Don’t Let Inlet Breakdown Shut You Down: The Importance of Flow path Inertness

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Application Engineers
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Outline for Today

The inlet and its supplies

    The front-end is crucial

Typical inlet maintenance routine

What is system good inertness?

Endrin/DDT

Other Actives

Other things to consider

Review

Q&A

Inlets

Inlet	Column	Mode	Sample Concentration	Sample to Column	Comments
Split/splitless	Capillary	Split Splitless	High Low	Very little All	Most commonly used inlet, very flexible
Multimode (MMI)	Capillary	Split Splitless Solvent vent	High Low Low	Very little All All	Flexibility of standard SSL inlet and PTV
Packed	Packed large capillary	N/A N/A	Any Any	All All	OK if resolution is not critical
Cool-on-column (COC)	Capillary	N/A	Low or labile	All	Minimal discrimination and decomposition
Programmed temperature vaporization (PTV)	Capillary	Split Splitless Solvent vent	High Low Low	Very little All All	Not great for hot injections Can concentrate analytes and vent solvent
Volatiles interface	Capillary	Direct Split Splitless	Low High Low	All Very little All	Purge and Trap/Headspace

Agilent Inert Flow Solution

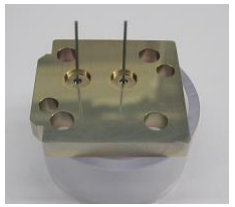
Agilent UltiMetal Plus inlet weldment shell

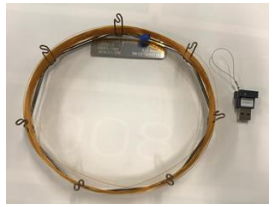
Agilent Ultra Inert inlet liner

Agilent UltiMetal Plus ferrules

Agilent UltiMetal Plus capillary flow technology devices, Ultimate union

Agilent UltiMetal Plus TCD, FPD, NPD/FID jets





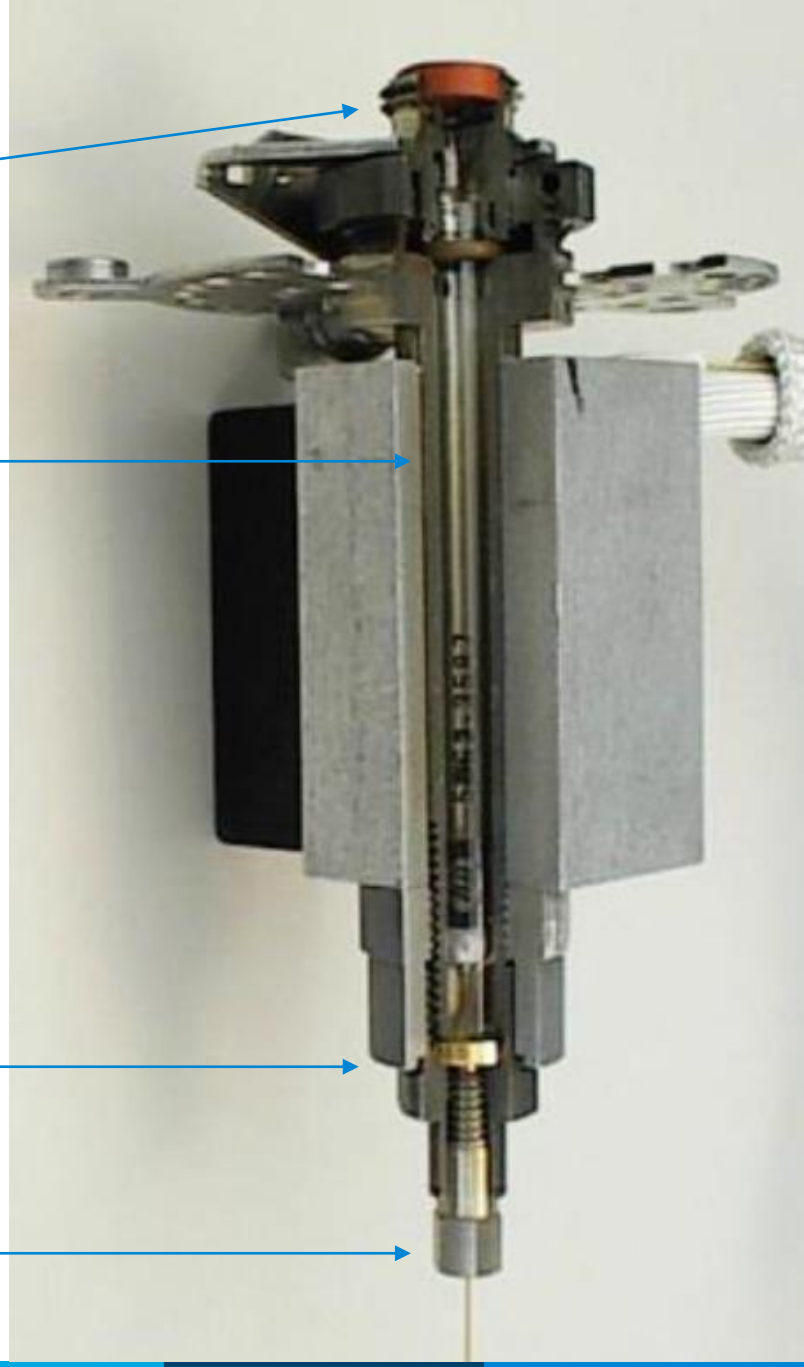
Agilent Ultra Inert gold seal

Agilent J&W Ultra Inert GC column

5990-8532EN brochure

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)
- **Most chromatography problems are "front-end" related**
- Many consumables to replace septa, liner, gold seal
- Inlet body must be cleaned/solvent rinsed periodically (**no steel brushes!**)



30+ Years of R&D Focused on Surface Deactivation

Two unique chemistries were developed to treat the surfaces in a GC flow path.

UltiMetal Plus

- Inorganic vapor deposition
- CFT device
- Ultimate union
- Flexible metal ferrule
- Inlet welding
- Detector parts
- GC tubing

Ultra Inert (UI)

- Organic vapor deposition
  - Ultra Inert inlet liners
  - Ultra Inert gold seals
- Unique proprietary deactivation process
  - UI columns

Septa

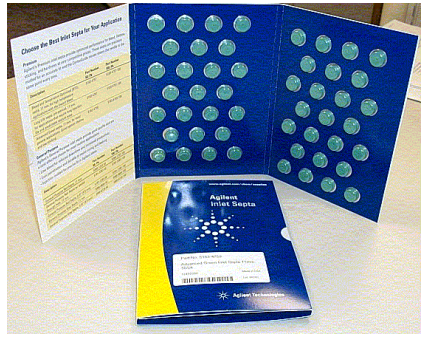
Summary of Premium Inlet Septum Characteristics

Septum Type	Bleed	Lifetime	Temperature Limits
Non-Stick BTO (Bleed and Temperature Optimized)	◆◆◆	◆	to 400°C injection port temp
Non-Stick Advanced Green	◆◆	◆◆	to 350°C
Non-Stick Long-Life	◆	◆◆◆	to 350°C

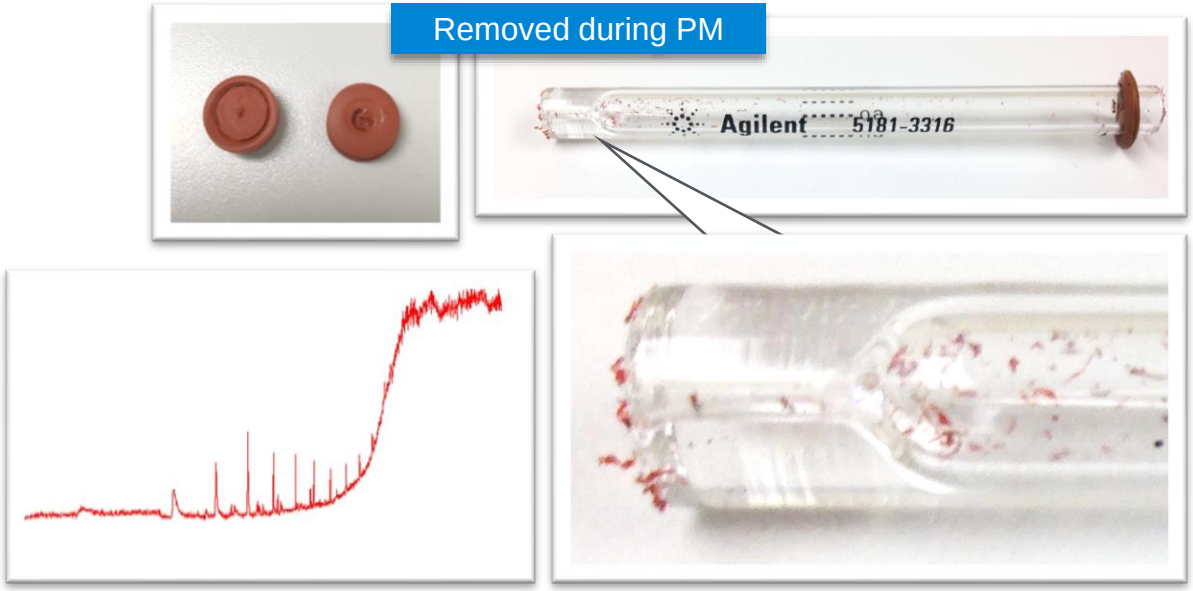
◆◆◆ = best ◆◆ = very good ◆ = good

Septa

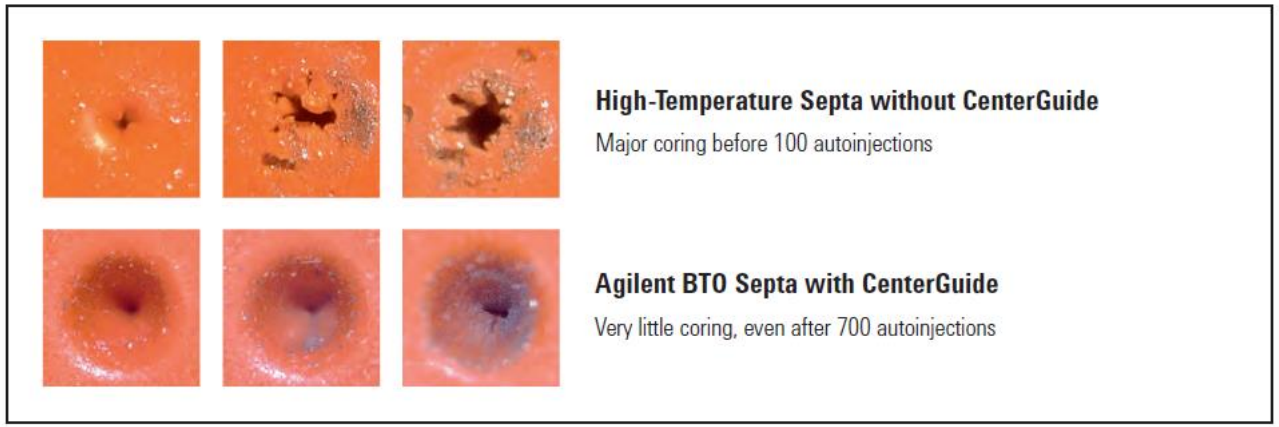
- Typical cost of one premium septum, ~$2.00
- Typical cost of one GC column, 30 m x 0.25 mm id, ~$600
- Proactively change inlet septa
- Agilent packaging eliminates contamination of septa
- “Center-Guide septa” puts less train on syringe compared to solid septa
- Do not overtighten septum nut; septum can begin to “bulge” out
- Should tighten nut until c-clamp on top stops turning, then ½ to ¾ turn more (gap = 1 mm)



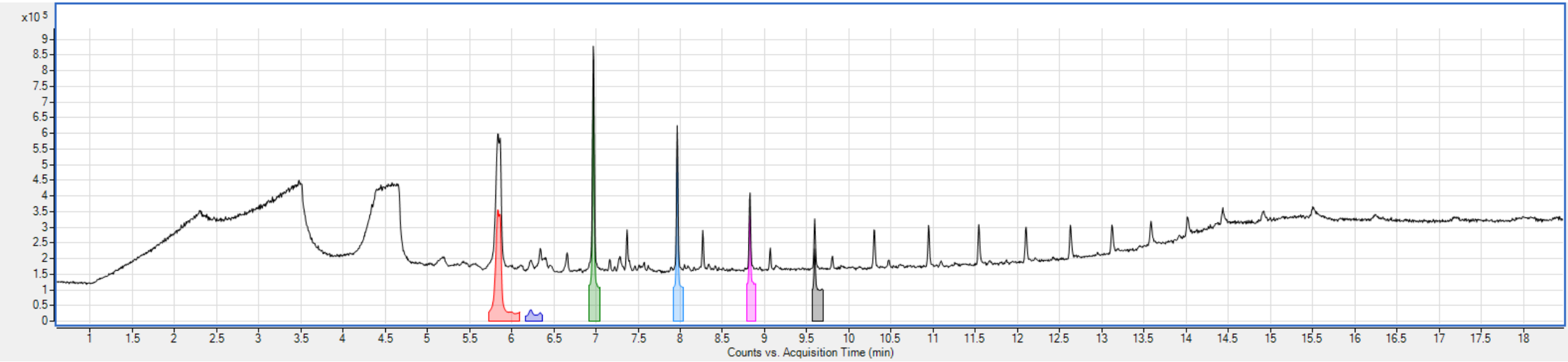
Septum nut

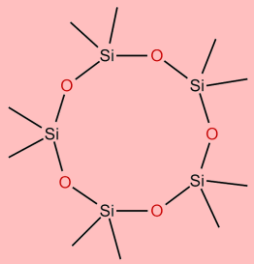
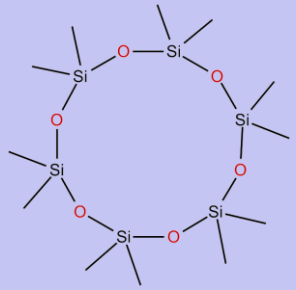
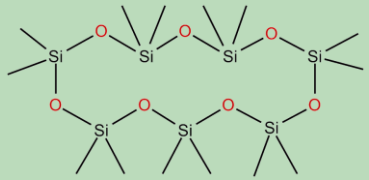
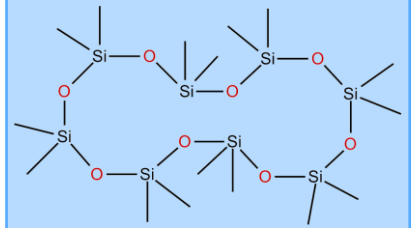
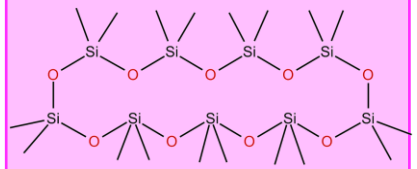
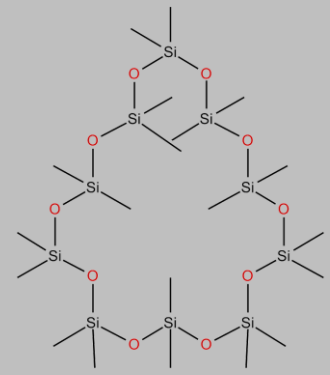


