Don’t Get Carried Away by Carryover: Troubleshooting GC Chromatography

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March 20, 2024

Agilent
Trusted Answers

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

“How do I troubleshoot?”

Track your actions/keep a logbook of events:

- Changed column, liner, septum, or syringe
- Injected samples, or used another method
- Carried out maintenance, cut column, or flushed inlet

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

Logical Troubleshooting

Troubleshooting starts with isolating the problem

- There are five basic areas that problems can arise from:
  - Injector
  - Flow
  - Column
  - Detector
  - Electronics

  Or…
  - A combination of all of these

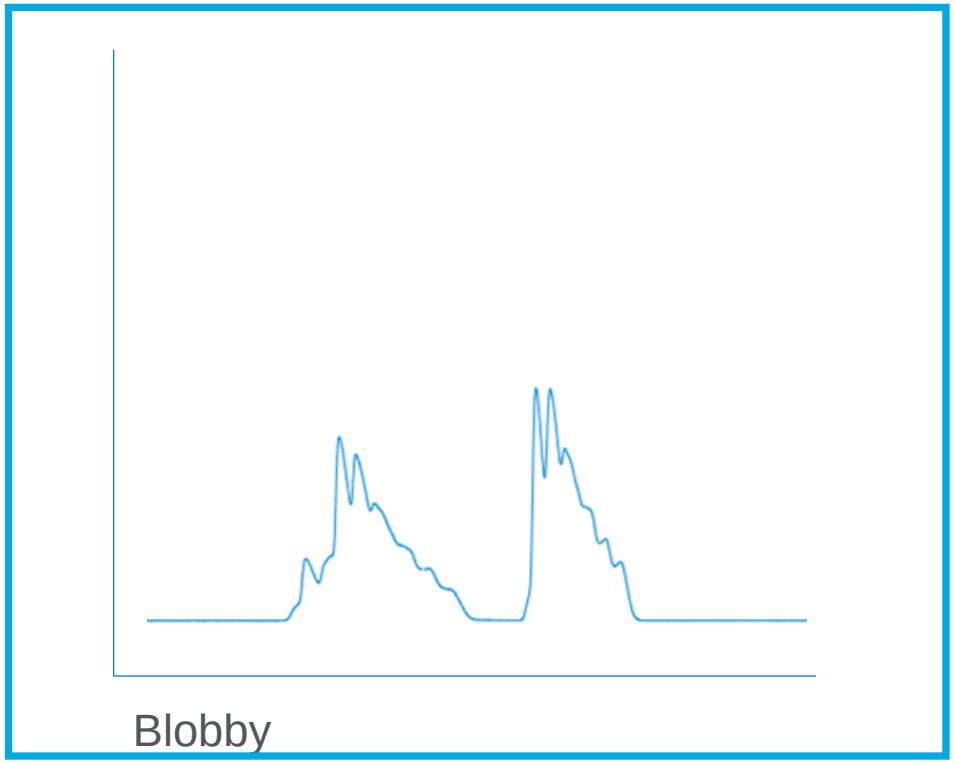
Knowing what can and cannot cause the symptoms is key, and most importantly **don’t panic.**

Common Peak Shape Issues

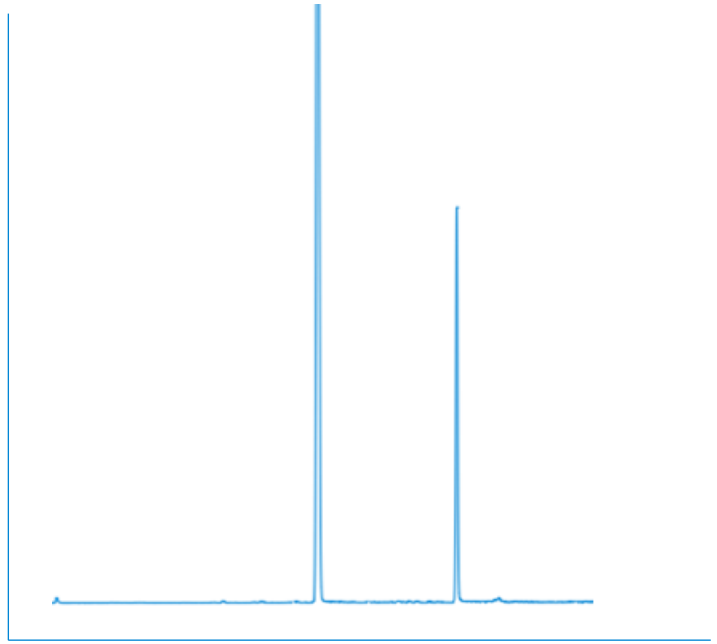
- **Peak tailing** – flow path or activity
- **Bonus peaks** – in sample or backflash (carryover)
- **Split peaks** – injector problems, mixed solvent
- **No peaks** – sample wasn't introduced, wasn't detected
- **Response changes** – activity, injector discrimination, detector problem
- **Peak fronting** – 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 issues
- **Noisy or spiking baseline** – electronics or contaminated detector
- **Quantitation problems** – activity, injector, or detector problems
- **Other**

Questions to Ask About These Extra Peaks

- Are the peaks nice and sharp at the same retention times?
- Do the peaks have the same response?
- Are the peaks broad/blobby? Are there inconsistent retention times?
- Are they your peaks of interest and do they match the expected retention time?



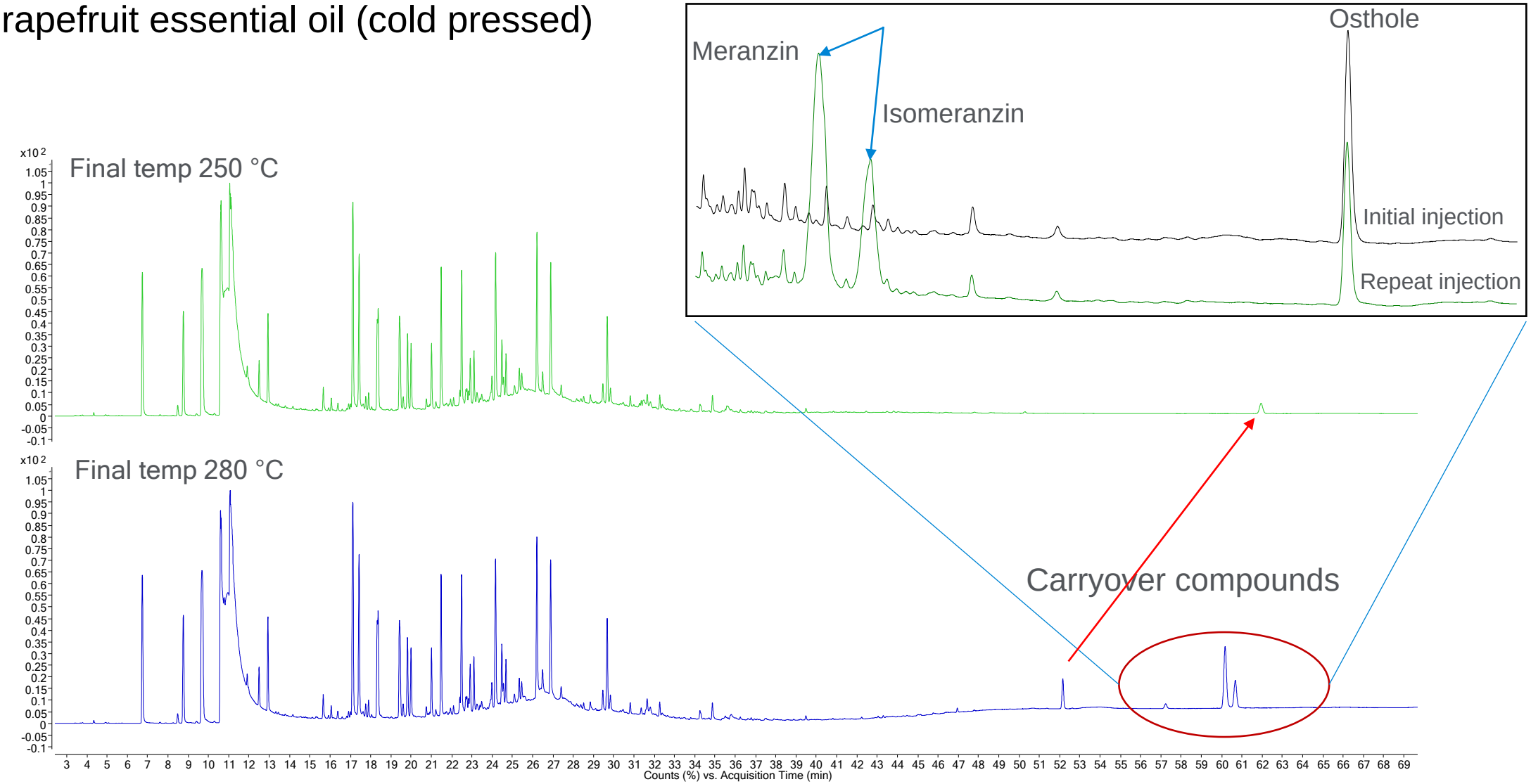
Blobby



Sharp

Higher Final Temperatures = Earlier Elution of Heavy Compounds, Sharper Late Eluting Peaks, and Reduced Carryover

Pink grapefruit essential oil (cold pressed)



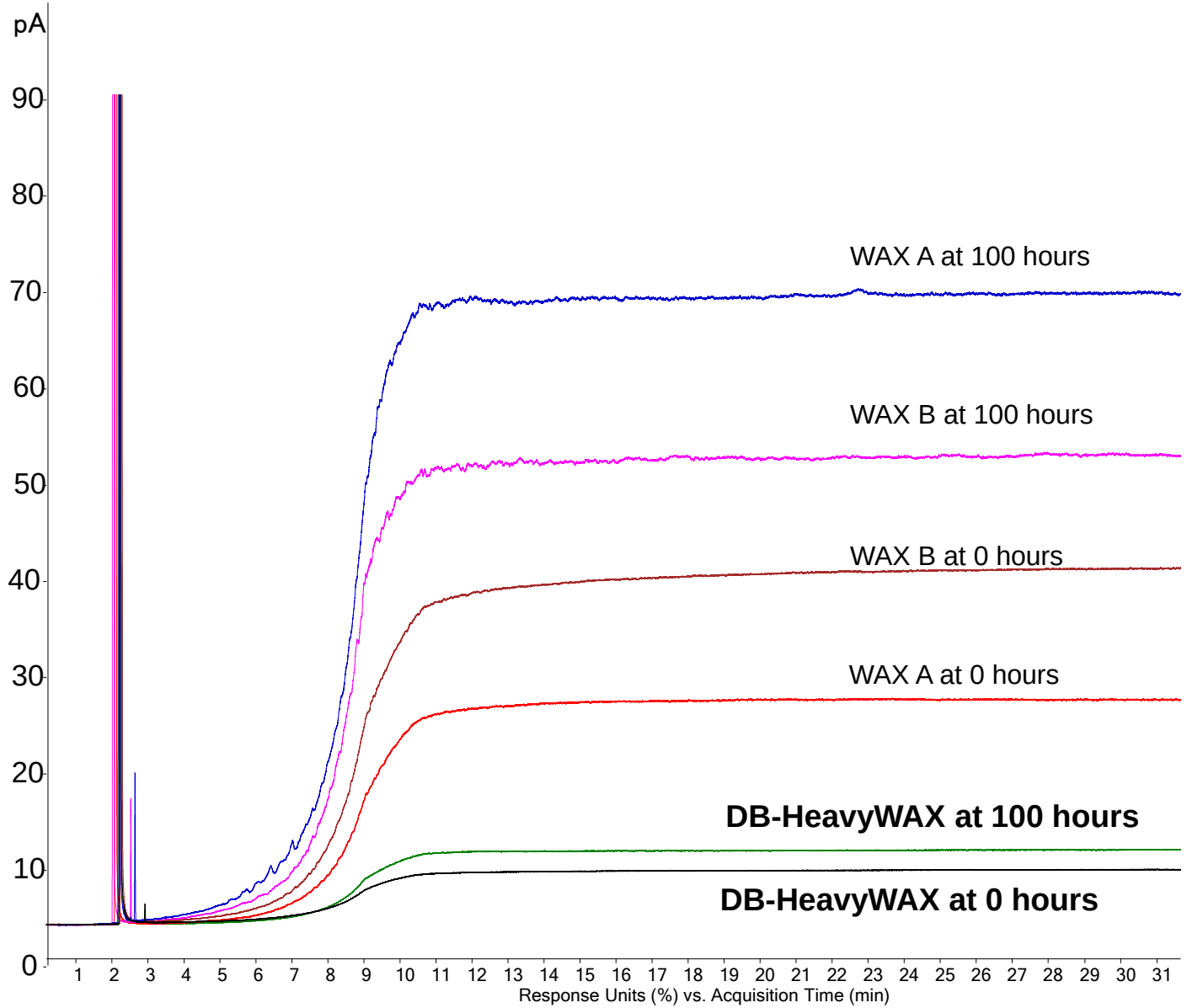
Application note 5991-9078EN

DB-HeavyWAX

- This is a WAX column with increased MAOT compared to existing columns on the market
  - 280 °C isothermal and 290°C programmed
- Provides increased thermal stability
- Has a low bleed level
- Advantages

General GC	GC/MS	GC×GC
• Shorter runtimes when late eluters are present • Better S/N ratio, improved detection • Better thermal stability • Faster column bake-out	• Desire for “zero” bleed • Avoid MS contamination from column bleed for longer system uptime and column lifetime • Improve detection limit	• Extended scope of compounds

