Installation, CARE and Maintenance of Capillary Gas Chromatography Columns

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June 28, 2018

"It's not what your column can do for you, but what you can do for your column"

Column Installation Procedure

- Install the column
- Leak and installation check
- Column conditioning
- Setting linear velocity or flow rate
- Bleed profile
- Test mix

"Getting off to a good start"

Column Installation

What type of ferrule should I use?

polyimide

polyimide/graphite

graphite

flexible metal

Composition	Re-use	Max T	Use	Limitation
Polyimide (Vespel)	yes	280	Easy seal	Shrink after heating causing leaks after thermal cycle; isothermal only
Graphite	yes	450	FID, NPD Inlets	Contamination, permeable to air – not for oxygen sensitive detectors
Polyimide/graphite (85% / 15%)	limited	350	MS, ECD, Inlets	Still shrink after thermal cycles creating leaks; need to retighten regularly
Flexible metal	no	450	Capillary Flow Technology (backflush, splitters,…)	May not seal well with damaged fittings or rough surfaces

"Short" ferrules for inlet and detector configurations on Agilent GC's

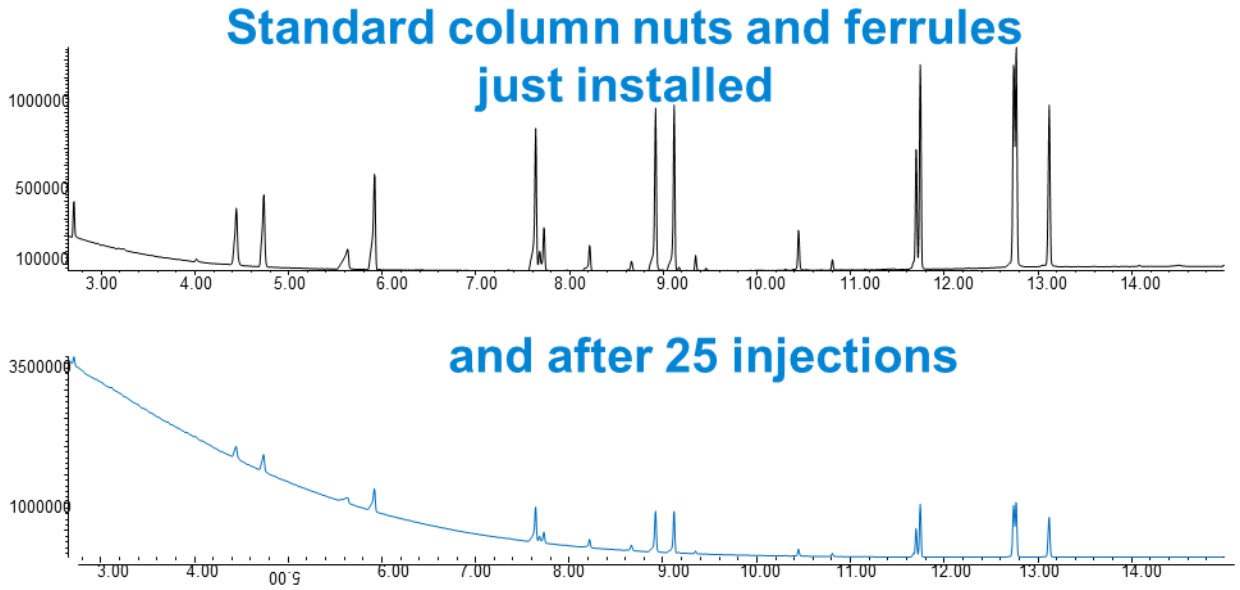
"Long" ferrules for MS transfer lines and MS interface nut

Graphite / Polyimide blend capillary ferrules

Unfortunately … leak following normal temperature program runs

Studies show the leaking continues with use of the ferrules -

Not just after the first one or two runs



Frequent re-tightening of the fitting is needed to maintain a leak-free seal – and system performance and productivity

Better Connections: Self Tightening Column Nuts

Designed for use with *short* graphite/polyimide blend ferrules –both at the inlet and the MS interface – so only one type of ferrule needed for both ends of the column!

For inlet or detector
P/N 5190-5233

For mass spec transfer line
P/N 5190-6194

Short ferrule exposes more thread of the fitting for better sealing

How do Self Tightening Column Nuts work?

- Ease of use – install in dark, small space in GC oven without wrenches
- Wing design for finger tight installation with graphite/polyimide blend ferrules
- No tools dramatically reduces force preventing over tightening or damage
- Robust stainless steel construction

Plus….

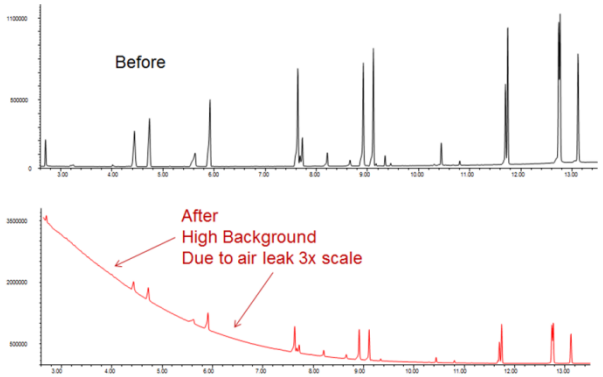
- Novel **spring driven piston** design that

  continuously presses against the ferrule

  to **maintain a leak-free fitting**

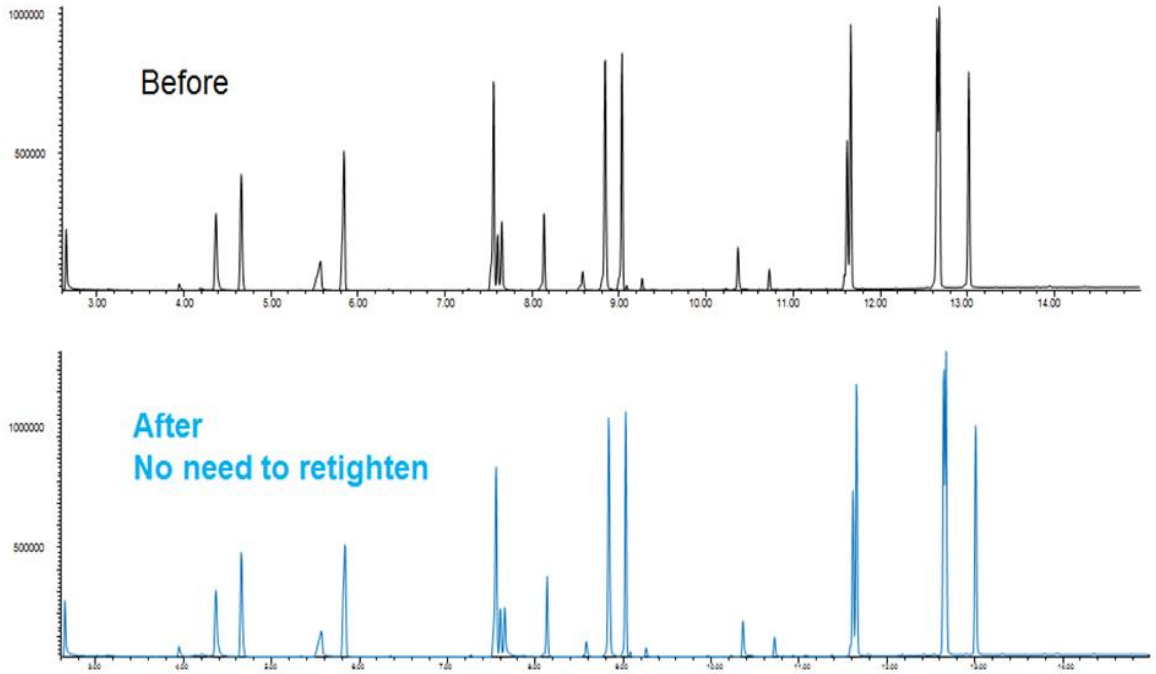
  even when the ferrule shrinks during temperature program!

Benefit of Self-Tightening Column Nuts



Take you from this….

…. to this!

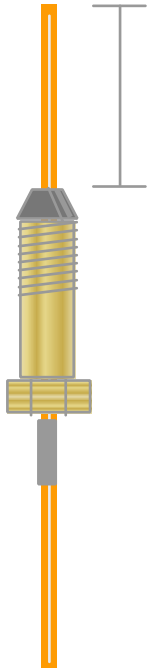


Without retightening, the baseline remains flat after 400 runs with no indication of leaks when using the Self tightening Column Nut

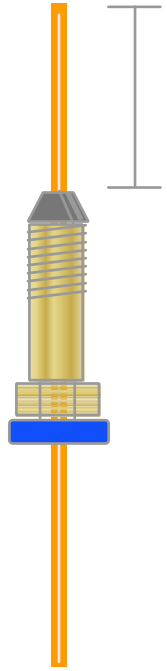
Ref. Tech note: 5991-3612EN

Column Installation
Measuring the right distance

White out



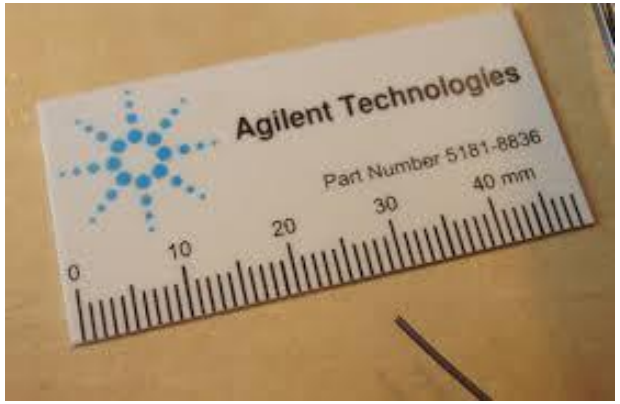
Septa



Extra septa

Cutting The Column

Gently scribe through the polyimide coating.
Do not attempt to cut the glass.



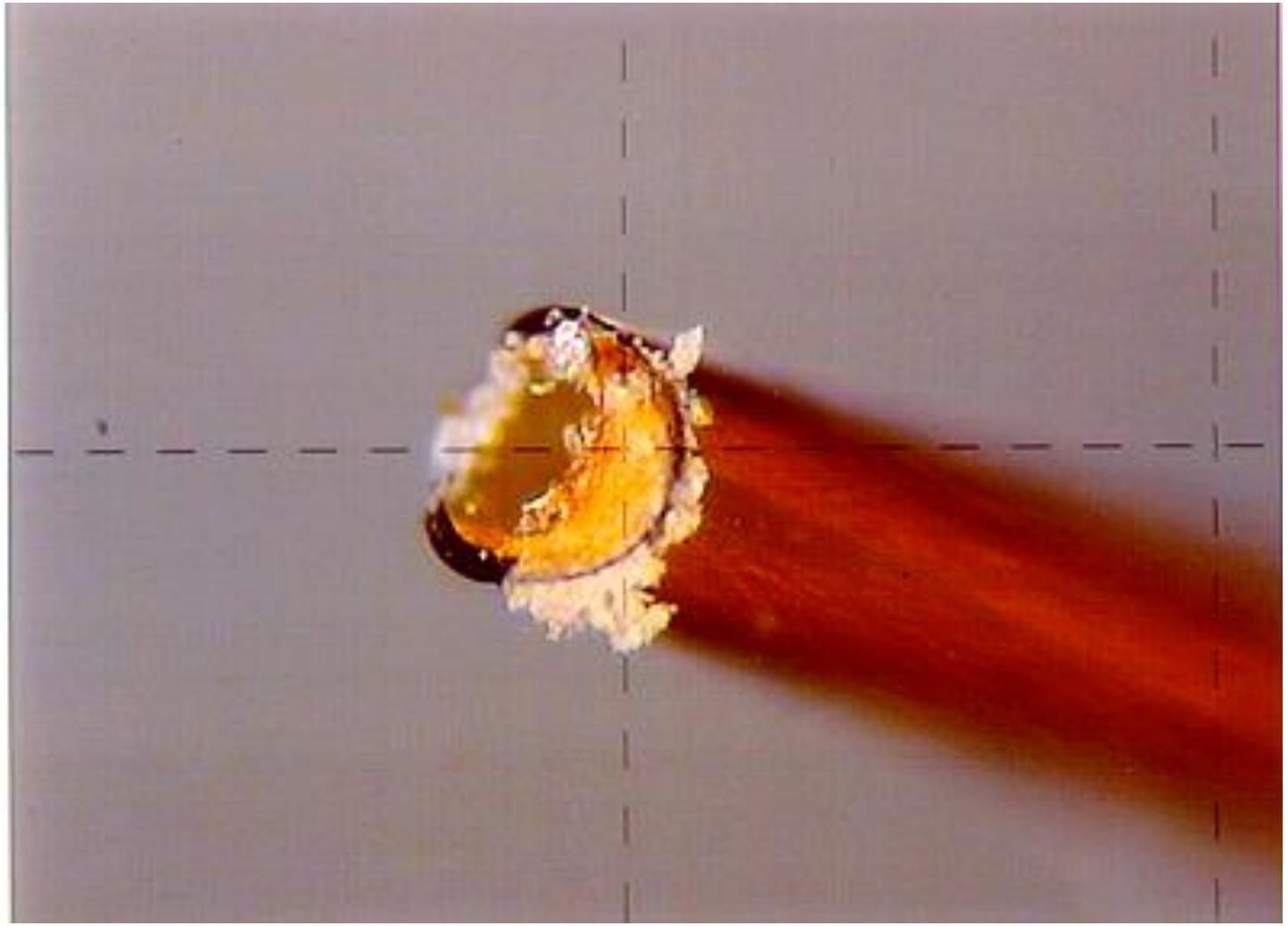
Recommended tools:

Diamond or carbide tipped pencil; or sapphire cleaving tool, ceramic wafer
Ocular

Do not use:

Scissors, file, etc.