Comparison of Coring, With and Without CenterGuide (30x magnification)



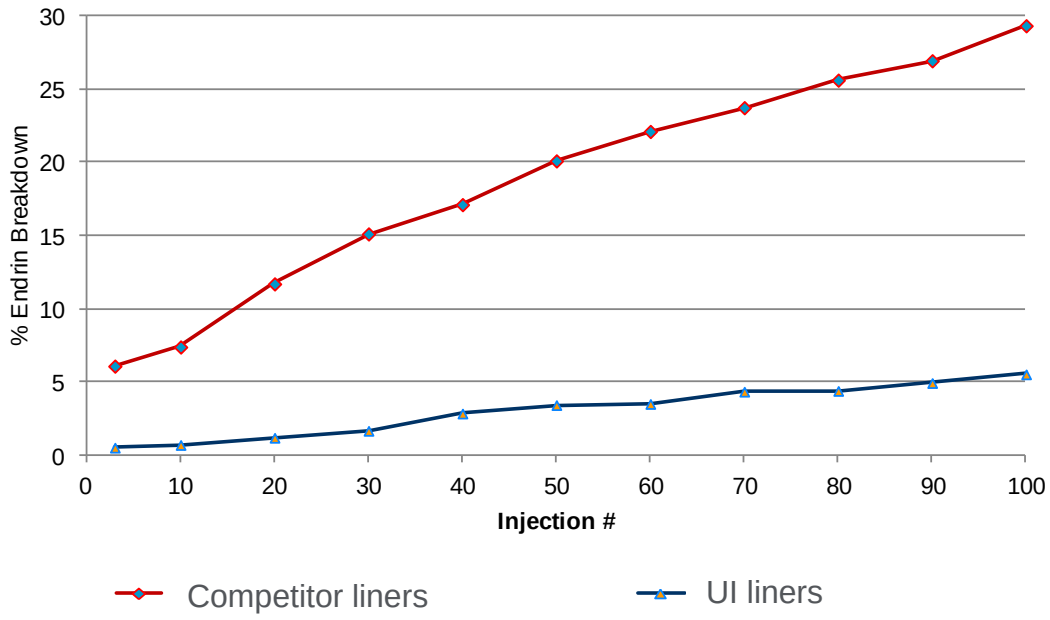
Septum Maintenance: Deconvoluted Inlet Septa Spectrum



10	12	14	16	18	20
					
Decamethyl cyclopentasiloxane	Dodecamethyl cyclohexasiloxane	Tetradecamethyl cycloheptasiloxane	Hexadecamethyl cyclooctasiloxane	Octadecamethyl cyclononasiloxane	Eicosamethyl cyclodecasiloxane

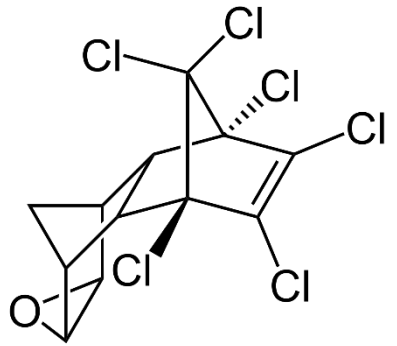
Ultra Inert Inlet Liners

1. Ultra Inert deactivated inlet liners provide higher response for sensitive compounds.
2. Ultra Inert **glass wool liners** deliver benefits of glass wool without loss of active compounds.
3. QC tested and certified for consistent performance



Productivity

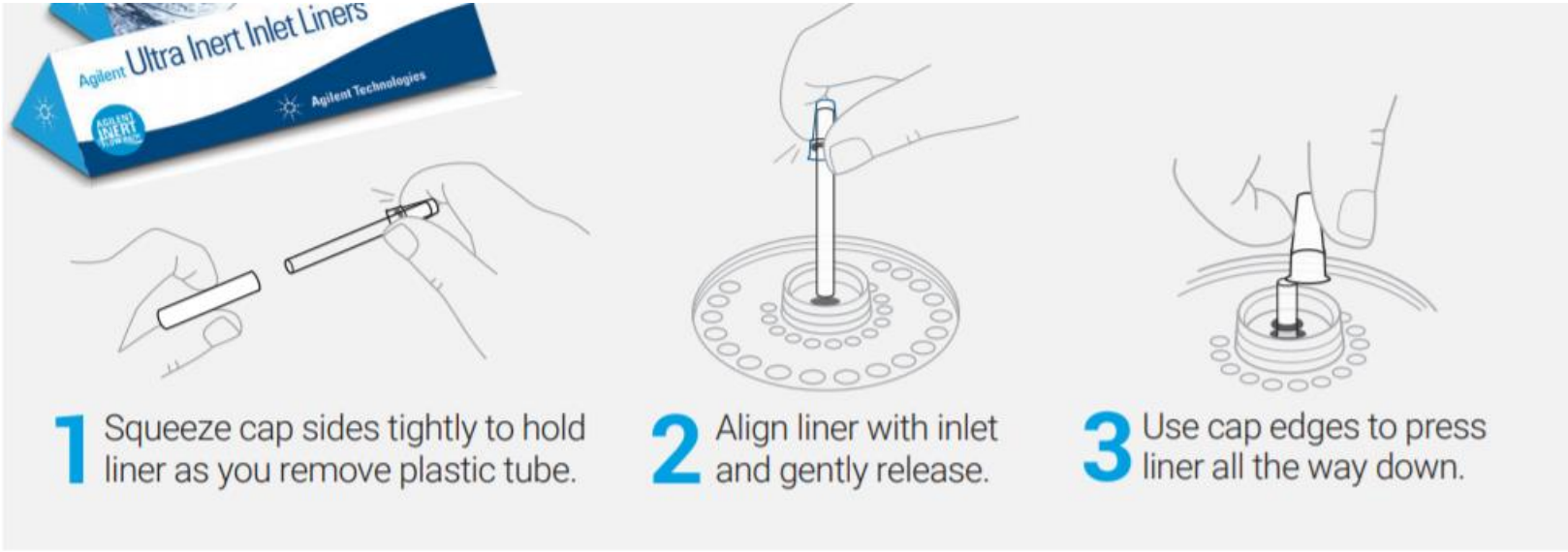
Touchless packaging with pre-installed O-ring: quick and easy hassle-free installation



Agilent Assurance

- UI liner, lot QC with demanding test probes (dinitrophenol and others)
- Assurance of consistent performance
- Label for p/n, batch, and lot testing
- Traceability
- Deactivation lot number is on certificate
  - Liner lot number (and part number) etched on glass

Comes with O-ring pre-installed



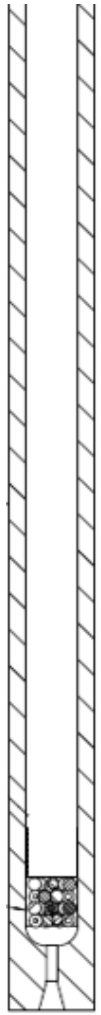
Modern Day Solution: The Glass-Frit

All the benefits of wool, but…
Frit is fixed and will stay in place
No wool fibers

Substituting glass wool for a sintered glass frit provides equivalent vaporization properties, while enhancing performance consistency and quality
Still acts as a filter to protect column
Frit is fixed so cannot move like GW can

Glass Wool Alternative Liners

Ultra Inert liners with sintered glass frits



All the benefits of wool, but…
Frit is fixed and will stay in place
No wool fibers

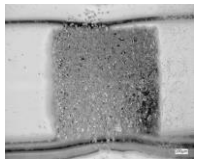
Low frit single taper liner

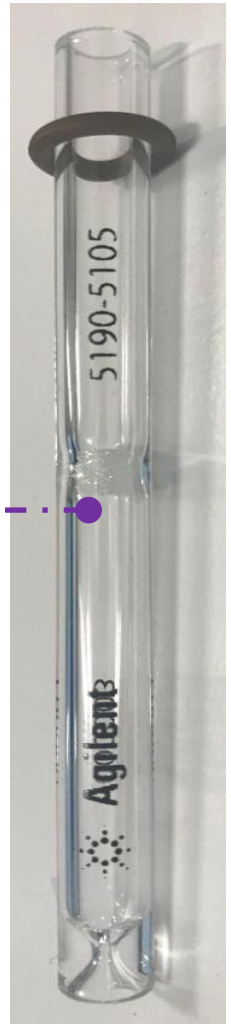
- **Designed for dedicated splitless analyses**
- **Ideal for SVOCs and OC/OP pesticides**

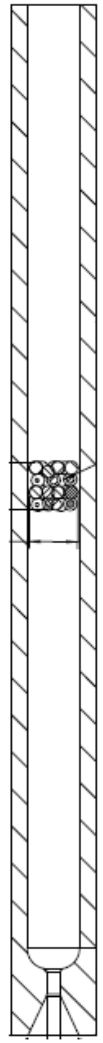
- **General purpose split/splitless liner**
- **Ideal for basic drugs analysis**

Mid frit single taper liner

Porous glass frit

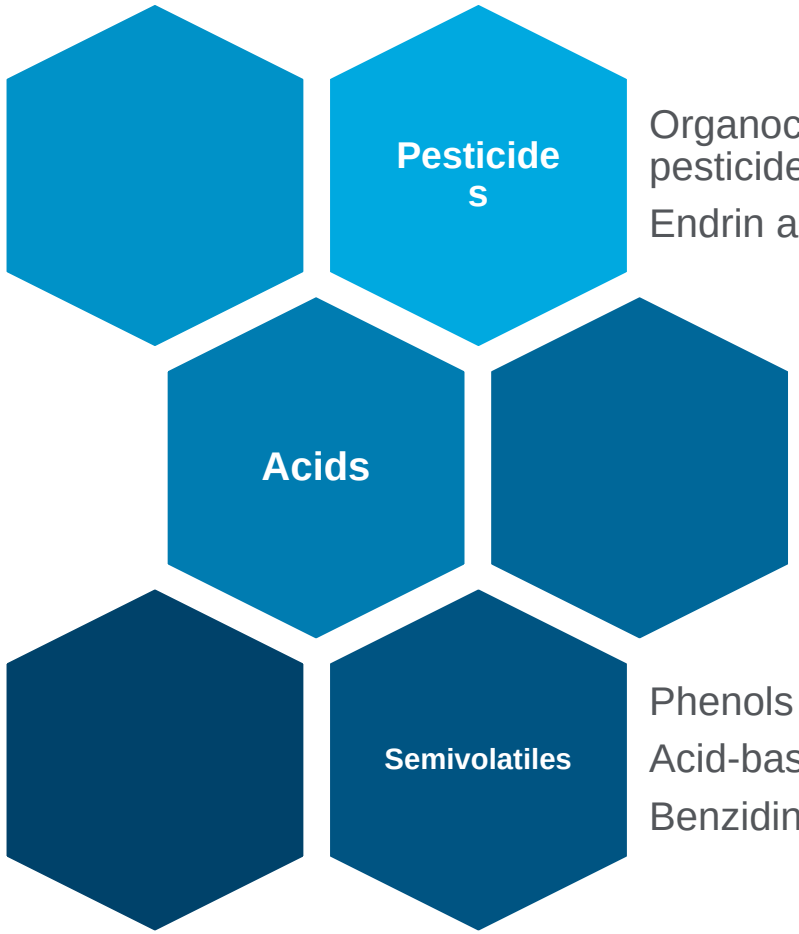






The Benefits of the Glass Frit

Dislodged glass wool fibers expose active sites that interact with sensitive analytes



