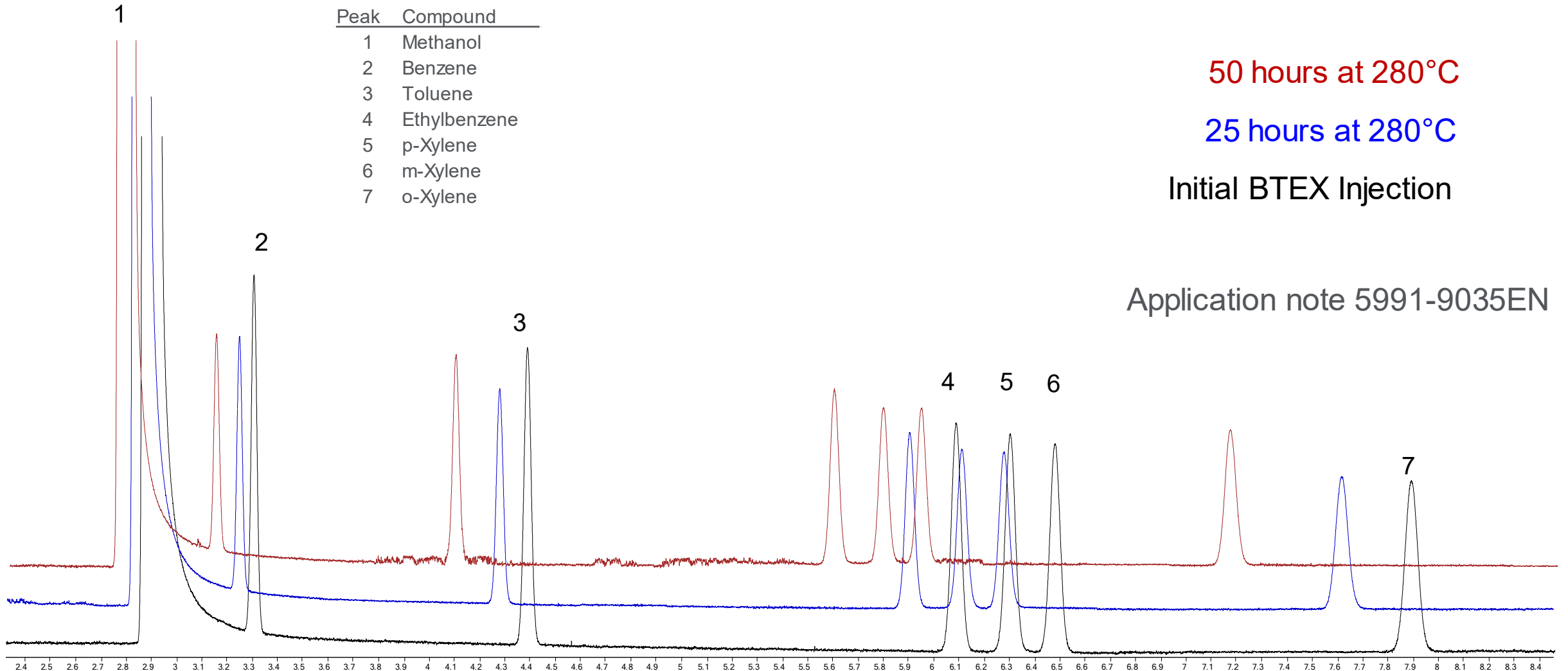
- Change in gas velocity

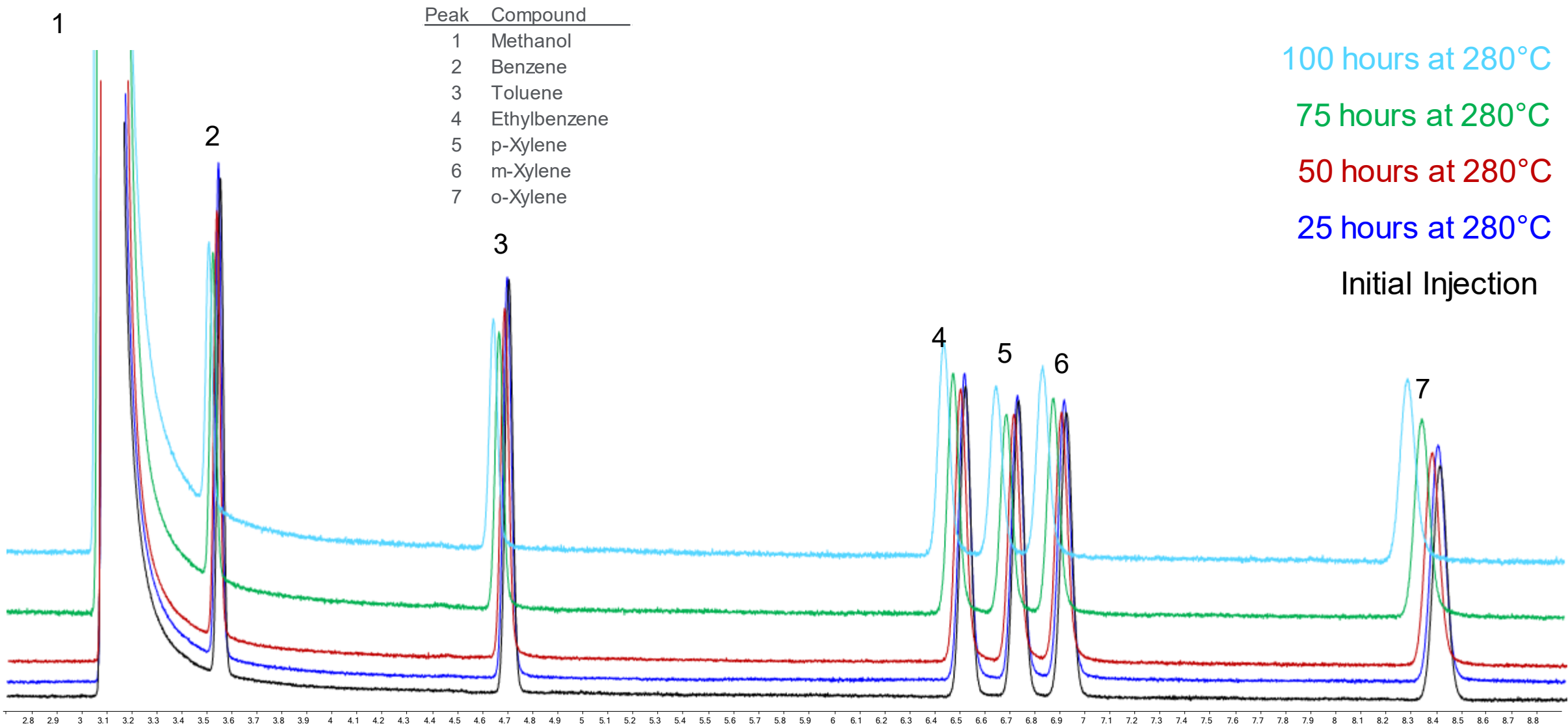
Column

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

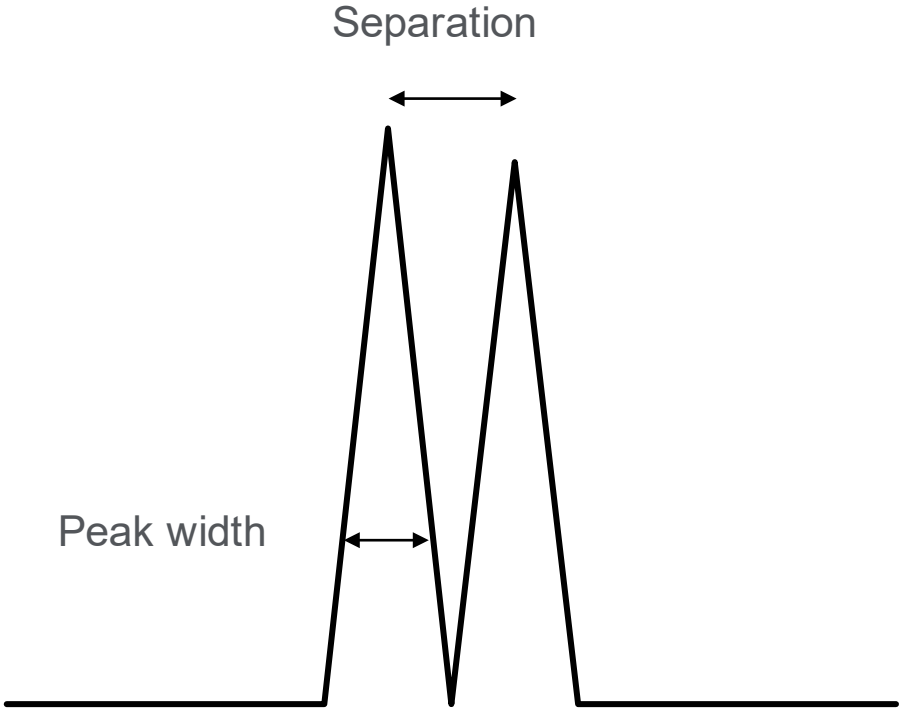


Thermal Stability and Retention Time Shifting on Standard WAX Column





Loss of Resolution



Resolution is a function of separation and peak width

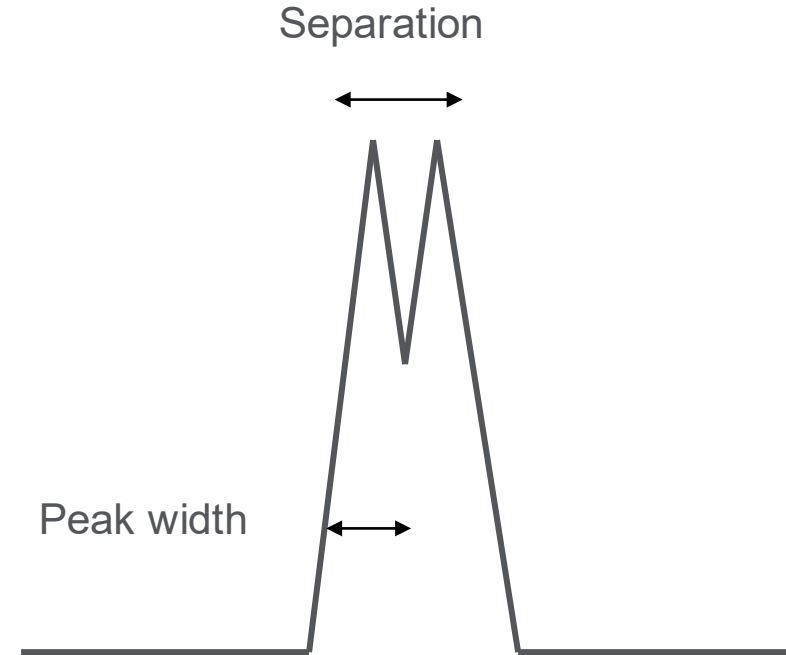
Loss of Resolution - Separation Decrease (RT's change)

Column

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

Flow

- Change in velocity?



Loss of Resolution - Peak Broadening (RT's unchanged)

Flow

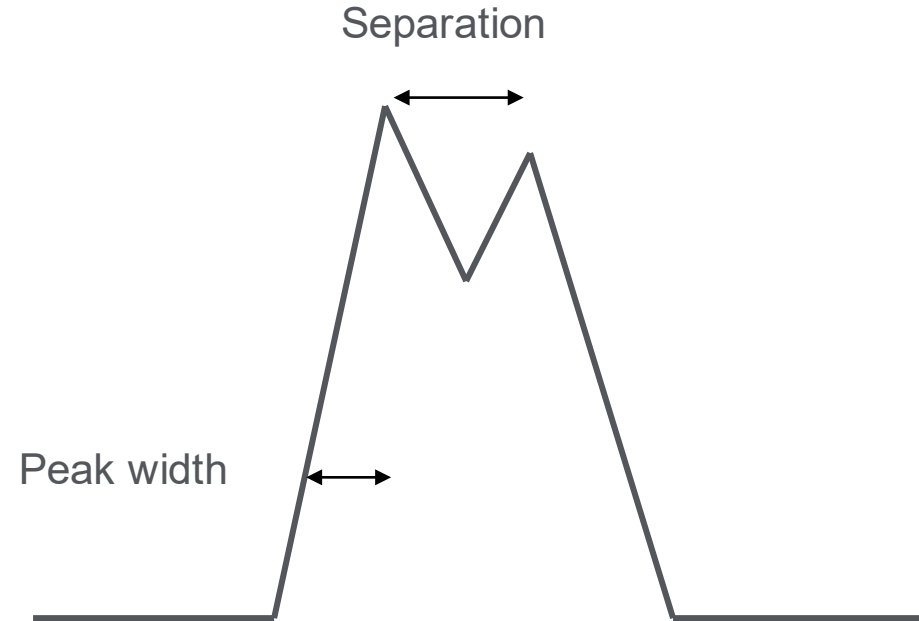
- Make-up gas

Column

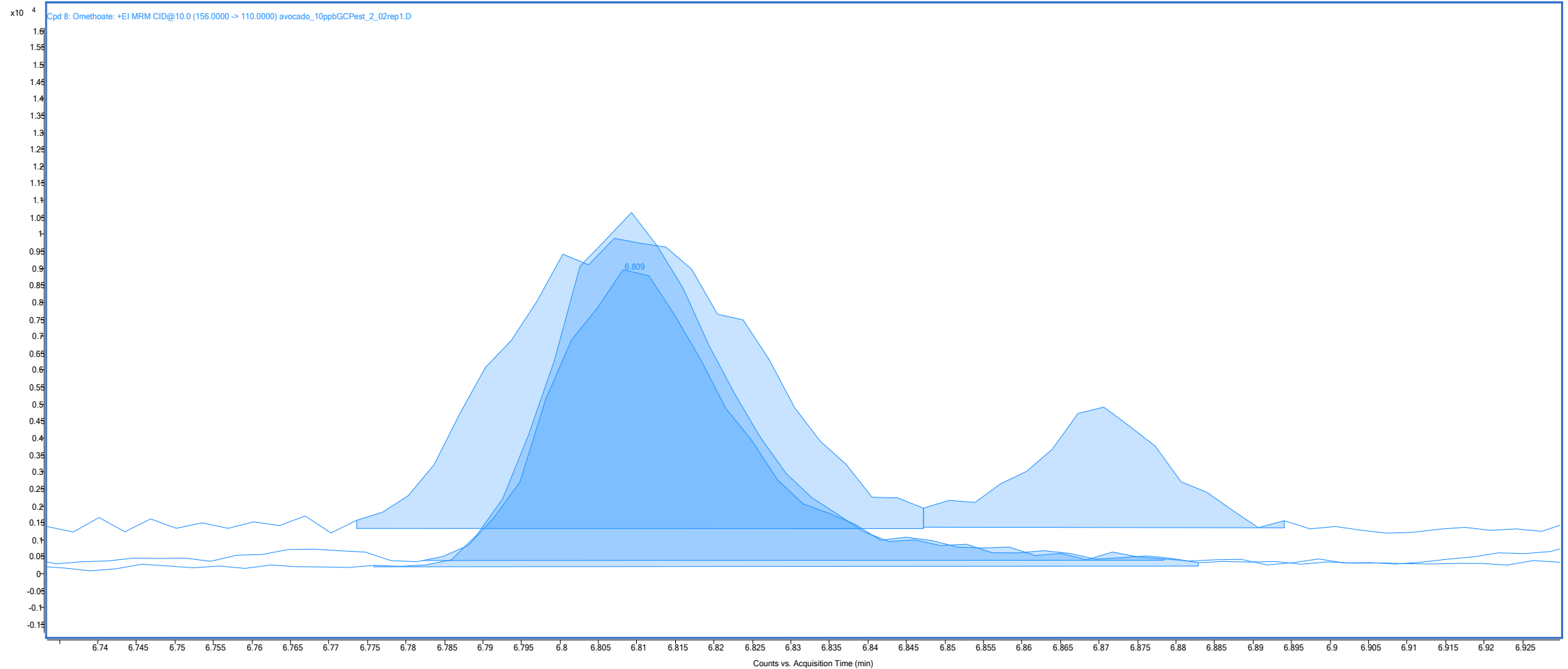
- Contamination
- Phase degradation

Injector (efficiency)

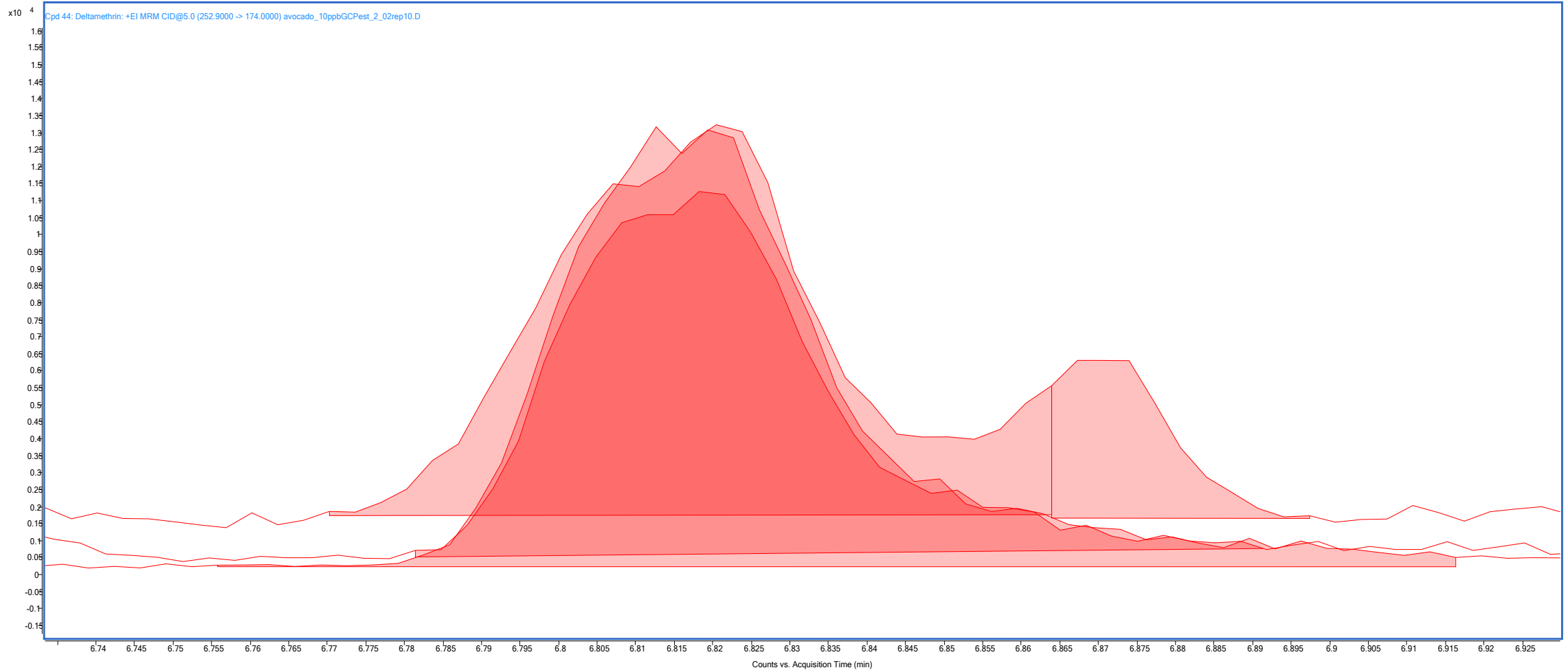
- Settings, liner, installation, etc.



