Lost and Found: Troubleshooting Missing Peaks in GC Analysis

Alexander Ucci February 22, 2022





DE8

Lost and Found

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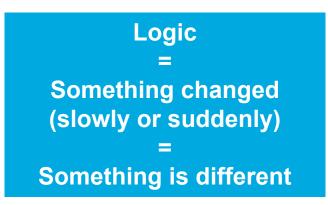
"Everything Was Just Fine... and Then This Happened!" "How do I troubleshoot?"

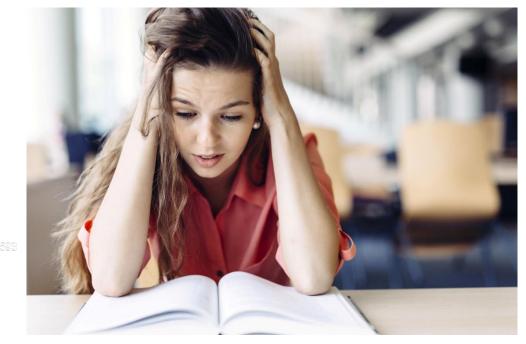
Track your actions/keep a logbook of events:

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

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Logical Troubleshooting

Troubleshooting starts with isolating the problem.

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

-Flow

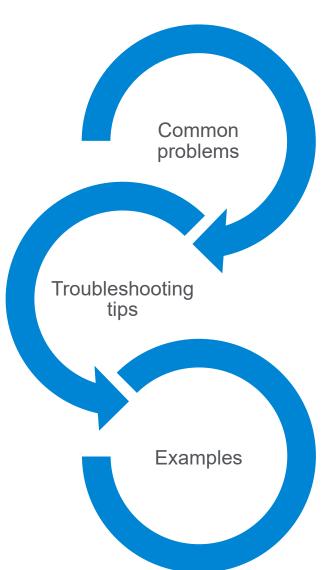
- -Column
- -Detector

-Electronics

Or...

- A combination of these

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







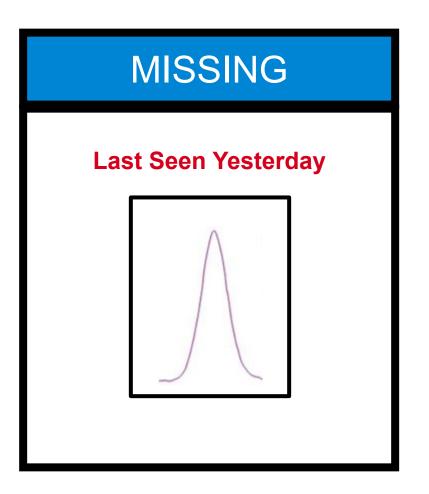
Agenda

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
- Other



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



New Agilent Universal Fit GC Detector Jets

- Easier column installation and jet replacement, reducing the risk of column damage
- Lubricant-free threads, reducing the risk of contamination
- Made from strong material, reducing the risk of deforming
- Universal fits in both capillary column and packed column (adaptable) FID detectors

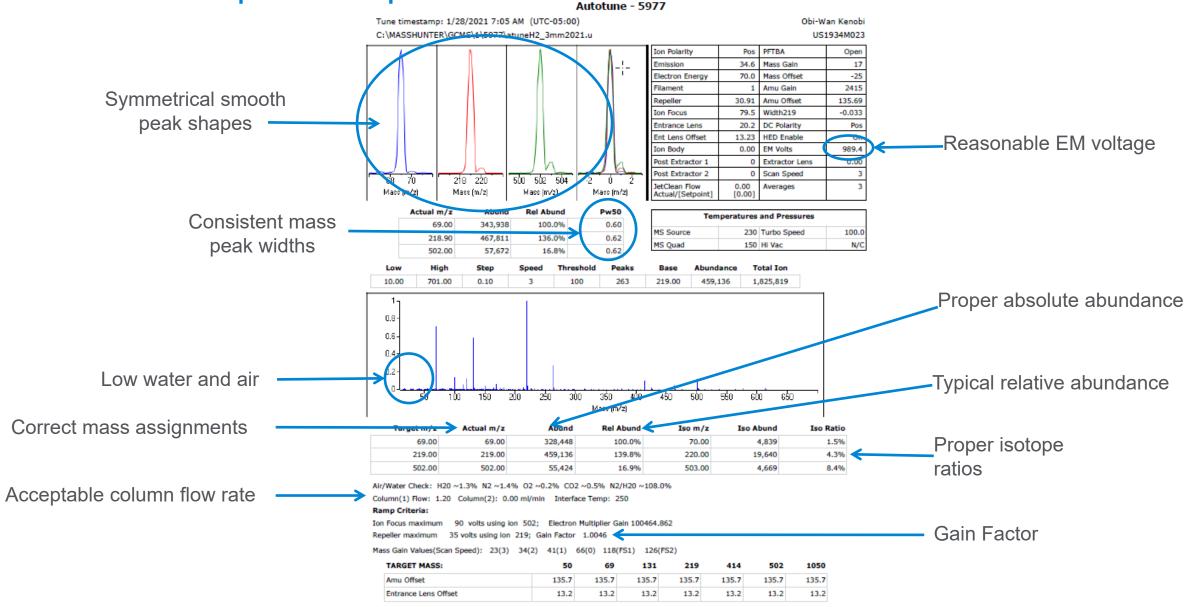


Previous Jets				New Universal Fit Jets			
Previous Jet PN	Jet Orifice ID (inch/mm)	Jet Length (inch/mm)	Fit of Detector Fitting Type	New Jet PN (use for re-order)	Jet Orifice ID (inch/ mm)	Jet Length (inch / mm)	Fit of Detector Fitting Type
19244-80560	0.011 / 0.29	2.4 / 62	FID, Adaptable	5200-0176	0.011 / 0.29	1.2 / 31	FID, Capillary & Adaptable
G1531-80560	0.011 / 0.29	1.7 / 43	FID, Capillary				
18710-20119	0.018 / 0.47	2.5 / 64	FID, Adaptable	5200-0177	0.018 / 0.47	1.2 / 31	FID, Capillary & Adaptable
19244-80620	0.018 / 0.47	2.4 / 62	FID, Adaptable				
G1531-80620	0.018 / 0.47	1.7/43	FID, Capillary				
18789-80070	0.030 / 0.76	2.5 / 64	FID, Adaptable	5200-0178	0.030 / 0.76	1.2 / 31	FID, Capillary & Adaptable
G1534-80580	0.011 / 0.29	2.0 / 52	NPD, Capillary	5200-0179	0.011 / 0.29	1.6 / 40	NPD, Capillary & Adaptable
G1534-80590	0.011 / 0.29	2.8 / 71	NPD, Adaptable				

Lost and Found

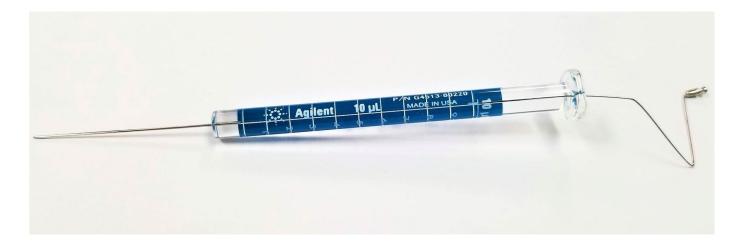


MS Tune Report Interpretation





Autosampler Issues











Troubleshooting

Problem: Bent Plunger or stuck syringe

Possible causes:

- Particles such as dust, salts, metal, leftover sample, or glass can fill the narrow gap between the plunger shaft and the inside wall of the barrel.
- Overtightened septum nut compresses septa, causing excessive resistance during injection

Suggested actions:

- Switch to a syringe with PTFE-tipped plunger
- Avoid using 5 µL syringes where possible
- If plunger movement feels "gritty", carefully remove plunger from barrel, flush with solvent, and wipe dry with lint-free cloth. Carefully reinsert plunger into barrel. Finally, submerge needle tip into container of solvent and cycle plunger to pull solvent into and out of the barrel.
- Never cycle the plunger in a dry syringe
- Do not "mix-and-match" plungers and barrels
- Immediately clean syringes after use
- Loosen septum nut



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Troubleshooting Problem: Bent needle

Possible causes:

- Improper needle alignment
- Narrow gauge needles (26 g) bend more easily than larger gauge (23 g) needles
- Needles tend to bend when inserted into sample vial, not the inlet. This can be caused by septa that are too "rough".
- Needles bent during installation into the autosampler are more likely to bend when pushed through the sample vial cap septum.
- On-column inlets wrong needle gauge
 - Use correct needle support

Suggested actions:

- Use syringes with 23 to 26 gauge tapered needles
- Re-align autosampler
- Check septum nut is not overtight

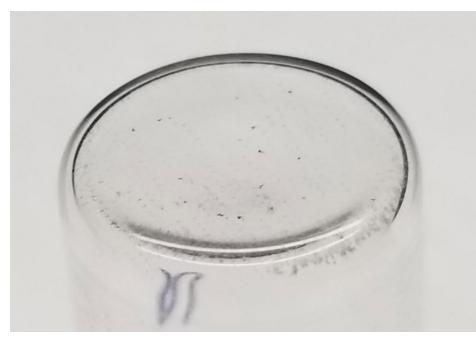




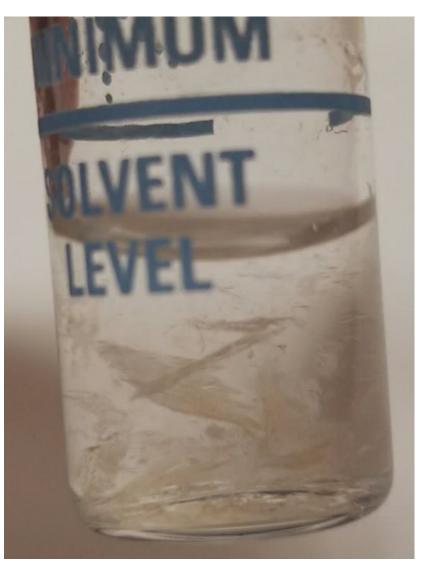
Washes and Pumps: Solvents

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 solvent





Washes and Pumps: Solvents

Choose a wash solvent or a series of solvents that make sense for the analysis

- Is the analyte soluble in the solvent?
- Wash solvent = sample solvent when possible
- If wash solvent *≠* sample solvent, are they miscible?
- If using a binary wash system, make sure solvents are miscible and rinse with the sample solvent last just before the sample
- Do not use acidic or alkaline solvents with syringes
- What other solvents are used/analytes determined in methods on the same GC?





