Mass Spectrometer Troubleshooting

What to Check When Your Results Go Wrong

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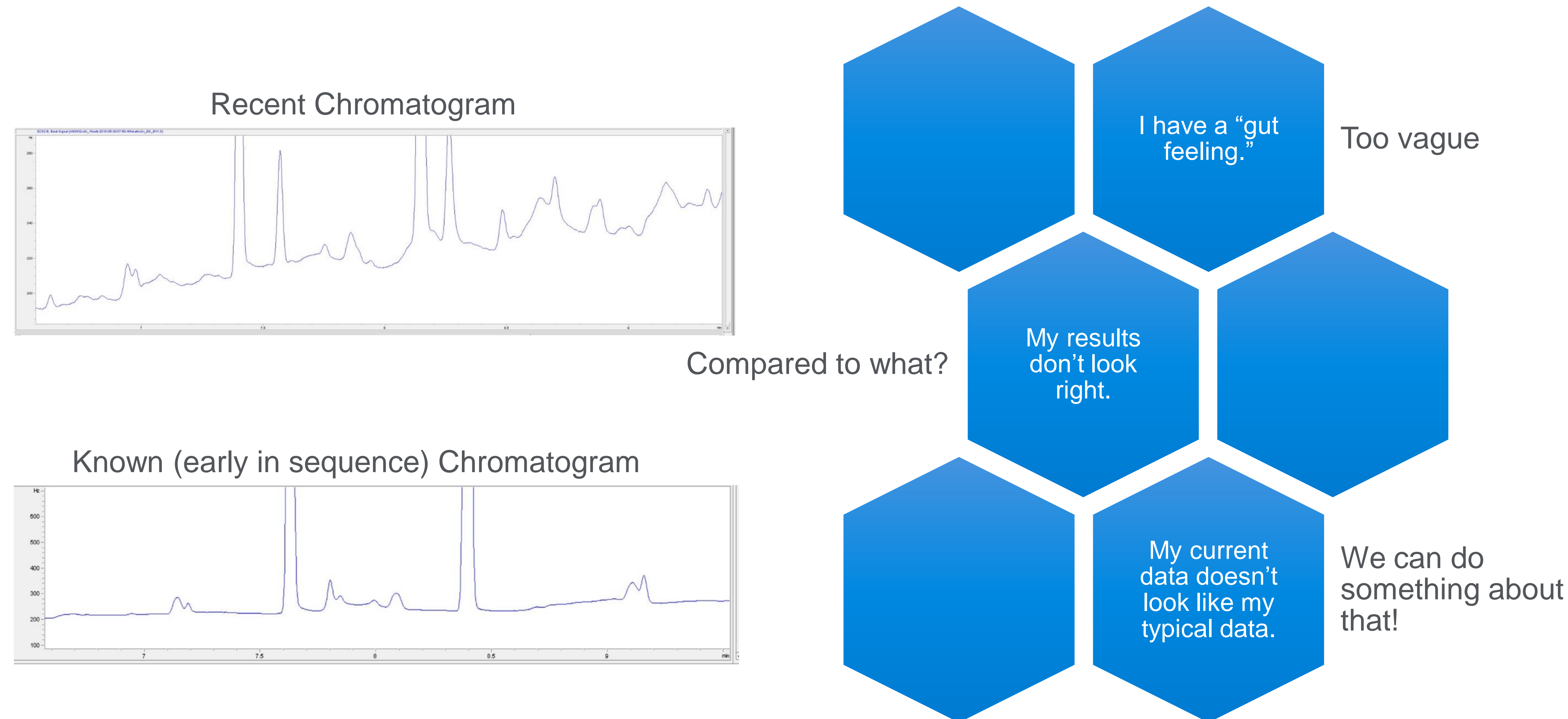
How do we know something is wrong?



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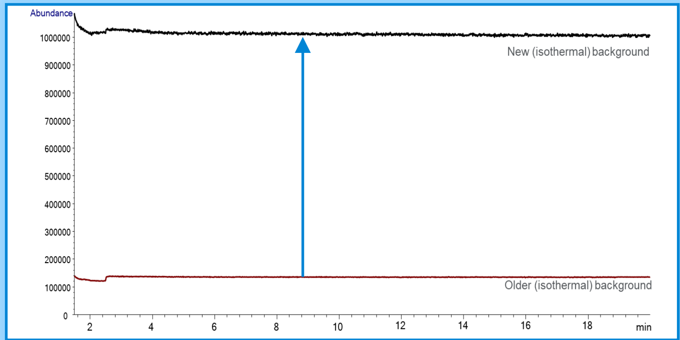
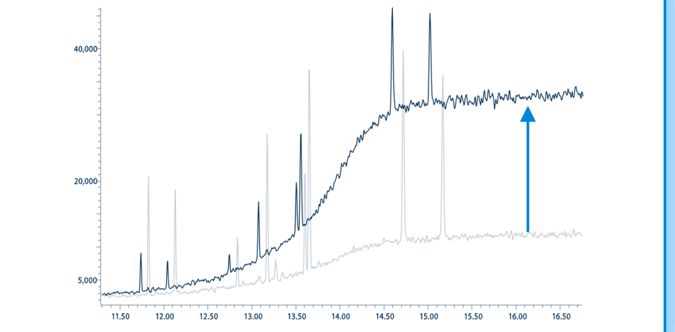
What to Check When Your Results Go Wrong: Common ailments of Mass Spec

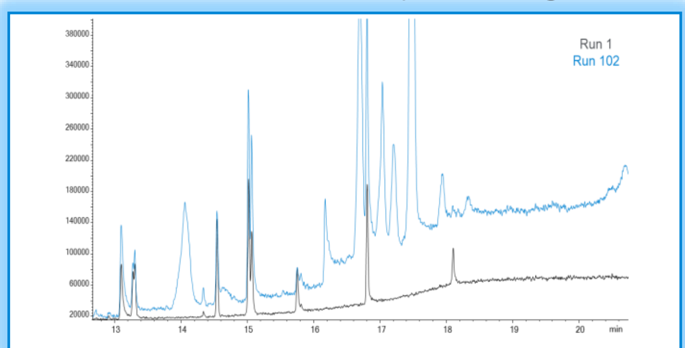
- High background
- Noisy background
- Too many peaks!
- Diminished peaks
- Few or no peaks
- Poor data quality (peak shape, etc.)

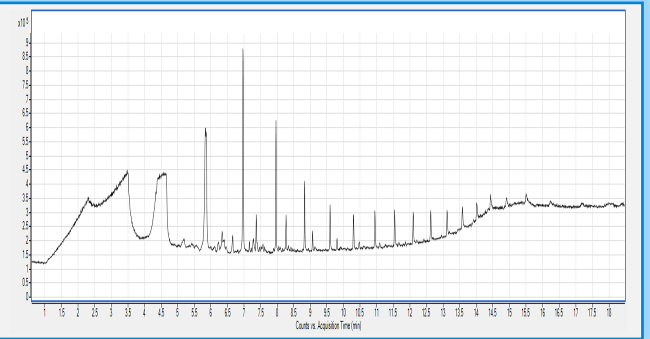
High backgrounds?



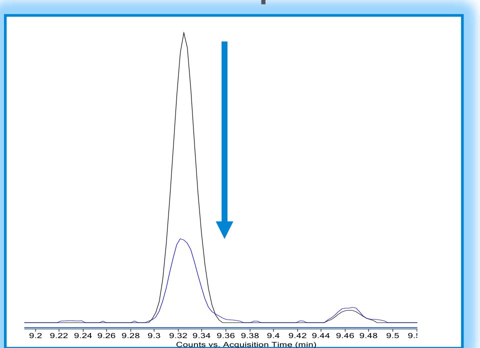


Noisy backgrounds or too many peaks?

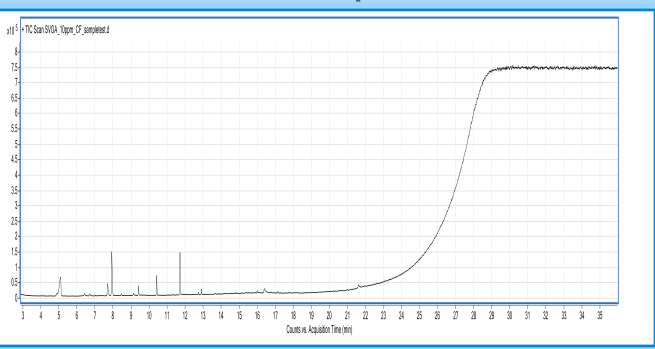




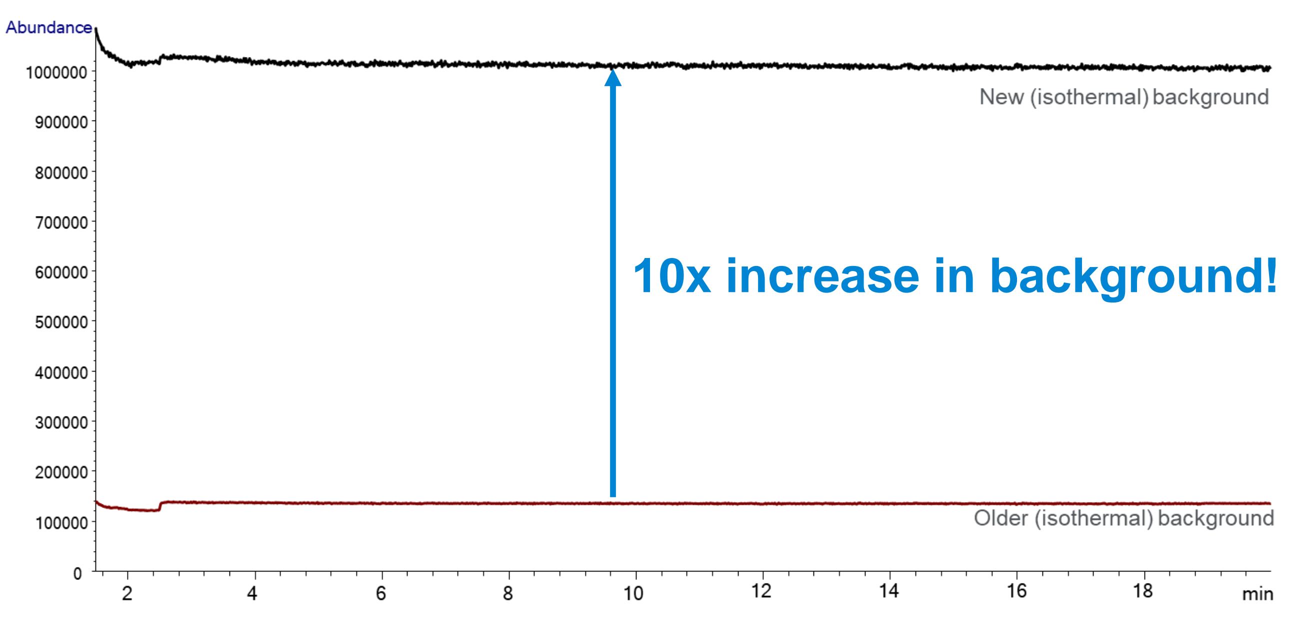
Lower response?



Few or no peaks?



My GC/MS has a high background!? What could be the culprit?



Questions to ask:

- Is it really a high background?
  - Did I just pump down the instrument?
- What gas quality do I have?
- Does the system have gas filters?
  - Have I checked them recently?
- What do my nitrogen, oxygen and water backgrounds look like?
  - What do they normally look like?
- Do I have any leaks?

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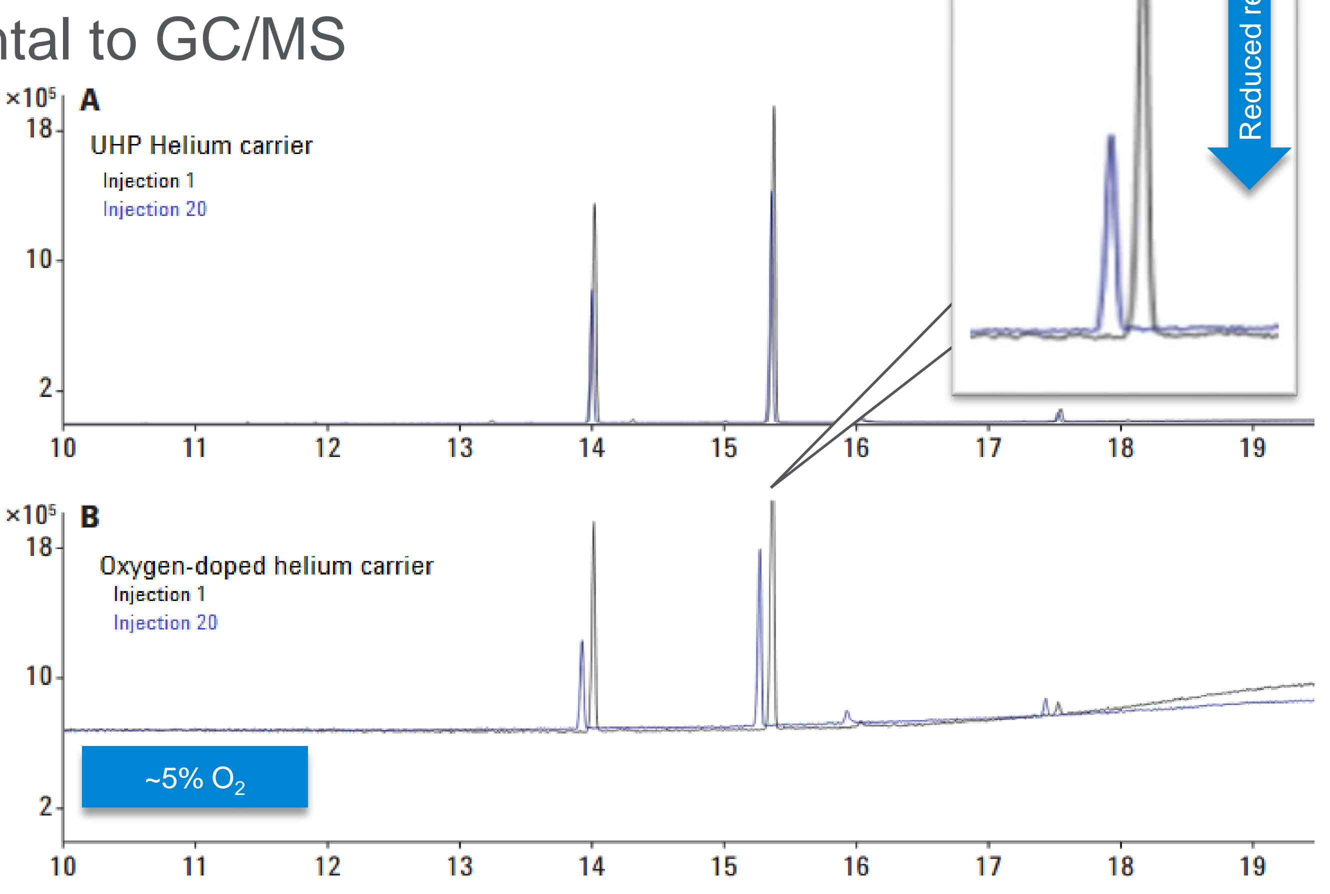
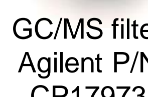
Let's talk about Gas Quality and Filters