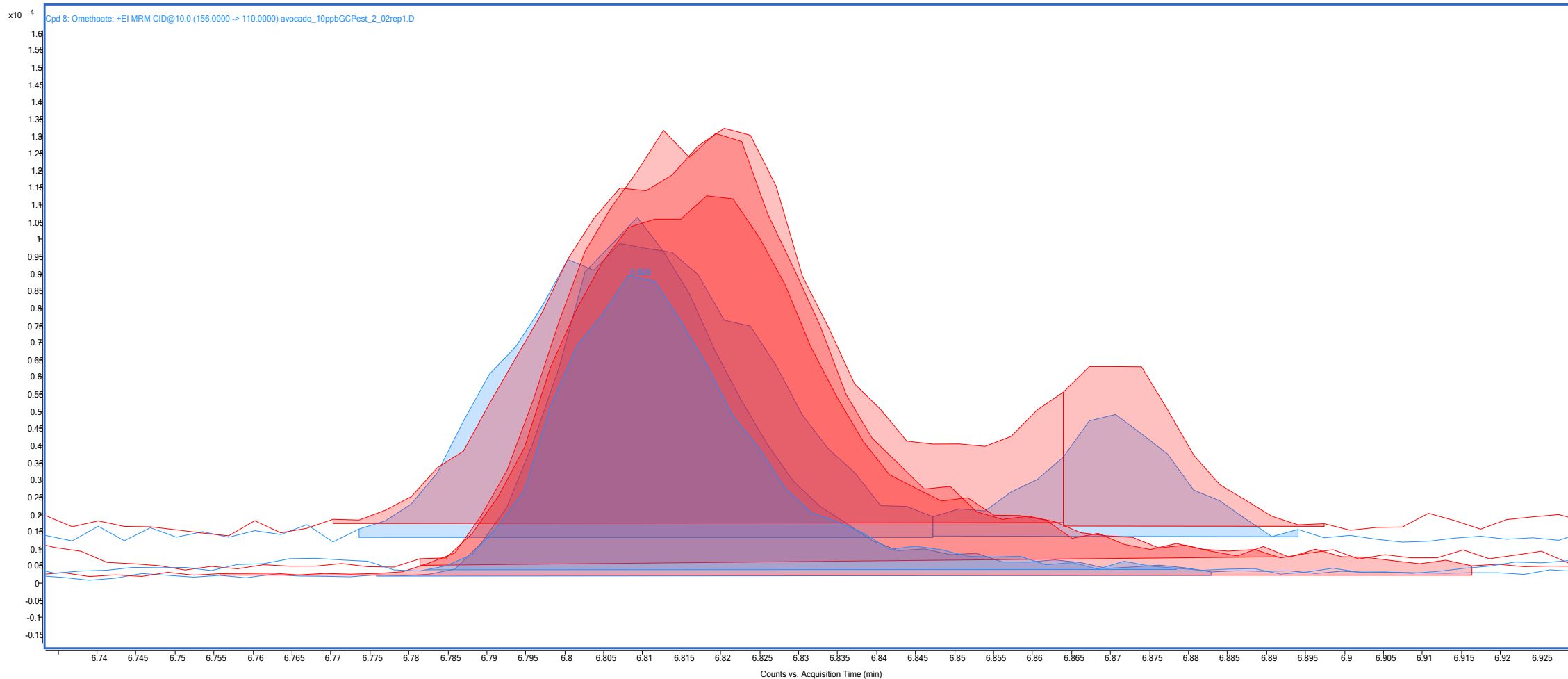
Peak Broadening: Omethoate in Avocado in Run 1



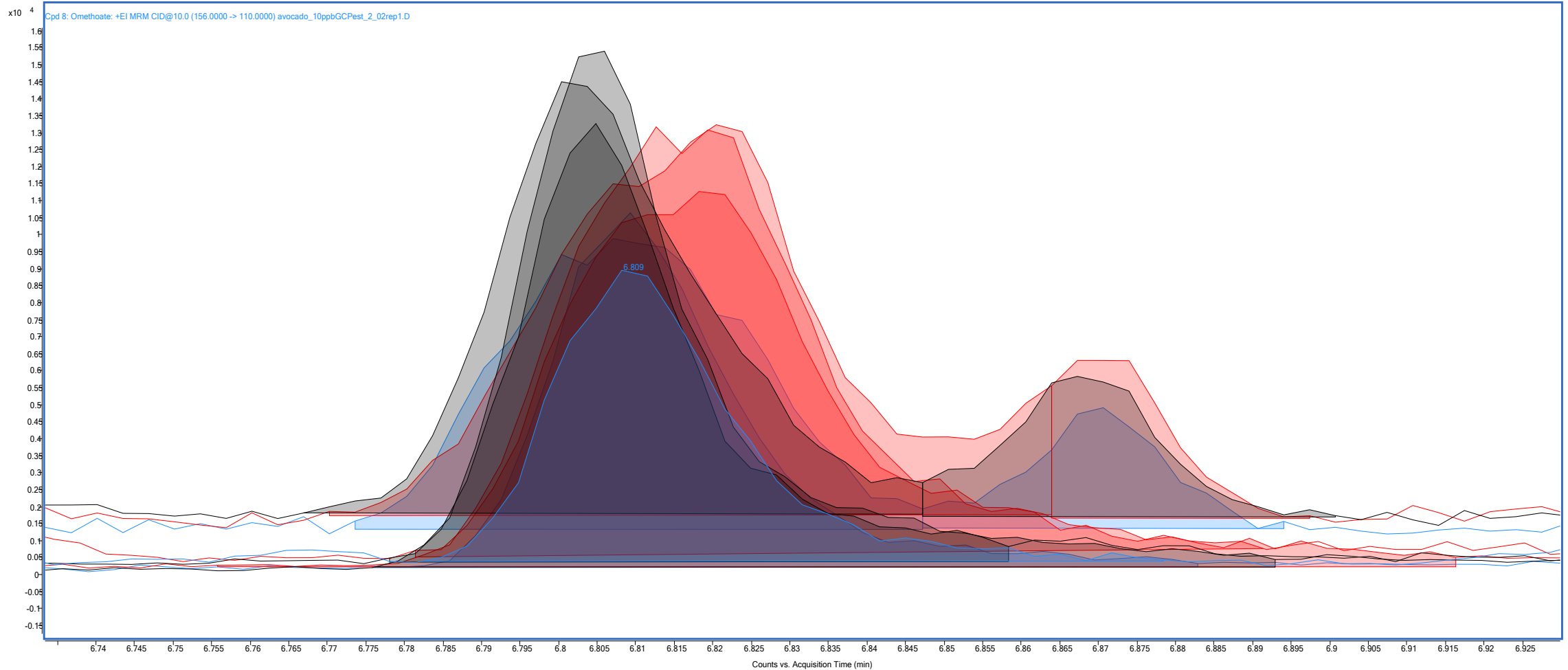
Peak Broadening: Omethoate in Avocado in Run 65



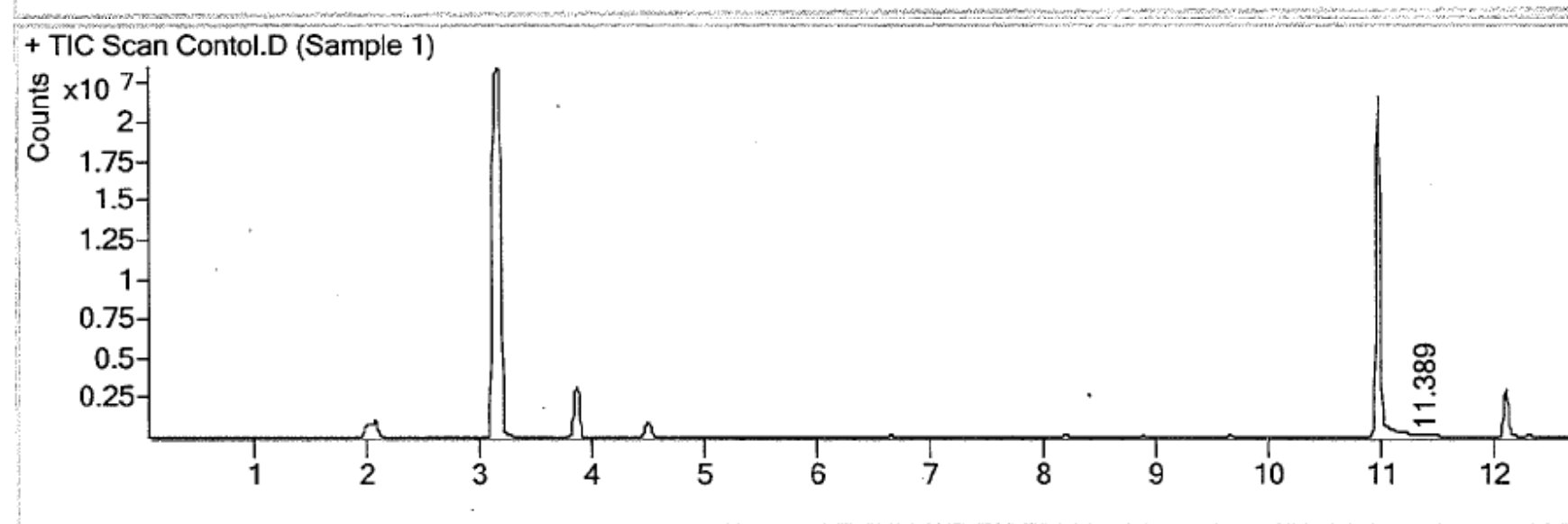
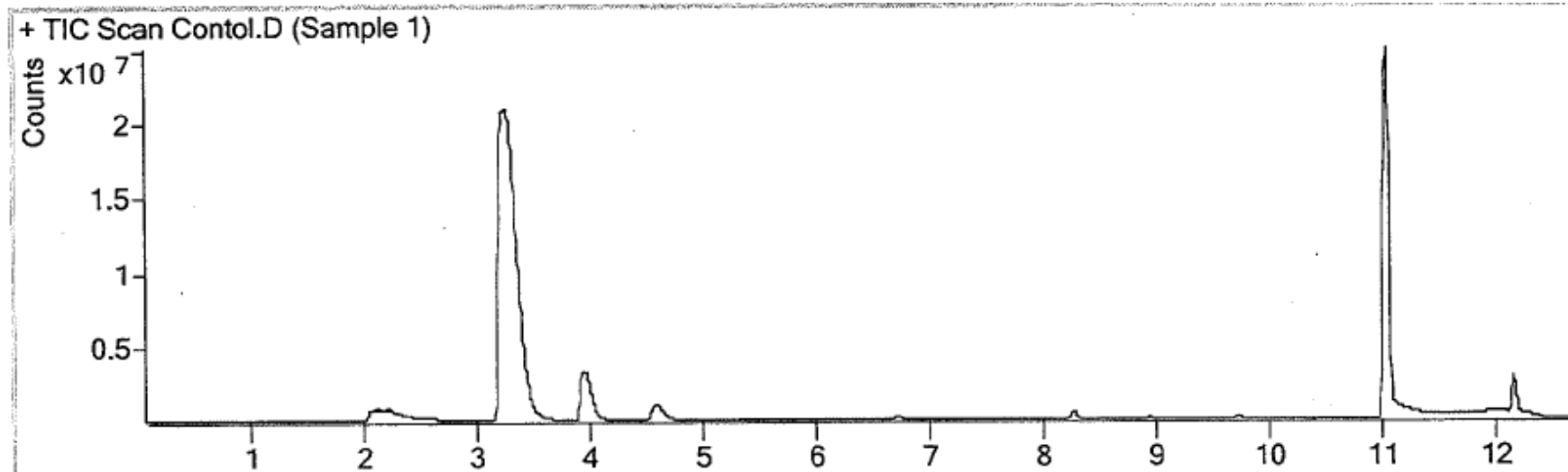
Peak Broadening: Omethoate in Avocado in Run 1 versus Run 65



Peak Broadening: Recover Peak Shape with New Liner



Peak Broadening: The Case of the Wrong Liner



Baseline Disturbances

Sudden changes, wandering, or drifting

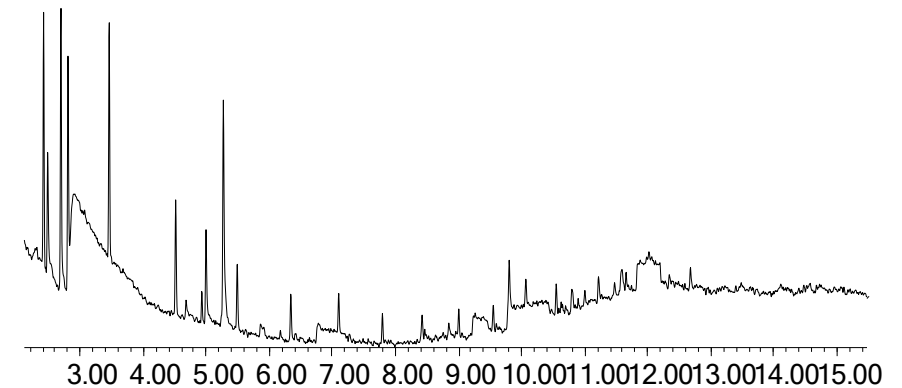
Drifting/wandering/weird disturbances

Column or detector

- Not fully conditioned or stabilized (electronics)
- Contamination

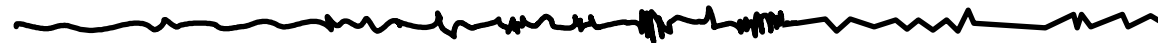
Flow

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

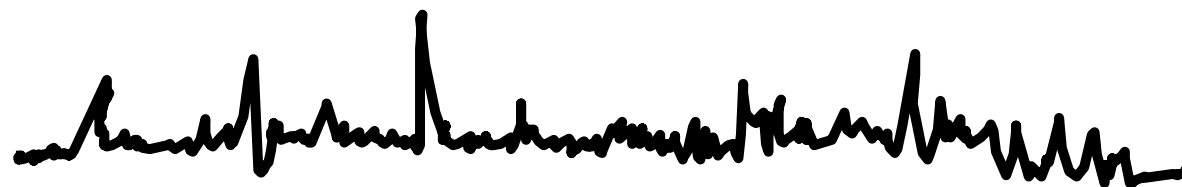


Noisy Baseline

Mild



Severe



Flow

- Contaminated gas
- Incorrect detector settings

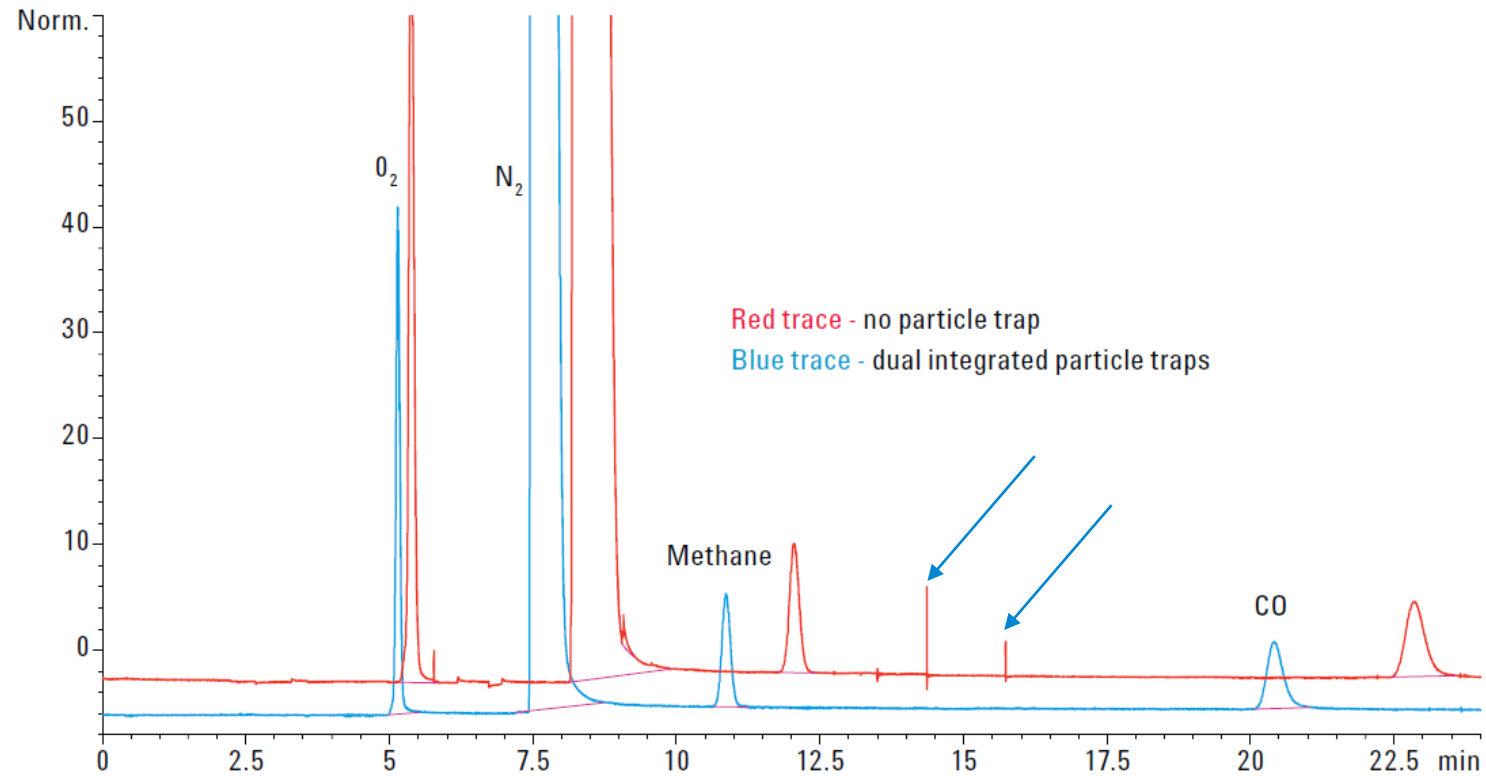
Detector

- Air leak - ECD, TCD
- Electronics malfunction

Column

- Bleed if at high temperature
- In detector flame (poor installation)

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
- Integration parameters

Activity (adsorption) in injector or column

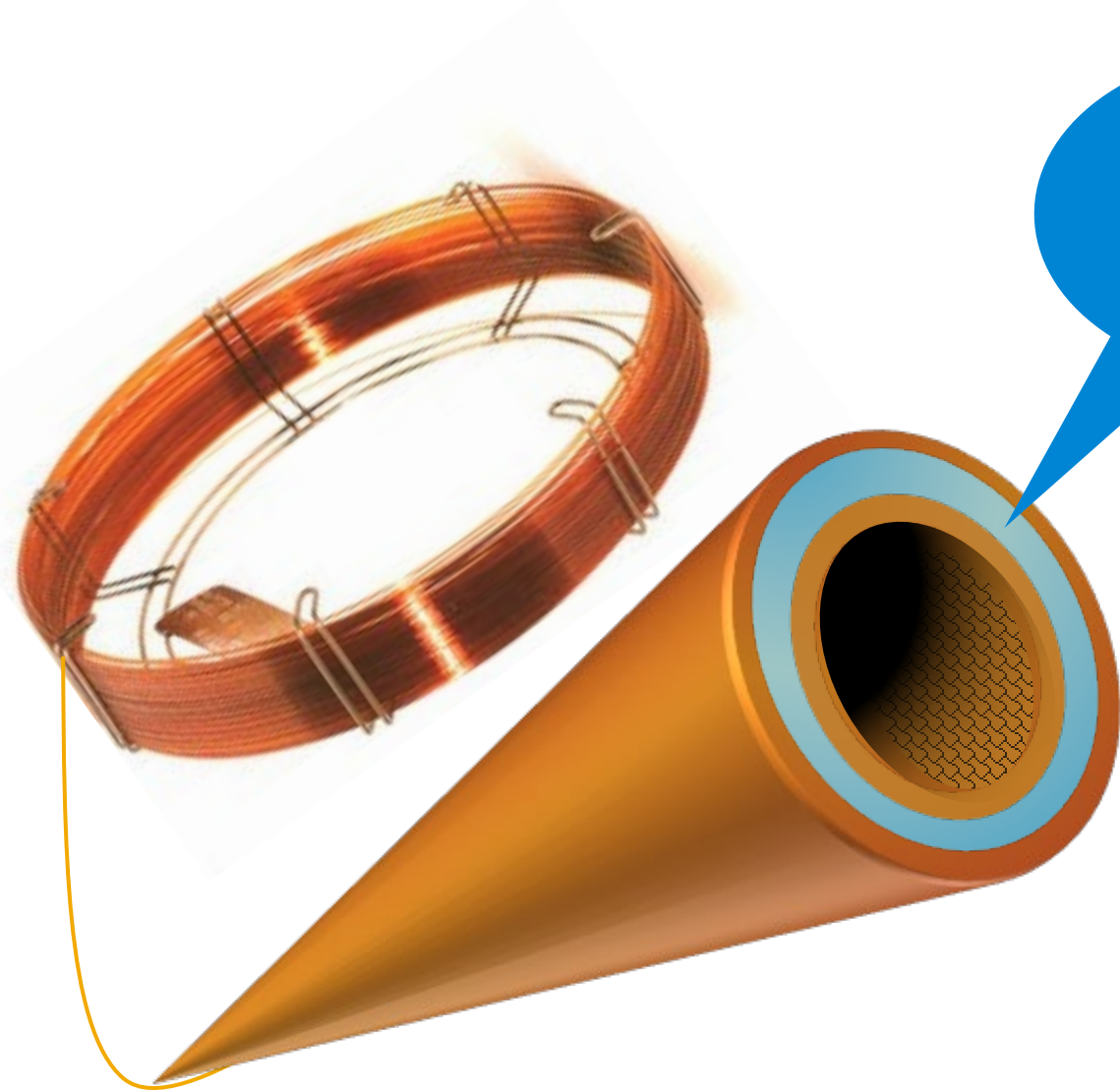
Injector

- Technique, settings, conditions
- Syringe worn

Other

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

What is Not Caused by a Column?