Bleed Summary at 280 °C Over 100 Hours



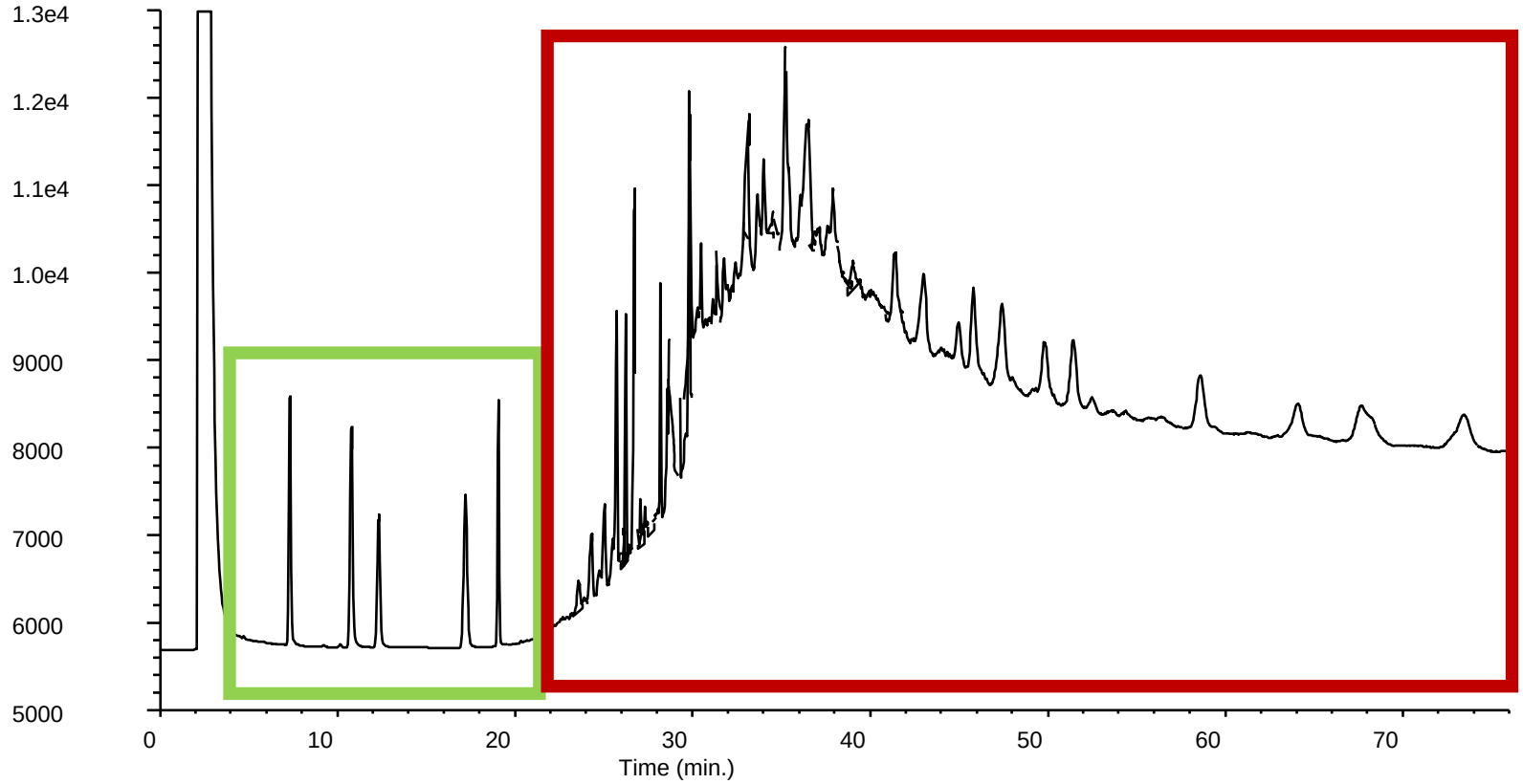
Conclusions for the J&W DB-HeavyWAX

- ✓ Increased thermal stability (280 °C isothermal, 290 °C programmed)
  - ✓ Stable retention times
  - ✓ Consistent peak order
- ✓ Deceased column bleed
  - ✓ Lower noise at higher temperatures for greater sensitivity with “heavier” compounds
  - ✓ Increased analyte range
  - ✓ Decreased analysis time
  - ✓ Safely bake out the column up to 290 °C
- ✓ DB-WAX selectivity
  - ✓ Simpler method translation

Example of Column Contamination

1.5 m removed*

QC test mix to upper temperature limit



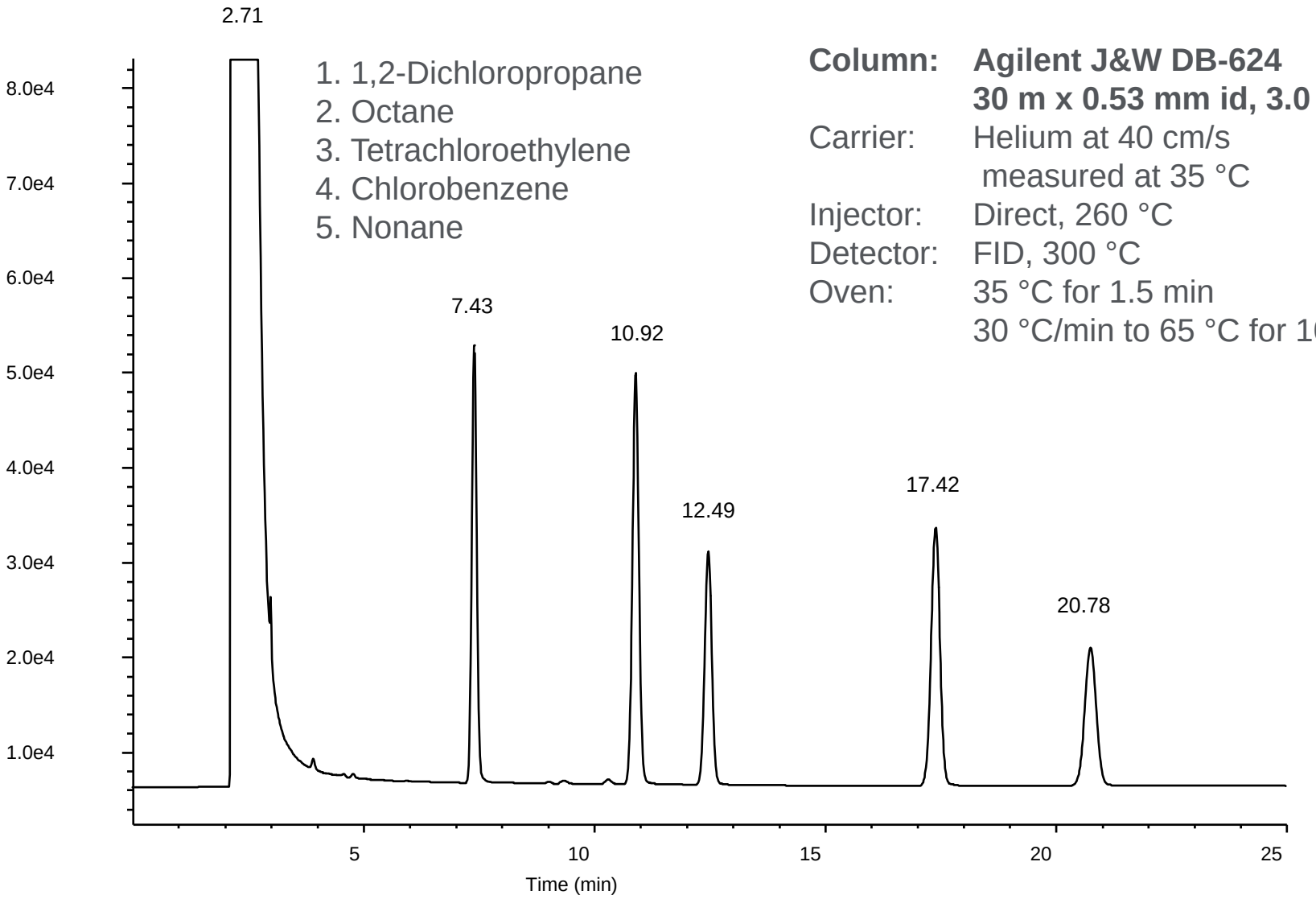
We have more semivolatile contamination

*Before column bake

Temperature program // 35 °C, hold 1.5 min // 30 °C/min to 65 °C, hold 15 min // 20 °C/min to 260 °C, hold 50 min

Agilent J&W DB-624 Column

QC test mix



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

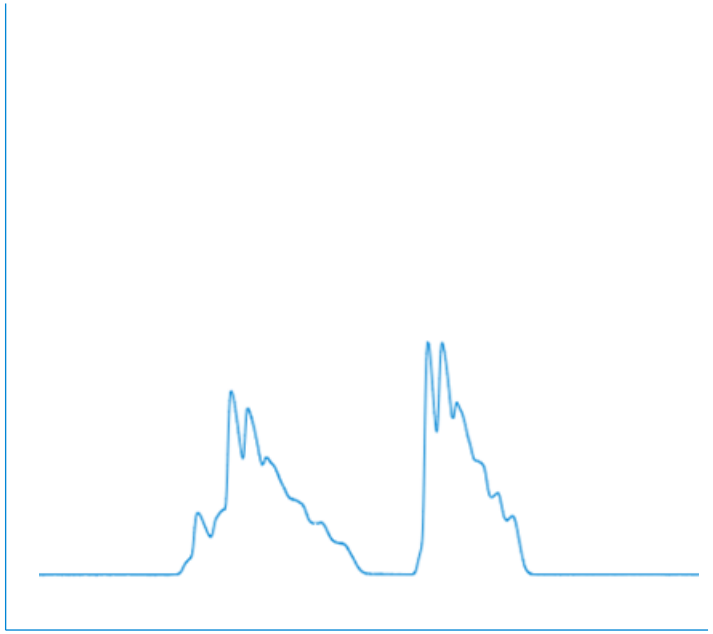
Column:	**Agilent J&W DB-624**
	30 m x 0.53 mm id, 3.0 µm
Carrier:	Helium at 40 cm/s
	measured at 35 °C
Injector:	Direct, 260 °C
Detector:	FID, 300 °C
Oven:	35 °C for 1.5 min
	30 °C/min to 65 °C for 10 min

What to Do to Address Carryover (from Sample)

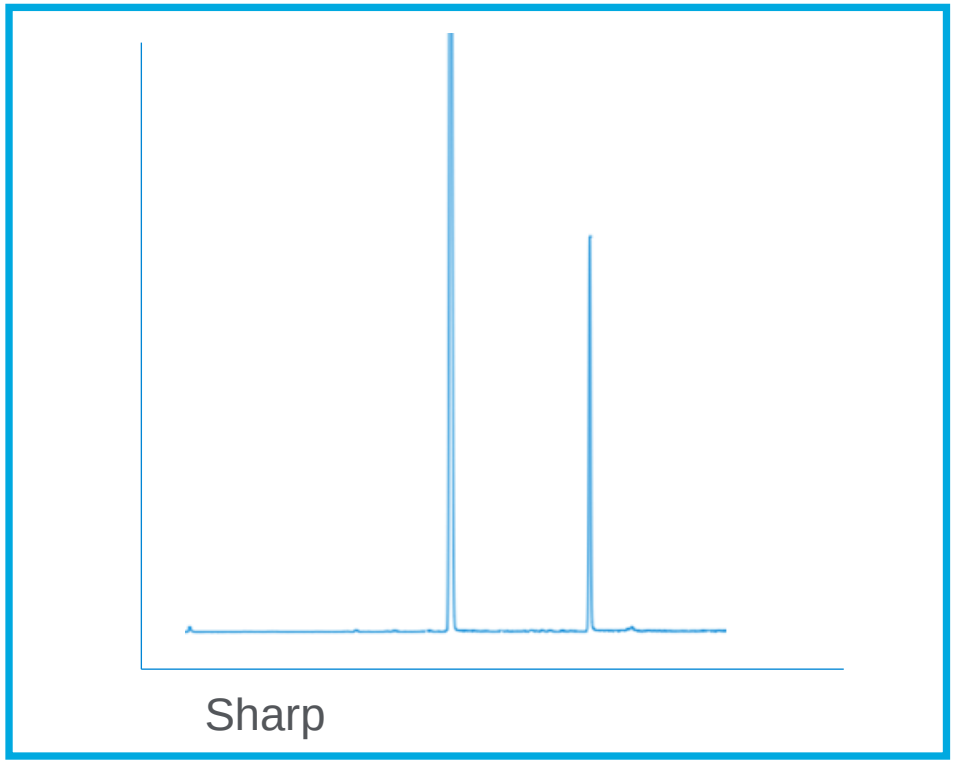
- Bake out the column
- Re-examine your temperature program
- Use a higher temperature column
- Trim the column, if contamination is severe
- If carryover is matrix, might need to do some sort of sample preparation

Questions to Ask About These Extra Peaks

- Are the peaks nice and sharp at the same retention times?
- Do the peaks have the same response?
- Are the peaks broad/blobby? Are there inconsistent retention times?
- Are they your peaks of interest and do they match the expected retention time?

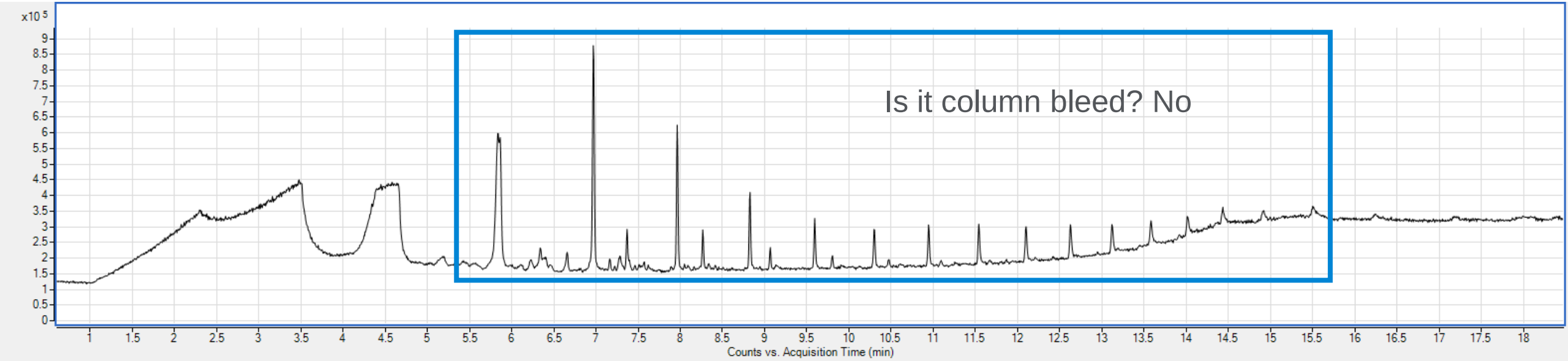


Blobby



Sharp

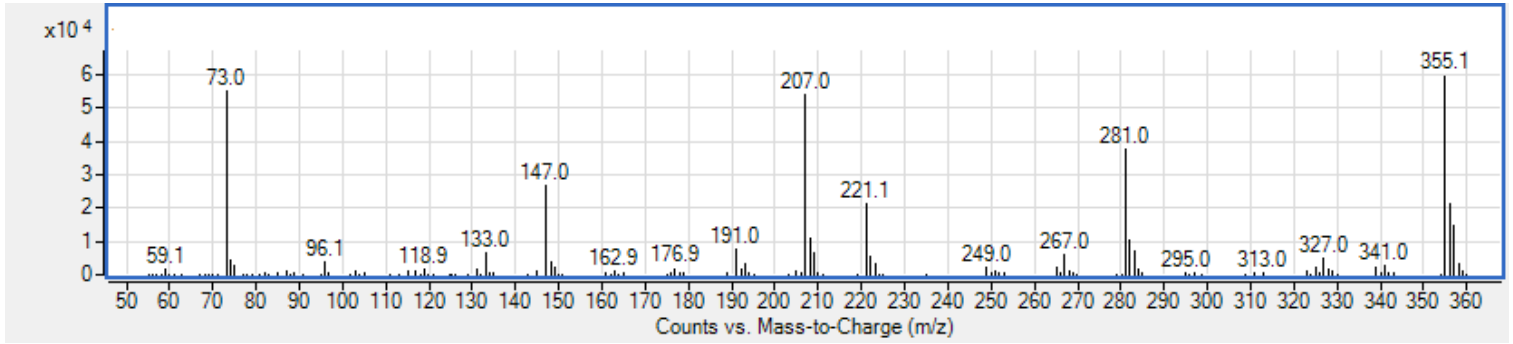
What Are These Repeating Peaks?



