

# Don't Fear the Ghost or Unexpected Peaks: Troubleshooting and Prevention

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# Exclusive Offer for Attending Agilent Chemistries Webinars

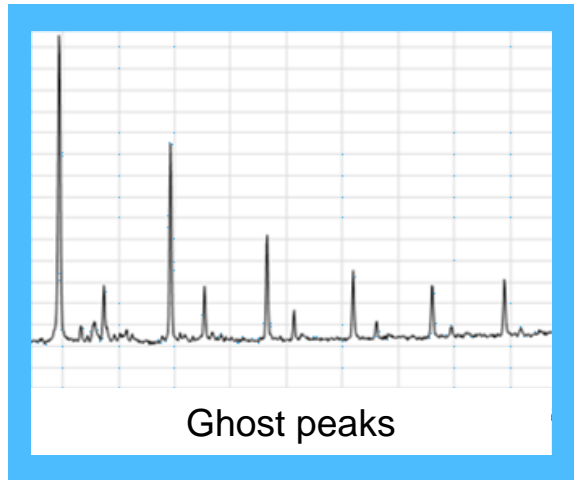
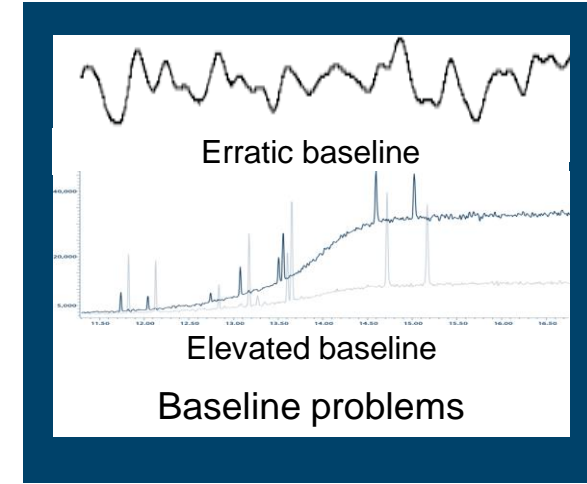
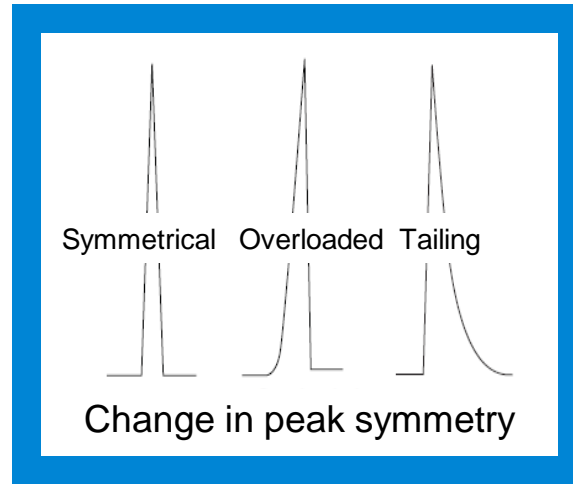
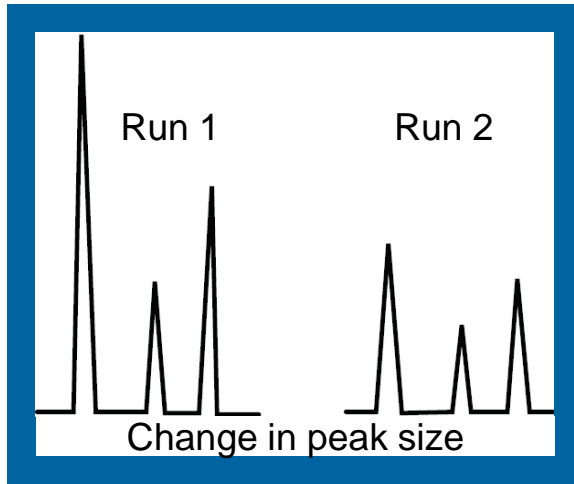
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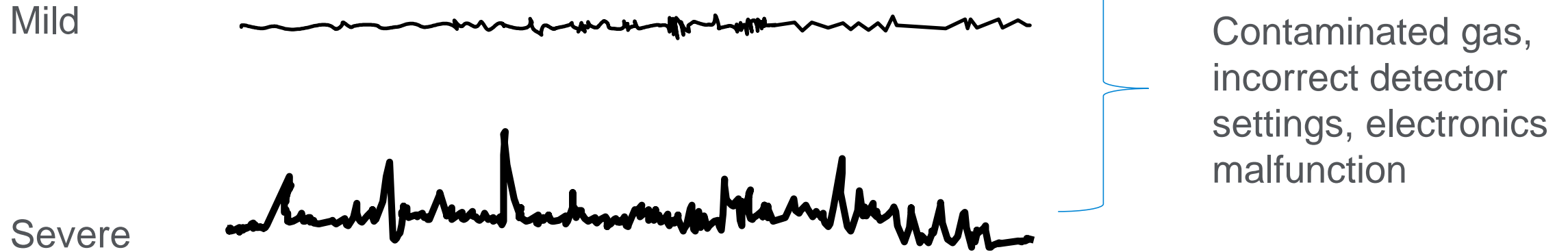
\*Some restrictions apply on supplies

# Examples of Poor Peak Shape



# What Isn't a "Peak"?

Baseline noise/erratic baseline



Severe

PLOT columns shedding, electronic/detector issues

Spiking



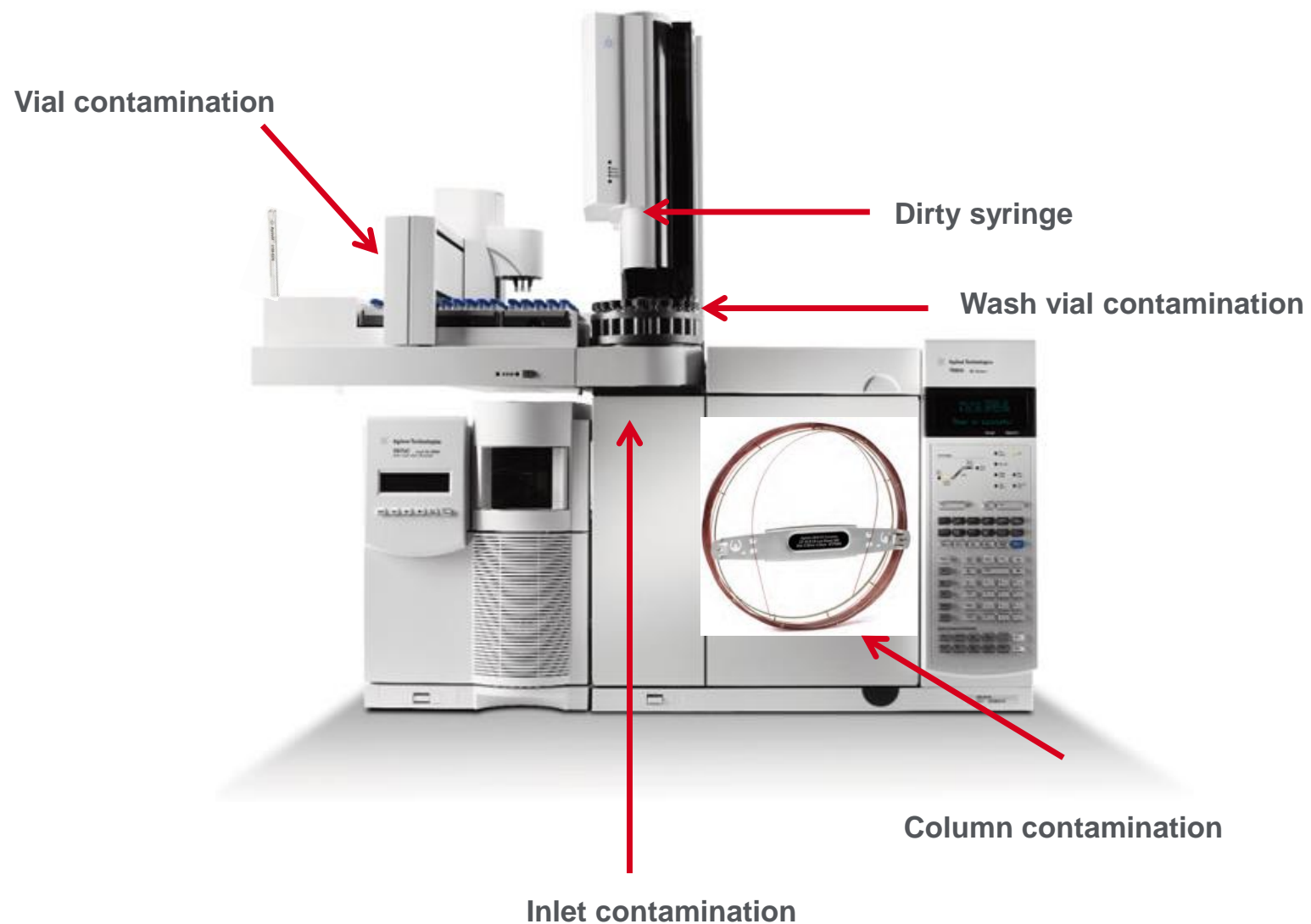
# Bad Gas Stinks

- Use ultrahigh purity carrier gas (99.9995% or greater)
- Use the appropriate gas traps
- Oxygen in carrier gas is detrimental to GC, resulting in:
  - Reduced response
  - Elevated background
  - Irreversible column damage
  - Premature filament failure
  - Excessive source maintenance
- Agilent has a wide range of gas filters
  - GasClean oxygen and moisture filters have indicators
    - Replace when needed
    - Correct any leaks present



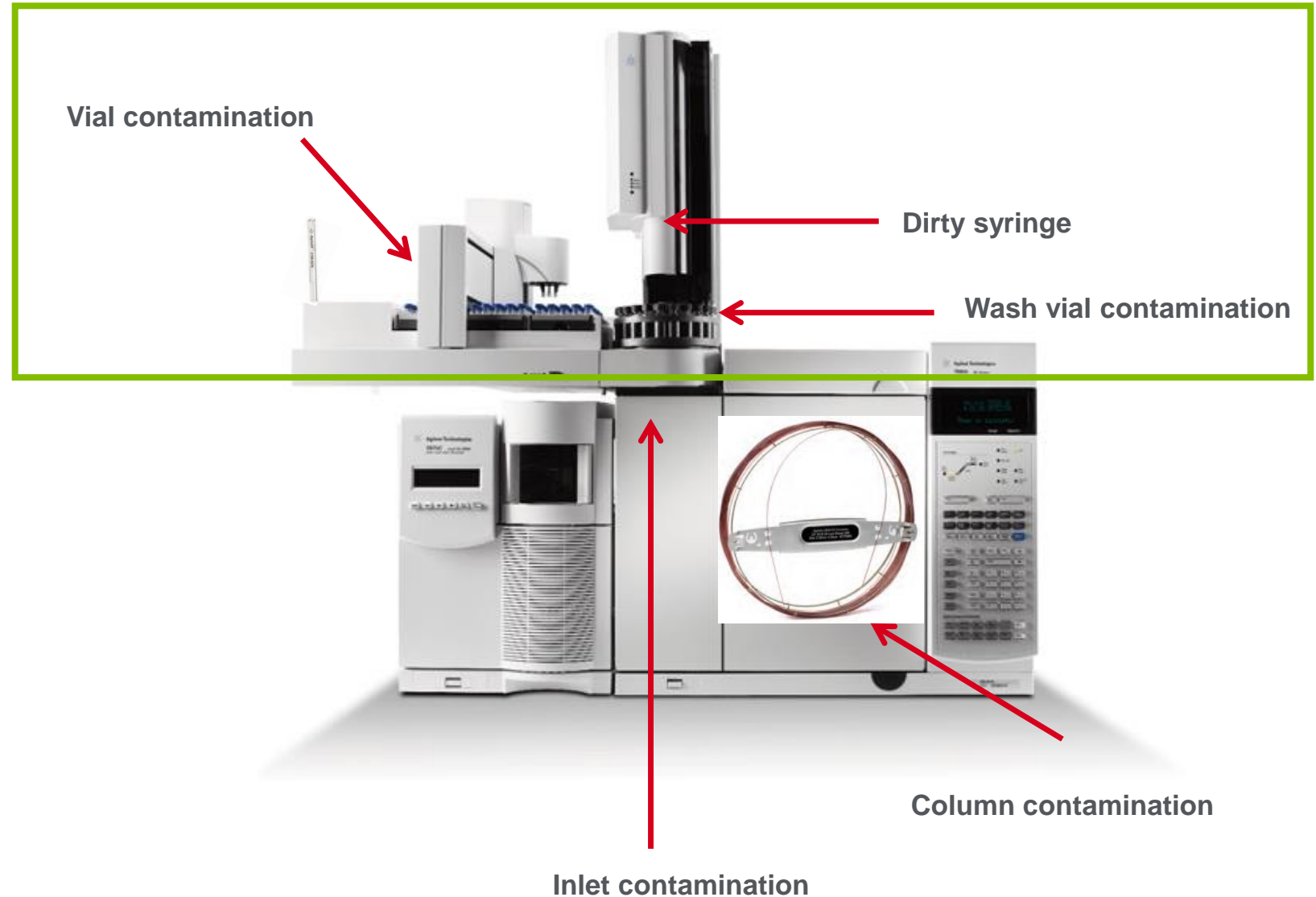
GC/MS filter  
Agilent p/n CP17973

# Where Can These Ghost Peaks Come From?



# Where Can These Ghost Peaks Come From?

## Autosampler



# Ghost Peaks from Your Syringe Carryover

## Possible causes

- Insufficient number or type of needle washes
- Solvent wash vial empty
- Syringe worn or dirty
- Sample/solvent mismatch
- Dirty autosampler needle guide

## Suggested actions

- Increase number or type of washes
- Rinse with a variety of solvents
- Rinse and refill solvent wash vial
- Clean or replace syringe
- Ensure samples and solvents, from one vial to the next are miscible
- Replace needle guide

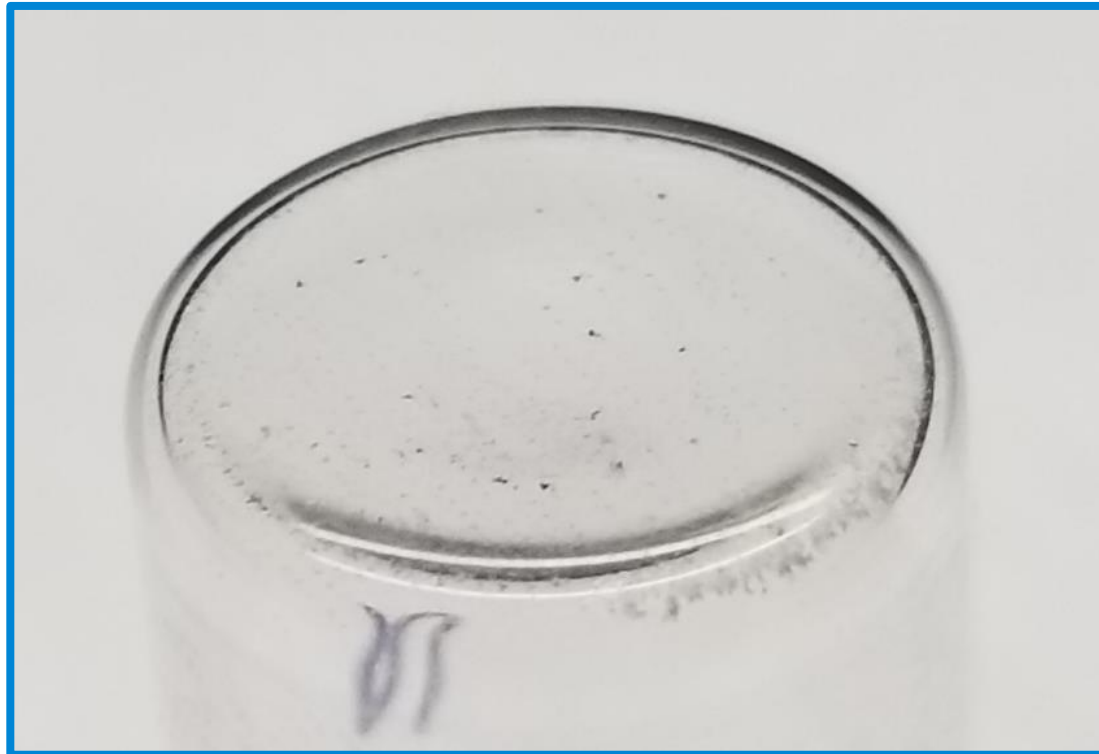




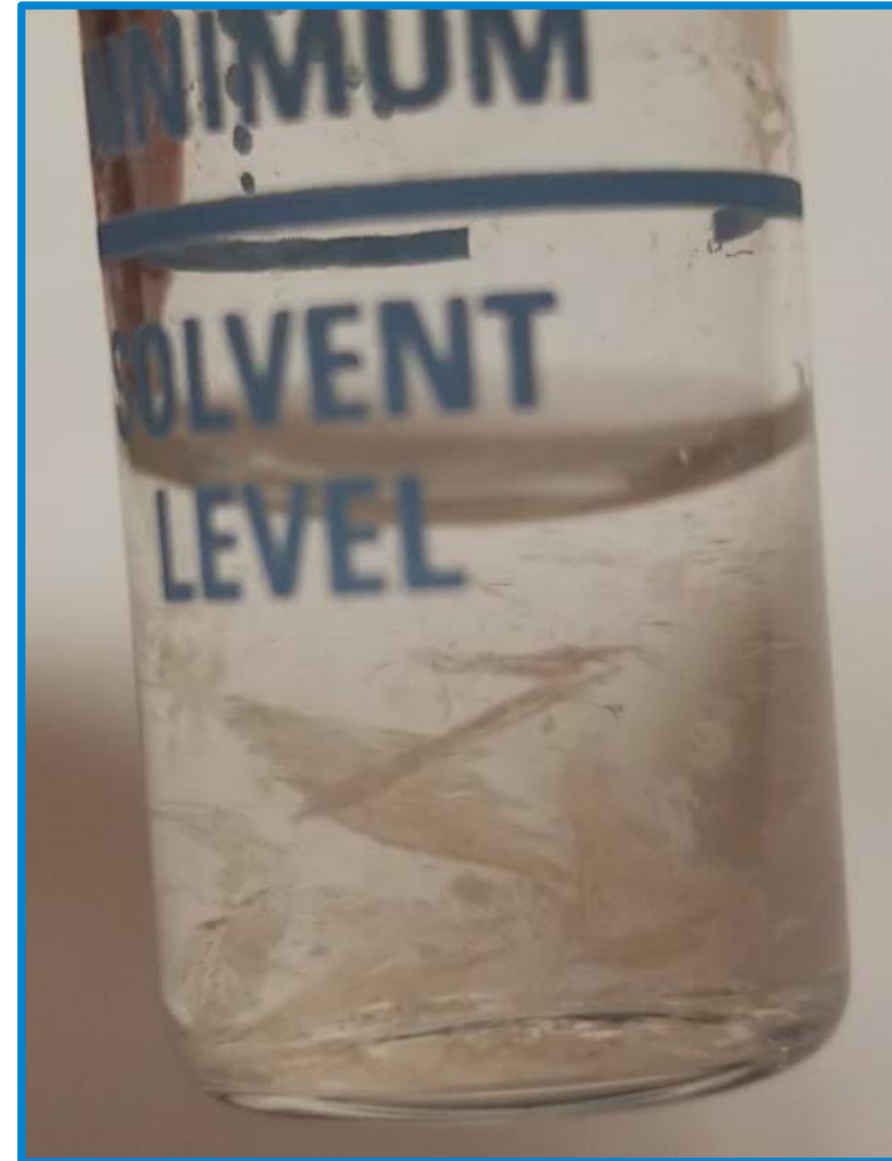
# Washes and Pumps

## 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

## “Diffusion caps”

- Diffusion caps are very important
- Reduce volatile solvent diffusion
- Good alternative to using septa, which could core, contaminate wash solvent vial, resulting in septum bleed peaks

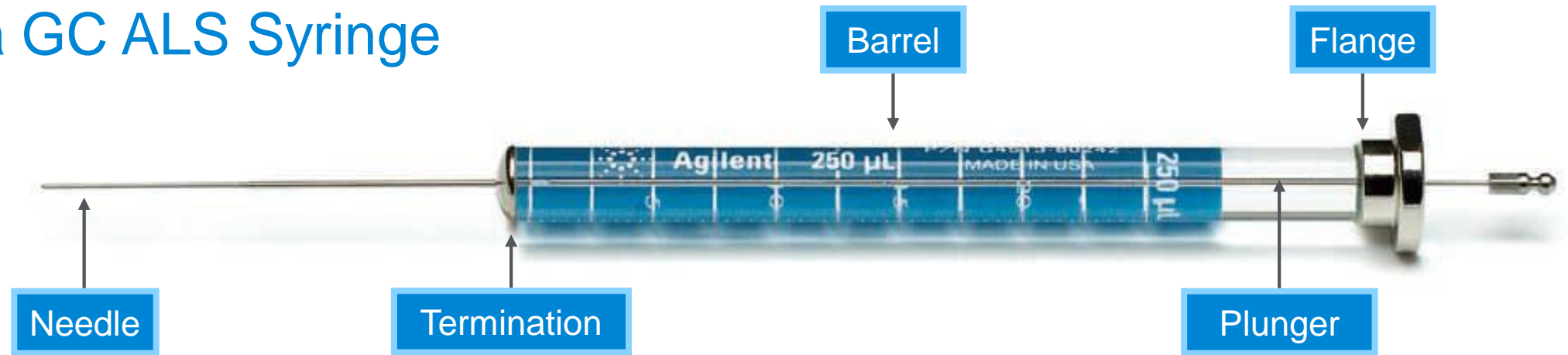


Diffusion inserts with black open top screw caps, 12/pk (p/n 07673-40180)