Not responsible

- Peaks
 - Any reproducible sharp chromatographed peak
- Siloxanes (even though it looks like bleed spectrally)
- Degradation product peaks: Endrin Aldehyde, endrin ketone, DDE, DDD...
- Carry-over of sample compounds
- Splitting of peaks

Troubleshooting Tools

Bleed profile (non-injection): *baseline problems*

Inject a nonretained peak: *peak shape problems*

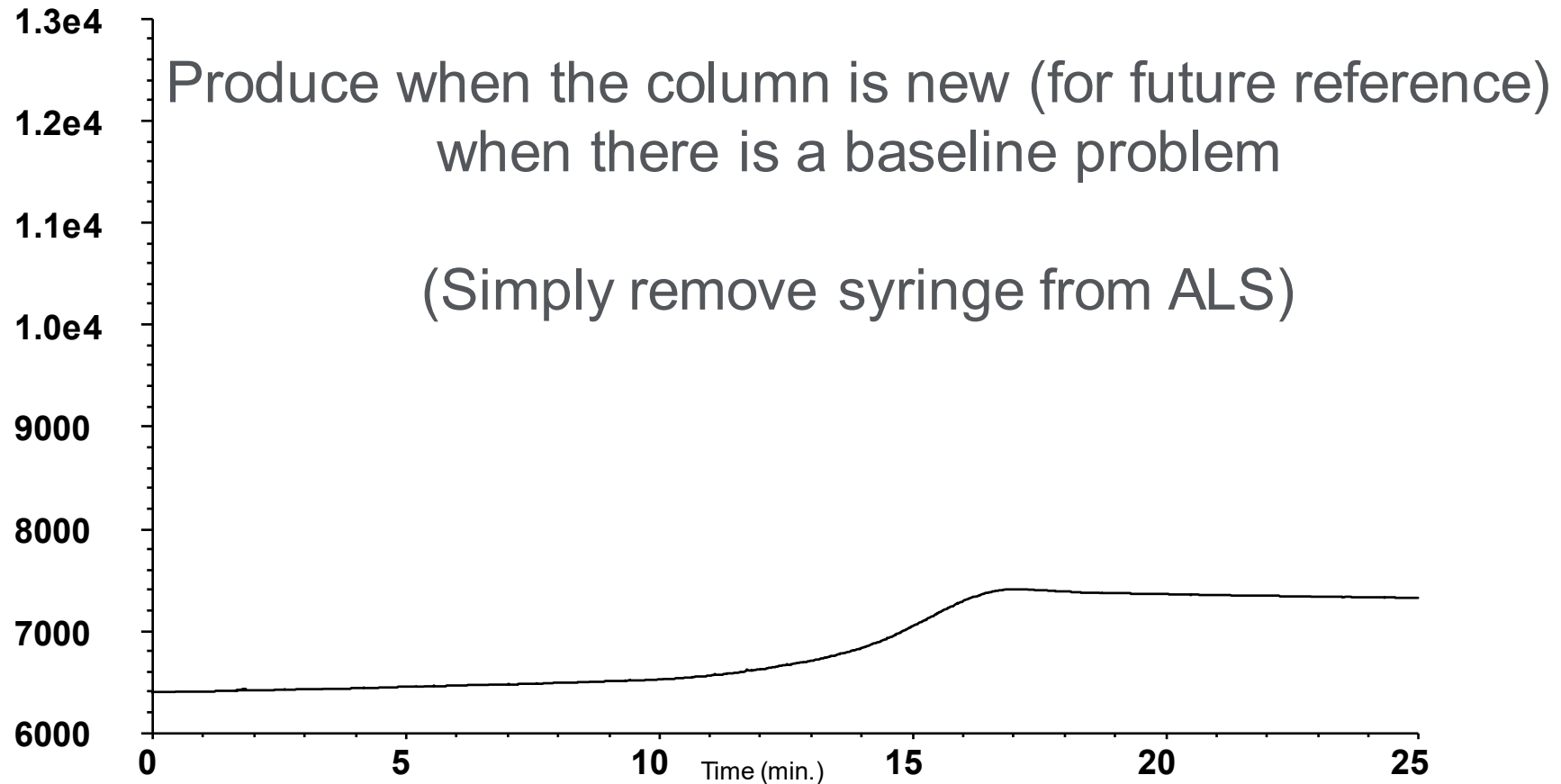
Test mix: *all problems*

Isolate the components: *all problems*

Condensation test: *baseline problems*

Jumper tube test: *baseline problems*

Generating a Bleed Profile

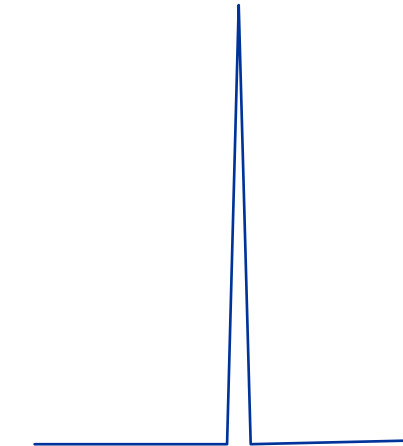


Agilent J&W DB-1, 30 m x 0.32 mm id, 0.25 μ m

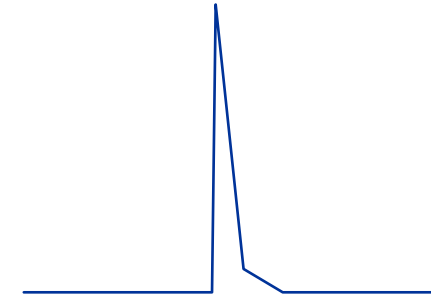
Temperature program // 40 °C, hold 1 min // 20 °C/min to 320 °C, hold 10 min.

Inject a Nonretained Compound to Check Flow Path

Used to check
flow path



Good Installation



Improper Installation or
Injector Leak

Potential explanations:

- 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!

A test mix is used to determine how “good” the column is, or if the problem is related to the chemical properties of the analytes.

It is simplest to use your own standard.



Compound	Purpose
Hydrocarbons	Efficiency Retention
Alcohols	Activity
FAME's, PAH's	Retention
Acids	Acidic Character Activity
Bases	Basic Character Activity

Test Conditions	
Inlet:	Split (250°C)
Detector:	FID(320°C)
Flow:	37.3 cm/sec (1.8 mL/min)
Carrier gas:	Hydrogen
Holdup compound:	Methane (0.671-min)
Temperature program:	Isothermal (110°C)

ULTRA Scientific is Now Part of Agilent Technologies

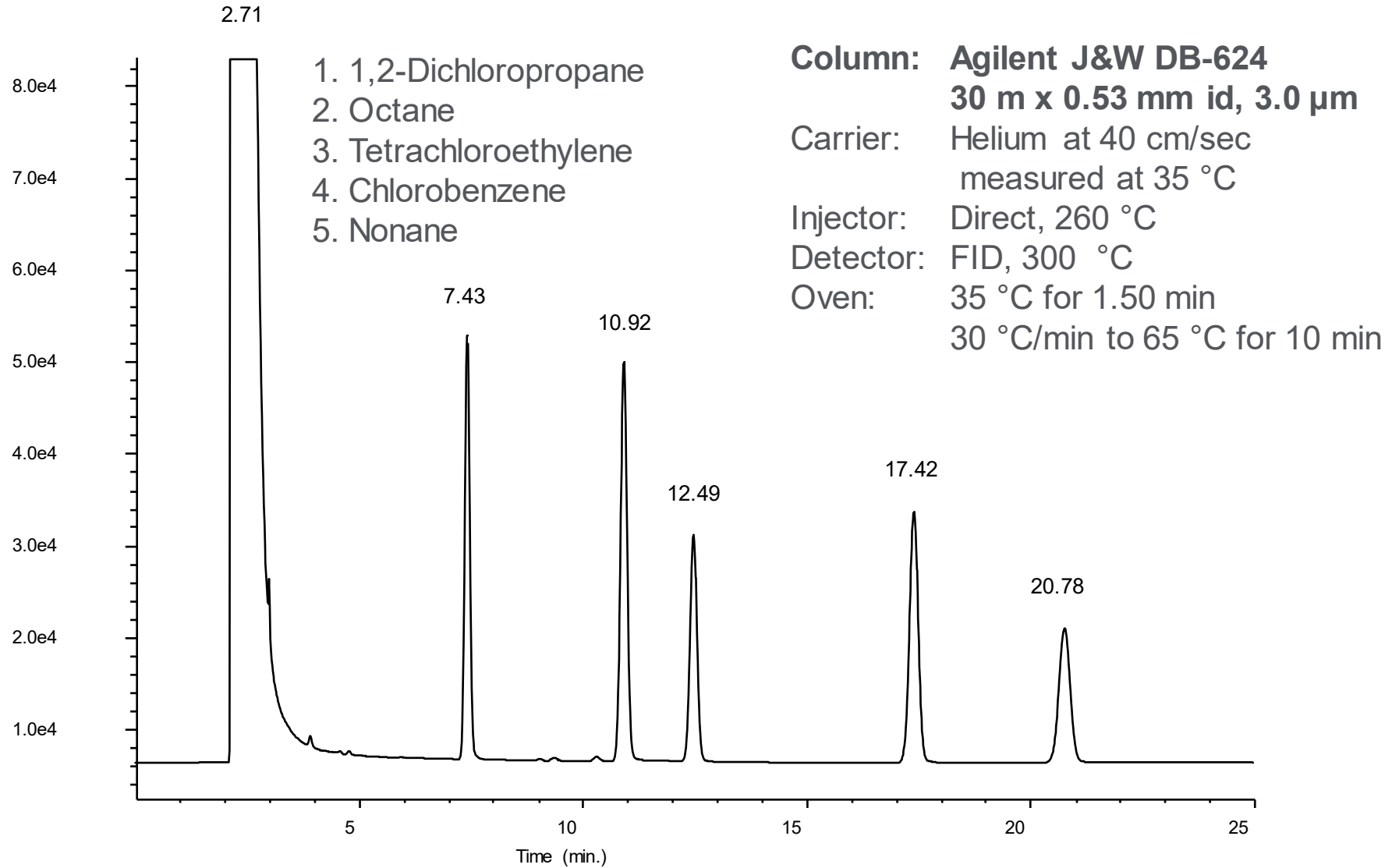
Agilent ULTRA Chemical Standards have:

- Best in class online search, compare, and ordering capabilities
- Rapid shipping: 99.9% of orders dispatched within 24 to 48 hours (continental US only as of now)
- Custom standard solutions including our *new* online custom quoting tool, enabling customers to upload recipe formulations to and to modify the recipe before submitting it
 - Tool will allow customers to see the quote pricing instantly and allow them to check quote pricing based on quantity range
 - Check it out at <https://www.agilent.com/en/product/chemical-standards>
- Rigorously tested and manufactured under ISO 9001, ISO 17025, and ISO 17034 certifications
- Sample preparation materials, columns, supplies, instrumentation, and reference materials from a single source



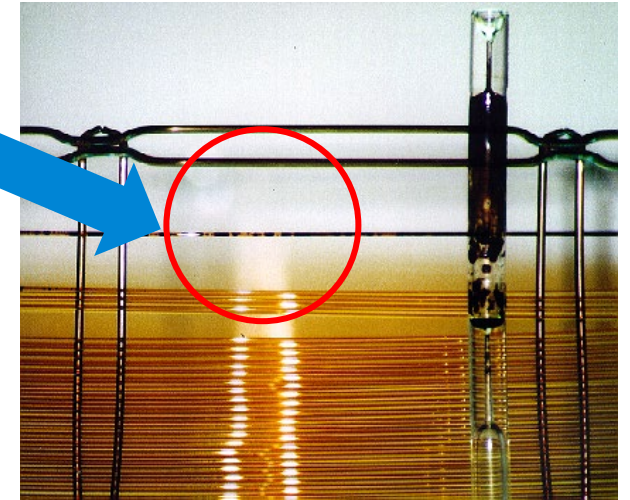
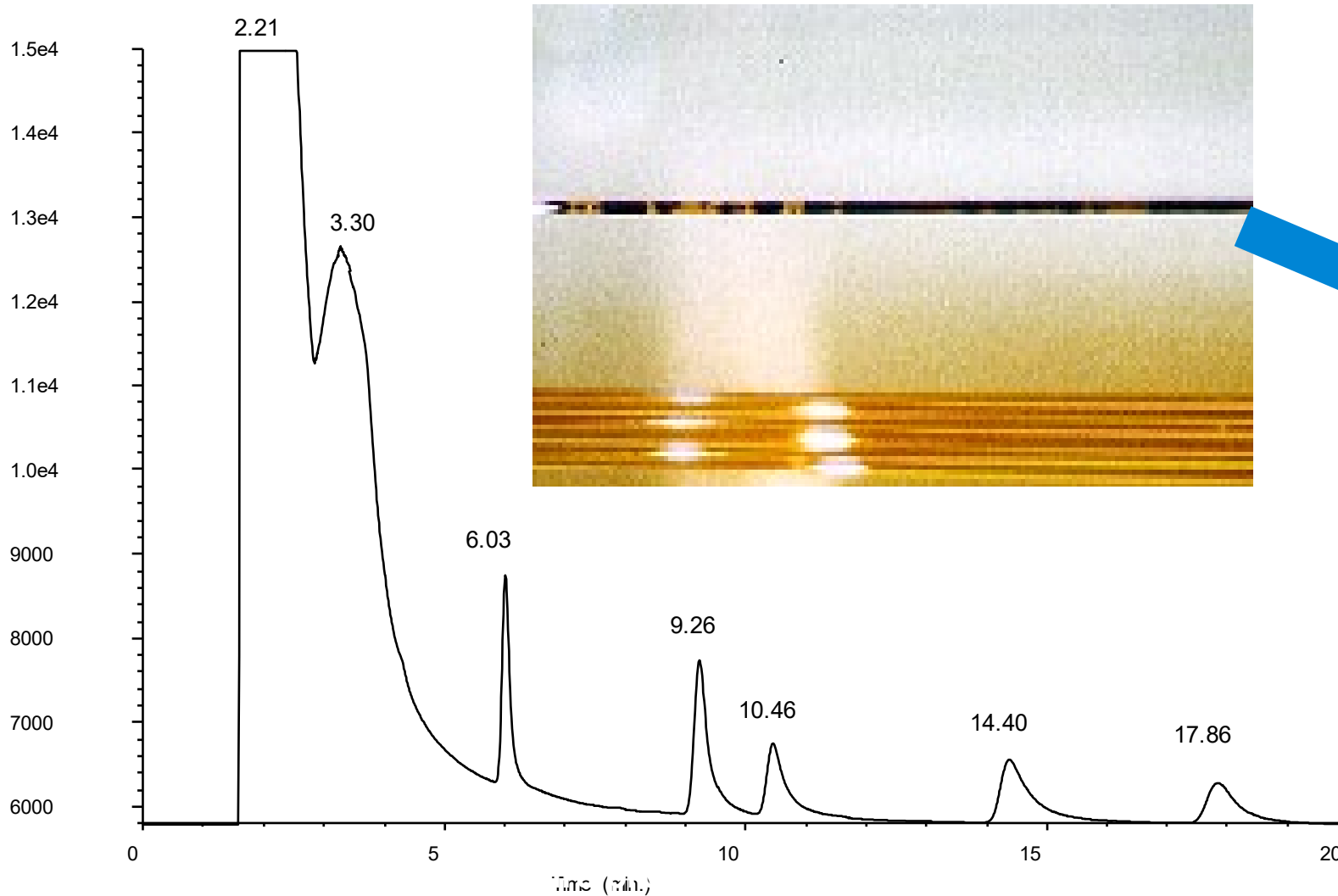
Agilent J&W DB-624 Column

QC Test Mix

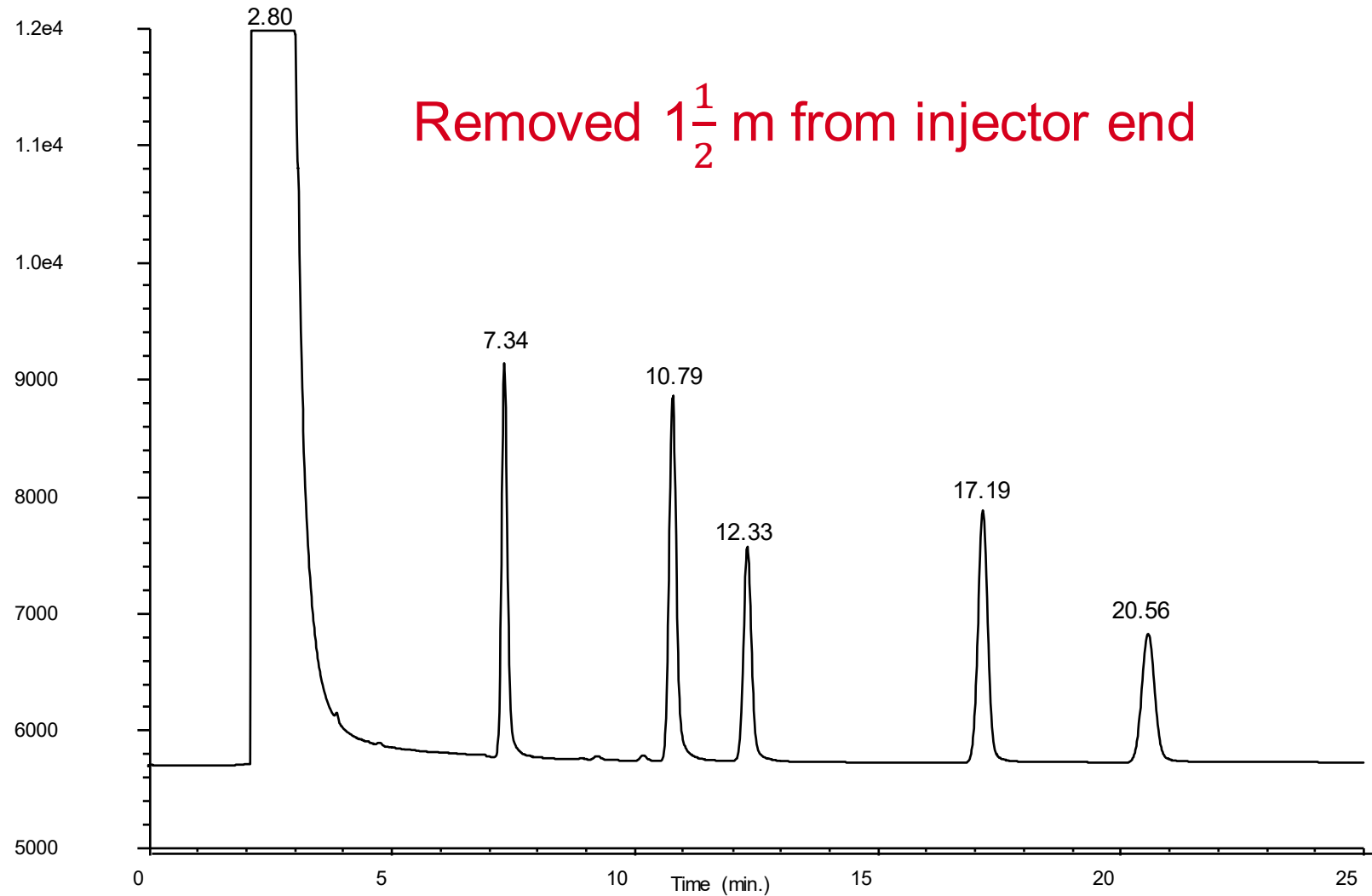


Example of Column Contamination

Agilent J&W DB-624 QC Test Mix
After 75 injections of oily sample



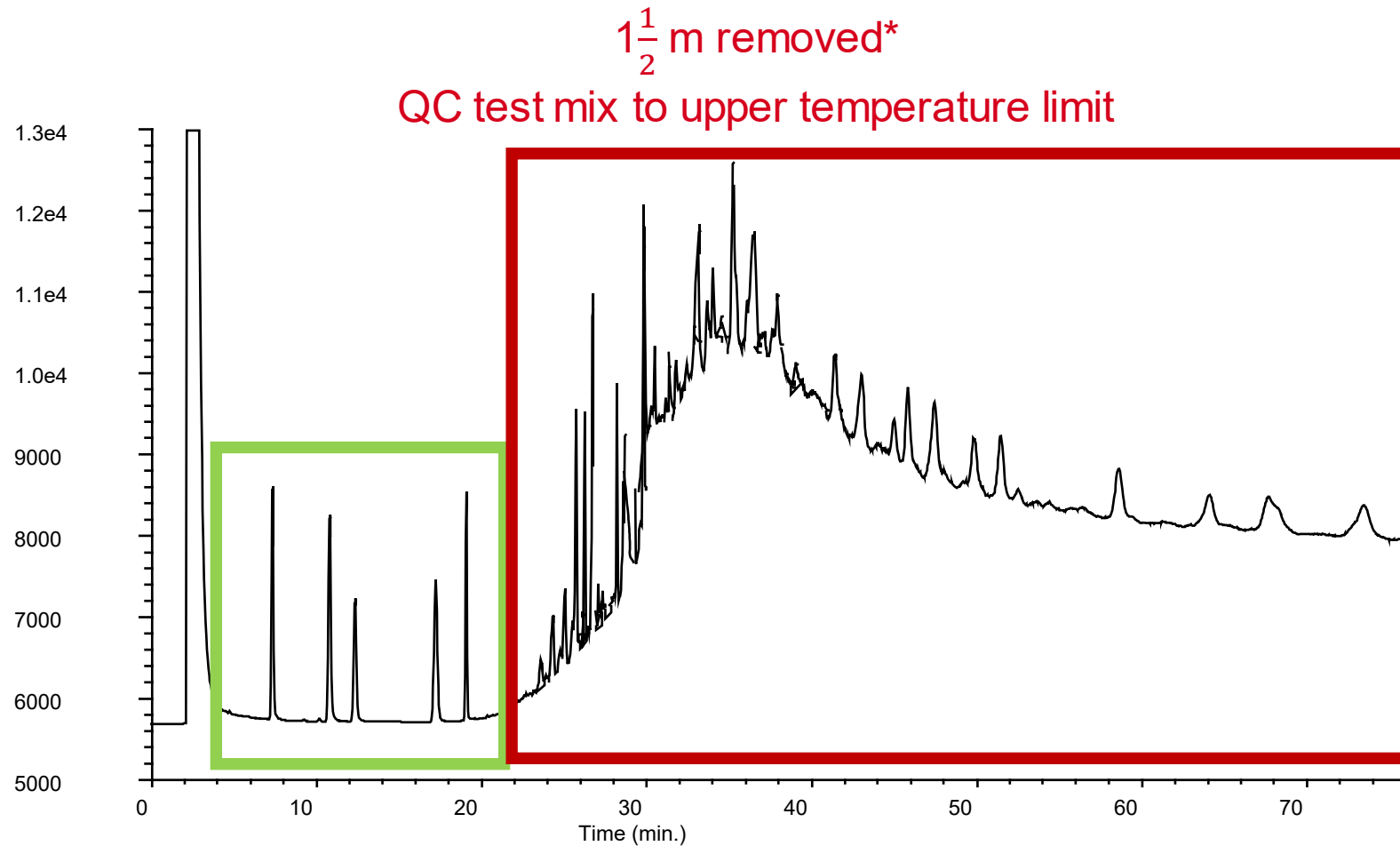
Example of Column Contamination



*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:

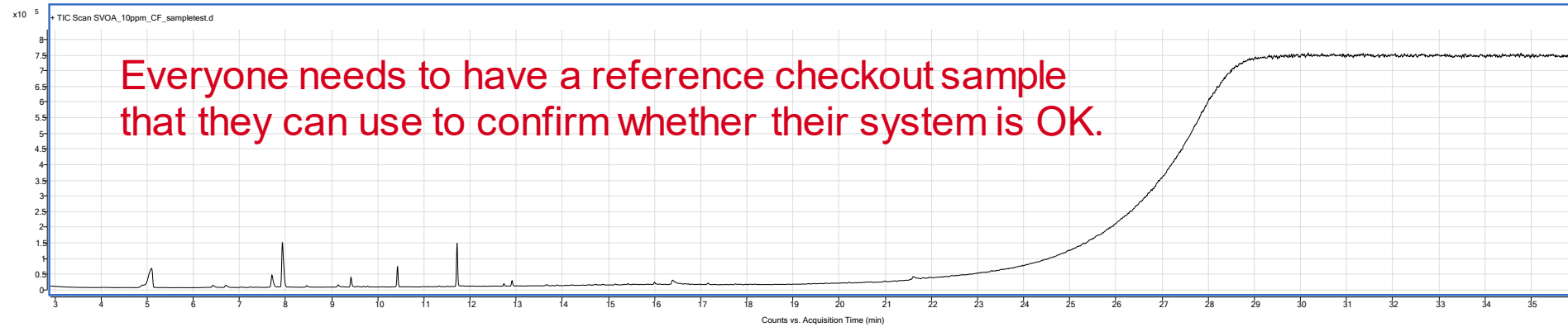
- Problem returns? It's the column
- Problem gone? Previous leak, solid debris, or installation problem

Troubleshooting Example

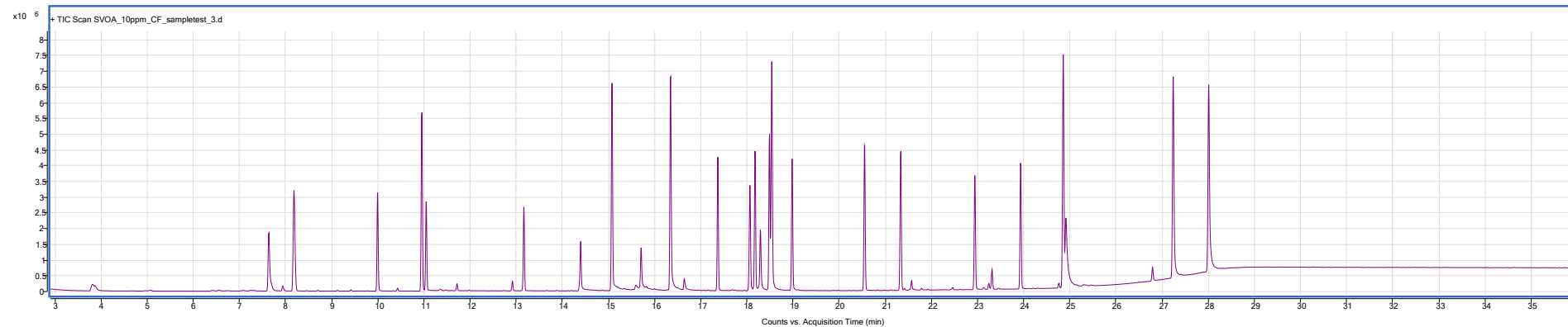


Problem: No Peaks with Semivolatiles Checkout Mixture

What my TIC looked like:



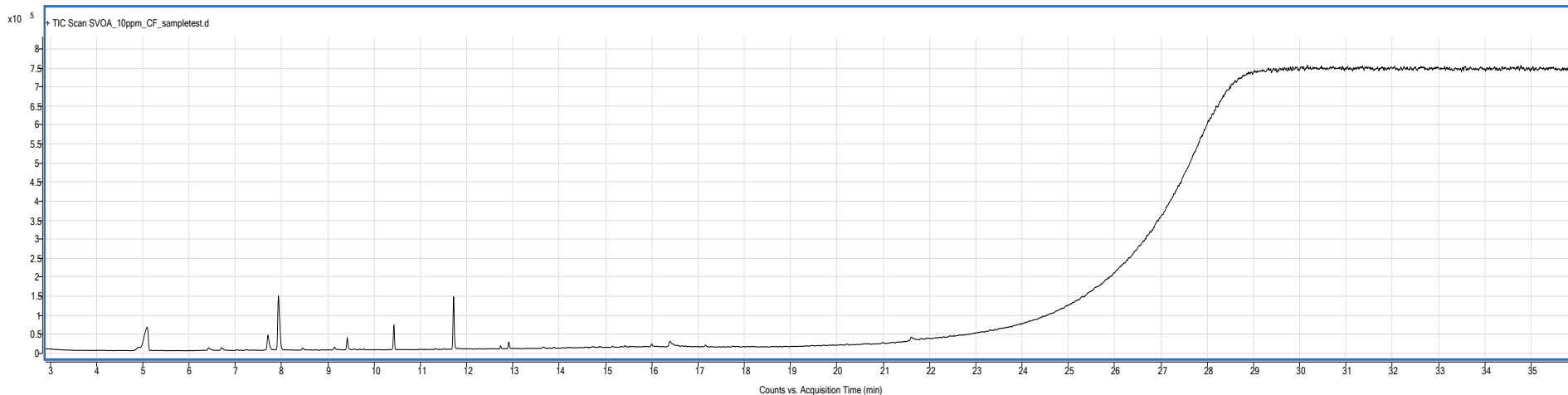
What my TIC should look like:



Problem: No Peaks with Semivolatiles Checkout Mixture

What could cause this?

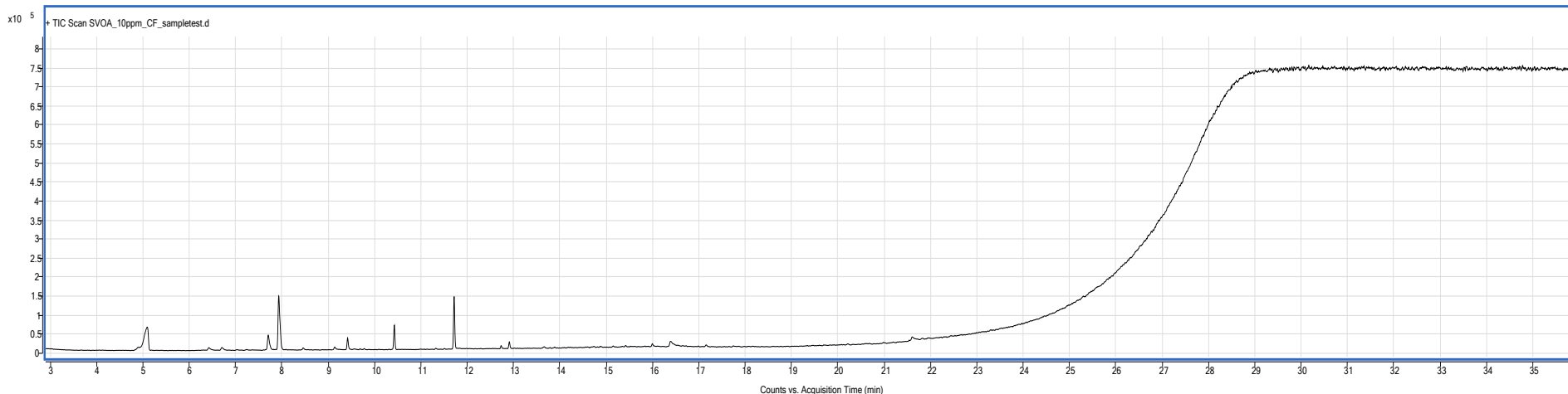
- The wrong vial was injected
- The sample has degraded
- The inlet is leaking
- The column is damaged



Problem: No Peaks with Semivolatiles Checkout Mixture

What could cause this?

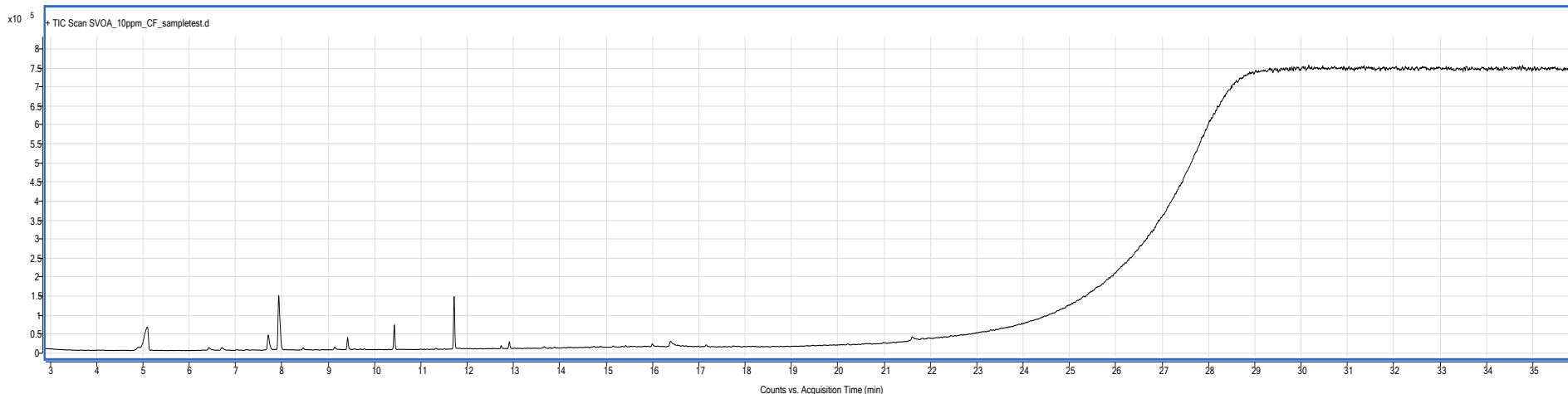
- The wrong vial was injected: **Sequence and vial checked, no problem found**
- The sample has degraded
- The inlet is leaking
- The column is damaged



Problem: No Peaks with Semivolatiles Checkout Mixture

What could cause this?

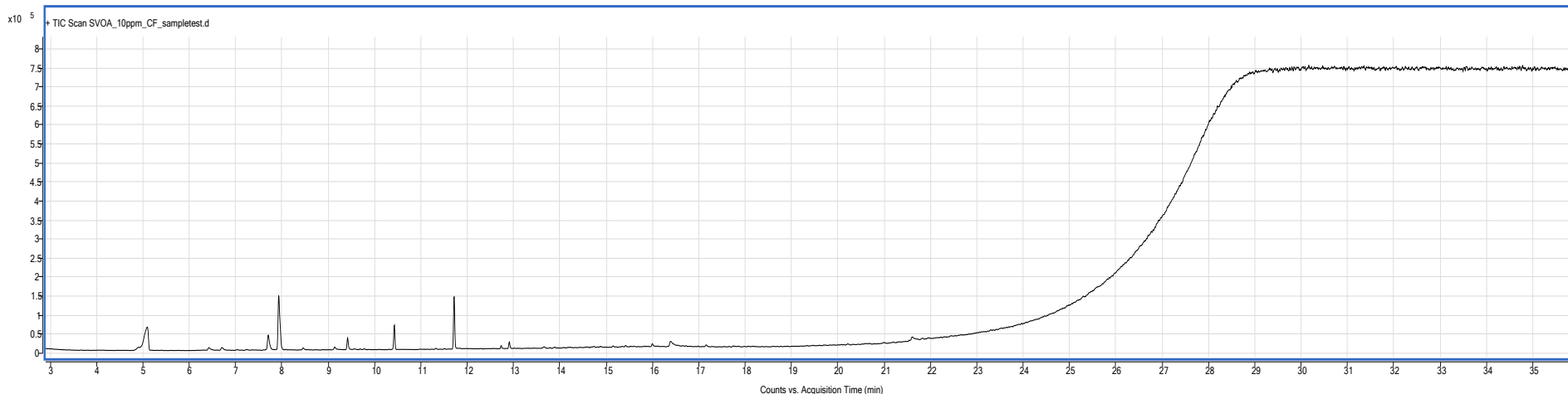
- The wrong vial was injected: **Sequence and vial checked, no problem found**
- The sample has degraded: **A new vial of standard was used, no difference observed**
- The inlet is leaking
- The column is damaged



Problem: No Peaks with Semivolatiles Checkout Mixture.

What could cause this?

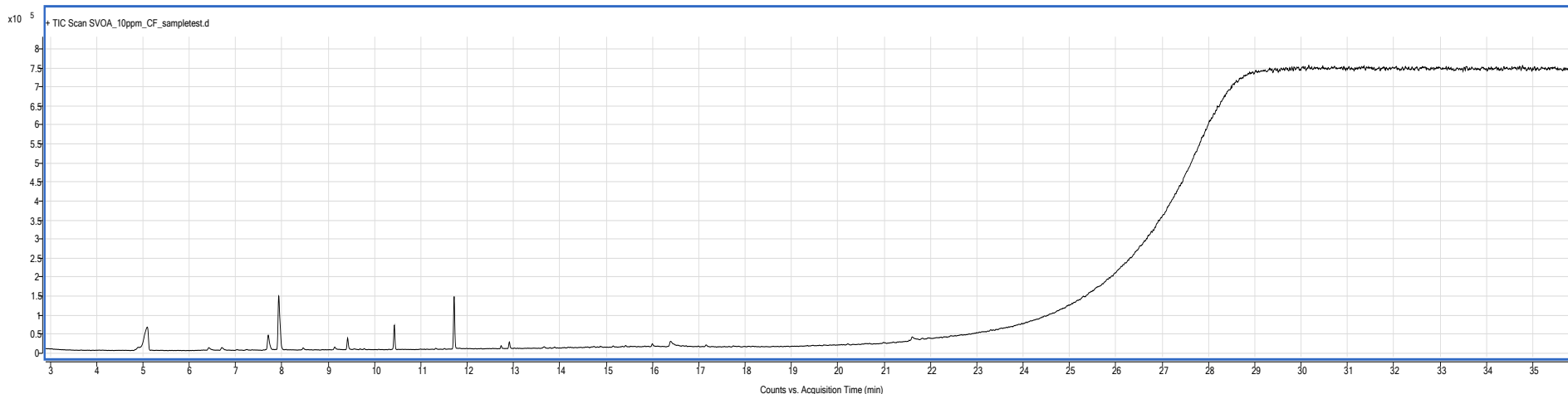
- The wrong vial was injected: **Sequence and vial checked, no problem found**
- The sample has degraded: **A new vial of standard was used, no difference observed**
- The inlet is leaking: **A tune was performed. O₂, N₂, and H₂O levels were normal**
- The column is damaged



Problem: No Peaks with Semivolatiles Checkout Mixture

What could cause this?

- The wrong vial was injected: **Sequence and vial checked, no problem found**
- The sample has degraded: **A new vial of standard was used, no difference observed**
- The inlet is leaking: **A tune was performed. O₂, N₂, and H₂O levels were normal**
- The column is damaged: **“Well, I guess I need to replace my column”**



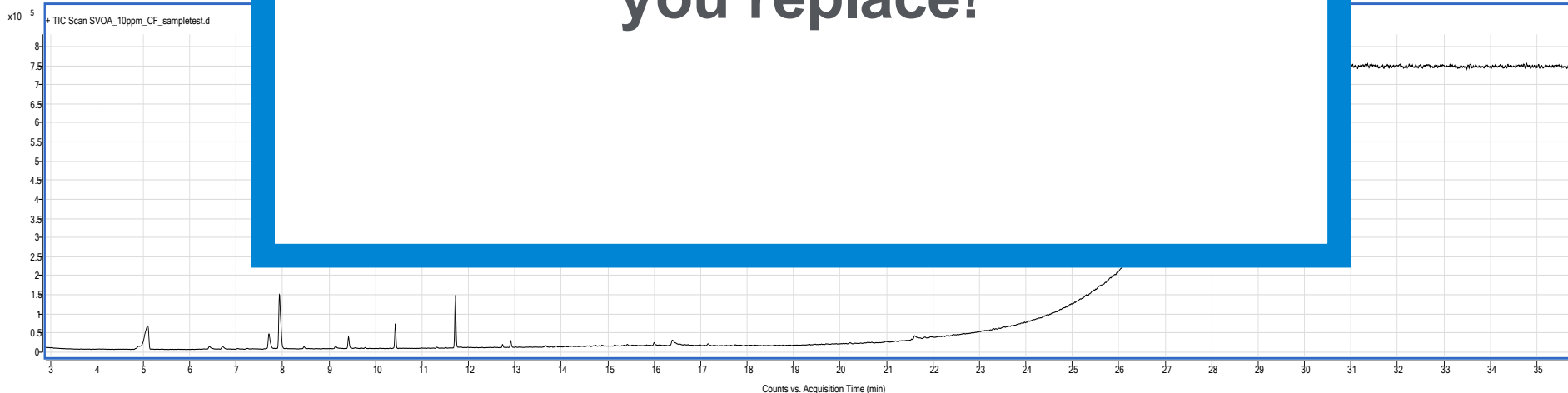
Problem: No Peaks with Semivolatiles Checkout Mixture

What could cause this?