Example of a Bad Cut

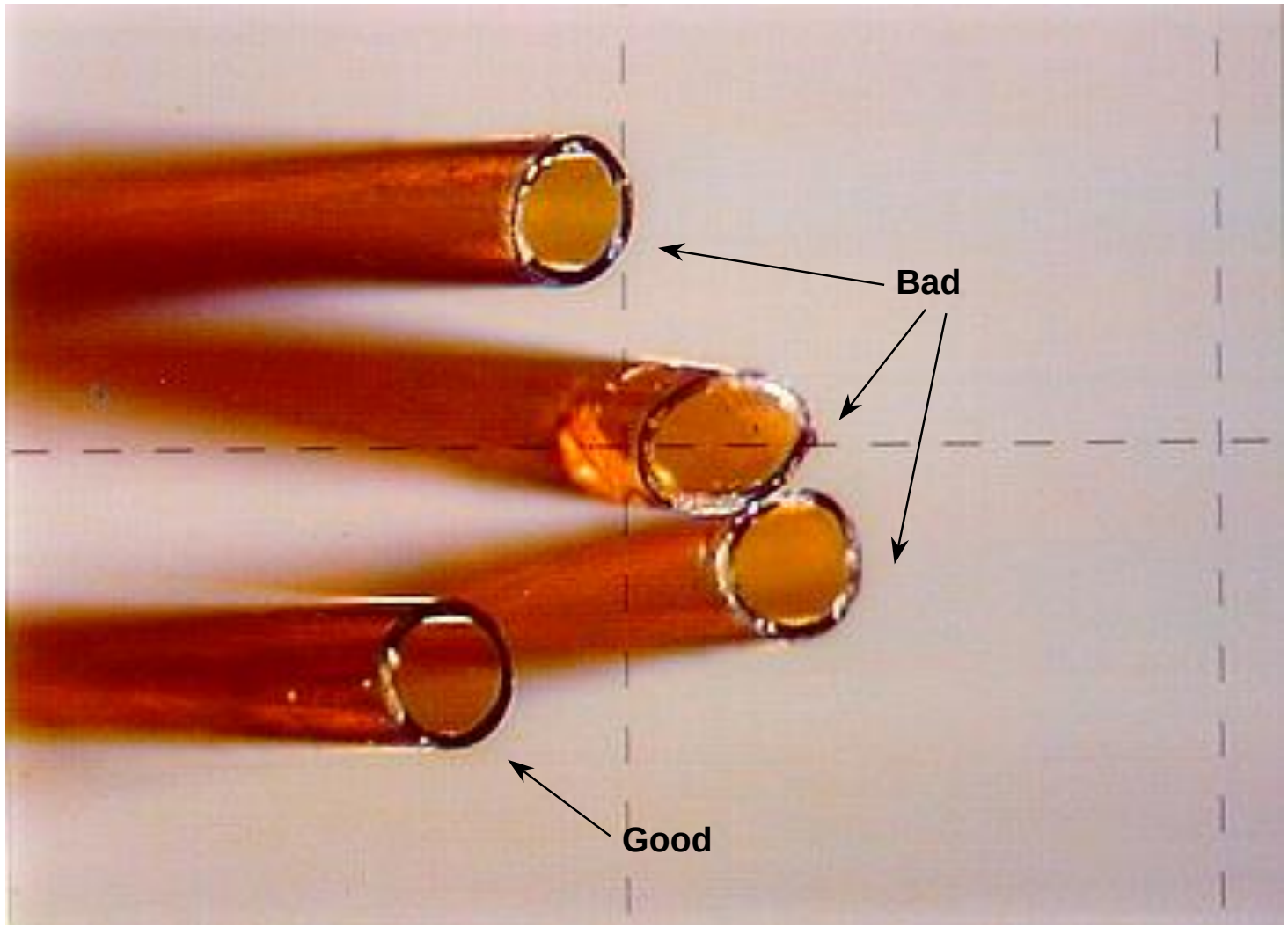


A poor cut will definitely get you off to a bad start

- Active sites created
- Poor peak shape/tailing

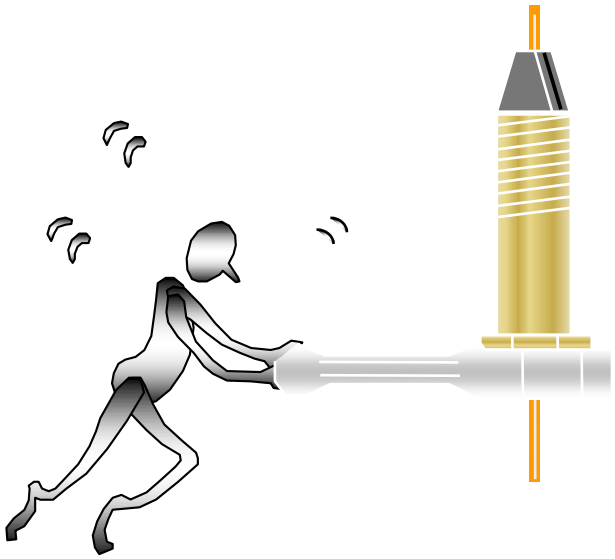
= UNHAPPY

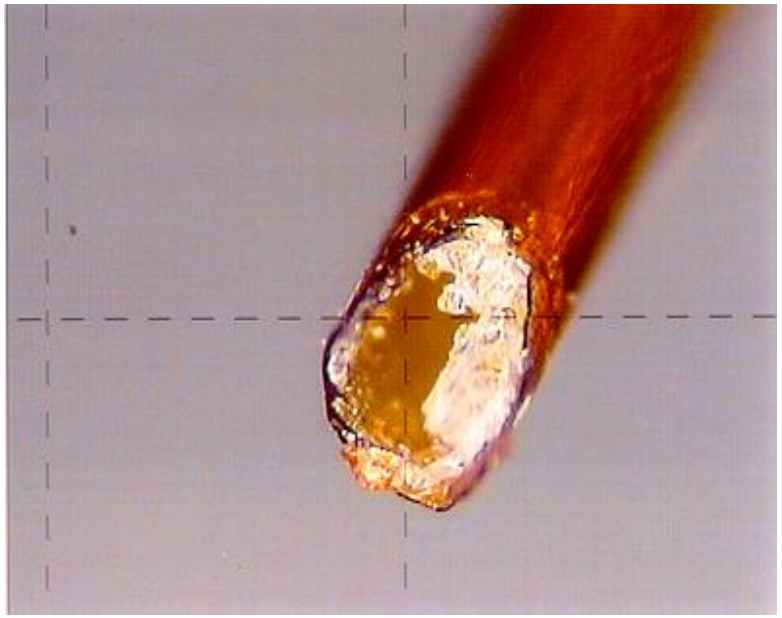
Examples of Column Cuts



Column Installation

How tight is tight?





Over-tightened Ferrule

Column Installation

Leak Check

DO NOT USE SNOOP

Electronic leak detector
IPA/Water
Inject a non-retained peak

G3388B Leak Detector

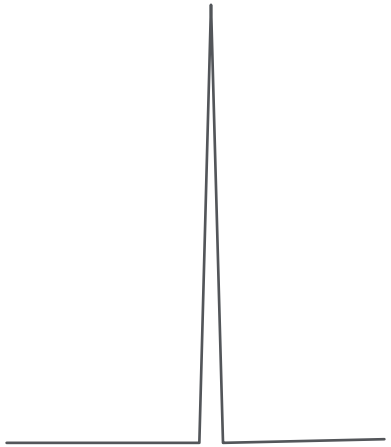
Leak and Installation Check

Inject a non-retained compound vs DB-1

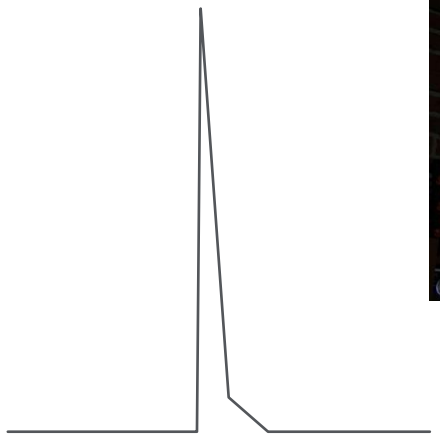
Detector	Compound
FID	Methane or Butane
ECD	$MeCl_2$ (headspace or diluted)
NPD	CH_3CN-acetonitrile (headspace or diluted)
TCD	Air
MS	Air or Butane

The peak should be sharp and symmetrical

Non-Retained Peak Shapes



Good Installation



Improper Installation or
Injector Leak

Check for: -Too low of a split ratio

-Injector or septum leak

-Liner problem:

(broken, leaking, misplaced)

-Column position in injector and detector

Calculating Linear Velocity

Inject a non-retained compound and obtain the retention time:

$$\bar{\mu} = \frac{L}{t_o}$$

$\bar{\mu}$ = Average linear velocity (cm/sec)
L = Column length (cm)
t_o = Retention time (sec)

He 20-40 cm/sec
H_2 35-55 cm/sec

μ is *dependent* on column temperature, but is *independent* of column dimensions

Calculating Flow Rate

Inject a non-retained compound and obtain the retention time:

$$\bar{F} = \frac{\pi r^2 L}{t_o}$$

$\bar{F}$ = Flow rate (mL/min)
r = Column radius (cm)
L = Column length (cm)
t_o = Retention time (min)

$\bar{F}$ is dependent on column temperature
Measuring flow with a flow meter is often inaccurate

Column Conditioning

System must be leak free before conditioning column

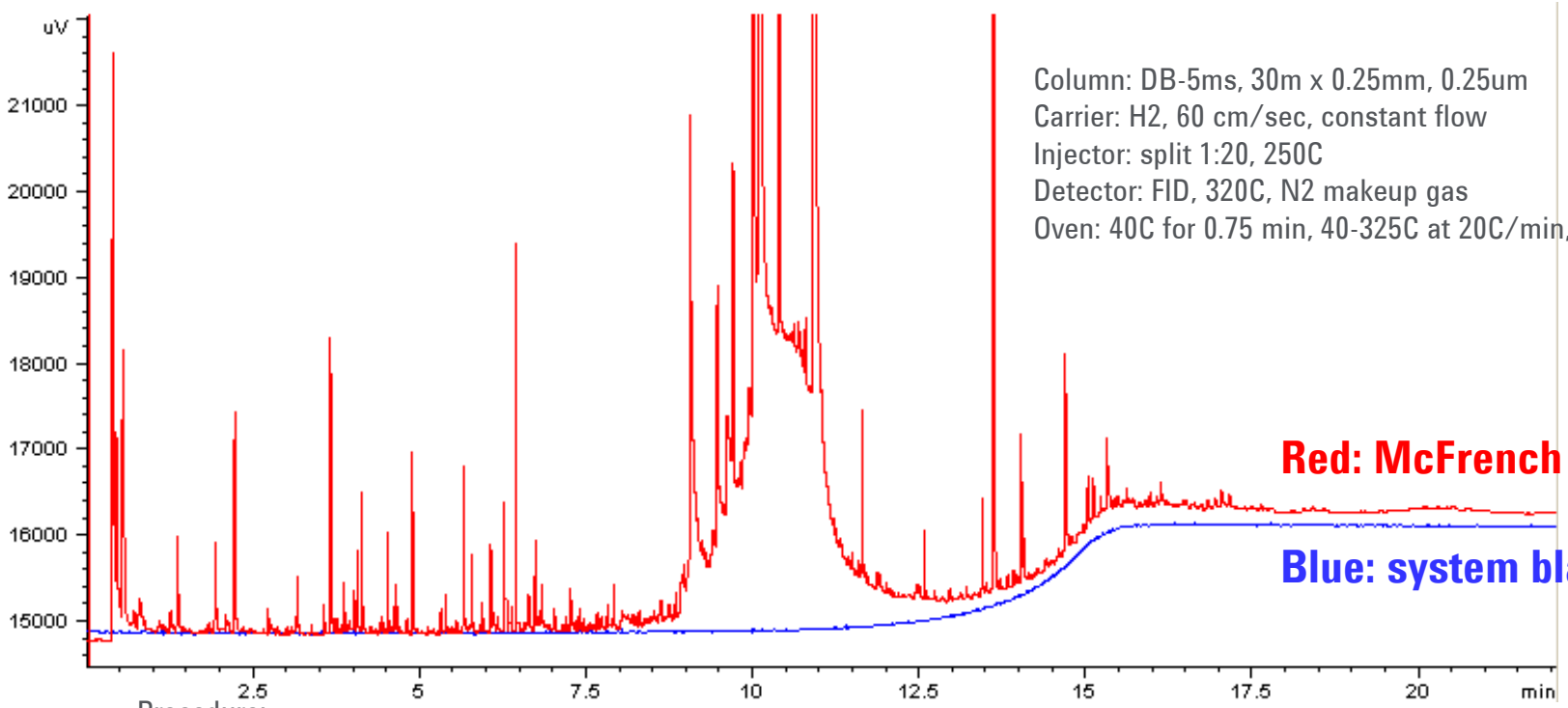
Heat the column to the lower of:

- Isothermal maximum temperature OR
- 20° to 30°C above highest operation temperature
- Temperature programming is not necessary

Stop conditioning when the stable baseline is obtained:

- 1 to 2 hours in most cases

Contamination from French Fry Grease



Column: DB-5ms, 30m x 0.25mm, 0.25um
Carrier: H2, 60 cm/sec, constant flow
Injector: split 1:20, 250C
Detector: FID, 320C, N2 makeup gas
Oven: 40C for 0.75 min, 40-325C at 20C/min, 325C for 30 min

Red: McFrench Fry

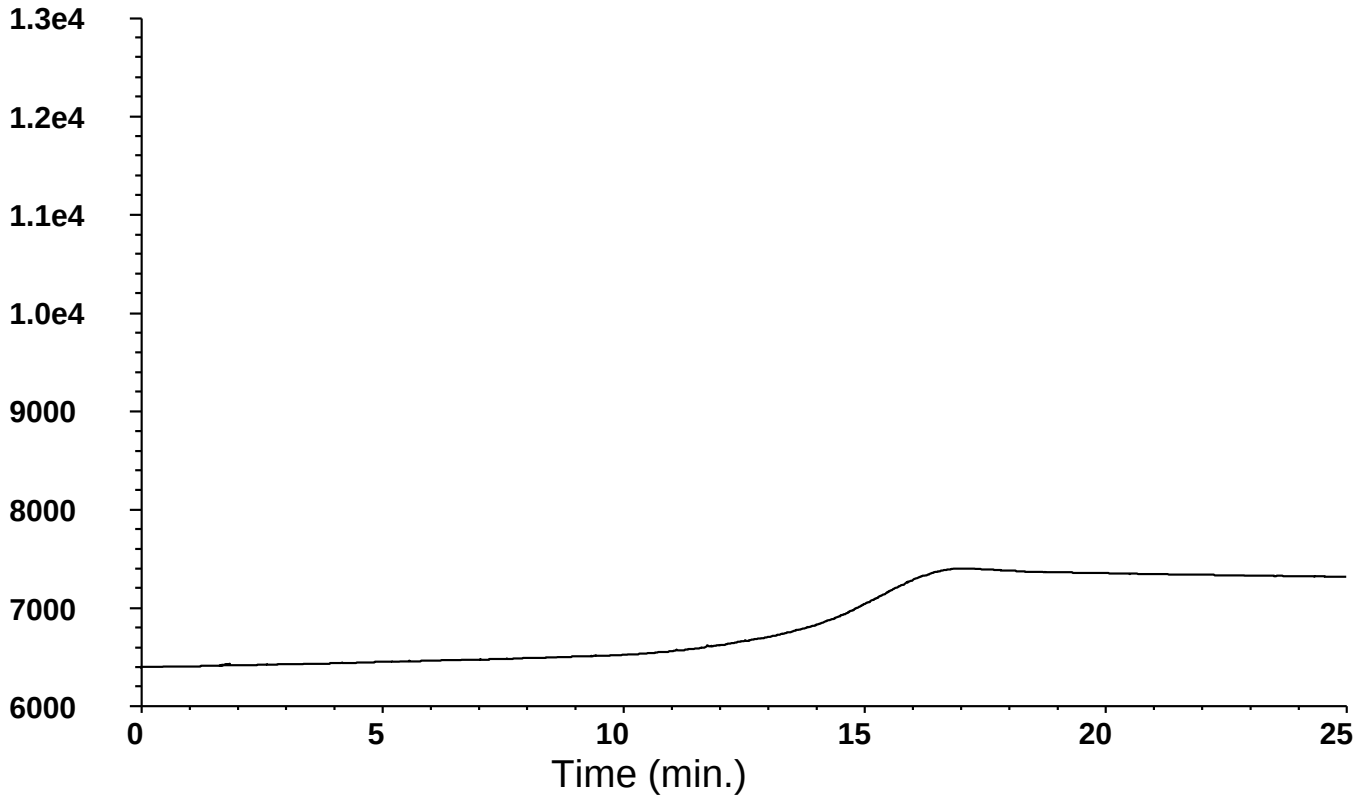
Blue: system blank

Procedure:

(1) Held french fry for 5 seconds.
(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 40C.
(6) Started oven temperature program as soon as oven reached 40C.