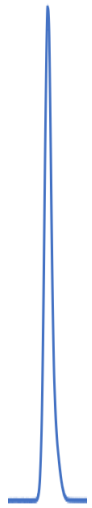
Organochlorine pesticides

Endrin and DDT

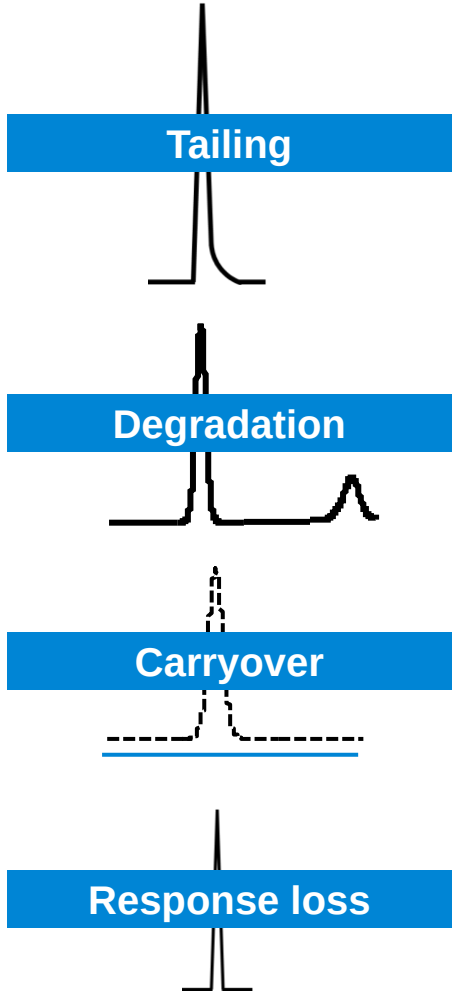
Phenols

Acid-base pairs

Benzidine

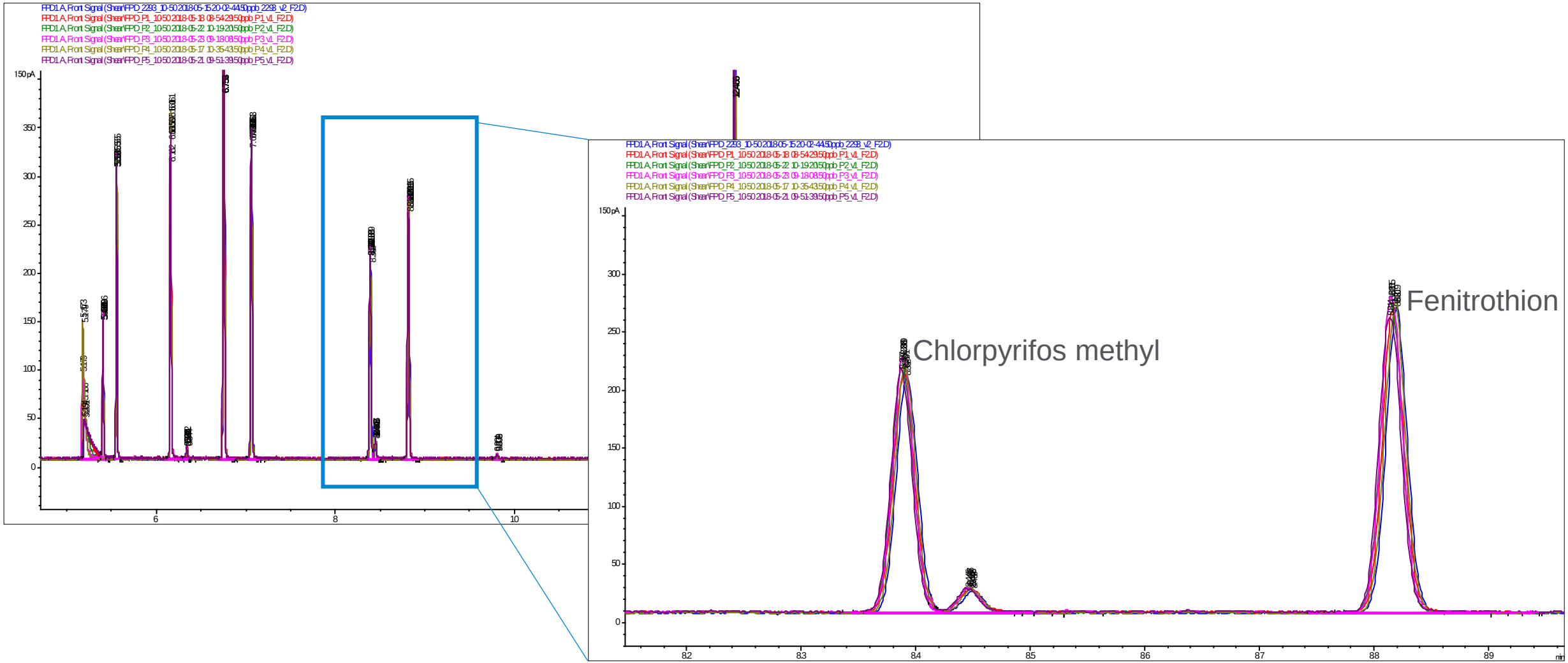


Interaction with active sites results in



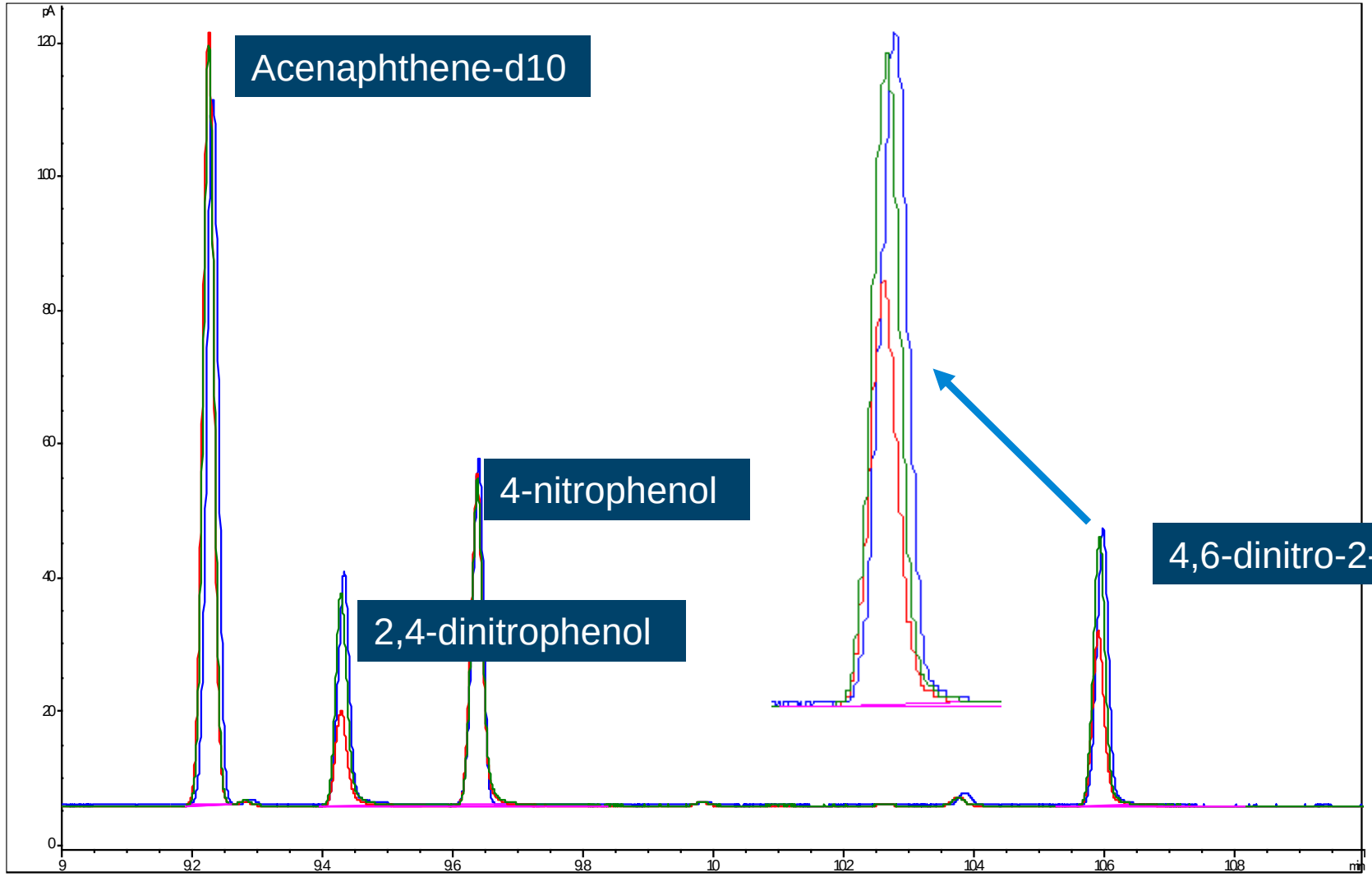
Pesticides by FPD

Results for fritted liner indicate highly inert, consistent response



EPA 8270 Chromatogram Overlay – Phenols/Low Frit Liner

Low frit liner gives excellent response and peak shape for tricky analytes like phenols

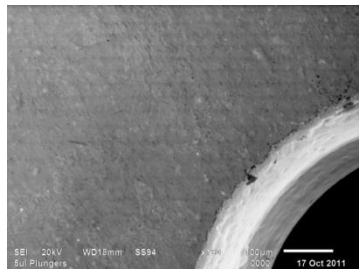


Blue = Initial injection
Red = After 100 matrix injections
Green = After liner replacement

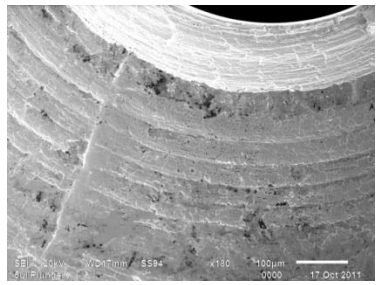
5 ppm, 1 µL splitless injection
FID detector

Agilent UI Gold Seal: Deactivated Gold Surface

- Soft gold plating is essential for proper sealing
- Ultra Inert chemistry blocks active sites (gold is **not** inert)
- Smooth surface does not leak (injection molded)
- Part numbers 5190-6144 ea. 5190-6145, 10/pk. 5190-6149, 50/pk.



Agilent MIM seal



Competitor's machined seal

Reliable ppb and ppt measurements require attention to the little things and the entire flow path

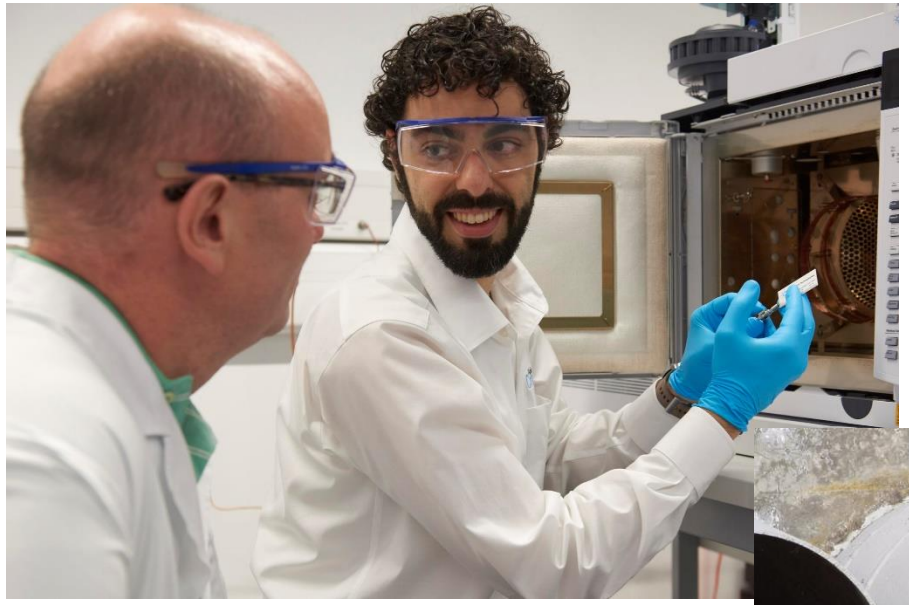
Agilent UltiMetal (UM) Plus Flexible Metal Ferrules

- Primarily used with CFT devices (unions, splitters, back-flush, etc
- Can be used for the column connection to the inlet…but…
  - Need to pre-swage the ferrule
- Touchless packaging

Metal Ferrules, G3440-80218

https://www.agilent.com/en/video/packageinstructions

Typical “Front-End” Maintenance Routine



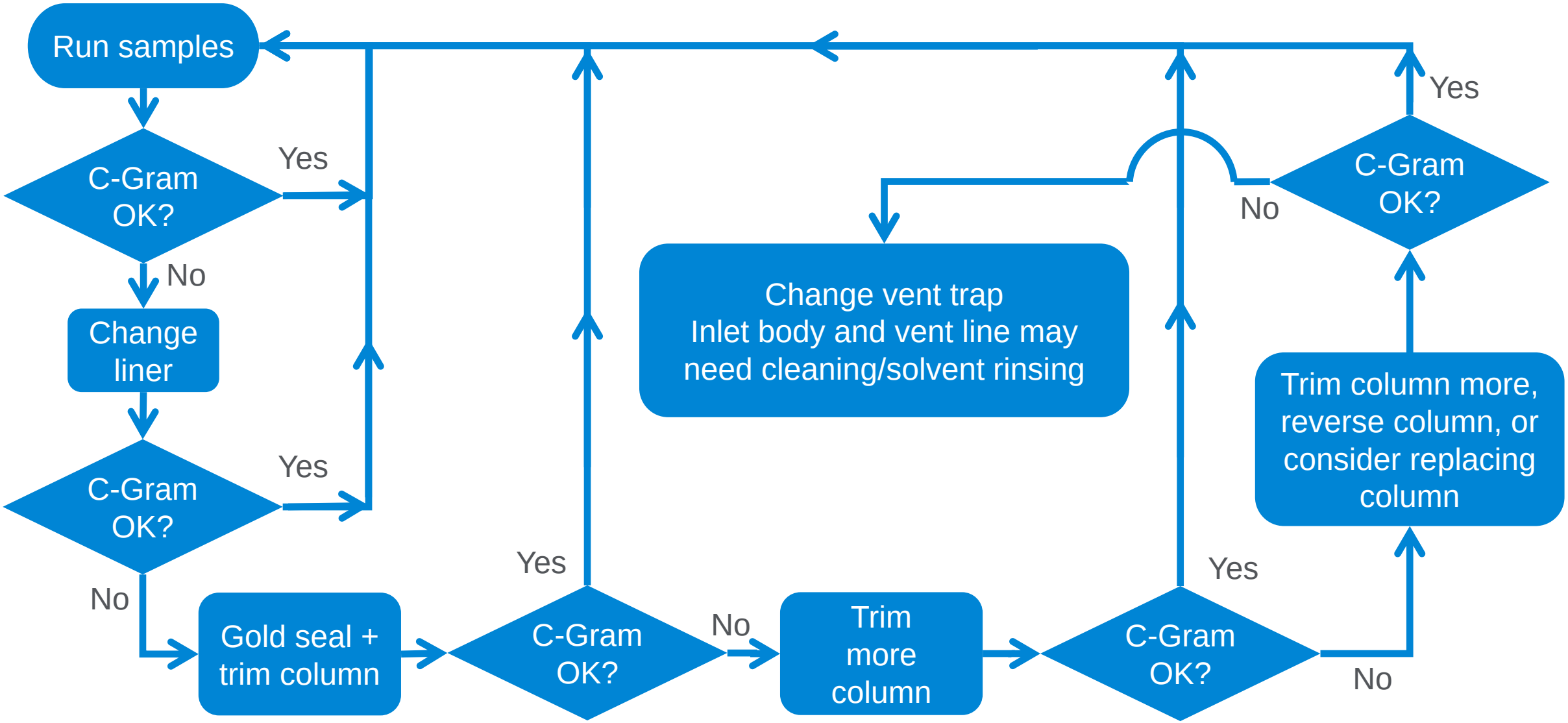
Ok so lets’ talk about “front-end” maintenance……