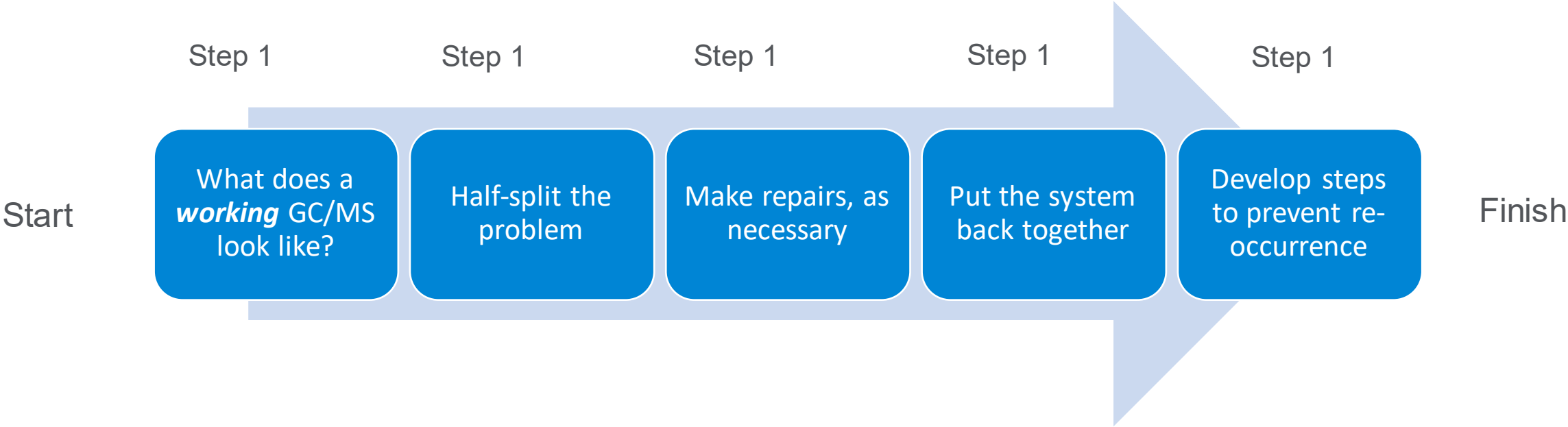
- The wrong vial was injected: **Sequence and vial checked, no problem found**
- The sample has degraded: **A new vial of standard was used, no difference observed**
- The inlet is leaking
- The column is old

WAIT
**Test (a few more things) before
you replace!**

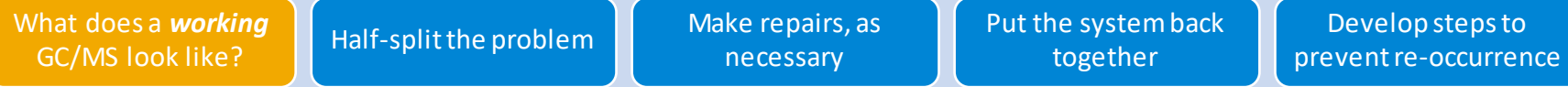
normal



Follow a Logical Troubleshooting Procedure!

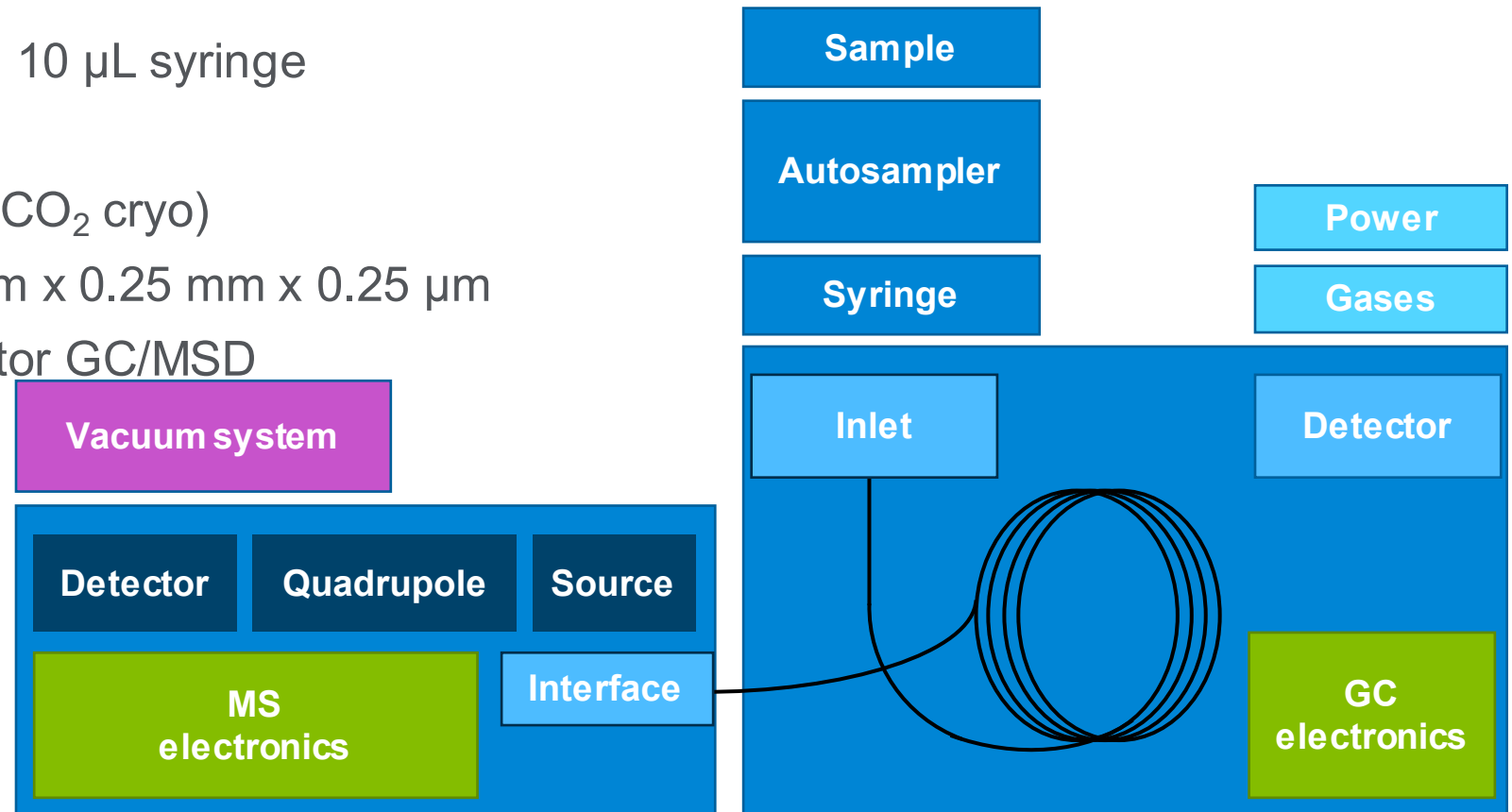


Troubleshooting Step 1: What is the “Working System”?

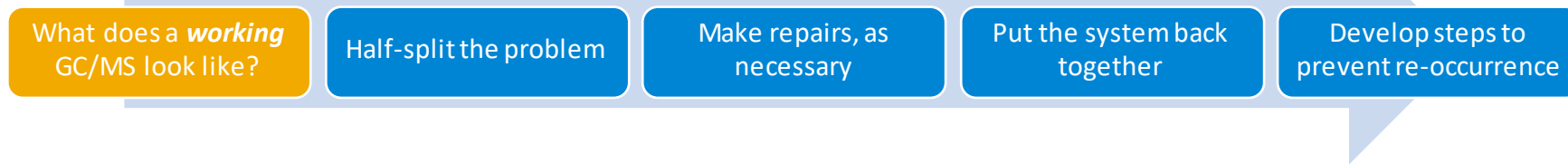


What are the components of the GC/MS system (follow the sample flow-path)

- Agilent 7693A autosampler + 10 μ L syringe
- Agilent 7890B GC
- Agilent MultiMode Inlet (with CO₂ cryo)
- Agilent J&W HP-5ms UI, 30 m x 0.25 mm x 0.25 μ m
- Agilent 5977A Series Extractor GC/MSD



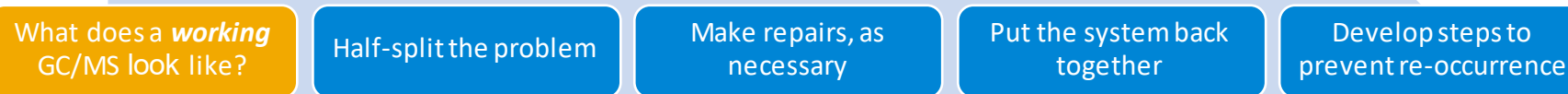
Troubleshooting Step 1: What is the “Working System”?



Compare your current data to known good data, when possible.
Use over-lay to zero-in on differences

- How does your background compare to normal?
- Does the problem occur for every run, every analyte, every method?
Only affects certain samples/analytes/Instruments?
- 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”?



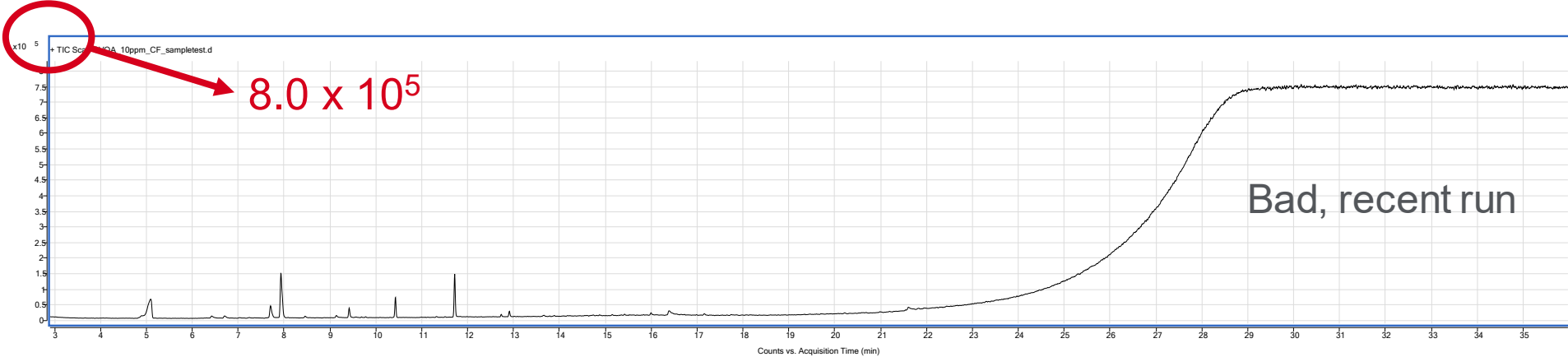
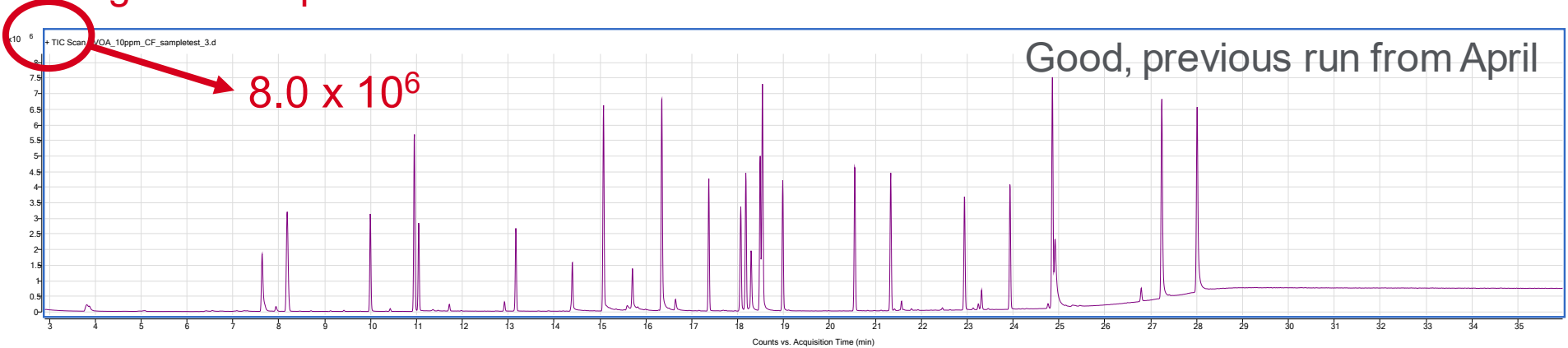
Compare your current data to known good data, when possible.

- How does your background compare to normal?
Background looked a LOT bigger than peaks in the good 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:

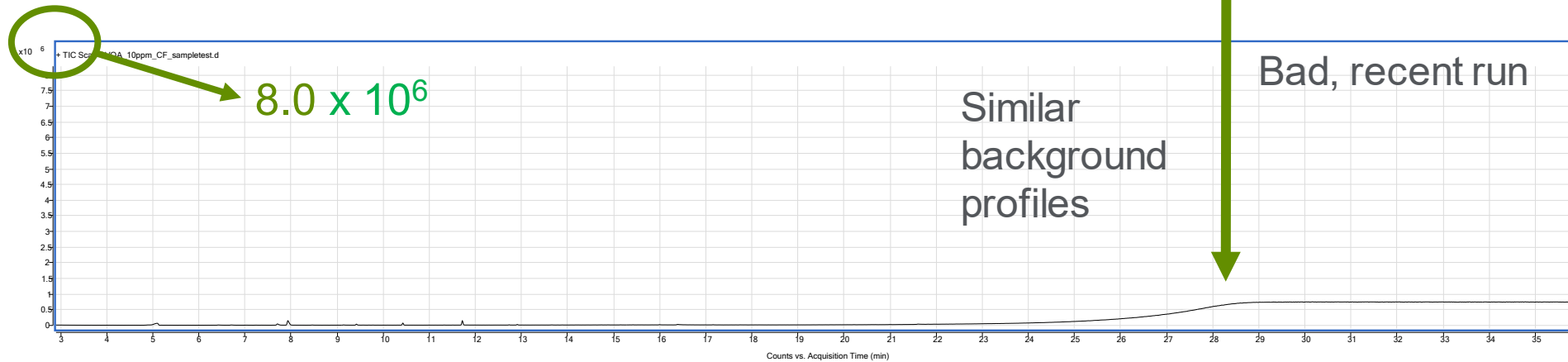
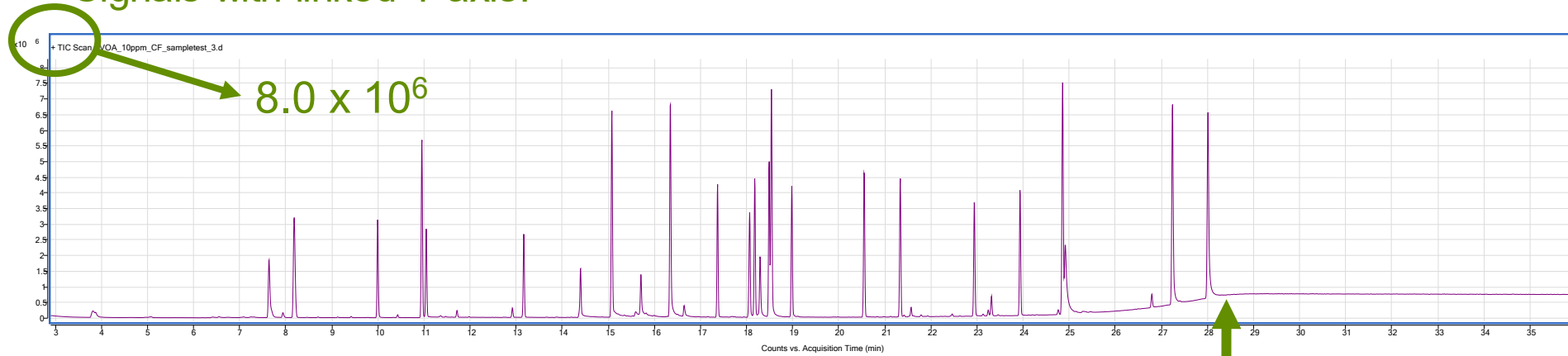


Troubleshooting Step 1: What is the “Working System”?

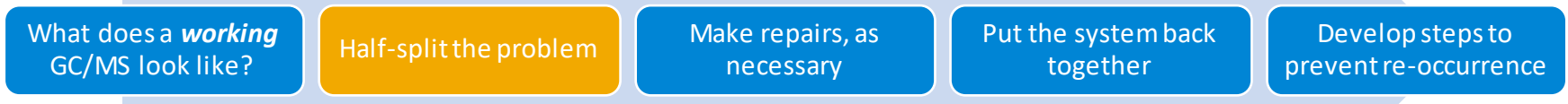
Compare your current data to known good data.

Now, the data is much clearer, and the background is not significantly higher.

Signals with linked Y axis:

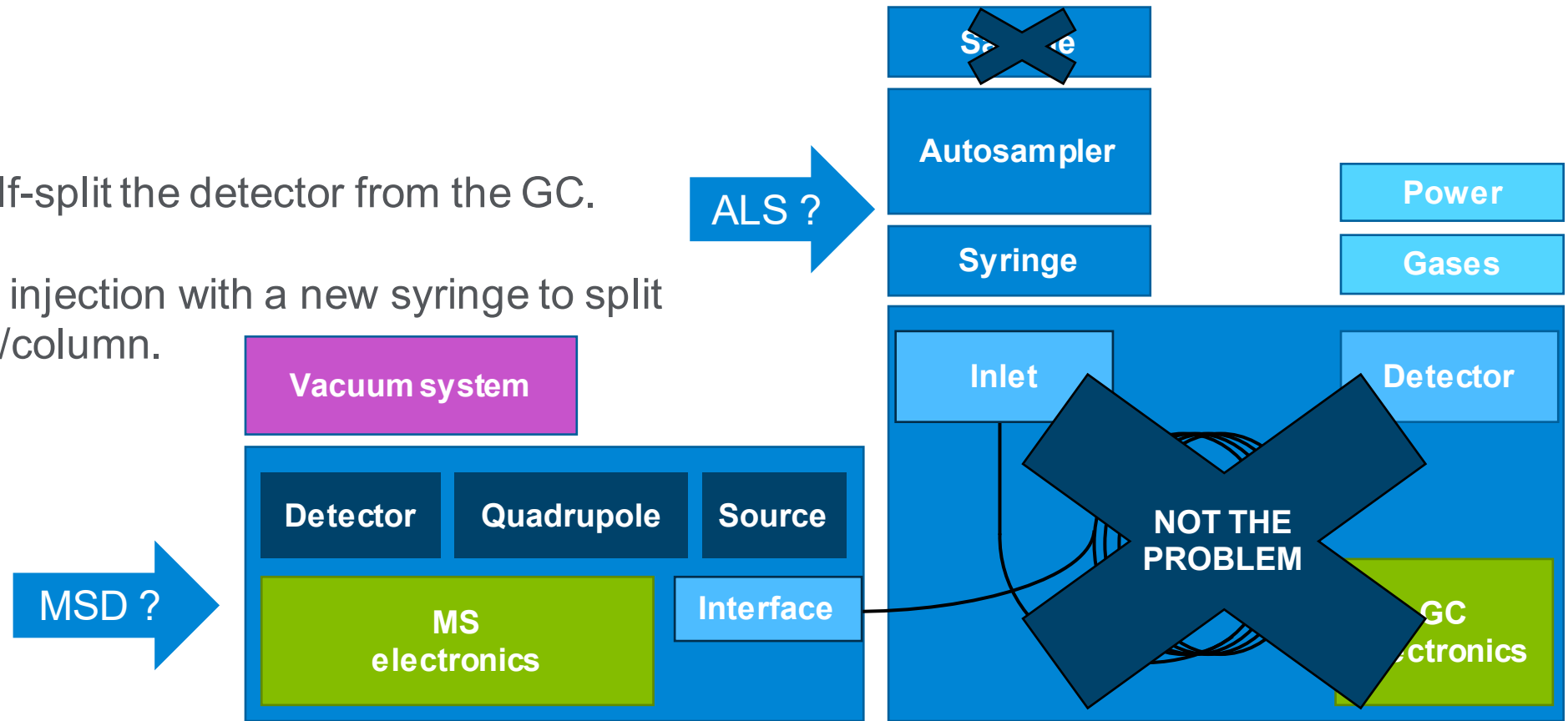


Troubleshooting Step 2: Break Apart (Half-Split) the Problem

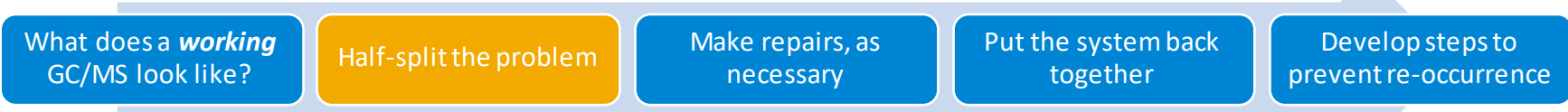


Think of a set of tests that will break the system into smaller pieces.

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.