Generating a Bleed Profile

Temperature program the column without an injection*



*DB-1 30m x .32mm I.D., .25µm
Temperature program // 40°C, hold 1 min // 20°/min to 320°C, hold 10 min.

Column Performance Summary

Catalog: 19091S-433UI
Serial: F542252H

Stationary Phase: HP-5MS UI
Description: 30m x 0.250mm x 0.25µm
Temperature Limits: -60°C to 325°C (350°C Pgm)

Performance Results

Theoretical Plates/Meter:

n-DECANE	3208

Retention Index:

n-PROPYLBENZENE	953.110
1-HEPTANOL	967.660

Resolution:

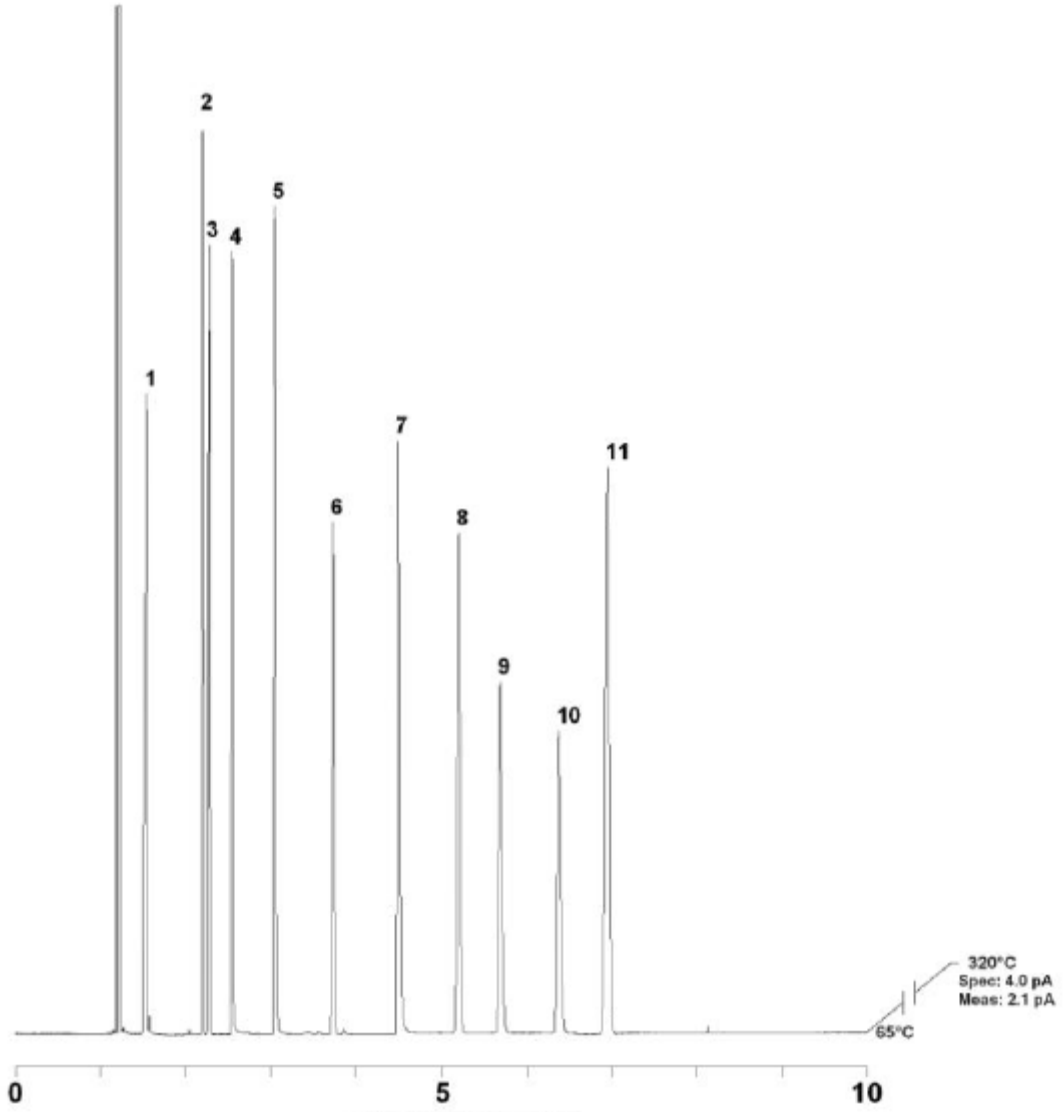
1-OCTENE, n-OCTANE	2.97

Compound Identification	Retent. Time	Part. Ratio	1/2-Width
1. PROPIONIC ACID	1.543	0.30	0.027
2. 1-OCTENE	2.203	0.86	0.015
3. n-OCTANE	2.282	0.92	0.016
4. 1,3-PROPANEDIOL	2.552	1.15	0.020
5. 4-METHYLPYRIDINE	3.051	1.57	0.021
6. n-NONANE	3.738	2.15	0.027
7. TRIMETHYLPHOSPHATE	4.482	2.78	0.033
8. n-PROPYLBENZENE	5.193	3.38	0.038
9. 1-HEPTANOL	5.682	3.79	0.041
10. 3-OCTANONE	6.368	4.37	0.047
11. n-DECANE	6.940	4.85	0.053

Test Conditions

Inlet: **Split** (250°C) **Detector:** FID (325°C)
Carrier Gas: Hydrogen **Flow:** 42.1 cm/sec (1.2 ml/min)
Holdup Compound: Pentane (1.187-min)
Temperature Program: Isothermal at 65°C

Chromatographic Performance



Test Mixture Components

Compounds	Purpose
Hydrocarbons	Efficiency
	Retention
FAME’s, PAH’s	Retention
Alcohols	Activity
Acids	Acidic Character
Bases	Basic Character

Own Test Mixture

- More specific to your application
- Selective detectors
- Concentrations specific to your application
- Use same instrument conditions
- Easiest to simply inject a calibration standard
- Store for future measure of column performance

An Ounce of Prevention.....

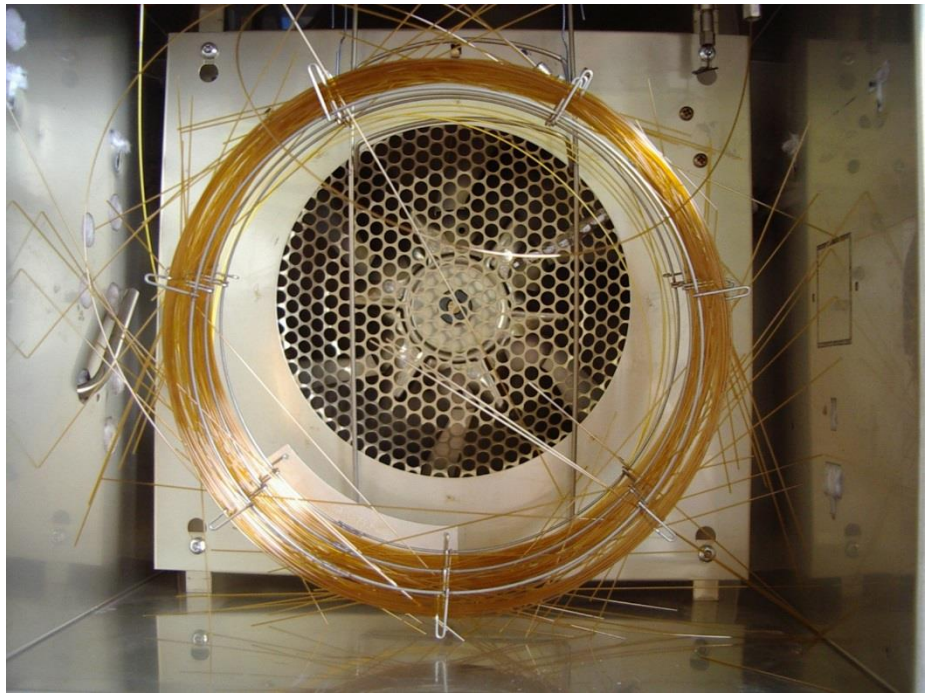
Agilent

Common Causes of Column Performance Degradation

- Physical damage to the polyimide coating
- Thermal damage
- Oxidation (O_2 damage)
- Chemical damage by samples
- Contamination

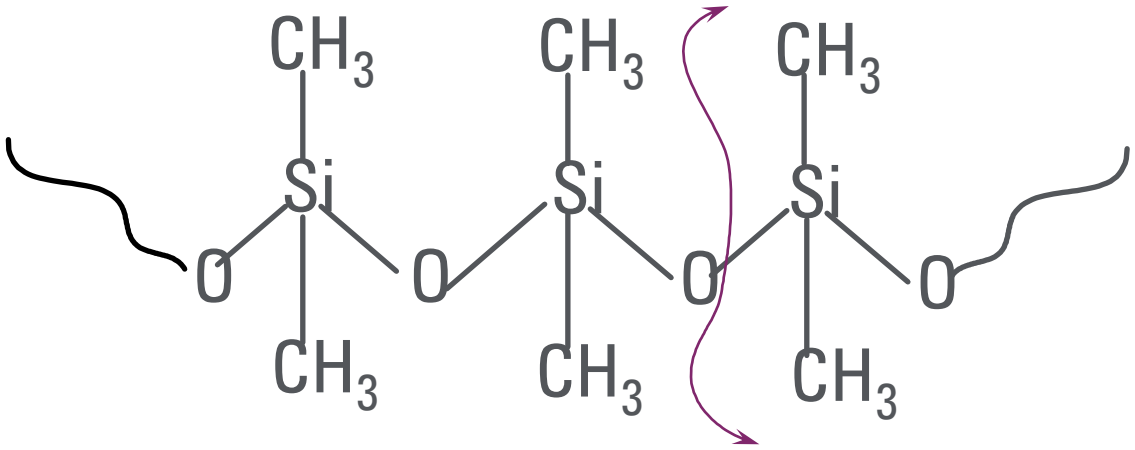
Physical Damage to The Polyimide Coating

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



Thermal Damage

Degradation of the stationary phase is increased at higher temperatures.
Breakage along the polymer backbone.



Dimethylpolysiloxane

- Rapid degradation of the stationary phase caused by excessively high temperatures

  Isothermal limit = Indefinite time

  Programmed limit = 5-10 minutes

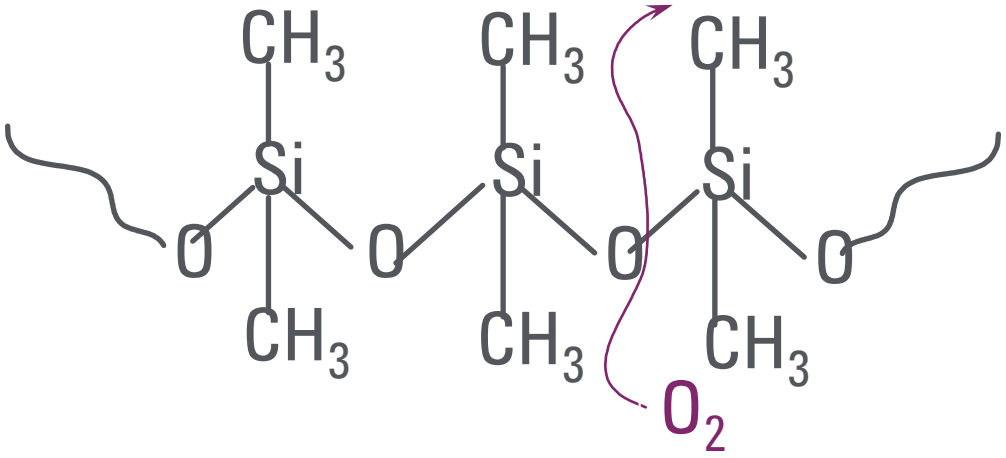
- Temporary "column failure" below lower temperature limit

Thermal Damage

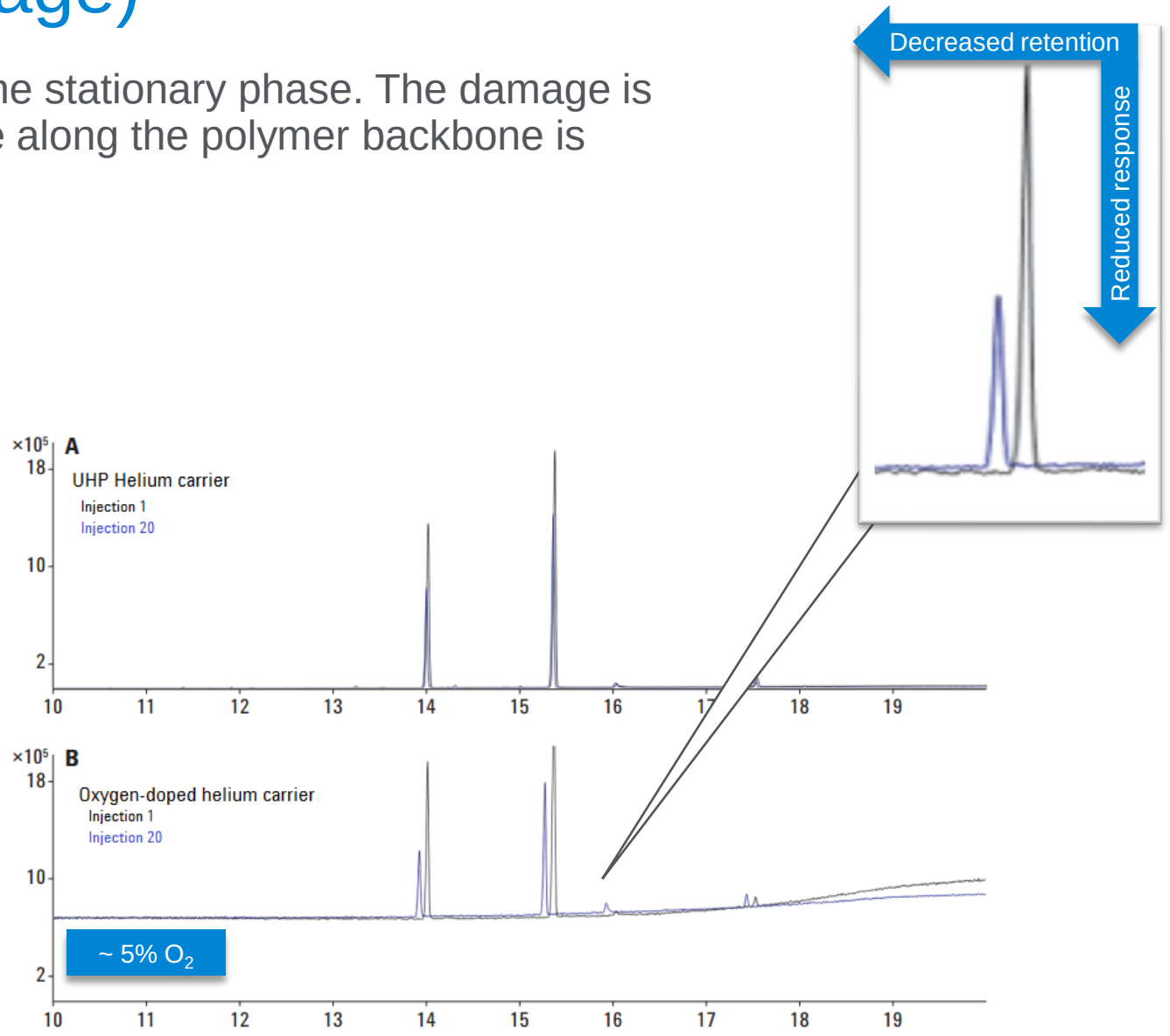
- Disconnect column from detector
- “Bake out” overnight at isothermal limit
- Remove 10-15 cm from column end

Oxidation (O2 Damage)

Oxygen in the carrier gas rapidly degrades the stationary phase. The damage is accelerated at higher temperatures. Damage along the polymer backbone is irreversible.