- Oxygen in carrier gas is detrimental to GC/MS
  - Reduced response
  - Elevated background
  - Irreversible column damage
  - Impaired electron multiplier function
  - Premature filament, liner lifetime

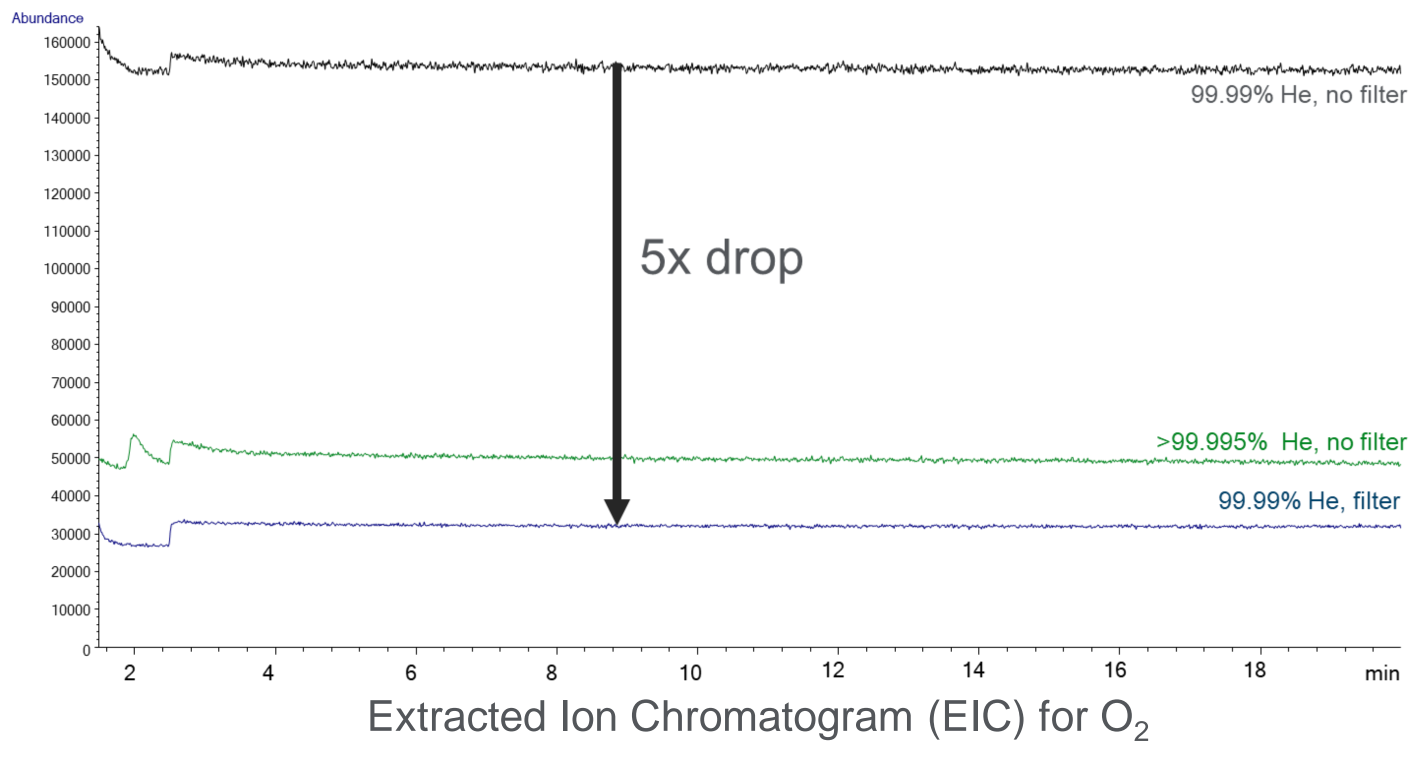
- Use UHP carrier gases
  - 99.9995% or greater
- Use GasClean carrier gas filters





GC/MS filter
Agilent P/N
CP17973

Let's talk about Gas Quality and Filters: If you used lower quality gases, how much O_2 could the filter clean up?



Extracted Ion Chromatogram (EIC) for O_2

Installing (and properly purging) the GasClean carrier gas filter lowered the O_2 signal by a **factor of 5**!

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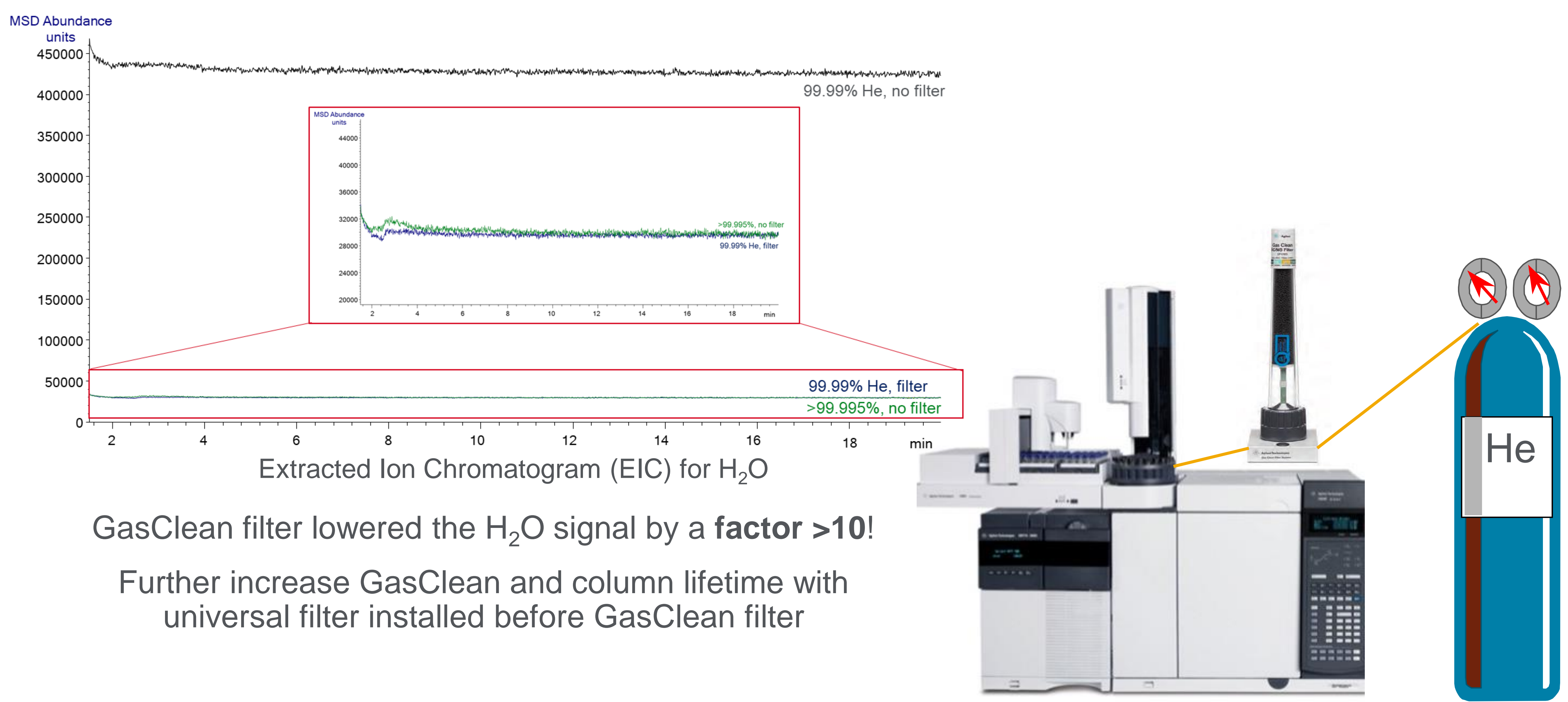
Let's talk about Gas Quality and Filters:
If you used lower quality gases, how much H_2O could the filter clean up?



Extracted Ion Chromatogram (EIC) for H_2O

GasClean filter lowered the H_2O signal by a **factor >10**!

Further increase GasClean and column lifetime with universal filter installed before GasClean filter

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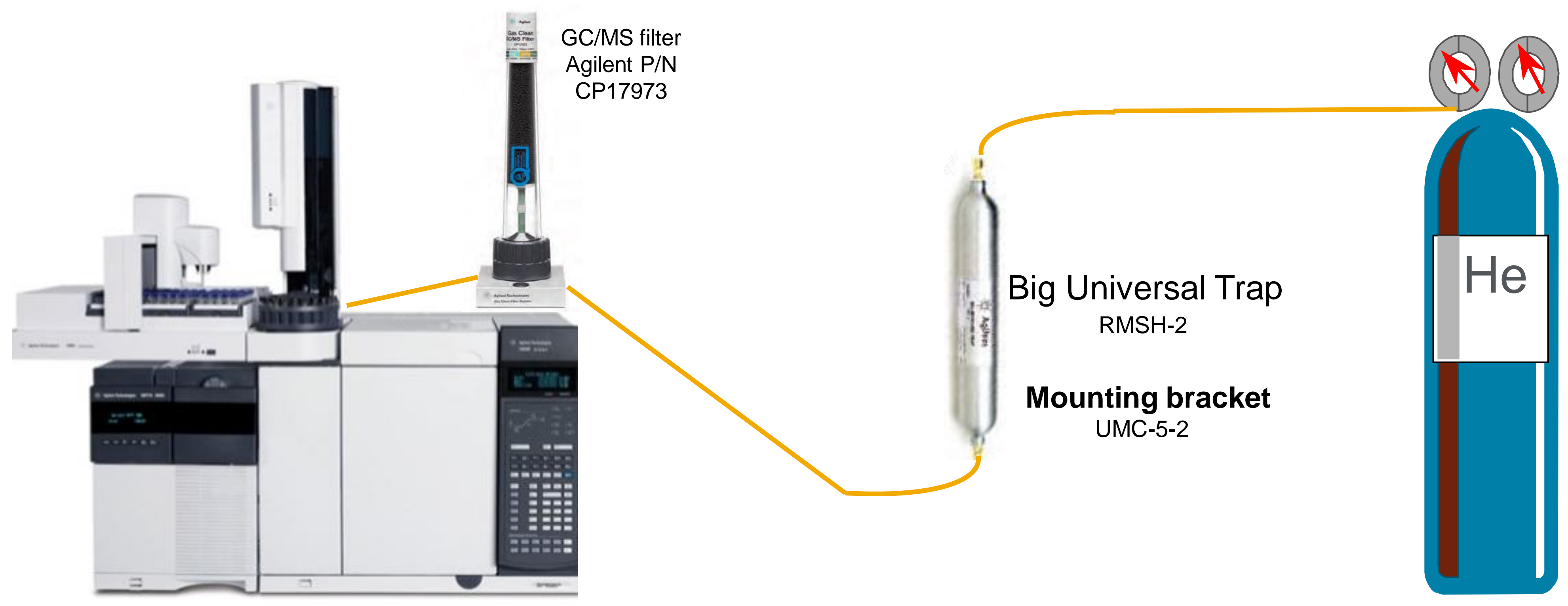
Let’s talk about Gas Quality and Filters:
If lower quality gases were used, how much background could the filter clean up?



- Install the Universal trap vertically – use the mounting bracket(s)
- Extend the lifetime of your GasClean (indicating) filter AND (most importantly) your column!