4 mL wash vials with fill markings and caps, 25/pk (p/n 5182-0551)

# Anatomy of a GC ALS Syringe

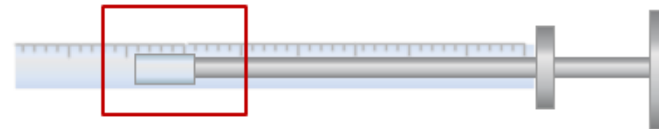


## Standard plungers

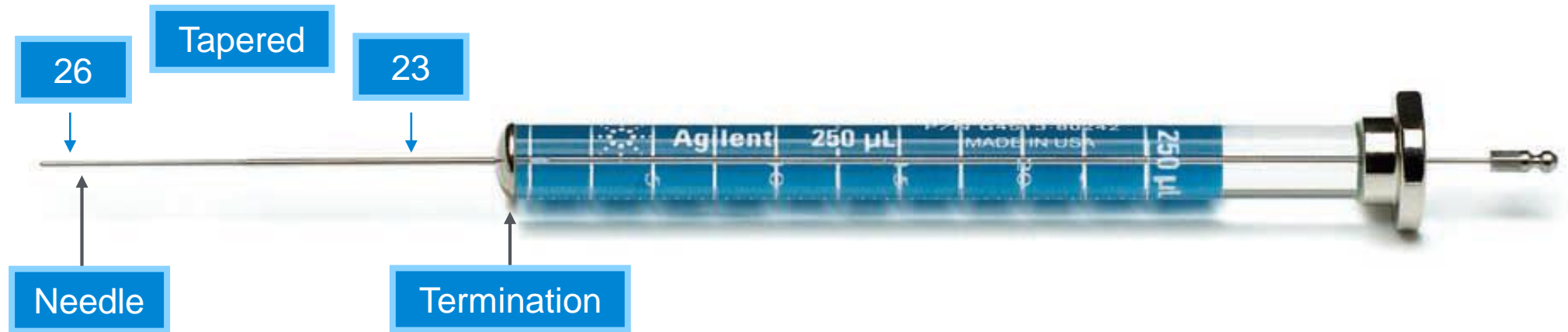
- Fit tightly within syringe barrel
- Limit loss of volatile sample
- Individually fitted to the syringe
- Not replaceable/not interchangeable
- Recommended for analysis of liquid samples

## PTFE-tipped (shown)

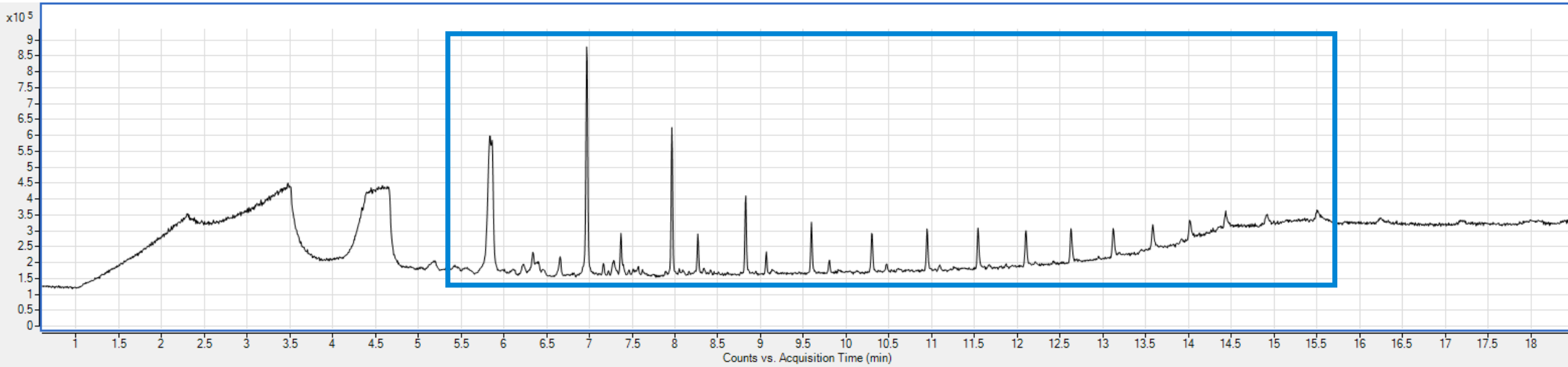
- Limit sample deposit adsorption
- **Forms gas-tight seal**
- Replaceable
- Requires maintenance to maintain PTFE seal
- Recommended for:
  - “Dirty” samples
  - Highly volatile samples
  - Gas injections
  - Chlorinated solvents



# Repeat Injections from the Same Vial



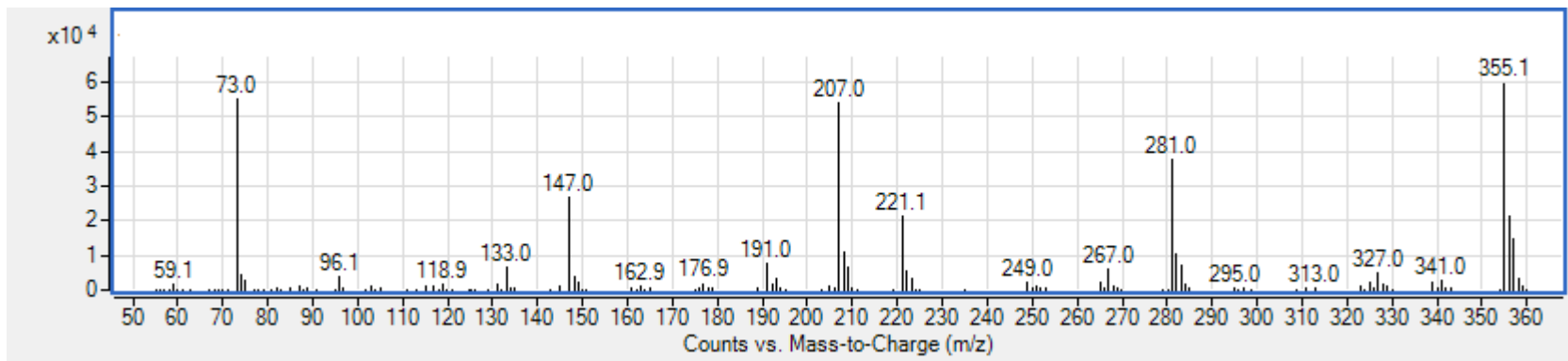
# Septum Maintenance: TIC of an Inlet Septum



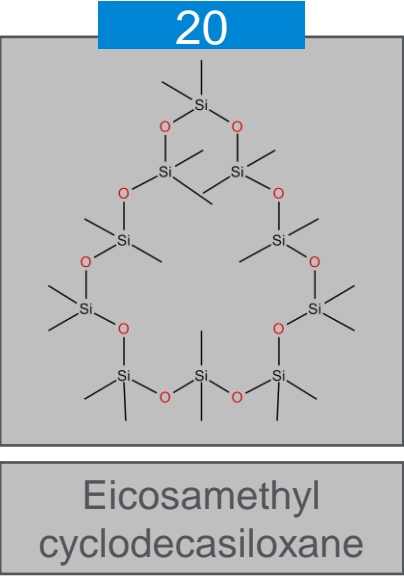
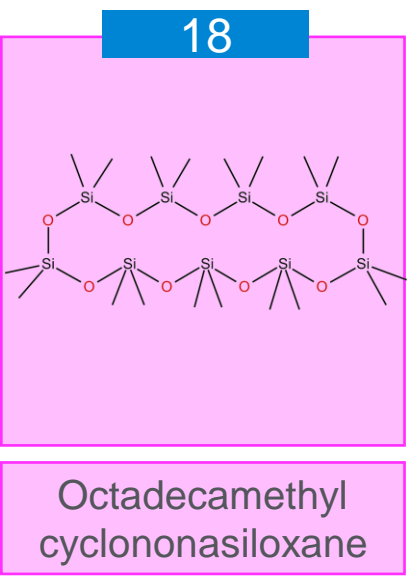
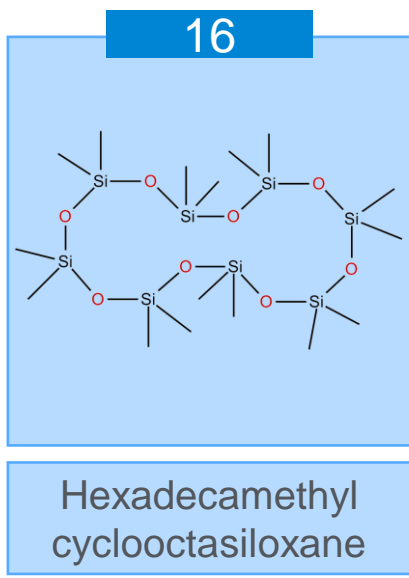
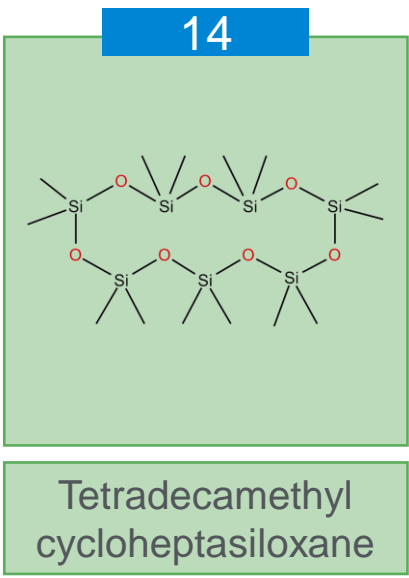
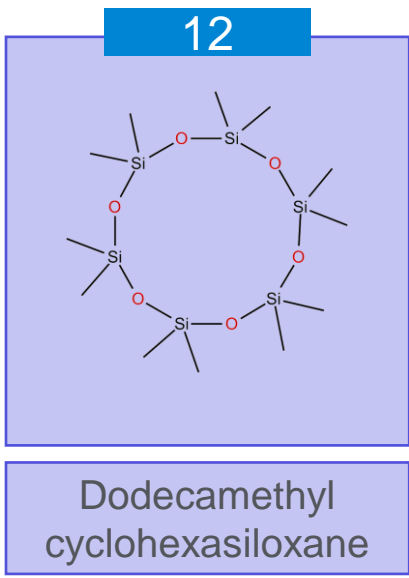
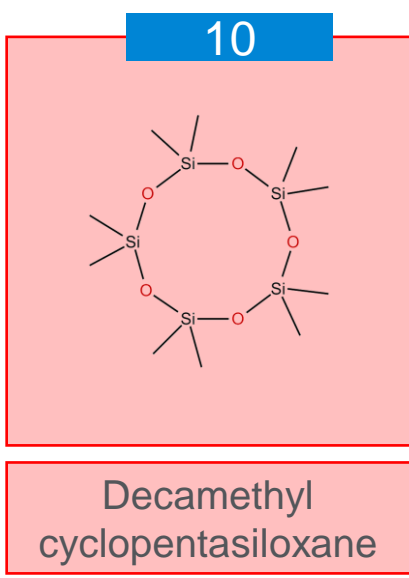
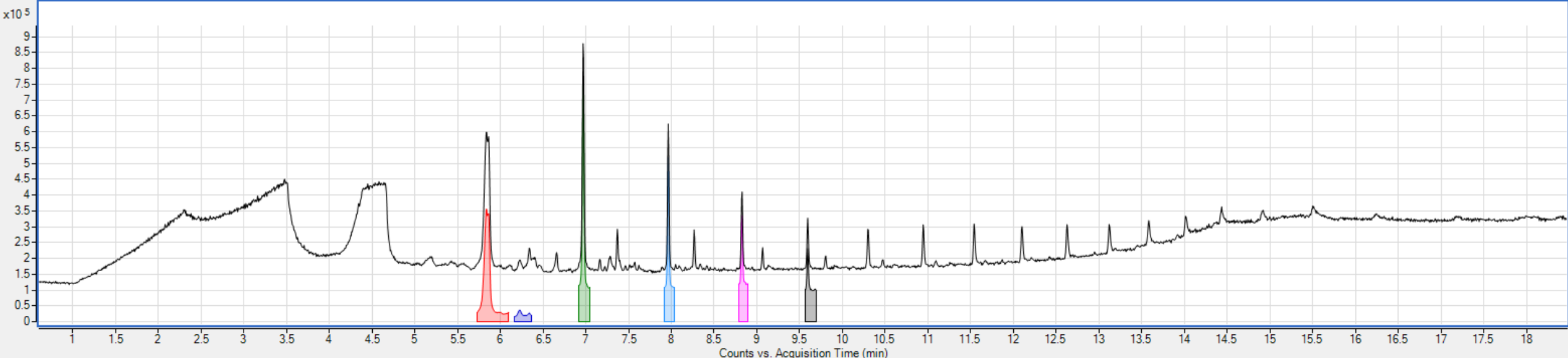
**Common ions for siloxane molecules**

- 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.

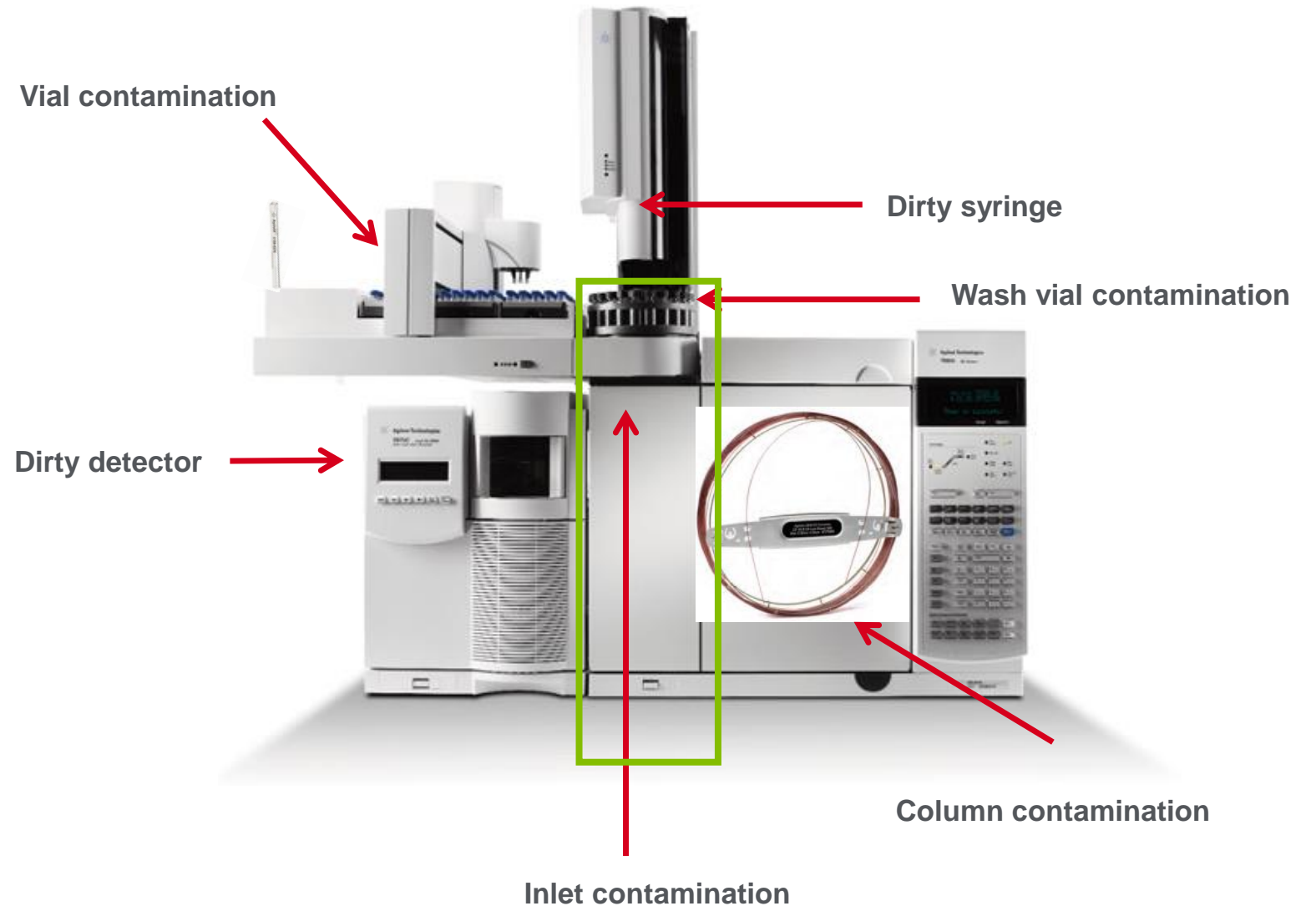


# Deconvoluted Inlet Septa Spectrum



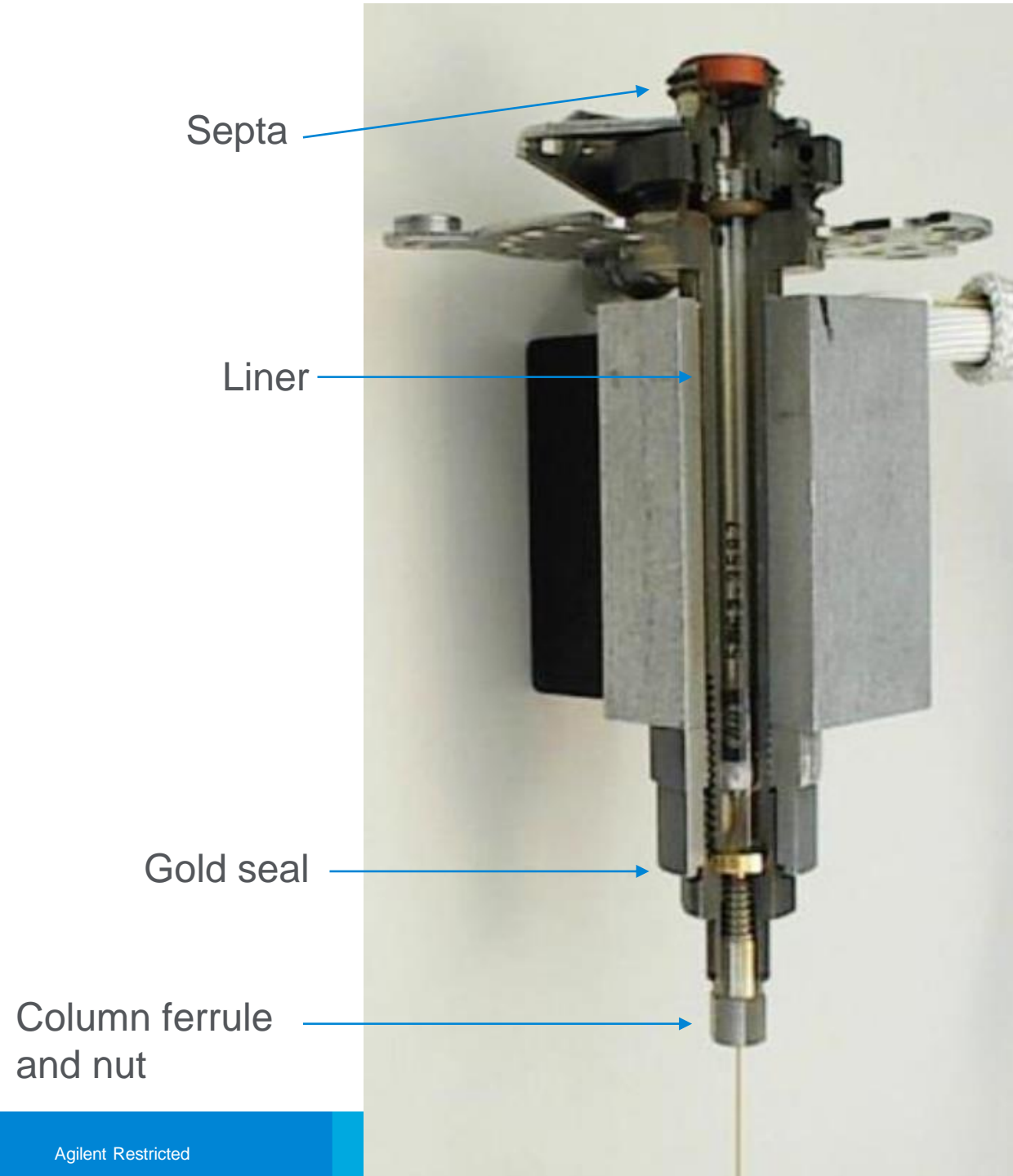
# Where Can These Ghost Peaks Come From?