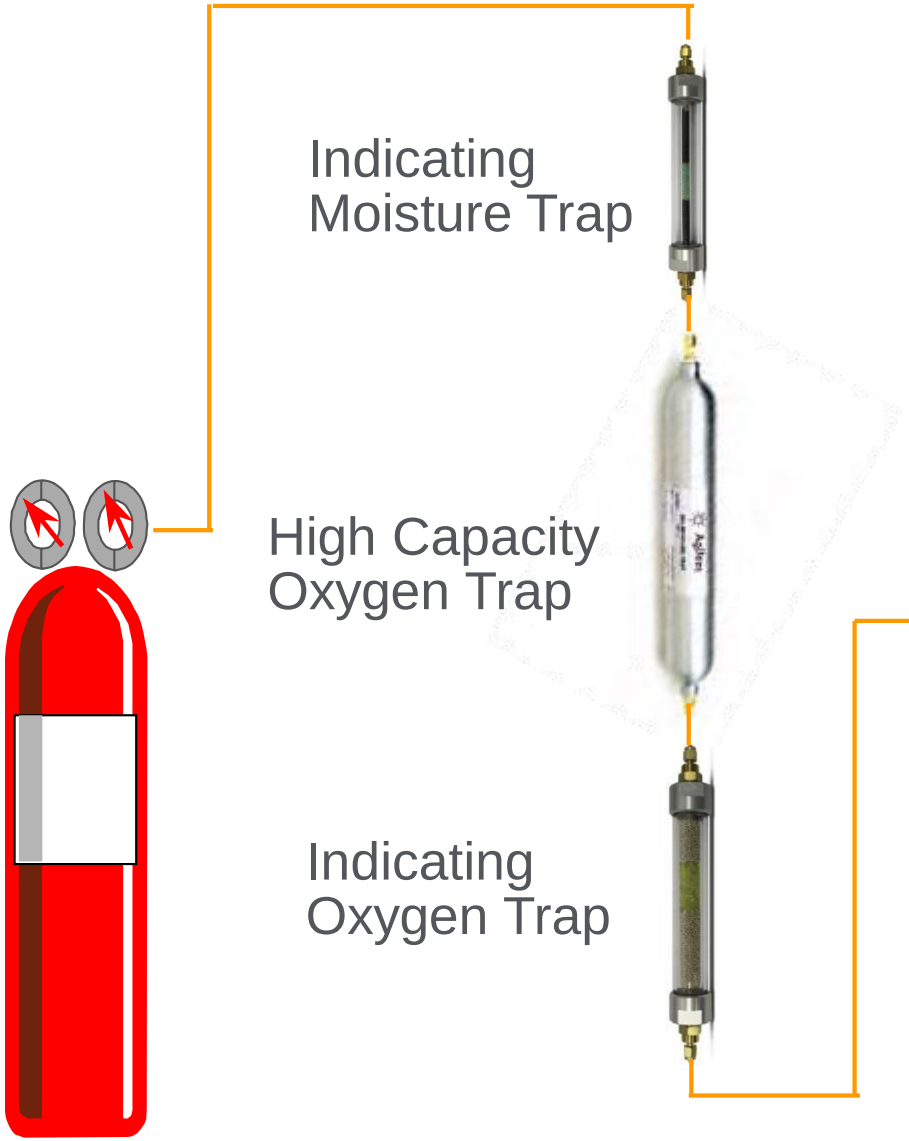
Dimethylpolysiloxane



How to Prevent Column Damage by Oxygen

- High quality carrier gas (4 nine's or greater)
- Leak free injector and carrier lines
  - Change septa
  - Maintain gas regulator fittings
- Appropriate impurity traps

Configurations for Carrier Gas Purifiers



Indicating
Moisture Trap

High Capacity
Oxygen Trap

Indicating
Oxygen Trap

Hydrocarbon
Indicating Moisture
Indicating Oxygen

Chemical Damage

Bonded and cross-linked columns have excellent chemical resistance except for inorganic acids and bases

HCl NH_3 KOH $NaOH$

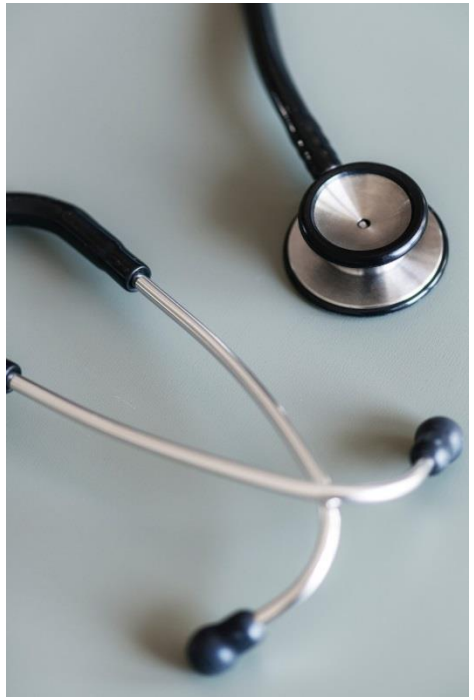
H_2SO_4 H_3PO_4 HF etc.

Chemical damage will be evident by excessive bleed, lack of inertness or loss of resolution/retention.

Chemical Damage

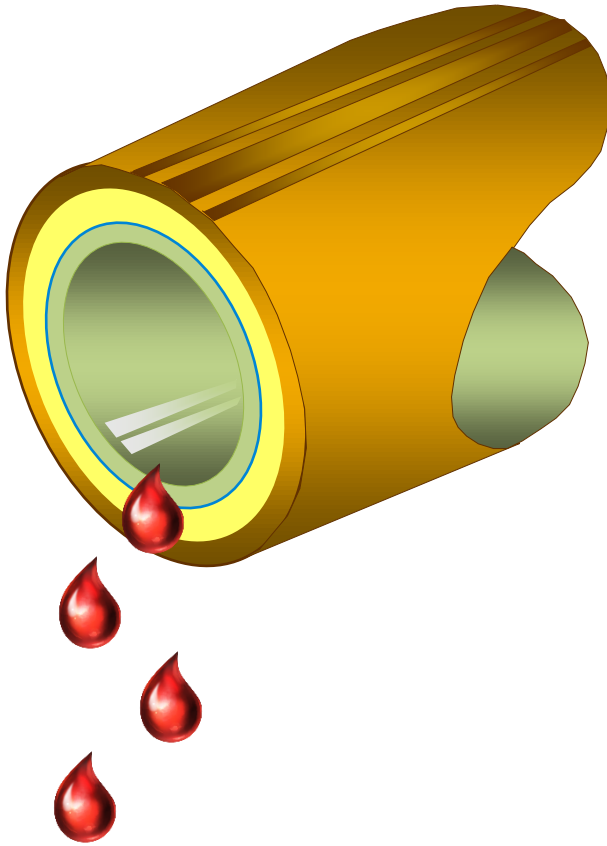
What To Do If It Happens

- Remove 1/2 - 1 meter from the front of the columns
- Severe cases may require removal of up to 5 meters

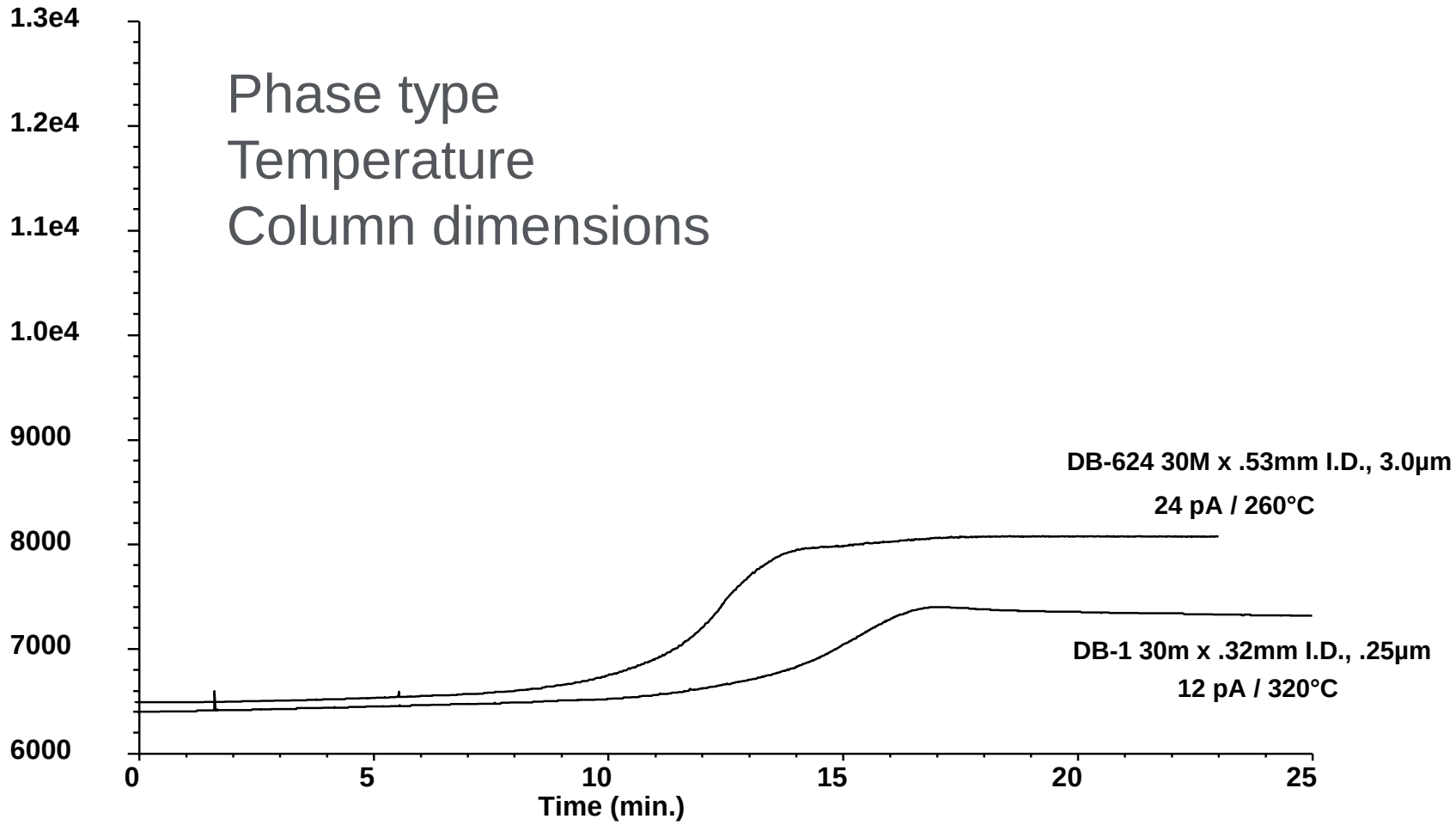


What is Normal Column Bleed

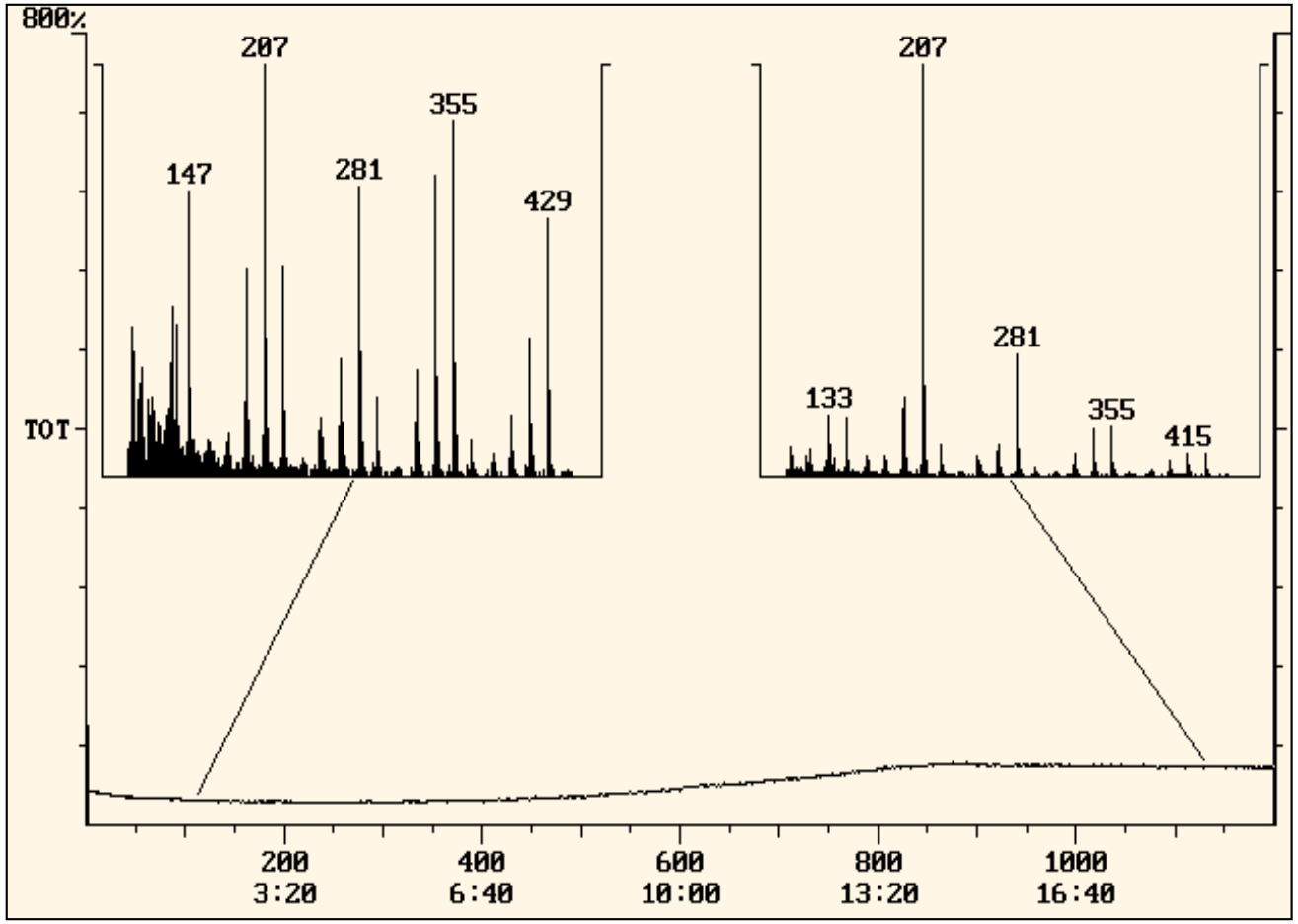
Normal background signal generated by the elution of normal degradation products of the column stationary phase



Column Bleed is Influenced by:



Mass Spectrum of Phenylmethylpolysiloxane Column Bleed (Normal Background)



Mass spectral library search is not always accurate

What is a Bleed Problem?