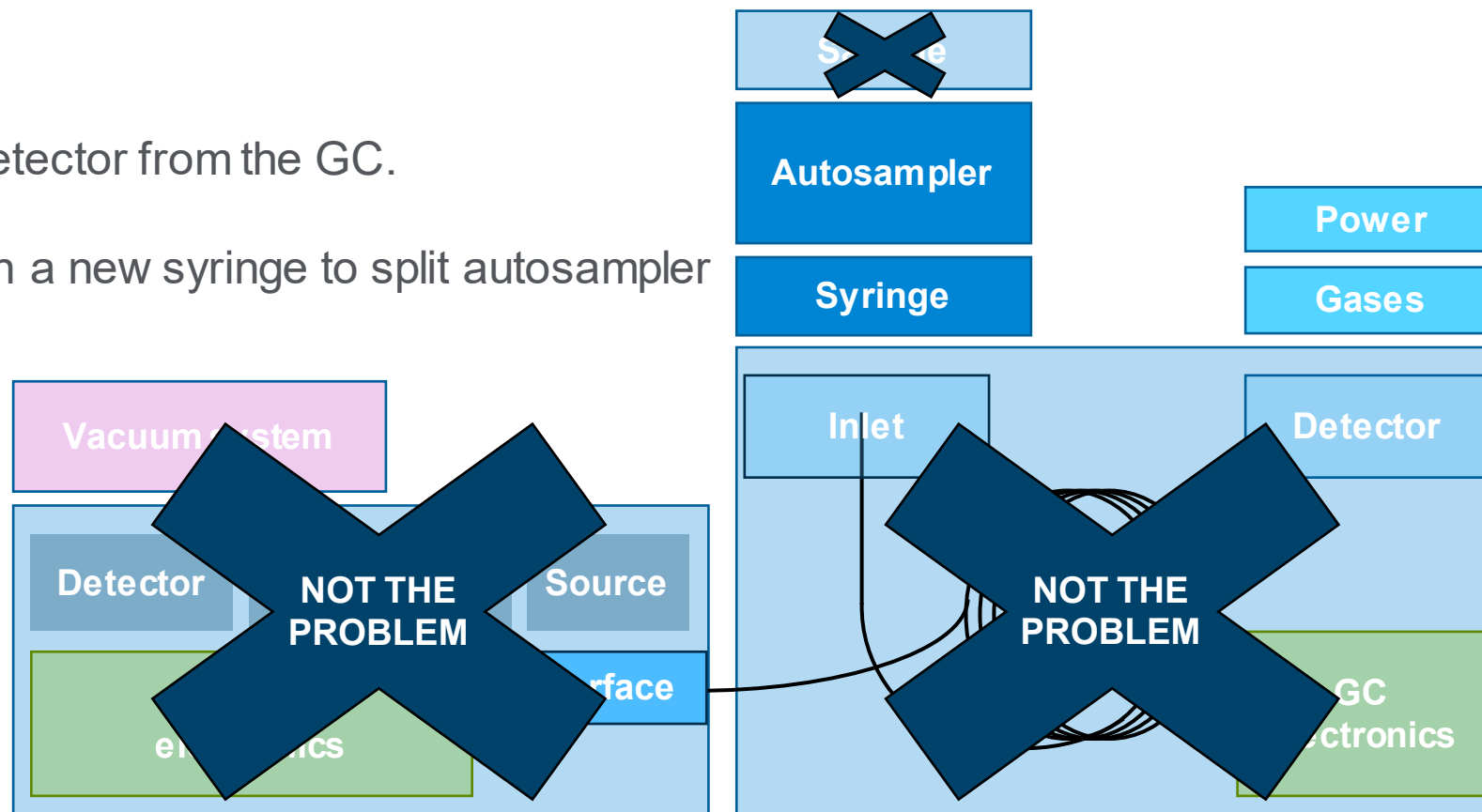


Troubleshooting Step 2: Break Apart (Half-Split) the Problem

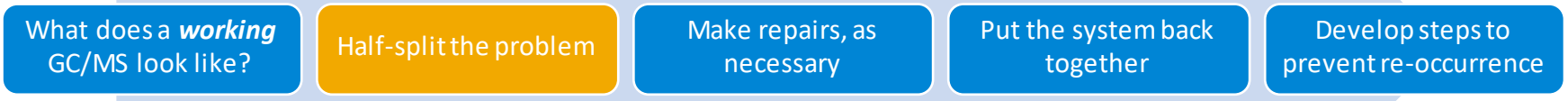


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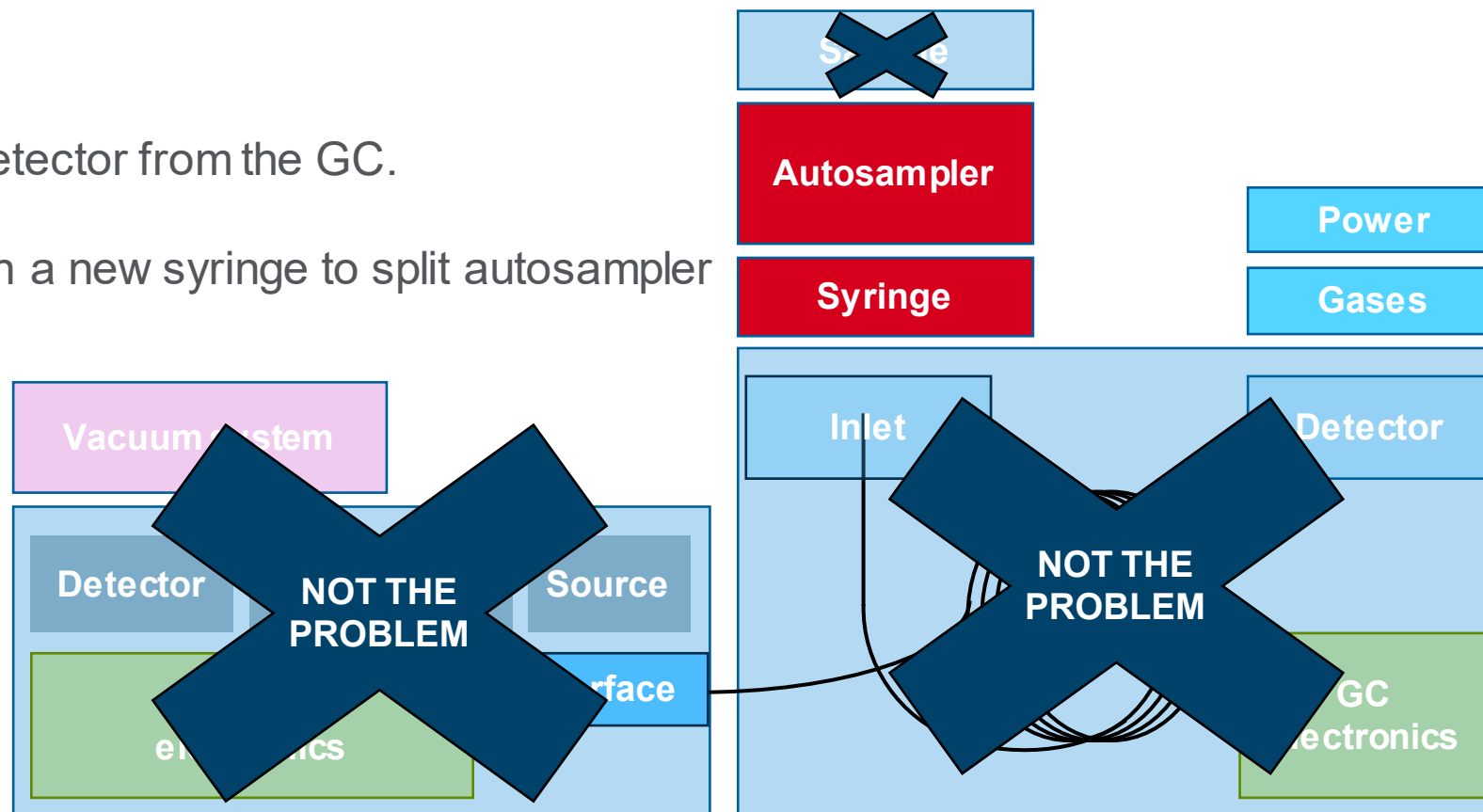


Troubleshooting Step 2: Break Apart (Half-Split) the Problem

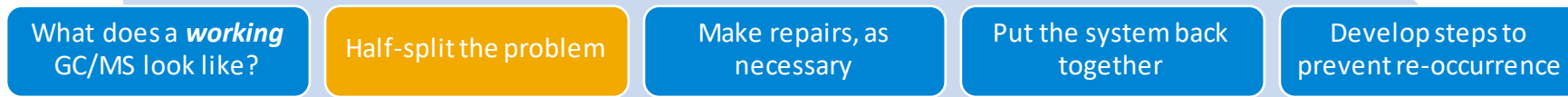


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1. Try a new sample.
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Troubleshooting Step 2: Narrow Focus of the Problem



Let's focus on the autosampler and syringe:

Autosampler

While sample was new, what is the solvent? Dichloromethane

Syringe

What kind of syringe? Agilent 10 μ L 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



PTFE plunger tip

Replace the syringe with a 10 μ L 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.

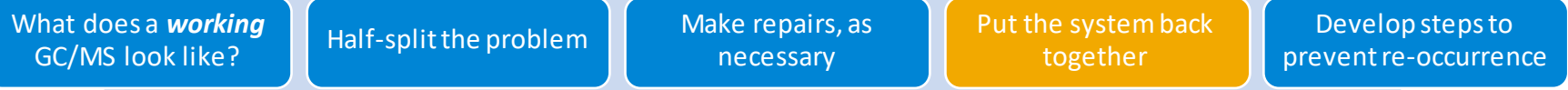
Proper syringe maintenance must still be performed. Clean and refill syringe wash vials frequently.

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

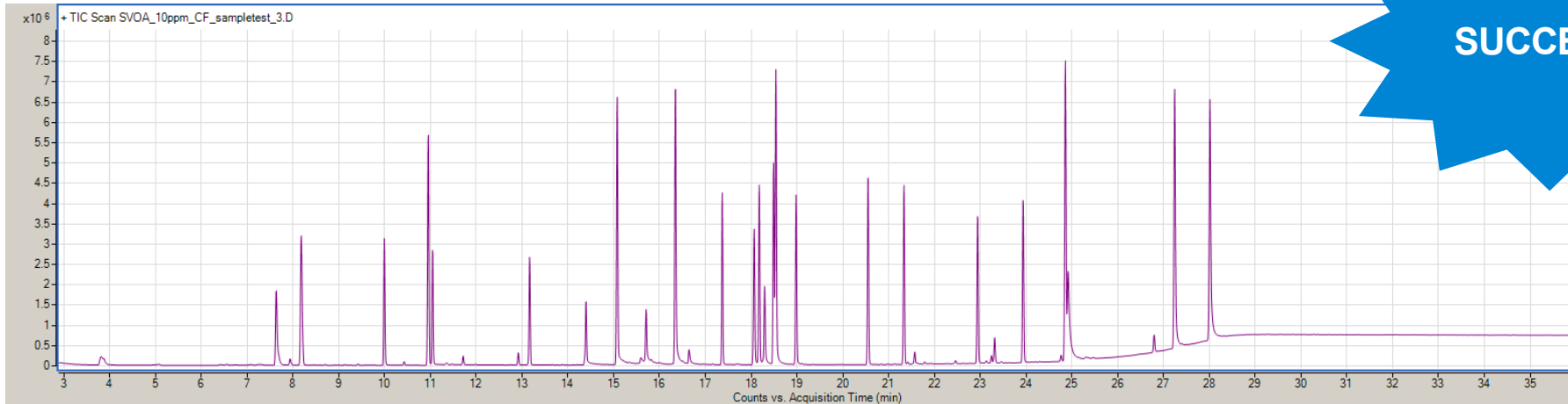
Syringe



Troubleshooting Step 4: Put the System Back Together



What happened with a new syringe?



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 – half-split the system into its component parts
(blank run, inject unretained 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 and GC/MS columns and supplies](#)

Option 2 for LC and LC/MS columns and supplies

Option 3 for sample preparation, filtration, and QuEChERS

Option 4 for spectroscopy supplies

Option 5 for chemical standards

Available in the USA and Canada 8–5, all time zones



gc-column-support@agilent.com

lc-column-support@agilent.com

spp-support@agilent.com

spectro-supplies-support@agilent.com

chem-standards-support@agilent.com

“Everything was Just Fine and then this Happened!”

“How do I go about Troubleshooting?”

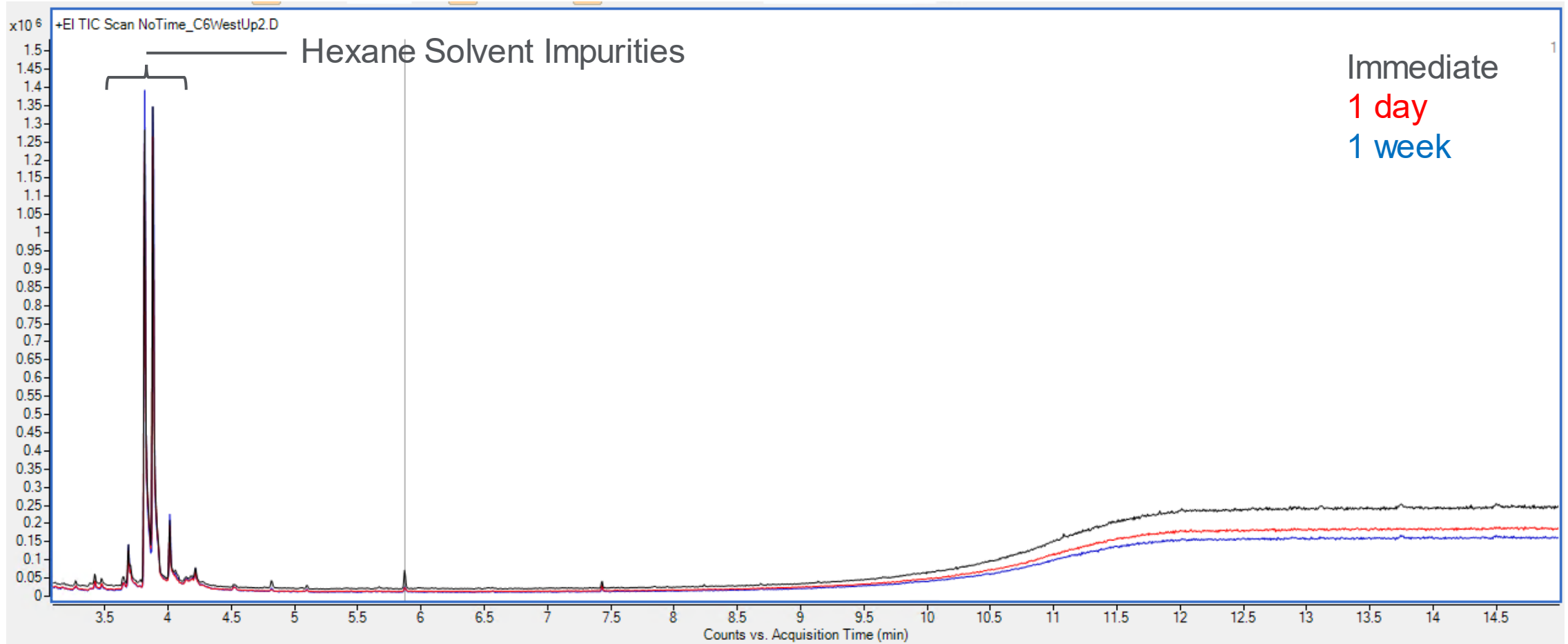


Track events- log book

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

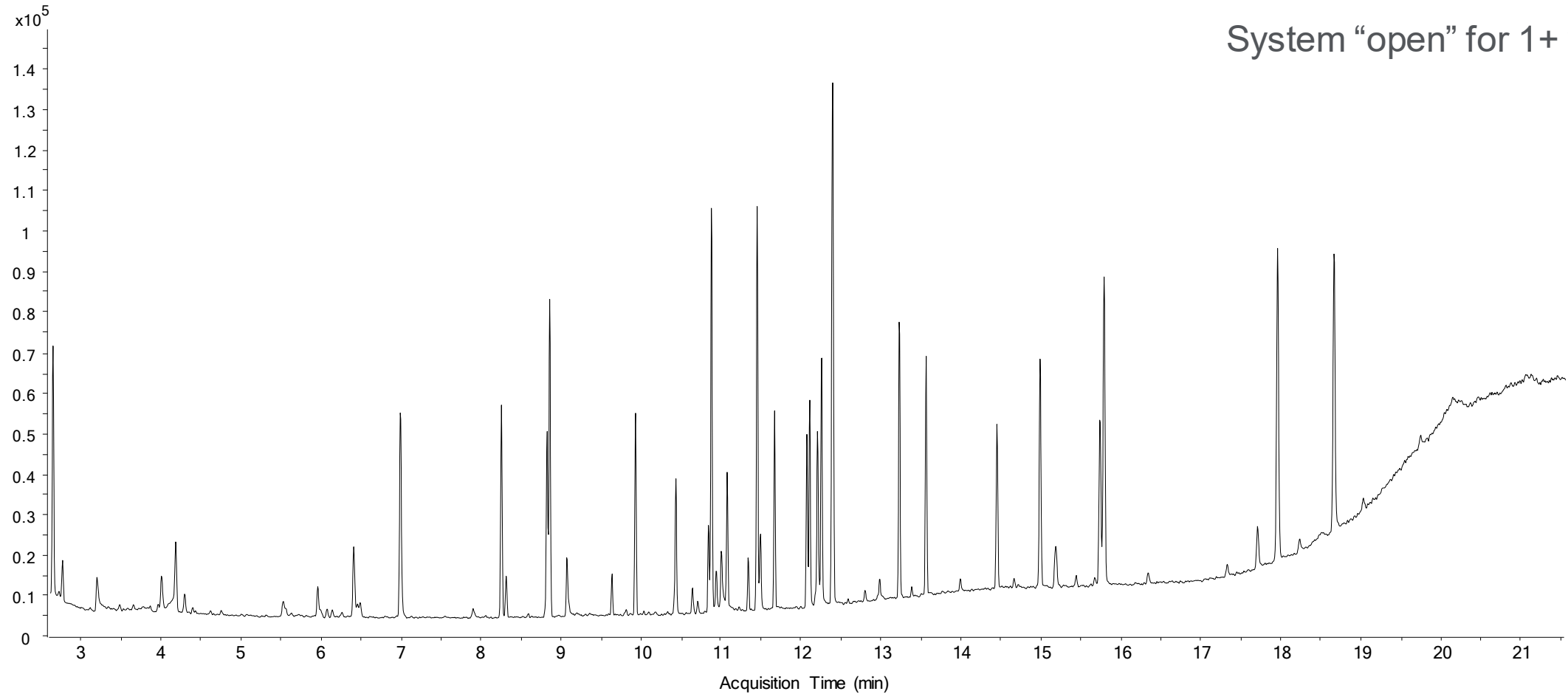
Logic = Something changed (slowly or sudden) =
Something is different

Hexane blanks (testing vial storage over time)



If GC/MS was off for 1+ week (no carrier gas flow)...

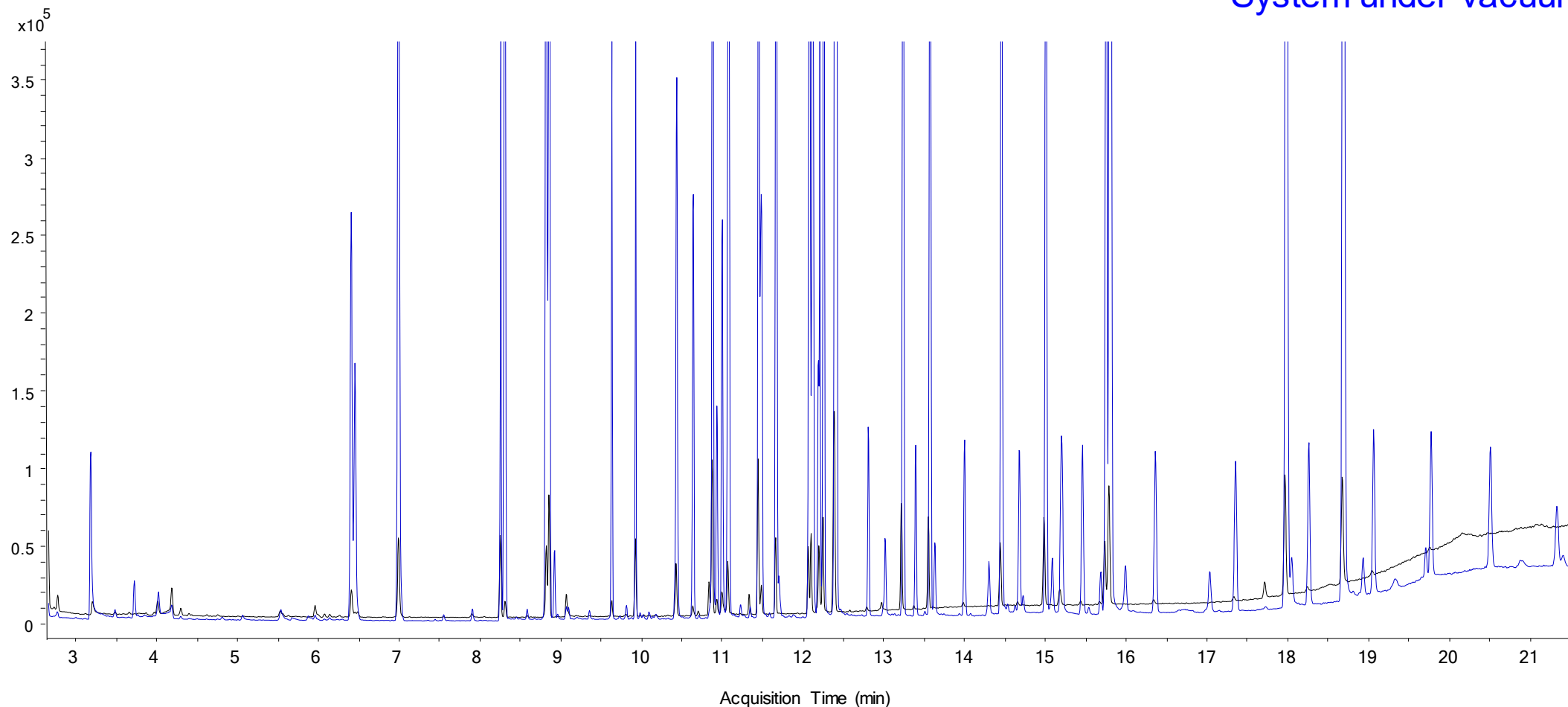
System "open" for 1+ week



TIC looks okay (I think). How does it compare to a previous run of the same sample?