## Inlet contamination



# Inlet

- Injection efficiency:
  - Main function of the inlet is to produce a narrow sample band at the head of the column
  - One of the most important aspects to any high resolution GC method
- Must be reproducible
- The liner volume must be large enough to accommodate the solvent's phase transformation into a vapor (backflash)
- The vast majority of chromatography problems are at the “front-end”
- Many consumables to replace: septa, liner, gold seal
- Inlet body must be cleaned/solvent rinsed periodically (**no steel brushes please**)





# Backflash

## Cause

- Vaporized sample expands 100 to 1000 times
- Portions may leave the liner
- Occurs when vapor volume > liner volume

## Potential problems:

- Loss of sample
- Baseline interferences
- “Ghost” peaks
- Tailing solvent front or major component

# Typical Solvent Expansion Volumes

Solvent	Vapor volume ( $\mu\text{L}$ ) of 1 $\mu\text{L}$ Liquid
Water	1010
Methanol	450
Carbon disulfide	300
Methylene chloride	285
Acetone	245
n-Hexane	140

# Backflash

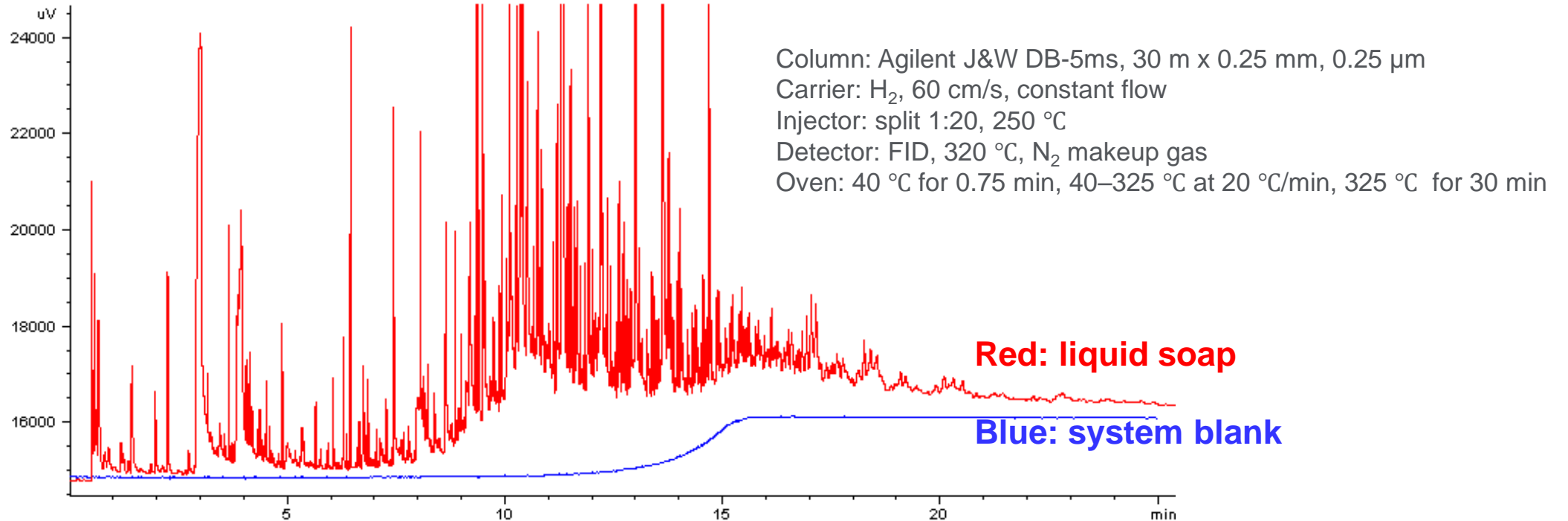
## Minimizing

- Large volume liner (possibly tapered)
- Small injection volume
- Low expansion solvent
- Low injector temperature
- High carrier gas flow rates
- High head pressures (possibly pulsed)
  - Smaller column diameters
- Usually start with 0.5  $\mu\text{L}$  or less

Use vapor volume calculator

<https://www.agilent.com/en/support/gas-chromatography/gccalculators>

# Contamination from Your Hands



## Procedure:

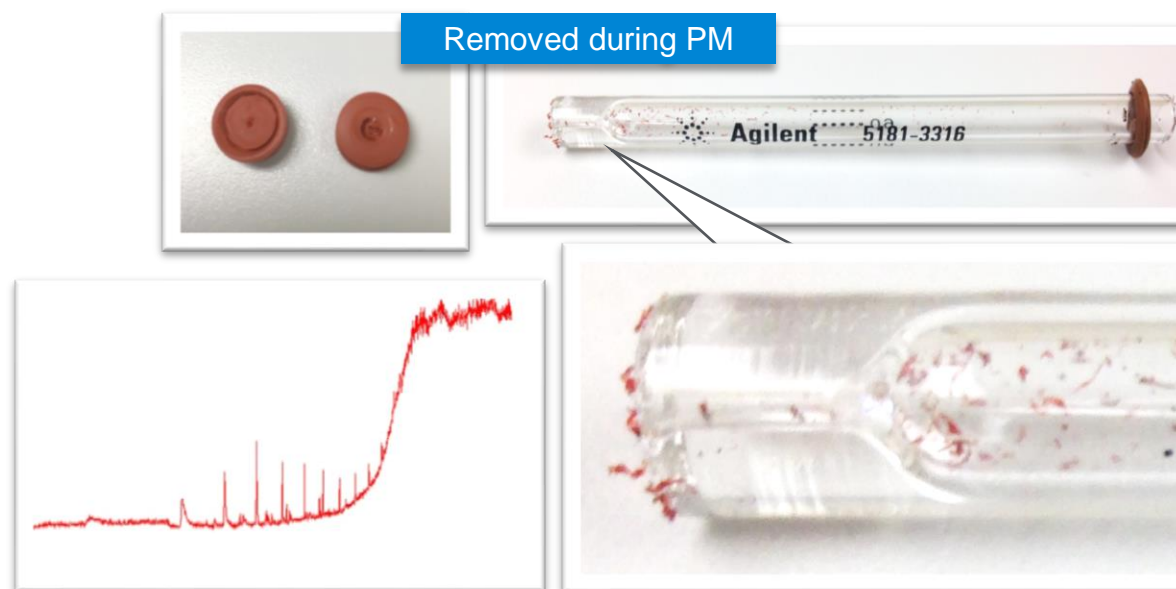
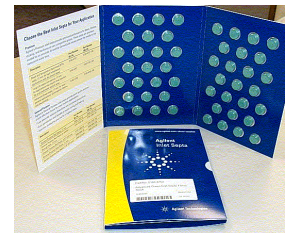
- (1) One very small drop of liquid placed on one fingertip.
- (2) Fingertip was wiped with paper towel to remove as much of the offending material as possible.
- (3) Lightly touched the part of the column sticking up above the ferrule.
- (4) Installed column into injector.
- (5) Set oven temperature to 40 °C.
- (6) Started oven temperature program as soon as oven reached 40 °C.

# More and More “Touchless” Packaging



# Septa

- Typical cost of one premium septum (list), \$1.25.
- Typical cost of one GC column, 30 m x 0.25 mm id, \$450.
- Proactively change inlet septa.
- Agilent packing eliminates contamination of septa.
- “Centerguide septa” puts less strain on syringe compared to solid septa.
- Do not overtighten septum nut, as septum can begin to “bulge” out.
- Should tighten nut until c-clamp on top stops turning, then ½ to ¾ turn more.



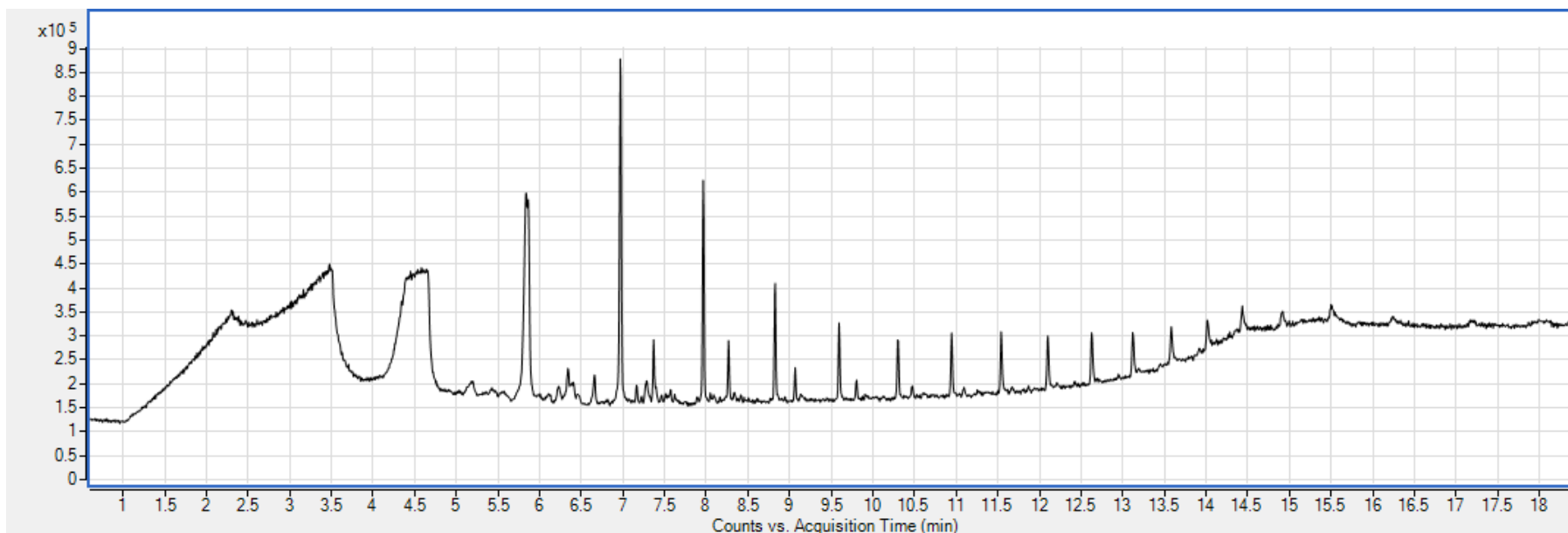
# Septum Maintenance: Septum Coring

- Pieces of rubber from the septum may break off and fall into inlet liner after many injections
- This is called septa coring
- Replace the inlet septa and liner frequently to prevent septa contamination
- Use a cone tipped syringe to reduce the chance of tearing the septum



Septum core placed in a clean liner, and a blank injection performed

- Inlet: 320 °C, split mode, 10:1 split ratio
- Oven: 35 °C to 300 °C at 20 °C per minute
- Detector: Single quadrupole EI Scan, 35 to 500 amu



# Split/Splitless Inlet

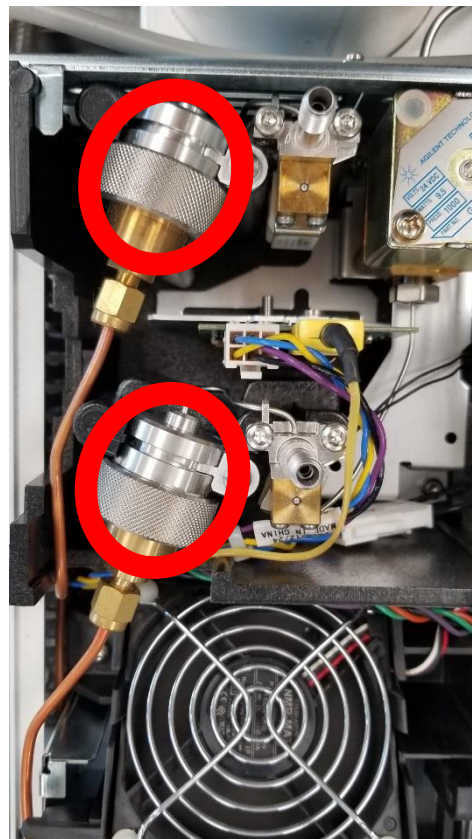
## The split vent trap

What is it?



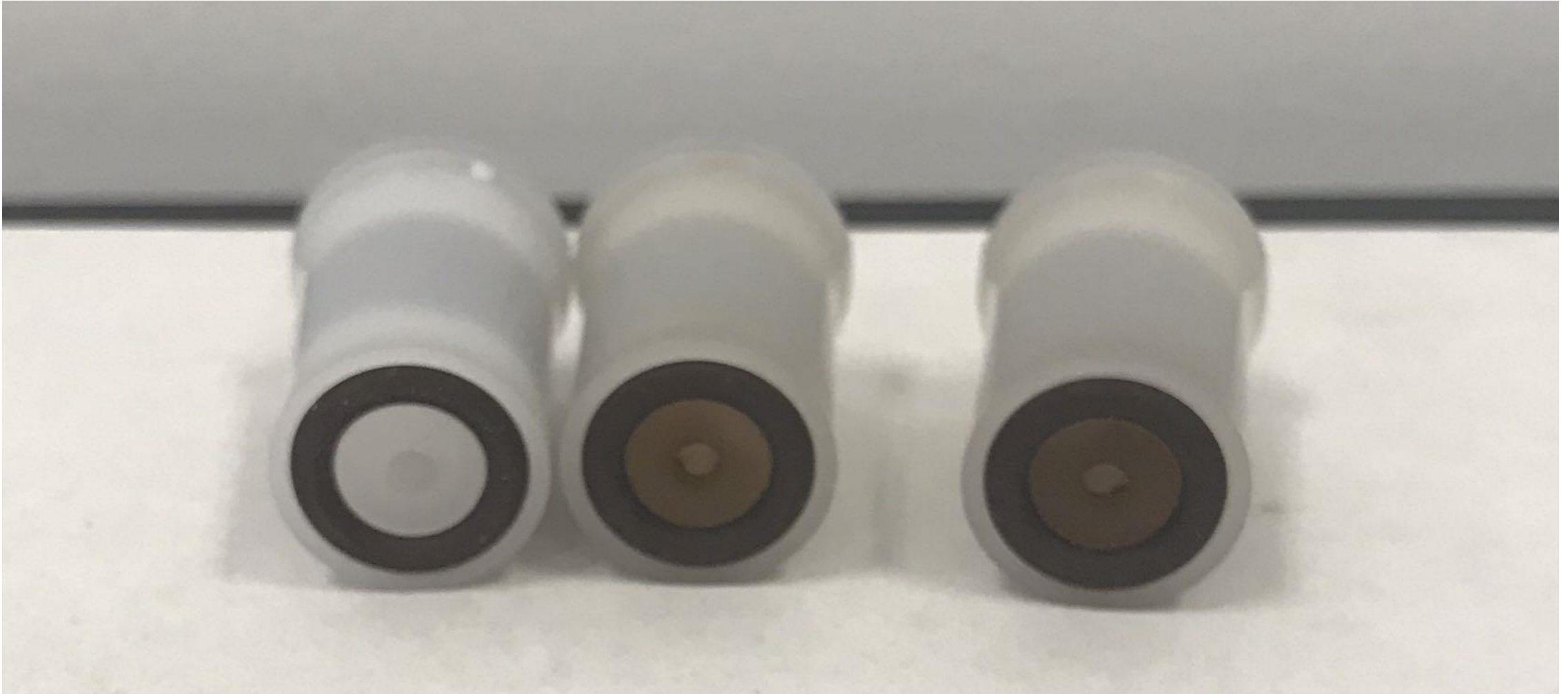
Where is it?

On a 7890 GC



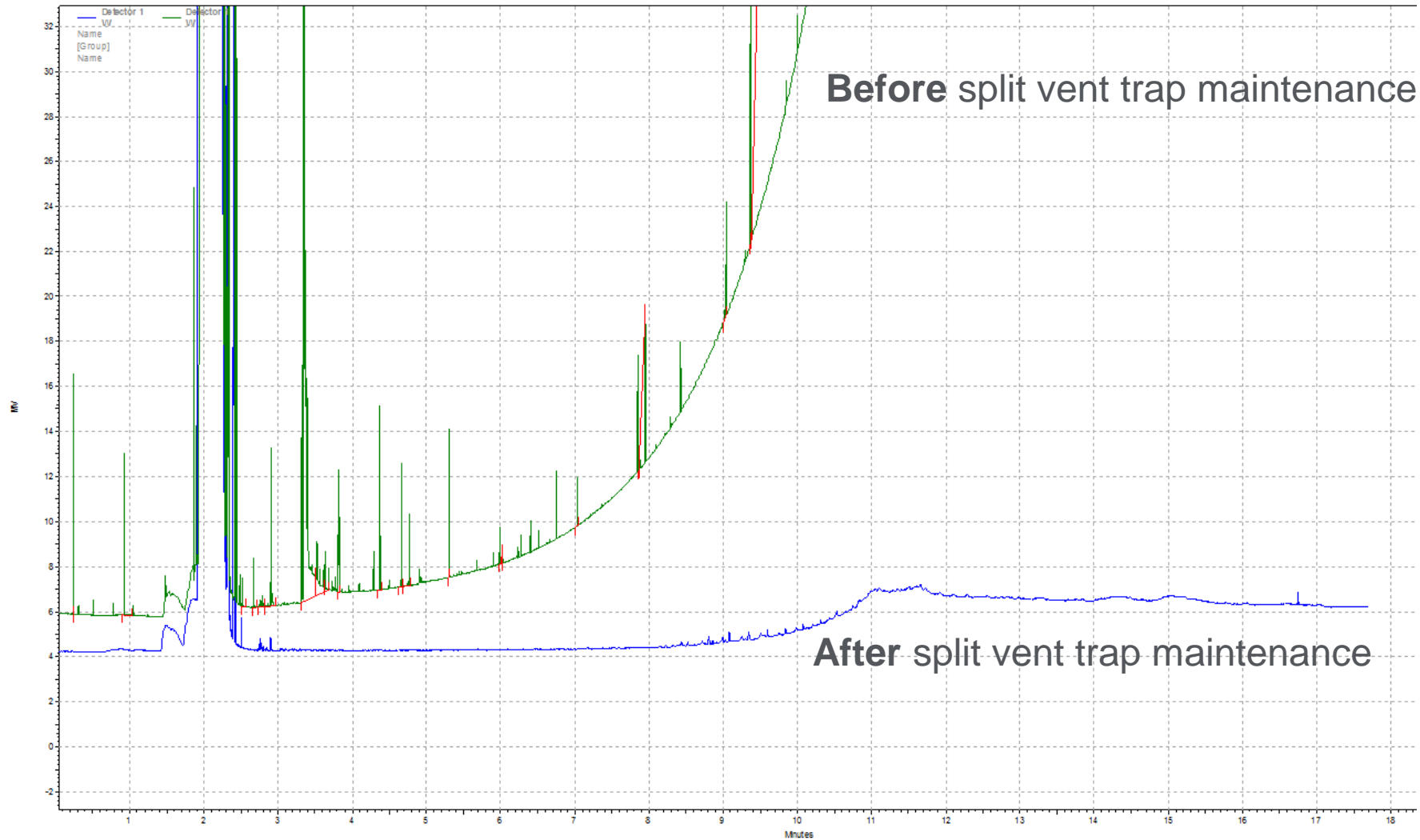


# What Your Split Vent Trap Should Not Look Like

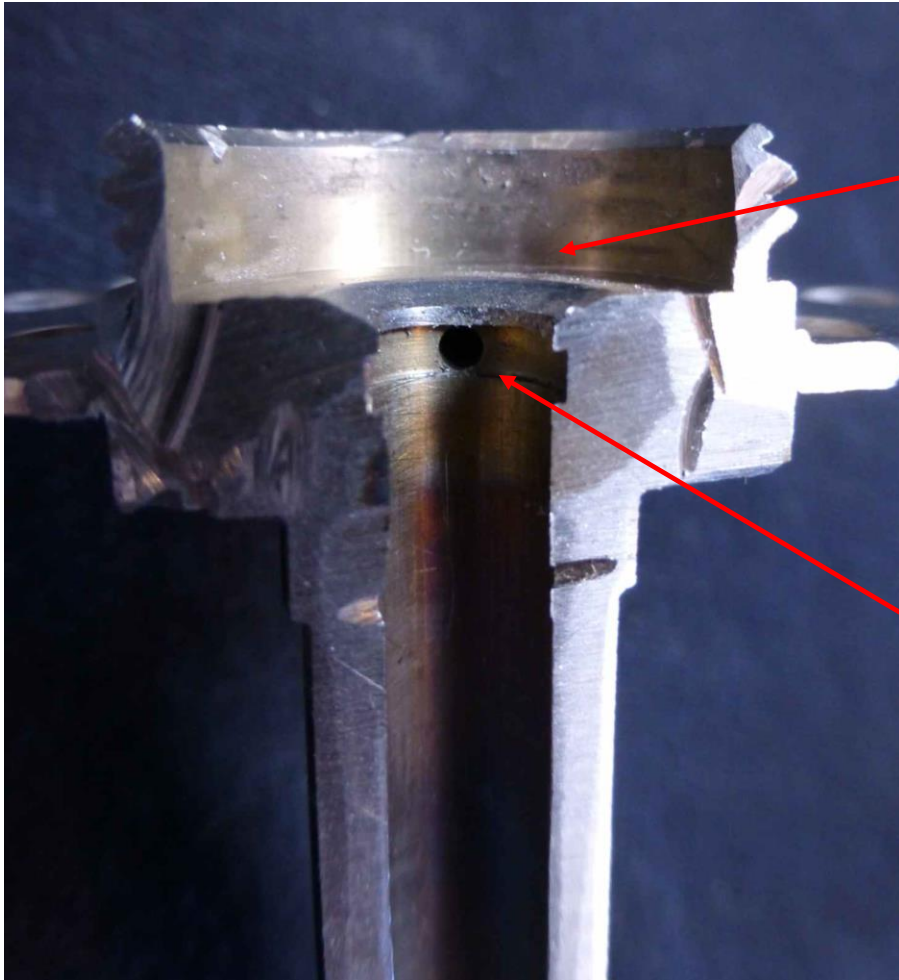


# Split/Splitless Inlet

## Split vent trap changed



# Cleaning the Split Vent Trap Lines



Inspect the liner O-ring sealing surface. It must not have any nicks, cuts, leftover O-ring, or stuck on sample residue. Clean with swabs and solvent.