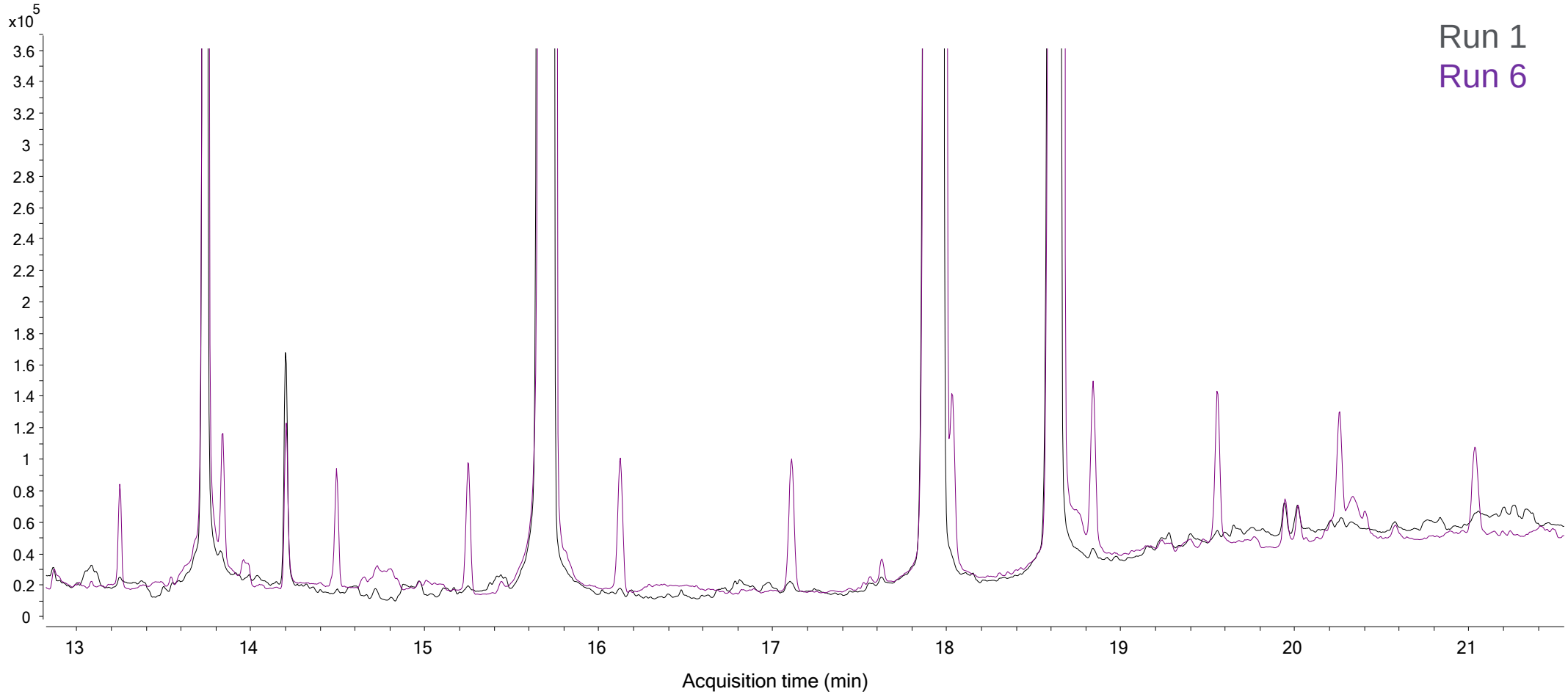
Common Ions for Siloxane Molecules
73
147
207
281
355

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 similar patterns.

Example spectrum:



Multiple Injections from the Same Vial: Siloxanes



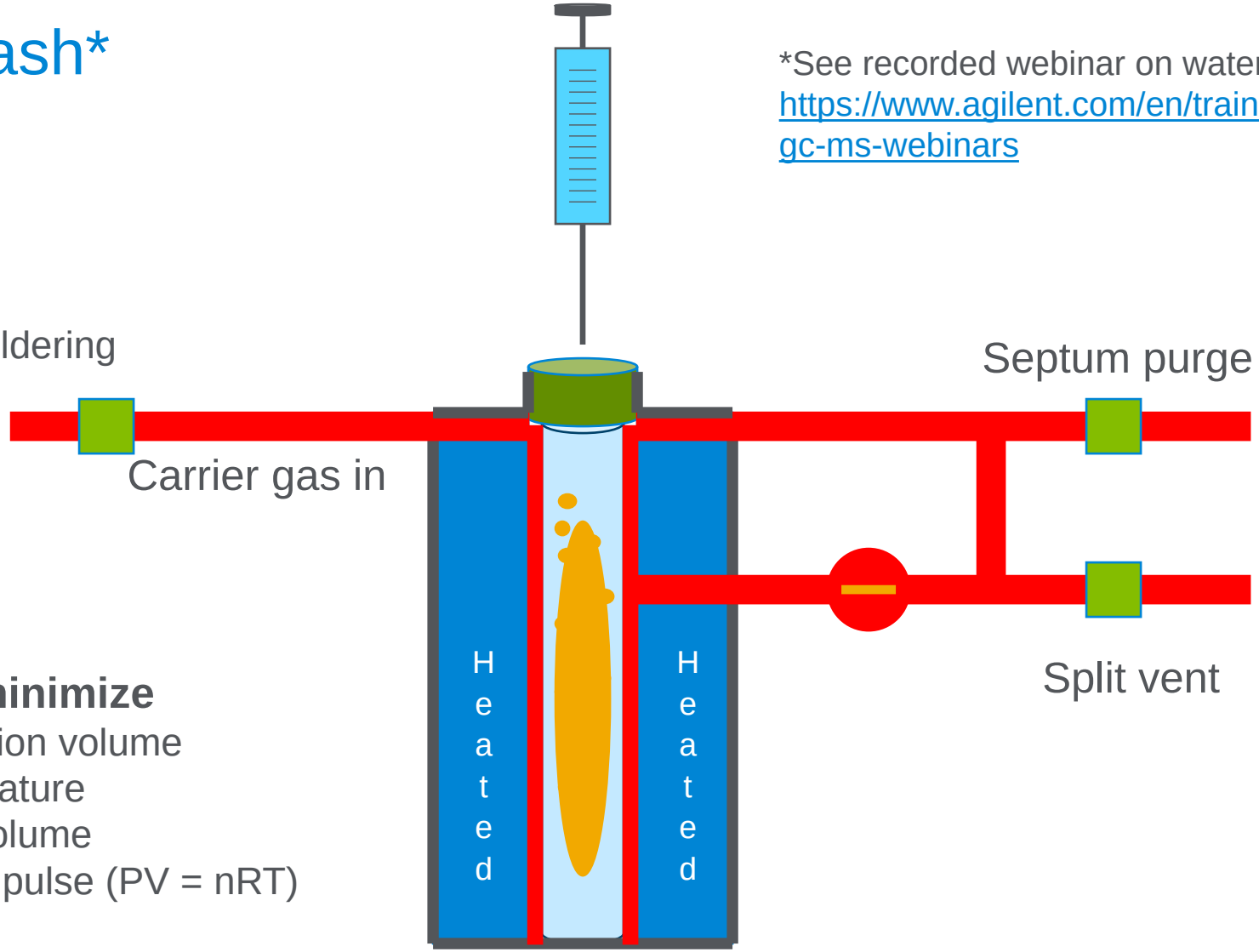
What If the Extra Sharp Peaks are Analyte Peaks?

Sample Backflash*

*See recorded webinar on water injections for more info: https://www.agilent.com/en/training-events/eseminars/gc-gc-ms-webinars

Negative effects

- Tailing
- Carryover
- Peaks splitting/shouldering
- Low response
- Poor reproducibility

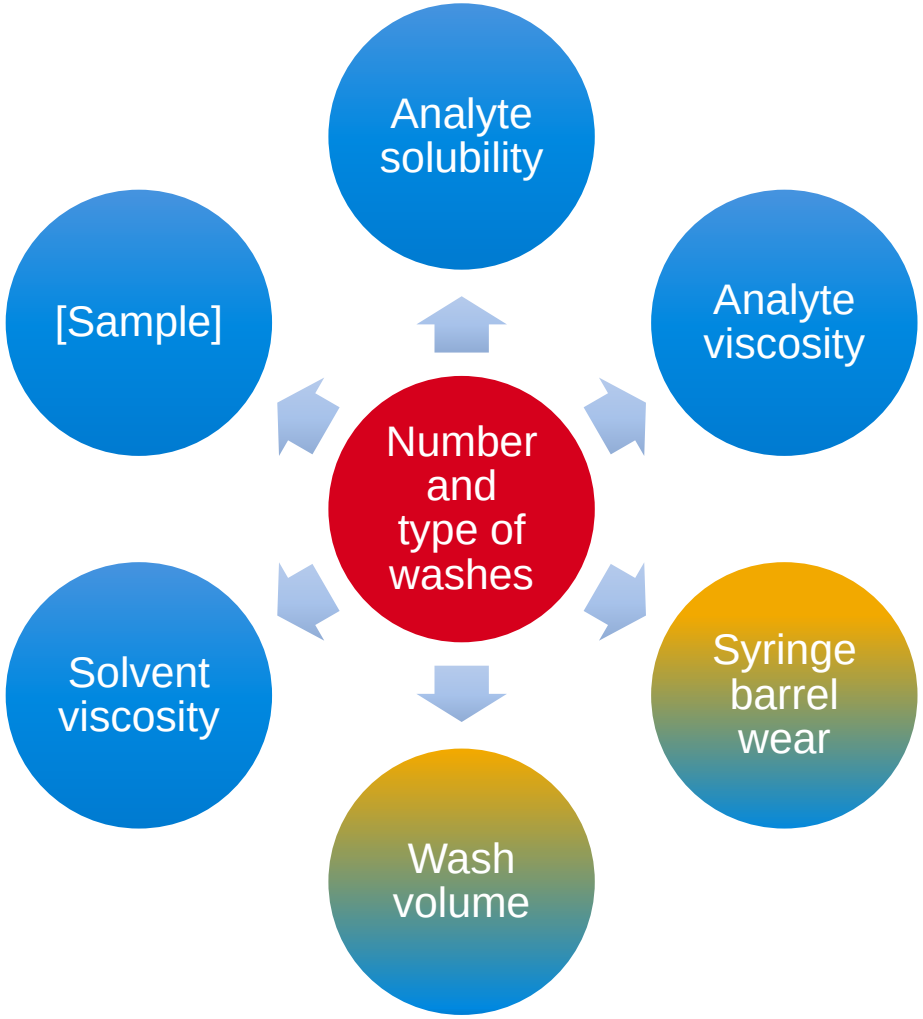


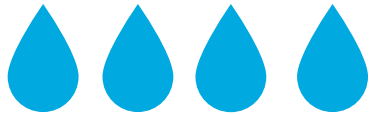
How to avoid/minimize

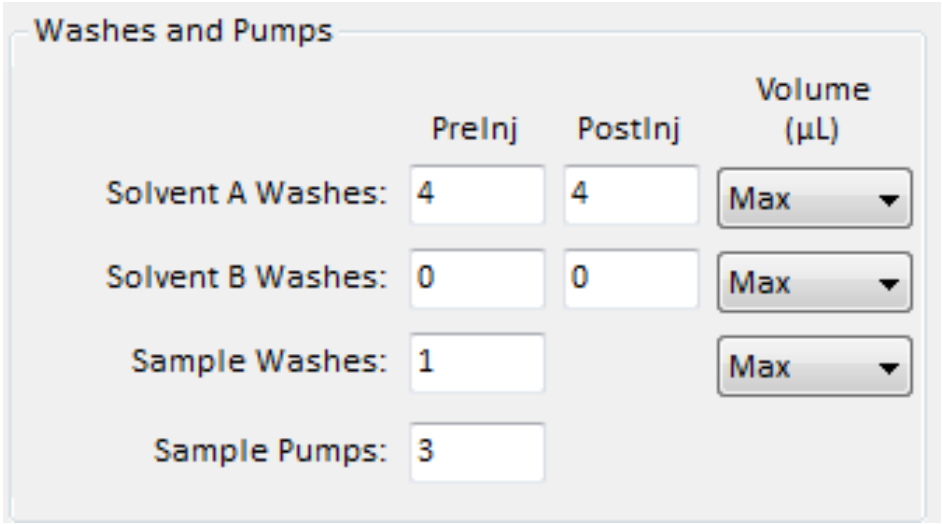
- Reduce injection volume
- Lower temperature
- Larger liner volume
- Use pressure pulse (PV = nRT)
- Tapered liner

Washes and Pumps: Solvents

- Four pre- and post-washes significantly reduces carry-over

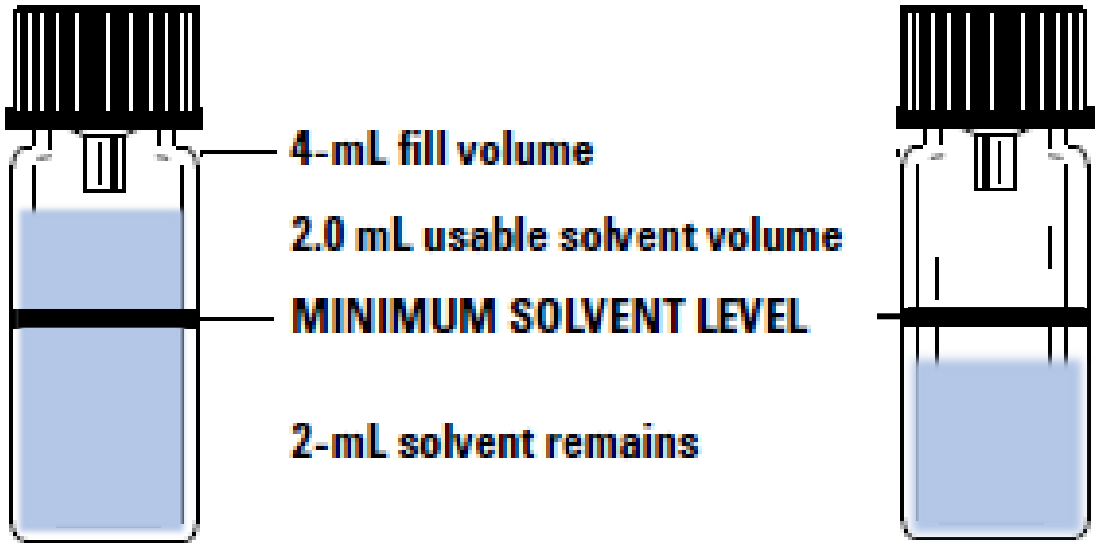






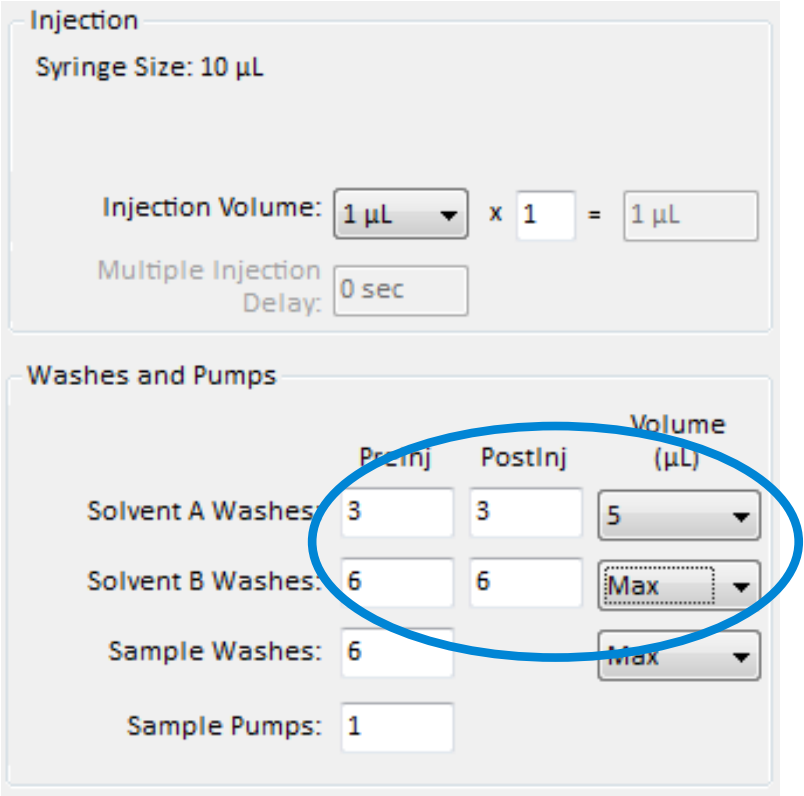
Wash Vial Volumes

$$Number\ of\ injections\ * \frac{(pre + post)\ washes}{injection} * wash\ volume = wash\ solvent\ used$$