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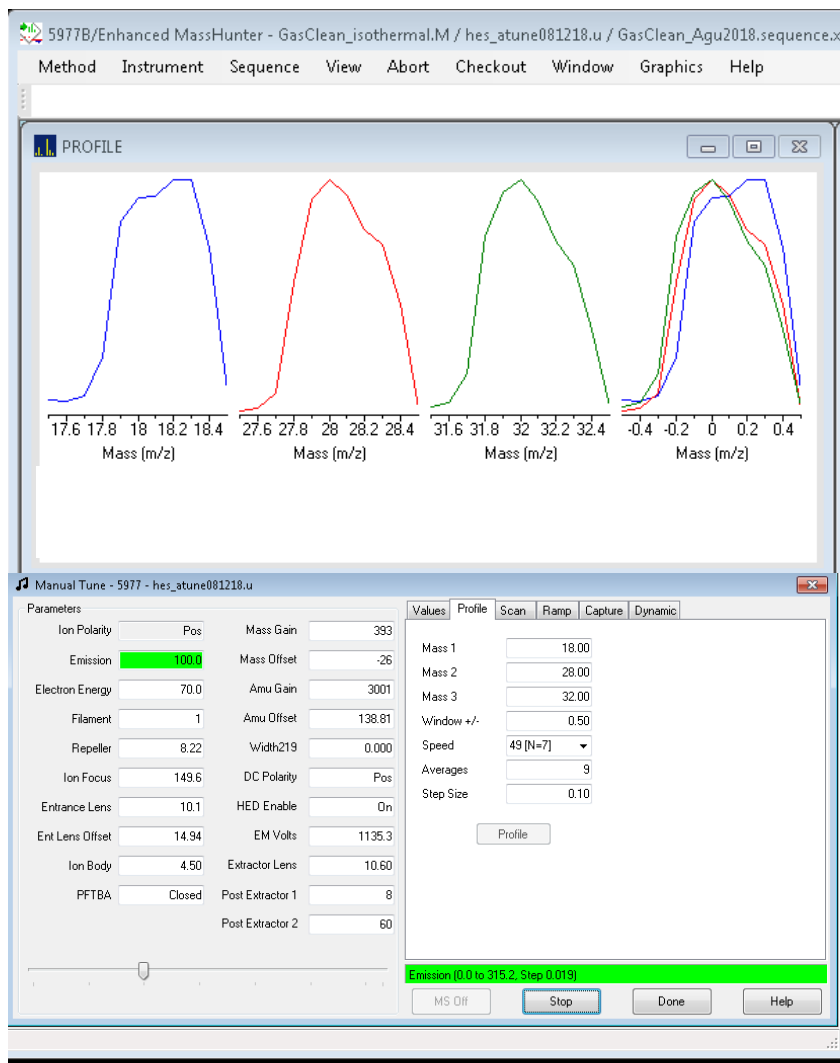
I have gas filters and high quality gas, and I still have a high background!



Use manual tune (before any experiments!) to check for leaks/background

- Ions 18, 28, 32 m/z

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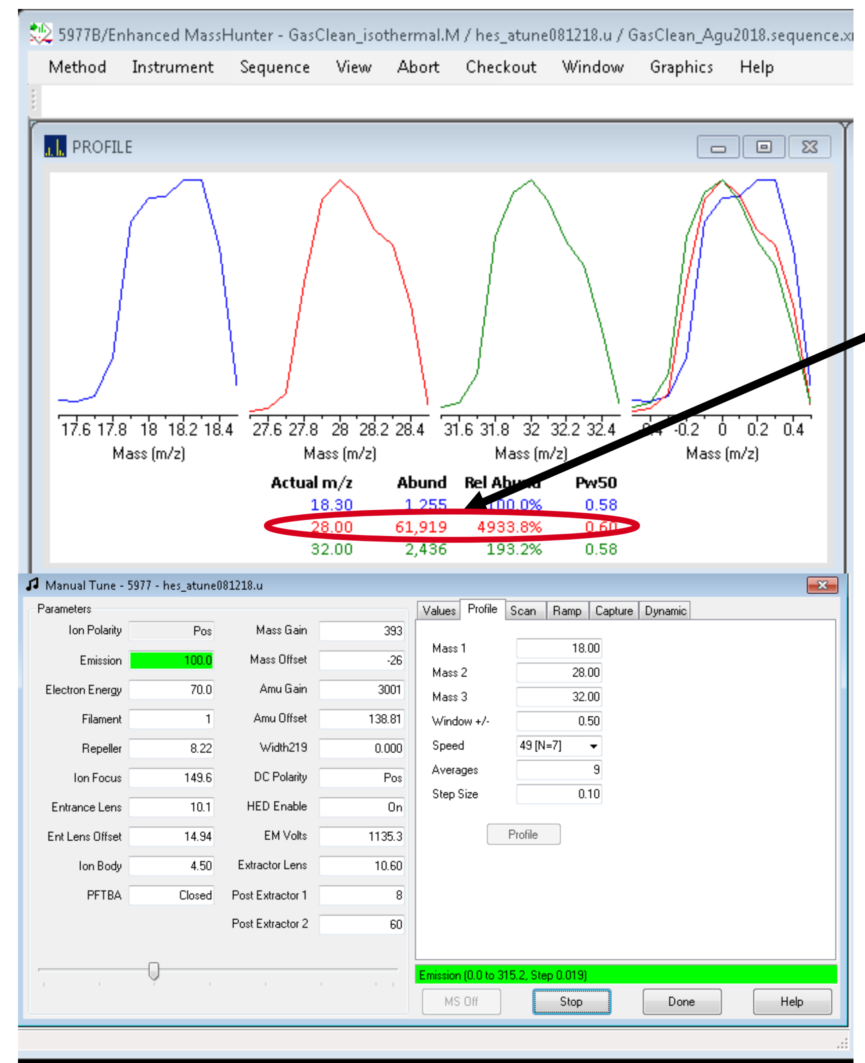
I have gas filters and high quality gas, and I still have a high background!



Use manual tune (before any experiments!) to check for leaks/background

- Ions 18, 28, 32 m/z

60,000 counts for N_2 is definitely high.

What to check:

1. Verify gas fittings are leak-free.

   Water/methanol mixture (external to GC/MS ONLY!)

   Leak detector

Agilent G3388B Leak Detector

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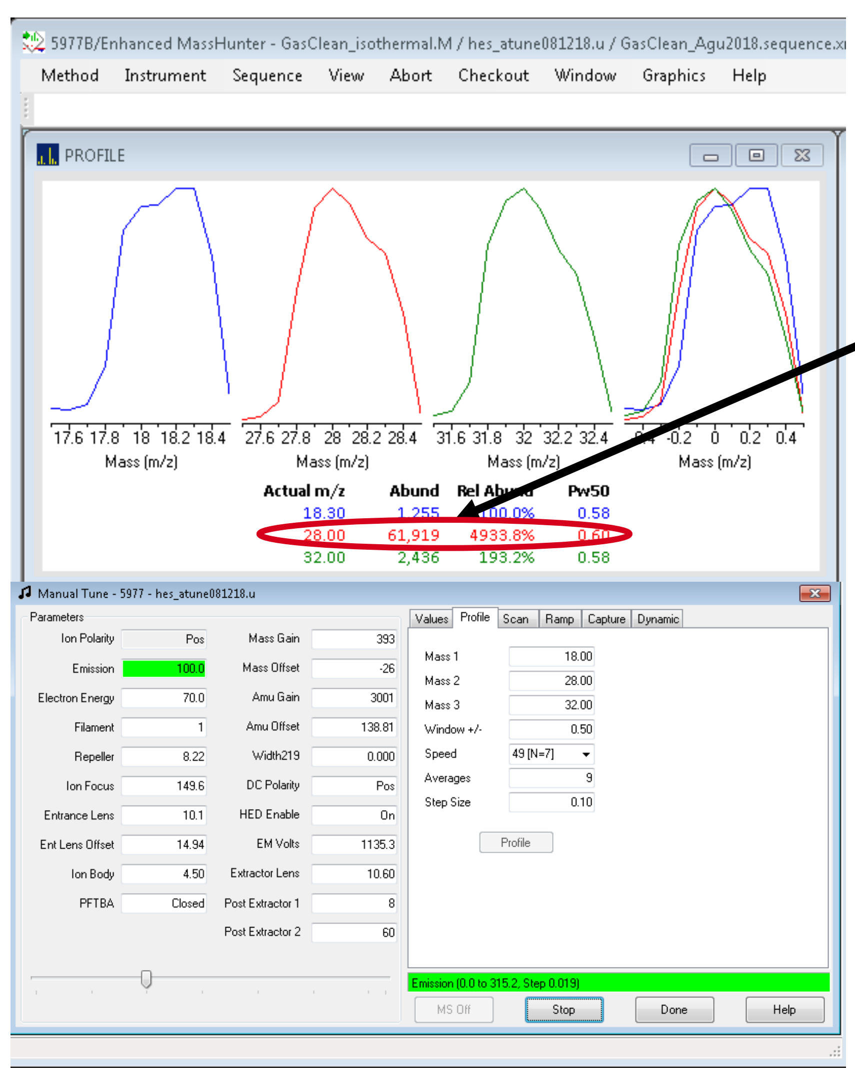
I have gas filters and high quality gas, and I still have a high background!



Use manual tune (before any experiments!) to check for leaks/background

- Ions 18, 28, 32 m/z

60,000 counts for N_2 is definitely high.

What to check:
1. Verify gas fittings are leak-free.

My fittings outside of the GC/MS are leak-free, what else is there to check?

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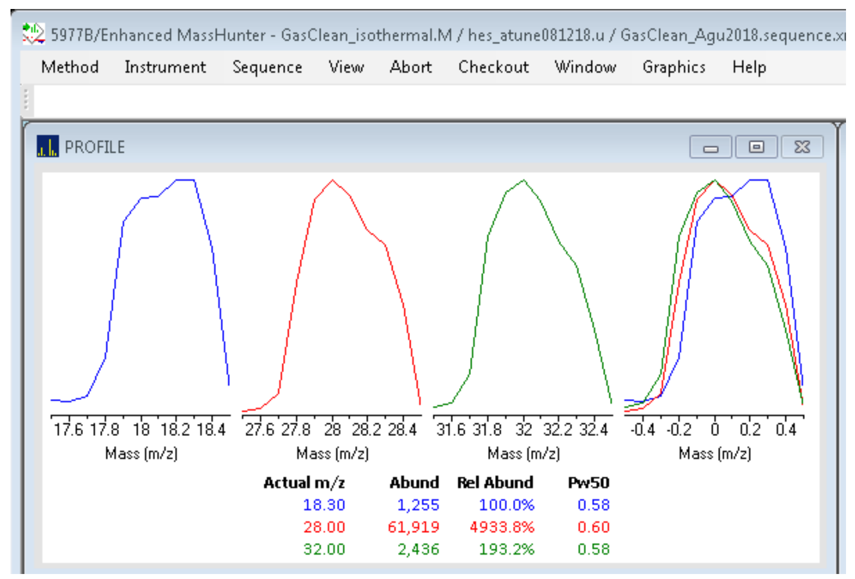
I have gas filters and high quality gas, and I still have a high background!



Use manual tune (before any experiments!) to check for leaks/background

- Ions 18, 28, 32 m/z

60,000 counts for N_2 is definitely high.

What to check:
1. Gas line fittings (Done)

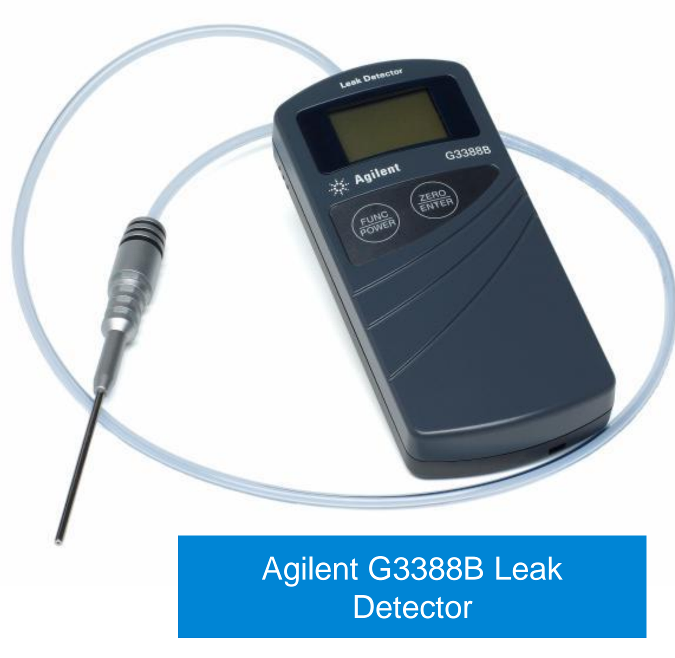
2. Check the vent valve, MSD transfer line nut and side door.

How?

Use leak detector and/or electronics duster to find your leaks!

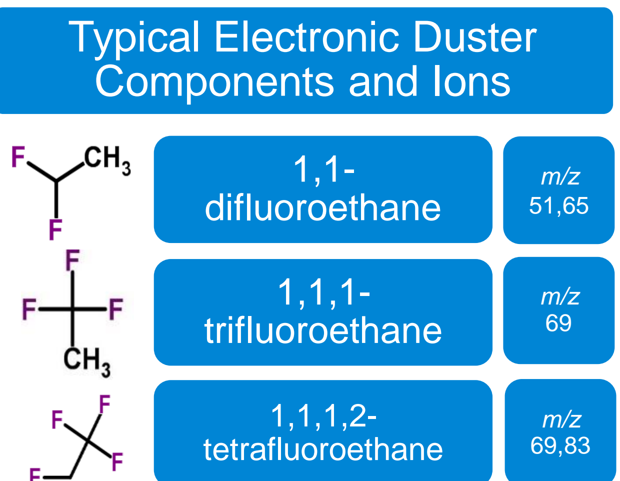
Why use a leak detector?