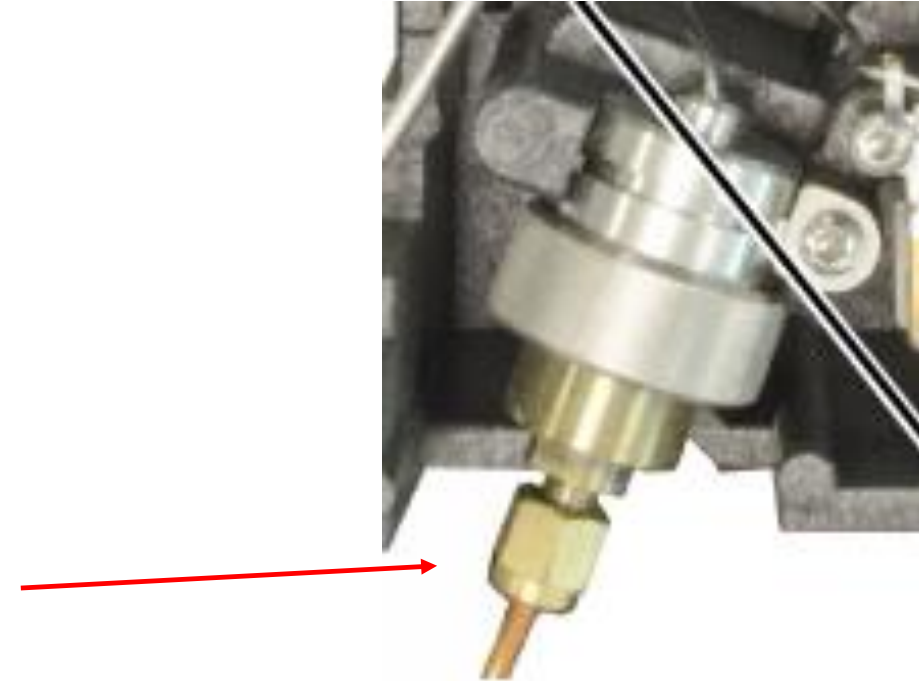
Inspect the split vent hole. It must not be blocked. This hole is hard to see. You can remove the copper split line fitting and push a small metal tool, like an Allen key, or a dead syringe needle through it to verify that it is open.

# Cleaning the Split Vent Trap Lines

But the best way to verify that the split vent line is clear is to backflush solvent from the entrance to the split vent trap back into the inlet.

Cool off inlet and oven. Wear safety glasses.

1. Remove liner and column.
2. Place a beaker in the oven underneath the inlet.
3. Disconnect 1/8 in copper line at split vent trap.
4. Squirt solvent into the copper line. It should squirt out the split vent hole in the inlet and drip down into the beaker in the oven.
5. Install new liner and septum.
6. Hold a gloved finger over the bottom of the inlet.
7. Pressurize inlet to blow out as much solvent as possible.
8. Reconnect the 1/8 in copper line to the trap.
9. Install column.



# When Do I Change Each Part?

Item	Typical Schedule	Comments
Septum nut	3-6 months	Septum nut can get worn and shed metal particles 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 to 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 to monthly	When experiencing chromatographic problems trim ½ to 1 meter of the front end of the column. Replace liner, septum, and gold seal.
Inlet septa	100 to 200 injections	Depends on septum type and manual/auto injections.

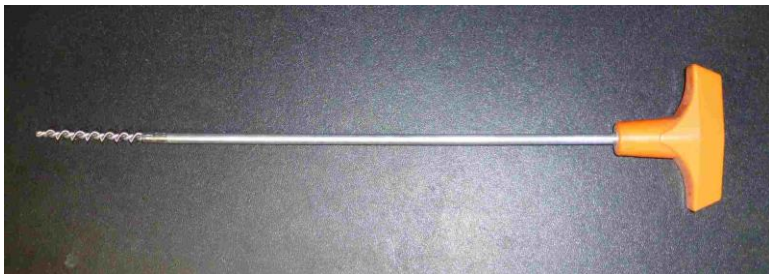
This schedule is an approximation of average use requirements. Actual frequency is application and sample specific. Use your chromatography as a guide to developing a normal maintenance schedule.

# Split/Splitless Cleaning

If the body needs cleaning, use a plastic burette brush and solvent in a cold inlet.



Scrubbing the inlet with a metal brush is not recommended. Most applications do not need the inside of the metal to be cleaned harshly.

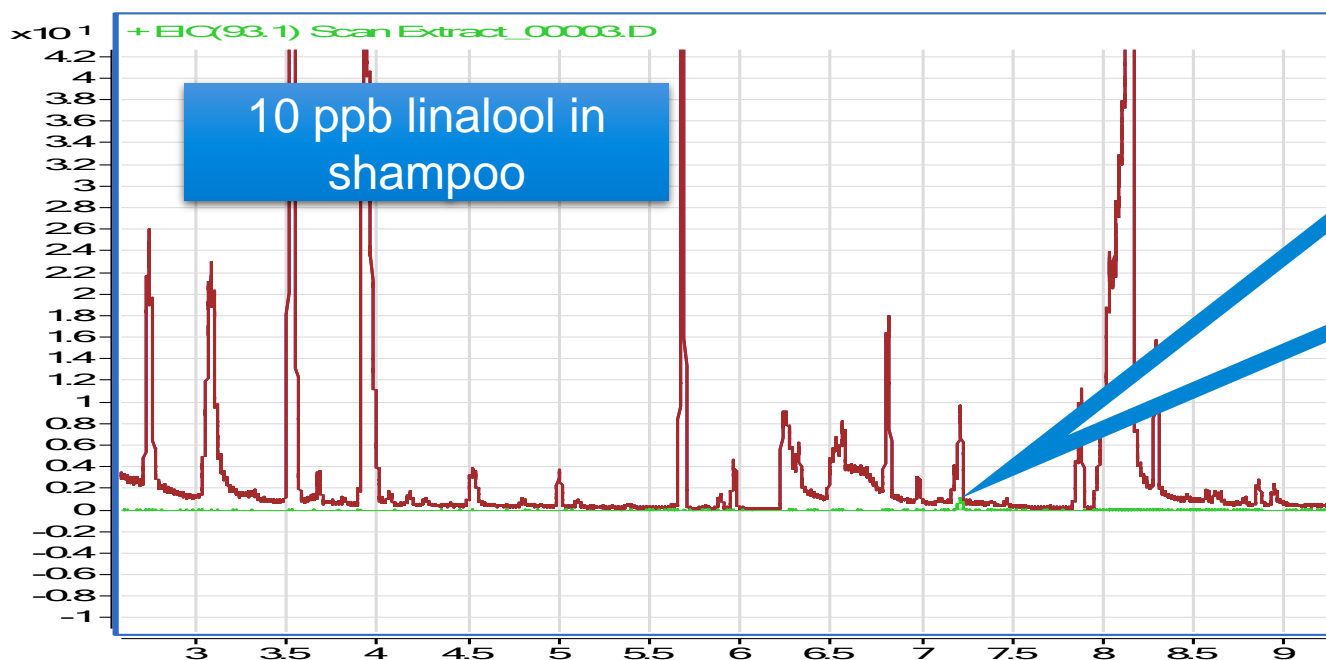
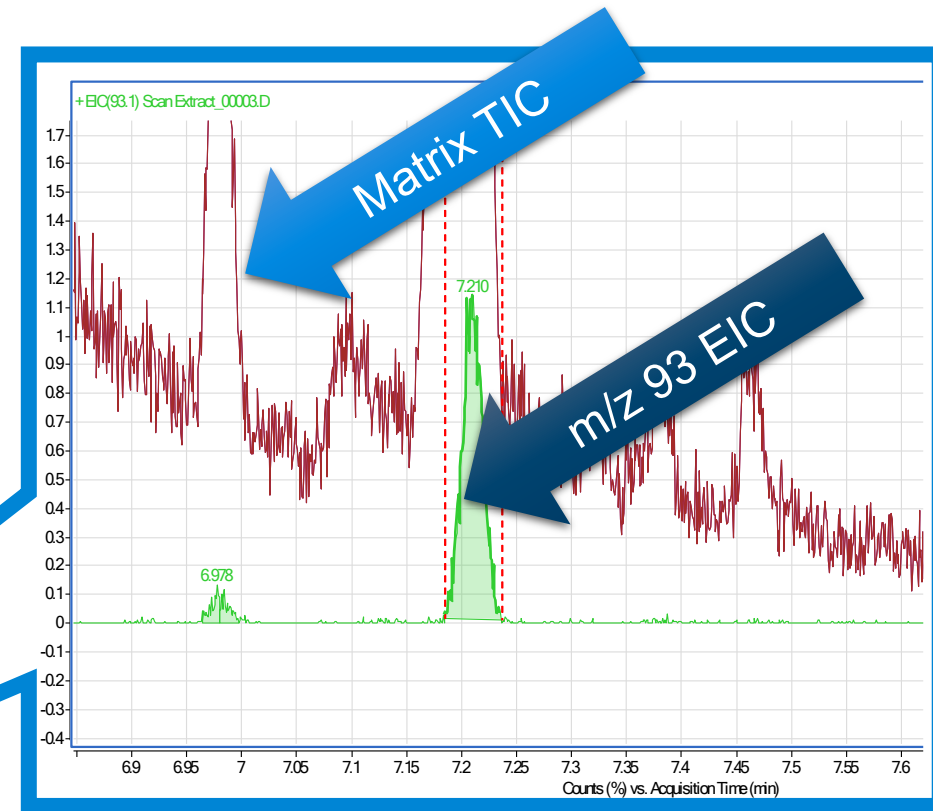


# How Did It Become Contaminated in the First Place?



# 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)



# Agilent Bond Elut Sample Cleanup Products

Solid Phase Extraction  
cartridges and plates



Synthetic Chem Elut S

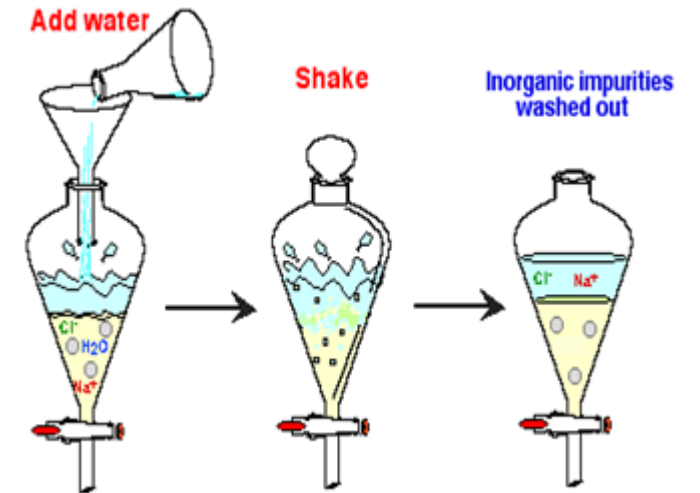
Filtration cartridges  
and plates



Captiva EMR Lipid

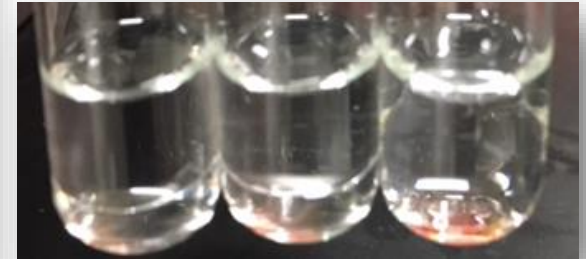
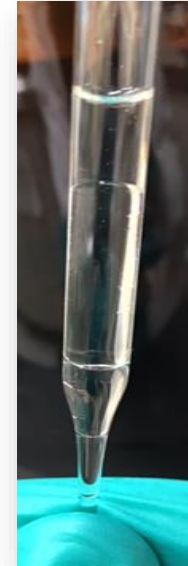
# Liquid/Liquid Extraction (LLE)

- LLE has been successfully used as a method of sample preparation for many years.
- It separates the more organic solvent-soluble compounds from the more water-soluble compounds using water immiscible organic solvents.
- It can remove many interfering substances, such as salts.
- Modulating pH can selectively extract or eliminate specific compound types.



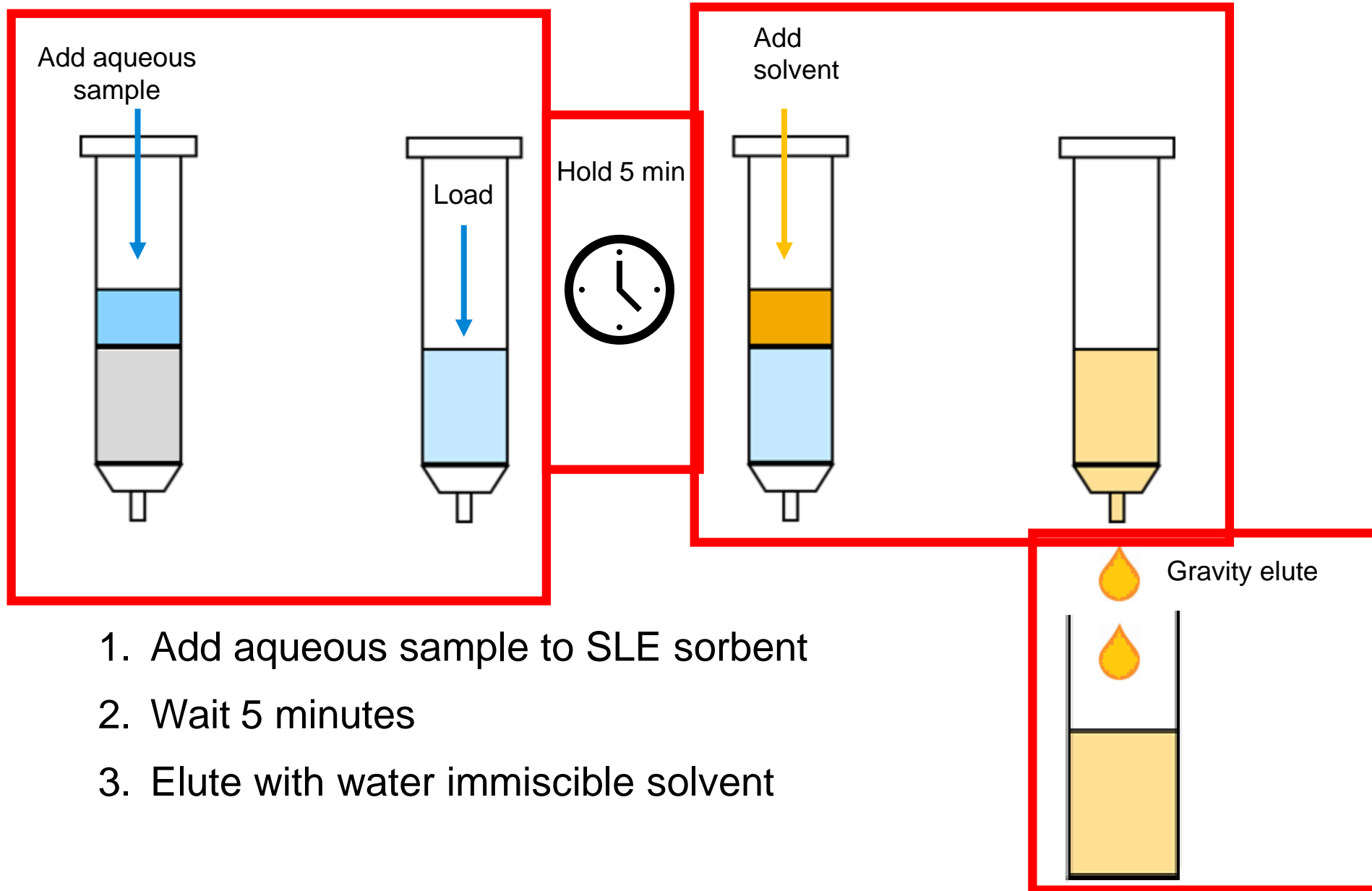
# Drawbacks of Liquid/Liquid Extraction

- LLE does have drawbacks
  - Inconsistent results from one analyst to another
    - Shaking time
    - Shaking motion
    - Determination of where to cut between layers
  - Emulsions
  - Labor intensive
  - Quite Tedious with small sample sizes (<5 mL)
  - Challenging with large numbers of samples
  - Difficult to automate for large numbers of samples



How many of these problems can be fixed with Solid Supported Liquid Extraction?

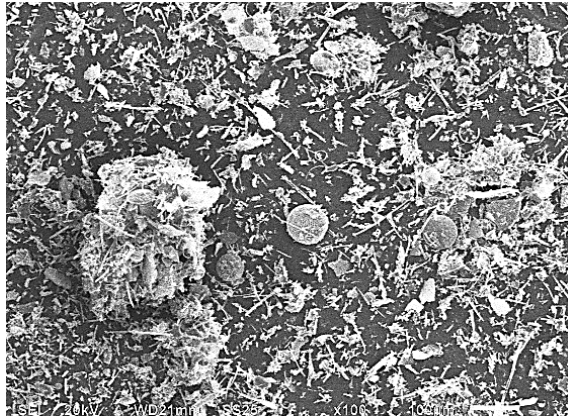
# How Does SLE Work?



1. Add aqueous sample to SLE sorbent
2. Wait 5 minutes
3. Elute with water immiscible solvent

# What Is SLE Sorbent?

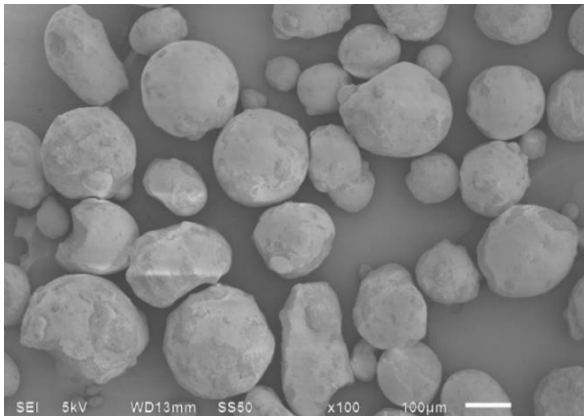
- There are two types of SLE media
  - Diatomaceous earth (DE) based products like our Chem Elut brand of SLE products
    - A mined fossil diatom material, which is heterogeneous and inconsistent from one mine to the next



## Diatomaceous earth in Chem Elut

- ✗ Naturally occurring; mined
- ✗ Broad particle size distribution
- ✗ Supplier reliability issues
- ✗ Poor lot-to-lot consistency

- Synthetic media we use in Chem Elut S
  - Controlled synthesis to be consistent from batch to batch



## Synthetic SLE sorbent

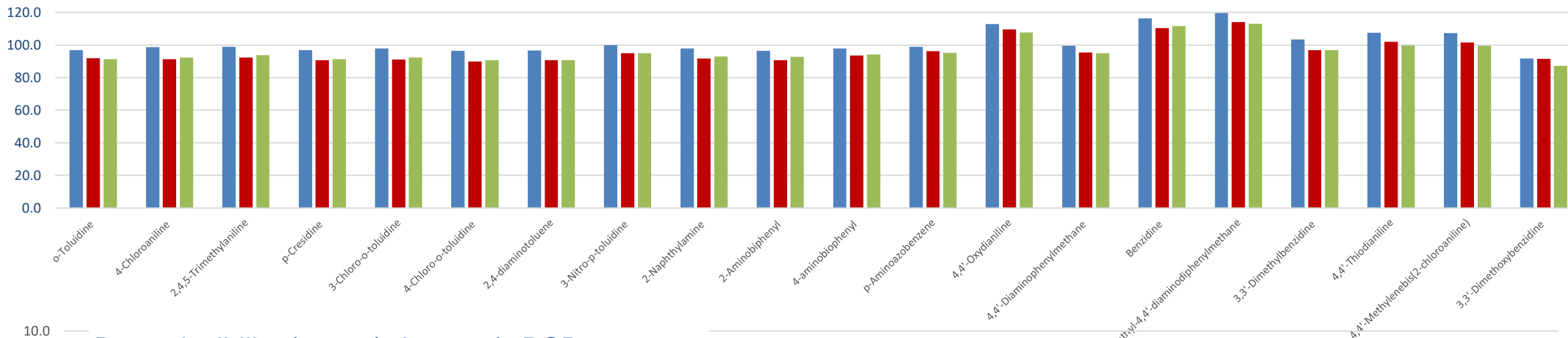
- ✓ Large scale synthesis
- ✓ Narrow particle size distribution
- ✓ Reliable supplier
- ✓ Controlled manufacturing

# Chem Elut S – 15 minute Hold Time

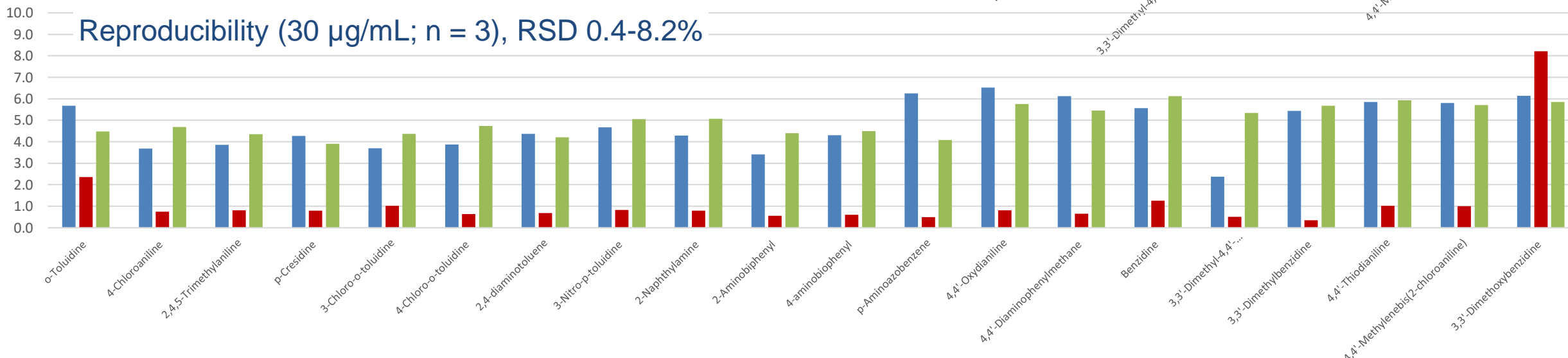
## Large scale format comparison with aromatic amines