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.



Agilent CrossLab CS (Cartridge System) No Peaks from Leaks

Features:

- Exchangeable cartridge with ADM Flow Meter
- Automatic Notification of Probe Filter Replacement
- Ergonomic and robust design
- Universal 3AA or USB power
- USB connects to web interface for added functionality and firmware updates
- Easy to view OLED Screen
- Kickstand



ADM Flowmeter cartridge



The Cost of Leaks

- Cost of gases
- Contamination from exposure
- Reduced consumable lifetime
- Reduced productivity from downtime
- Detector noise and elevated baselines
- Time in troubleshooting

It is critical that every customer checks for leaks. They should have the best tool for the job! Check valves, fittings, and traps for leaks after every maintenance, and after thermal cycling as these can loosen some types of fittings.



Assets Available for Launch

- Agilent.com CrossLab CS Leak Detector
 www.agilent.com/chem/gas-leak-detector
- Agilent.com ADM Flow Meter
 Agilent CrossLab CS Cartridge System | Agilent
- Installation manual

Agilent CrossLab CS Electronic Leak Detector manual Part number: G6693-90000

The installation manual is available on Agilent.com.

Innovation minute video

The video is available on Agilent.com.

• Technical overview Agilent CrossLab Cartridge System (CS) Electronic Leak Detector Publication number: 5994-4262EN

The technical overview is available on Agilent.com

• Brochure GC Troubleshooting in the Palm of Your Hand Publication number: 5994-3607EN

The brochure is available on Agilent.com

• Flyer Is a Leak Causing Your Inaccurate Results? Publication number: 5994-4202EN

The flyer is available on Agilent.com



Ordering Guide

1 year warranty

- G6693A CrossLab CS Electronic Leak Detector
- G6694A Electronic Leak Detector Cartridge
- G6699A CrossLab CS Bundle: ADM Flow Meter and Electronic Leak Detector
 - The bundle will include 1 handheld, 2 cartridges, and a free carrying case.
- G6694-60005 Replacement Probe Filter
- G6691-40500- Carrying Case



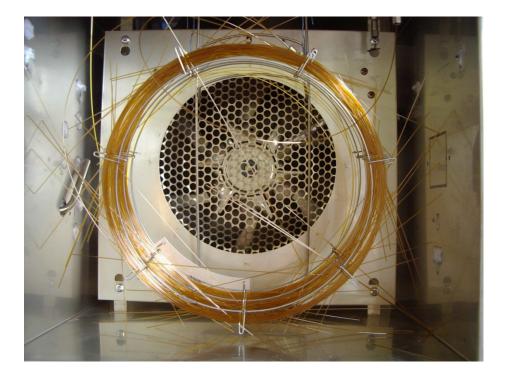
Existing products:

- G6691A CrossLab CS ADM Flow Meter
- G6692A ADM Flow Meter Cartridge*
 - Note that the ADM Flow Meter cartridge is ordered annually for calibration. The Electronic Leak Detector does not need to be recalibrated!



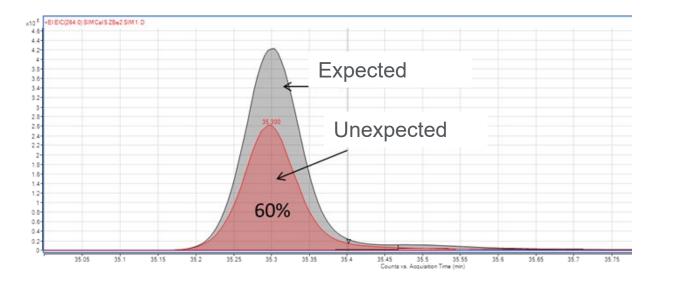
Physical Damage to the Polyimide Coating

- The smaller the tubing diameter, the more flexible it is
- Avoid scratches and abrasions
- Immediate breakage does not always occur upon physical damage





Peak Response All change in size



Injector

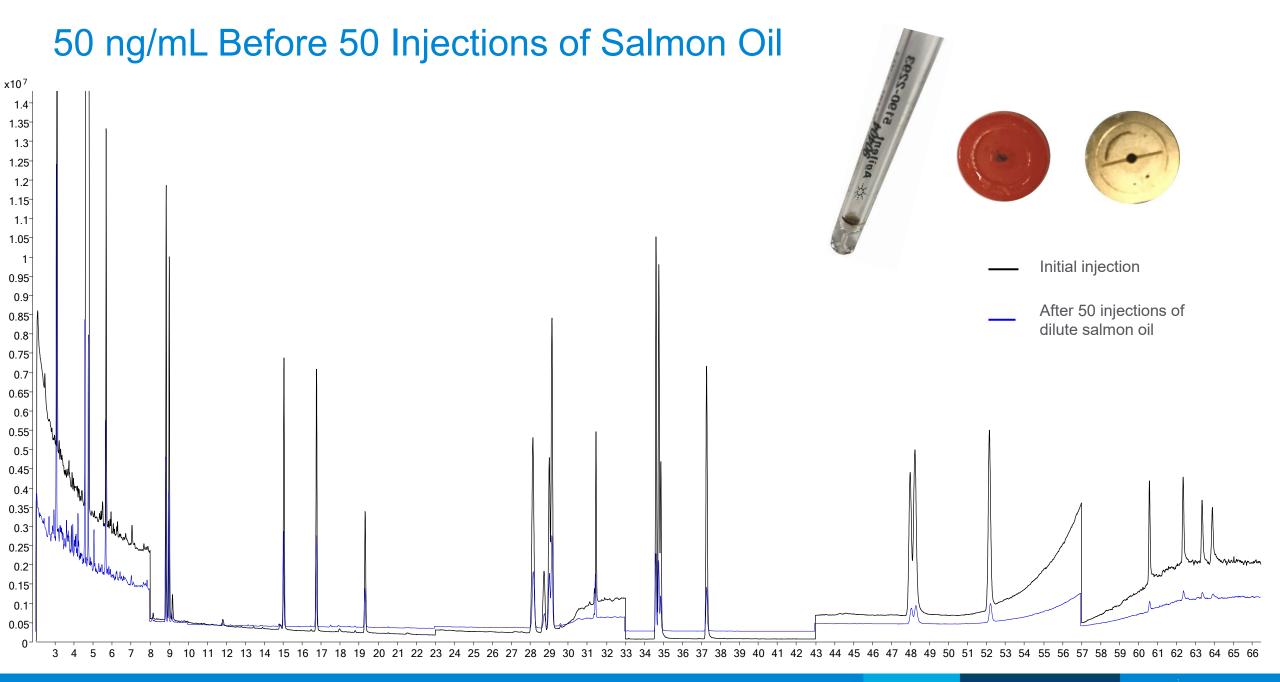
- Leaky syringe
- Split ratio set incorrectly
- Wrong purge activation time
- Septum purge flow too high
- Injector temperature too low*

Detector (response problem)

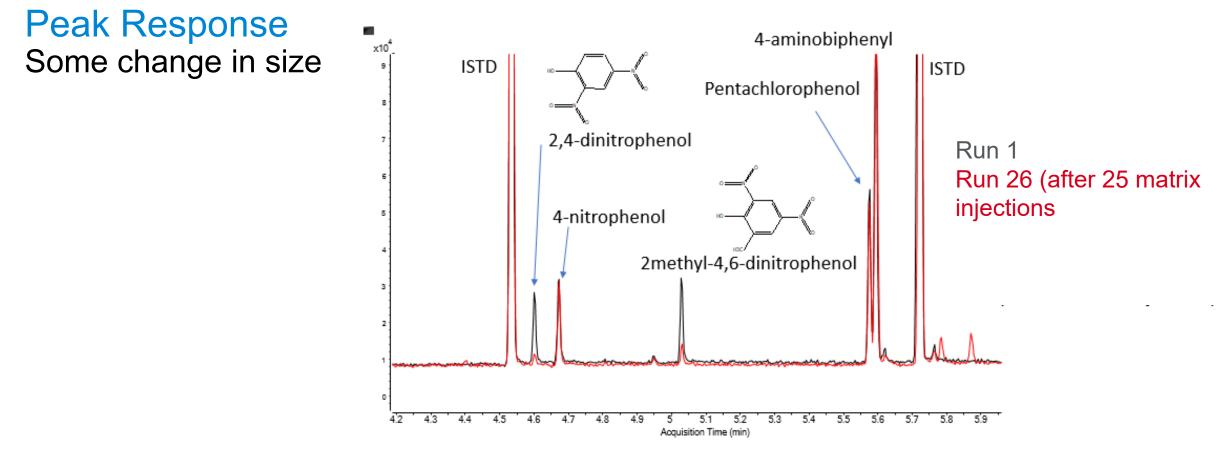
- Settings or flows changed
- Electronics failing