>2 mL ✓

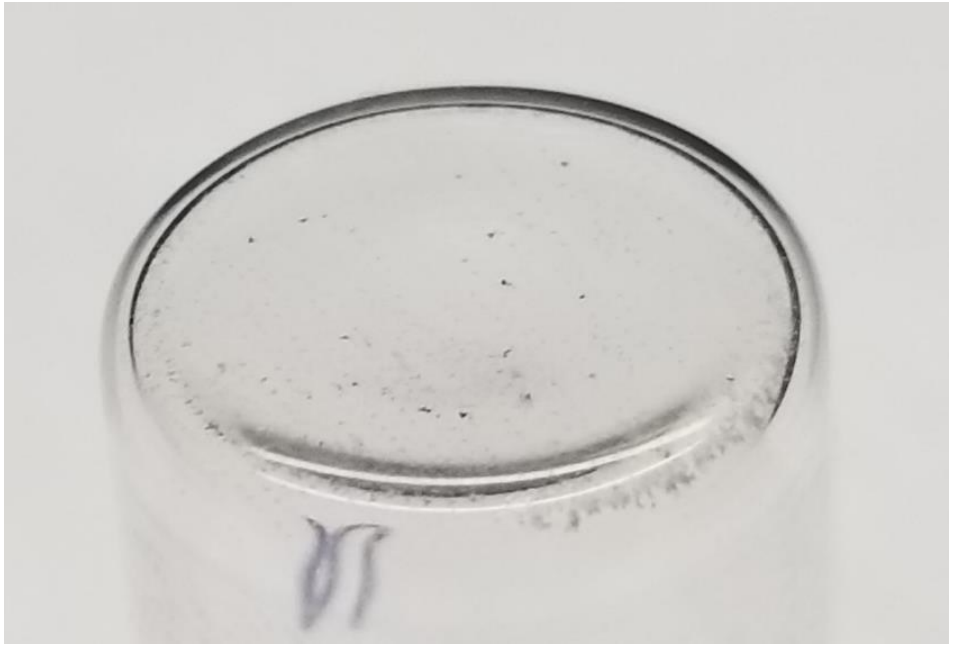
<2 mL ✗



Washes and Pumps: Solvents

Frequently clean or replace wash vials

- Traces of previous samples will accumulate over time
- Do not refill or “top-off” the vial, instead empty, rinse, and replace solvent
- Use a cotton swab to remove particulates from the glass surface



Contaminated wash vial bottom

Contaminated wash solvent

Washes and Pumps: Solvents

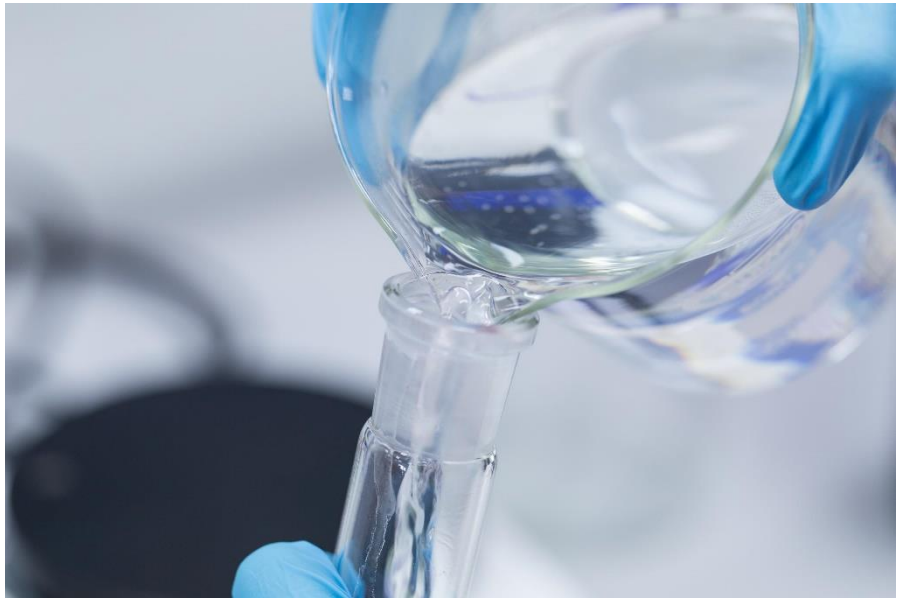
Choose a wash solvent that makes sense for the analysis

- Is the analyte soluble in the solvent?
- Wash solvent = sample solvent when possible
- If using a binary wash system, make sure the solvents are miscible and rinse with the sample solvent last, just before the sample is injected
- Do not use acidic or alkaline solvents with syringes

- Use both A and B wash vials
  - Second wash vial will be cleaner than first
  - Second wash vial should never be water (rust)

Avoid viscous solvents and solvents with high vapor expansion volumes. Use the vapor volume calculator to make sure it will not overload the inlet liner.

Miscibility Chart



	o-Xylene	Water	Toluene	Tetrahydrofuran (THF)	Pentane	Methyl Ethyl Ketone	Methyl t-butyl Ether	Methanol	Isopropanol (IPA)	Iso-Octane	Hexane	Heptane	Ethylene Dichloride	Ethyl Ether	Ethyl Alcohol	Ethyl Acetate	1,4-Dioxane	Dimethyl Sulfoxide (DMSO)	N,N- Dimethylformamide	Dichloromethane (DCM)	Cyclohexane	Chloroform	n-Butyl Alcohol	Acetonitrile (ACN)	Acetone	
Acetone																										
Acetonitrile (ACN)					■					■	■	■									■					
n-Butyl Alcohol		■																								
Chloroform		■																								
Cyclohexane		■						■										■	■					■		
Dichloromethane (DCM)		■																								
N,N- Dimethylformamide	■				■					■	■	■									■					
Dimethyl Sulfoxide (DMSO)	■				■					■	■	■		■							■					
1,4-Dioxane																										
Ethyl Acetate		■																								
Ethyl Alcohol																										
Ethyl Ether		■																■								
Ethylene Dichloride		■																								
Heptane		■						■										■	■					■		
Hexane		■						■										■	■					■		
Iso-Octane		■						■										■	■					■		
Isopropanol (IPA)																										
Methanol					■					■	■	■									■					
Methyl t-Butyl Ether		■																								
Methyl Ethyl Ketone		■																								
Pentane		■						■										■	■					■		
Tetrahydrofuran (THF)																										
Toluene		■																								
Water	■		■		■	■	■			■	■	■	■	■		■				■	■	■	■			
o-Xylene		■																■	■							

■ Immiscible

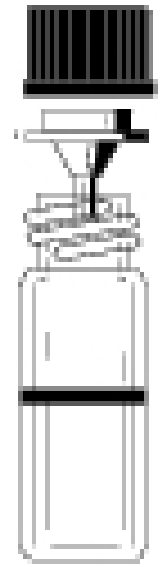
□ Miscible

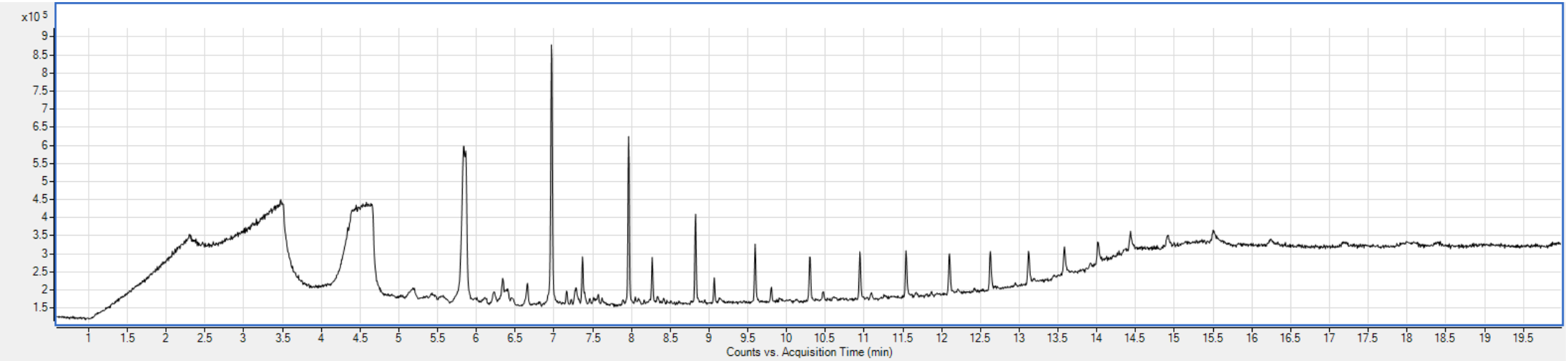
Washes and Pumps: Diffusion Caps

Diffusion caps are important

- Reduce volatile solvent diffusion
- Better alternative than using vial septa, which will core, contaminate wash solvent vial → septum bleed peaks

5182-0551: 4 mL wash vials with fill markings and caps, 25/pk
07673-40180: Diffusion inserts with black open top screw caps, 12/pk





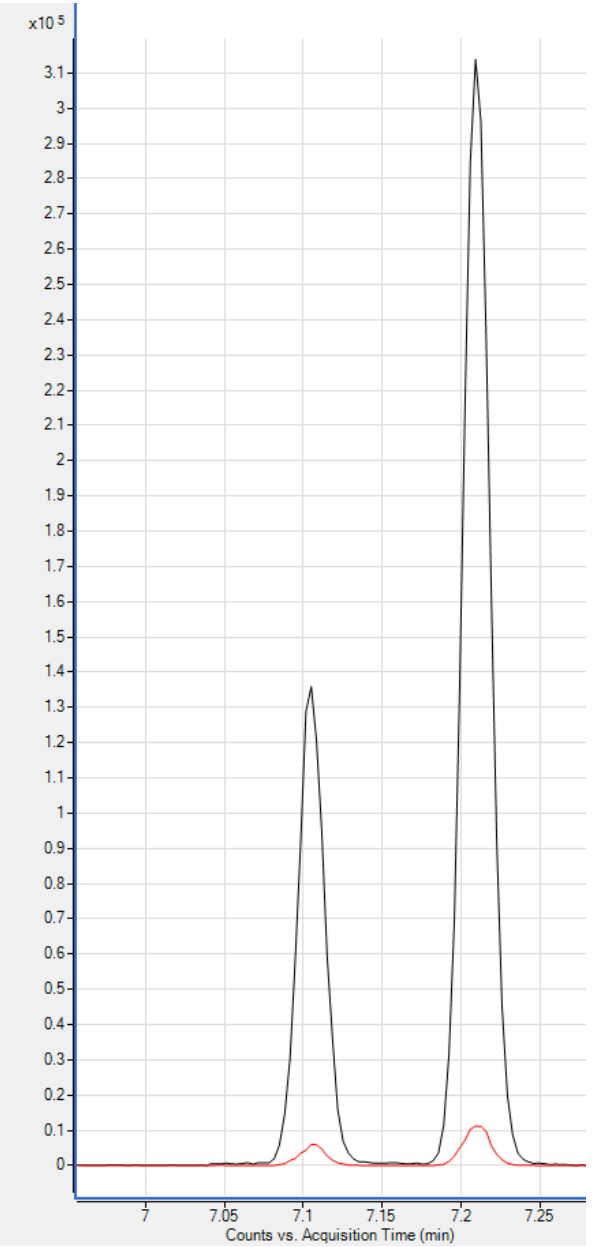
Troubleshooting

Problem: Sample carryover

Possible causes:

- Insufficient number of washes
- Solvent wash vial empty
- Wrong wash solvent
- Dirty ALS needle guide
- Dirty septum nut

ALS needle guide, G4513-40525



Suggested actions:

- Increase number or type of washes
- Rinse with a various polarity solvents
- Clean or replace syringe
- Ensure samples and solvents are miscible
- Occasionally replace needle guide (or “needle foot”)
- Check septum nut for sample residue

Other Miscellaneous Carryover Pointers

Headspace Carryover

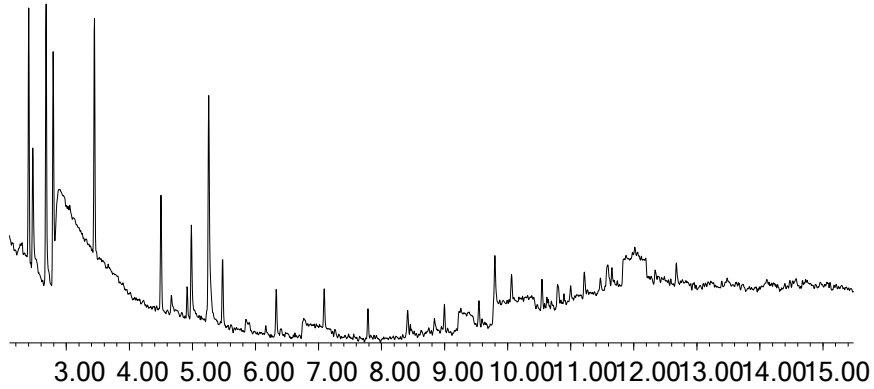
- Carryover results when sample condenses on the flow path or is trapped in any unswept areas of the flow path
- If you are experiencing carryover, try increasing the loop purge flow or loop purge time to sweep any residual sample vapors from the system
- Verify temperatures in the flow path, including the transfer line
  - Make sure temperature is hot enough and increases from vial to inlet