- High sensitivity
- Recommended for leak detection in gas plumbing and fittings



Agilent G3388B Leak Detector

link



Typical Electronic Duster Components and Ions		
F, CH_3, F	1,1-difluoroethane	*m/z* 51,65
F, F, F, CH_3	1,1,1-trifluoroethane	*m/z* 69
F, F, F, F	1,1,1,2-tetrafluoroethane	*m/z* 69,83

Use electronics duster

- Hold can upright (don't spray liquid!)
- Spray short bursts around possible leak points
- "Live" tune profiling for ions to pinpoint leak

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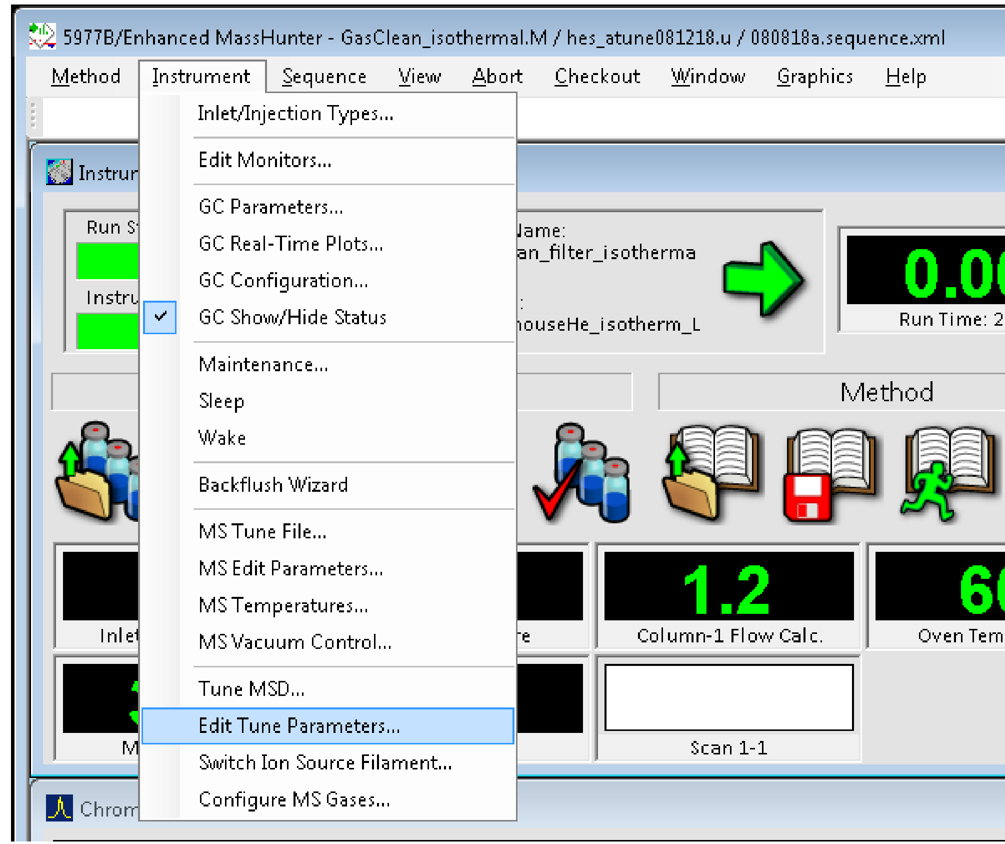
Using electronics duster to find system leaks: Manual Tune



MassHunter Data Acquisition

Navigate to MSD Manual Tune in the Data Acquisition

- Instrument > Edit Tune Parameters

Or

- View > Tune Vacuum Control
  - Parameters > Manual Tune

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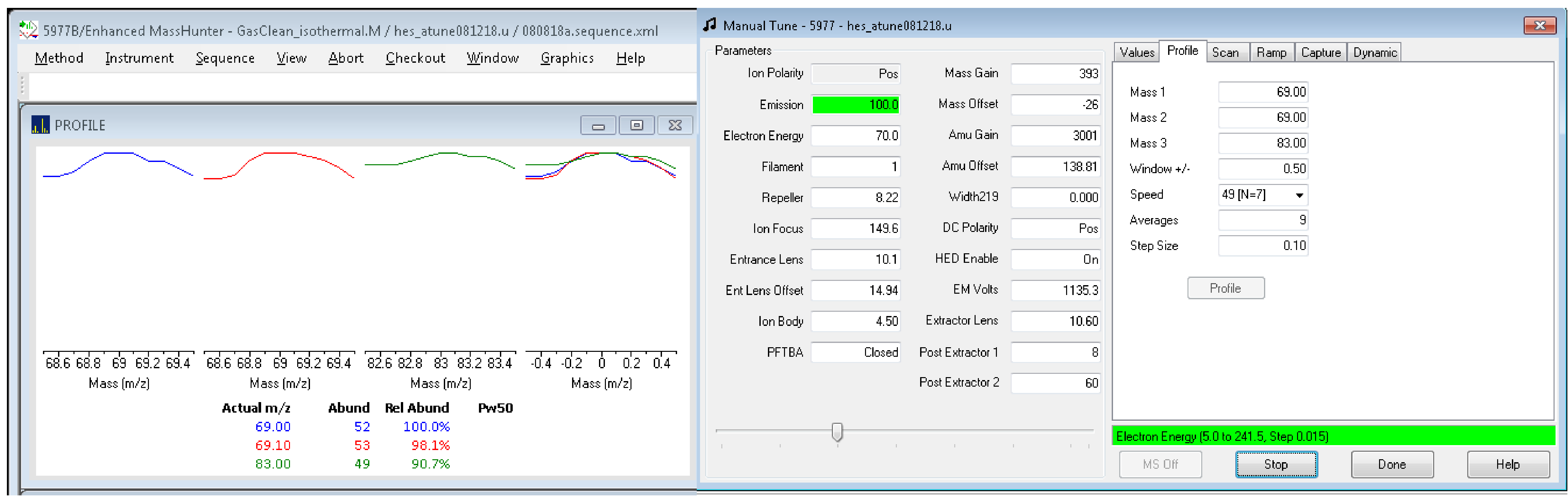
Using electronics duster to find system leaks: Manual Tune



- Use Profile tab to watch the main ions (69 and 83 m/z for my electronics duster)
- Spray short bursts at vent valve, transfer line, and side door

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Forgetting to retighten - or overtightening - the vent valve

- How does this happen?
  - Easily overlooked
  - Urge to overtighten fittings
- Why is it bad?
  - Overtightening compresses o-ring
  - Prevents leak-free seal formation
- How is it fixed?
  - After venting, retighten vent valve before maintenance activity
  - Tighten gently to avoid cross-threading
  - If leaks are persistent, replace vent valve o-ring

Agilent MSD
Vent Valve o-ring
P/N 0905-1014
link

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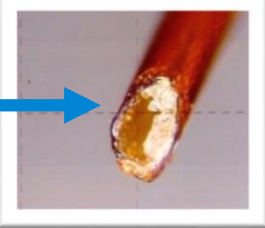
Check the Transfer Line Nut for Leaks

- Using a graphite/vespel ferrule, right?
  - Installed with the flat end of ferrule facing the MSD?
- Nut "loosens"* with heat cycles
- If you find a leak:
  - Tighten in small increments and then check again until no leak
  - Try to not overtighten the nut
    – If you have to apply a lot of pressure, vent and check the ferrule/threads

Be careful with the transfer line nut!

- Over-tightening damages transfer line threads, column
- Audible squeaking → overtightening

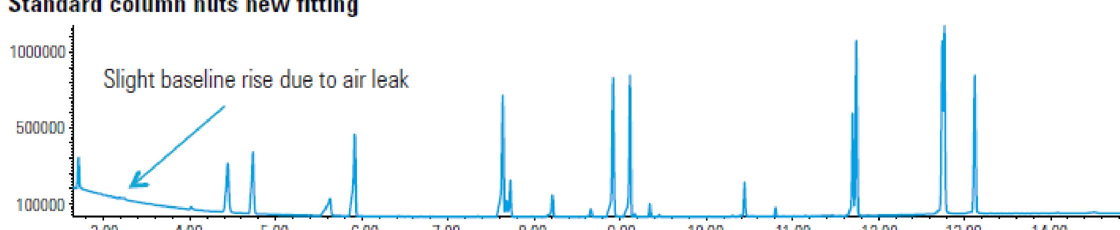
Crushed end of column

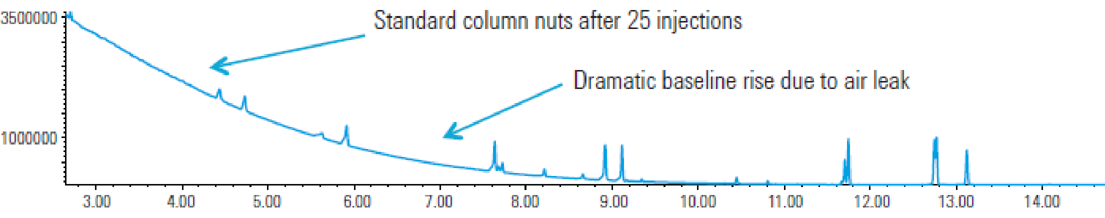


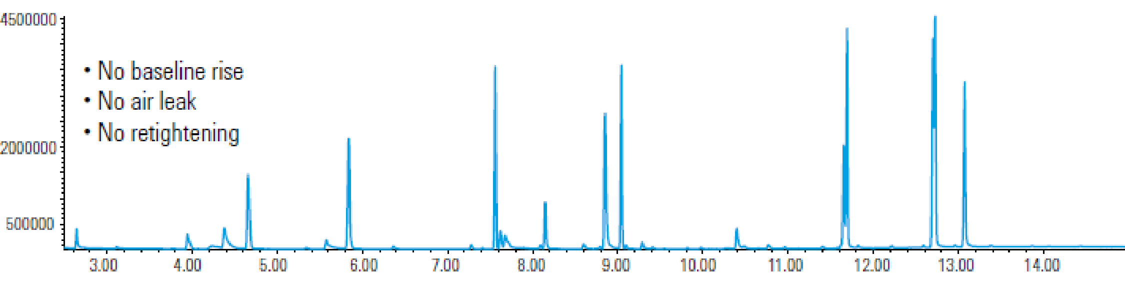
I always forget to check my transfer line nut the morning after a pump down….

- Switch to self-tightening MS column nuts
  - Spring-driven piston **continuously** presses against ferrule
  - Easily installed by hand
  - **Longer column lifetime, lower column bleed**
  - ONLY use the short graphite vespel ferrules
    - Install with flat edge TOWARDS MSD

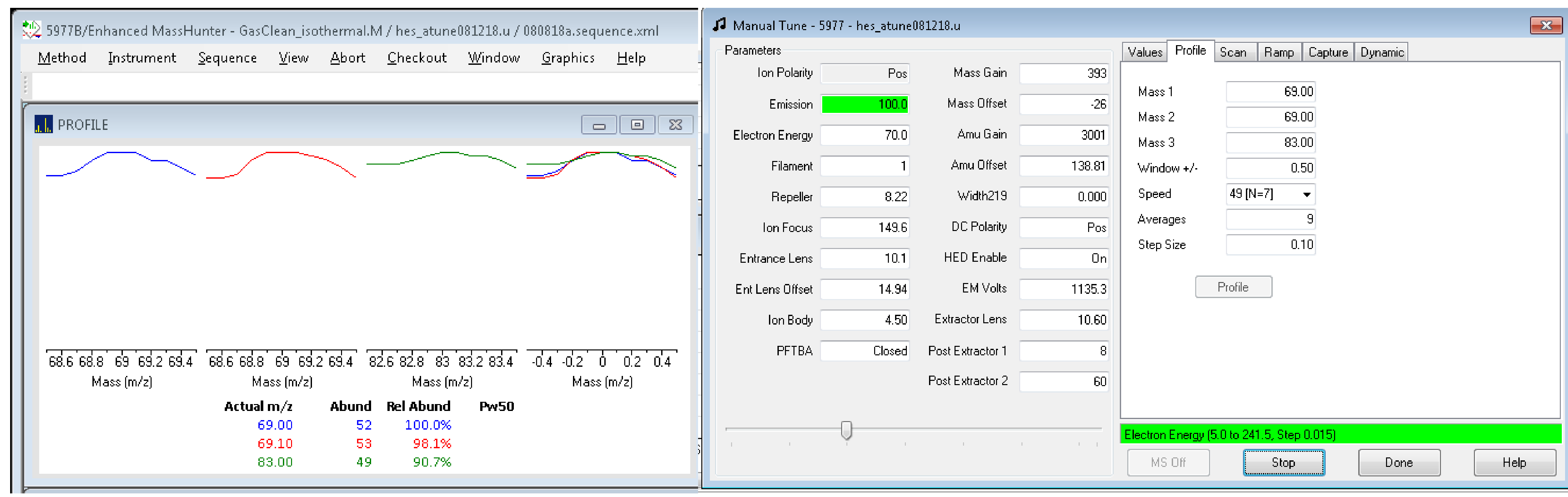
Self-tightening Nut for Agilent MS transfer line: P/N 5190-5233
0.25mm column short graphite vespel ferrules: P/N 5181-3323







We’ve checked the vent valve and MSD transfer line, is there anywhere else?



Don’t forget the side door!

…if leaks are found at side door, you will need to vent.

Good Habit Tip # 1: Check the side panel BEFORE you close the door

Side door o-ring was dislodged; how do I prevent an unnecessary vent?