Typical “Front-End” Maintenance Routine

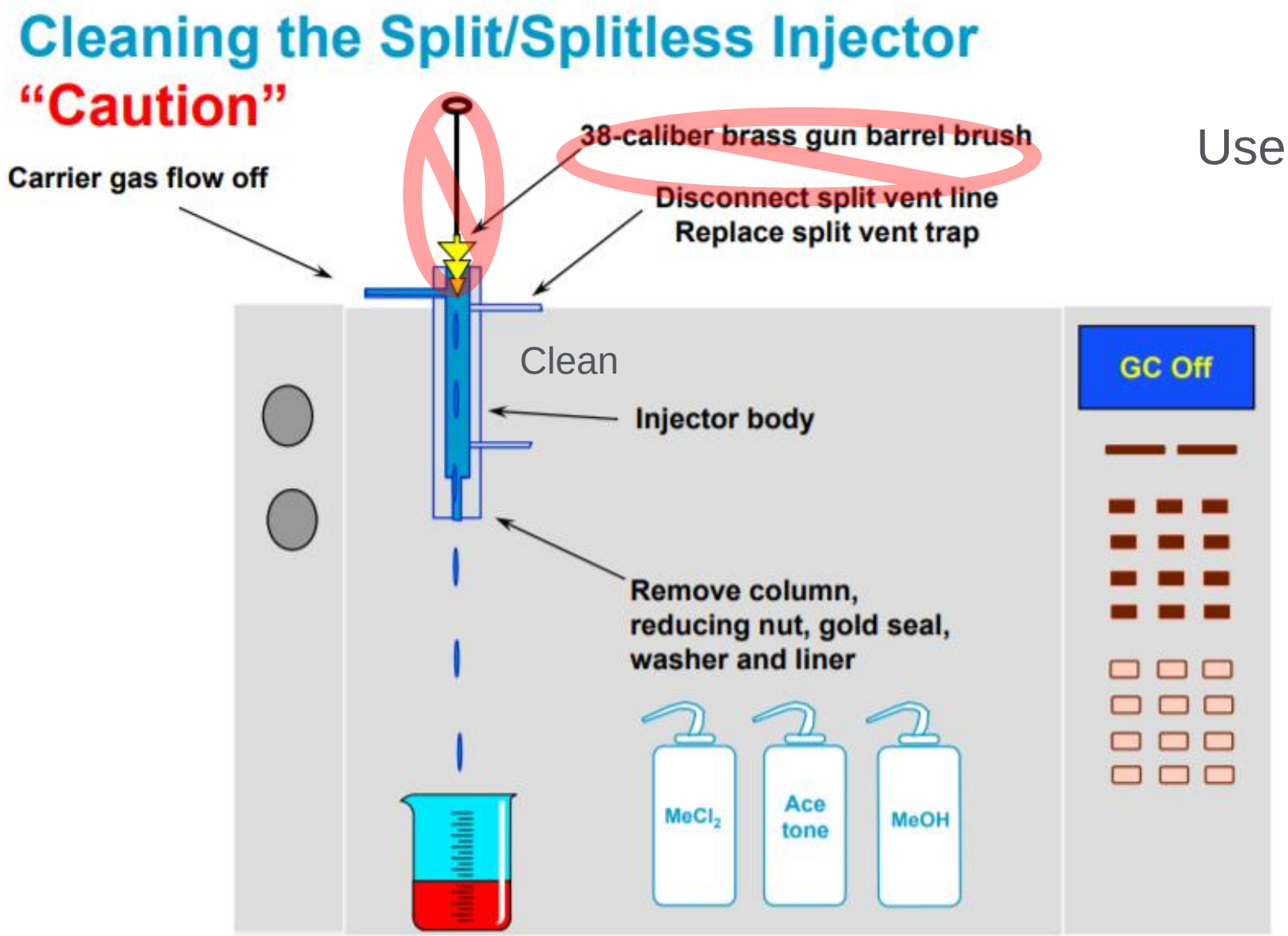
- Million-dollar question: *How long do consumables/columns last?*
  - It depends…mostly on sample matrix and number of injections
- Follow the sample flow path
  - Septum → liner → gold seal → column (front 0.5 to 1 m / 1 to 2 loops)
  - Generally, each process is performed less frequently as you proceed down the flow path

- Change the septum regularly, based on the number of injections
- Run samples until conditions start to deteriorate, then change the liner and continue down the follow sample flow path…see flow chart next slide….

Inlet Maintenance Flow Chart



Inlet Cleaning/Solvent Rinse



Use a swab instead!

GC System Inertness
What do we mean?

What Does GC System Inertness Look Like?

Easier question: What does poor inertness look like?

Symptoms of poor GC system inertness:

* Tailing peaks
* Reduced peak response
* No peak response
* Extra (aka bonus) peaks! (Endrin → E-aldehyde + E-ketone, etc.)
* Poor linearity of a peak – usually at low concentrations
* Unstable detector baseline

GC System Inertness
What do we mean?

Problems with poor inertness almost always limited to “active” solutes.

For example:
Alcohols & Diols (-OH), Phenols, Amines (-NH3), Acids (COOH),
Thiols & Sulfur in general like to tail.

Thermally labile and structurally “strained” solutes will breakdown or rearrange, e.g., DDT, Endrin, Carbamates, Nitroglycerines.

Tailing or breakdown of “benign” solutes is symptomatic of a more generalized system problem, usually related to gross contamination.

Possible Inertness Problem Areas

Inlet

– liner, liner packing, gold seal, stainless steel

Other Consumables

– septa, syringe, vial, caps, inserts, solvents

Column

GC Detector

– source geometry, material, column interface, acquisition rates

Temperatures

– inlet, transfer line, source, quads, oven

Other method factors i.e. samples and standards preparation

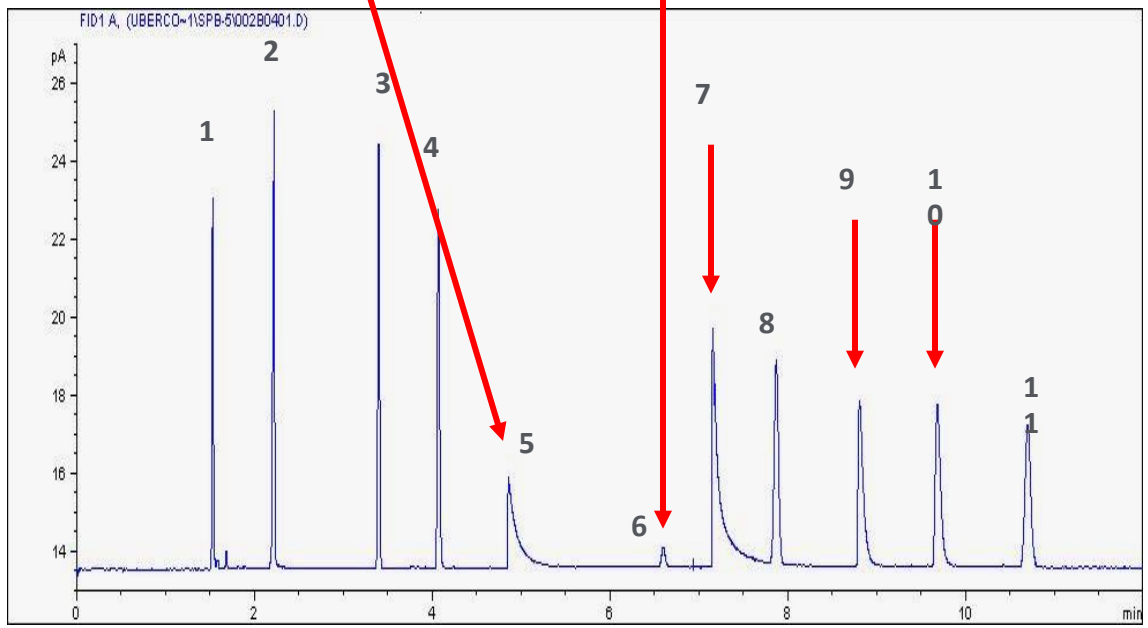
What Does System Activity Look Like?

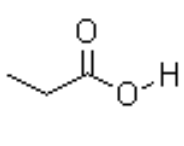
Tailing, and loss of response.

Loss of peak height, but same area counts

Loss of peak height and area counts

	Probe	0.5 ul inj, 1:50 split, (ng)
1	Methane	?
2	**Propionic Acid**	7.0
3	Octane	2.4
4	Nitrobutane	4.9
5	**4-Picoline**	2.7
6	**Trimethyl Phosphate**	12.7
7	**1,2-Pentanediol**	5.4
8	Propylbenzene	2.2
9	**1-Hetpanol**	3.3
10	3-Octanone	3.0
11	Decane	2.2





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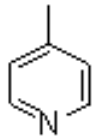

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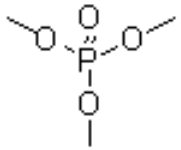

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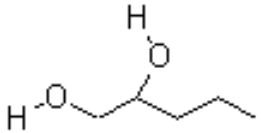

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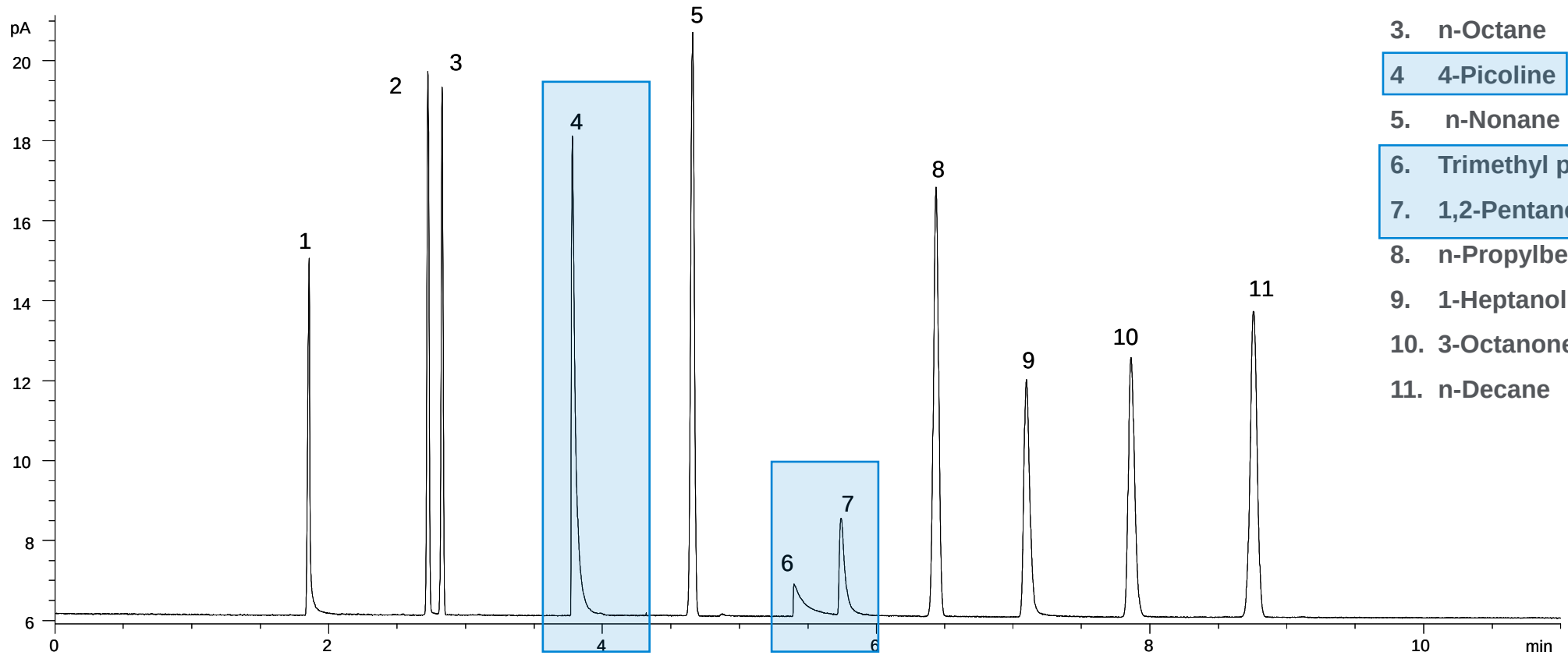
Ultra Inert Test Mix

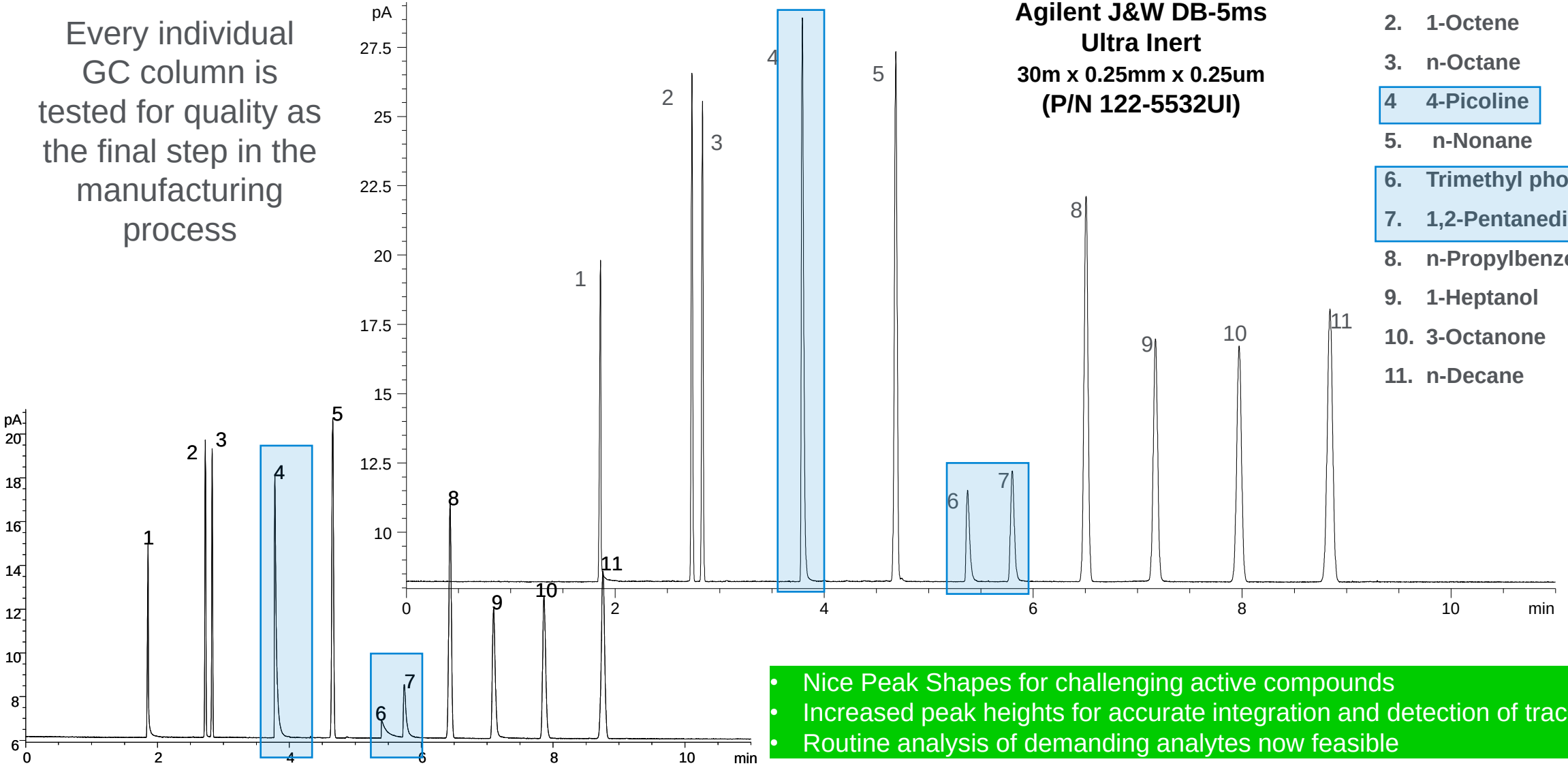


1. 1-Propionic acid
2. 1-Octene
3. n-Octane
4. 4-Picoline
5. n-Nonane
6. Trimethyl phosphate
7. 1,2-Pentanediol
8. n-Propylbenzene
9. 1-Heptanol
10. 3-Octanone
11. n-Decane