***Tip:** Ask yourself, is it all of them or some of them? If all, then injector or detector?









Injector or column is active/contaminated

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

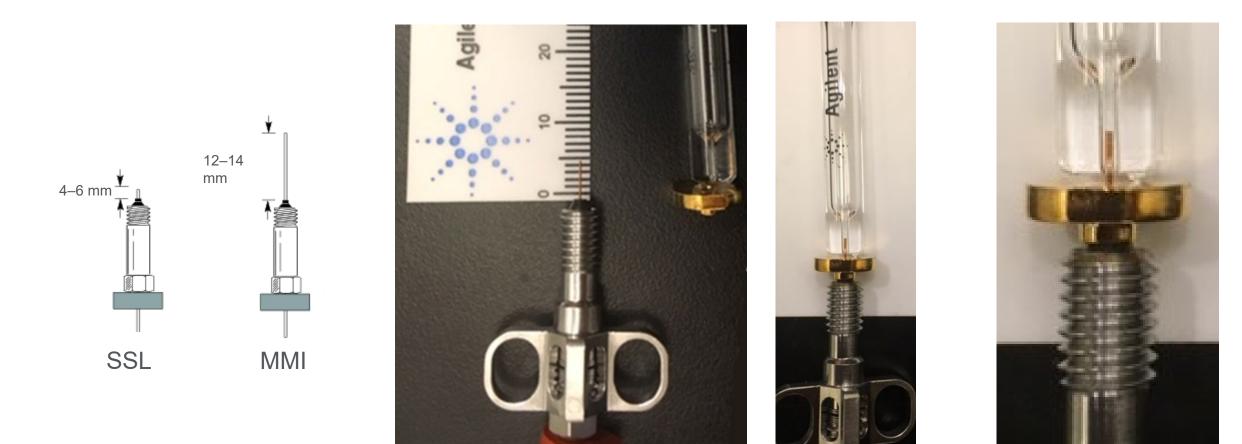
Decomposition of sample

- Temperature change discrimination
- Evaporation from sample

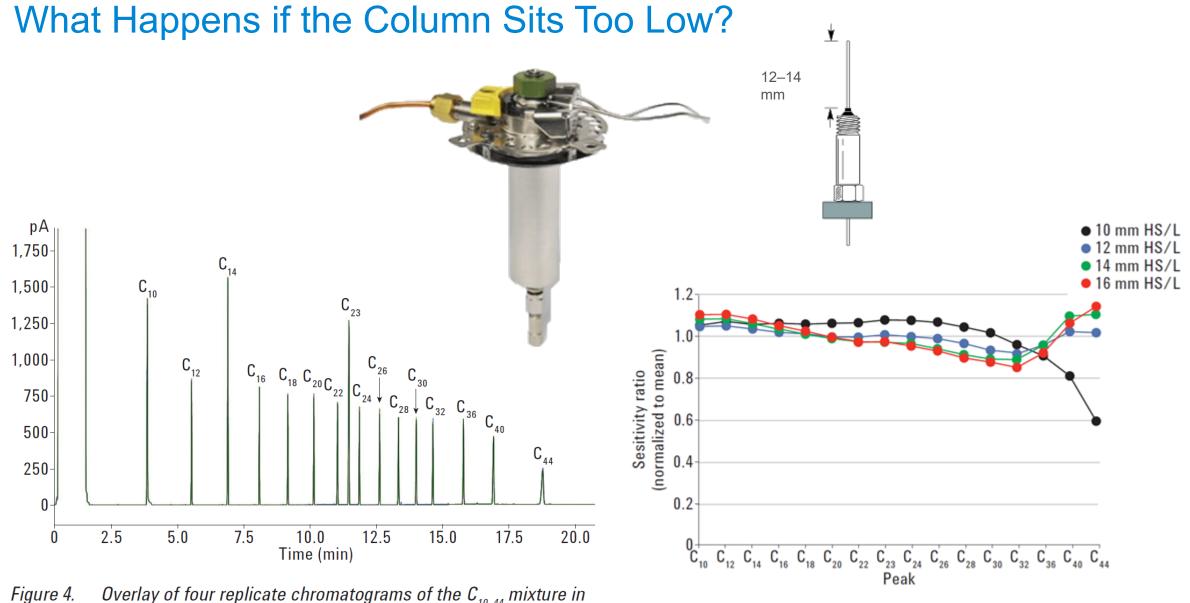


Why Does the Length Above the Ferrule Matter?

The tip of the column enters the bottom of the liner but does not pass the taper





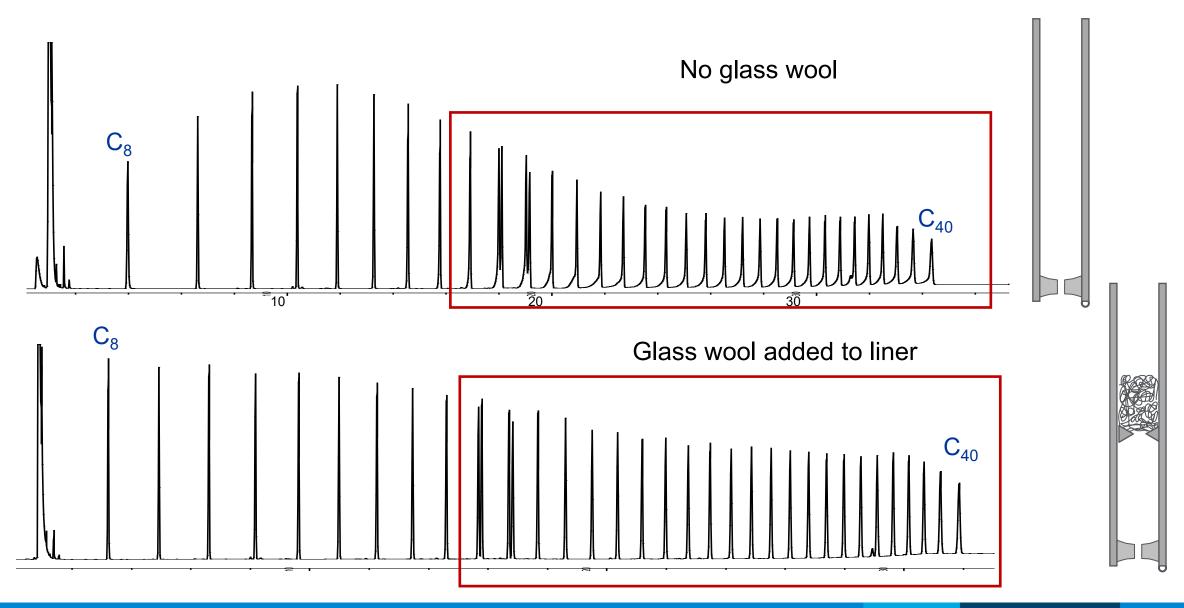


gure 4. Overlay of four replicate chromatograms of the C_{10–44} mixture in hot splitless mode at 14 mm install length.

Agilent Publication 5991-7619EN

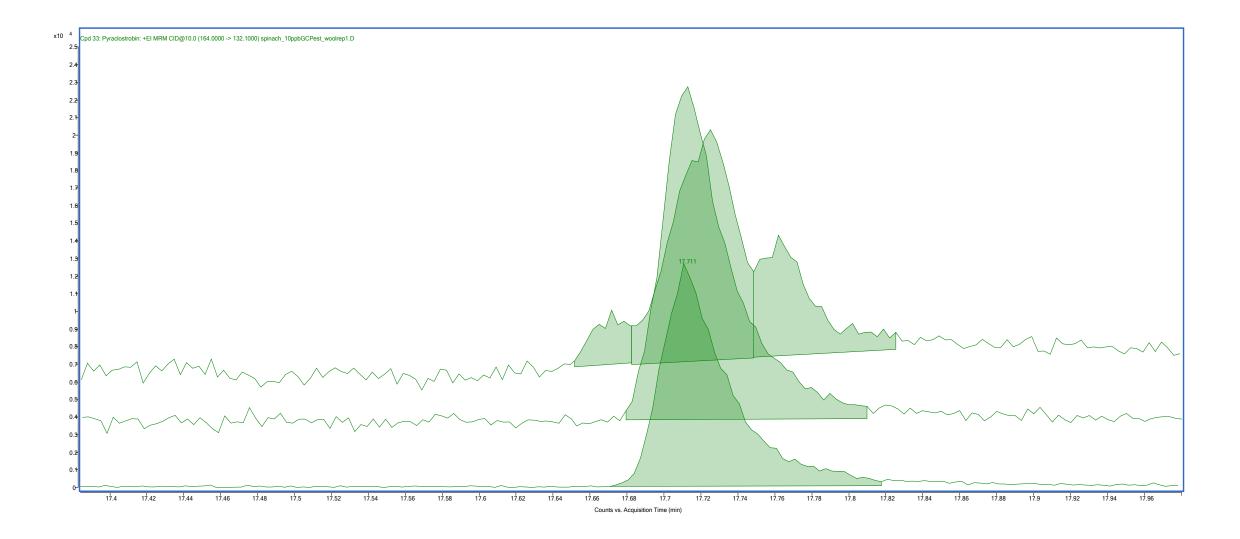
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What Does Mass Discrimination Look Like?