An abnormal elevated baseline at high temperature

IT IS NOT

A high baseline at low temperature

Wandering or drifting baseline at any temperature

Discrete peaks

Column Contamination

- Fouling of GC and column by contaminants
- Mimics nearly every chromatographic problems

Symptoms of Contamination

- Poor peak shape
- Loss of separation (resolution)
- Changes in retention
- Reduced peak size
- Baseline disturbances (semi-volatiles only)

Typical Samples That Contain a Large Amount of Residues

Biological (Blood, Urine, Tissue, Plants)

Soils	Foods
Waste Water	Sludges

All samples contain residues!! (even standards!)

Other Sources of Contamination

- Septum and ferrule particles
- Gas and trap impurities
- Unknown sources (vials, syringes,etc.)

Non-Volatile Residues

Any portion of the sample that does not elute from the column or remains in the injector.

Semi-Volatile Residues

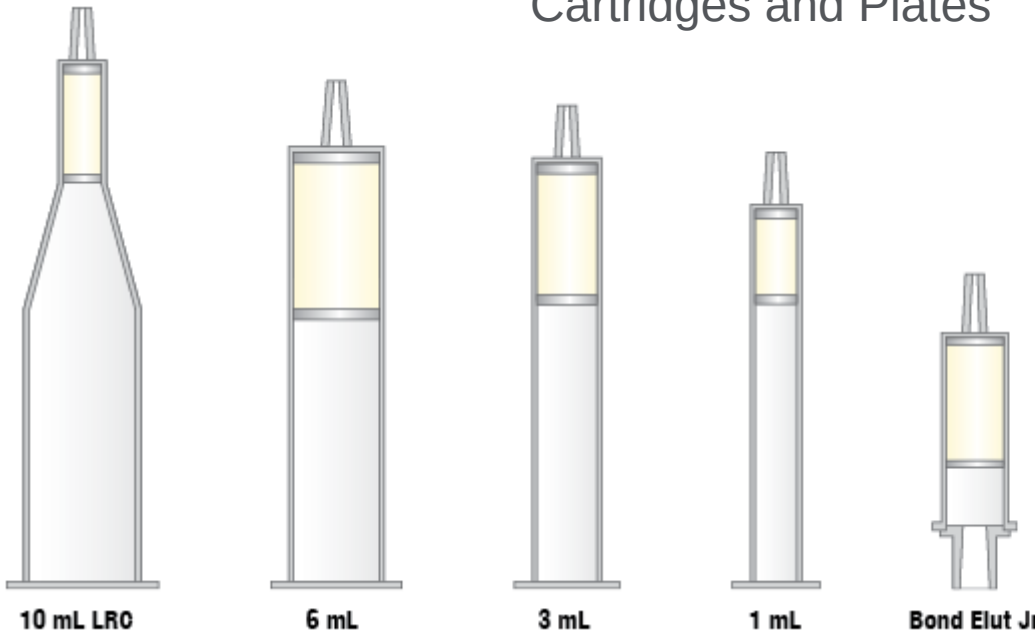
Any portion of the sample that elutes from the column after the current chromatographic run.

Methods to Minimize Non-Volatile Residue Problems

- Sample cleanup
- Packed injection port liners
- Guard columns

Agilent Bond Elut Sample Clean-Up Products

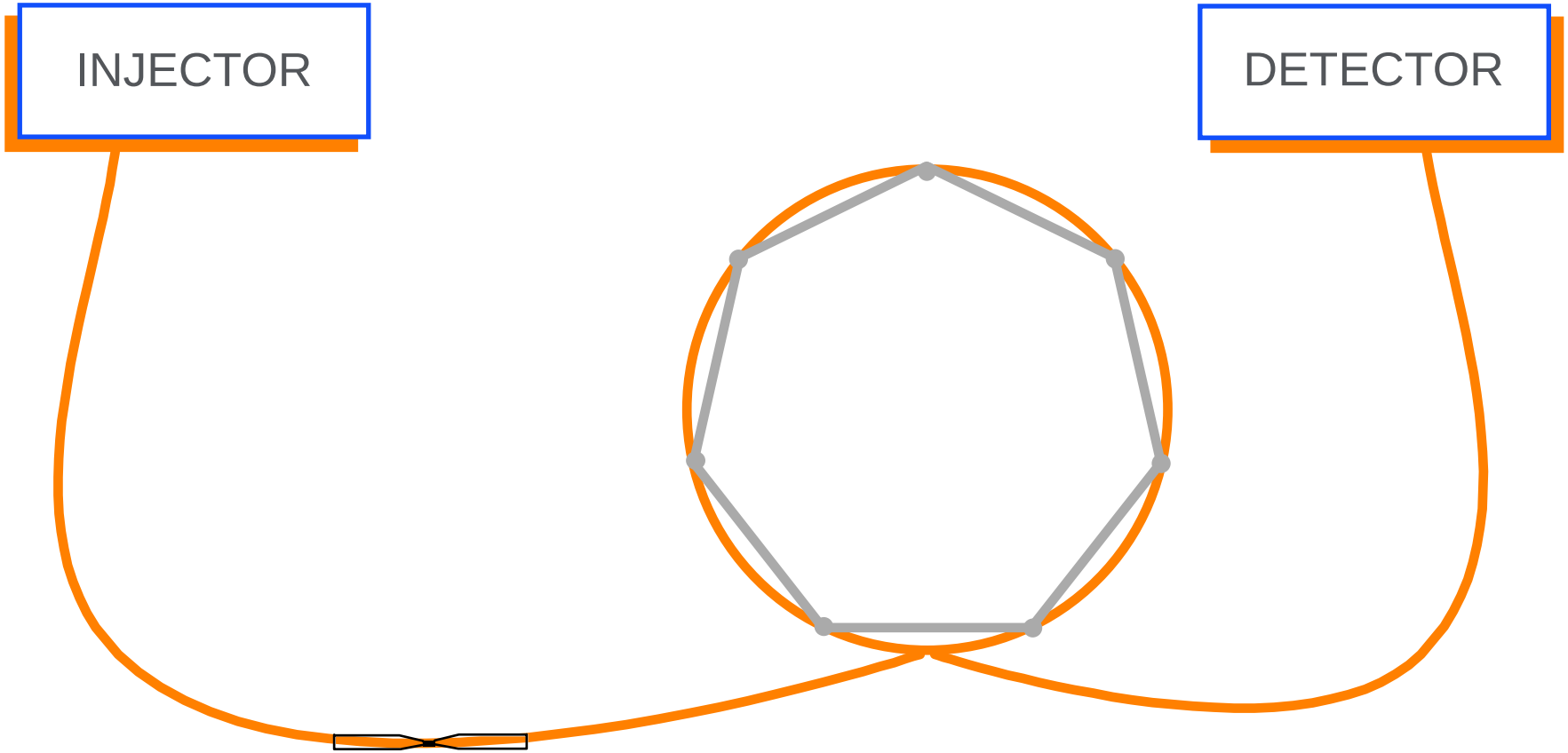
Solid Phase Extraction Cartridges and Plates



Filtration Cartridges and Plates

Captiva EMR Lipid

Guard Column or Retention Gap



The guard column is 3 - 5 meters of deactivated fused silica tubing with the same diameter as the analytical column. It is connected with a zero dead volume union.

Non-Volatile Contamination

What To Do If It Happens

- Do not “bake out” the column
- Front End Maintenance
  - clean or change the injector liner
  - clean the injector
  - cut off 1/2 -1 meter of the front of the column
- Turn the column around
- Solvent rinse the column (see appendix)
- Cut the column in half

Semi-Volatile Contamination

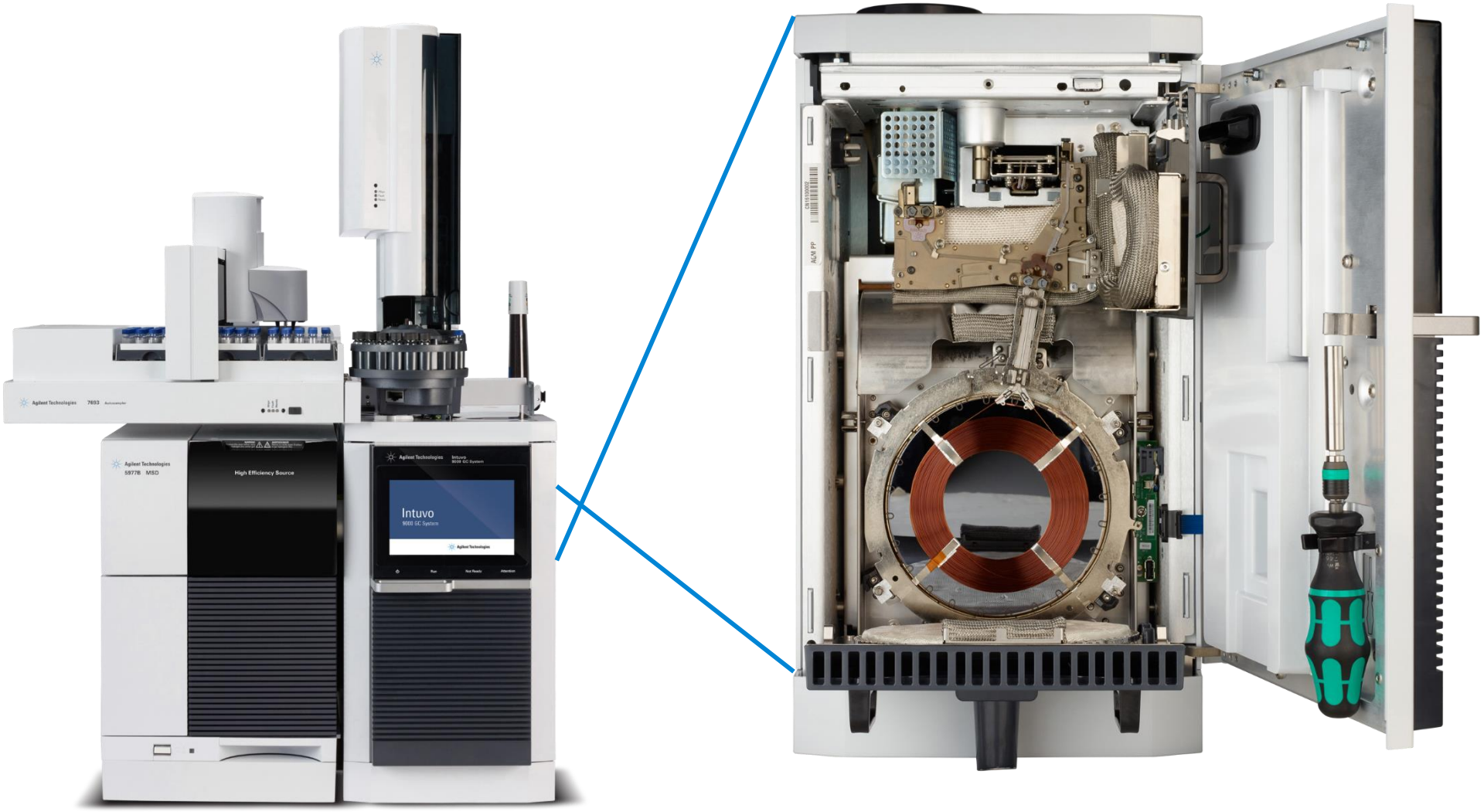
What To Do If It Happens

- “bake out” the column
  - Limit to 1-2 hours
  - Longer times may polymerize some contamination and reduces column life
- Solvent rinse the column

Column Storage

- Place septa over the ends
- Return to column box

The New Agilent Intuvo 9000 GC System



Common frustrations with GC

- Measuring column length correctly
- Cutting your column correctly
- How tight it too tight?
- Clipping columns to deal with active sites, then updating retention times

Common Care and Maintenance Scheme for GC Columns

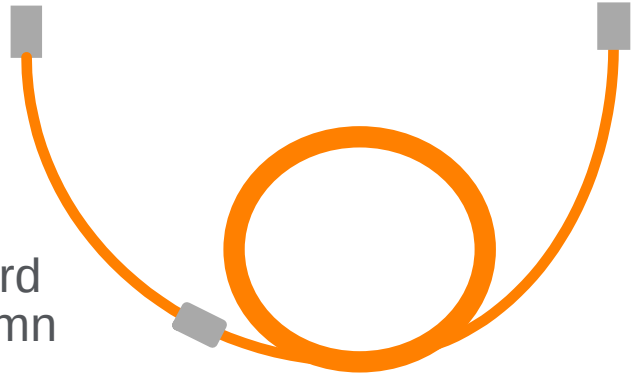
1. Cut off 6”-1ft of the inlet end of the column.