All highlighted peaks have poor peak shape – due to poor column deactivation

Ultra Inert Test Mix on Agilent J&W DB-5ms Ultra Inert

Every individual GC column is tested for quality as the final step in the manufacturing process

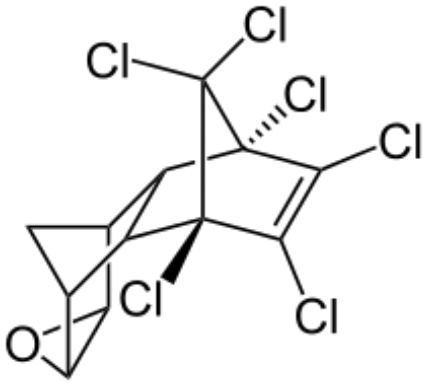


Agilent J&W DB-5ms Ultra Inert
30m x 0.25mm x 0.25um
(P/N 122-5532UI)

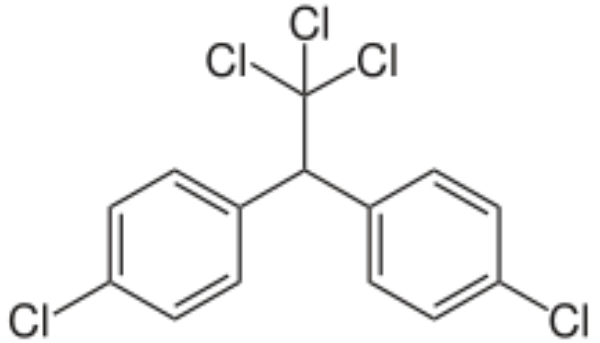
1. 1-Propionic acid
2. 1-Octene
3. n-Octane
4. 4-Picoline
5. n-Nonane
6. Trimethyl phosphate
7. 1,2-Pentanediol
8. n-Propylbenzene
9. 1-Heptanol
10. 3-Octanone
11. n-Decane

- Nice Peak Shapes for challenging active compounds
- Increased peak heights for accurate integration and detection of trace samples
- Routine analysis of demanding analytes now feasible

Endrin and DDT

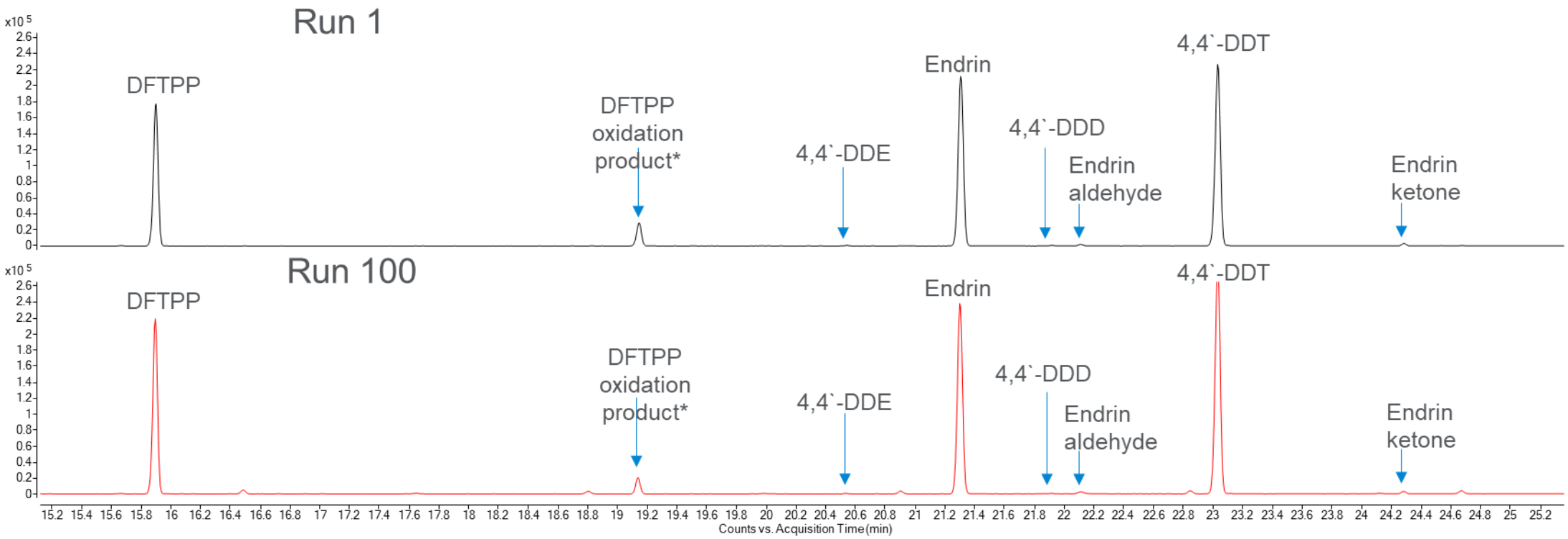


ENDRIN



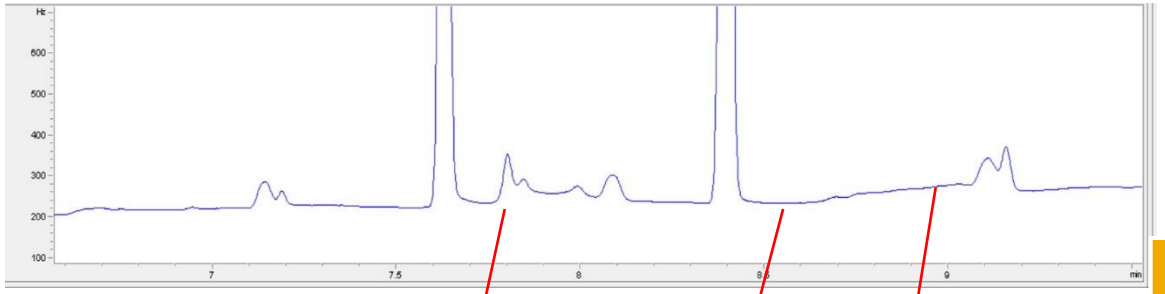
DDT

If Only All Results and Sequences Looked Like This



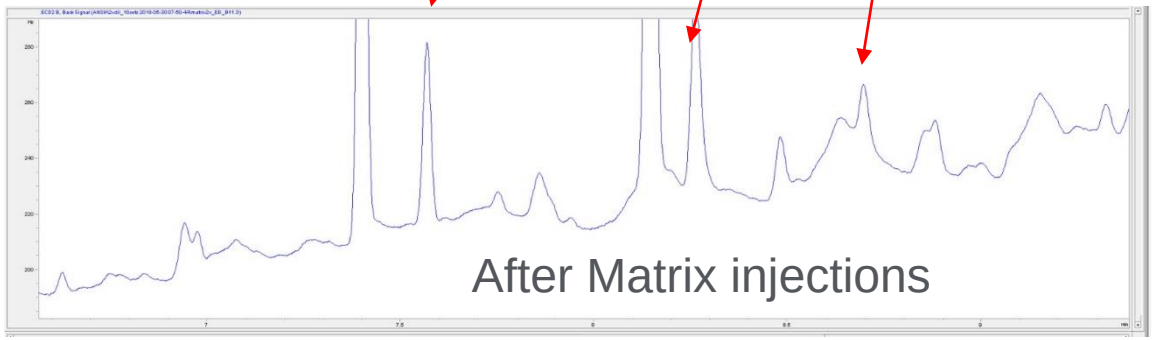
But, More Often, We Probably See…

Early chromatogram in Sequence

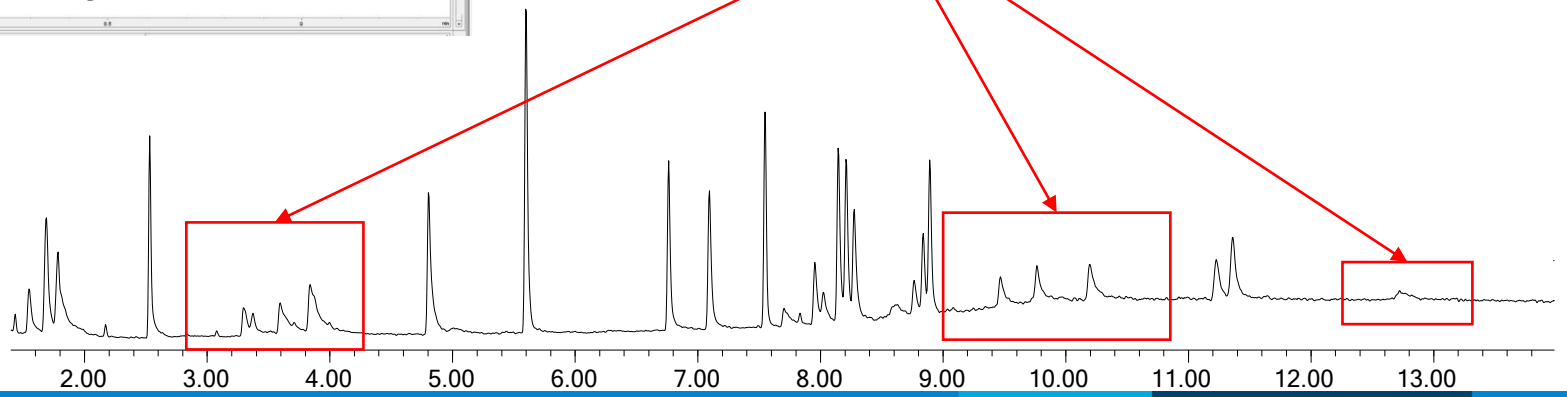


This is all due to matrix build-up
(primarily at the “front-end”)

Increase in degradation products



Tailing and/or peak response loss



What Analyses Might Use Endrin or DDT Breakdown?

Why do we care about the breakdown?

Semivolatile organic compound analysis

- Environmental and pesticide analyses
  - GC/ECD
    - EPA 8081
    - EPA 608
  - GC/MS
    - EPA 525
    - EPA 8270D/E

Why does it matter?

Sensitive to inlet and column contamination and temperature

Endrin isomerization:

High temperatures (inlet), active sites (matrix, non-deactivated silica)

DDT degradation:

Active sites (for example, non-deactivated metal, matrix, debris)

Indicators for other active/sensitive compounds

How Much % Breakdown is acceptable?

What are “good” values for starting % breakdown?

Endrin: ≤10%

Great system: ≤5%

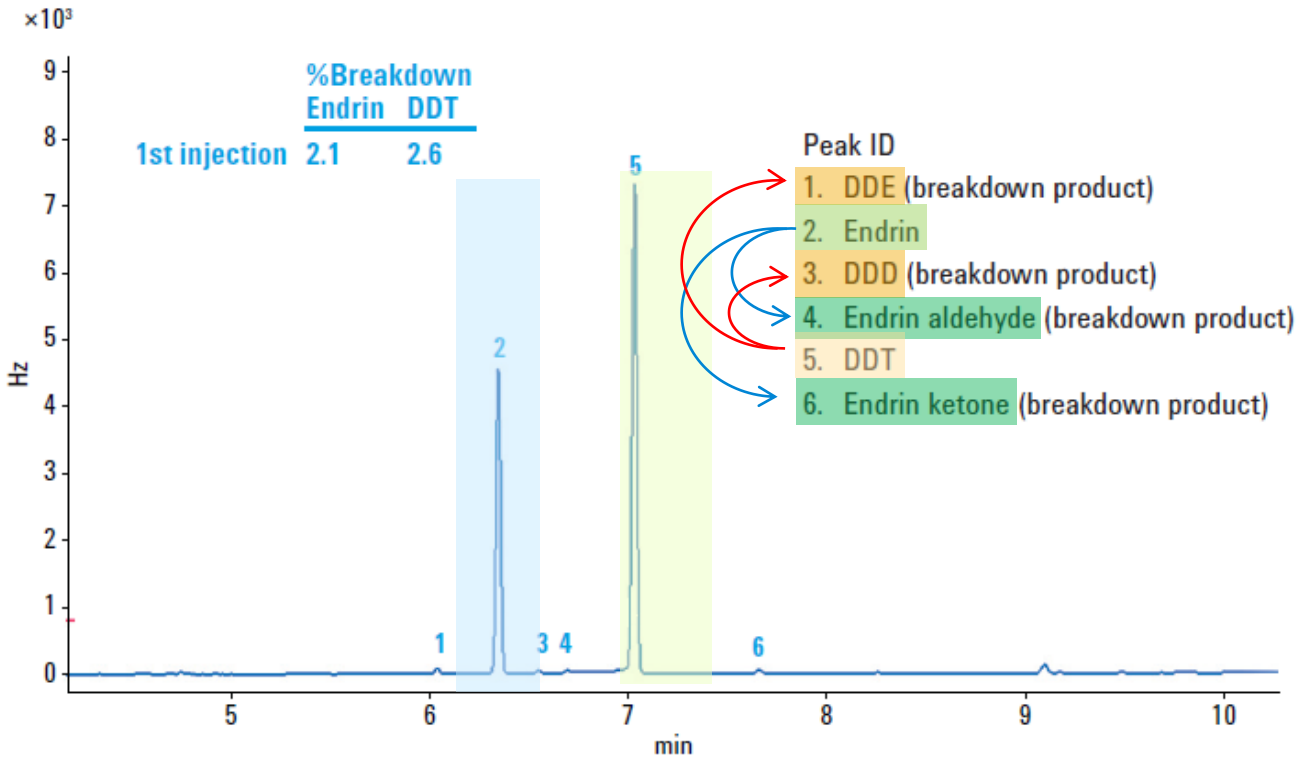
DDT %: ~2 to 3%

Great system: ≤1%

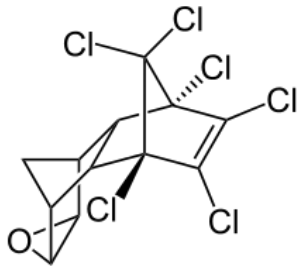
What are the method limits for % breakdown?