- Using gloves, run finger along o-ring in groove
- Visually inspect seal integrity prior to pump down
- Wipe o-ring perimeter with lint-free tissue
- Replace worn or damaged o-rings

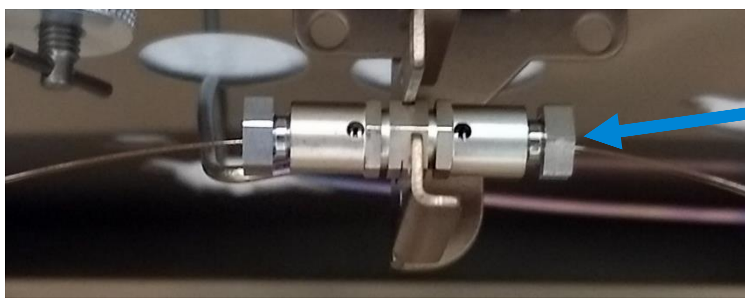
Other good habits

- Don't use the side panel thumbscrews
  - Only use during shipment/relocation
- Close vent valve before opening side panel door
- Periodically vacuum beneath outer covers
- Proper cooling for turbo pump and boards

Agilent GCMS Side plate o-ring
P/N 0905-1442
link

Notch for o-ring removal

Good Habit Tip #2: Check other connection points in the GC



- Check the inlet
  - Installed with a quarter turn with a wrench?
- Inlet nut may need slight tightening after heating cycles
  - Or, pre-swage flexi-metal ferrules
  - Or, use Self-tightening column nut for inlet
    - Only use graphite/vespel ferrules with self-tightening nuts!
- Check CFT connections

For inlet or detector
P/N 5190-6194

- Use leak detector or electronics duster
  - Short bursts of electronics duster at each connection point

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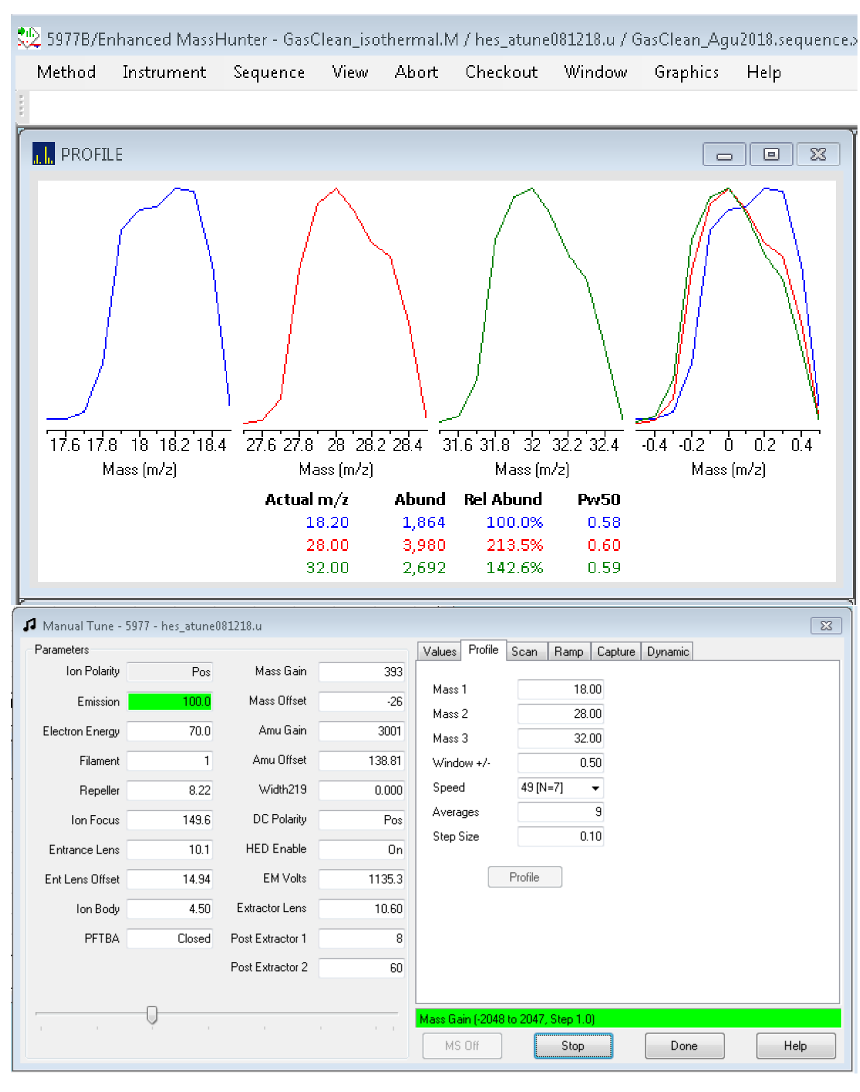
If my system is leak-free, what should my air ion abundances be?



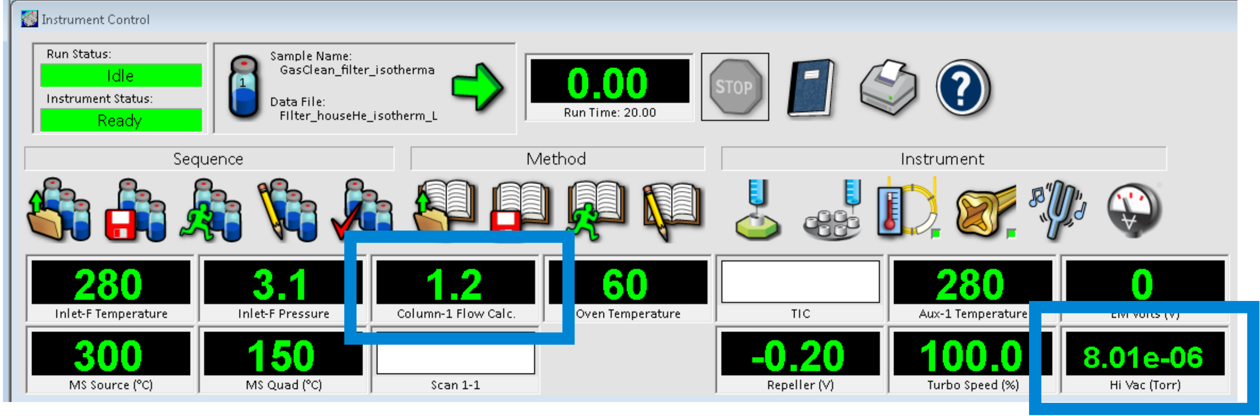
- These are just estimates!
  - H_2O: ~2,000 counts (less is ok)
  - N_2: ~10,000 counts(less is ok)*
  - O_2: ~3,000 counts (less is ok)

*Make sure to purge your GasClean filter

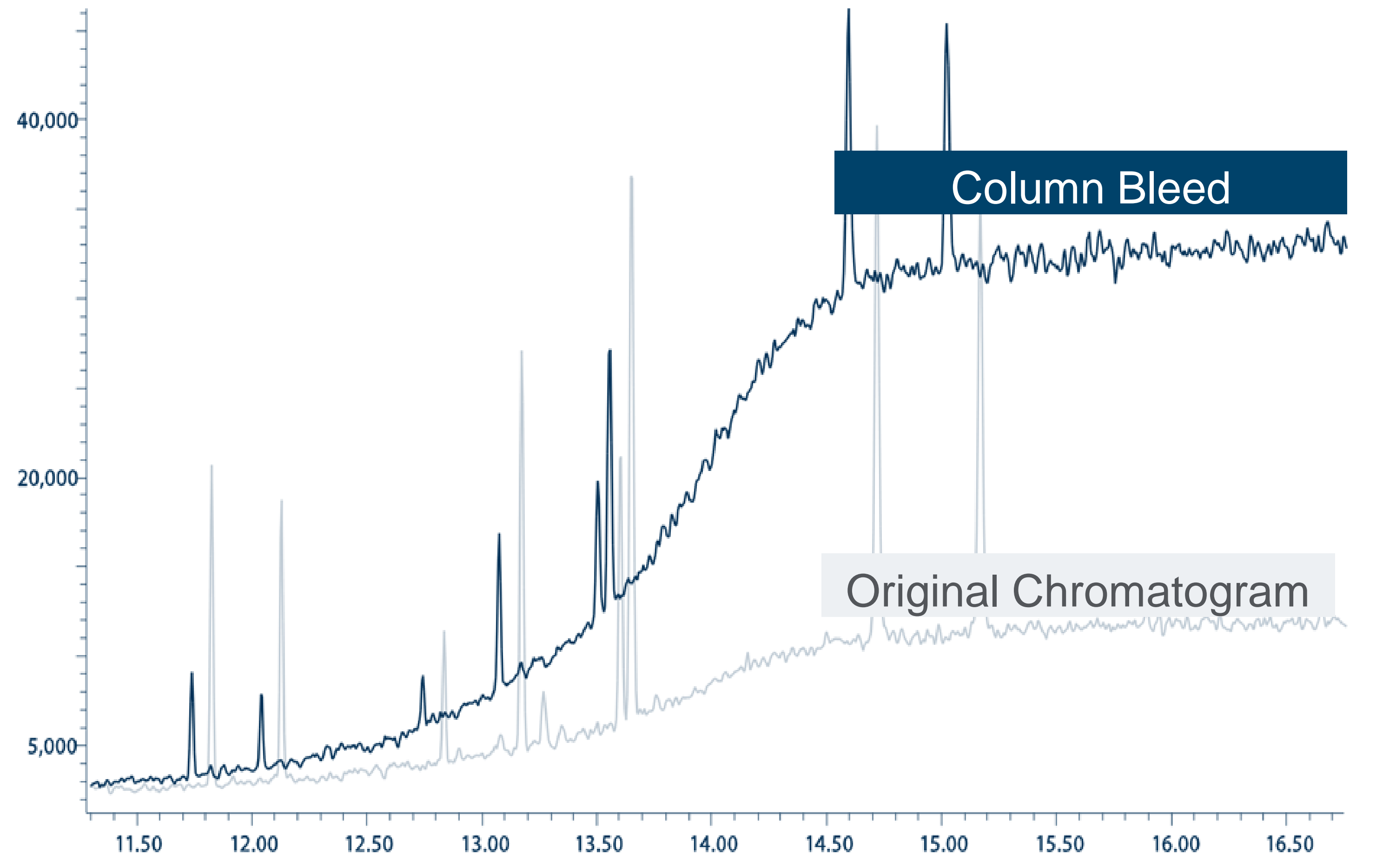
High vacuum gauge pressure (for SQ):

~1 x10^{-5} torr†

† dependent on flow rate



One Last High Background discussion: Troubleshooting Column Bleed



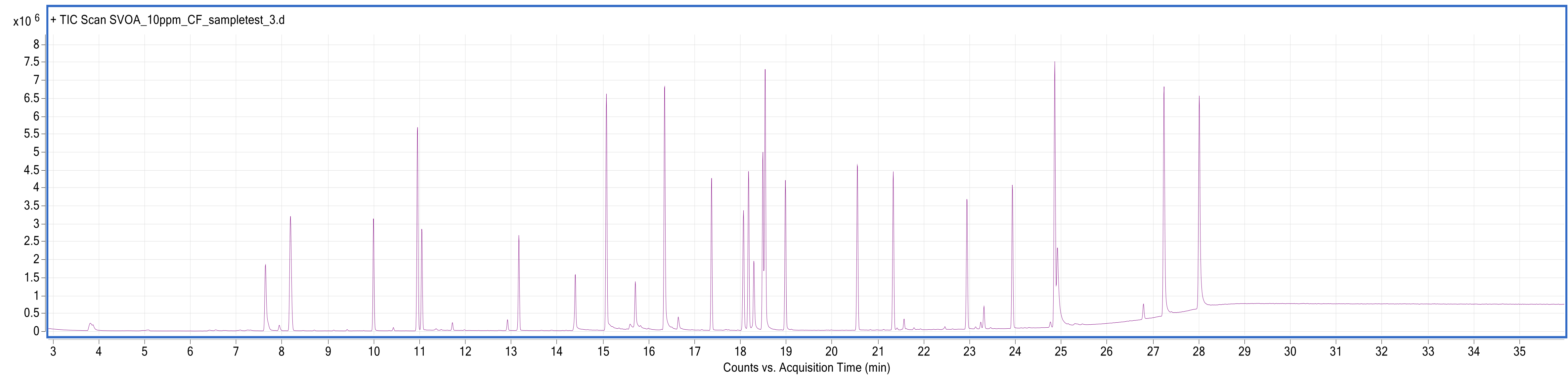
- Have you installed and/or conditioned the column?
- Are you exceeding the column's upper temperature limit?
- Is your column's film size too thick?
- Could leaks be present in your flow path, or are your carrier gases contaminated with air?
- Do you need to change your split vent trap?

Good Habit Tip #3: Save a chromatogram from immediately after the column was installed. Overlay the problematic chromatogram with your reference chromatogram to determine whether column bleed may be a problem.

“Noisy” Background and Too Many Peaks

How do you know it’s noisy?

Good habit tip #4: Have system “baselines”



Known standard: Agilent Semivolatiles Checkout Standard 5190-0473

What kind of system baselines?