2. Bake out the column for no more than 2 hours.

3. Cut off more column. (repeat as necessary)

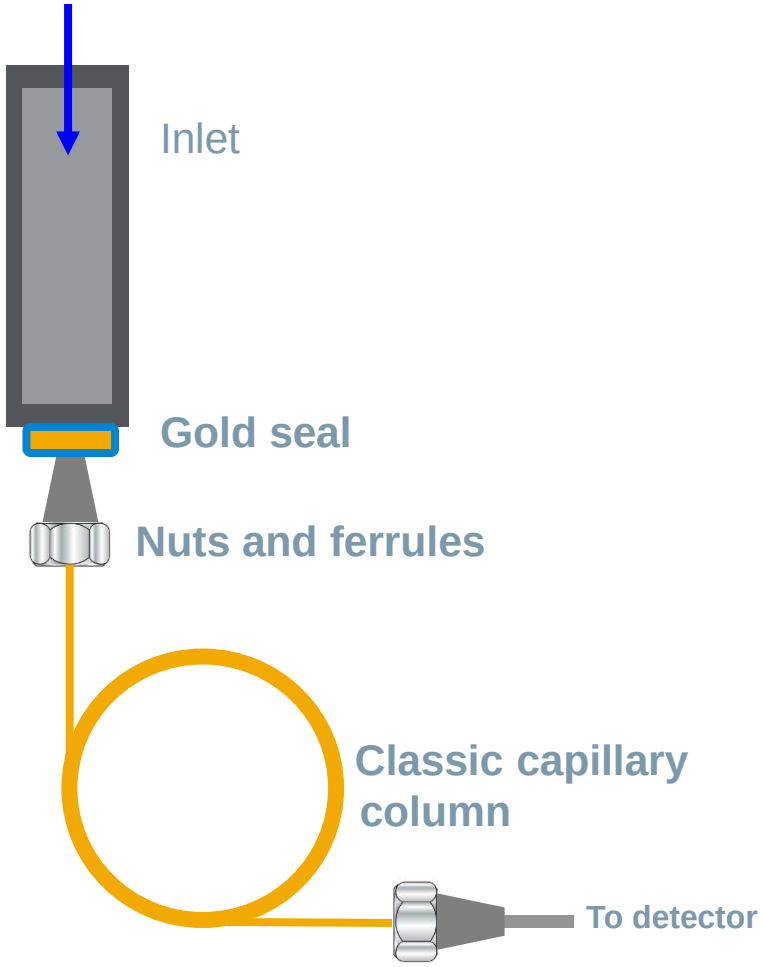
Intuvo…

Change the guard chip

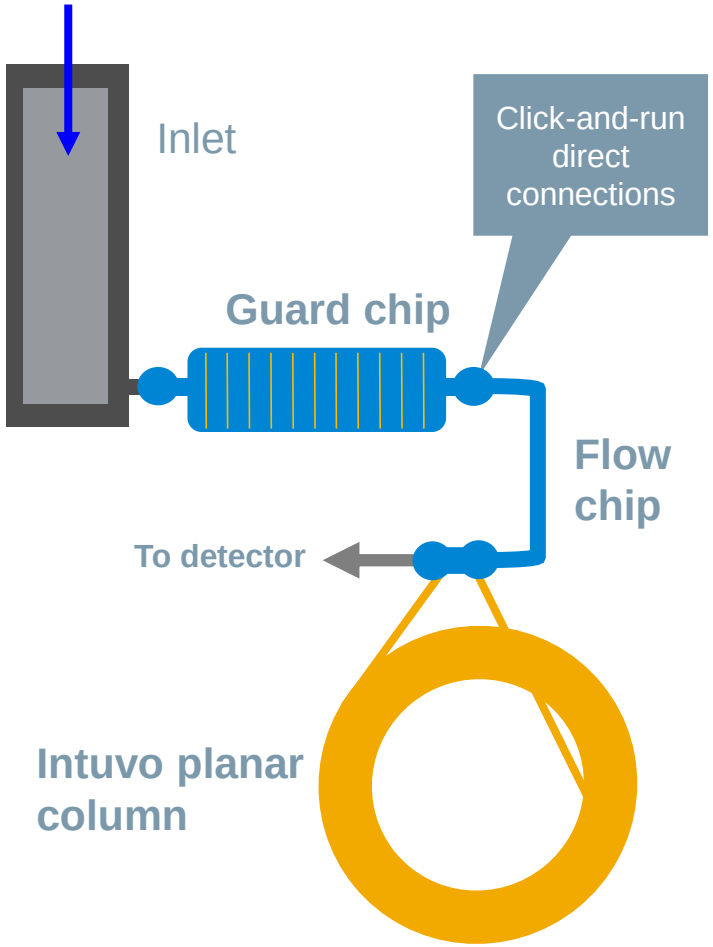


Innovating the GC Flow Path

Conventional flow path

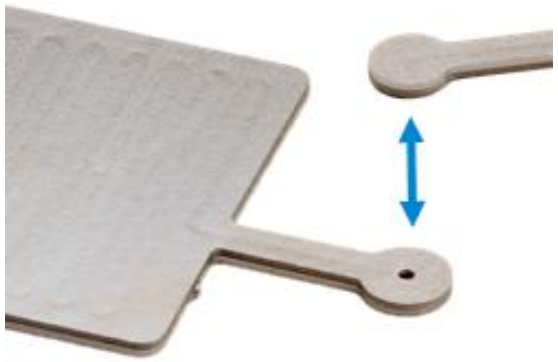


Intuvo flow path



Easier and faster maintenance with Intuvo

- No more ferrules
- Direct face seal connections
- Audible and tactile click lets you know connection is made
- Less unplanned downtime
- Fewer batch reruns and precious samples lost

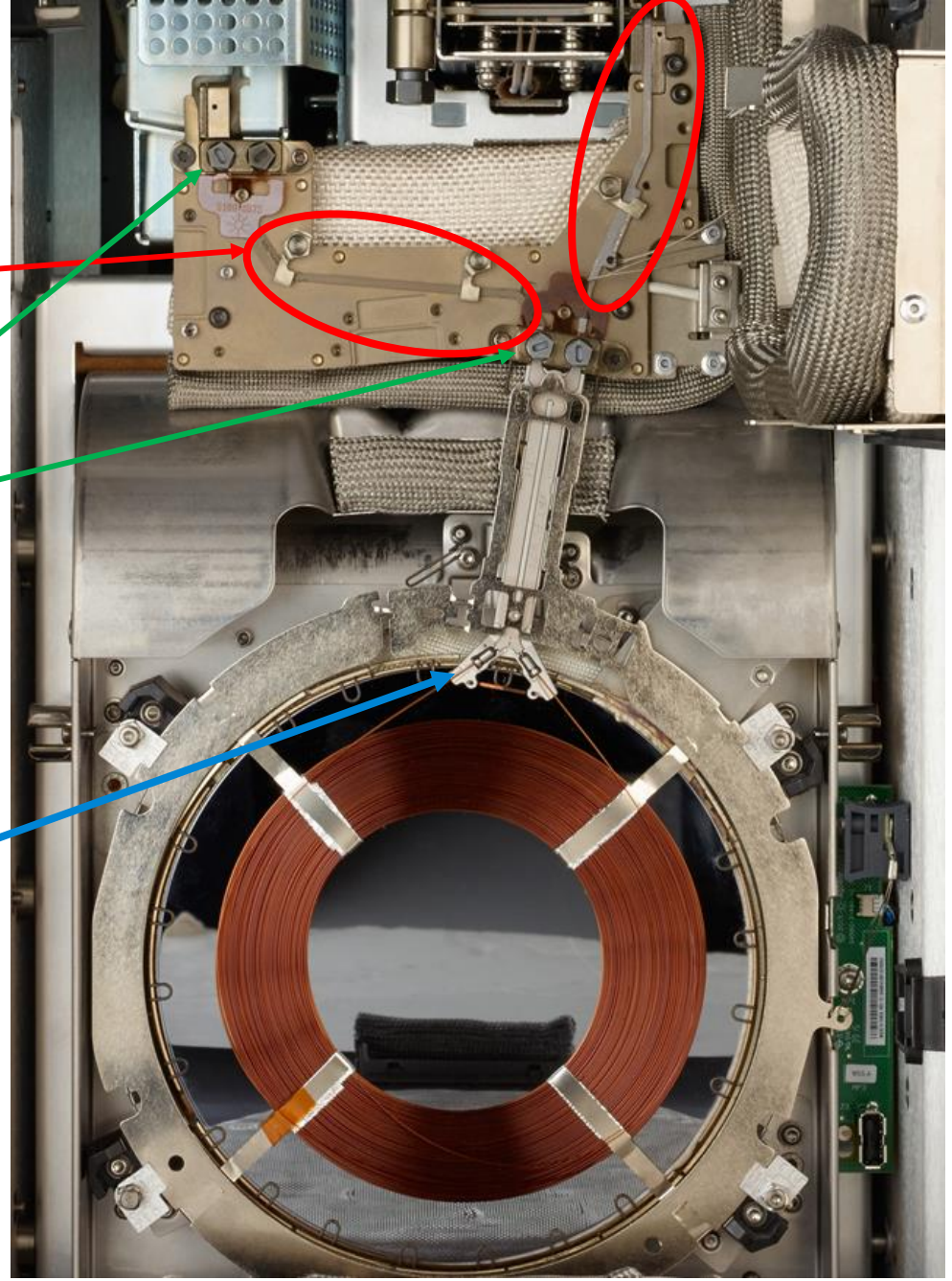


No More

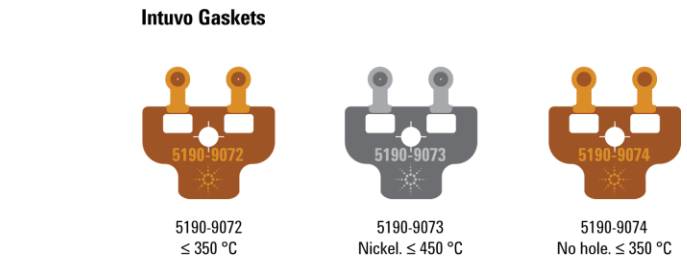
- measuring

- over-tightening

- trimming



A New Portfolio of GC Consumables



UI Inlet Liners

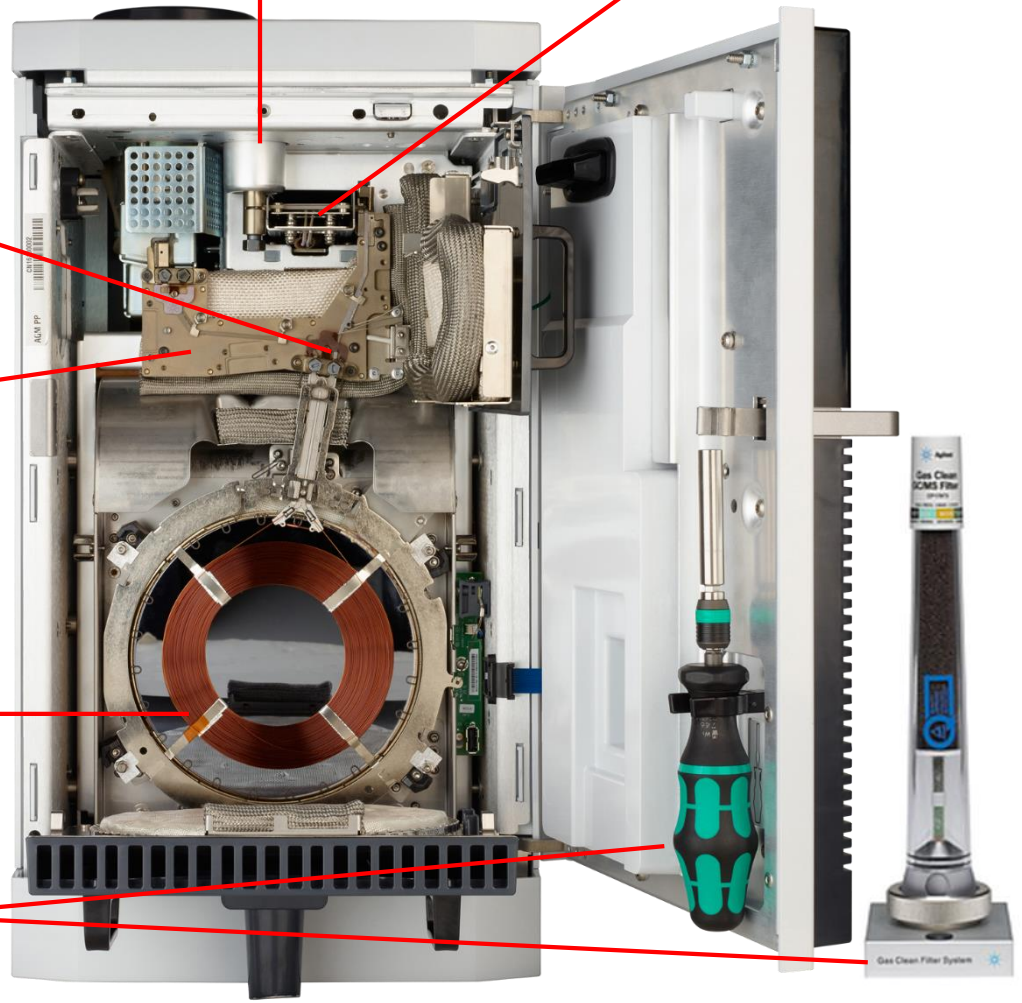
Guard Chip

Smart Keys

Intuvo Flow Chips

No-trim column

Tools & Accessories

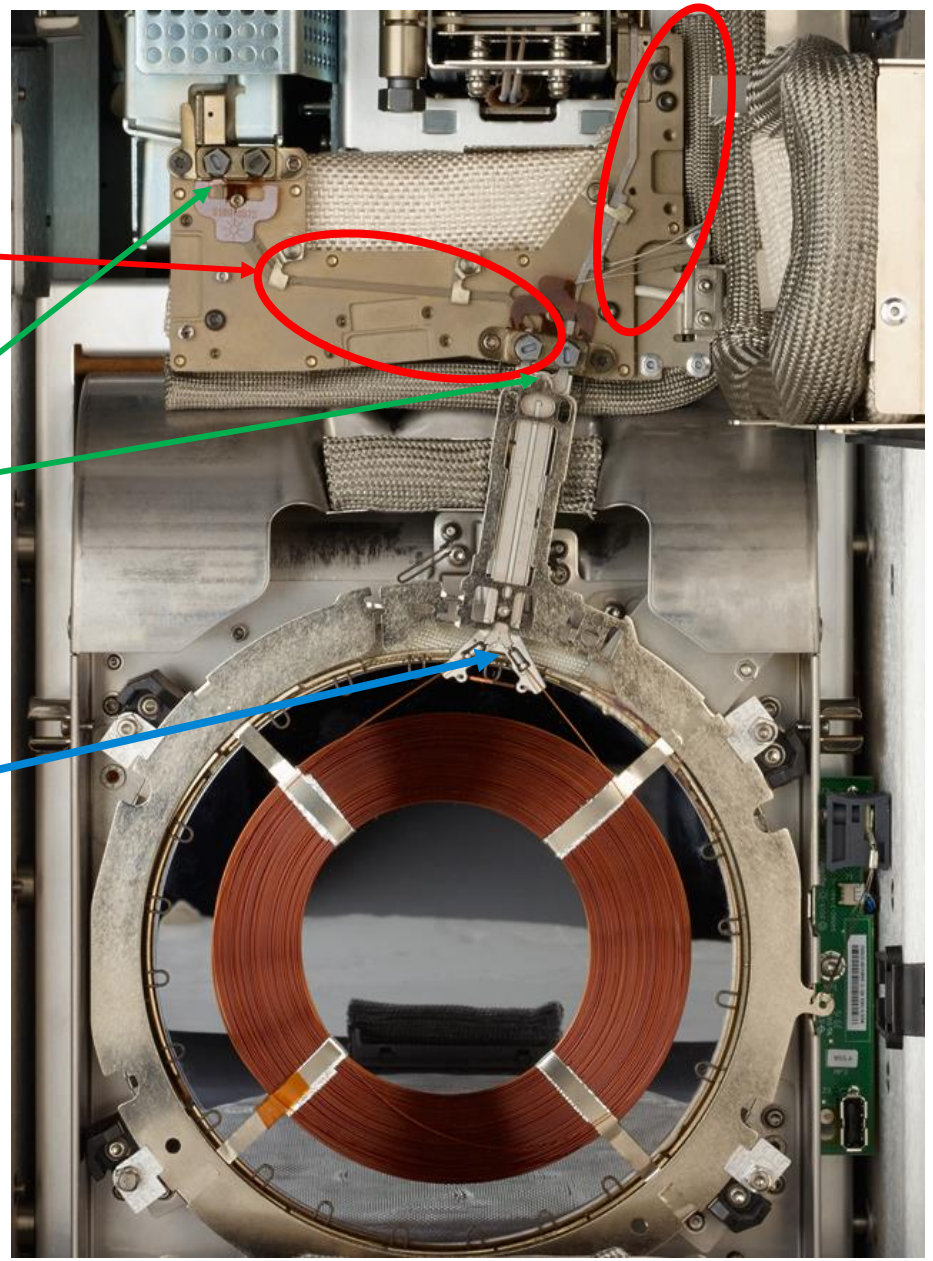


No More:

- Measuring

- Over-tightening

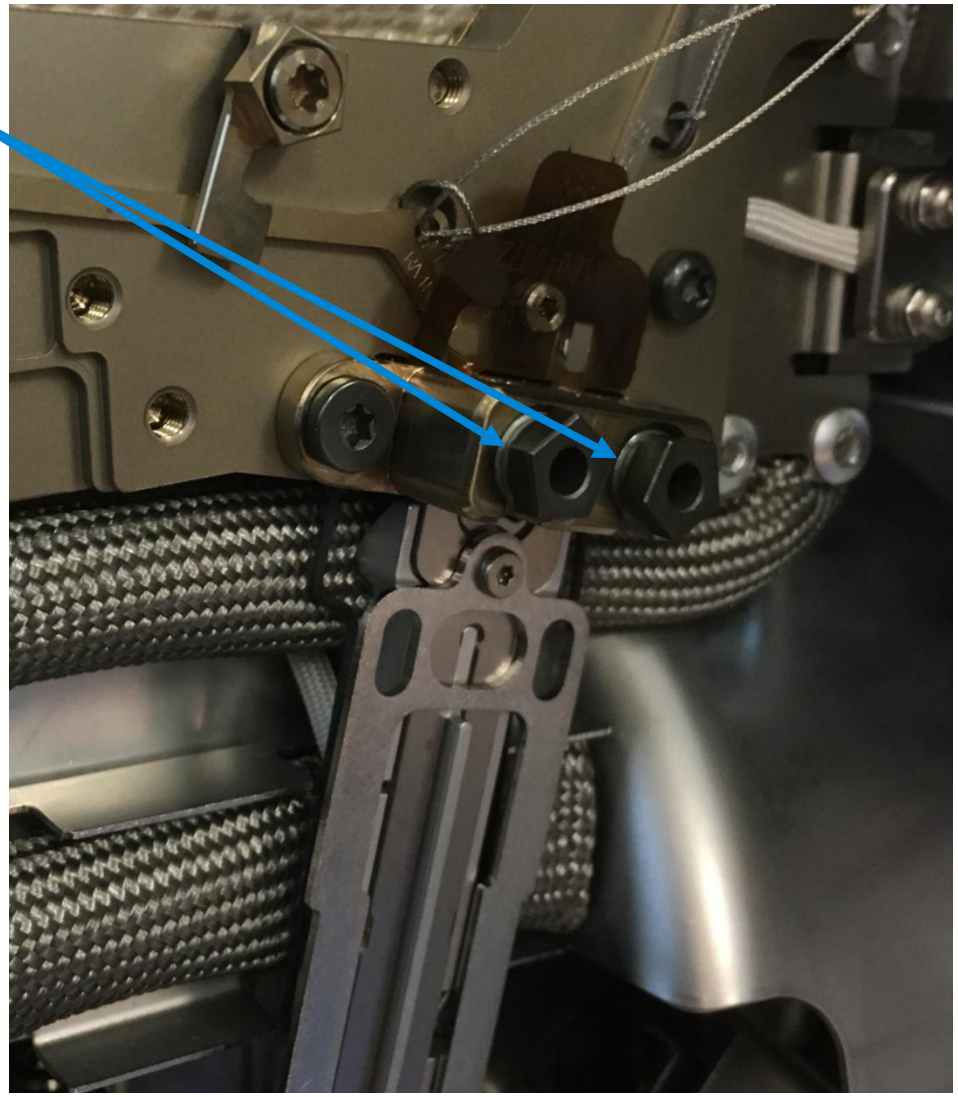
- Trimming



Tips to Assure a Good Column Installation

1. Finger tighten until only 1 thread on each of the two nuts is showing.

If more than 1 thread is showing, wiggle or re-position the column into place to further finger tighten the nuts to 1 thread.

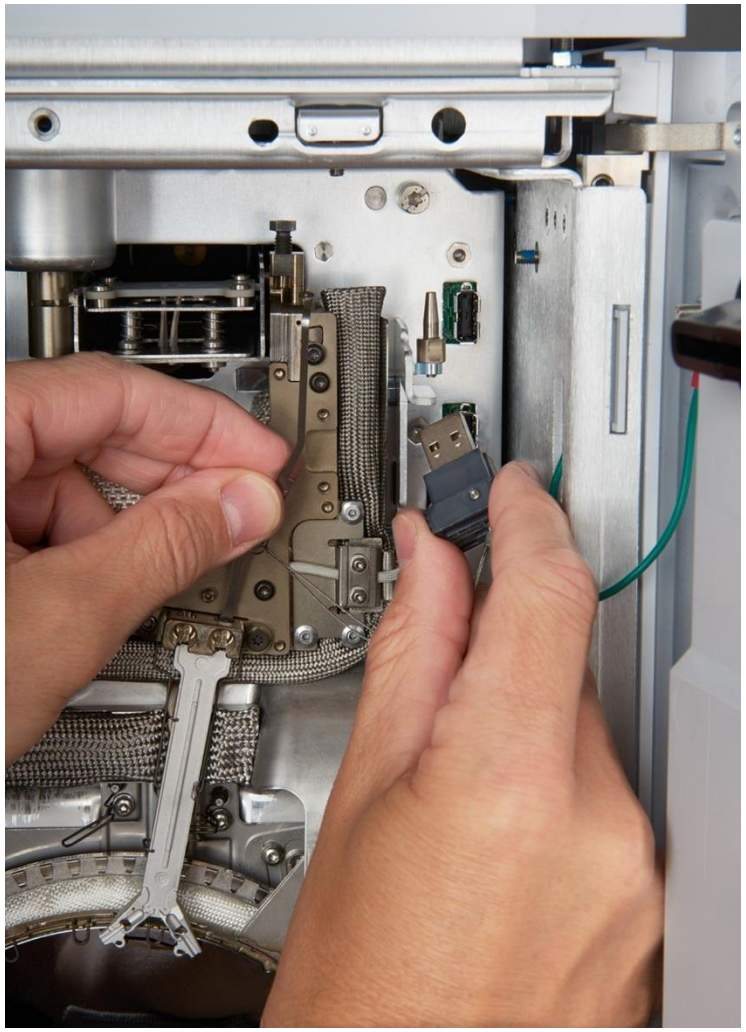


Tips to Assure a Good Column Installation

2. Check that the small integrated column nuts on the column are in form fitted place on the heater in the instrument.
3. Click and Run!!

Smart Key Technology

- Smart chip tells your Intuvo what you have
- Sets temperature limits for you
- Keep track of performance with read/writeable Smart Key



Where Does it Get Dirty?

Here

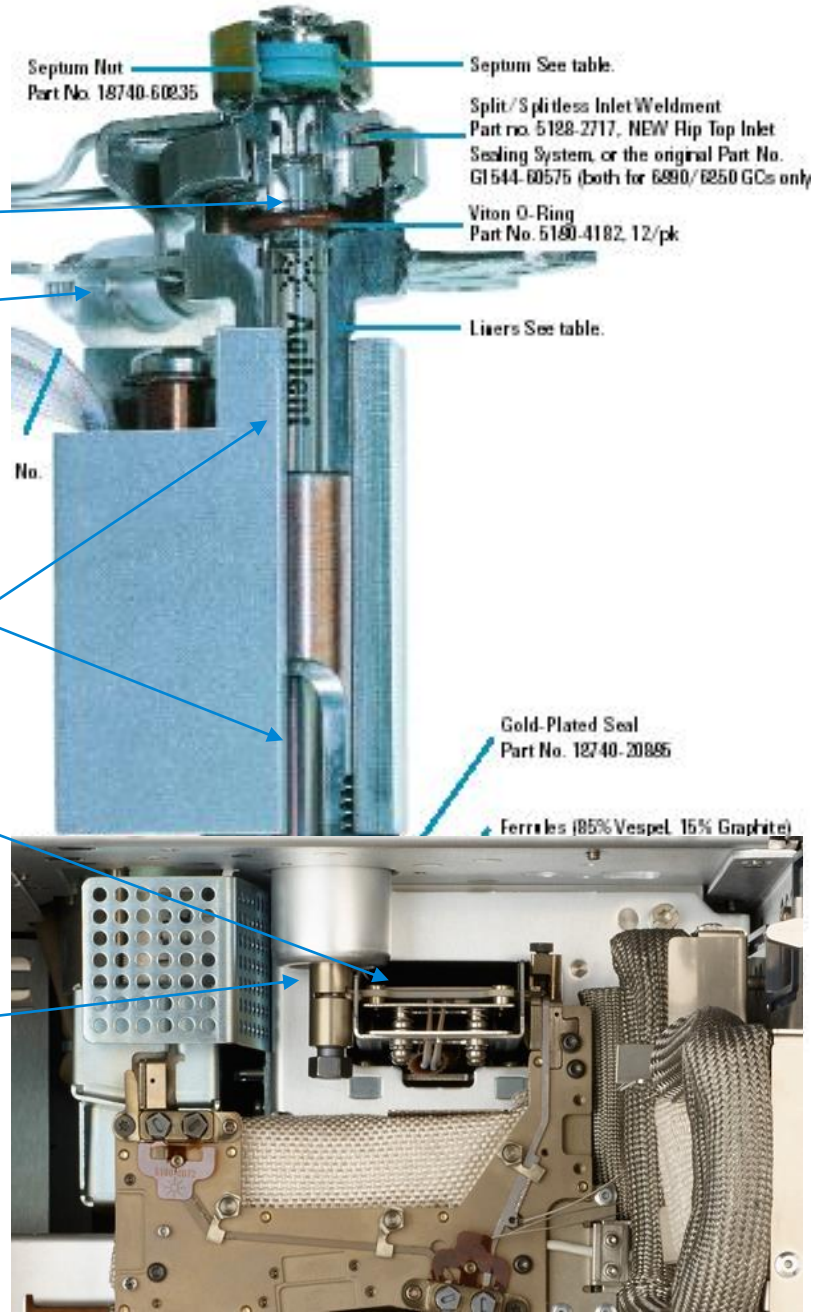
Here

Here

Here

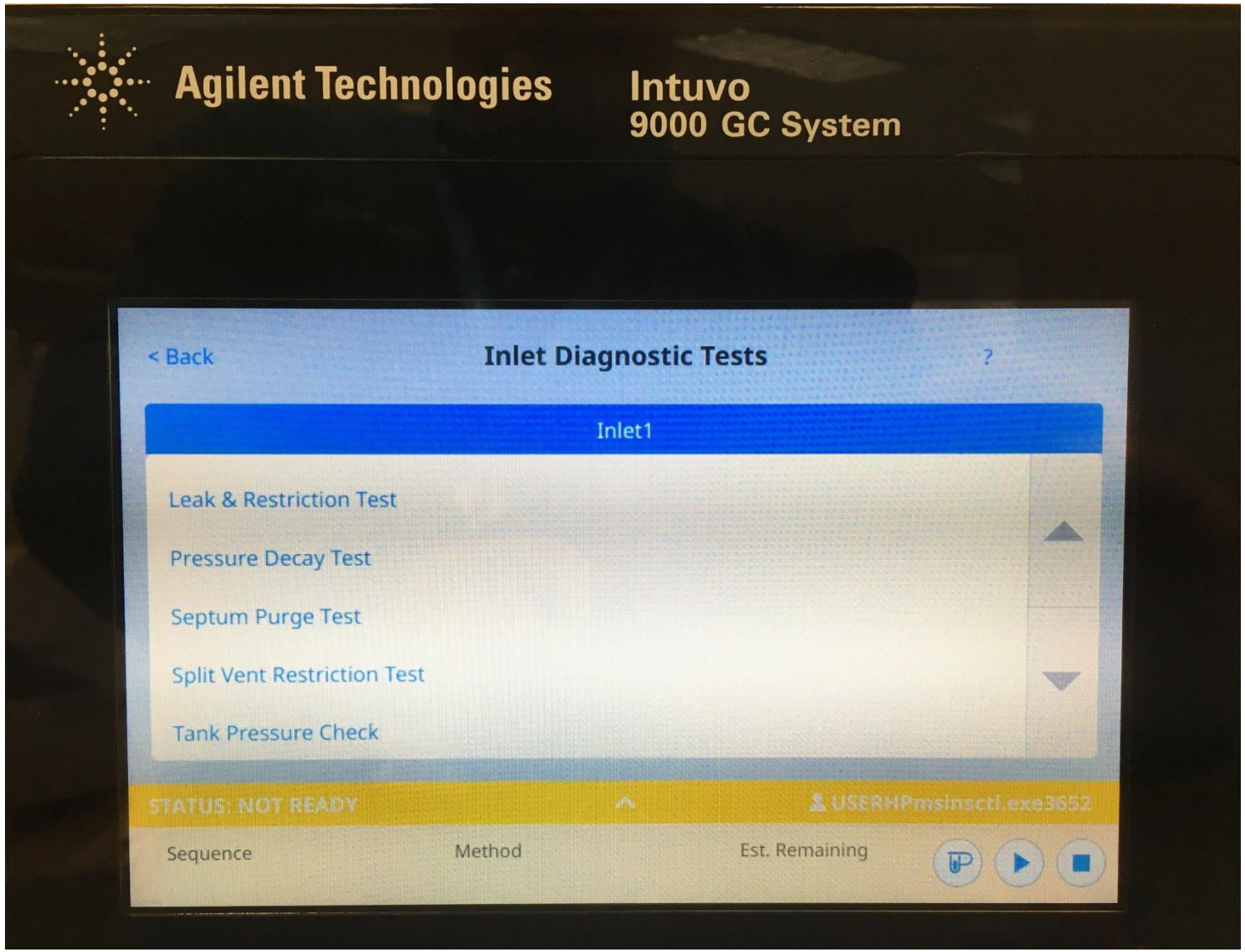
Here

Here



Not the Column!

New Autonomous Leak Checking



Agilent Intuvo 9000 Videos:

The Agilent Intuvo 9000 GC System – Environmental Science Corporation (ESC)

Discover higher GC productivity with the Agilent Intuvo 9000 GC system.