- EPA 8081 and 525.2
  - 20% total breakdown (or 15% of single compound)
- EPA 8270D/E and 525.3
  - 20% DDT breakdown

Environmental Pesticides Probes: Endrin/DDT Breakdown



Endrin

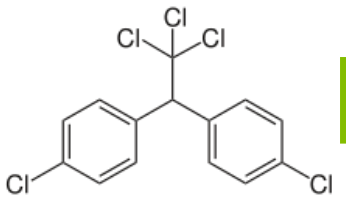


Exposure to heat or surface contaminants

Endrin aldehyde
Endrin ketone

$$\% \text{ Endrin breakdown} = \frac{(\text{Peak area}_{EA} + \text{Peak area}_{EK})}{(\text{Peak area}_{EA} + \text{Peak area}_{EK} + \text{Peak area}_{Endrin})} \times 100$$

DDT

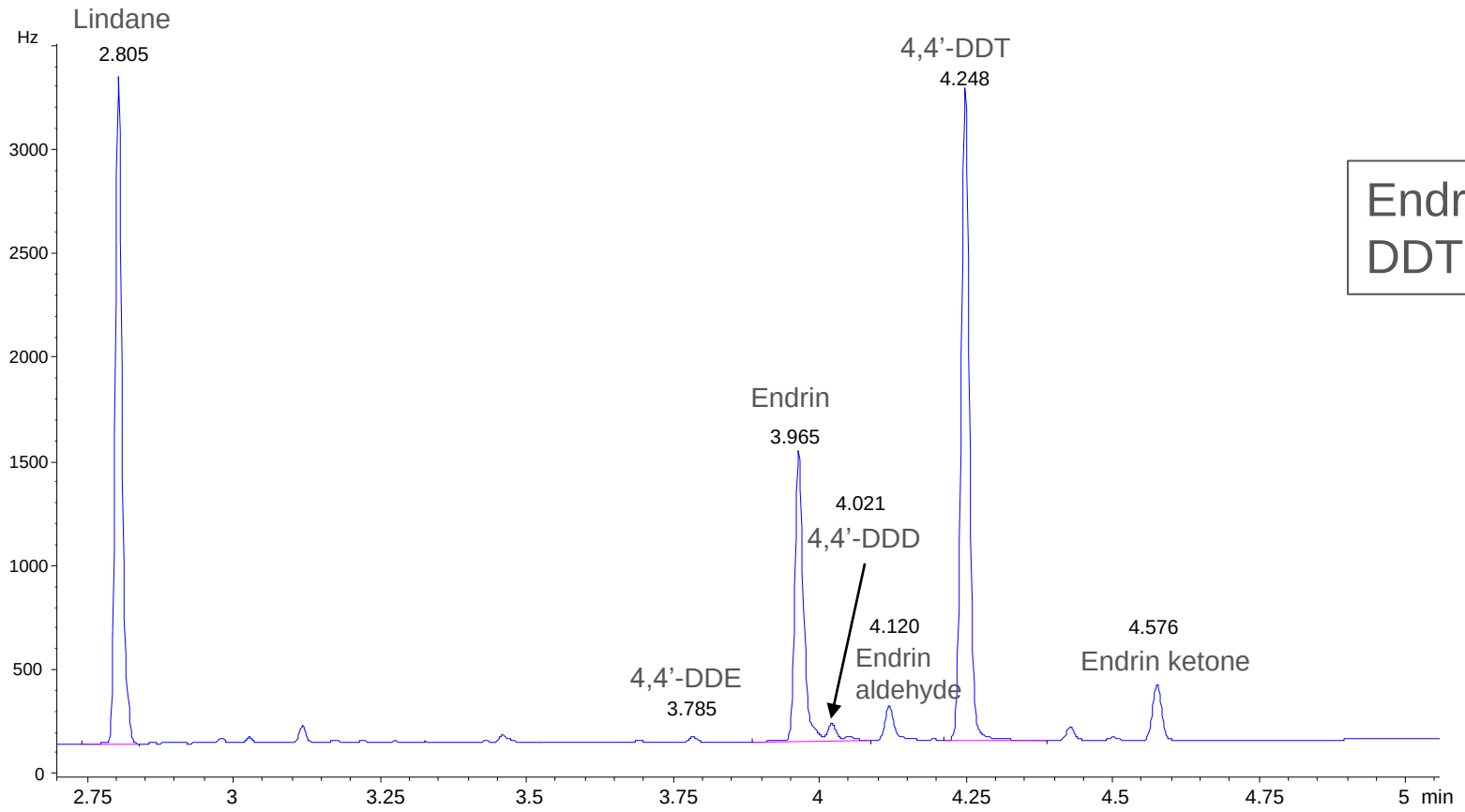


Exposure to metal surfaces or contaminants

DDE
DDD

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

System Has High % Breakdowns



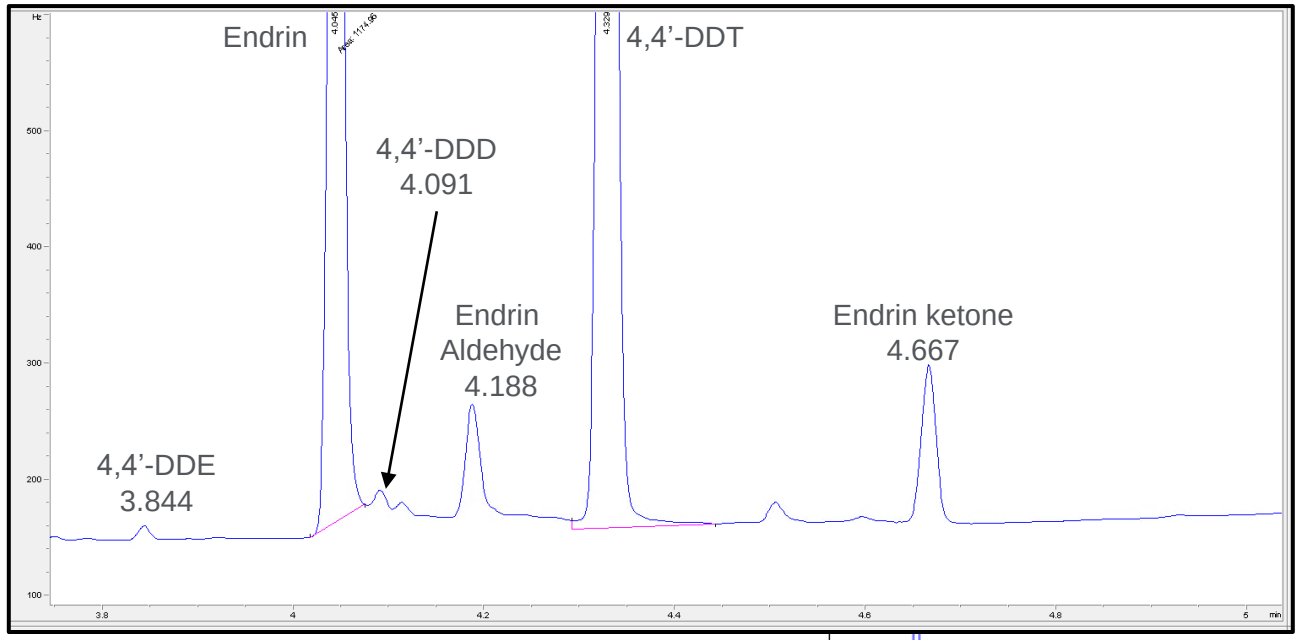
Endrin % breakdown: 27.03%
DDT % breakdown: 4.31 %

That's a problem…
What are the culprits for this failure?

How Can We Mitigate Compound Breakdown in Inlets?

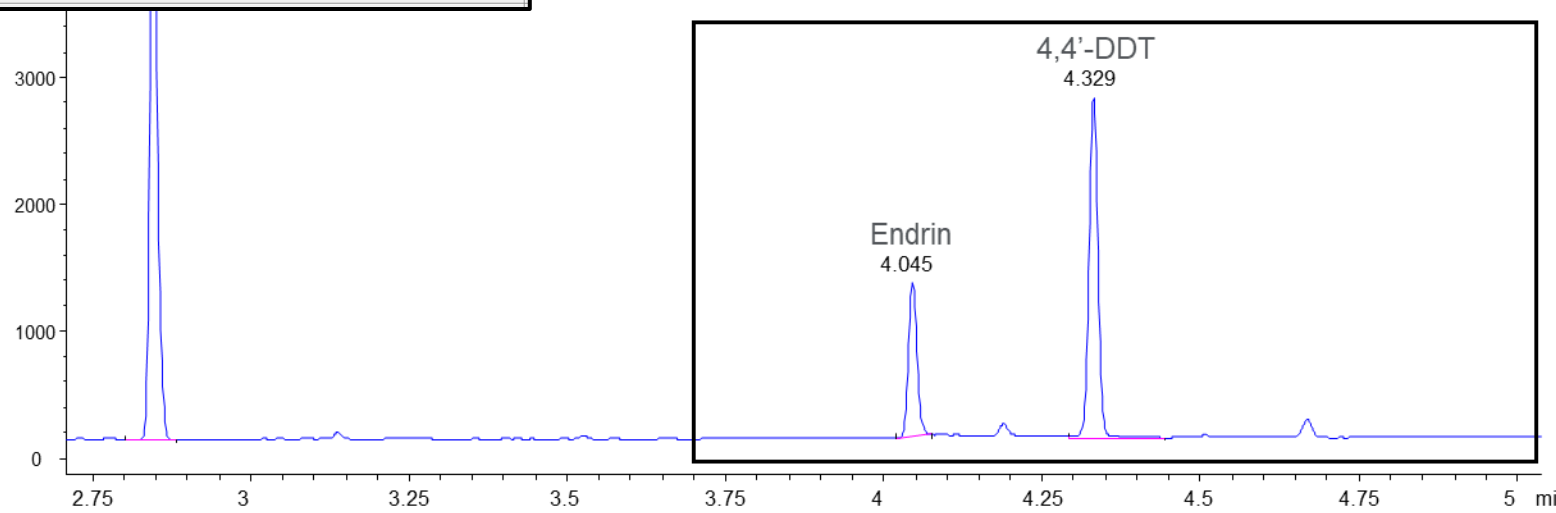
- Lower inlet temperature
  - Requires testing to identify optimal temperature across analysis
  - Too low may be a problem for less volatile solutes
- Smaller injection
  - This reduces over-all level of matrix deposits
    - (more injections before maintenance)
- Get sample onto the column quickly
  - Pulsed splitless injection (reduces residence time in the inlet)
- Use an inert flow path
  - Deactivated/UI liners and columns
- Try frit liners
  - Good alternative to glass wool
  - If matrices are clean, you may not need glass wool in the 1st place
- Inlet maintenance
  - Septum, liner, gold-seal, trim column

Let’s Lower the Inlet Temperature to 200 °C



Endrin % breakdown: 27.03%
DDT % breakdown: 4.31%

Endrin % breakdown: 18.19%
DDT % breakdown: 0.79%



Pulsed Injection

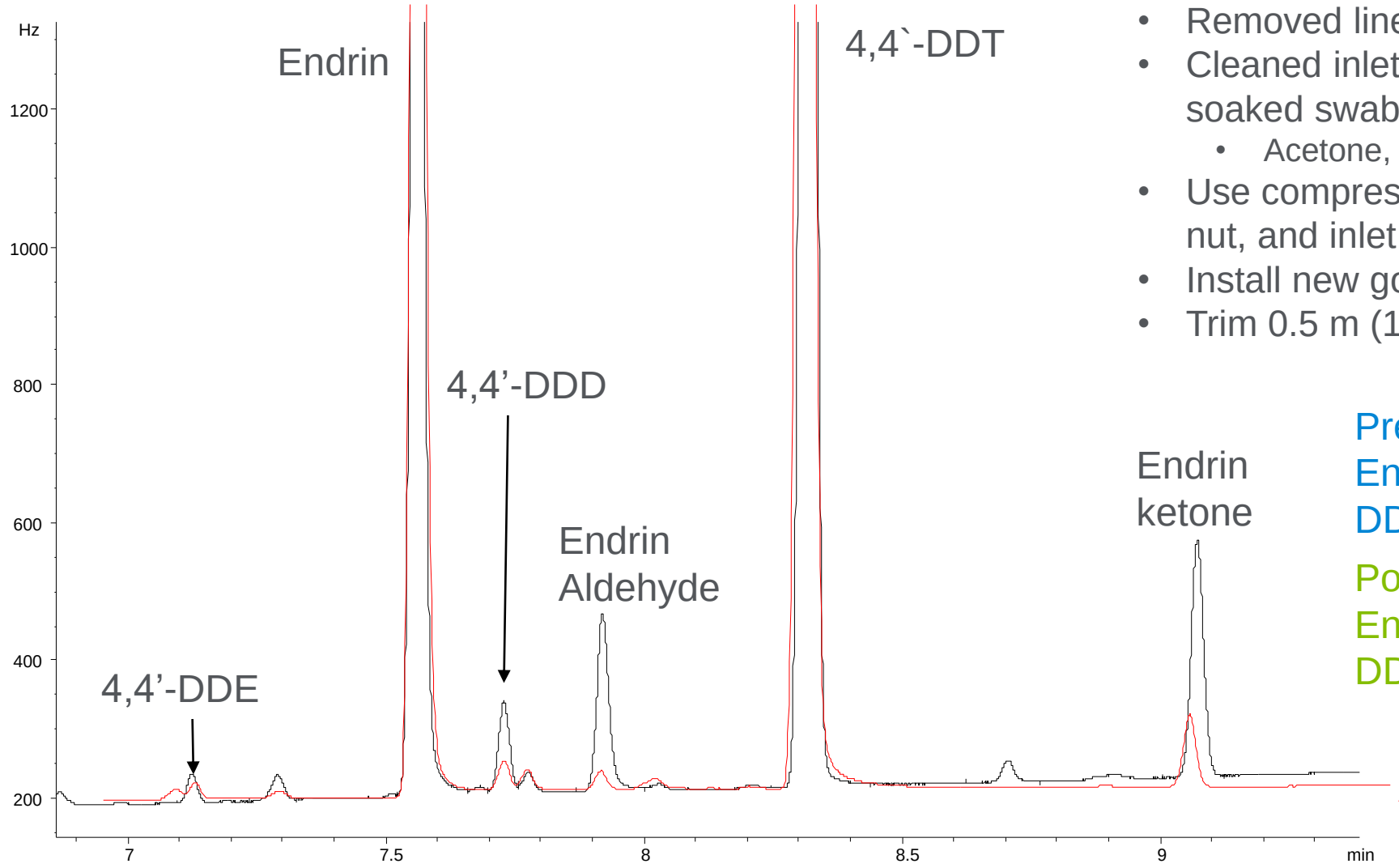
Running a pressure pulse reduces the residence time in the inlet which, in-turn reduces break-down.