Contaminated Gas

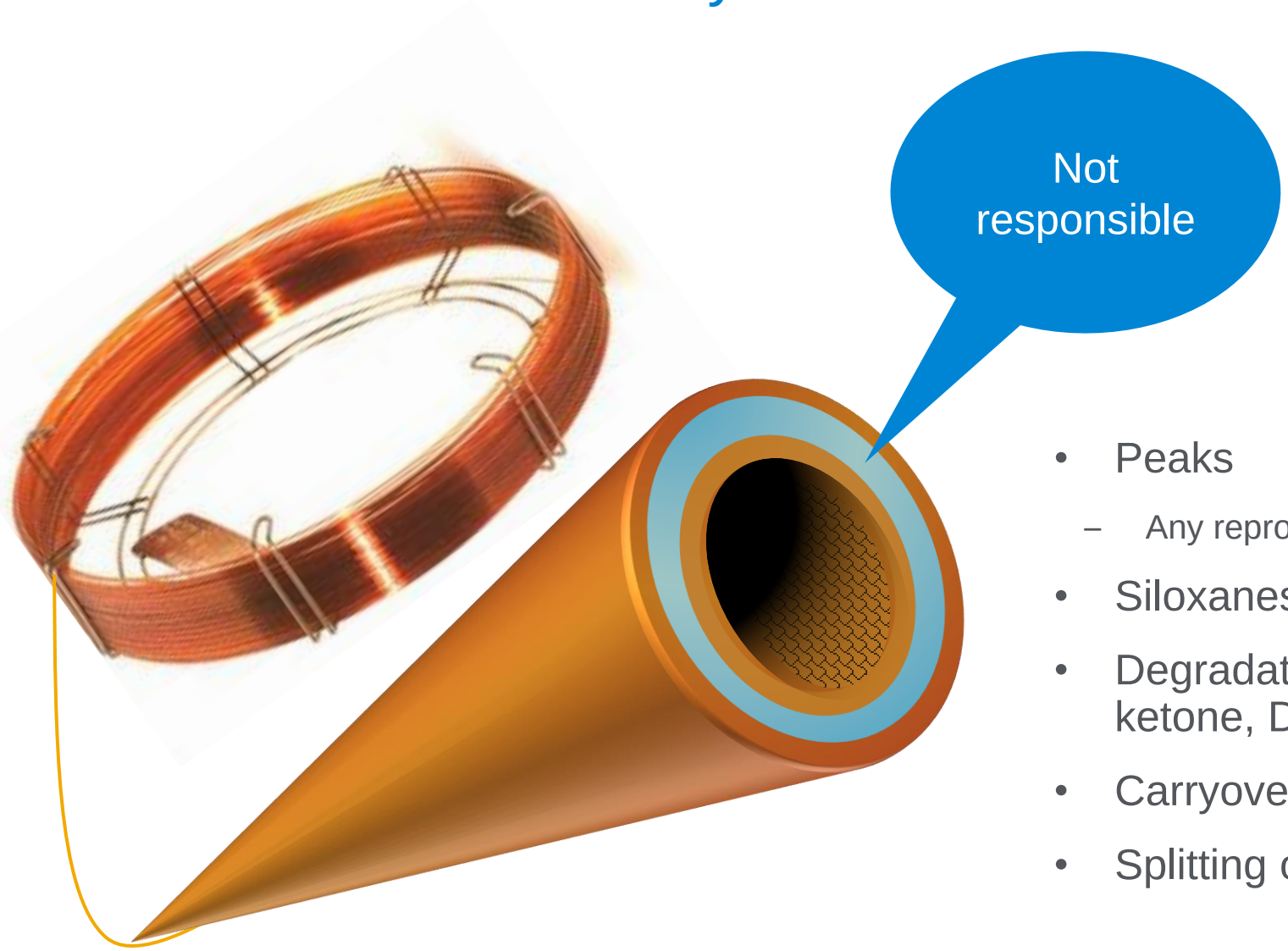
Sudden changes, wandering, or drifting

Drifting/wandering/weird disturbances

- Possible gas contamination
- Verify the quality of your gas
- Use gas traps
- Perform a “condensation test”



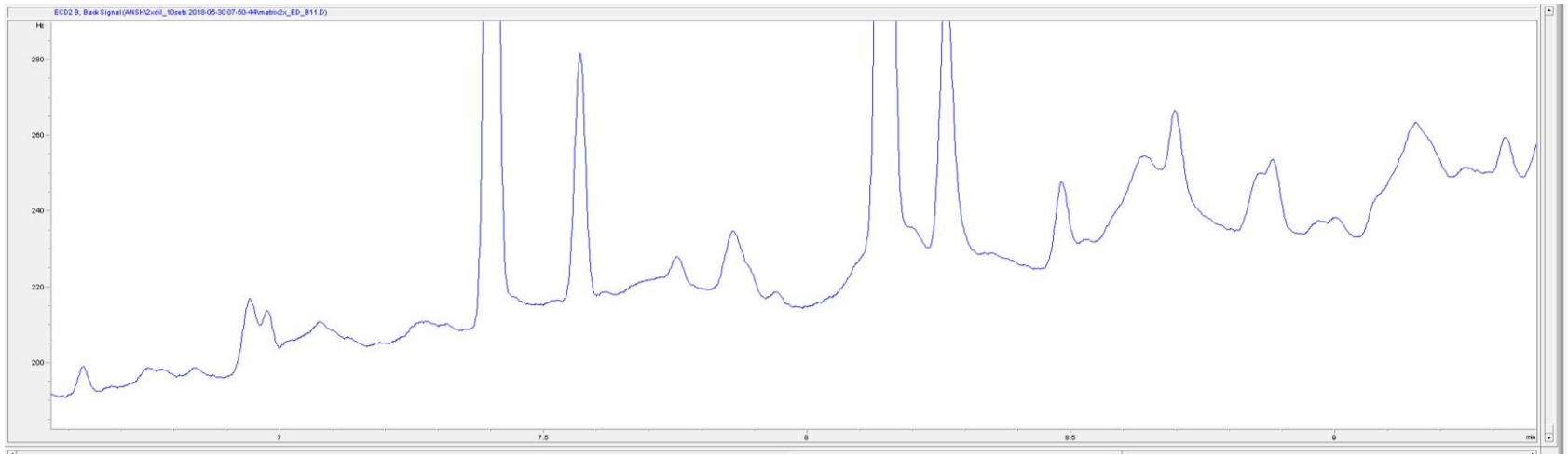
What Is Not Caused by a Column?



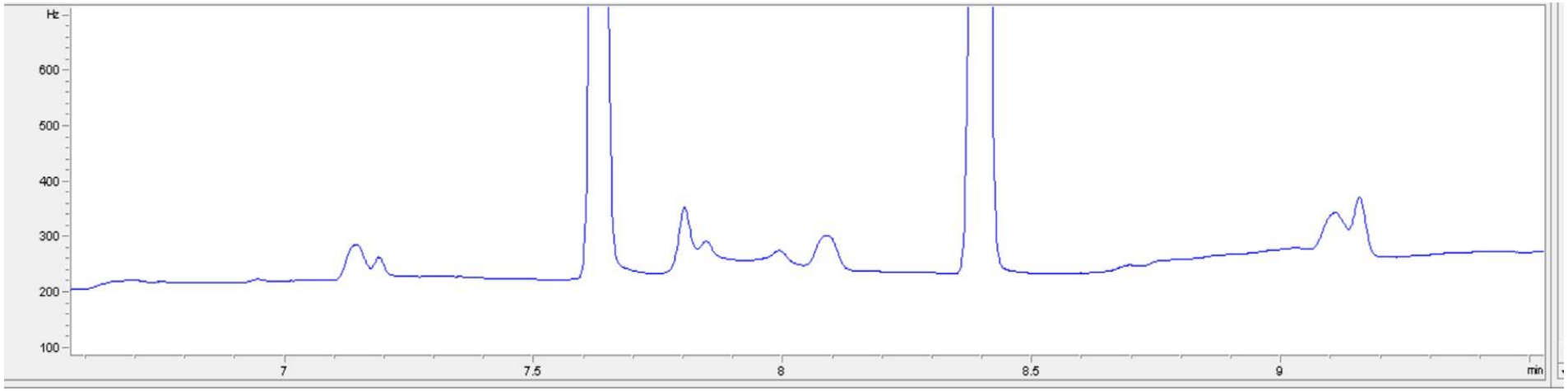
- Peaks
  - Any reproducible, sharp chromatographed peak
- Siloxanes (even though it looks like bleed, spectrally)
- Degradation product peaks: endrin aldehyde, endrin ketone, DDE, DDD
- Carryover of sample compounds
- Splitting of peaks

How Can We Prevent Carryover or Extra Peaks in the Future?

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

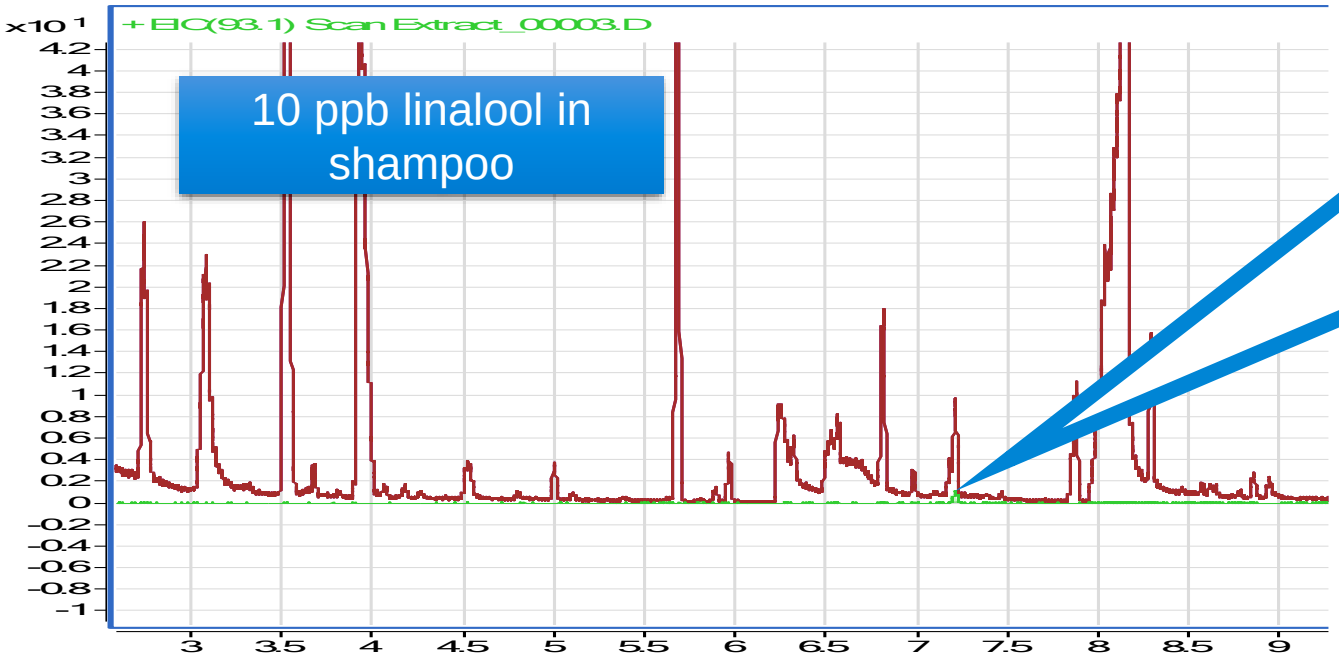


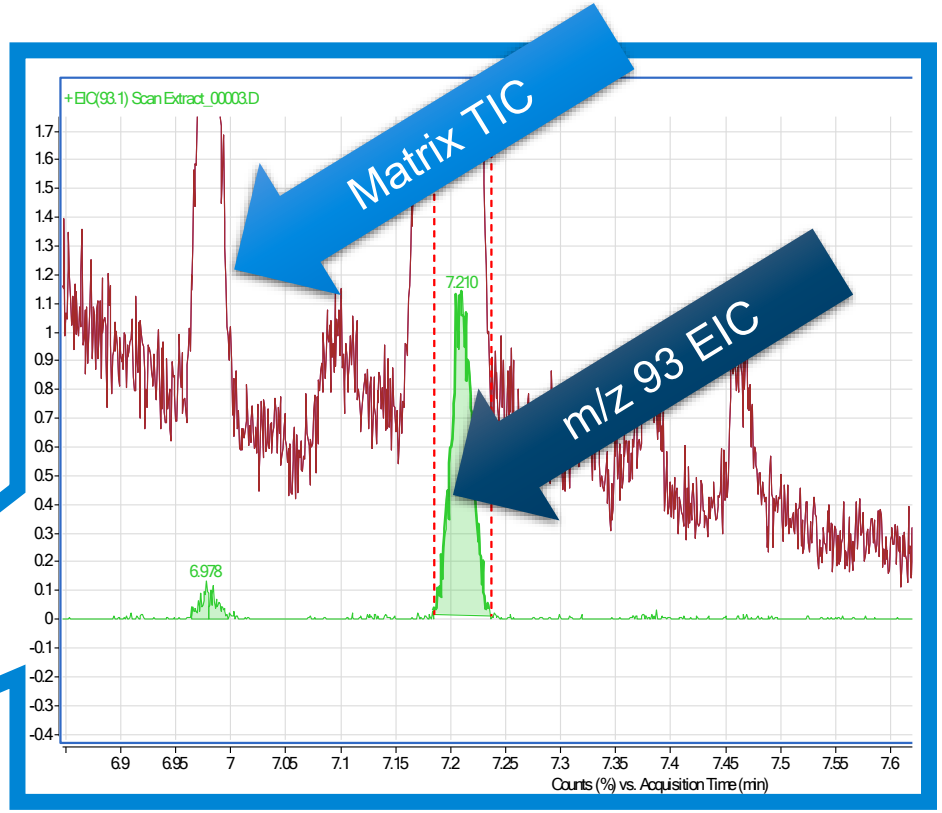
When it *should* look like…



The Matrix

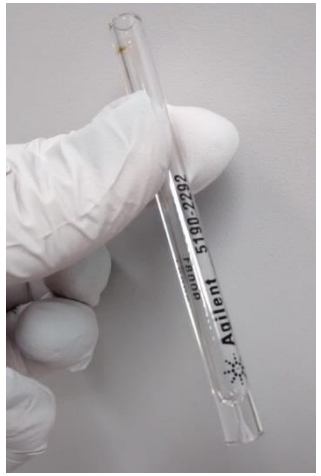
If your target ions are buried beneath matrix peaks, it might be time to trim the column or do sample cleanup