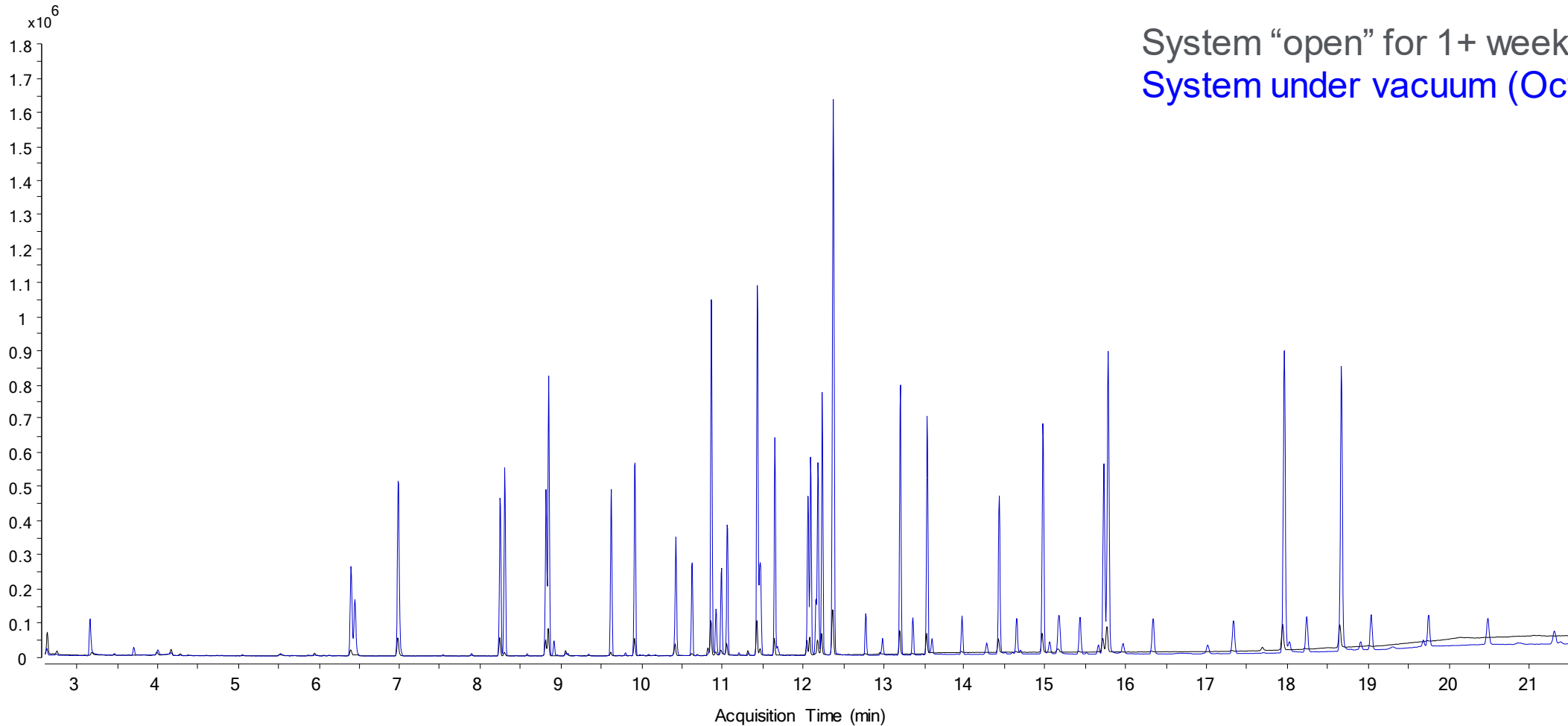
If GC/MS was off for 1+ week (no carrier gas flow)... zoom out

System "open" for 1+ week (Dec)
System under vacuum (Nov)



“Open system” TIC is ~10x lower than good run in the previous month. What happens if we replace the column and line

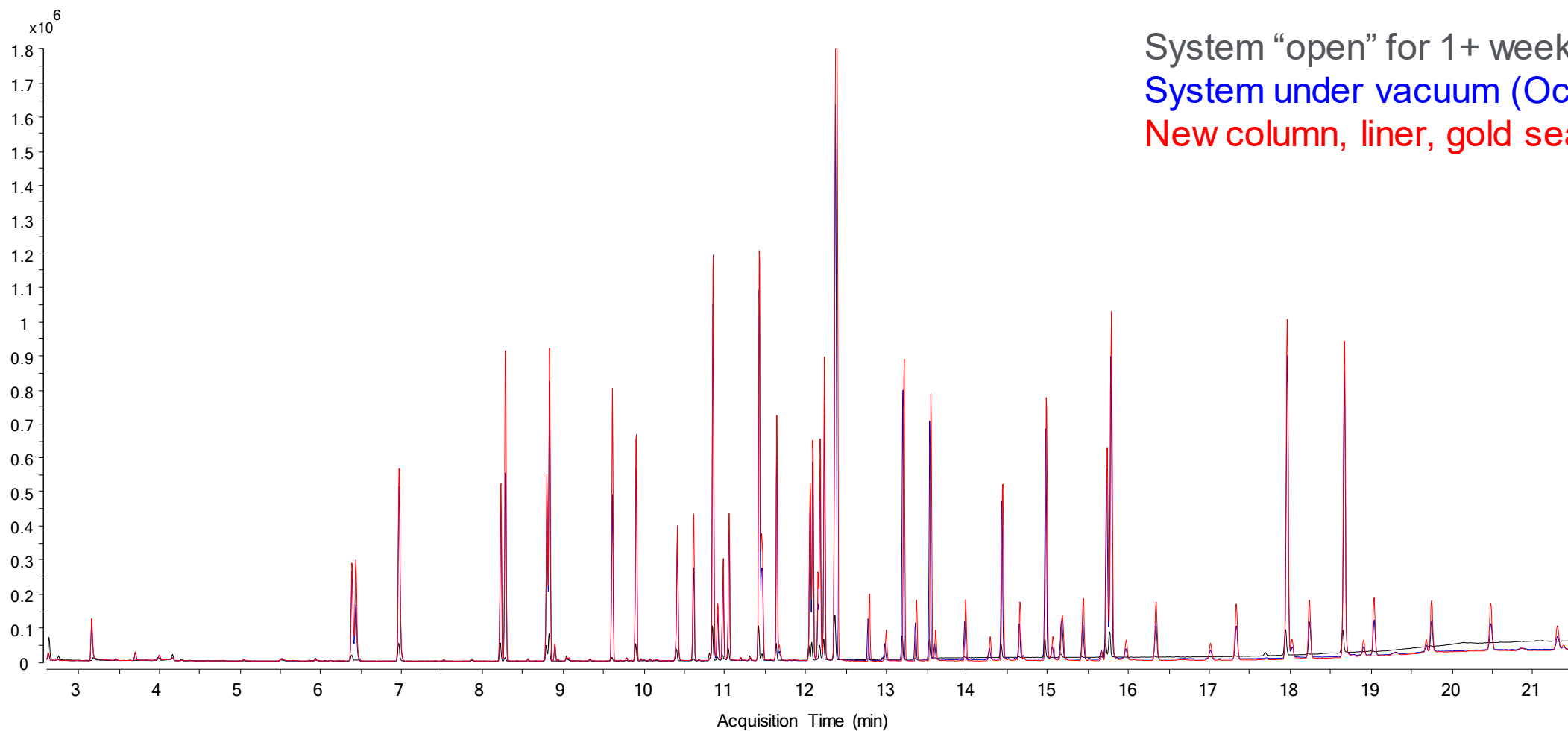
If GC/MS was off for 1+ week (no carrier gas flow)...



System "open" for 1+ week (Dec)
System under vacuum (Oct)

“Open system” TIC is ~10x lower than good run in the previous month. What happens if we replace the column and liner?

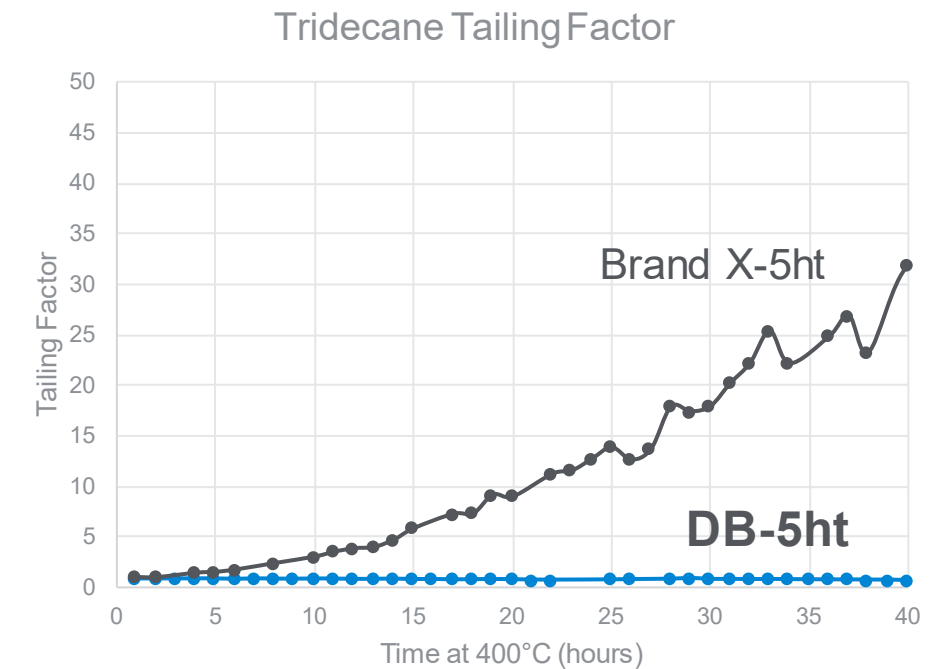
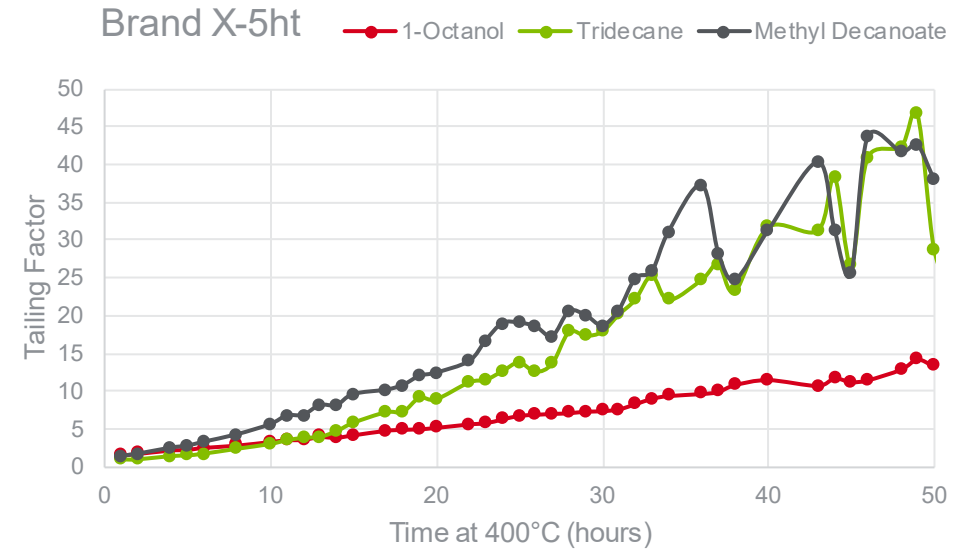
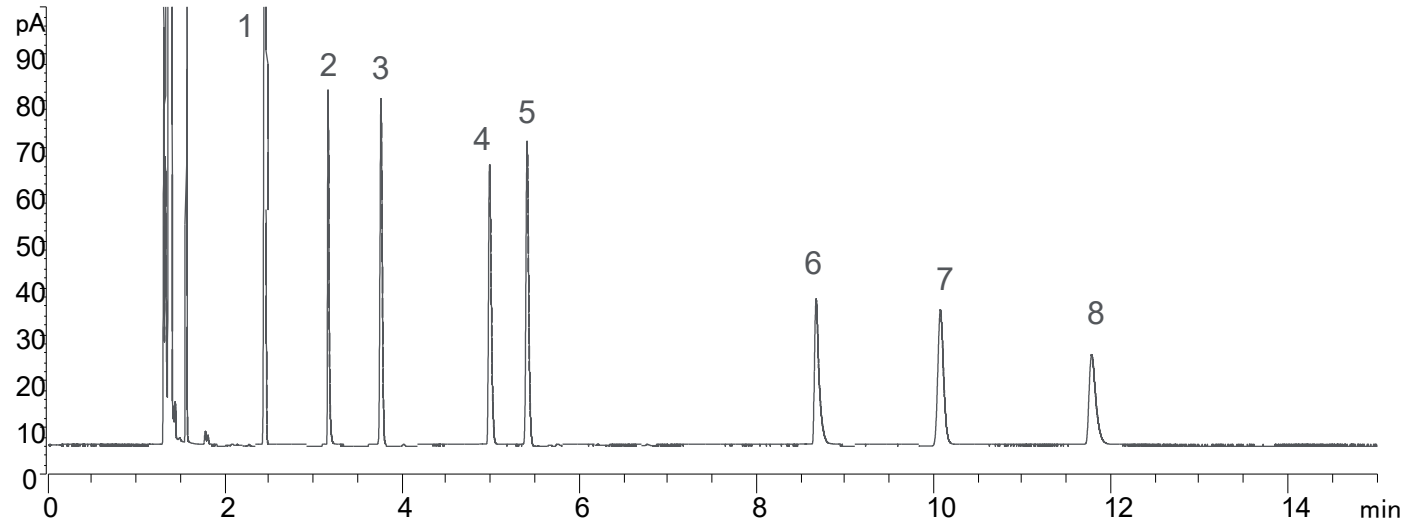
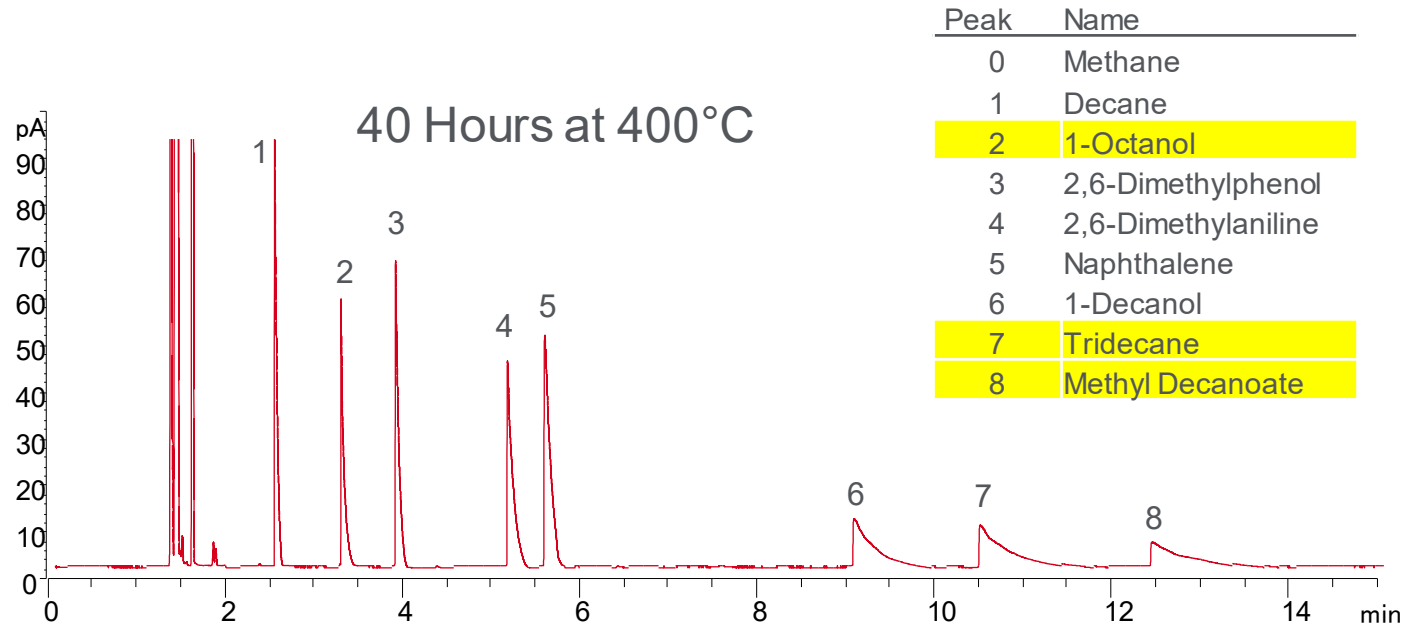
Recover peak response with new column and liner



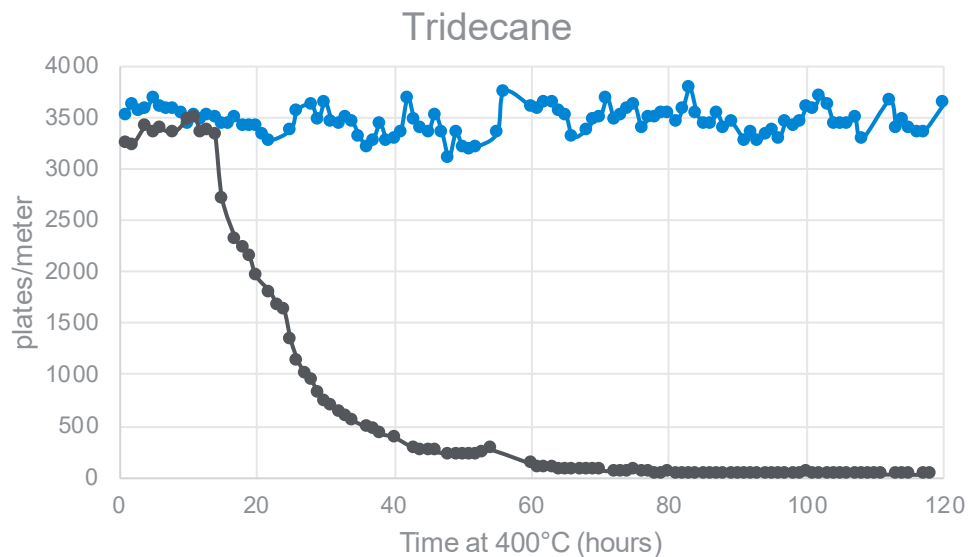
System "open" for 1+ week (Dec)
System under vacuum (Oct)
New column, liner, gold seal (Dec)

Try a new liner and re-conditioning column first.
If response doesn't recover, a new column may be required.