Generally want to set the PULSE PRESSURE to 2 or 3 x's "normal" pressure
PULSE TIME should be slightly off-set from PURGE TIME either just before or just after (I prefer just *after*, but experiment to see what works best for you)

Splitless	
Endrin % breakdown	DDT % breakdown
25.9 %	2.4 %

Pulsed Splitess	
Endrin % breakdown	DDT % breakdown
14.3 %	1.6 %

Can Inlet Maintenance Help Endrin/DDT Breakdown?...Absolutely!



Maintenance steps:

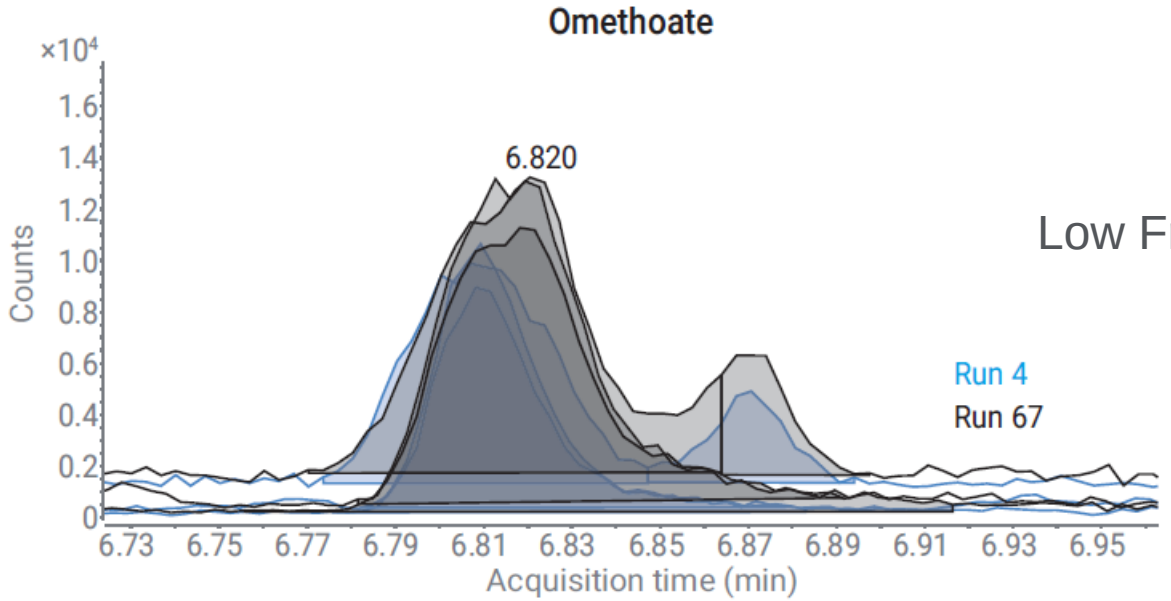
- Removed liner, septum, gold seal
- Cleaned inlet, turn-top with solvent soaked swabs
  - Acetone, isopropanol, methanol
- Use compressed air on turn-top, septum nut, and inlet
- Install new gold seal, liner, septum
- Trim 0.5 m (1 loop) from head of column

Premaintenance:
Endrin % breakdown: **17.6%**
DDT % breakdown: 2.6**%**

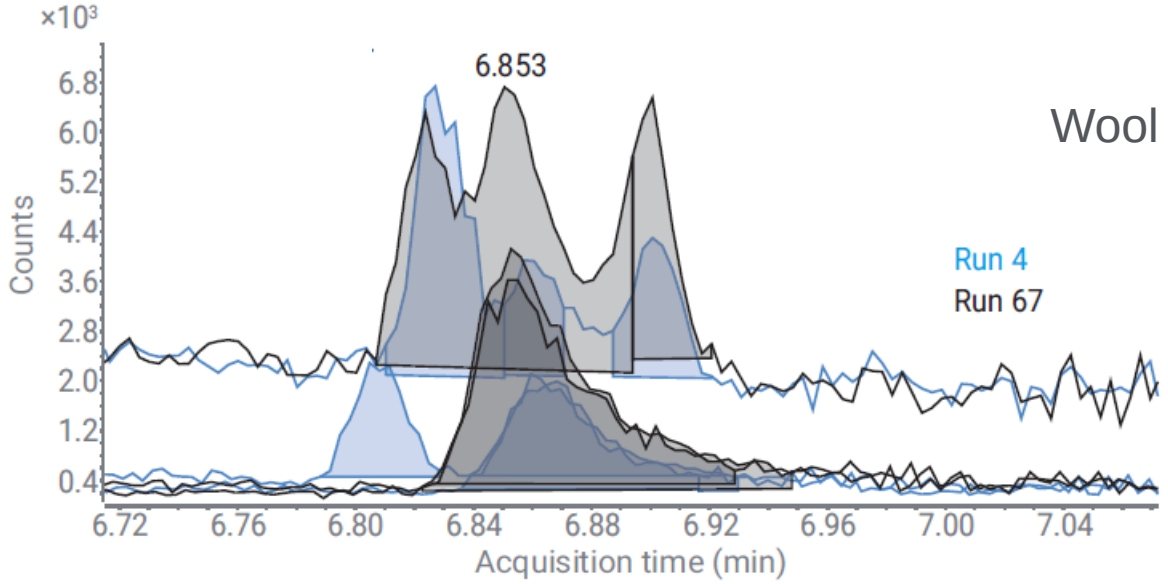
Postmaintenance:
Endrin % breakdown: 3.2**%**
DDT % breakdown**:** 0.85%

Before inlet maintenance
After inlet maintenance

Fritted Liner



Low Frit Liner

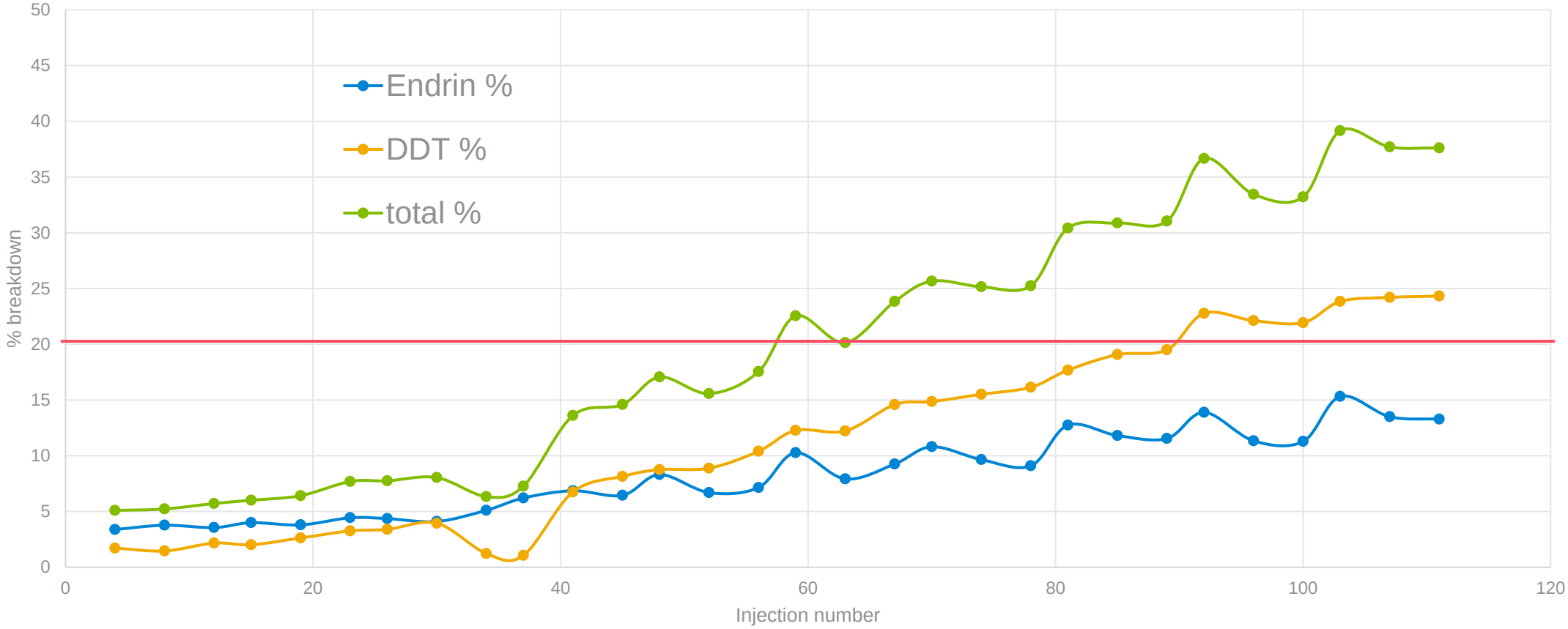


Wool Liner

https://www.agilent.com/cs/library/applications/application-pesticides-fritted-liner-gc-ms-ms-5994-1473en-agilent.pdf

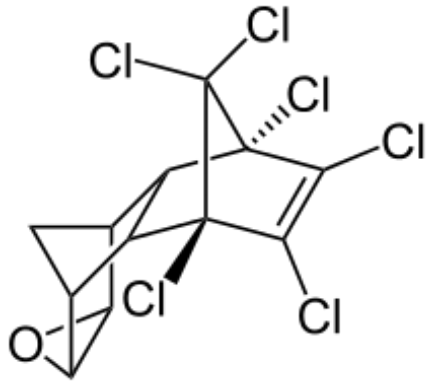
How Does Endrin/DDT Breakdown Typically Progress in a Sequence with Matrix?

Expected % breakdown progression

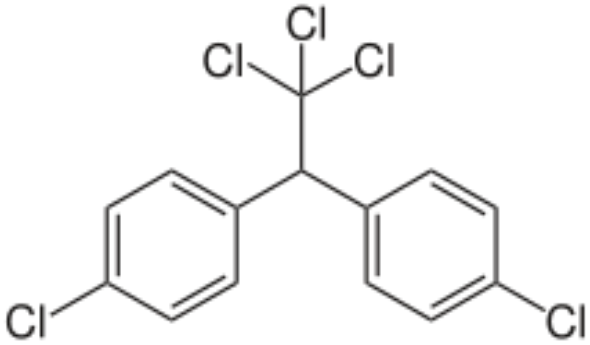


- Both gradually increase over time and may pass 20% criteria
- This is “normal” and directly related to matrix build-up on the “front-end”

Endrin and DDT – Special Case



ENDRIN



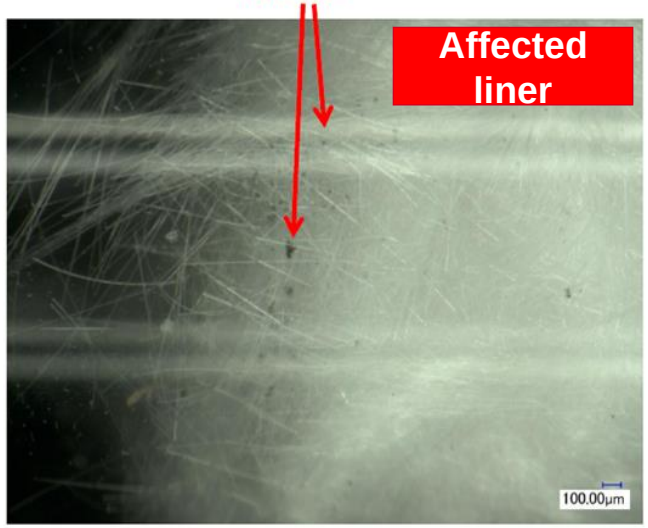
DDT

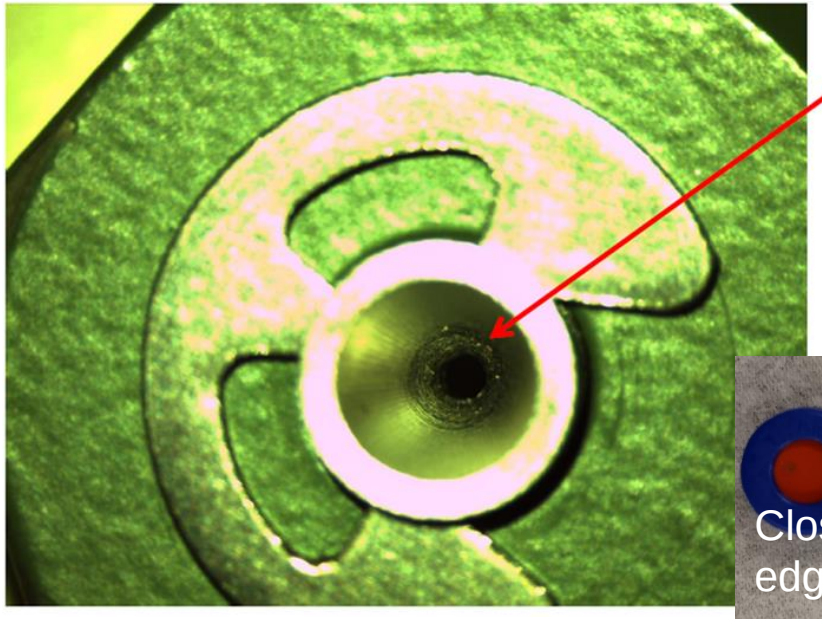
Addressing Endrin DDT Breakdown

- Check vial caps – is the syringe puncture near the center of vial cap? Or closer to the edge?
  - Check alignment of ALS tower and look at syringe needle
- Do you see black/gray color around the puncture mark in vial cap? Syringe may be impacting septum nut needle guide cone
  - Replace syringe guide (“foot”), syringe and/or septum nut

Part	Agilent Part Number	Take Action/Replace If
Septum nut	18740-60835	• Gouge marks are observed • Contamination buildup
ALS syringe	10 µL tapered 5181-1267 10 µL tapered, PTFE tip 5181-3354	• Gouge marks observed on septum nut • Needle is bent or looks pitted
ALS needle guide	7693 ALS G4513-40525	• Plastic looks worn or deformed • Contamination buildup

Top of wool





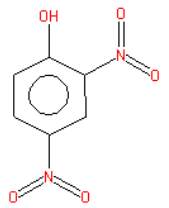
Sensitive Compounds (Other than Endrin/DDT) in EPA 8270D/E and Pesticides

Nitrophenols, pentachlorophenol, benzidines

More Sensitive Compounds in EPA 8270

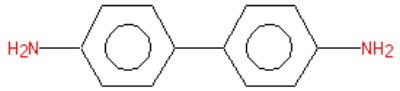
Nitrophenols:

- 2,4-dinitrophenol
- 4 nitrophenol
- 2-methyl-4,6-dinitrophenol



Benzoic acid

Benzidine



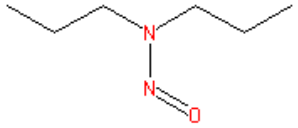
3,3'-dichlorobenzidine

Polychlorinated compounds

- Hexachlorocyclopentadiene

Nitrosamines:

- N-nitrosodimethylamine
- N-Nitrosodipropylamine
- N-nitrosobutylamine



What happens to them?