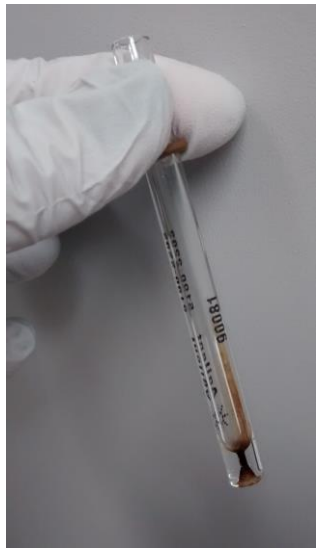


…or *improve* your sample cleanup

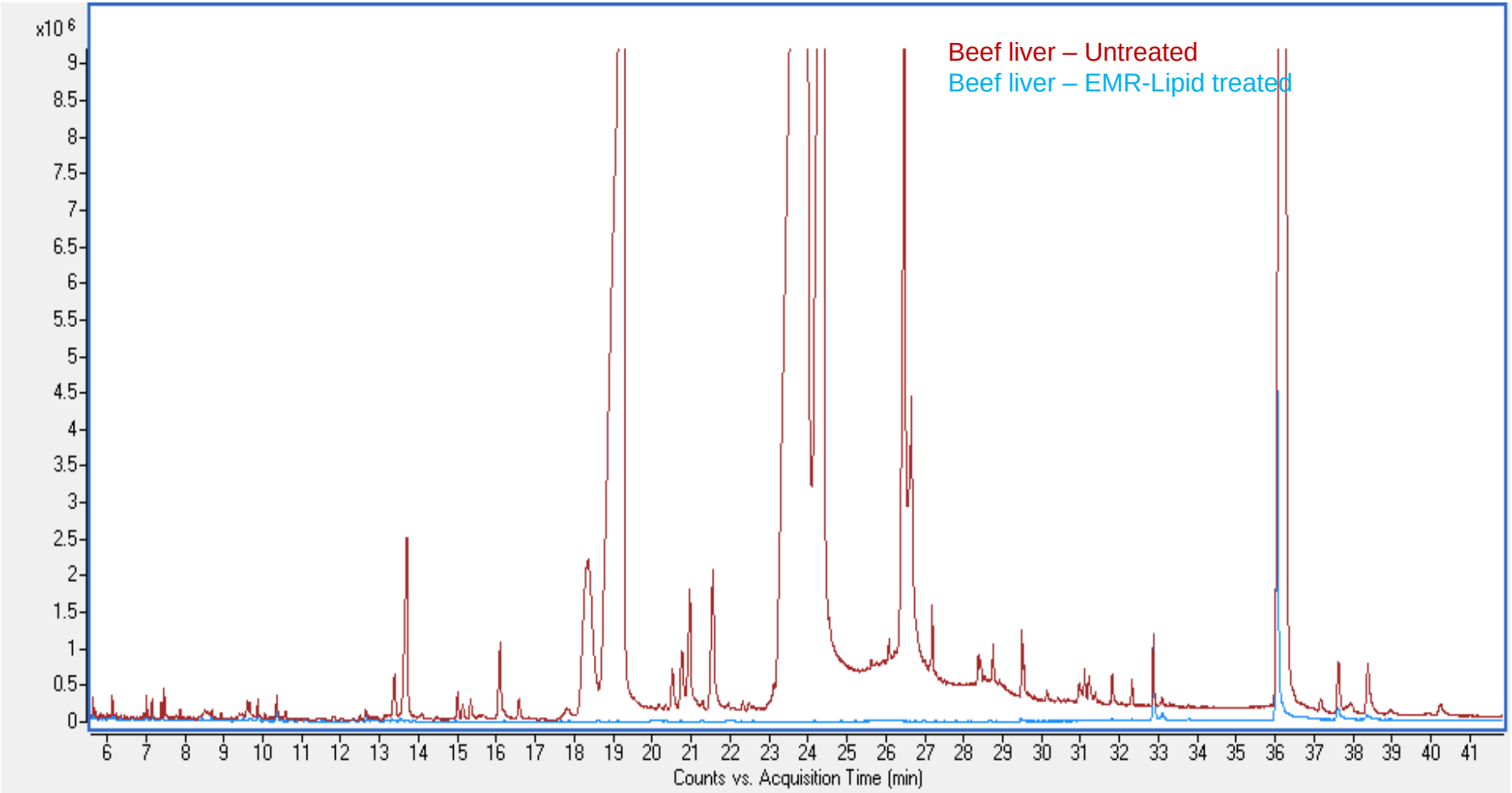
The Importance of Sample Cleanup



50 samples
with cleanup

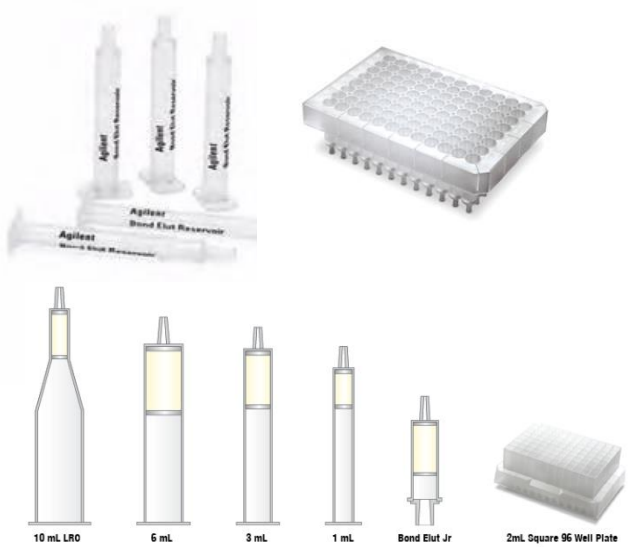


50 samples
without cleanup



For sample cleanup help, please contact us at spp-support@agilent.com.

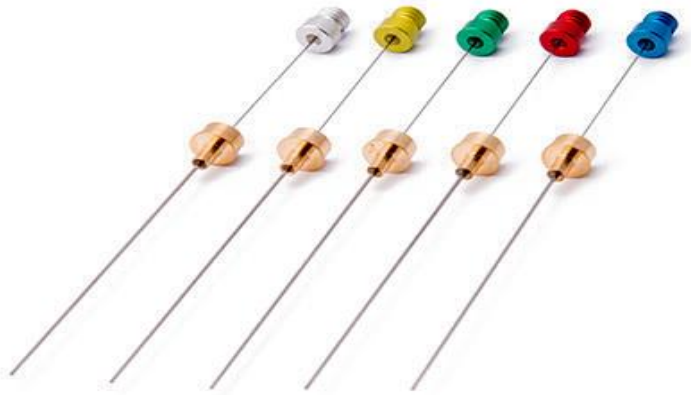
Offline Options for Sample Matrix Removal