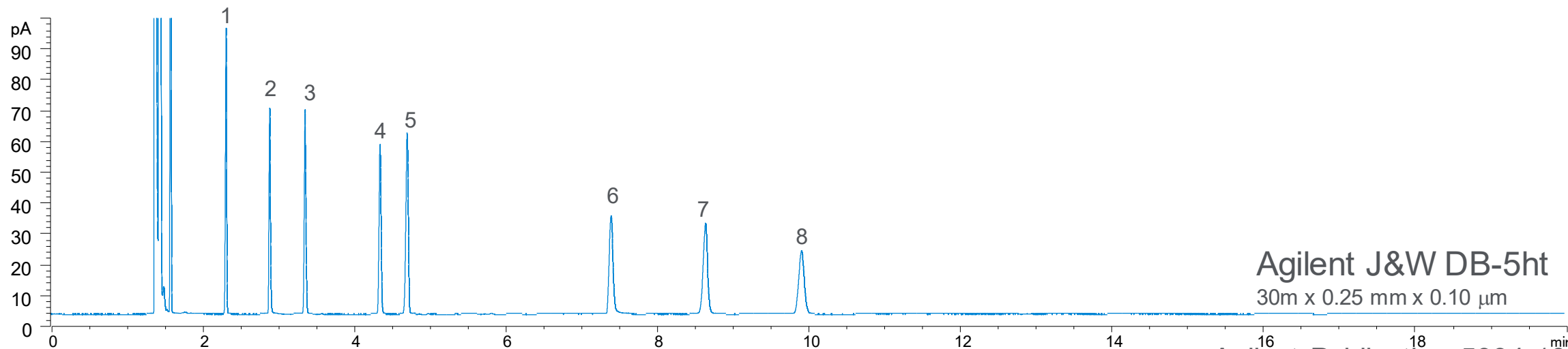
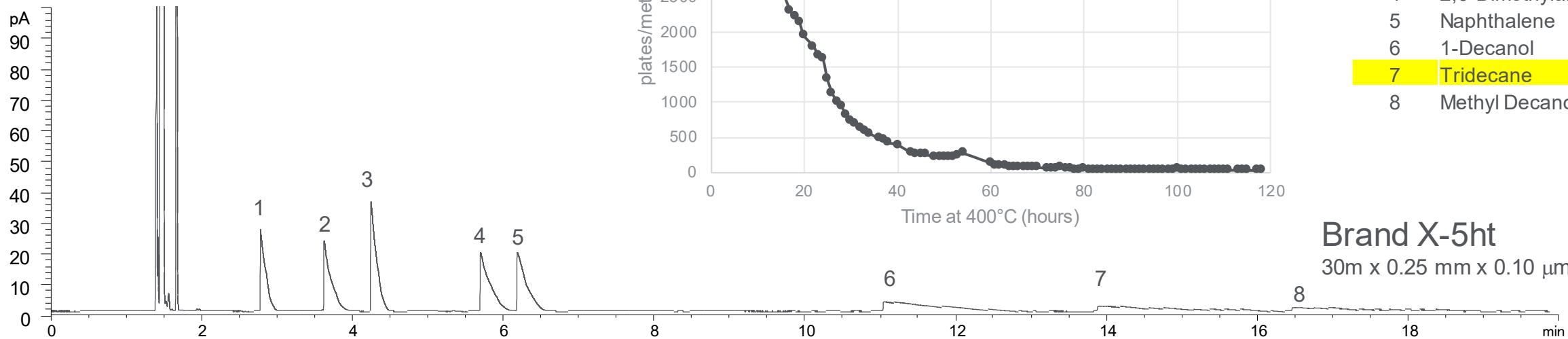
Brand X-5ht Peak Symmetry Degradation



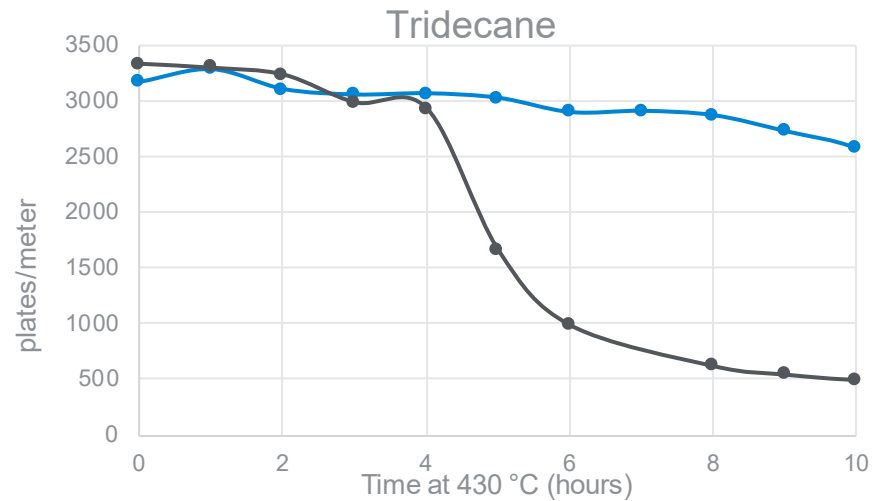
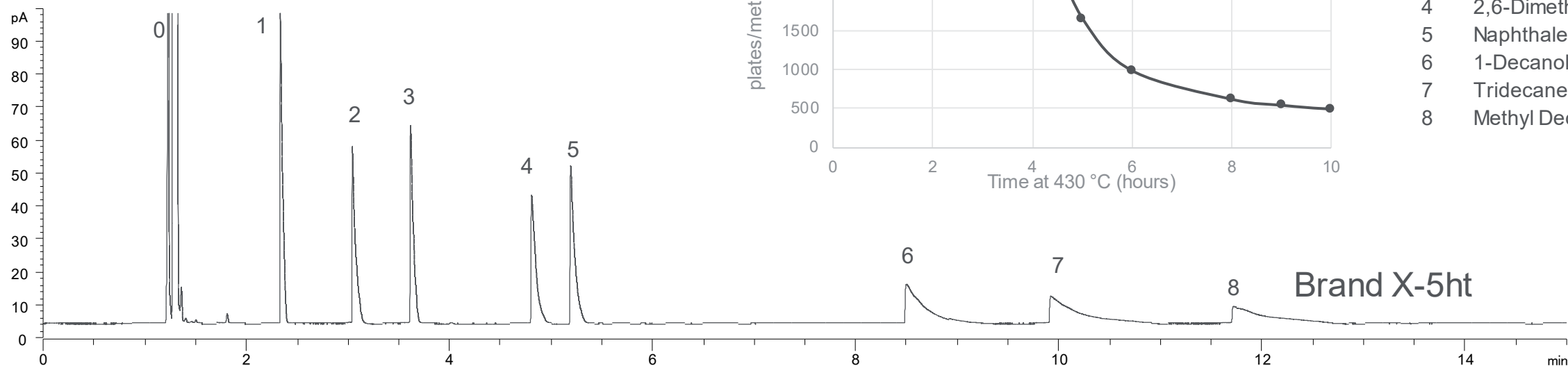
Column Efficiency Over 120 hours at 400°C



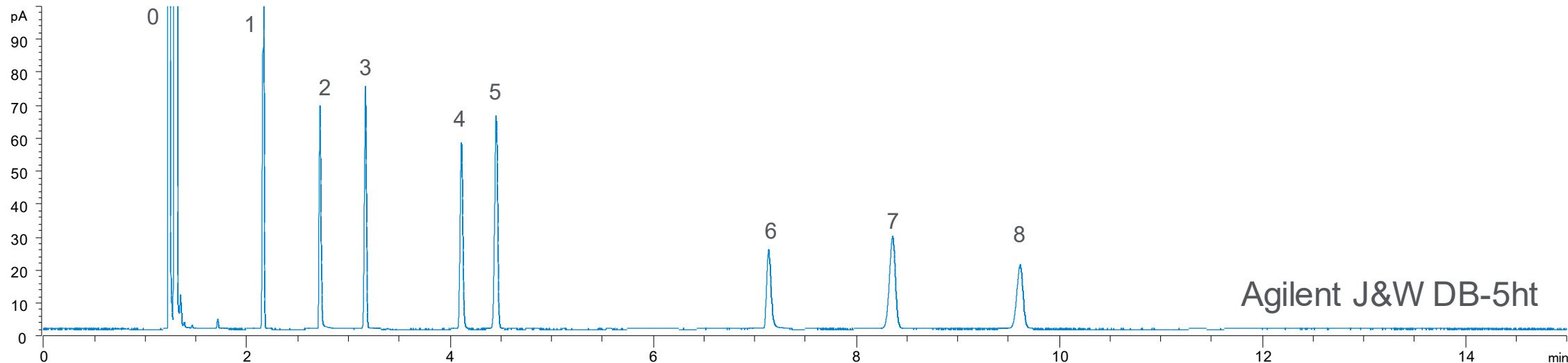
Peak	Name
0	Methane
1	Decane
2	1-Octanol
3	2,6-Dimethylphenol
4	2,6-Dimethylaniline
5	Naphthalene
6	1-Decanol
7	Tridecane
8	Methyl Decanoate



Column Efficiency at 430°C



Peak	Name
0	Methane
1	Decane
2	1-Octanol
3	2,6-Dimethylphenol
4	2,6-Dimethylaniline
5	Naphthalene
6	1-Decanol
7	Tridecane
8	Methyl Decanoate

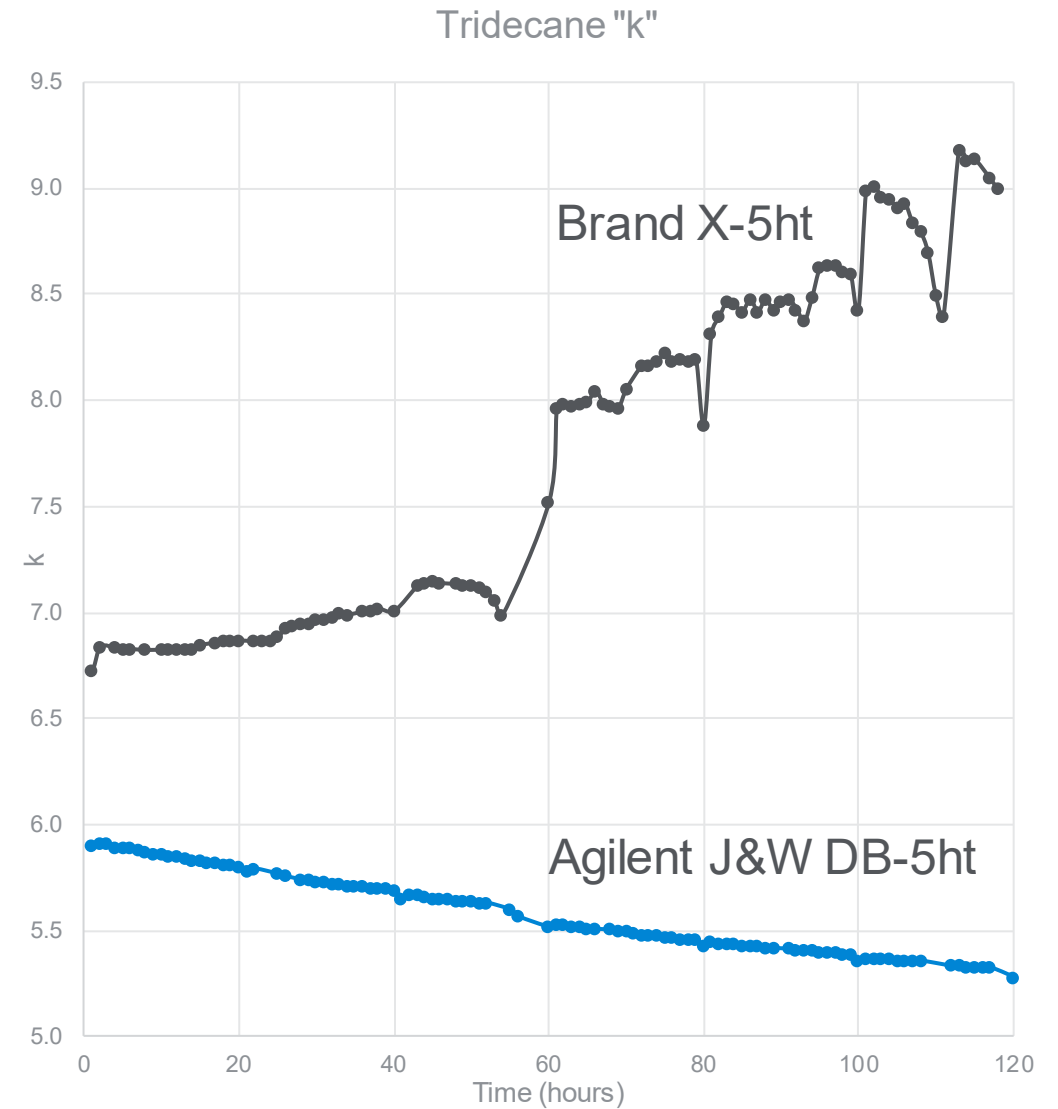
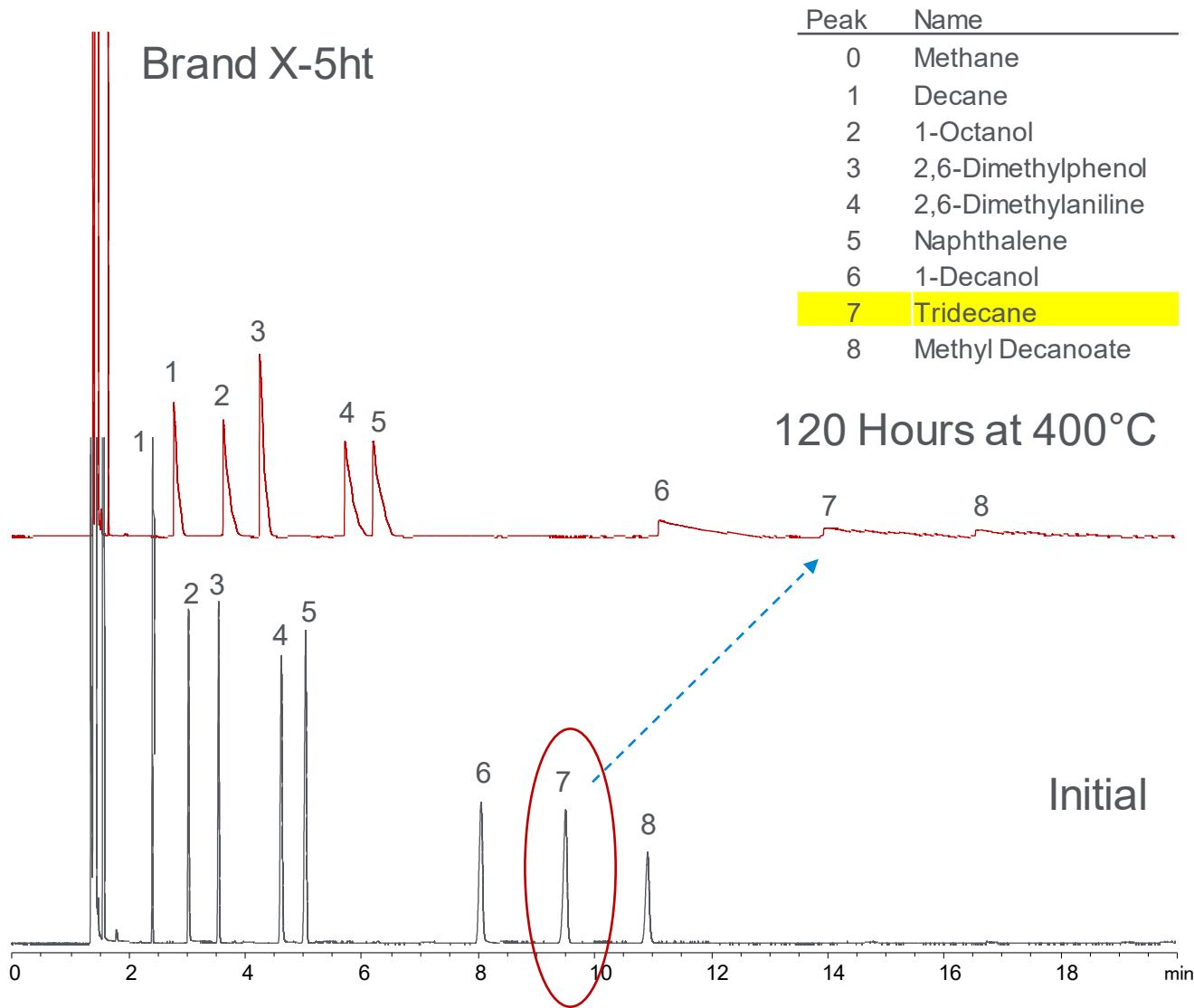


Agilent J&W DB-5ht

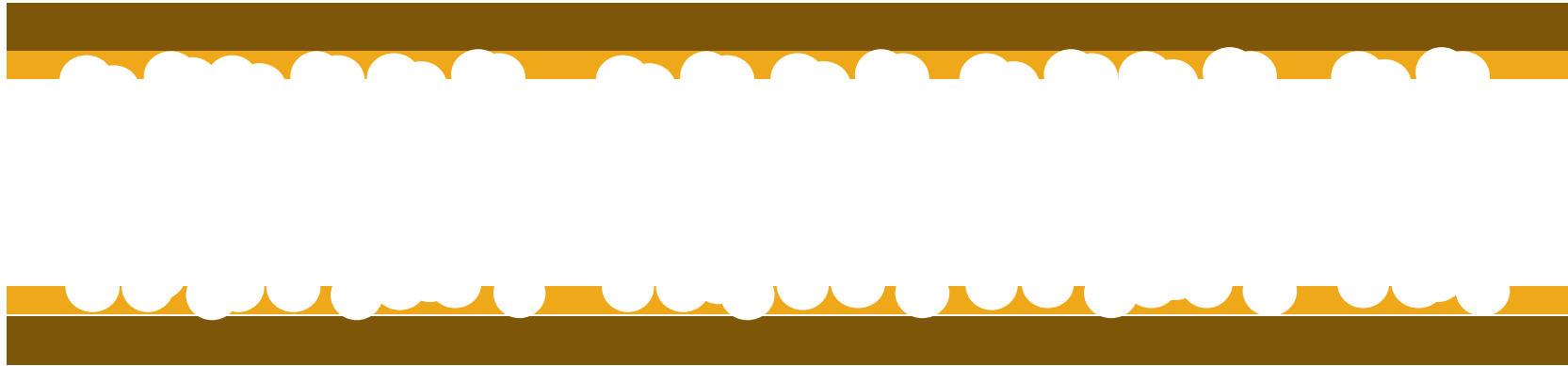
Agilent Publication 5994-1013EN

Phase Degradation Increases Retention

$$k = \frac{t_{\text{compound}} - t_{\text{methane}}}{t_{\text{methane}}}$$



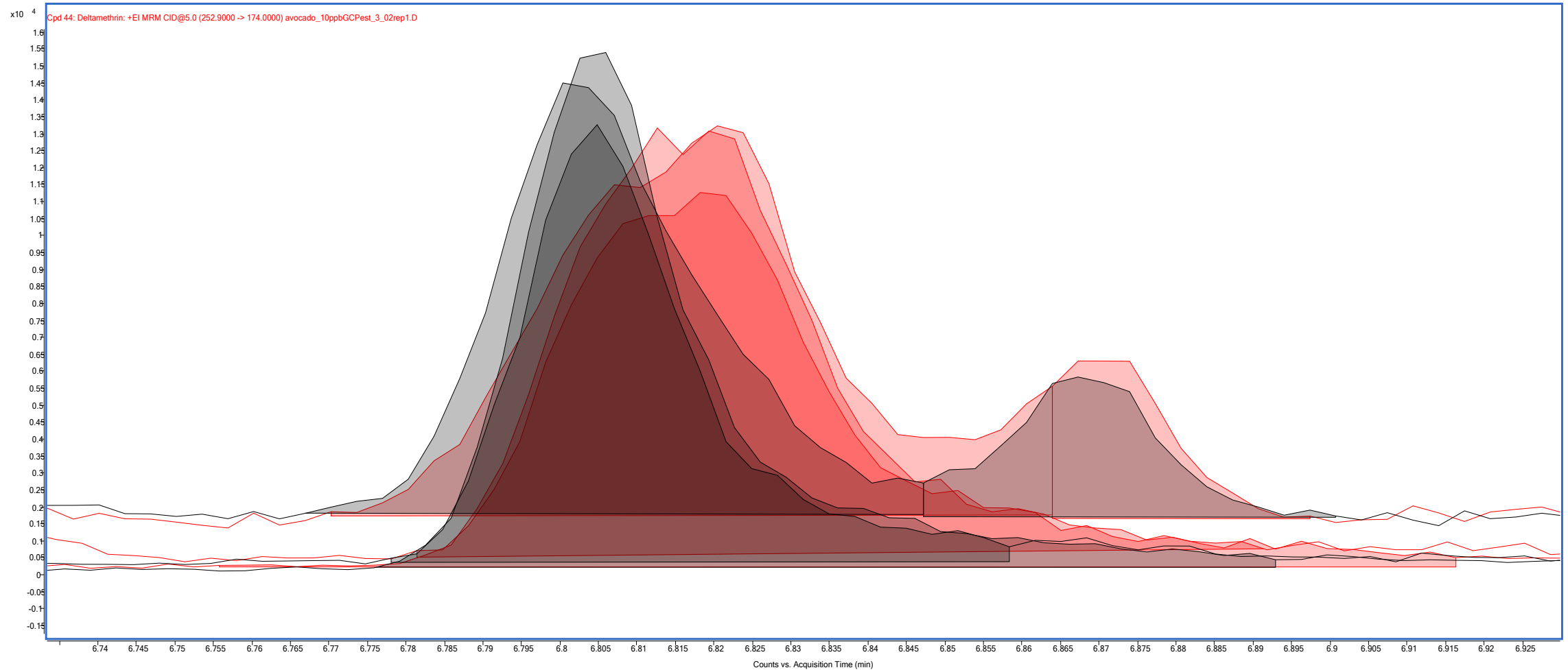
“Potholes” Created as the Phase Degrades Raw Fused Silica exposed.....



The more heat you add the more “potholes” you create



Recover peak shape with new liner (black)



Recover peak shape with new liner (black)