Recovery (30 µg/mL; n = 3), 87-119%

Chem Elut S 5 mL Chem Elut S 10 mL Chem Elut S 20 mL



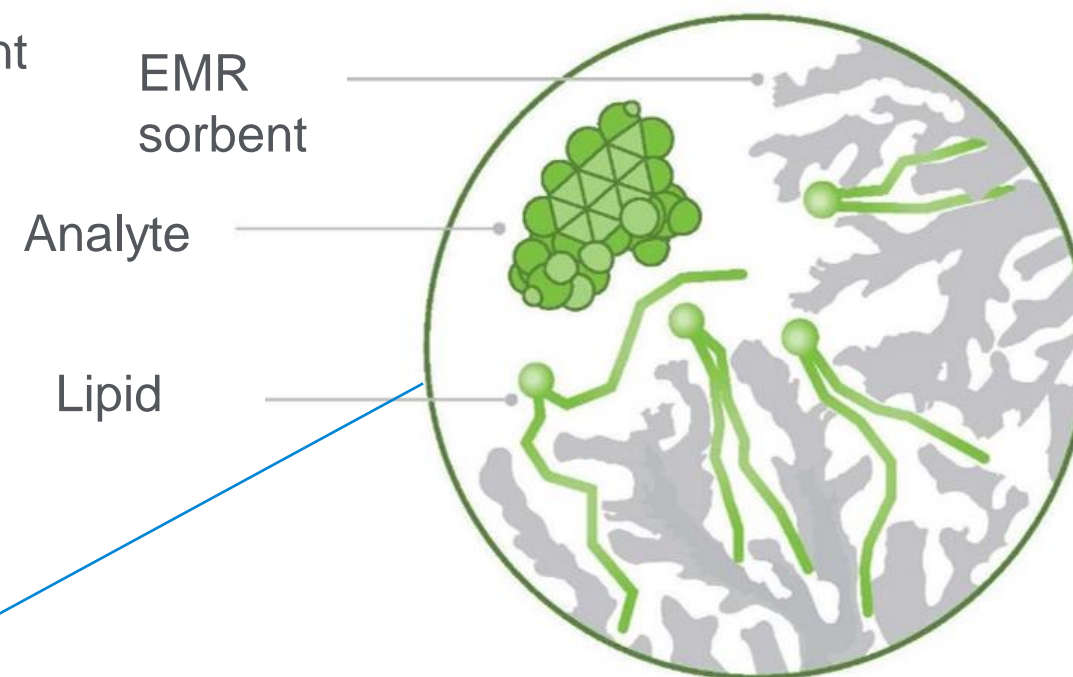
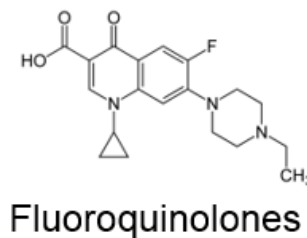
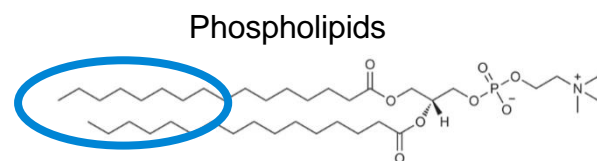
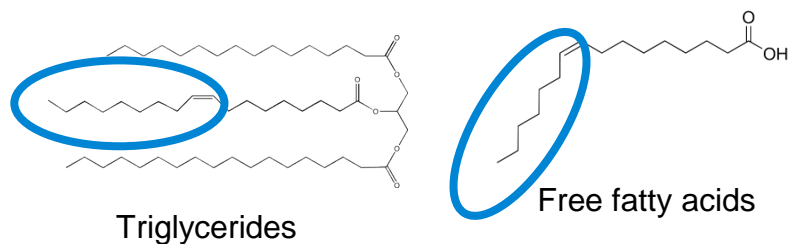
Reproducibility (30 µg/mL; n = 3), RSD 0.4-8.2%



# Enhanced Matrix Removal: Agilent Captiva EMR-Lipid

EMR-Lipid sorbent technology effectively traps lipids through two mechanisms:

- Size exclusion – unbranched hydrocarbon chains (lipids) enter the sorbent; bulky analytes do not
- Sorbent chemistry – lipid chains that enter the sorbent are trapped by hydrophobic interactions



# Application Case – Pesticides in Edible Oil by GC/MS/MS

Classification	Pesticides	Classification	Pesticides	Classification	Pesticides
<b>Organophosphate</b>	Dichlorvos	<b>Organochlorine</b>	Lindane	<b>Sulphamide</b>	Dichlorfluamid
	Trichlorfon		Aldrin		Tolyfluanid
	Sulfotep		Endrin	<b>Phthalimide</b>	Captan
	Diazinon		Endosulfan		Folpet
	Chlorpyriphos-methyl		DDT		Captafol
	Phosmet		Oxychlorthane	<b>Dicarbosimide</b>	Procymidone
	Coumaphos		Mirex	<b>Pyrimidinol</b>	Bupirimate
	Malathion		<b>Pheonl</b>	<b>Dicarboximide</b>	Iprodione
	Parathion		<b>Dinitroaniline</b>	<b>Pyrethroid</b>	Permethrin
	Dimethoate		<b>Chloronitrile</b>		Deltamethrin
	Fenamiphos	<b>Pyridazinone</b>	Esfenvalerate		
	Terbufos sulfone	<b>Pyridine</b>	Fenvalerate		
	Chlorpyriphos	<b>Triazine</b>	Thiazopyr	Bifenthrin	
<b>Oxazole</b>	Vinclozolin		Atrazin	<b>Strobilurin</b>	Pyraclostrobin
<b>Uracil</b>	Bromacil		Prometryne	<b>Carbamate</b>	Thiobencarb
			Propazine	<b>Diphenyl ether</b>	Nitrofen



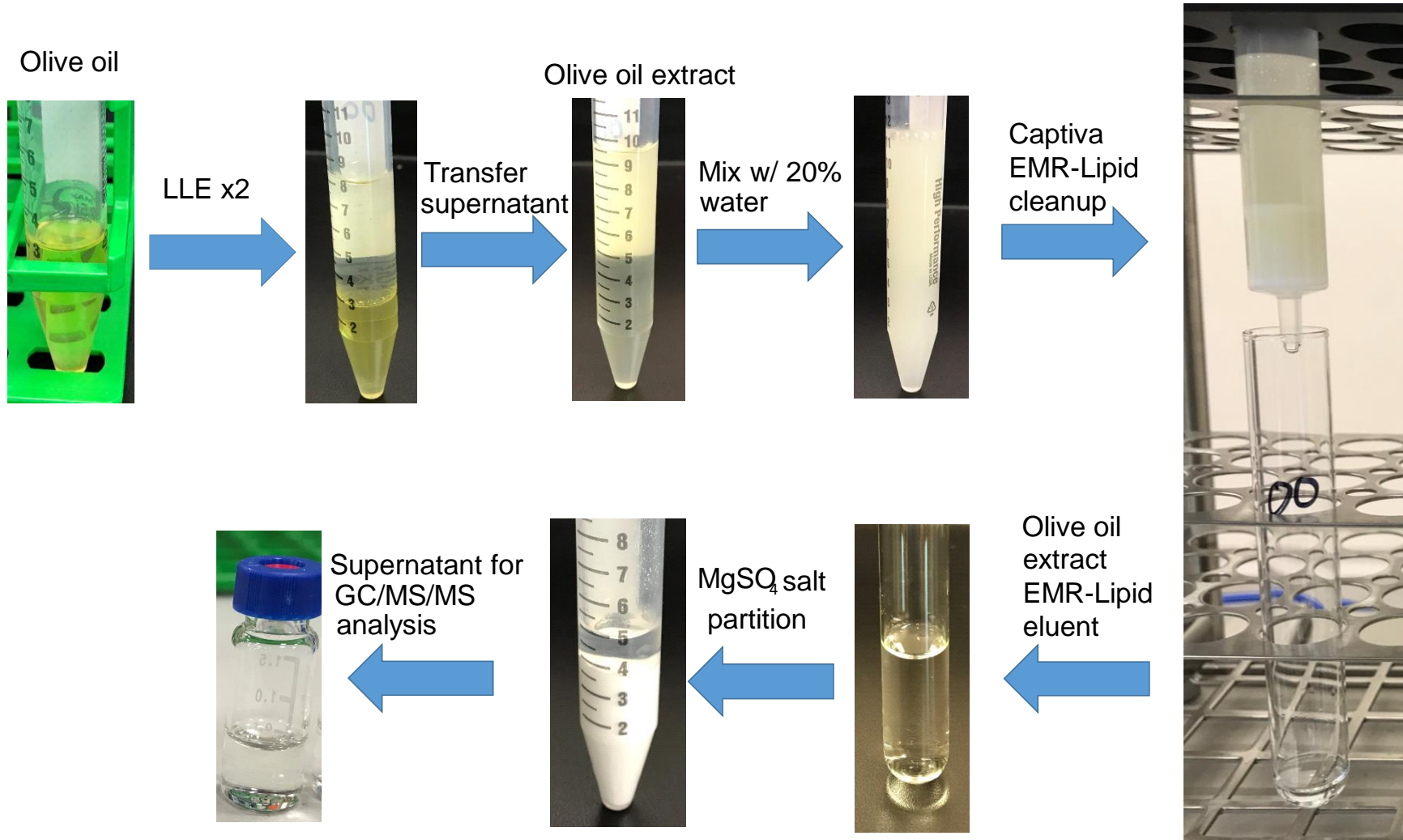
Captiva EMR-Lipid  
6 mL cartridge



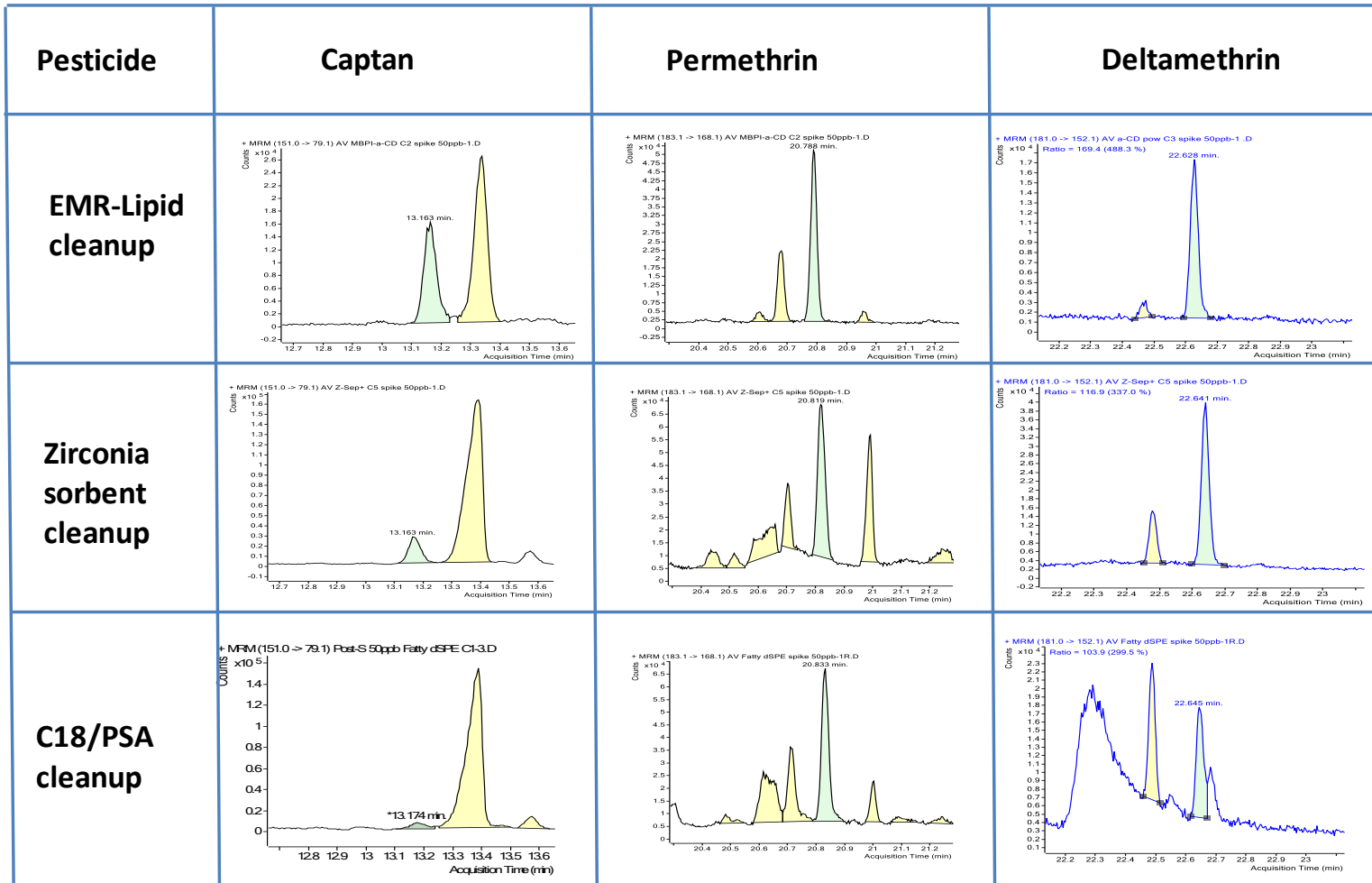


# Pesticides in Edible Oil by GC/MS/MS

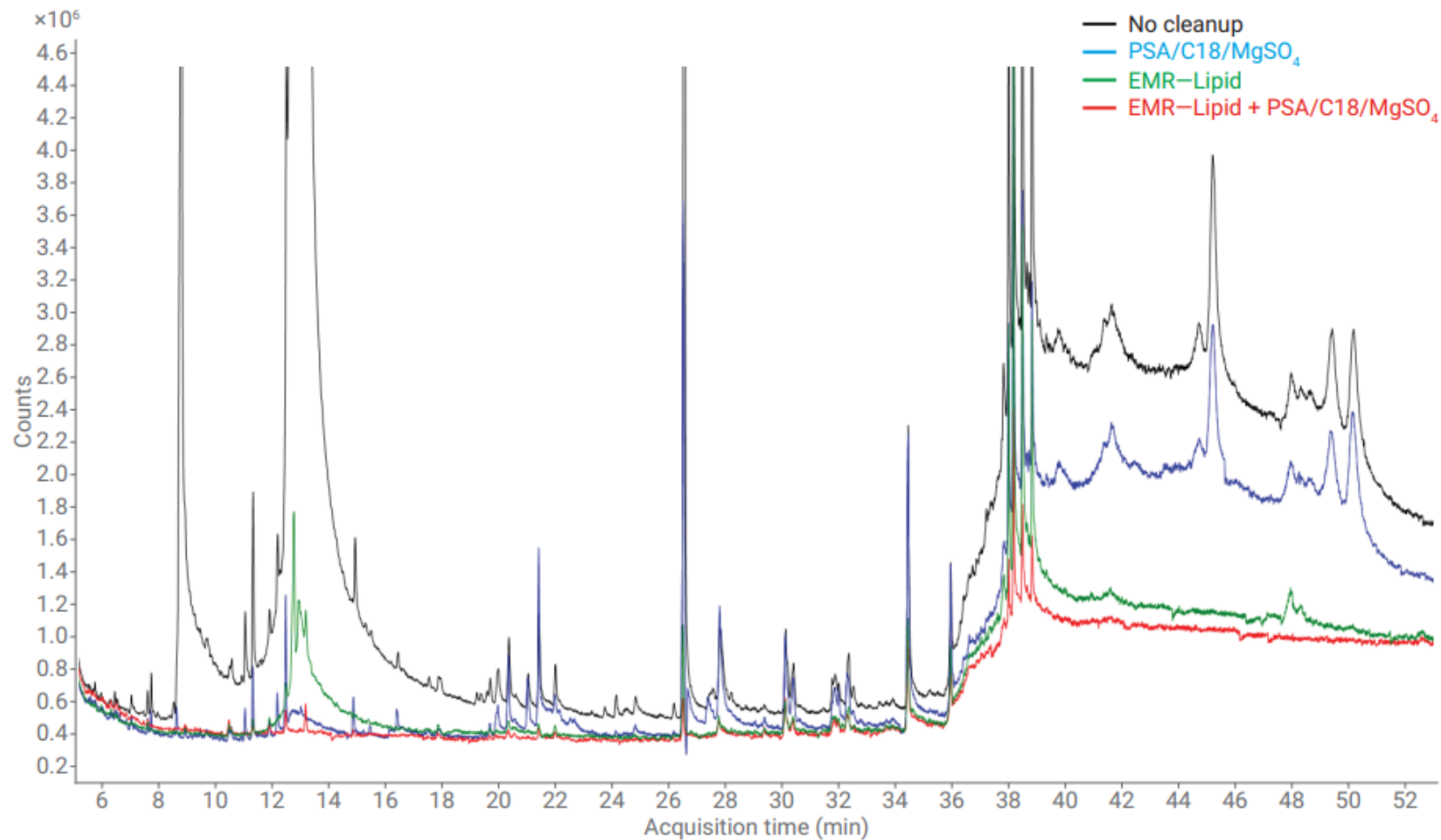
## Sample preparation procedure visual



# Captiva EMR-Lipid Cleanup Improves Analytes S/N Ratio and Integration Accuracy on GC/MS(/MS)

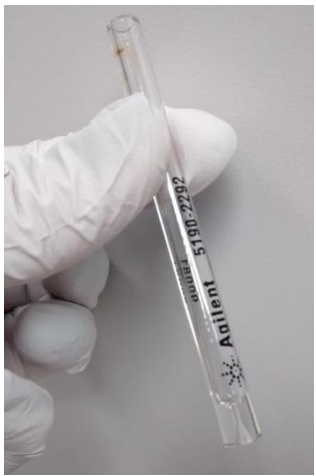


# EU Priority PAH Analysis in Pumpkin Seed Oil Using Bond Elut EMR-Lipid Cleanup by GC/MS/MS

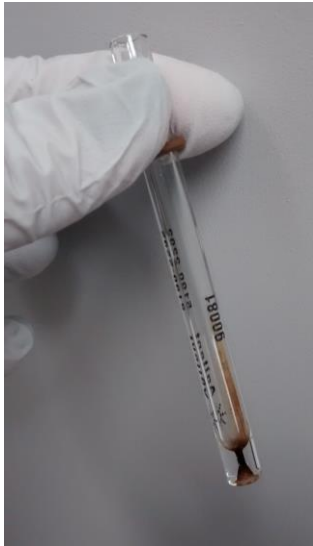


Application note 5994-0593EN

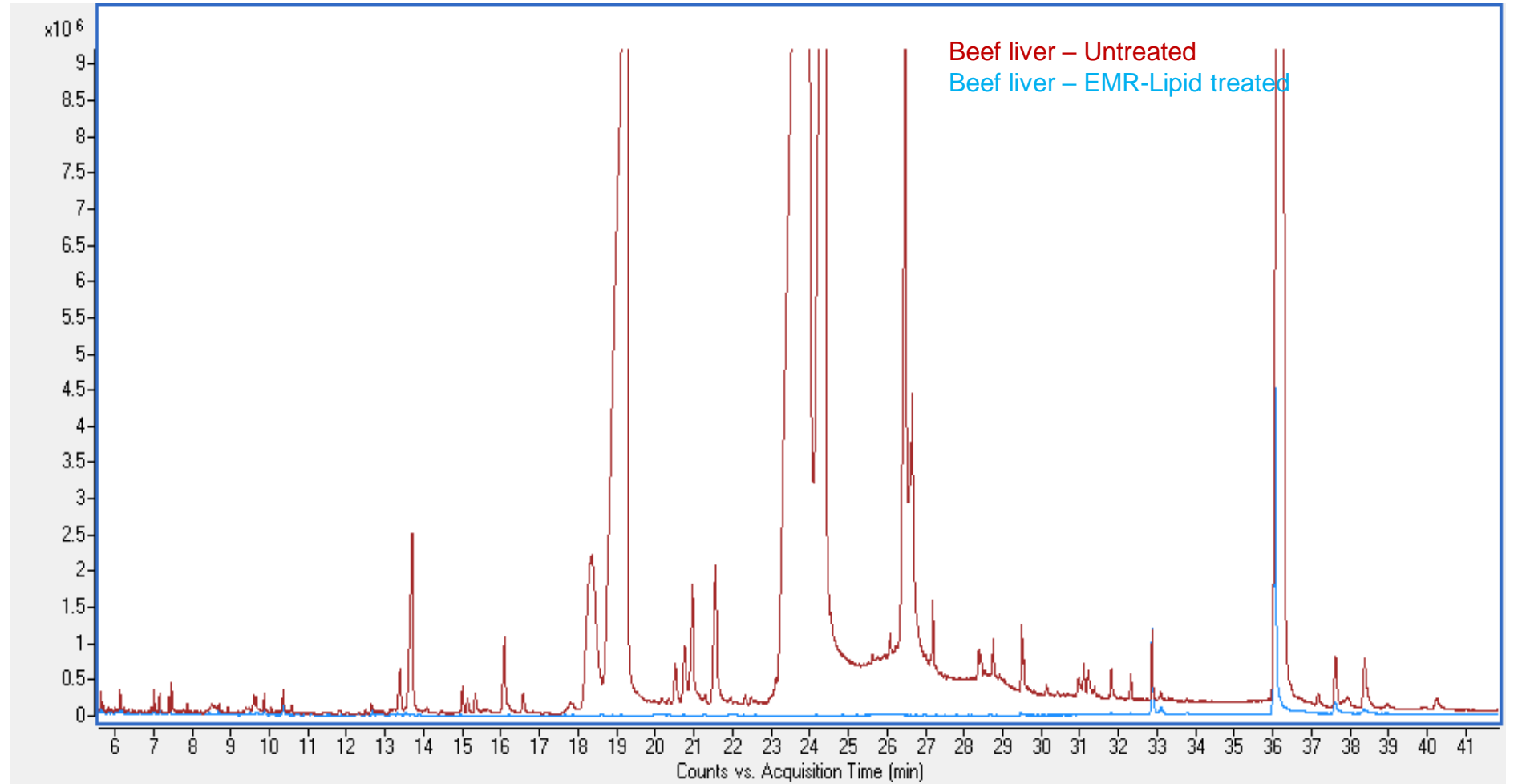
# The Importance of Sample Cleanup



50 samples  
with cleanup



50 samples  
without cleanup



For sample cleanup help, please contact us at [spp-support@agilent.com](mailto:spp-support@agilent.com)