Playing Time: 4:00

The Agilent Intuvo 9000 GC System Story

Learn more about the Agilent Intuvo 9000 GC System

Playing Time: 2:21

The Agilent Intuvo 9000 GC System: Return on Investment. Return on Innovation

A testimonial regarding the return on investment on the Agilent Intuvo 9000 GC System

Playing Time: 4:17

Always Remember to:

- Start with a good installation
- Maintain an oxygen free system
- Avoid physical, thermal, and chemical damage
- Take steps to prevent contamination

Contact Agilent Chemistries and Supplies Technical Support

1-800-227-9770 Option 3, Option 3:

Option 1 for GC/GCMS Columns and Supplies

Option 2 for LC/LCMS Columns and Supplies

Option 3 for Sample Preparation, Filtration and QuEChERS

Option 4 for Spectroscopy Supplies

Available in the USA 8-5 all time zones

gc-column-support@Agilent.com

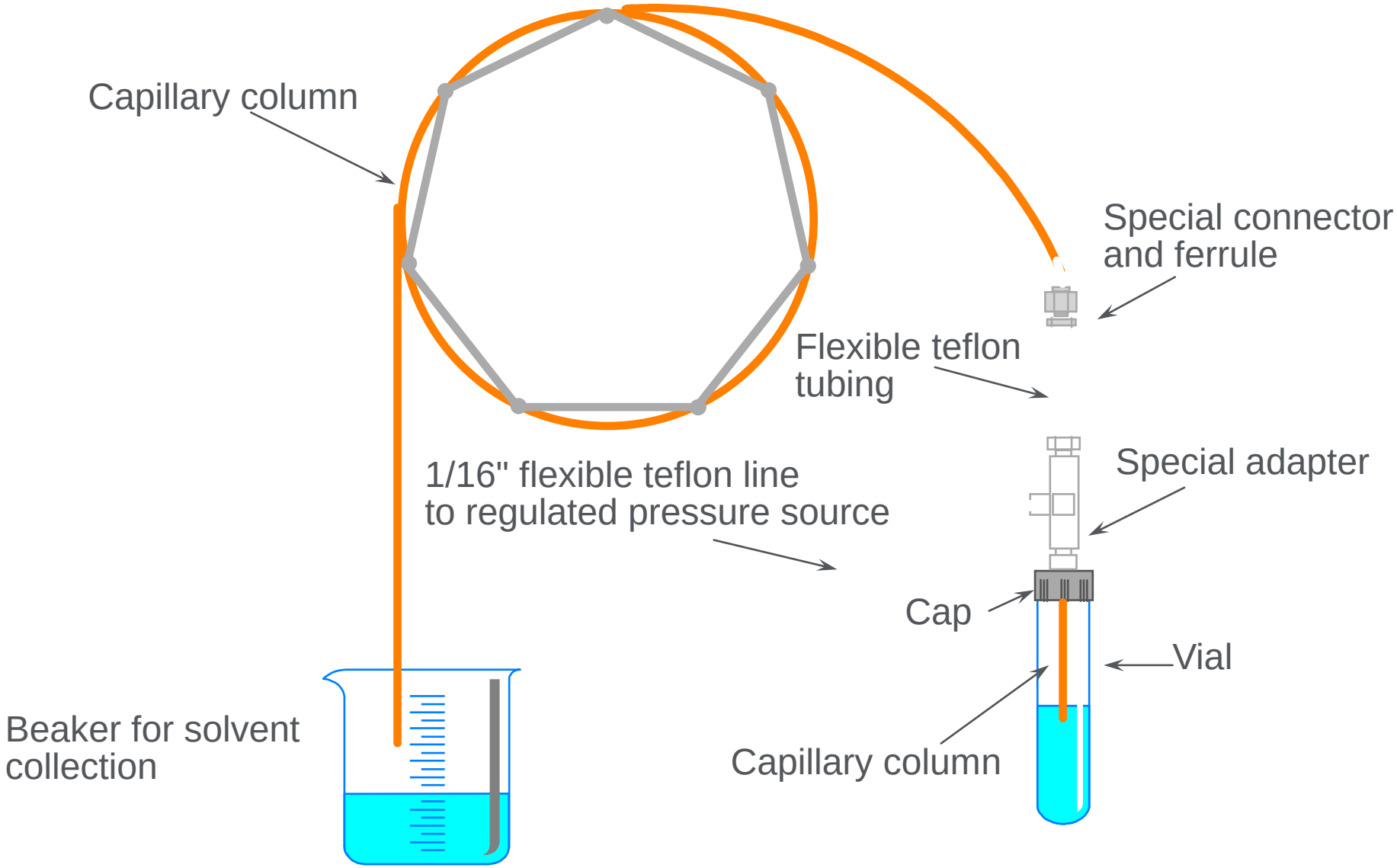
lc-column-support@agilent.com

spp-support@agilent.com

spectro-supplies-support@agilent.com

Appendix

Column Rinse Kit



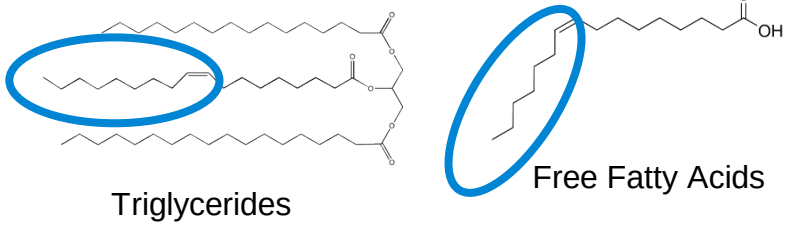
Captiva EMR-Lipid

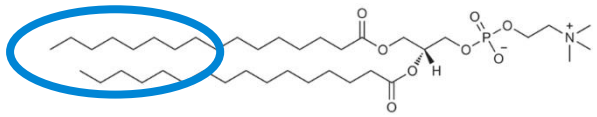
- One of Agilent's newest products with a 2 in 1 benefit of removing proteins and lipids
- Simple pass-through format
- Solvent-retention frit in 1 mL cartridge/96-well plate format for in well protein precipitation (*in situ*)
  - Unique cartridge/well construction minimizes clogging – and **ensures protein and lipid removal** (no cloudy samples)
- 3 and 6 mL cartridge format for larger samples
  - Do not contain solvent retain frit which allow for gravity flow
  - Protein precipitation performed offline (QUECHERS, etc.)
- Unique cartridge/well construction minimizes clogging – and **ensures protein and lipid removal** (no cloudy samples)
- High analyte recoveries
- Effective use will reduce ion suppression, increase analyte sensitivity, and detection, and extend the lifetime of your analytical column

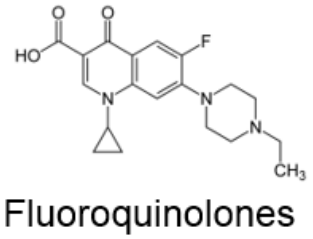
Enhanced Matrix Removal: EMR-Lipid

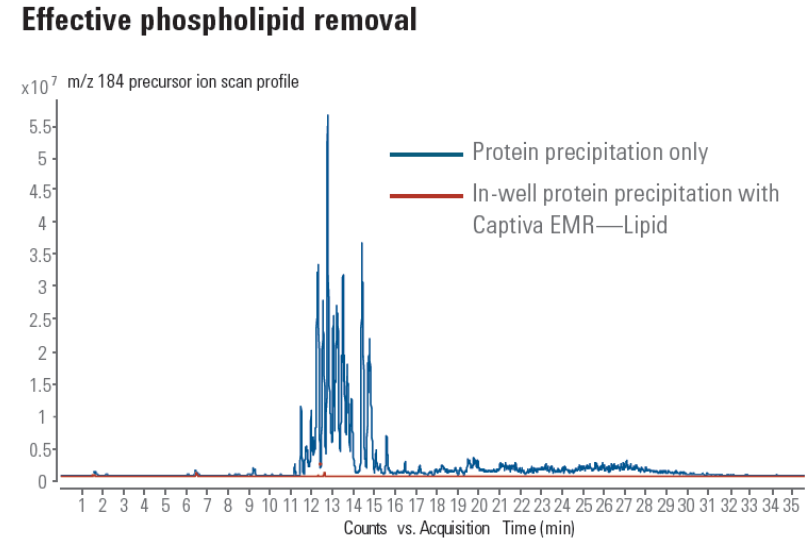
EMR-Lipid sorbent technology effectively traps lipids through two mechanisms:

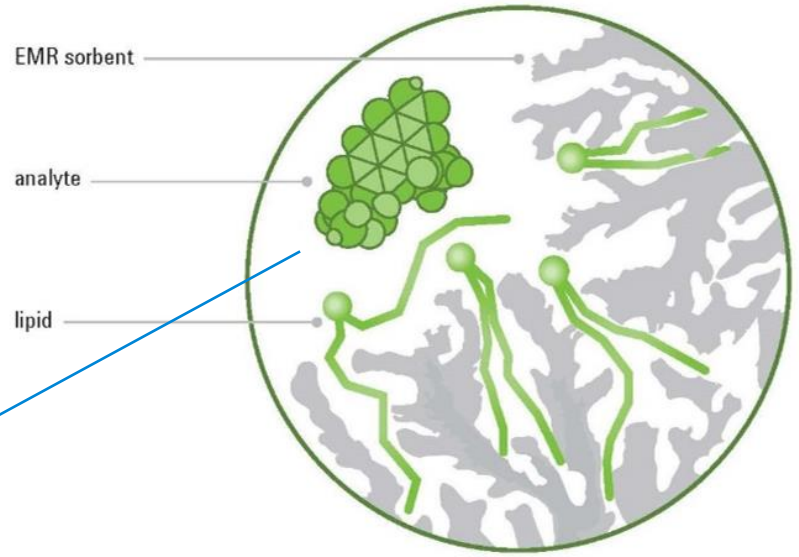
- ✓ **Size exclusion** – Unbranched hydrocarbon chains (lipids) enter the sorbent; bulky analytes do not
- ✓ **Sorbent chemistry** – Lipid chains that enter the sorbent are trapped by hydrophobic interactions





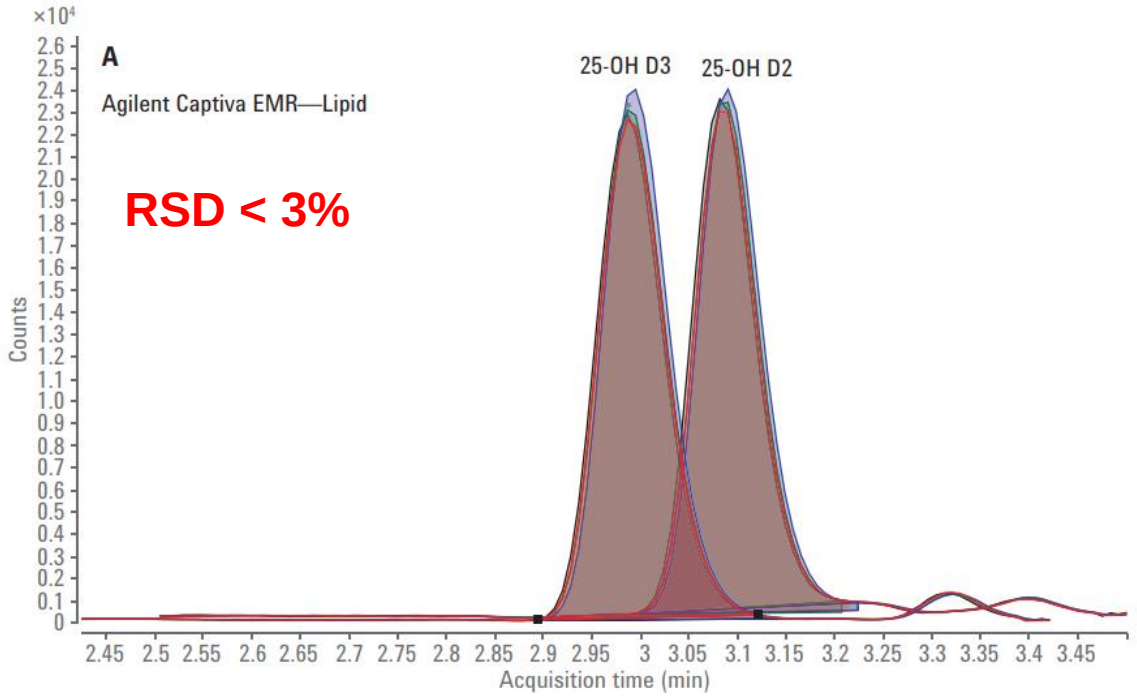




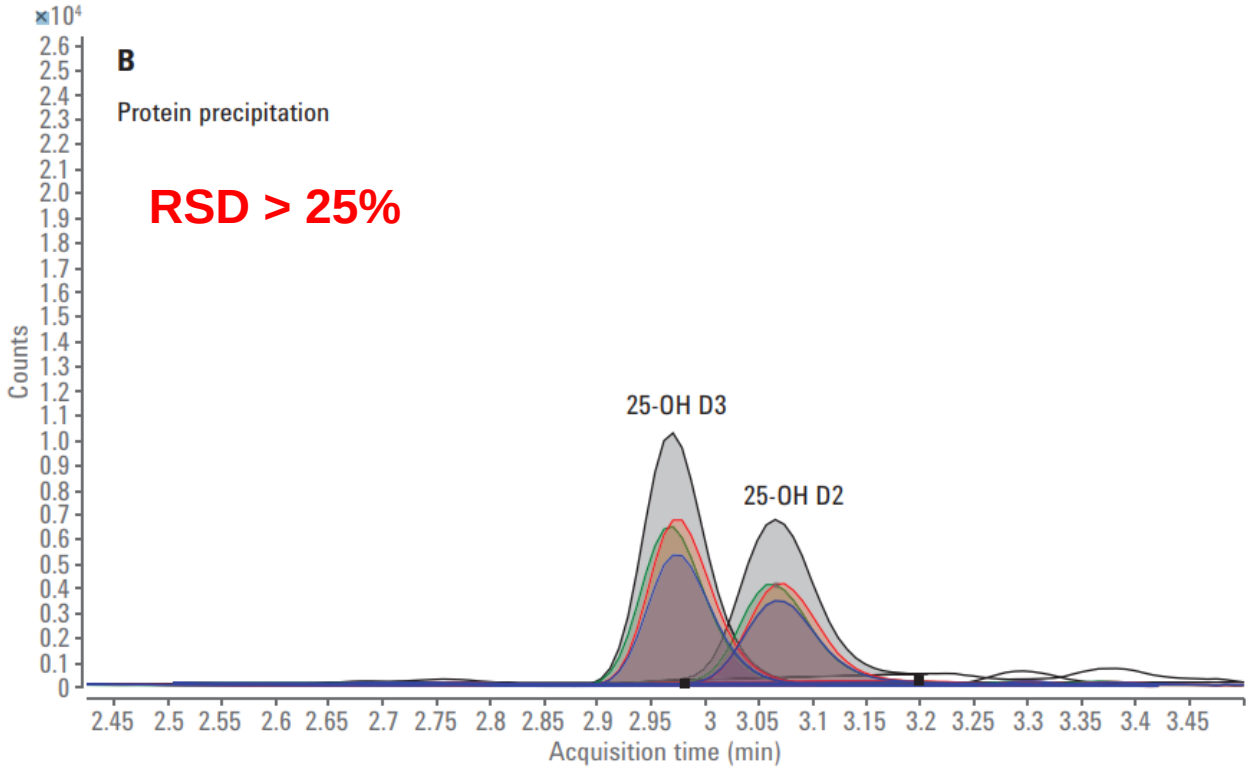


Protein Precipitation vs. Captiva EMR-Lipid RSD and Peak Area

Captiva EMR-Lipid



Protein Precipitation



Lipids cause reproducibility problems resulting in high RSD values

Using Captiva EMR-Lipid → low RSD values and higher peak areas

Higher peak area due to less ion suppression → can lead to lower detection limits