Bond Elut Solid Phase Extraction cartridges and plates

Filter vials

QuEChERS



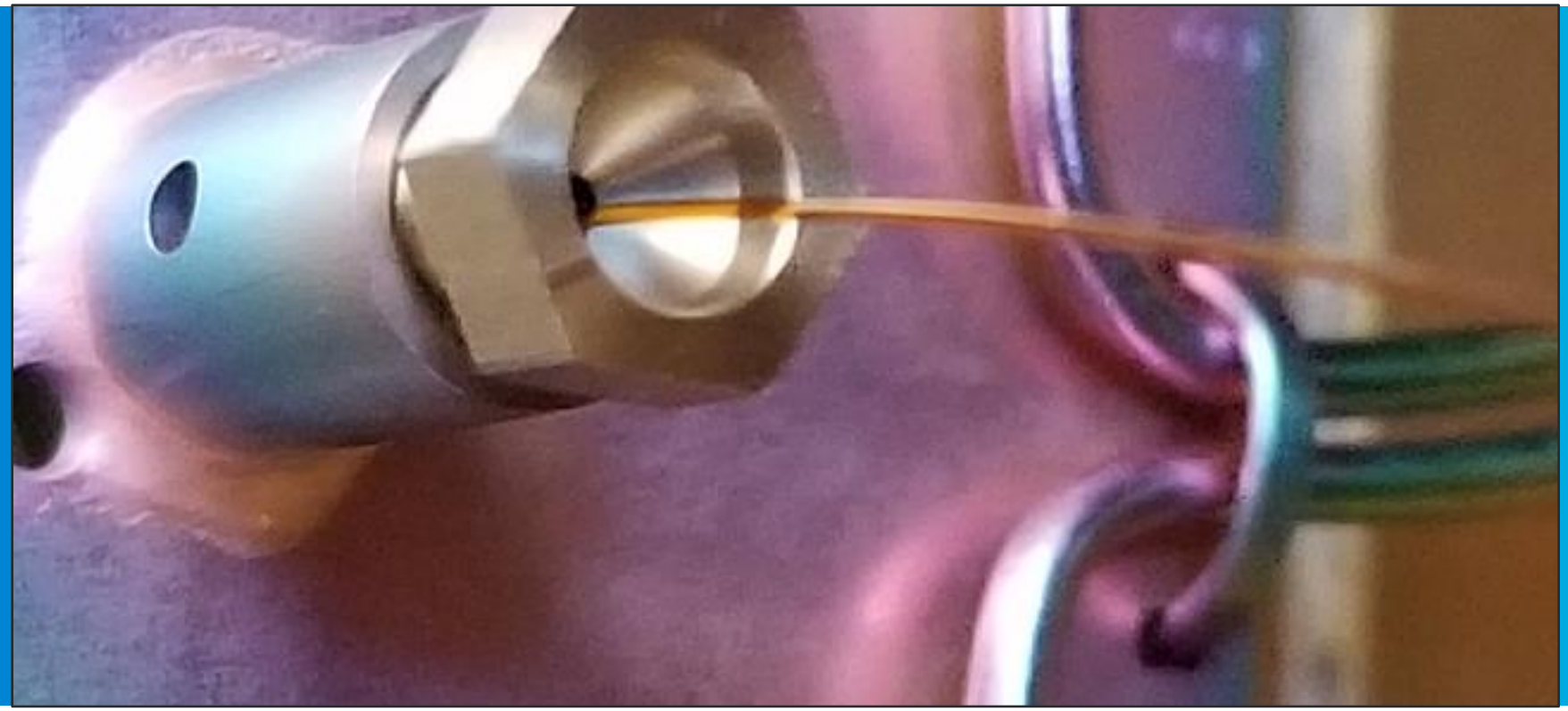
SPME

Captiva EMR-Lipid filtration cartridges and plates

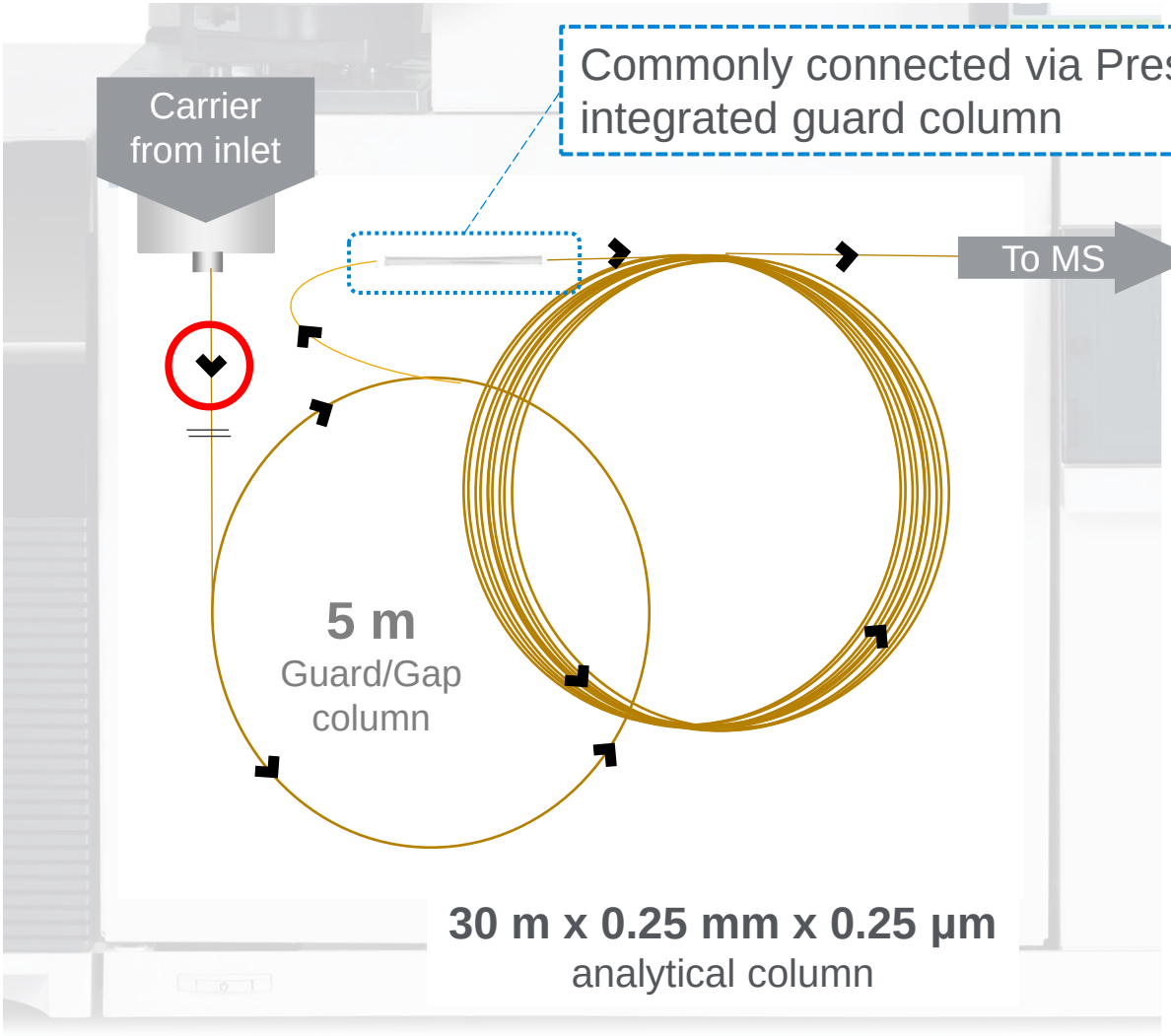
Chem Elut S

Captiva syringe filters

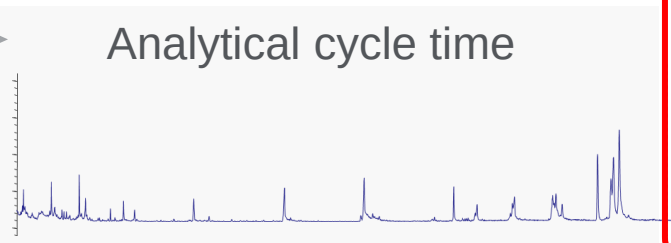
An Introduction to Backflush Techniques

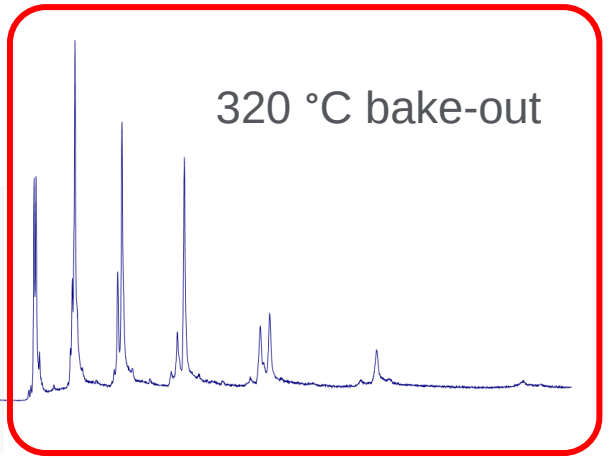


Conventional Single-Column Analysis



Analytical cycle time

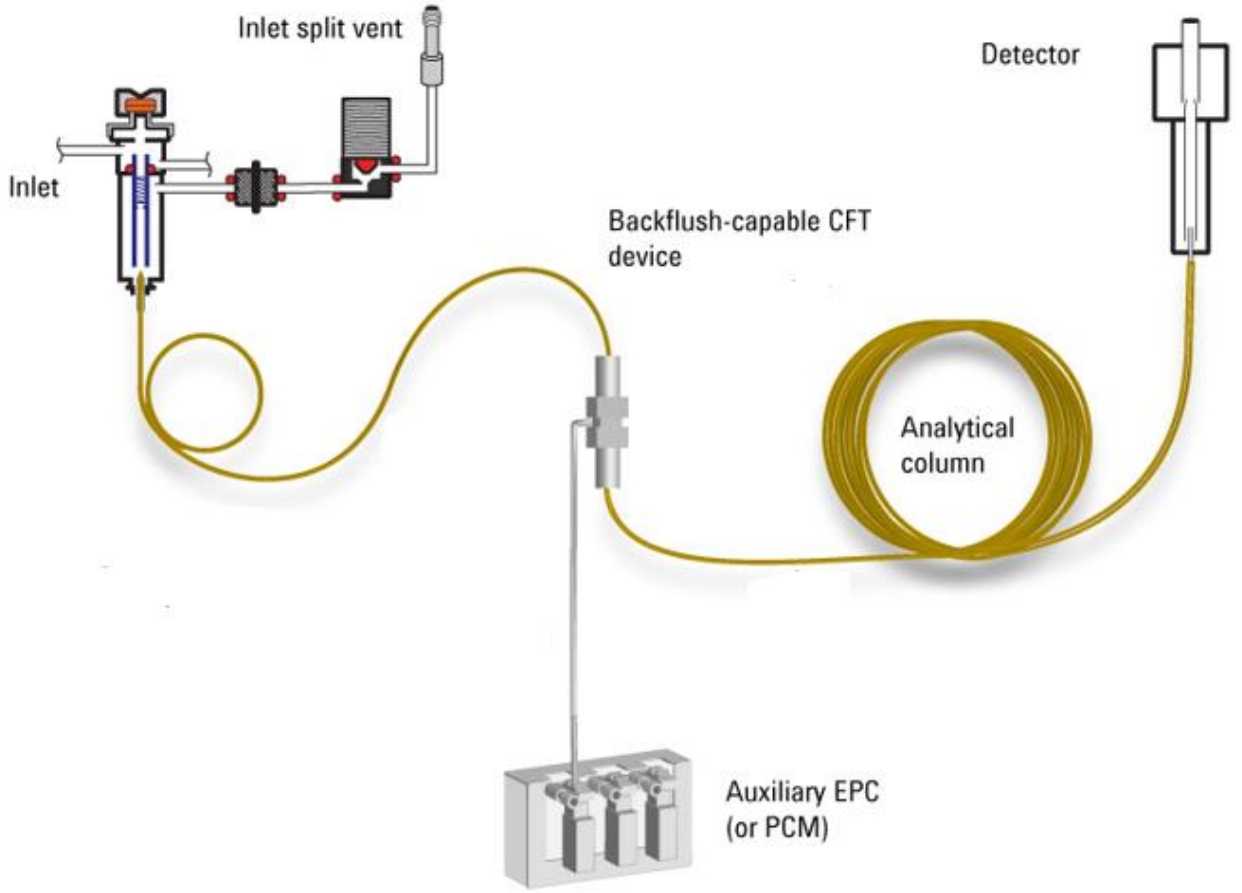




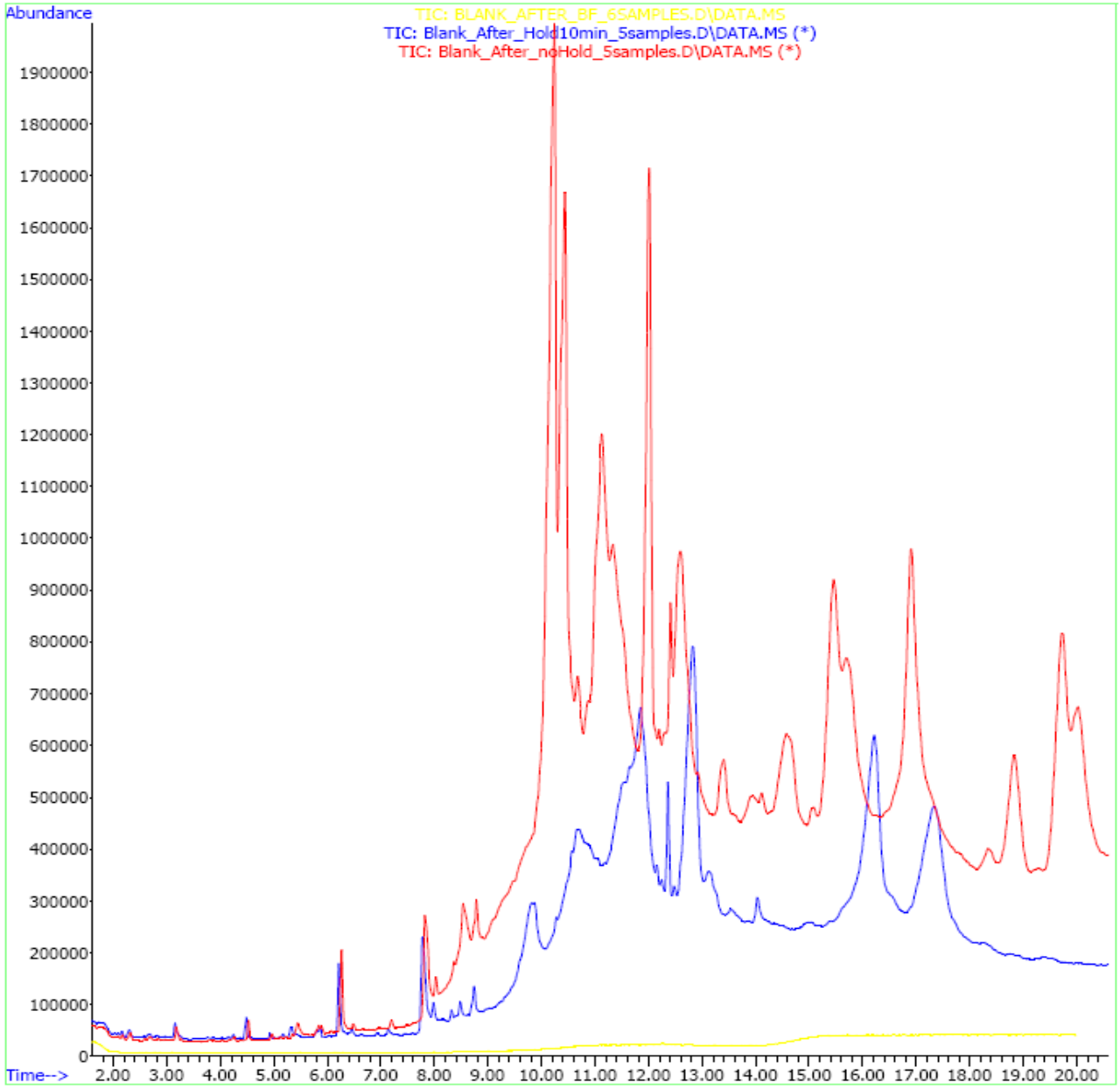
High(er) boiling matrix collects deteriorating data quality and results in a column trimming maintenance event (downtime).

Why Use Backflush?

- Avoid unwanted sample components from entering the analytical column
- Avoid heavy compounds from reaching the detector
- Generates shorten run times and increases sample throughput



Why Use Backflush?

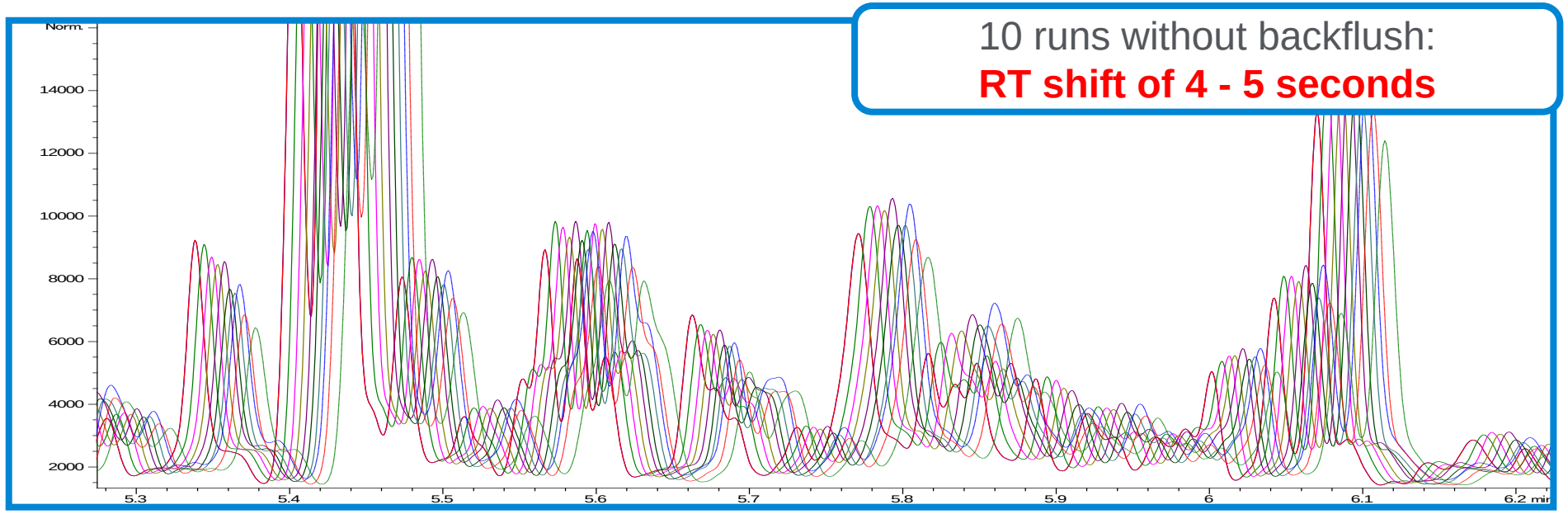


Blank after five injections (normal method)

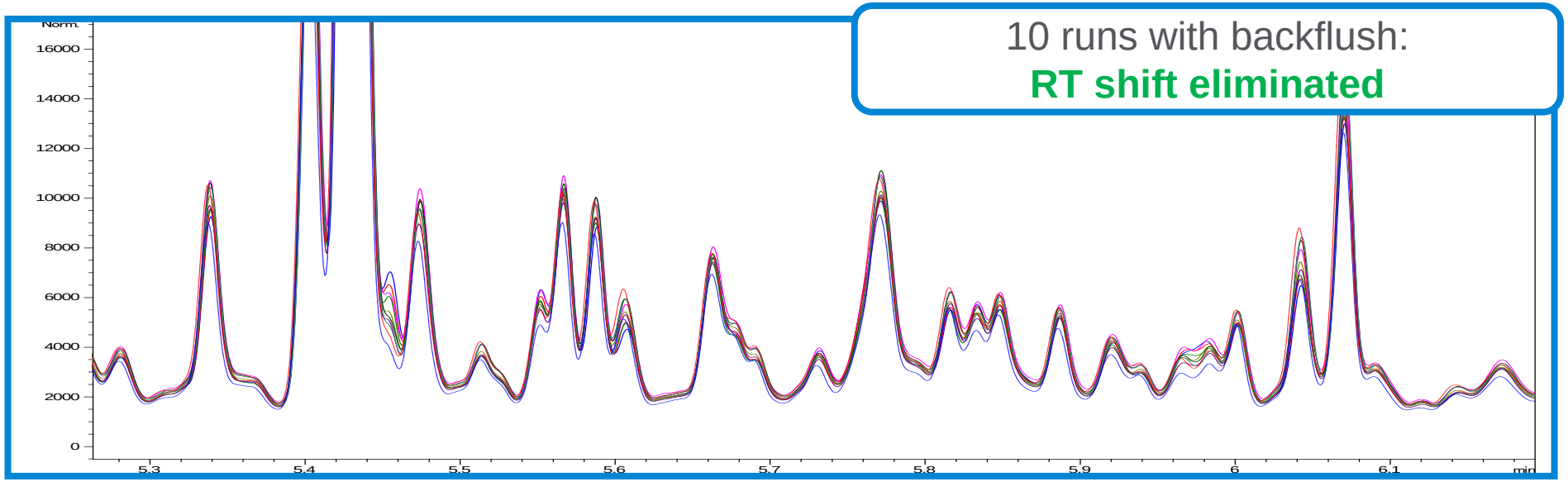
Blank after five injections (with 10 minute bake at 280 °C)

Blank after five injections (2 minute backflush, 50 PSI at 280 °C)

Why Use Backflush?