# The Benefits of Glass Wool in Your Liner

## What is glass wool used for?

### Filtration

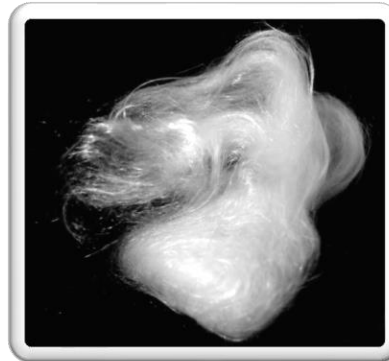
- Prevents nonvolatile matrix from entering column

### Vaporization

- Provides volatilization surface for liquid injections, promotes mixing with carrier gas

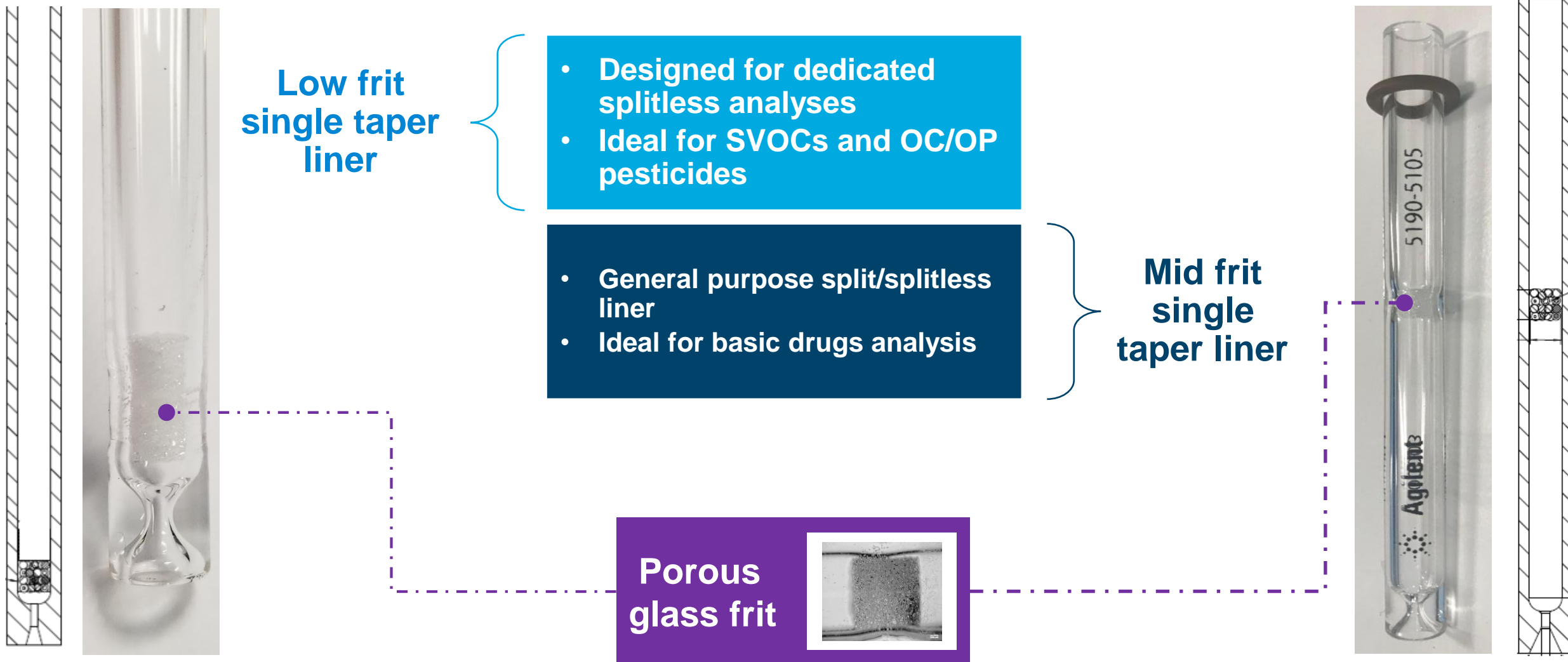
### Needle wiping

- Increases reproducibility by wiping needle after injection



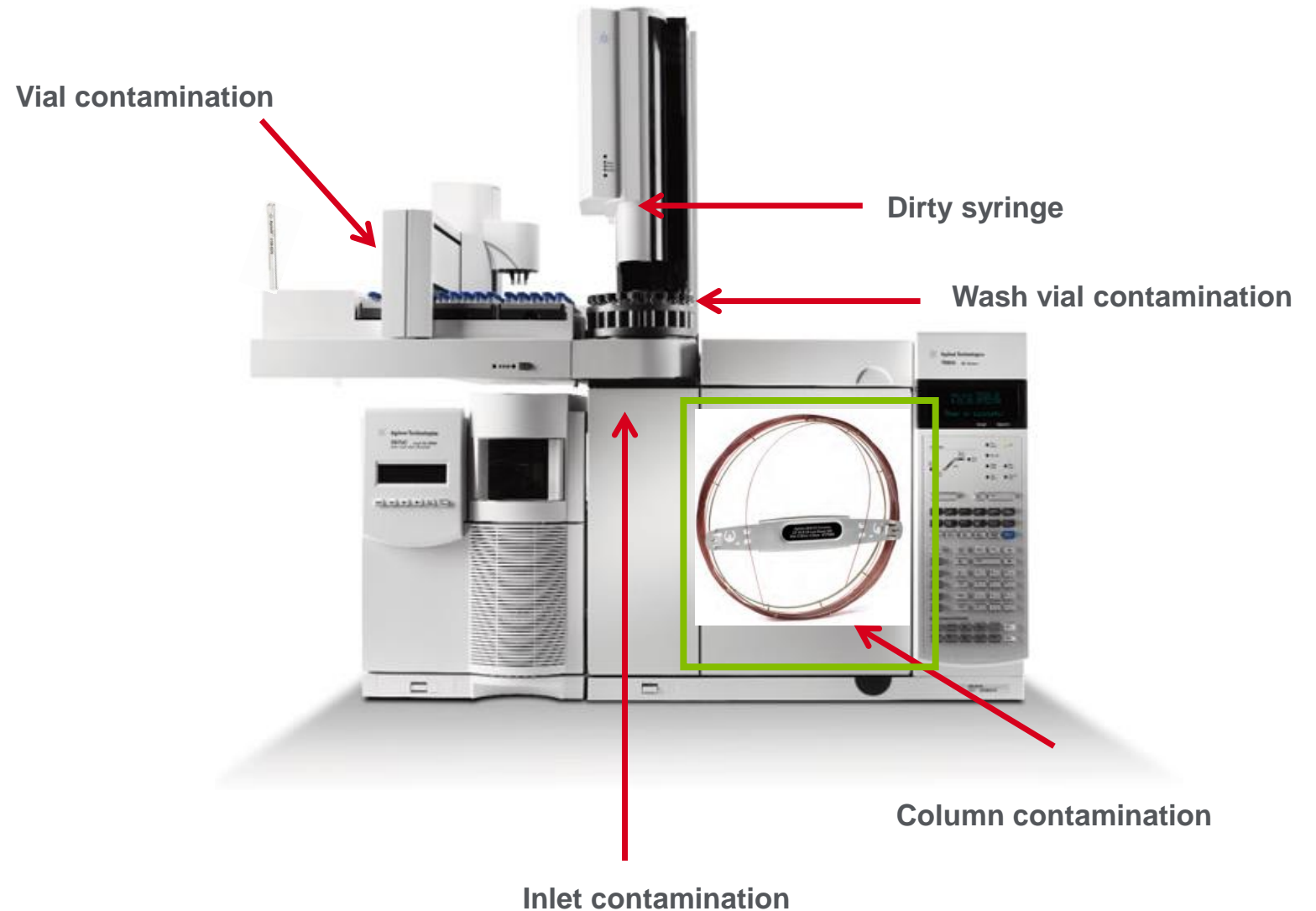
# What's New? Glass Wool Alternative Liners

## Ultra Inert liners with sintered glass frits

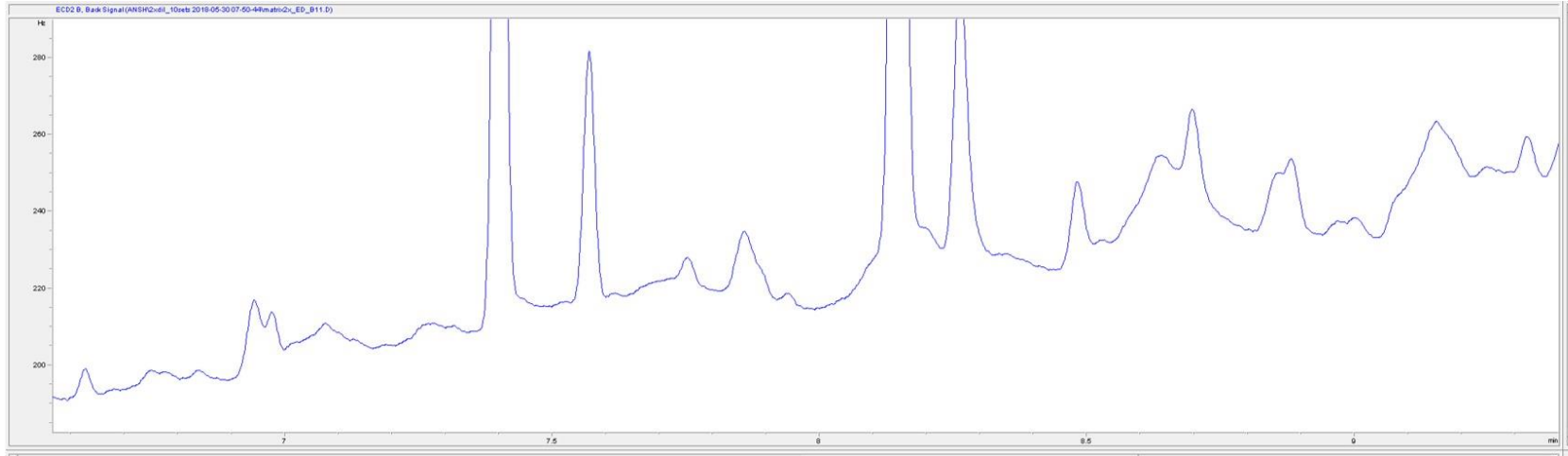


# Where Can These Ghost Peaks Come From?

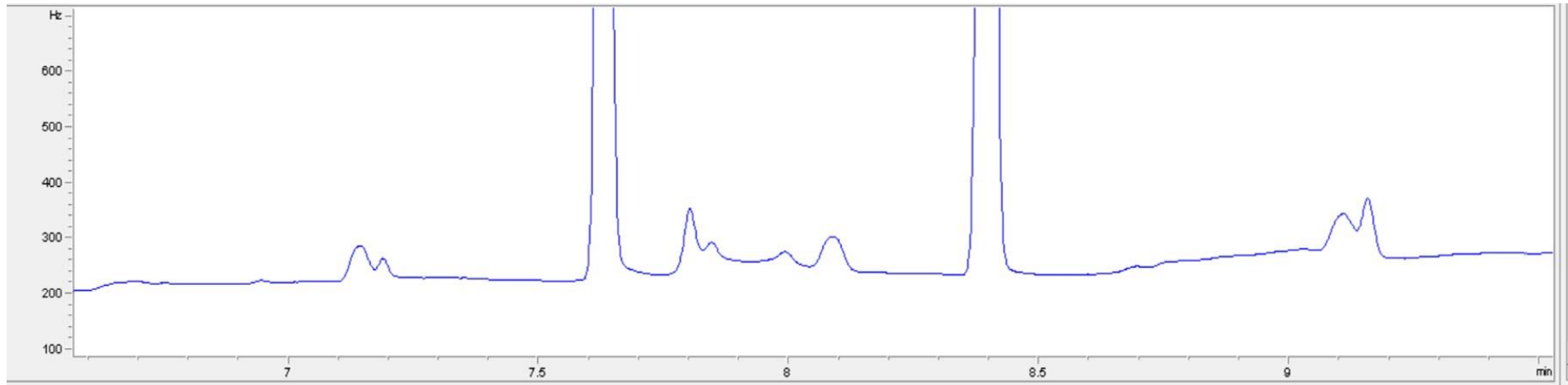
## Column contamination



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

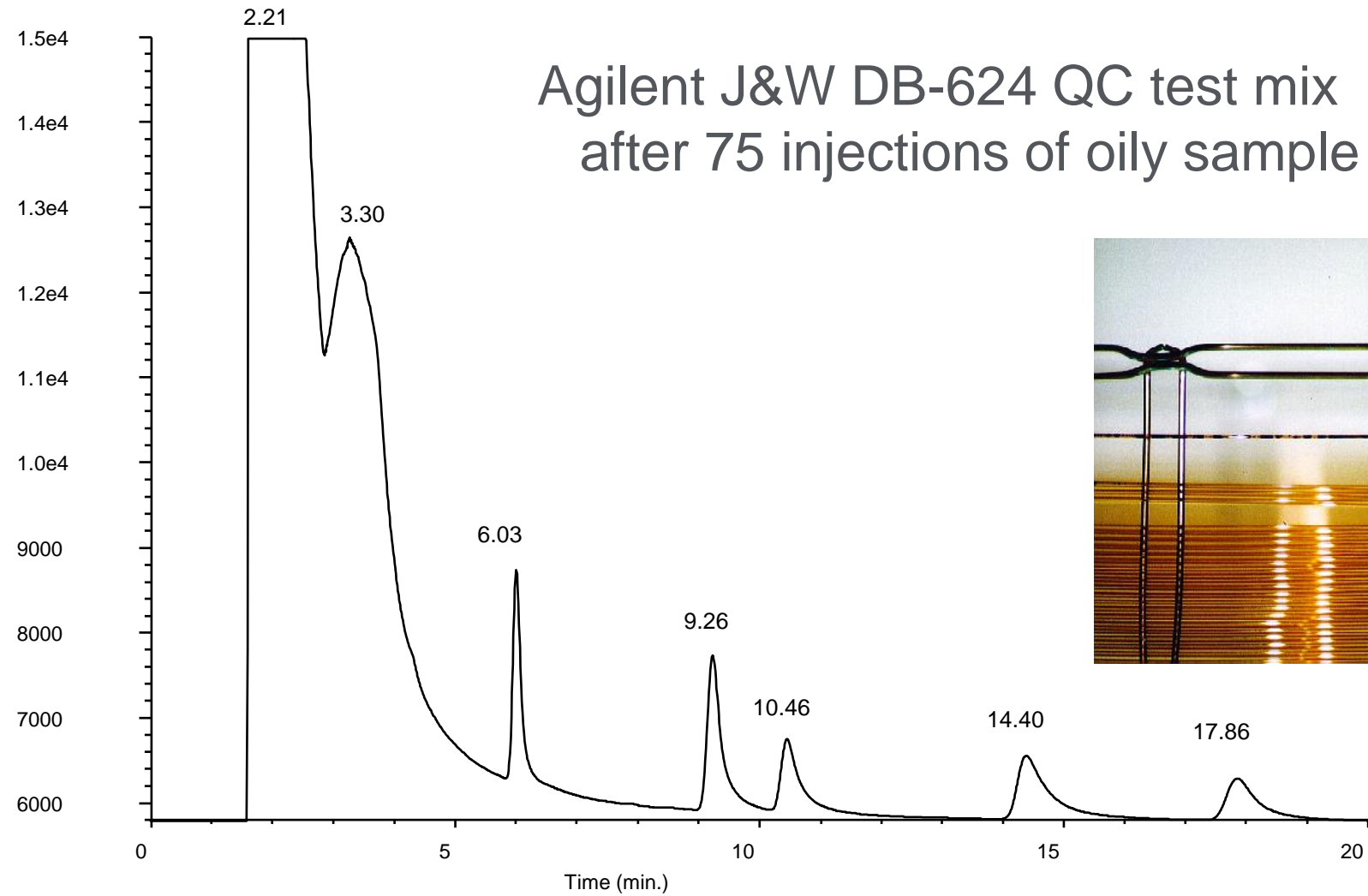


When it should look like:



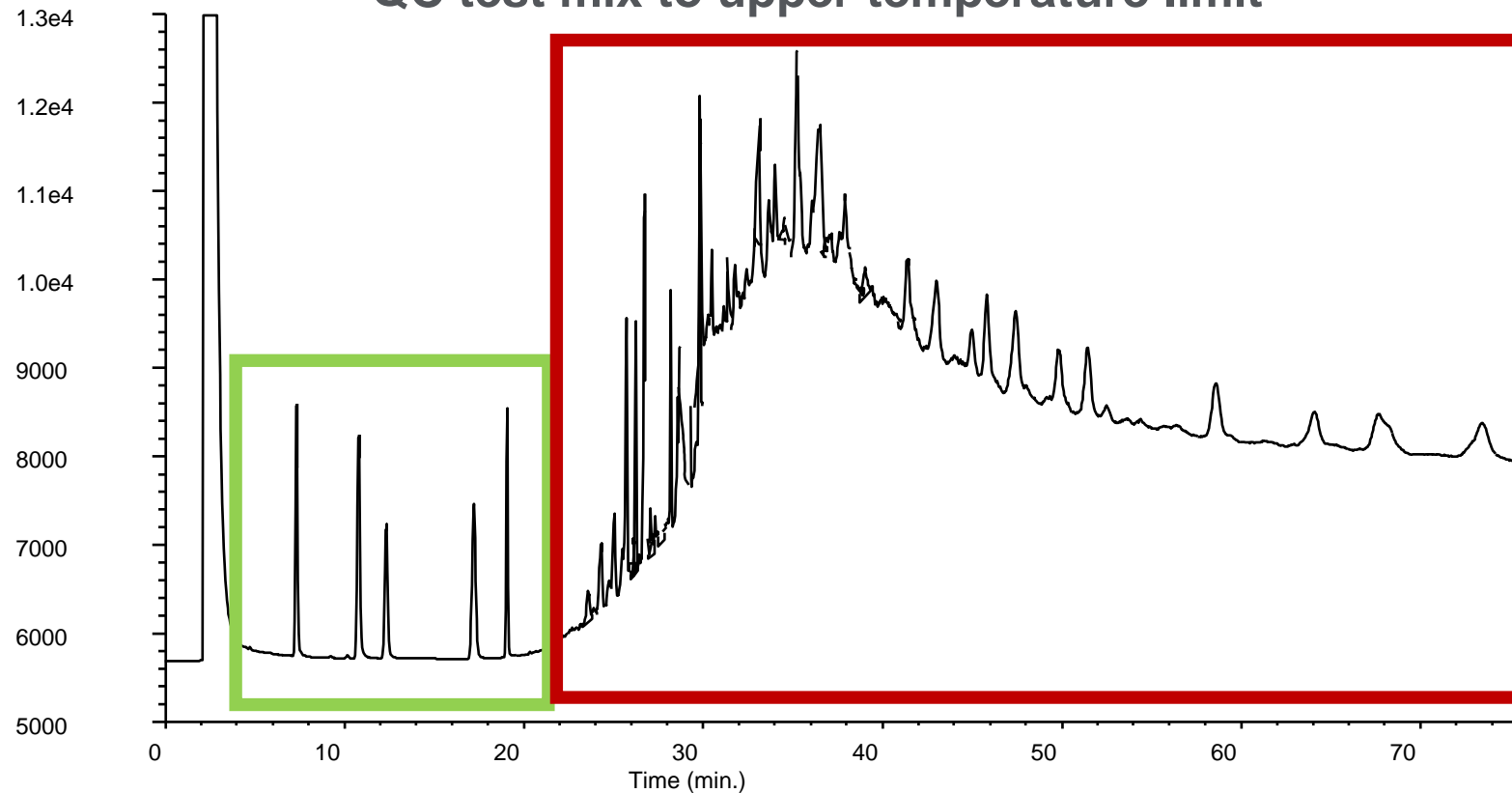


# Example of Column Contamination



# Example of Column Contamination

1 1/2 meters removed\*  
QC test mix to upper temperature limit

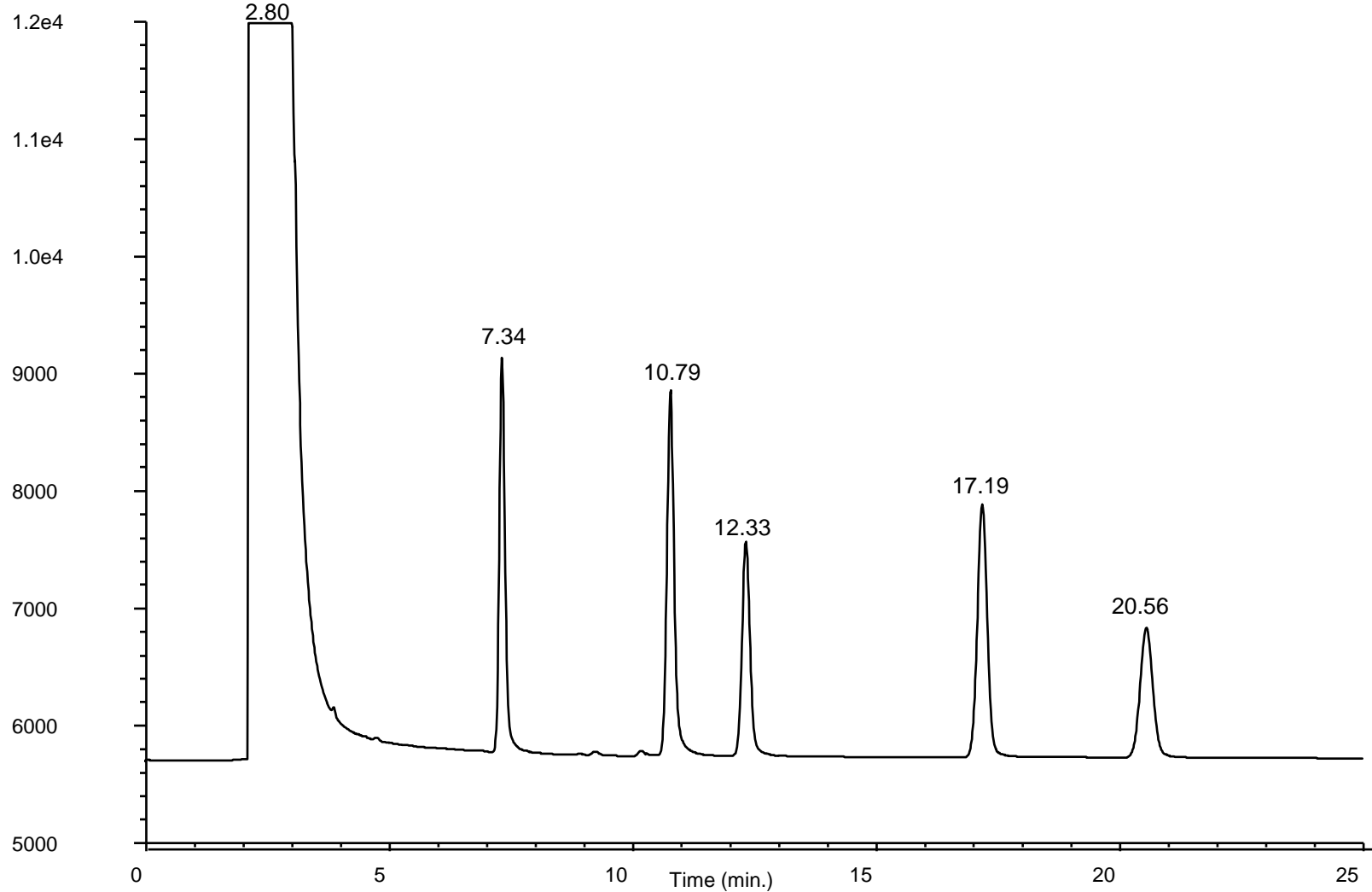


We have more  
semivolatile  
contamination

\*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

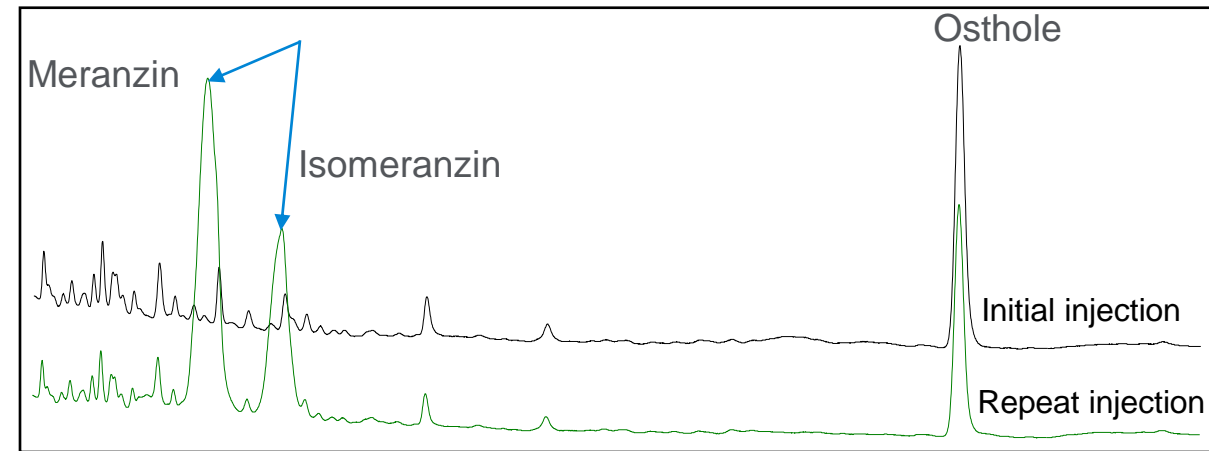
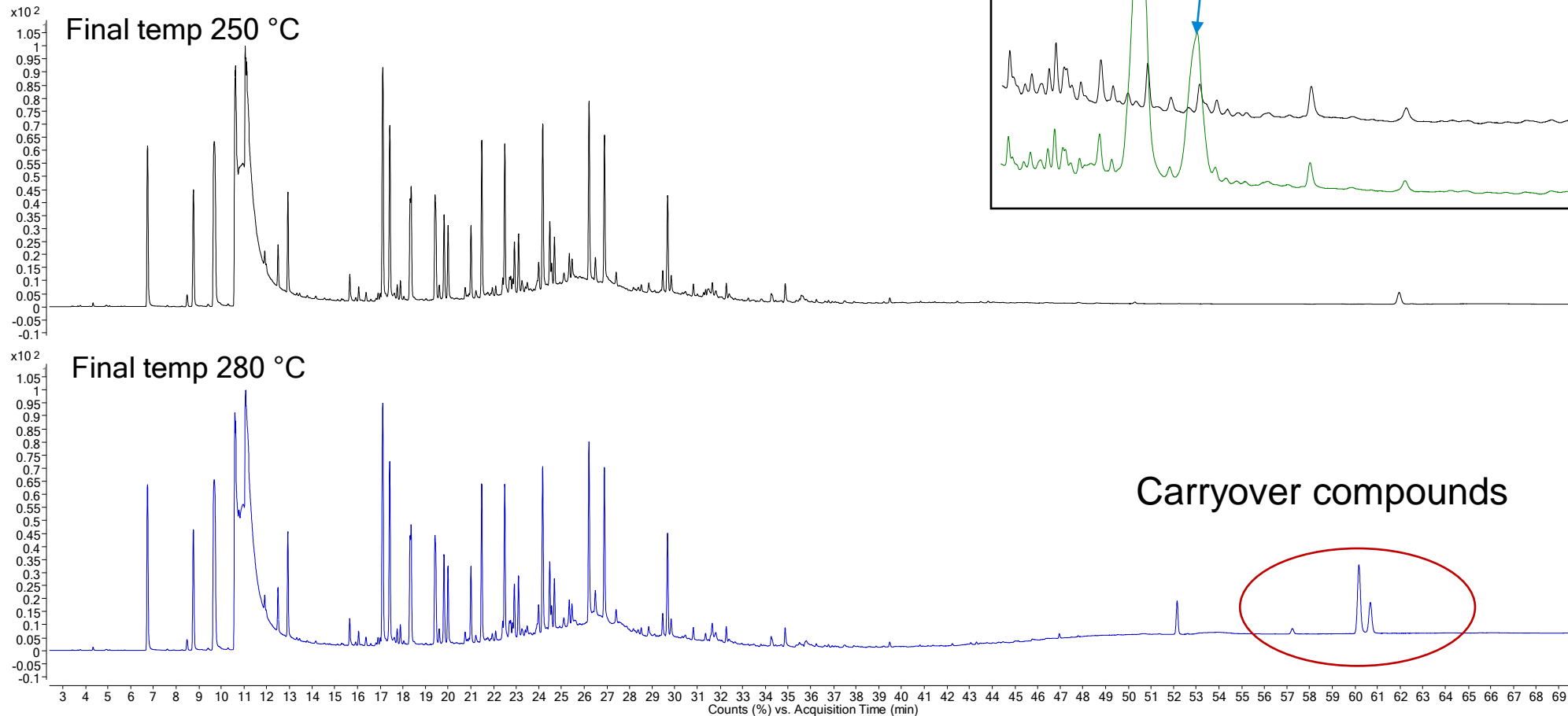
# Example of Column Contamination



\*Before column rinse and bake

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

# Carryover Ghost Peaks



Application note 5991-9078EN

# Another New Column: J&W DB-HeavyWAX

The WAX column you've been waiting for

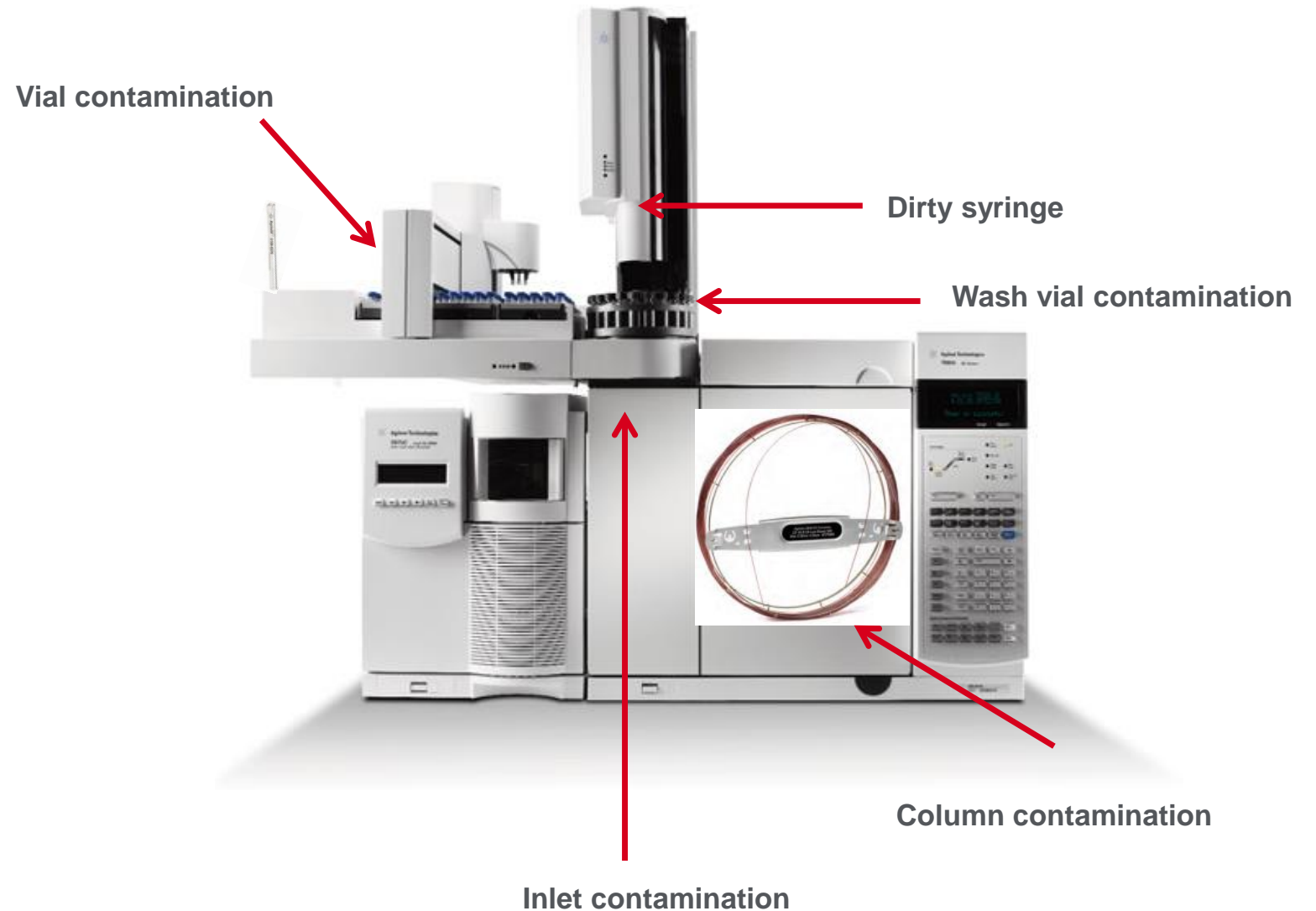
- Increased temperature range
  - **280 °C isothermal**
  - **290 °C programmed**
- Increased thermal stability
  - Stable retention times
  - Consistent peak order
- Lower bleed
  - Greater sensitivity for “heavier” compounds
  - Increase analyte range
  - Decrease analysis time
  - Safely bake out column



[www.agilent.com/chem/db-heavywax](http://www.agilent.com/chem/db-heavywax)

# Where Can These Ghost Peaks Come From?

## Other Sources



# Inlet Breakdown

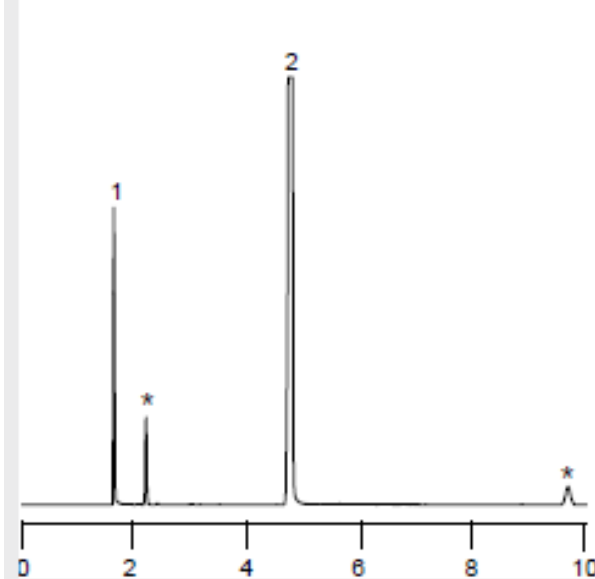
- Thermally labile compounds
- Very “active” compounds
- Derivatize compounds
- Inert flow path helps

## Formaldehyde

### Underivatized

**Column:** DB-WAX  
30 m x 0.32 mm I.D., 0.5 µm  
**J&W P/N:** 123-7033  
**Carrier:** Helium at 36 cm/sec,  
measured at 35°C  
**Oven:** 35°C isothermal  
**Injector:** Split 1:100, 200°C  
**Detector:** FID, 300°C  
Nitrogen makeup gas at 30 mL/min

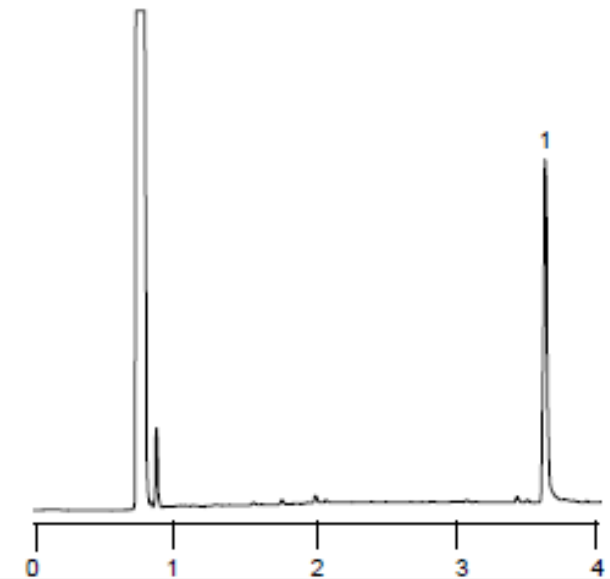
1. Formaldehyde
  2. Methanol
- \* Formaldehyde by-products



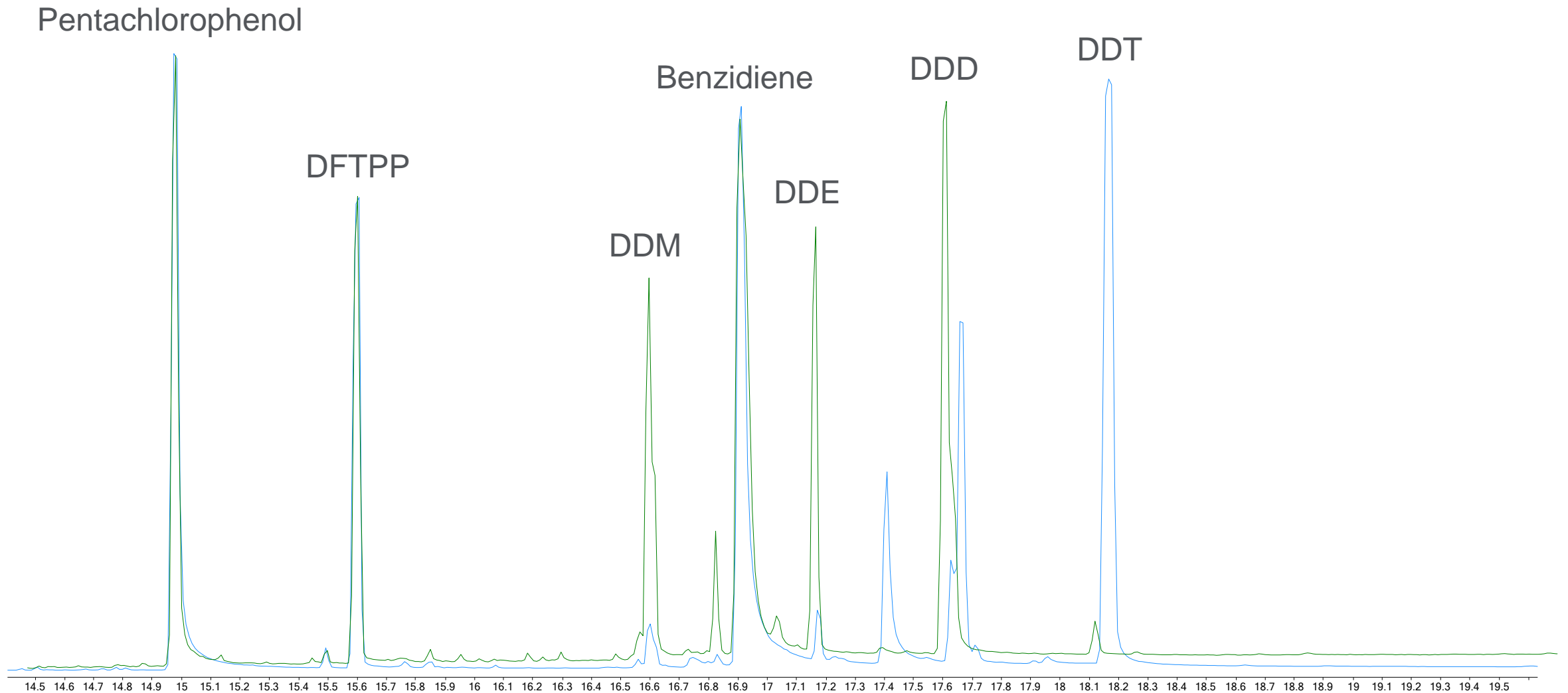
### DNPH Derivative

**Column:** DB-1  
15 m x 0.32 mm I.D., 0.25 µm  
**J&W P/N:** 123-1012  
**Carrier:** Helium at 35 cm/sec,  
measured at 150°C  
**Oven:** 150-250°C at 20°/min  
**Injector:** Split 1:100, 300°C  
**Detector:** ECD, 375°C  
Nitrogen makeup gas at 35 mL/min

1. Formaldehyde-DNPH



# Breakdown of DDT





# Agilent Inert Flow Solution

Agilent Ultimet Plus inlet weldment, shell and transfer lines



Agilent Ultra Inert inlet liner



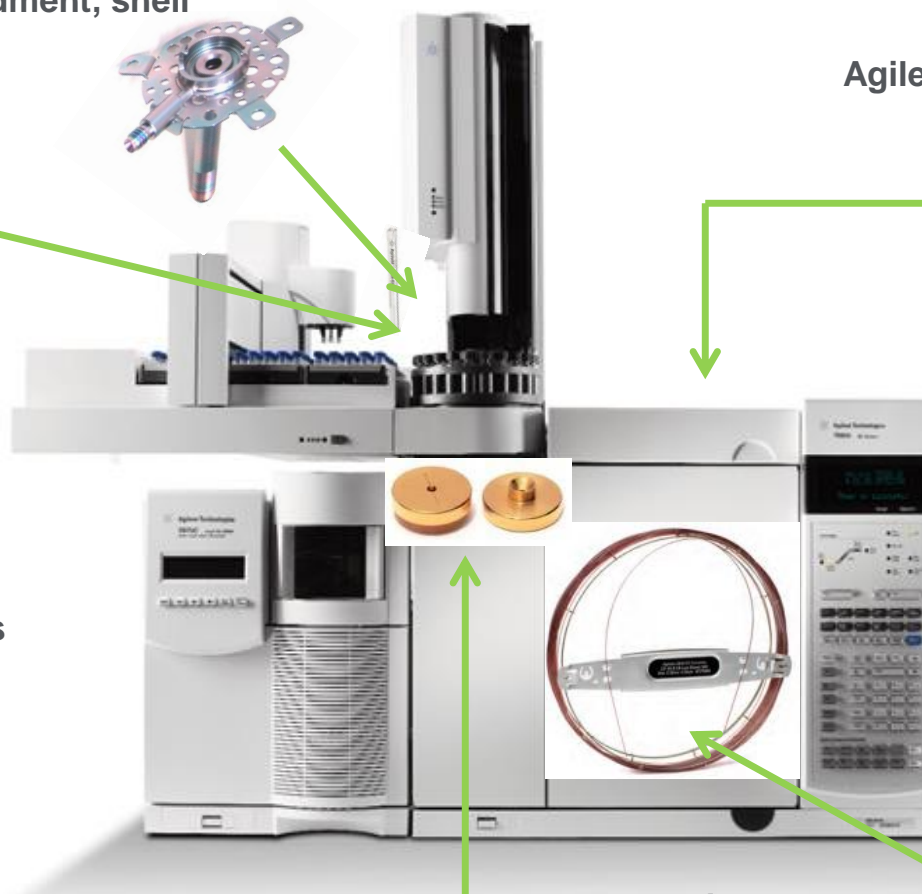
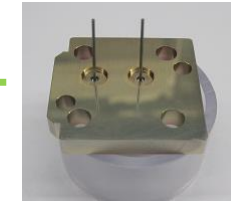
Agilent Ultimet Plus ferrules



Agilent Ultimet capillary flow technology devices, Ultimate union



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



Agilent Ultra Inert gold seal

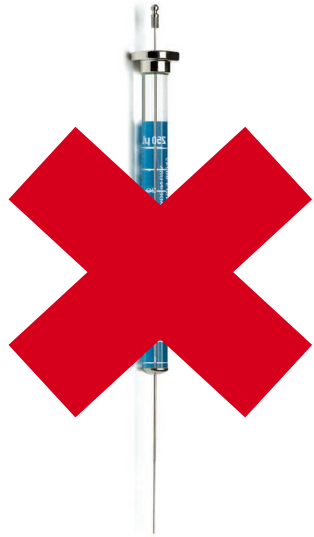
Agilent J&W Ultra Inert GC column

See Agilent brochure 5990-8532EN for more details.