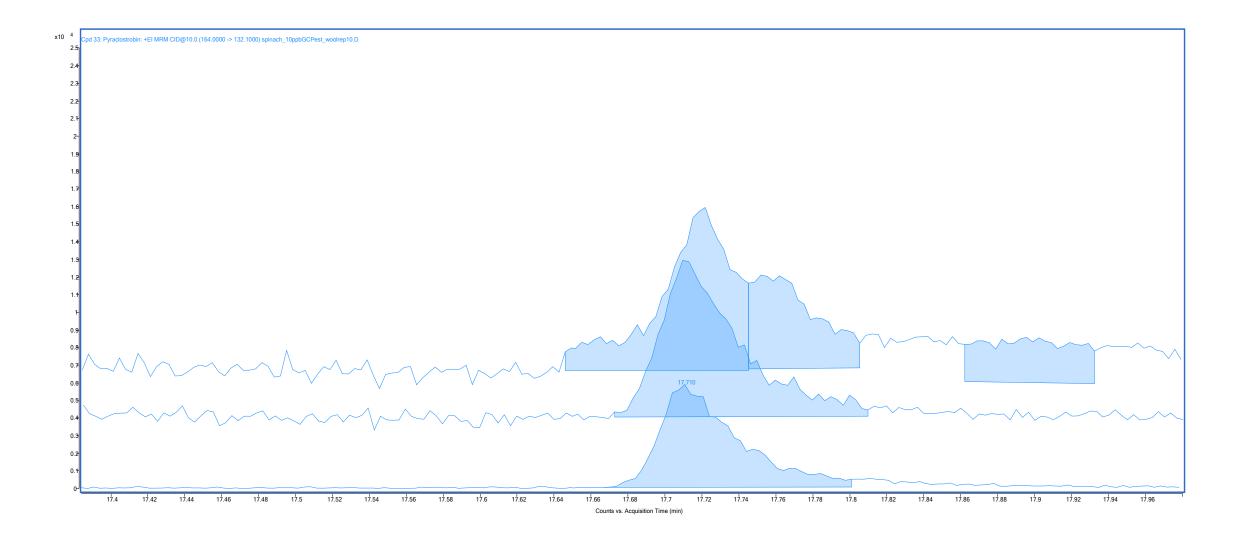


Change in Response: Pyraclostrobin in Spinach on Run 1





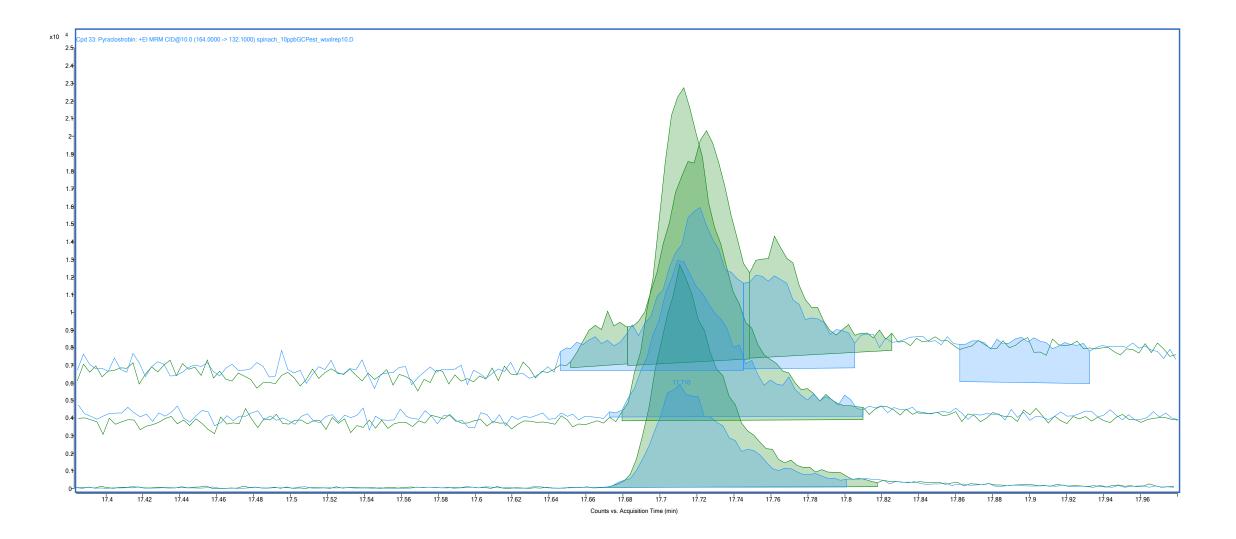
Change in Response: Pyraclostrobin in Spinach on Run 65







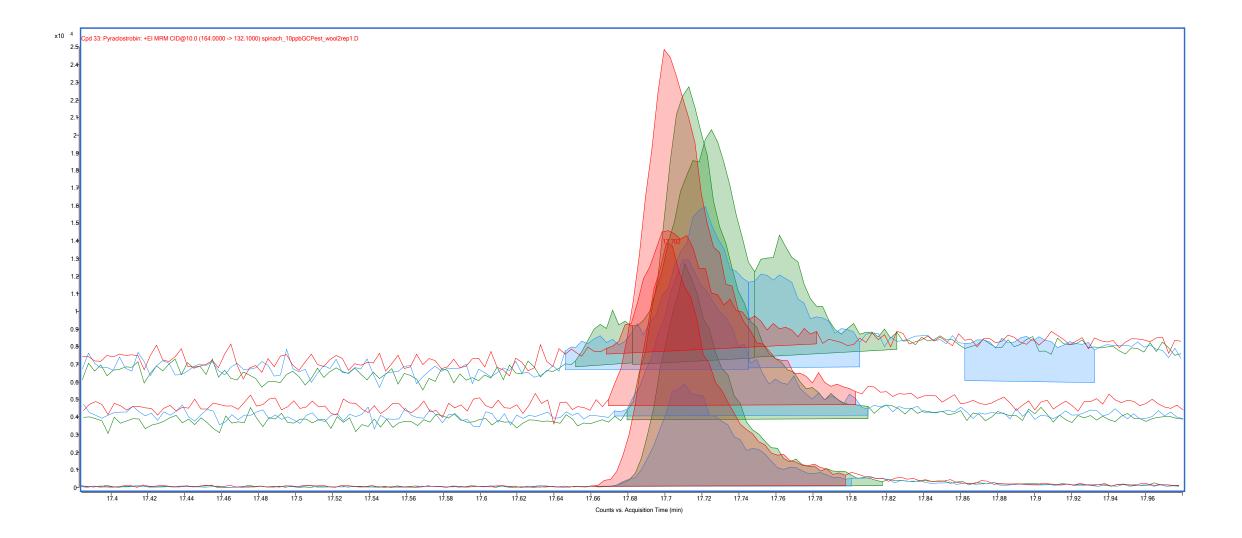
Change in Response: Pyraclostrobin in Spinach on Run 1 vs Run 65





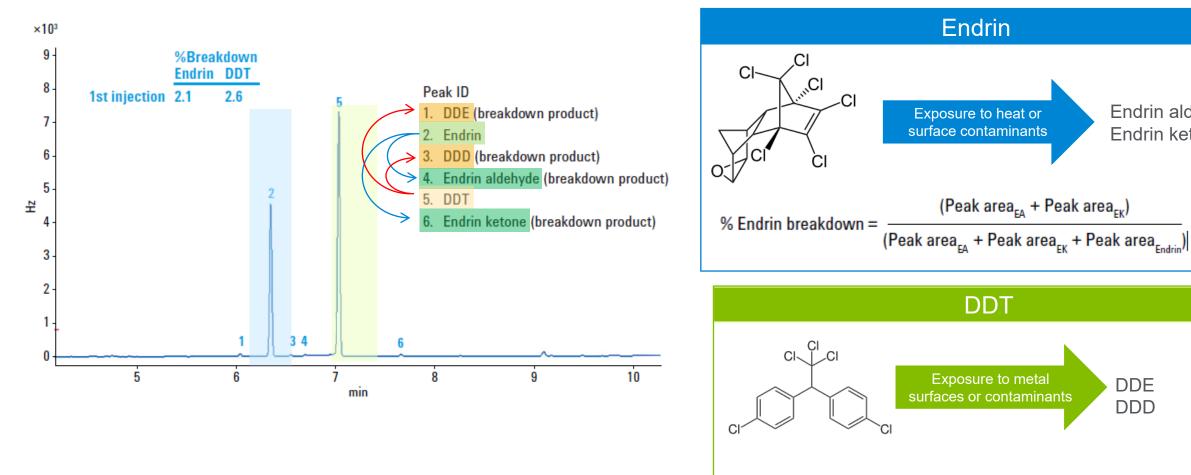


Change in Response: Pyraclostrobin in Spinach with New Liner





Environmental Pesticides Probes: Endrin/DDT Breakdown



% DDT breakdown =
$$\frac{(\text{Peak area}_{\text{DDE}} + \text{Peak area}_{\text{DDD}})}{(\text{Peak area}_{\text{DDE}} + \text{Peak area}_{\text{DDD}} + \text{Peak area}_{\text{DDT}})} \times 100$$



Endrin aldehyde

× 100

Endrin ketone

DDE

DDD

Pesticides Can Be Very Difficult Compounds (Detection in Food Matrices)

Varied reactions to different types of matrices

- Enhanced response
- Decrease response
- Interference for transition and matrix

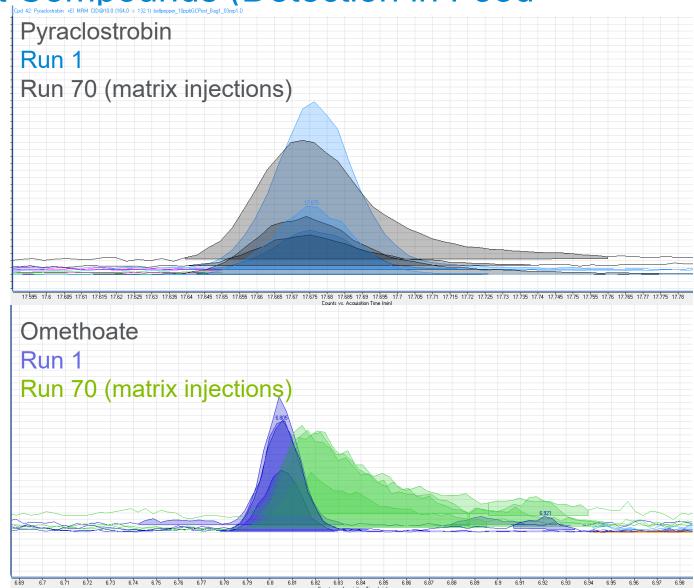
Which pesticides are sensitive to inertness (or lack of)?