- System blank – Should only see a rise in baseline with temperature (column bleed)
- Solvent blank – May contain contaminant peaks (e.g. phthalates, siloxanes, etc.)
  - Best practice: Use the same bottle of solvent that was used for any dilutions/extractions
- Known Standard – GC/MS checkout standard, DFTPP tuning mix, or known calibration standard at easily detectable level for your system (e.g. 1-10ppm)

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Good Habit Tip #5: Run a manual scan to review background peaks

Understand what the normal scan range background looks like

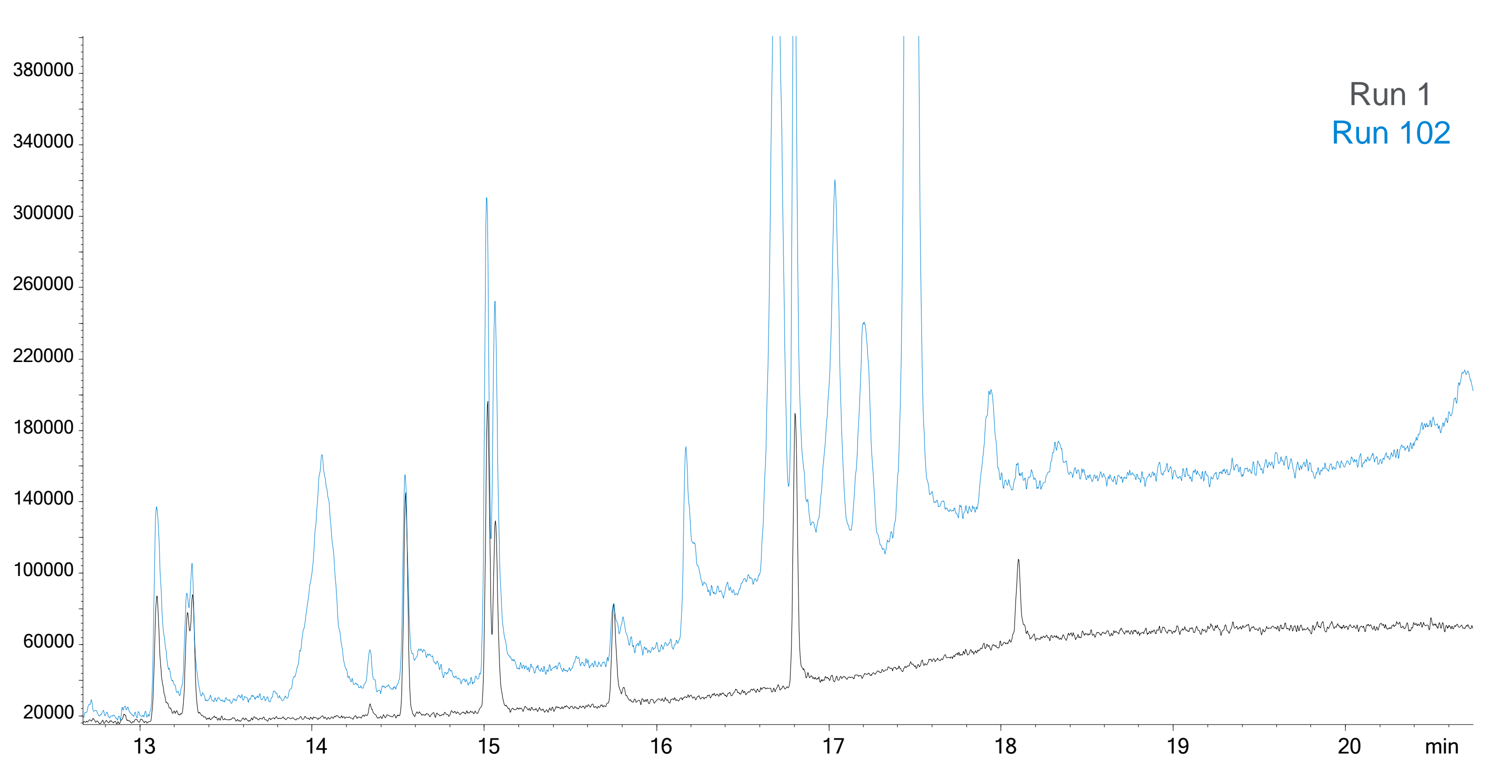
- Look at your normal scan range and an extended scan range
- 35-500 and 10-700
- Use your normal threshold
- Perform the scan after initial set-up and after major changes (new column, source clean, etc).
  - Track the results in notebook or on computer
- Normal # of peaks in manual scan: ~100-250 peaks

Does the noisy background negatively affect my results?

- Do compounds fall the quantitative criteria?
- Can you distinguish target compounds from matrix?
- Can you integrate the peaks from EICs?

- If your results fail criteria, something(s) is/are dirty

Does this mean I have to clean my source?

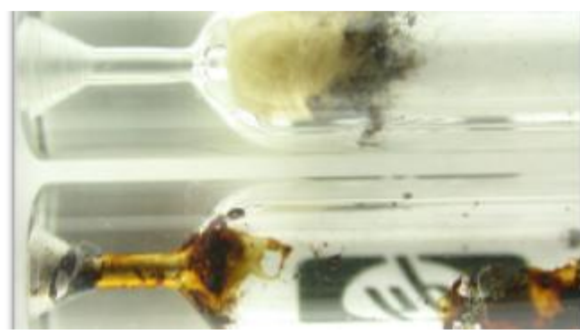


Check these before you vent for the source….

- Do the easy steps first!

- Liner and inlet septum
  - Change these first
  - Did the chromatography improve on your check sample?
- Trim head of column or install new guard column
  - Trim ~0.5m from front of column
  - Use a guard column
  - Did the results improve on the check sample?
- If chromatography hasn't improved, it may be time to check and clean the source….

Does your liner look clean or dirty?



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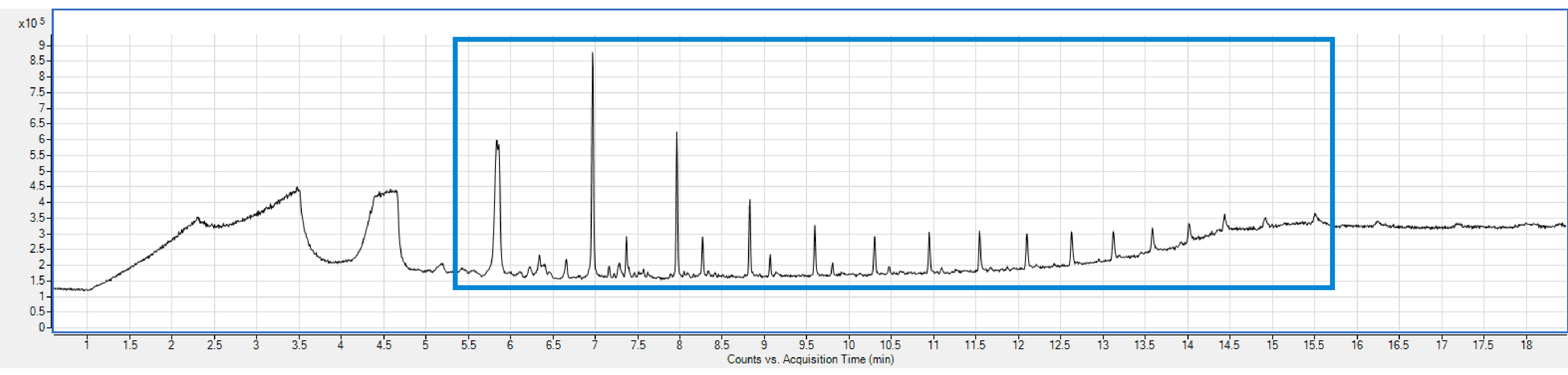
Too Many Peaks Case Study: What are these repeating peaks?



Is it column bleed?

Are these peaks from my solvent?

Septum maintenance: TIC of an inlet septum

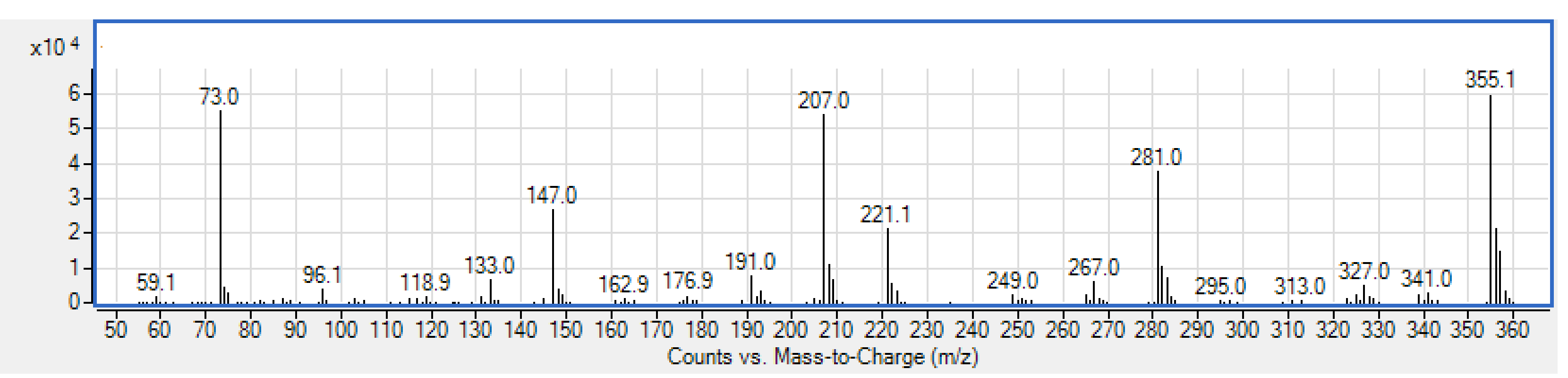
x10 5

Counts vs. Acquisition Time (min)

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:

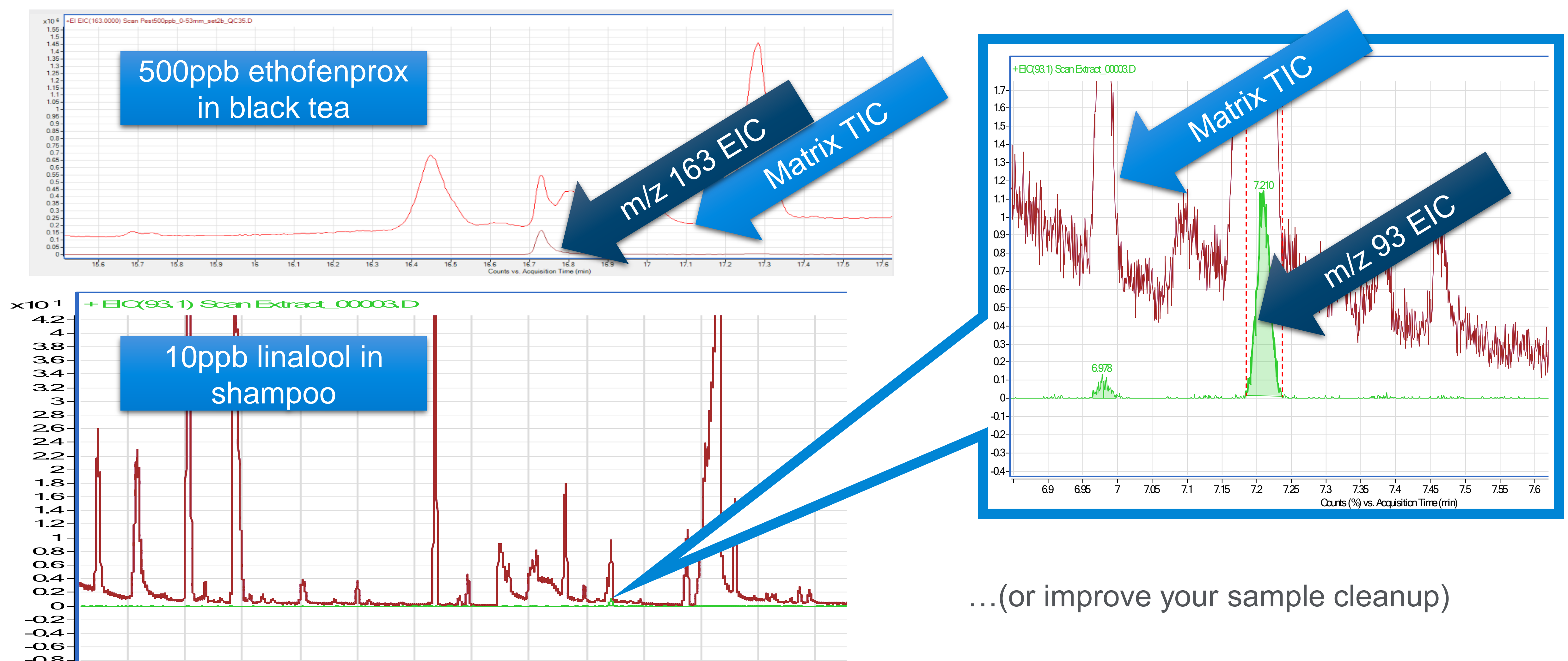


My peaks are much smaller than I expected; what's going on?