# Additional Troubleshooting Techniques



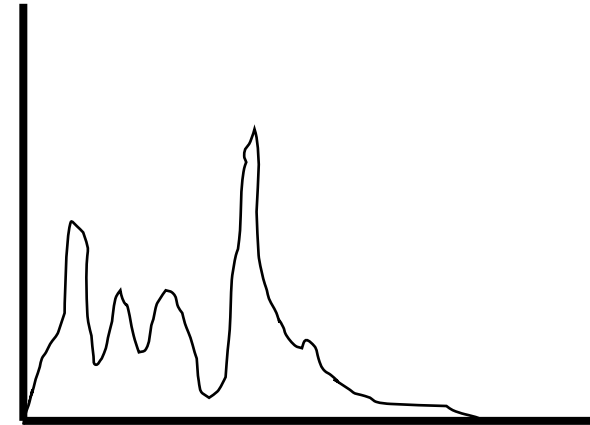
# Perform a Noninjection “Blank”



Remove syringe  
from autosampler



Run your program

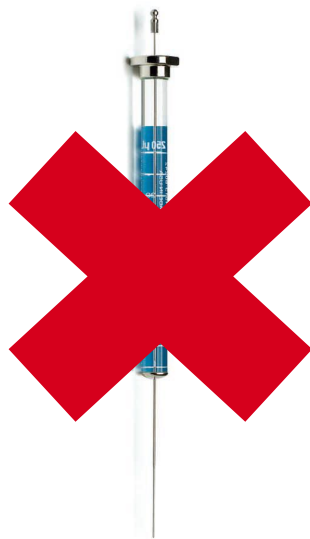


If you see peaks, you have  
some sort of inlet  
contamination likely

# Condensation Test Procedure



Leave GC at 40–50 °C  
for >8 hours



Remove syringe  
from autosampler

Blank 1

Blank 2

Run two blank runs  
and compare the two

# Condensation Test

## Results

### First blank run is worse

- Contaminants (from injector, lines, traps or carrier gas) carried into the column

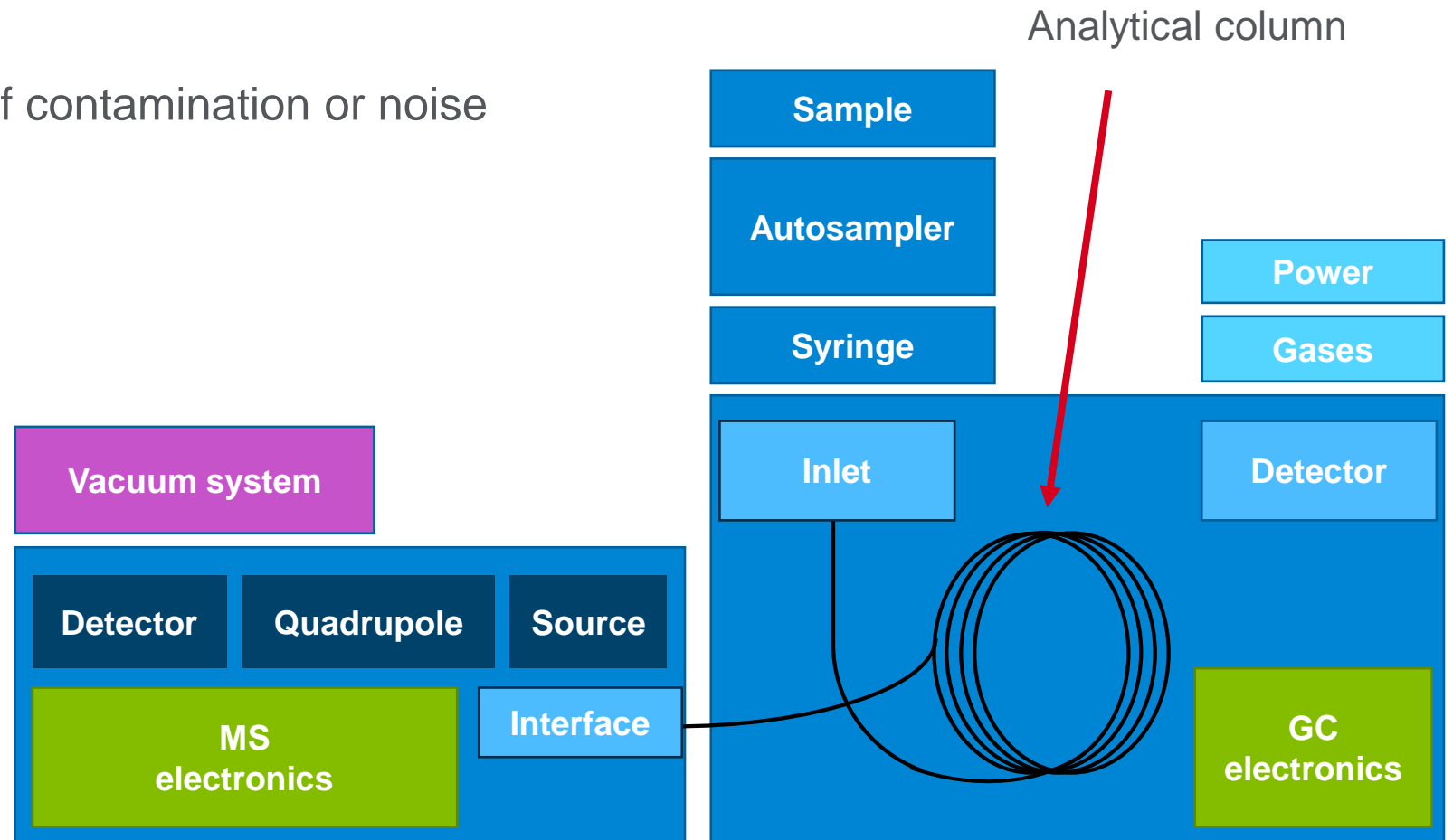
### Blank runs the same

- Contaminants are not strongly focused on the front of the column

# Jumper Tube Test

## Purpose

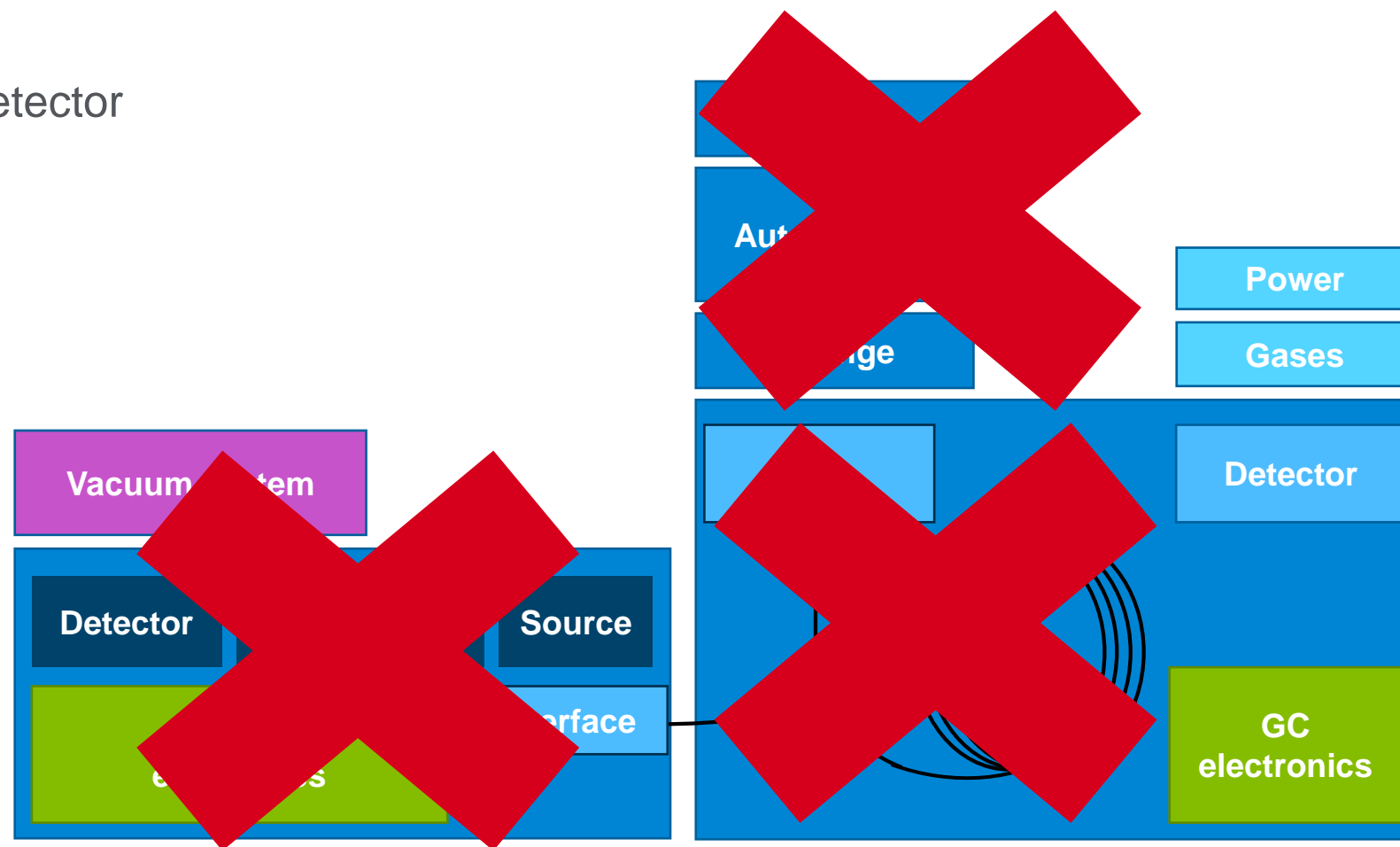
- Helps to locate the source of contamination or noise
- Isolates GC components



# 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

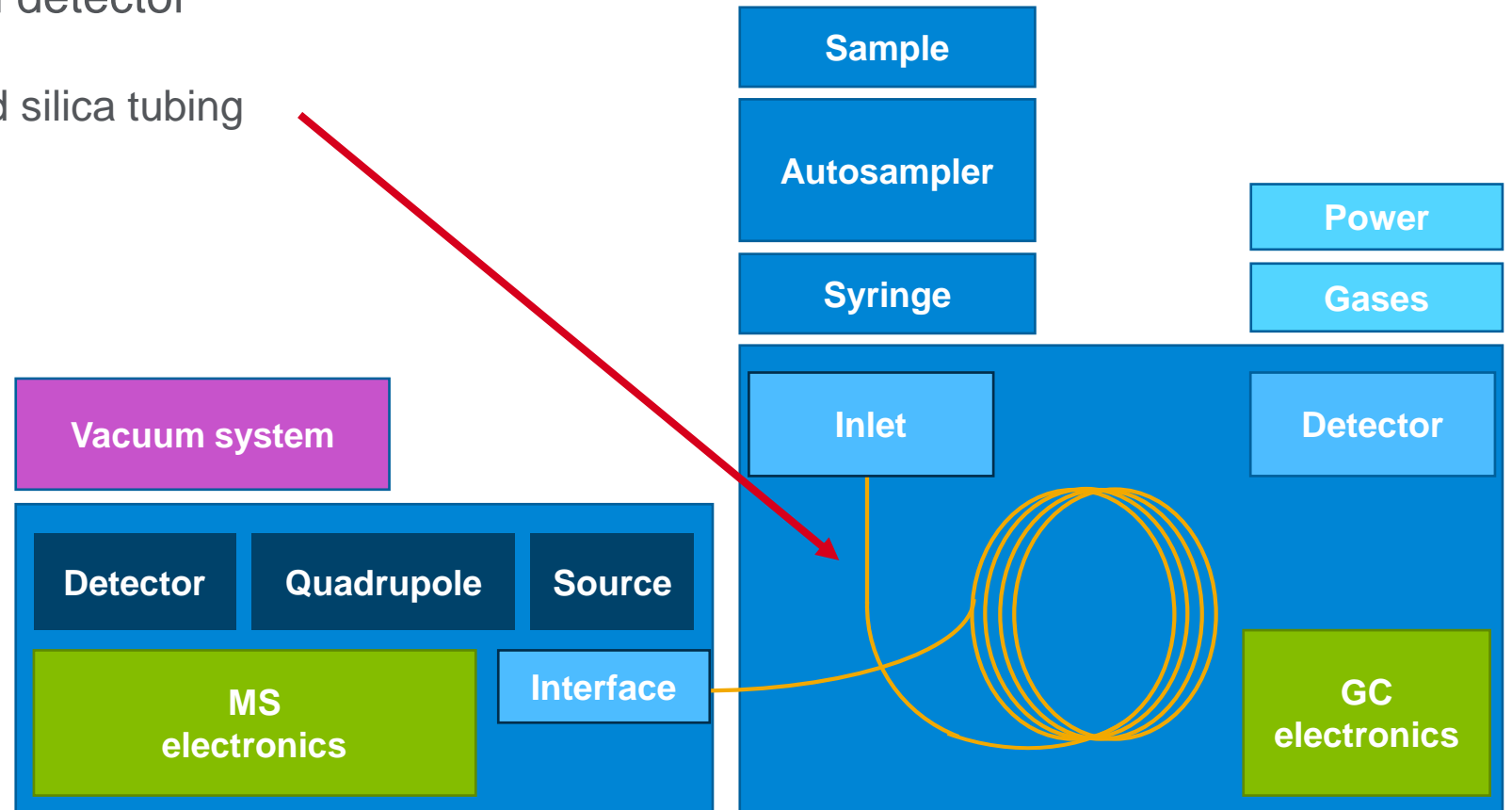
Detector is the problem



# Jumper Tube Test

## Isolate the injector

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



# Jumper Tube Test

## Isolate the Injector



## Results

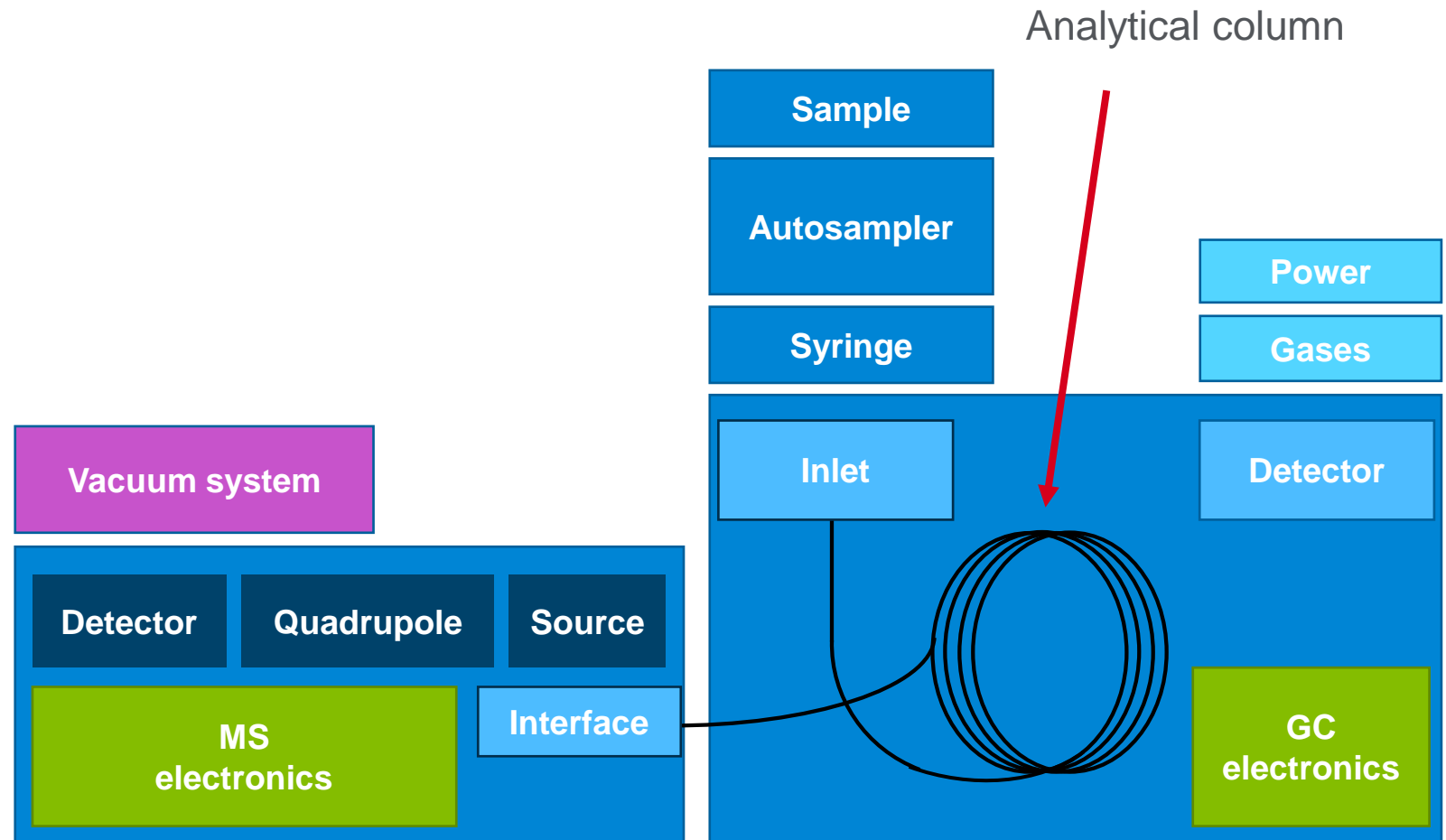
Injector OK

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

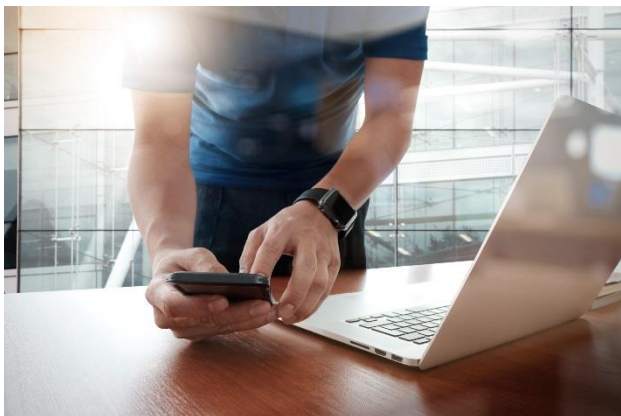
- Problem returns: It's the column
- Problem gone: Previous leak, solid debris, or installation problem

# Conclusion

- Distinguish between a “peak” and “nonpeak”
- Isolate different components to hunt down the ghost peaks (autosampler, inlet, column, and so on)
- Perform sample cleanup when necessary
- Proactively change consumables
- Please reach out to us for any assistance you might need



# 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](mailto:gc-column-support@agilent.com)

[lc-column-support@agilent.com](mailto:lc-column-support@agilent.com)

[spp-support@agilent.com](mailto:spp-support@agilent.com)

[spectro-supplies-support@agilent.com](mailto:spectro-supplies-support@agilent.com)

[chem-standards-support@agilent.com](mailto:chem-standards-support@agilent.com)