The answer is most.

Examples include...

- Omethoate
- Deltamethrin
- Methacrifos
- Pyraclostrobin
- Folpet
- Atrazine

The list can continue for a long time!





How Do We Mitigate Pesticide Breakdown, Loss of Response?

Most compounds may lose some response with repeated matrix injections

Use:

Matrix matched calibration curves and quant methods

• Does not fix breakdown, but user better knows what to expect for target analytes

Use a deactivated liner with barrier

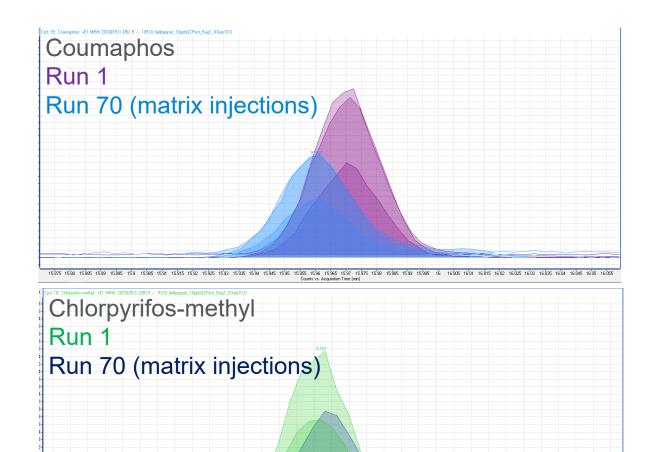
• Ultra Inert frit or glass wool liners

Preemptive maintenance

• Have a standard QC check and criteria for inlet maintenance

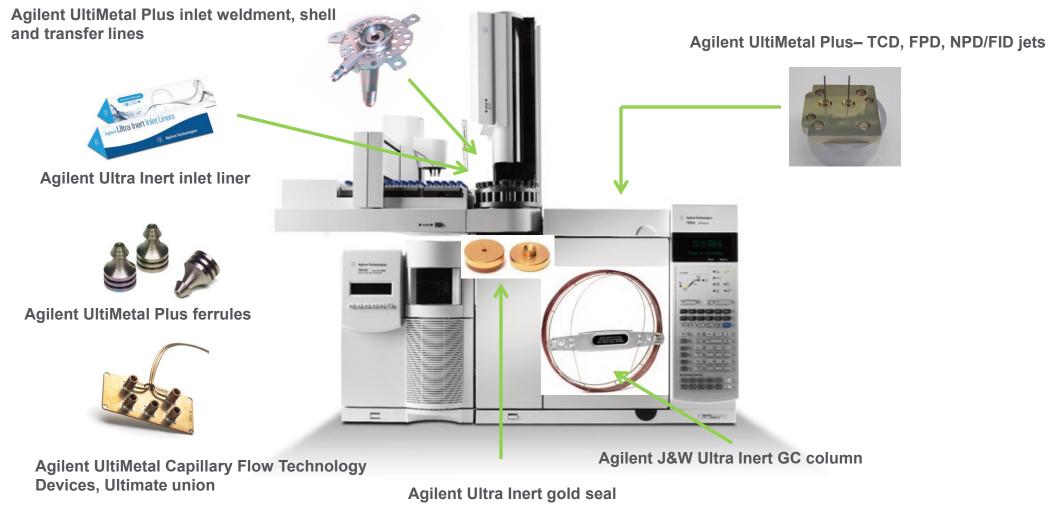
Use (mid column) backflush

• Prevent matrix from migrating as far onto column **and** allows you to trim or swap first column without venting MSD





Agilent Inert Flow Solution



5990-8532EN brochure





Offline Options for Sample Matrix Removal





QuEChERS



Captiva EMR-Lipid filtration cartridges and plates

Bond Elut Solid Phase Extraction cartridges and plates



Captiva syringe filters



Filter vials





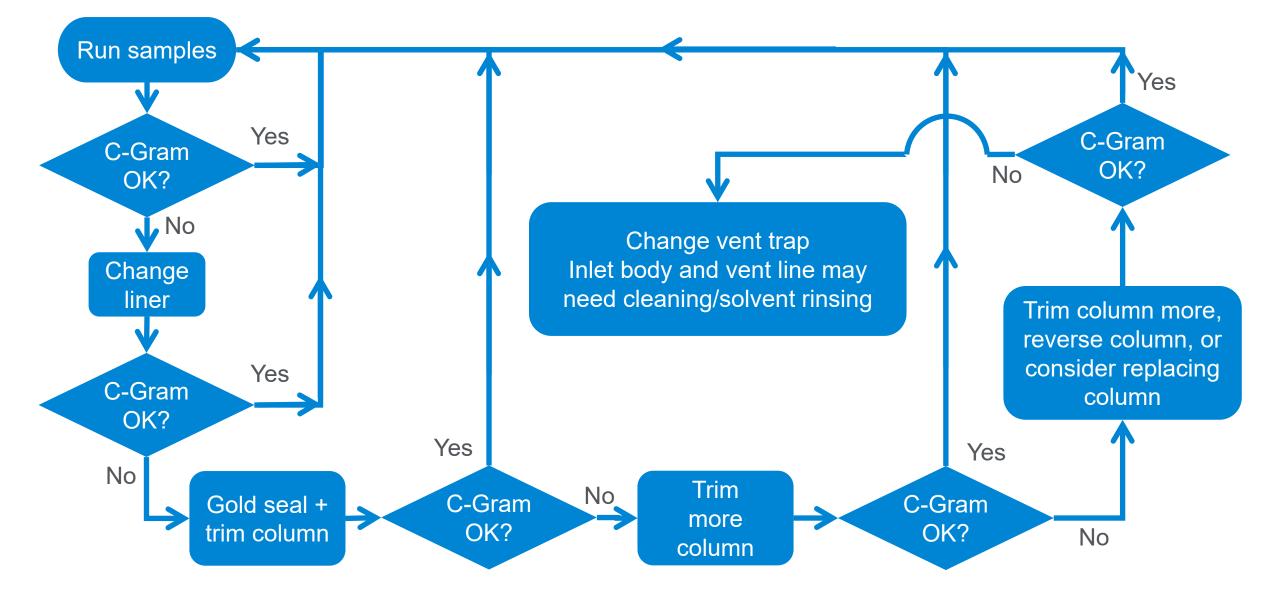
When Do I Change Specific Parts?

Item	Typical Schedule	Comments		
Septum Nut	3-6 months	Septum nut can get warn and shed metal particle into the liner. Replace to minimize activity in the inlet/liner.		
Syringe	Every 3 months	Check movement of plunger and replace if it does not move freely and cannot be cleaned.		
Gold Seal	Monthly	At a minimum replace when trimming the front end of the column		
Split Vent Trap	6 months-1 year	Often forgotten. Can also cause retention instability.		
Liner	Weekly	The liner takes the brunt of the sample load/residues. Replace often to help prevent unwanted down time.		
Trim/Replace column	Weekly-Monthly	When experiencing chromatographic problems trim ½ to 1 meter of the front end of the column. Replace liner, septum and gold seal.		
Inlet Setpa	100-200 injections	Depends a bit on septum type and manual/auto injections.		

Schedule is an approximation of average usage requirements. Actual frequency is application and sample specific. Use your chromatography as a guide to developing a normal maintenance schedule.



Inlet Maintenance Flowchart





Troubleshooting Techniques



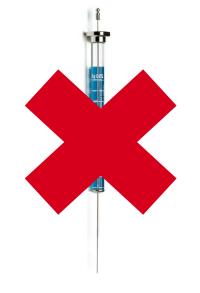


Troubleshooting Tools

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



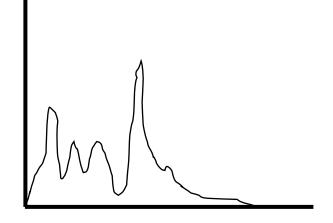
Perform a Noninjection "Blank"



Remove syringe from autosampler



Run your program



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



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, or 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 whether a 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)
	37.3 cm/sec
Flow:	(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 are dispatched within 24 to 48 hours (continental U.S. only, currently)
- Custom standard solutions including our online custom-quoting tool, enabling you to upload recipe formulations and modify the recipe before submitting it.
 - This tool allows you to see the quote pricing instantly and allow them to check quote based on quantity range
 - Check it out at www.agilent.com/en/product/chemical-standards
- Rigorously tested and manufactured under ISO 9001, ISO 17025, and ISO 17034 accreditations
- Sample preparation materials, columns, supplies, instrumentation, and reference materials are all from a single source.





Not Getting the Response You Expect?