Diminished peaks

Remember the Matrix: SIM or MRM can hide a lot

When you only look at EICs, you can miss a large picture



…(or improve your sample cleanup)

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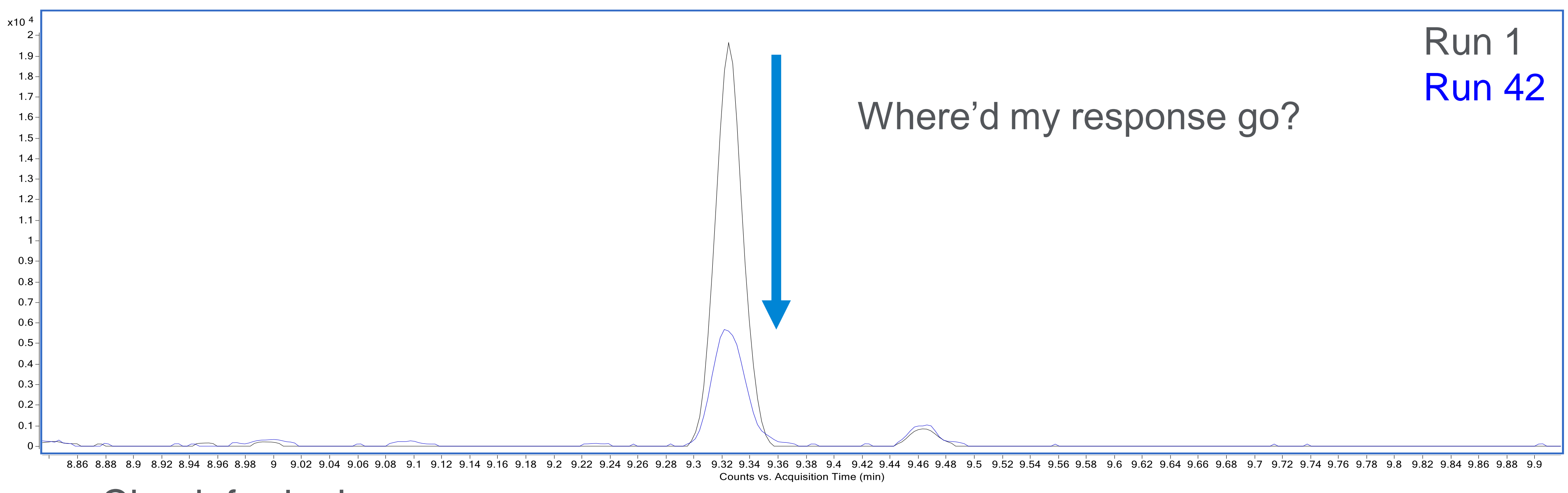
What should I check when my peak response is diminished?



- Check for leaks
- Liner and inlet septum
- Trim head of column
  - If column bleed has significantly increased or matrix interferences cannot be removed from column, replace the column
- If chromatography hasn't improved, it may be time to check and clean the source
- While the system is open, take a look at your filaments….

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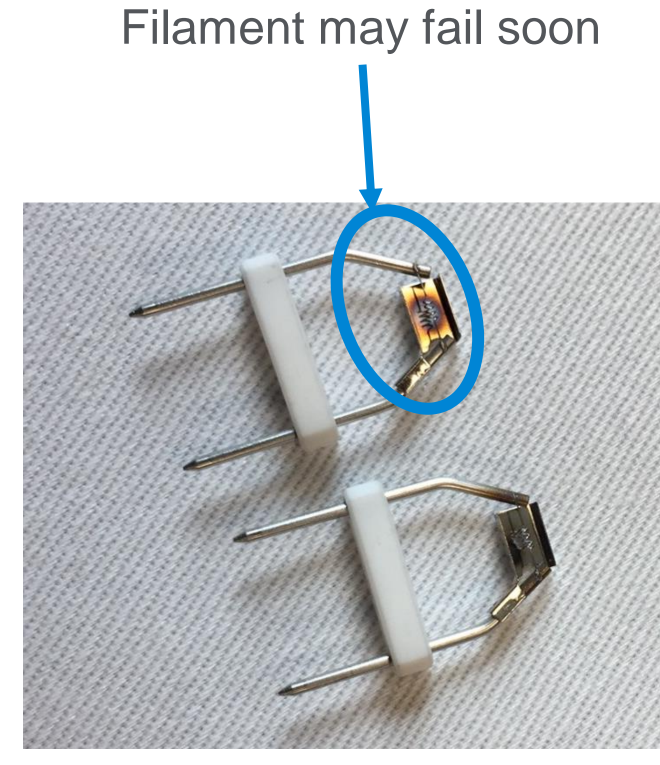
Good habit tip #5: Filament care: Have an extra pair (or two) on hand

- Have (at least) 2 extra filaments on hand
  - More than 1 GC/MS system? Keep >2 on hand, depending on the number of systems.
- Check filaments when you clean the source
  - Look for discoloration behind the filament and unraveling of the coil
  - Replace them as a pair
- End-of-life filaments may cause diminished response or odd artifacts in TIC
  - Keep them, just in case the problem is not the filaments

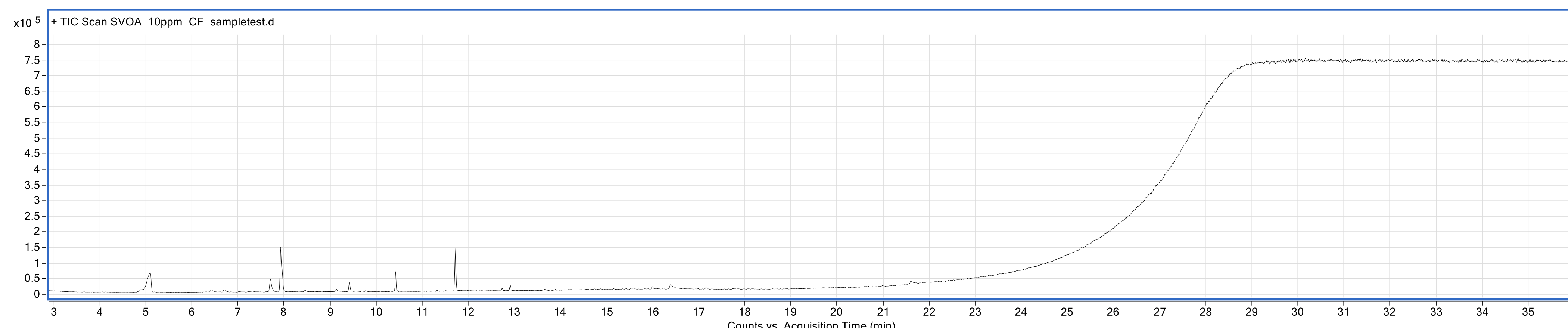


Agilent 5977 InertPlus, Extractor, & 5975 Filament Assemblies: G7005-6001

Careful! High Efficiency source (5977B HES single quad MS and 7010 HES tandem quad MS/MS) have different filament designs from 5977B InertPlus, extractor source and older MSD designs!

Fewer peaks than expected or no peaks: What should we check first?

- Easy stuff first!
  - Check syringe, inlet septum and liner
  - Check the vacuum reading
    - Is it normal?
  - Verify column is intact
  - Verify all cables, etc. are connected and method parameters are normal
  - Do the filaments turn on?
  - Run the known standard
    - Does it look normal?
  - If these steps don't work, then it's probably time to call for the lab manager and/or Online Support



Data quality

How do I know if my flow conditions, gain, scan speed, etc. is right?

Data quality: What are the best flow conditions to use for MSD?

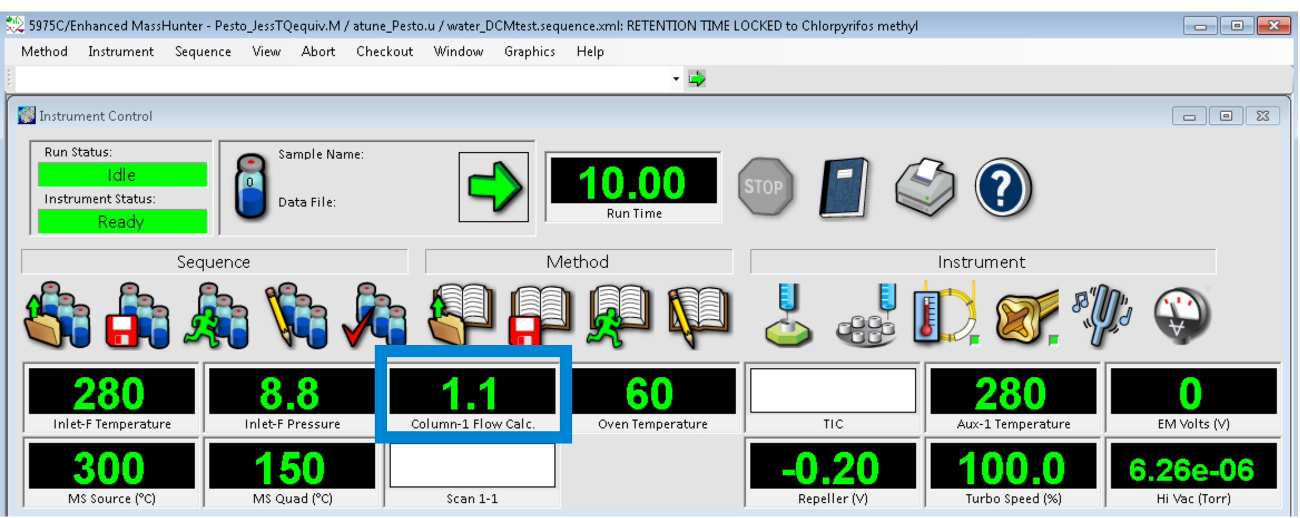
Use constant flow methods!

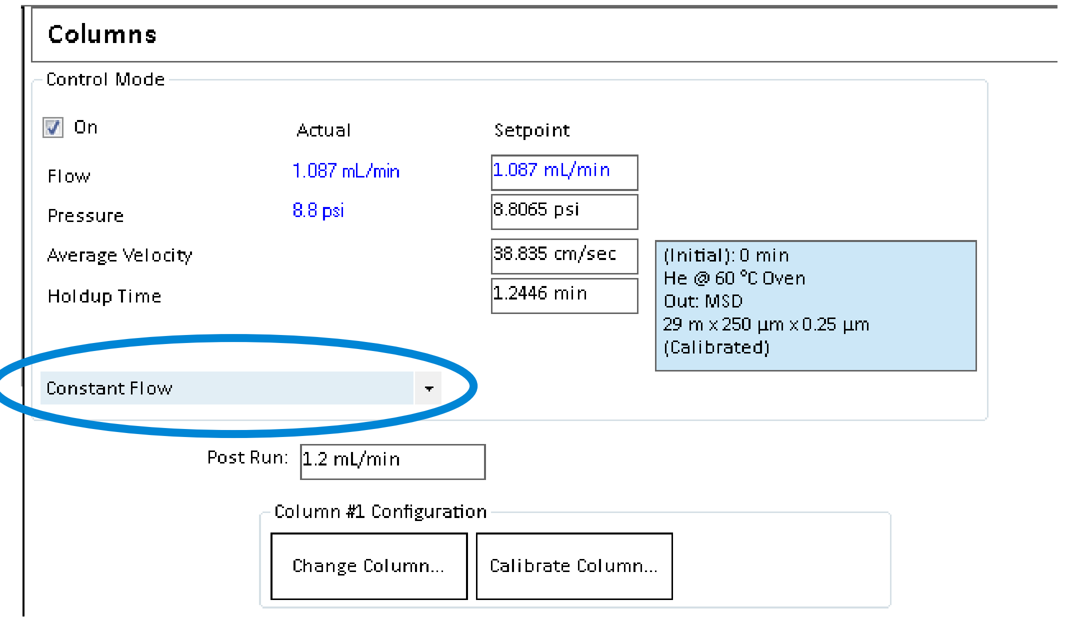
5977 Extractor, InertPlus, or older sources

- Suggested flow rate range: 1 - 2 mL/min
- Optimal flow: 1 - 1.2 mL/min

5977B High Efficiency (HES) source

- Suggested flow rate range 1 - 1.5 mL/min
- Optimal flow: 1 - 1.2 mL/min



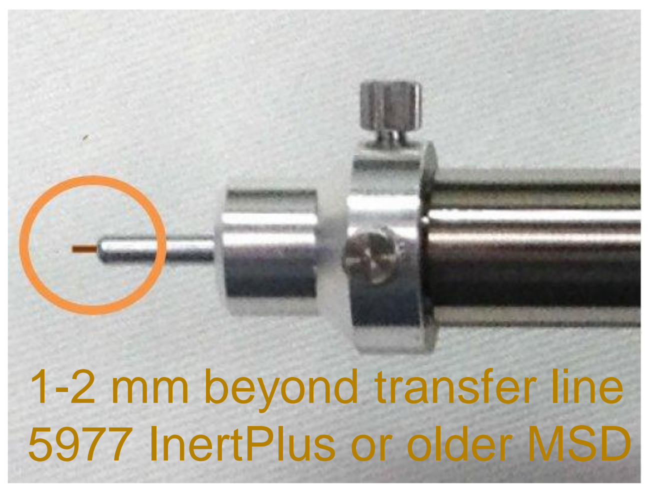


Data quality: How do I know if my parameters are right?

What is the proper column installation into MSD?