- "Disappear" in inlet/column
  - Inlet temperature
  - Residence time in inlet
  - Active sites in flow path

What does it do to the data?

- Loss of response
  - Low-level calibration standards "drop off"
- Calibration curves may need to be linear regressions
  - Drop lowest calibration levels
  - Preferred calibration method is using average response factors
- Loss (or gain) of response with matrix buildup

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

Varied reactions to different types of matrices

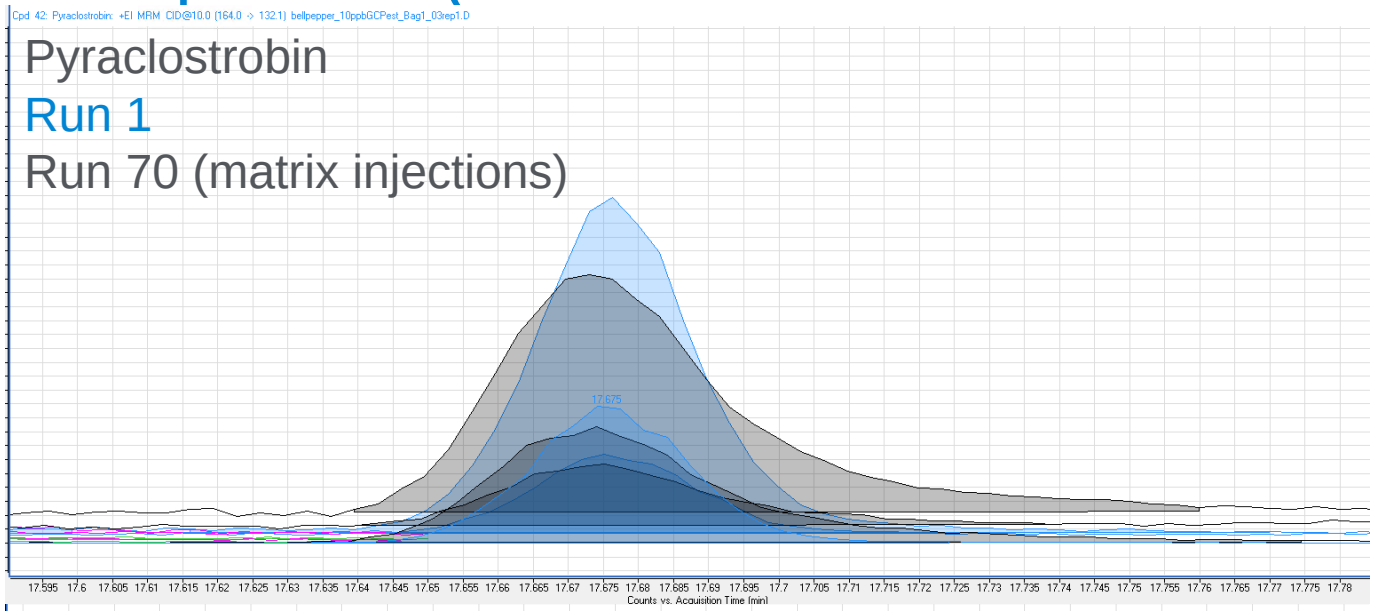
- Enhanced response
- Decrease response
- Interference for transition and matrix

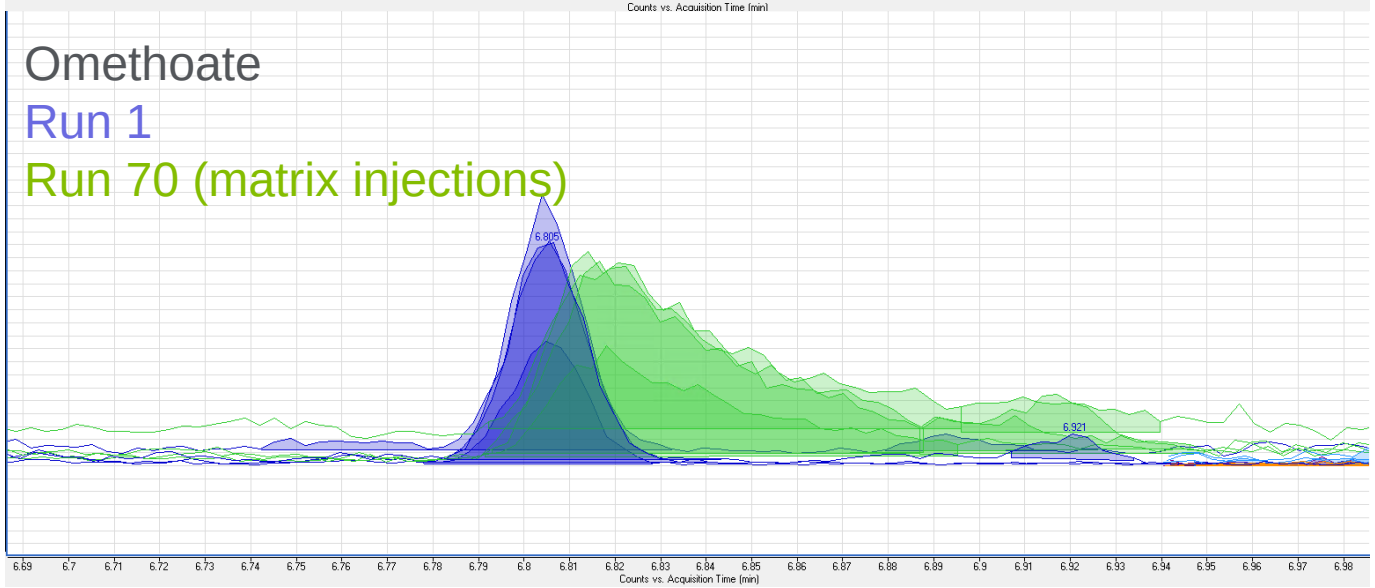
Which pesticides are sensitive to inertness (or lack of)?

Most…

- 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

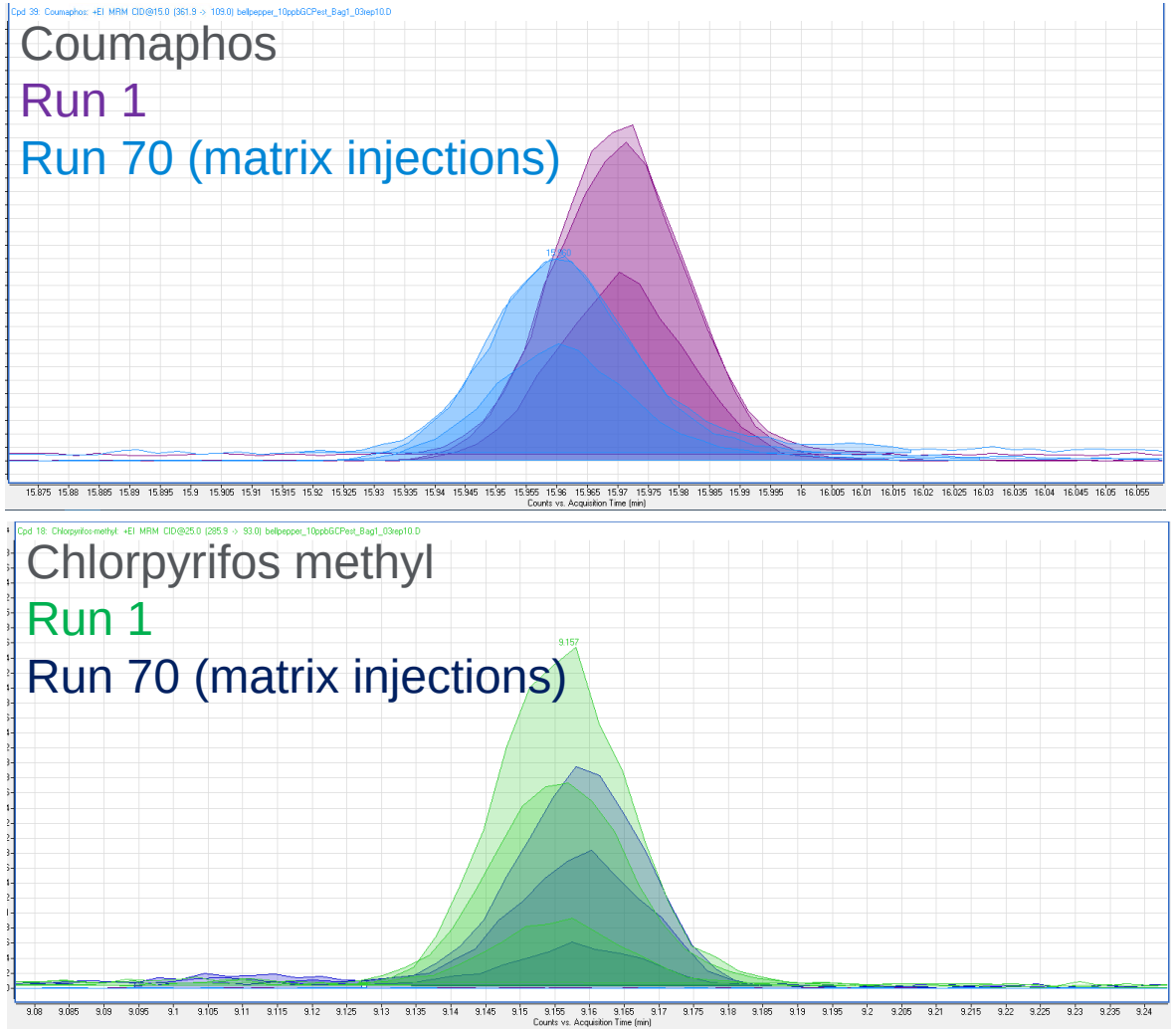
- Ultra Inert frit or glass wool liners

Pre-emptive 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



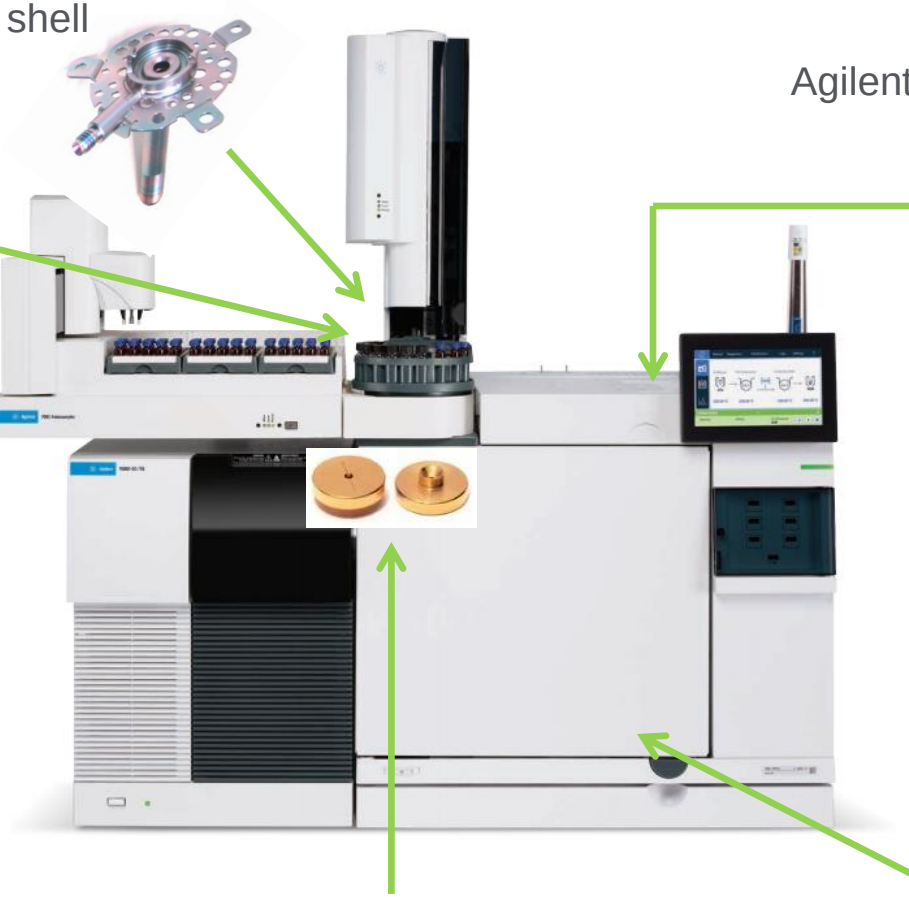
Agilent Inert Flow Solution

Agilent UltiMetal Plus inlet weldment shell and transfer lines

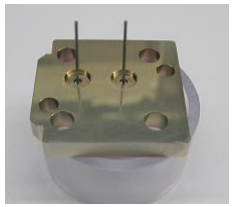
Agilent Ultra Inert inlet liner

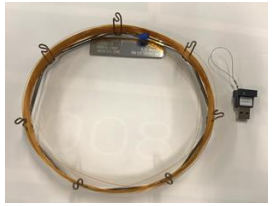
Agilent UltiMetal Plus ferrules

Agilent UltiMetal Plus capillary flow technology devices, Ultimate union



Agilent UltiMetal Plus TCD, FPD, NPD/FID jets





Agilent J&W Ultra Inert GC column

Agilent Ultra Inert gold seal

5990-8532EN brochure

Summary

- Three most important things as it relates to break-down:
  - 1-The front-end
  - 2-The front-end
  - 3-The front-end
    - Front end must be regularly maintained
- Choose the most inert consumables possible for the best start
  - Ultra inert (UI): Liner, Gold-seal, Column
- Try different liners
  - Frit Liners
  - Liners without wool

- Adjust GC method parameters
  - Temperatures (inlet)
  - Inlet parameters (i.e. pulsed injections)
- Look out for the small things
  - Metal shavings from column nut
  - Other foreign material finding its way to the inlet
- Proactively maintain the entire GC focusing on the “front-end”
  - Follow the flow path – (flow chart slide)
  - GC inlet will need to be solvent rinsed occasionally
  - Don’t forget the split vent line and trap

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