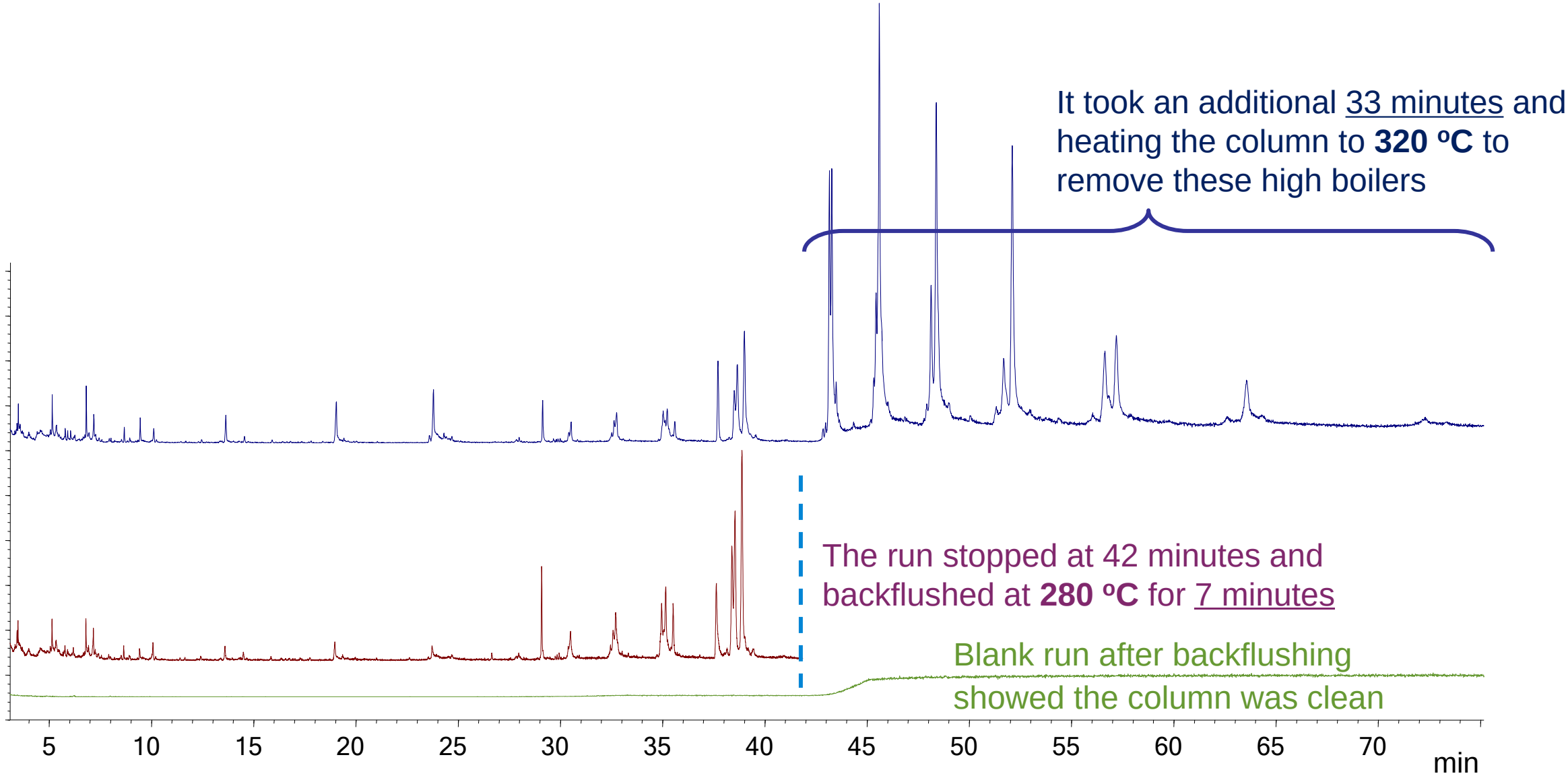


10 runs without backflush:
RT shift of 4 - 5 seconds

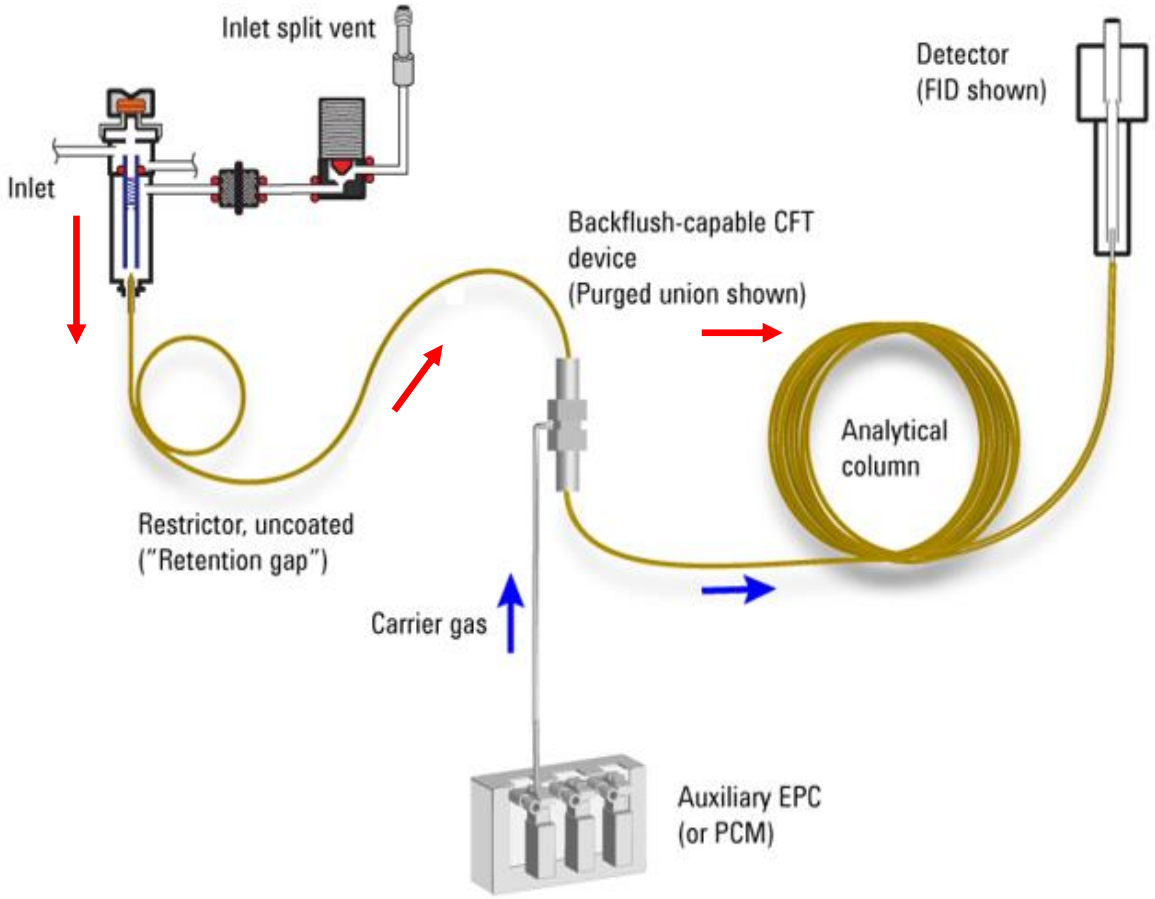


10 runs with backflush:
RT shift eliminated

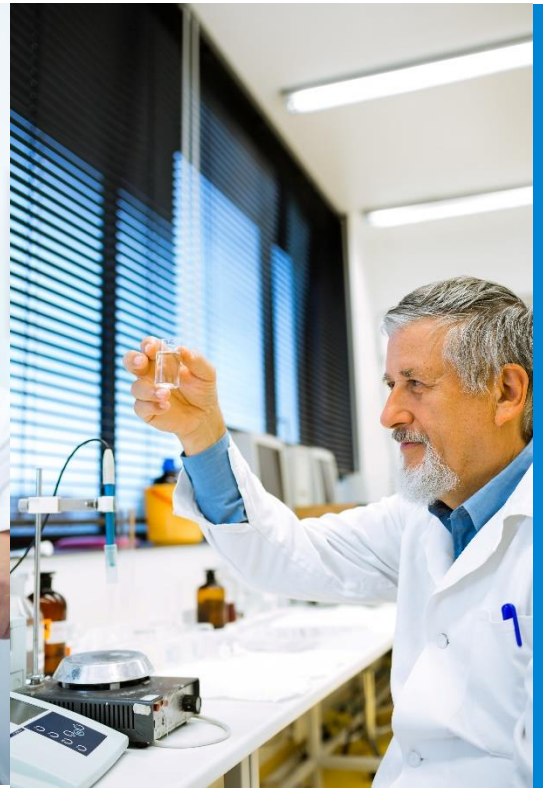
Why Use Backflush?



Why Use Backflush?



Additional Troubleshooting Techniques

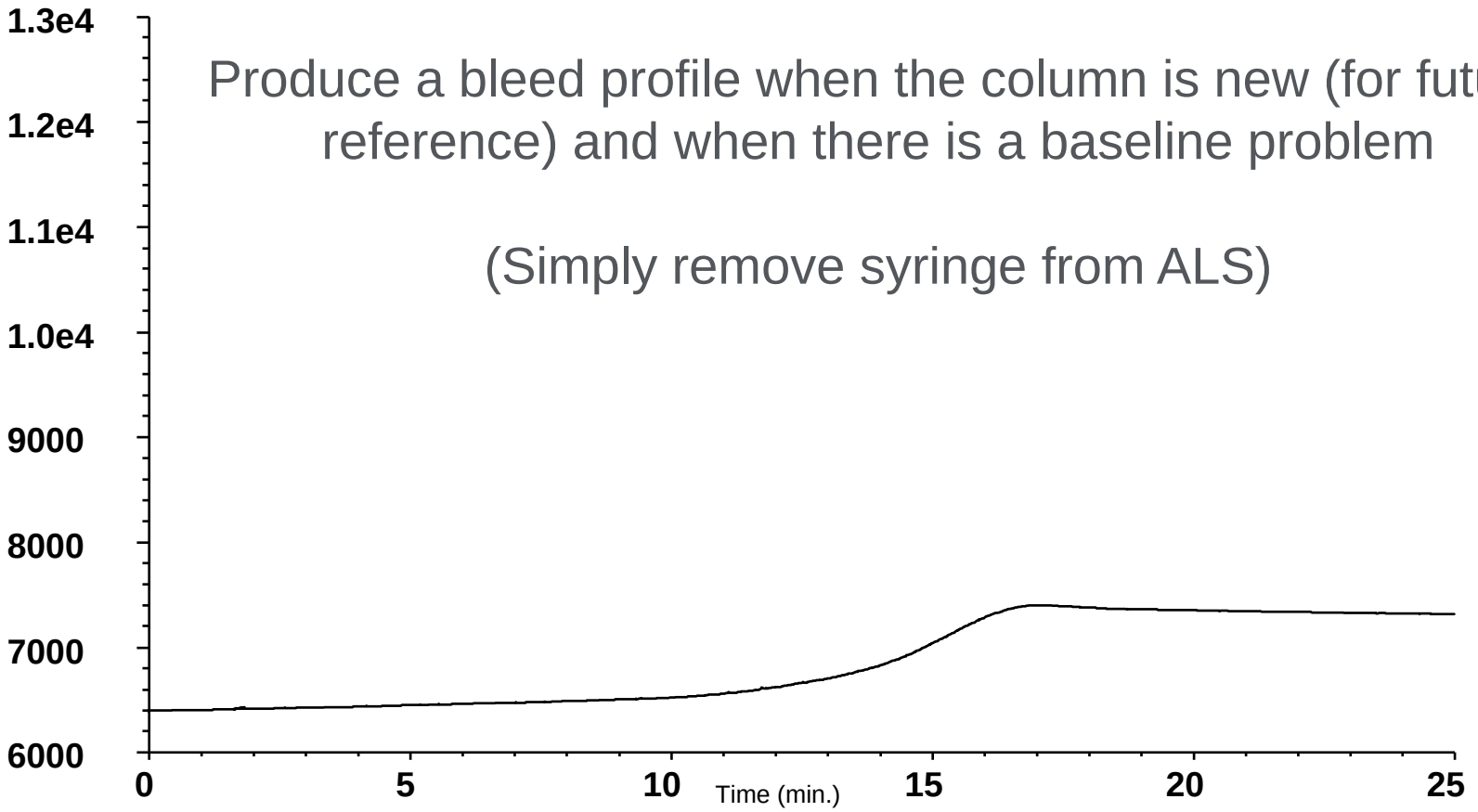


Troubleshooting Tools

Bleed profile (noninjection): *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



Produce a bleed profile when the column is new (for future reference) and when there is a baseline problem

(Simply remove syringe from ALS)

Agilent J&W DB-1, 30 m x 0.32 mm id, 0.25 µm
Temperature program // 40 °C, hold 1 min // 20 °C/min to 320 °C, hold 10 min

Test Mix – Make Your Own

A test mix is used to determine how “good” the column is, or whether 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
FAMEs, PAHs	Retention
Acids	Acidic character activity
Bases	Basic character activity

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

Standards Selection

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- Rigorously tested and manufactured under ISO 9001, ISO 17025, and ISO 17034 accreditation
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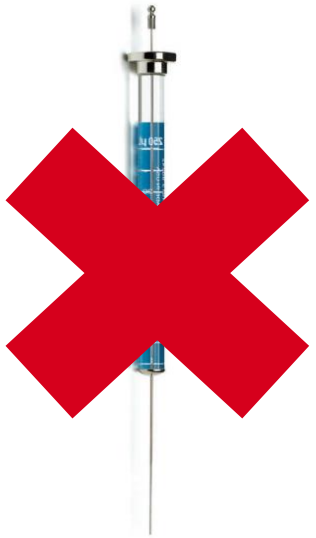
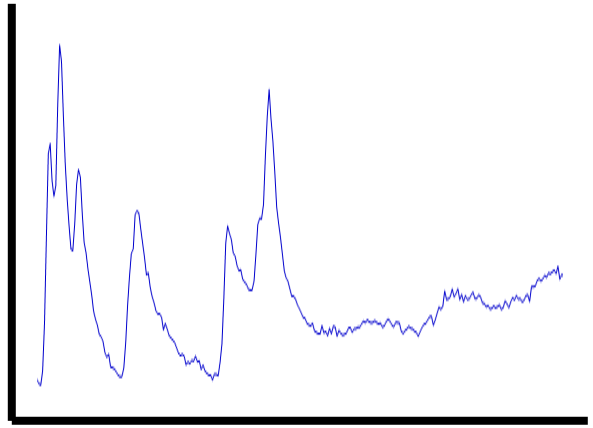
Perform a Noninjection “Blank”



Remove the syringe from the autosampler

Run your program



If you see peaks, it is likely that inlet contamination exists

Condensation Test

A condensation test is used to isolate the cause of:

- Erratic baselines
- Ghost peaks or carryover

For use when problems are worse after periods when the GC is not in use

Condensation Test

Procedure

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

Condensation Test

Results

- If the first blank run is worse: contaminants (from the injector, lines, traps, or carrier gas) have been carried into the column.
- If the blank runs are 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

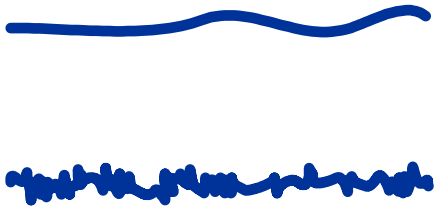
Isolate the detector

- Remove the column from the detector
- Cap the detector and turn it on
- Do a blank run

Jumper Tube Test

Isolation of detector – results:

Detector is OK



Detector is the problem

Jumper Tube Test

Isolate the injector

- Connect the injector and detector
  - 1 to 2 m of deactivated fused silica tubing
- Turn on the carrier gas
- Do a blank run

Jumper Tube Test

Isolate the injector – results:

Injector OK

Injector, lines, or carrier gas are contaminated

Jumper Tube Test

Isolate the column

- Reinstall the column
- Set up as before
- Do a blank run

Jumper Tube Test

Isolate the column – results:

- If the problem persists, it’s the column
- If the problem is gone, a previous leak, solid debris, or installation is the issue

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 with troubleshooting.

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Remember

Complete system = carrier gas + injector + column + detector + data system

- Multiple causes and effects
- 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

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