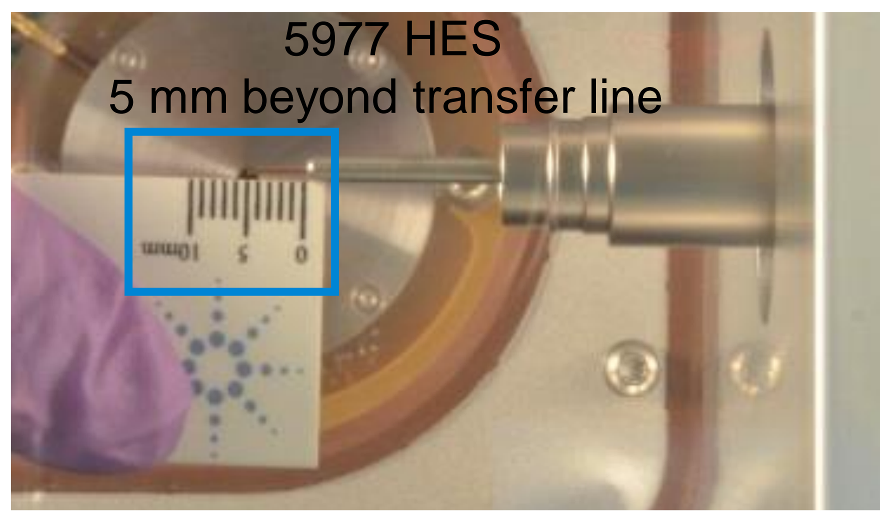
5977 Extractor or InertPlus, or 5975 MSD

Installation length: 1-2mm beyond end of transfer line



5977B HES

Installation length: 5 mm beyond end of transfer line



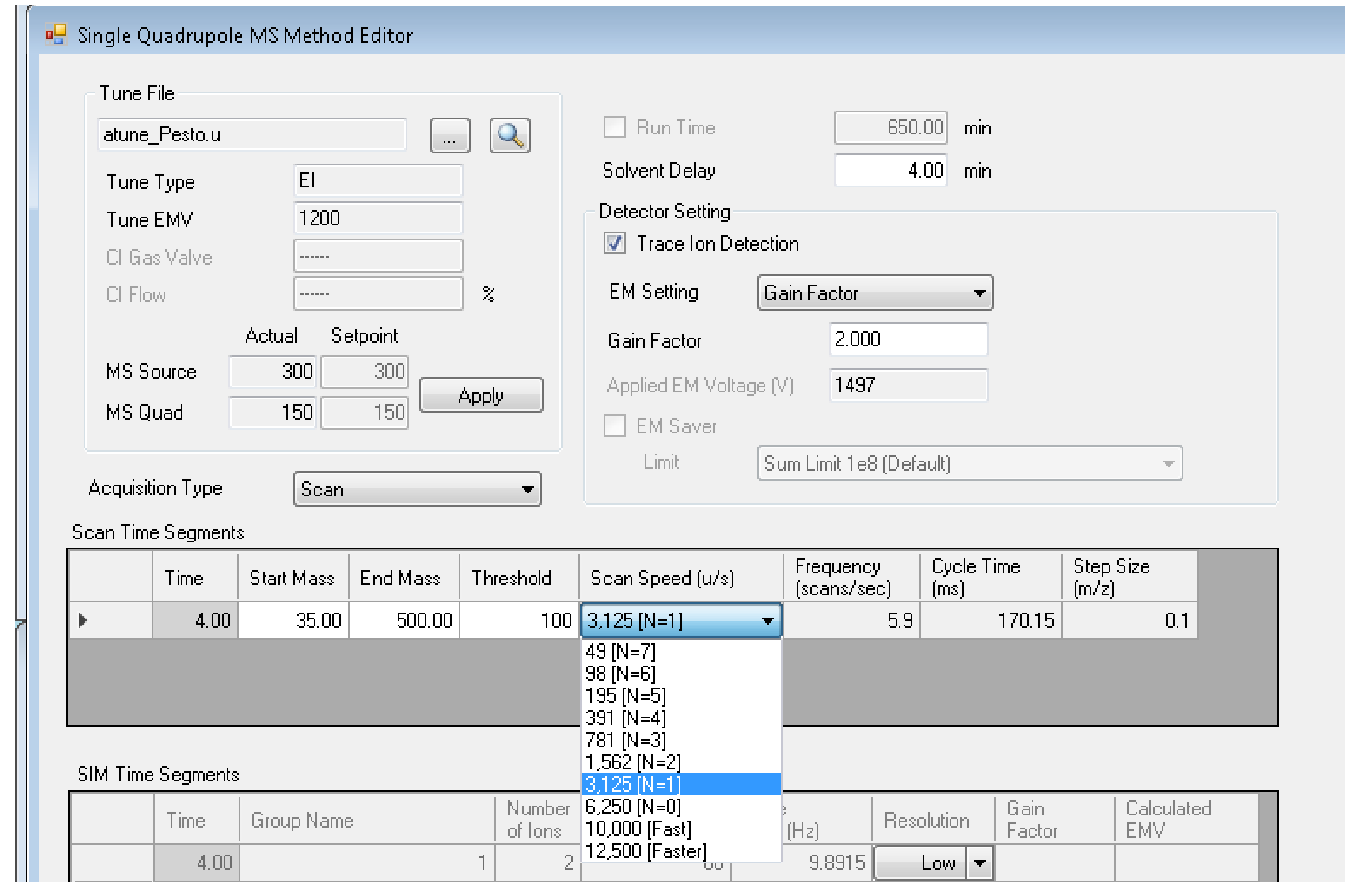
Remember to re-install the ceramic tip, spring, and nut after column installation!

Ceramic tip for 5977 and 70x0 series
G3870-20542

Transfer line spring
G7005-20024

Transfer line cap
G3870-20543

Data quality: How do I know if my scan speed is right?



Best acquisition frequency

- ~ 2.5 to 5 Hz

Optimal # of points across peak

- 8-12+ points across a peak

Set scan speed at N=1 or N=2

- Frequency in 2.5-5 Hz range
- Generally most samples will have 8-12+ points across a peak

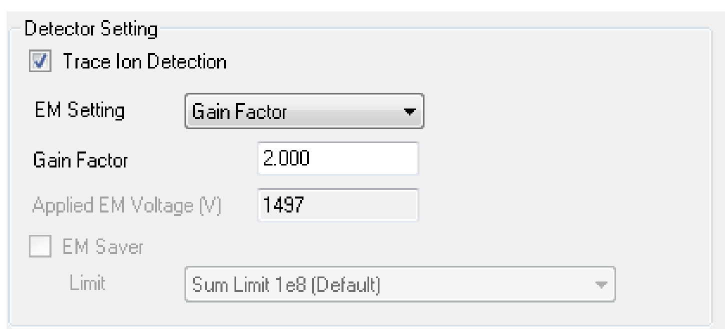
What about SIM dwell time?

- Default 100 ms = good start
- Depends on number of ions in time segment
- Watch Frequency (scan/sec) as you add ions and/or change dwell time

Gain selection: https://www.agilent.com/cs/library/technicaloverviews/public/5991-2105EN.pdf

Data quality: How do I know if my gain is right?

- Good practice: Start at gain 1
- Best Practice: Choose lowest gain factor for detection of most and least intense ions over target concentration range
  - Ideal gain could be < 1
  - Lowest suggestion gain: 0.3
- Avoid high gain for long periods of time
  - High gain can shorten life of EM

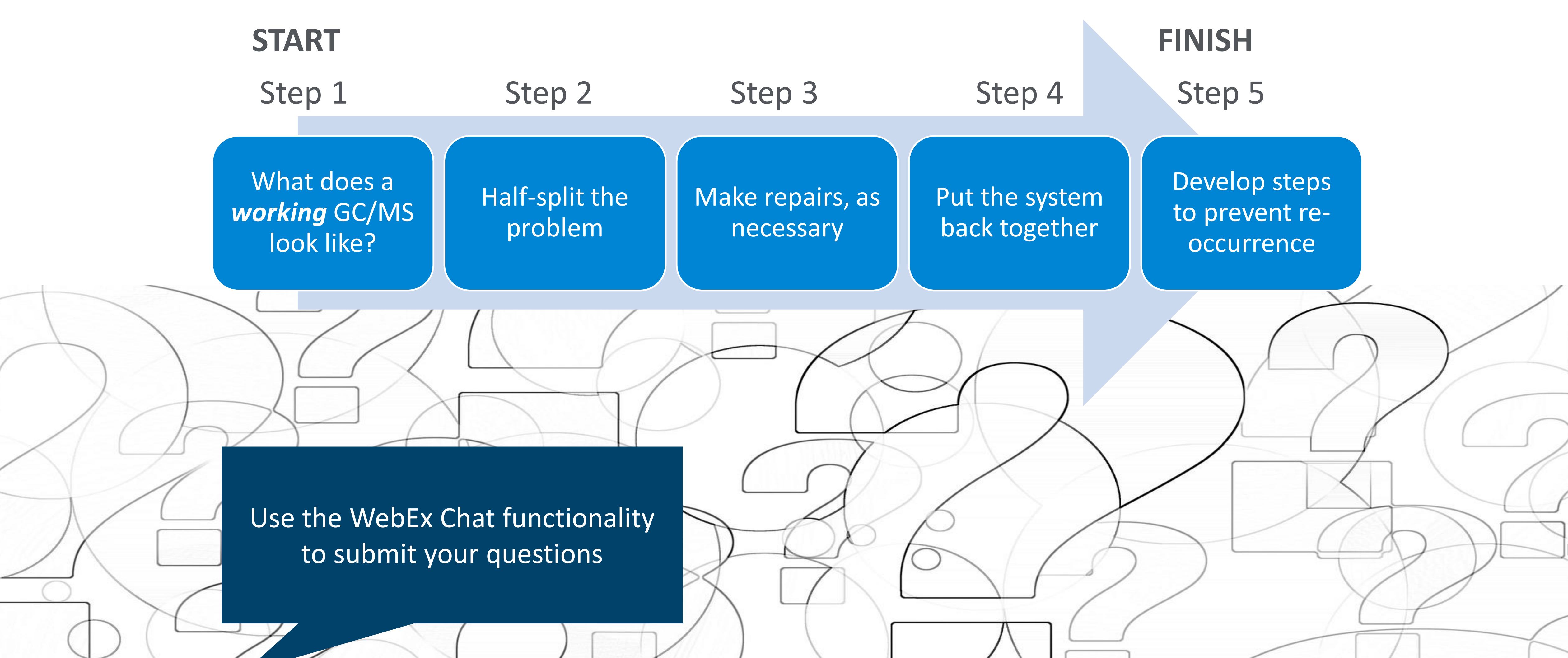


Process for testing gain levels: Do I need to increase my gain?

- Test gain factor in highest and lowest concentration standards
  - Choose most intense compound/ion
  - Run known standard at highest concentration with Gain = 1 (or current gain)
  - Is largest ion peak between $3x10^6$ – $6x10^6$ counts?
  - If yes, run lowest concentration standard
    - Are all compounds detectable?
  - If no, try a different drawout lens or increase/decrease gain (e.g. from gain 1 to gain 2)
    - Repeat process

Gain selection: https://www.agilent.com/cs/library/technicaloverviews/public/5991-2105EN.pdf

Thank you for your attention!



Column Installation: Self-Tightening Column Nut

- Spring driven piston continuously presses against ferrule
- Automatically retightens when ferrule shrinks
- No leaks! No downtime! No frustration!
- Wing design for finger tightening
- No tools needed!
- No polymer materials for durability
- Compatible with ONLY short Graphite
- Vespel ferrules

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What steps should I follow when troubleshooting?

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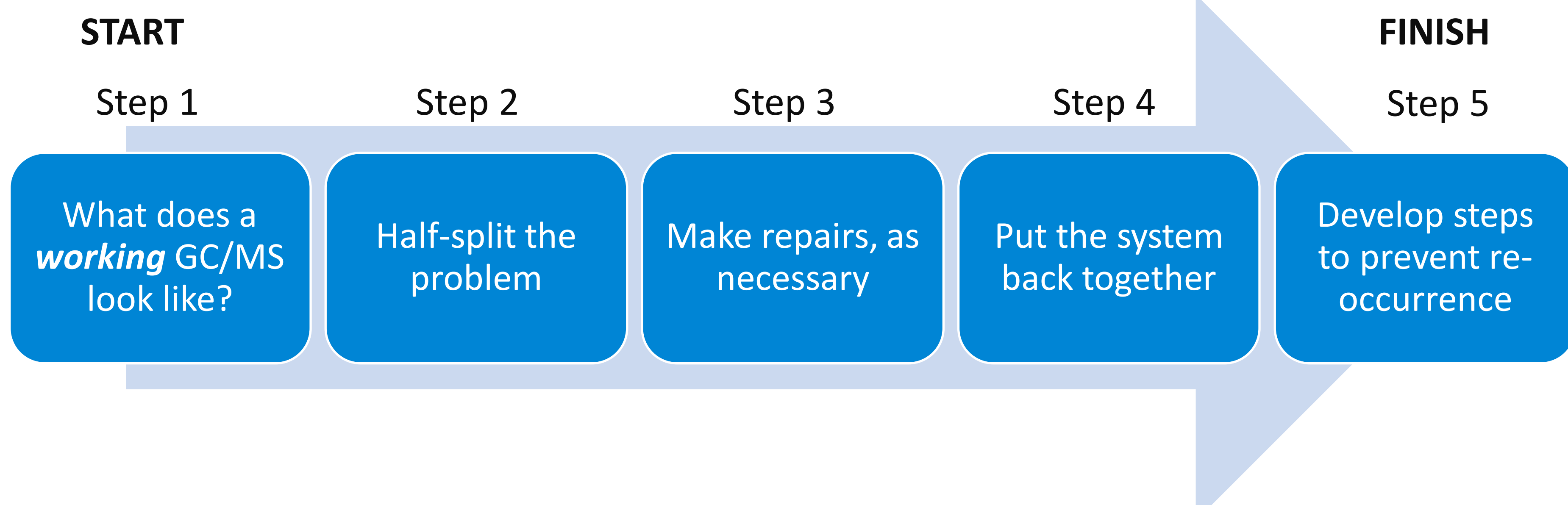
Follow a logical troubleshooting procedure



Troubleshooting starting points

Check parameters

- Do method parameters match what you see in/on the system

Autosampler

- Are sample/wash vials dirty or have particulates?
- Watch syringe moving/make an injection, or remove syringe and manually pull up solution.
- Try a manual injection to rule out the autosampler

Recent Maintenance?

- Determine if users have completed normal maintenance recently
- Change liner, inlet septum, syringe, trim column

Quick MSD check

- Generate a tune report to see if the current tune is working. This does not change any parameters.

Sample you know

- Inject a checkout sample you know
- Do you observe the expected peaks in the expected sizes at the expected retention times?