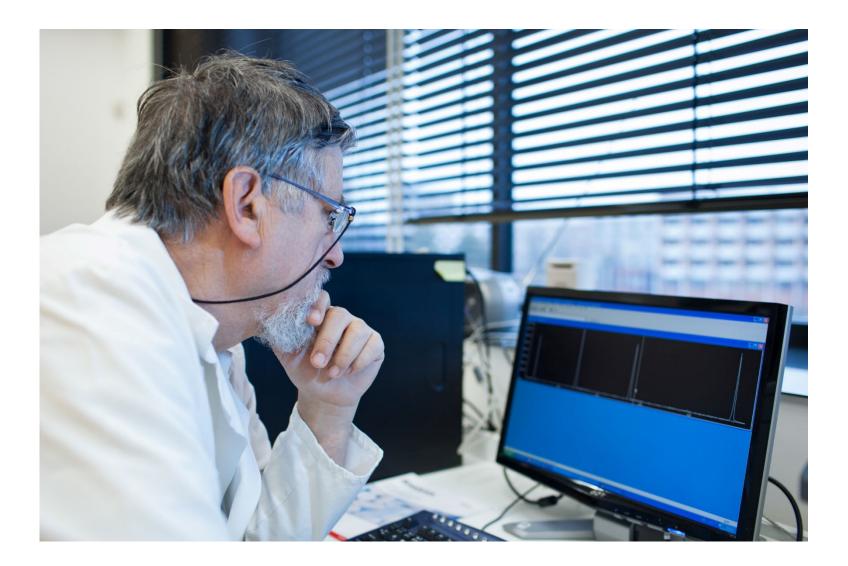
- If you are seeing a reduction in response or see no peaks, try injecting a much higher concentration
- Inject something simple as well





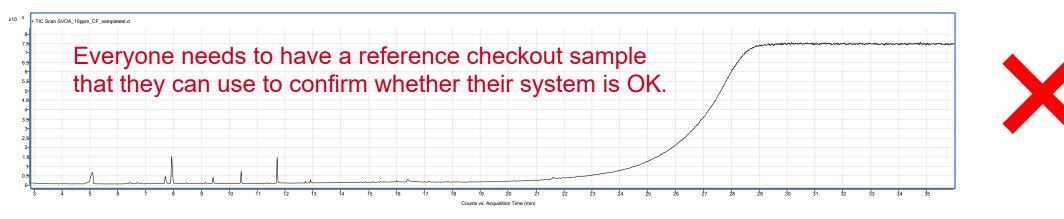


Troubleshooting Example

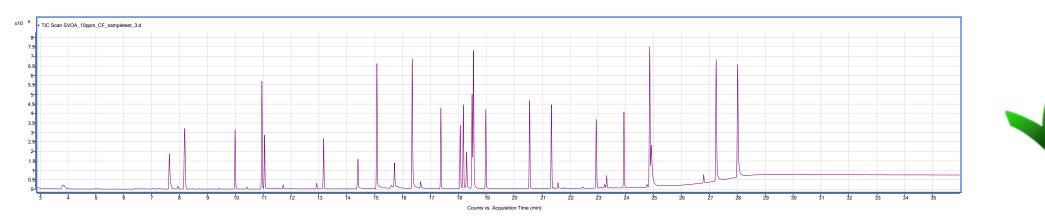




What my TIC looked like:

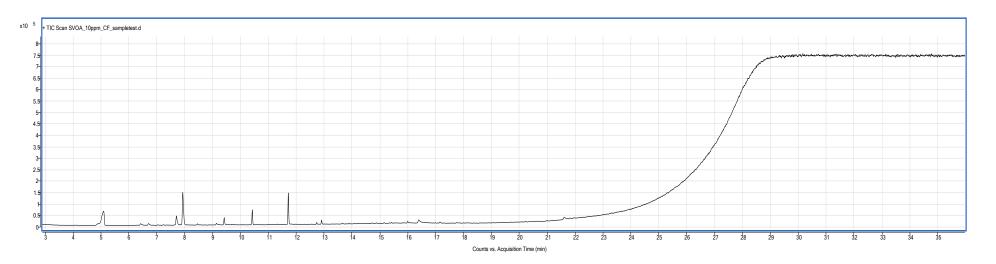


What my TIC should look like:



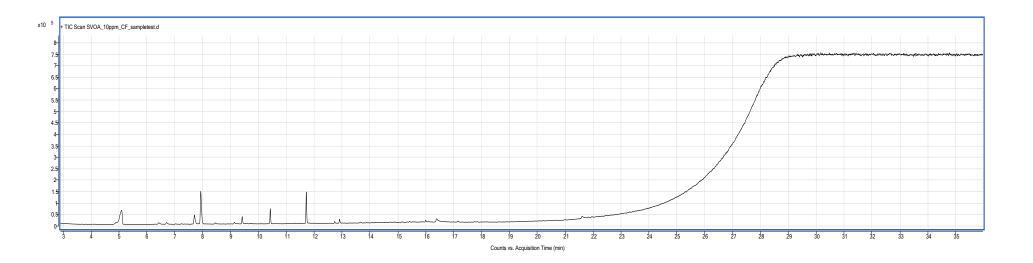


- The wrong vial was injected
- The sample has degraded
- The inlet is leaking
- The column is damaged

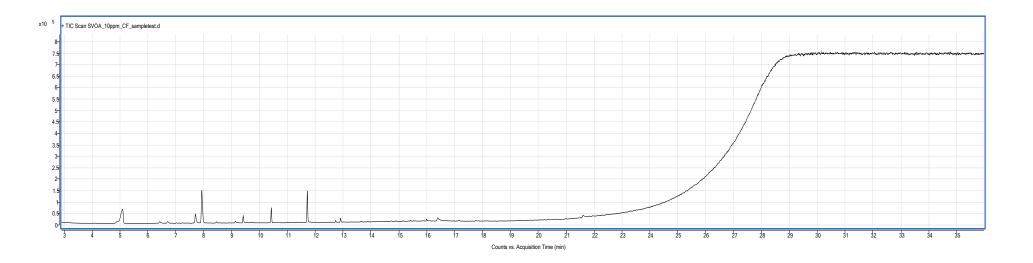




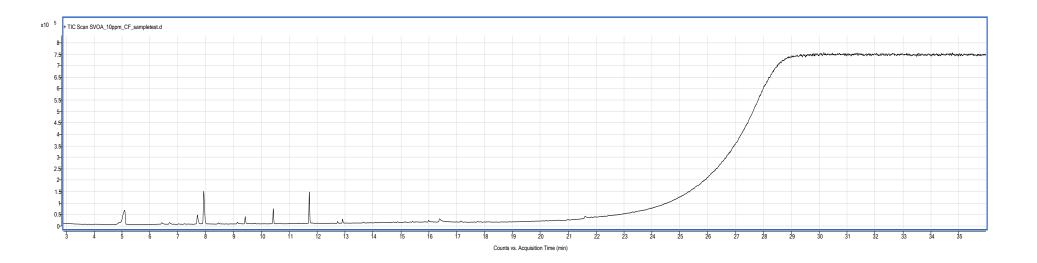
- The wrong vial was injected: Sequence and vial checked, no problem found
- The sample has degraded
- The inlet is leaking
- The column is damaged



- 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

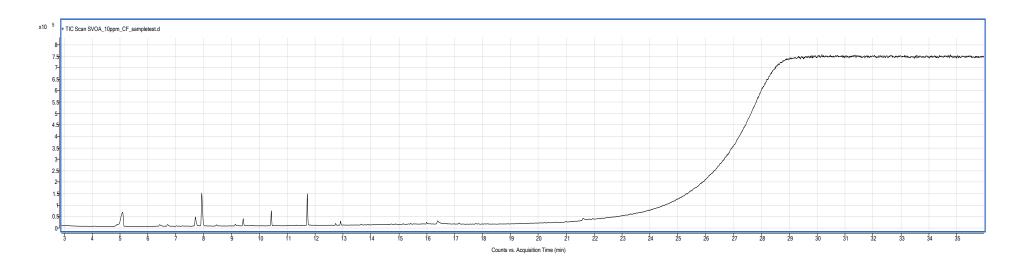


- The wrong vial was injected: Sequence and vial checked, no problem found
- The sample has degraded: A new vial of standard was used, no difference observed
- The inlet is leaking: A tune was performed. O₂, N₂, and H₂O levels were normal
- The column is damaged





- 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"

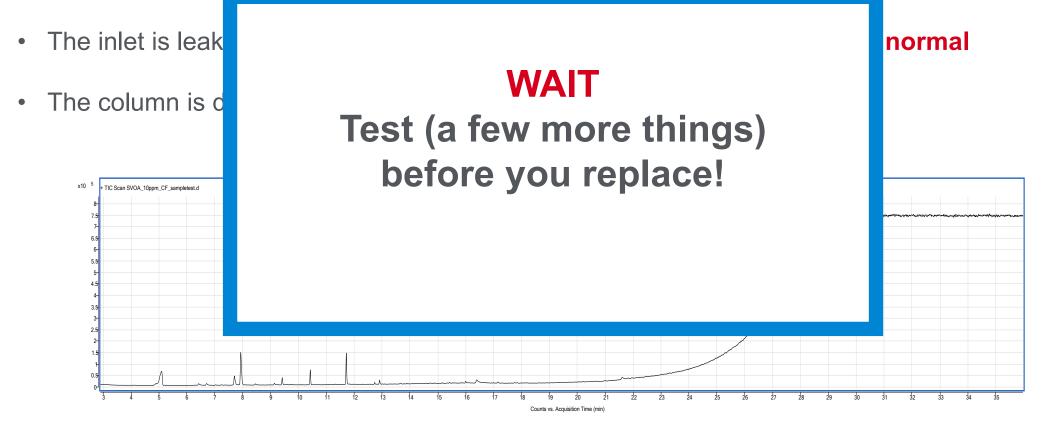






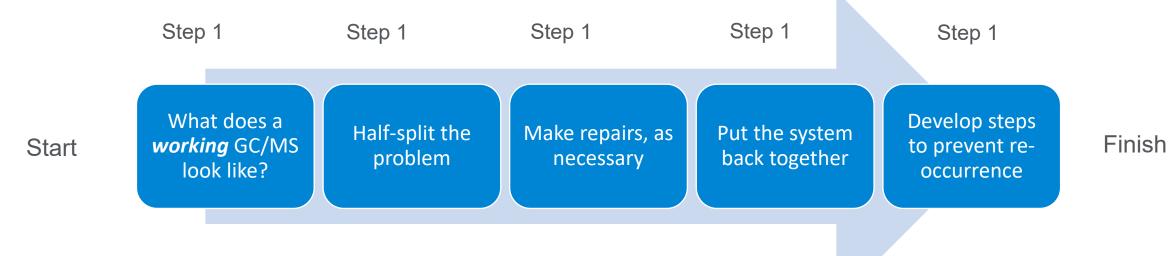
What could cause this?

- 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

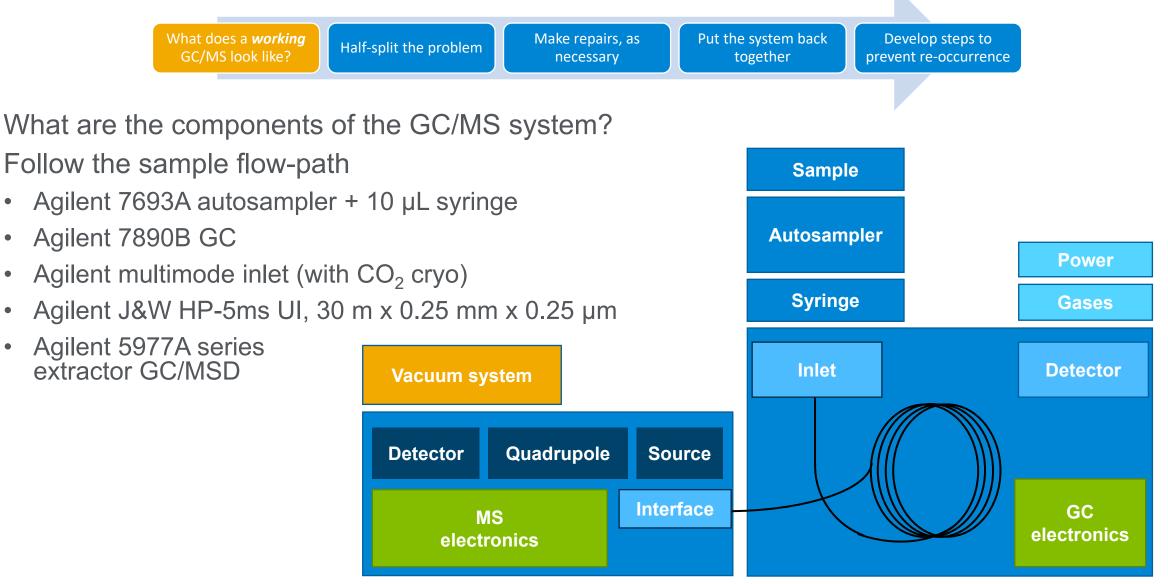


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Follow a Logical Troubleshooting Procedure!







•



Half-split the problem

Make repairs, as

necessary

Put the system back

together

Develop steps to

prevent re-occurrence

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

• How does your background compare to normal?

What does a working

GC/MS look like?

- 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?



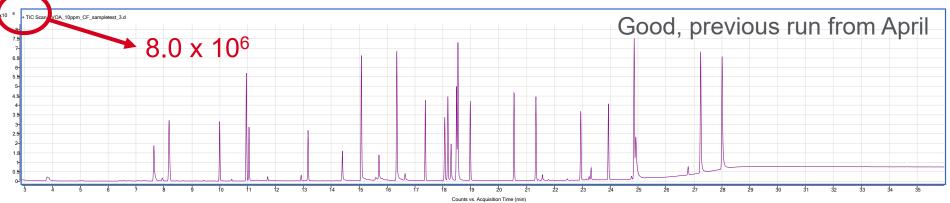
 What does a working GC/MS look like?
 Half-split the problem
 Make repairs, as necessary
 Put the system back together
 Develop steps to prevent re-occurrence

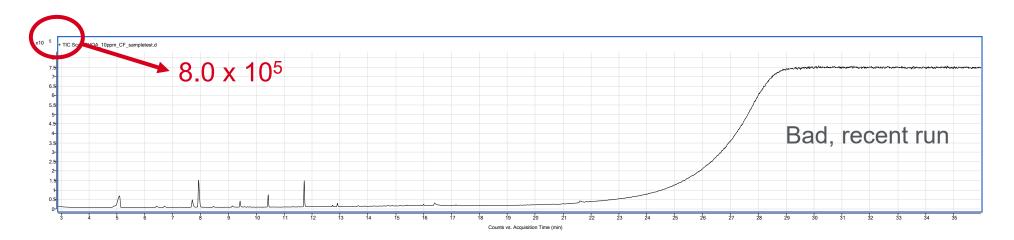
Compare your current data to known good data, when possible.

- How does your background compare to normal? Background looked much 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



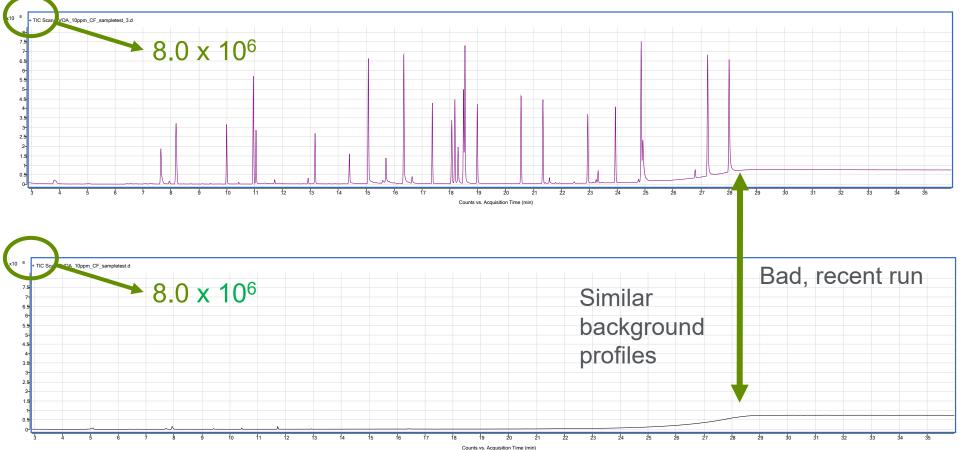
Compare your current data to known good data. Now, the data is much clearer, and the background is not significantly higher. Signals in separate scales:





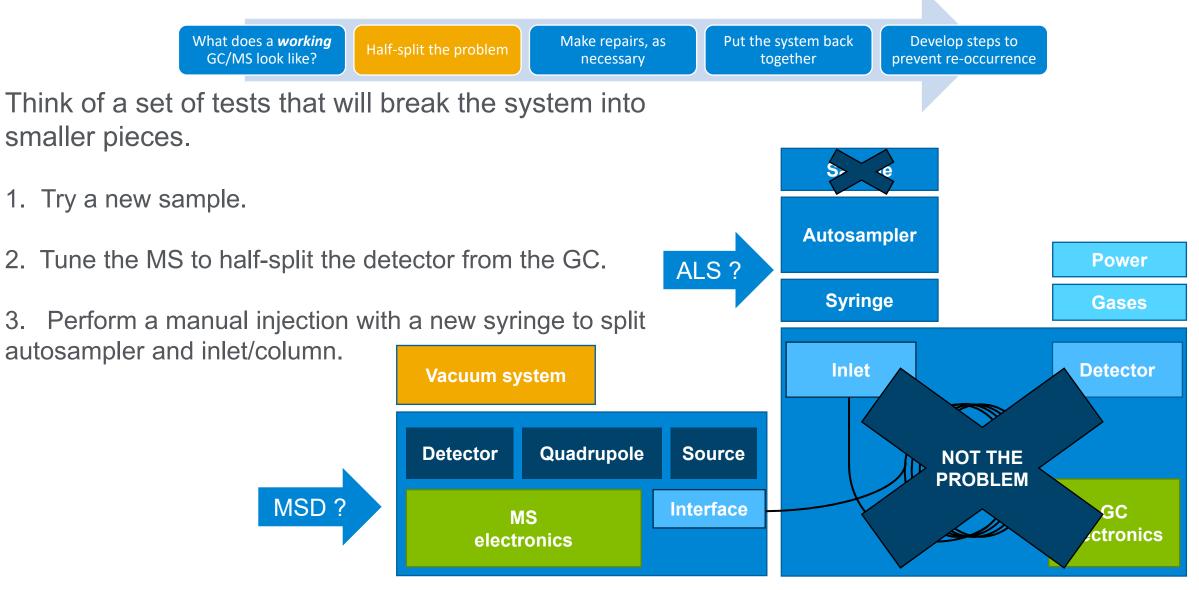


Compare your current data to known good data. Now, the data is much clearer, and the background is not significantly higher. Signals with linked Y axis:





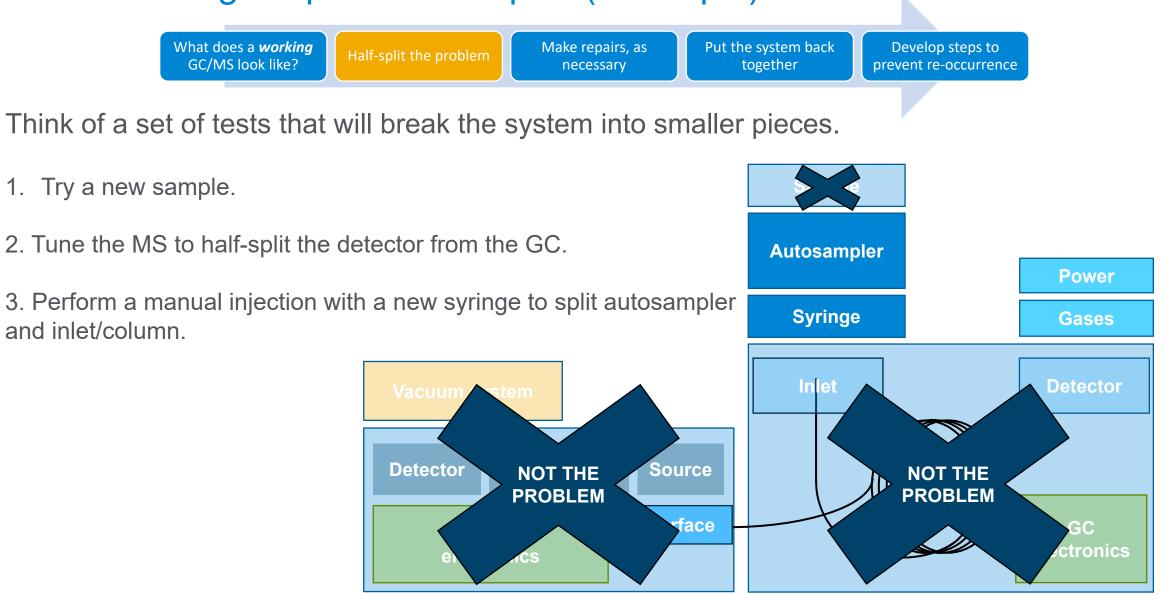
Troubleshooting Step 2: Break Apart (Half-Split) the Problem



3.

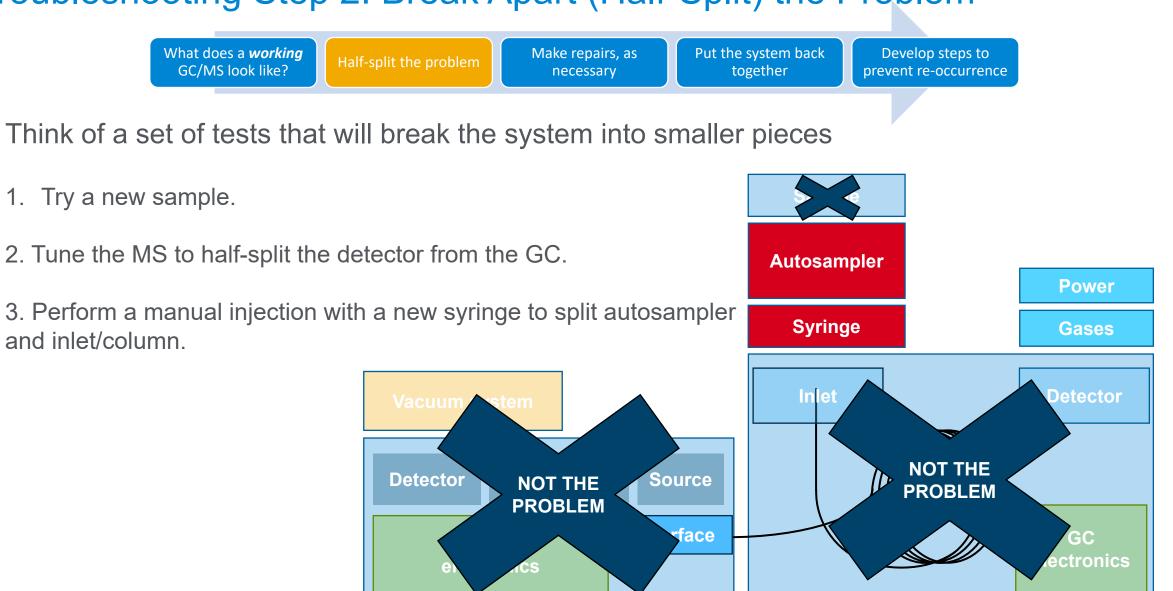


Troubleshooting Step 2: Break Apart (Half-Split) the Problem

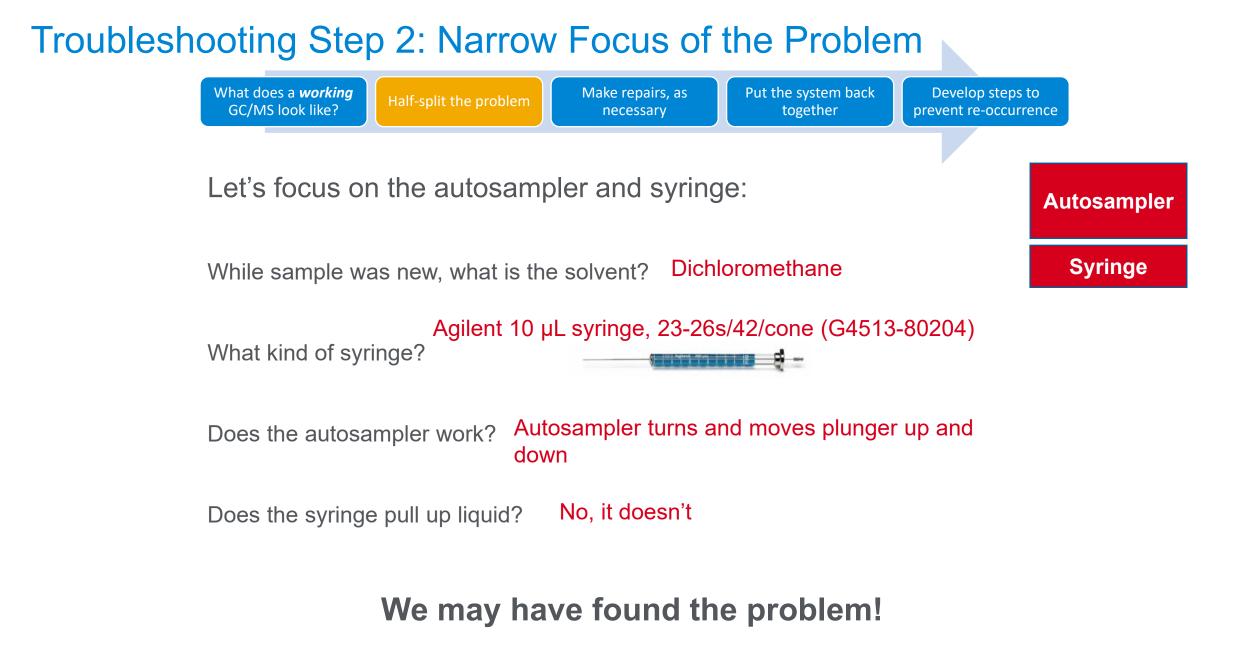




Troubleshooting Step 2: Break Apart (Half-Split) the Problem

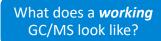








Troubleshooting Step 3: Make the Repair



Half-split the problem

Put the system back together

Develop steps to prevent re-occurrence



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

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

PTFE plunger tip

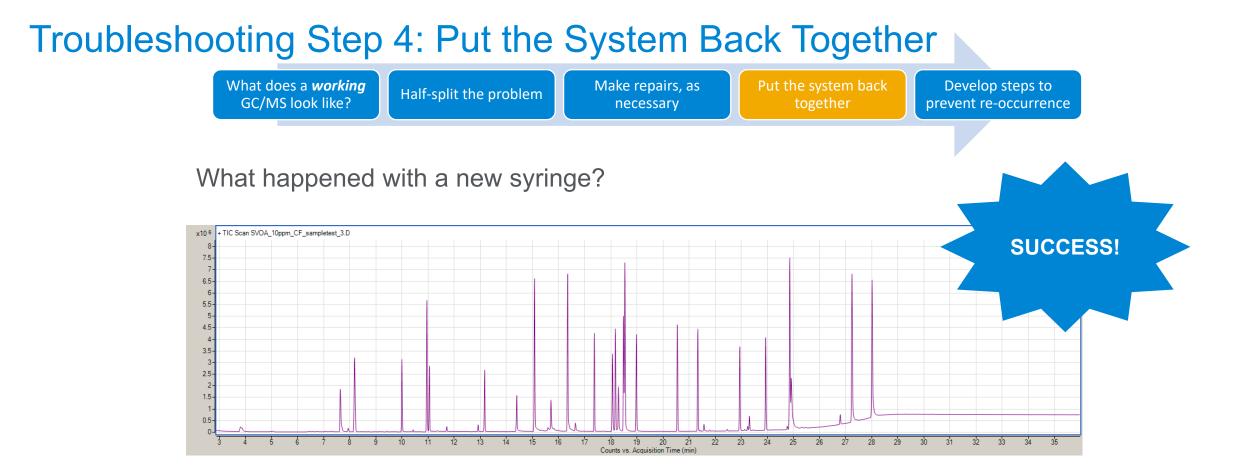
Proper syringe maintenance must still be performed. Clean and refill syringe wash vials frequently.

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



Syringe







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!





Agilent University

Why training? What can we help with?

Agilent University:

- Trained over 38K students FY19
- 98% customer recommended ٠
- 4.6 out of 5 customer satisfaction •
- 94% excellent and very good •

Labs who want faster and more efficient learning options to help overcome training challenges

Overtasked staff

Staff turnover

Pressure to improve quality and productivity

Daily consistency with output and results

Reduce costs associated with lab operations

Flexible and convenient training options when and where you need them:

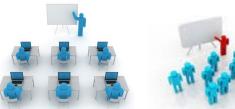


instructor led

Virtual training

E-learning self-paced

In-person training



Classroom



virtual

Onsite or

Trust Agilent for answers leveraging up-to-date knowledge and generally accepted practices for all your training needs



Troubleshooting Tips

1. Isolate the problem

(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 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 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

