Understanding your GC Inlet and How to Maintain it.

What's going on in there anyway & how do I stay in control?

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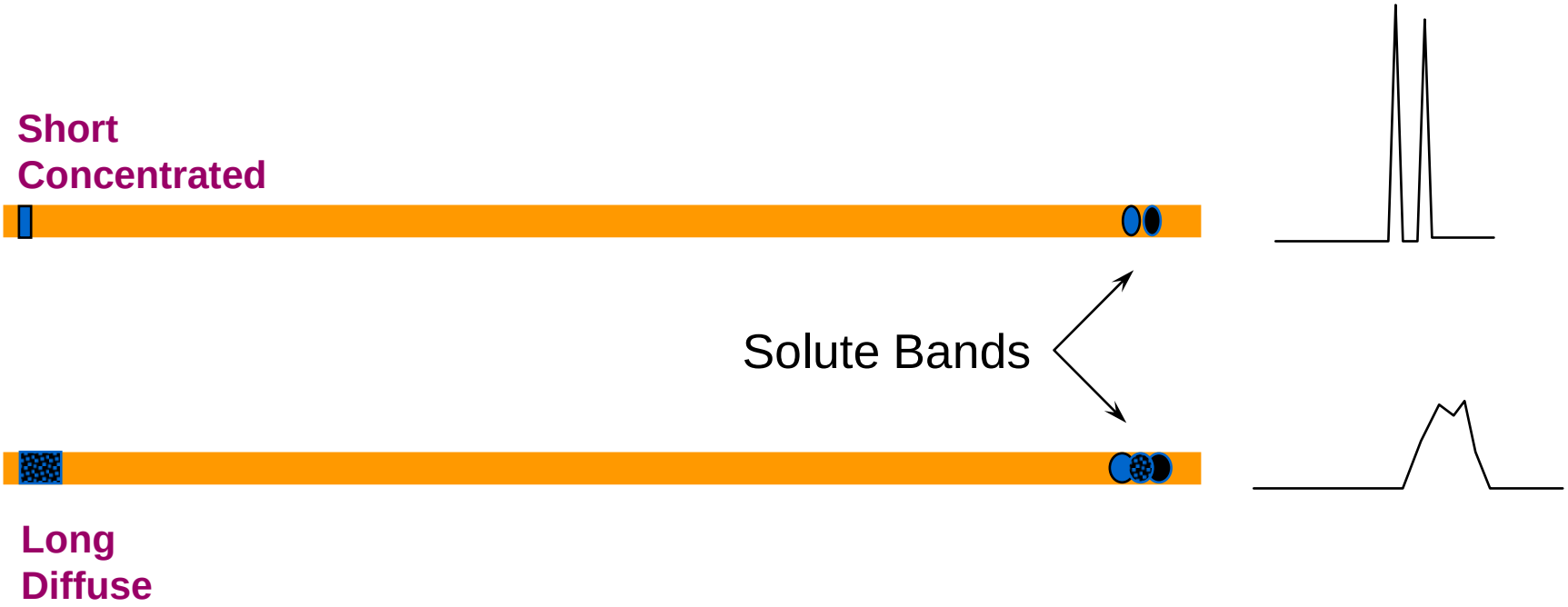
CSD Application Engineer

SAMPLE INJECTION
Goals

- Introduce sample into the column
- Reproducible
- No efficiency losses
- Representative of sample

Influence of Injection Efficiency



Same column, same chromatographic conditions

Inlet Choices

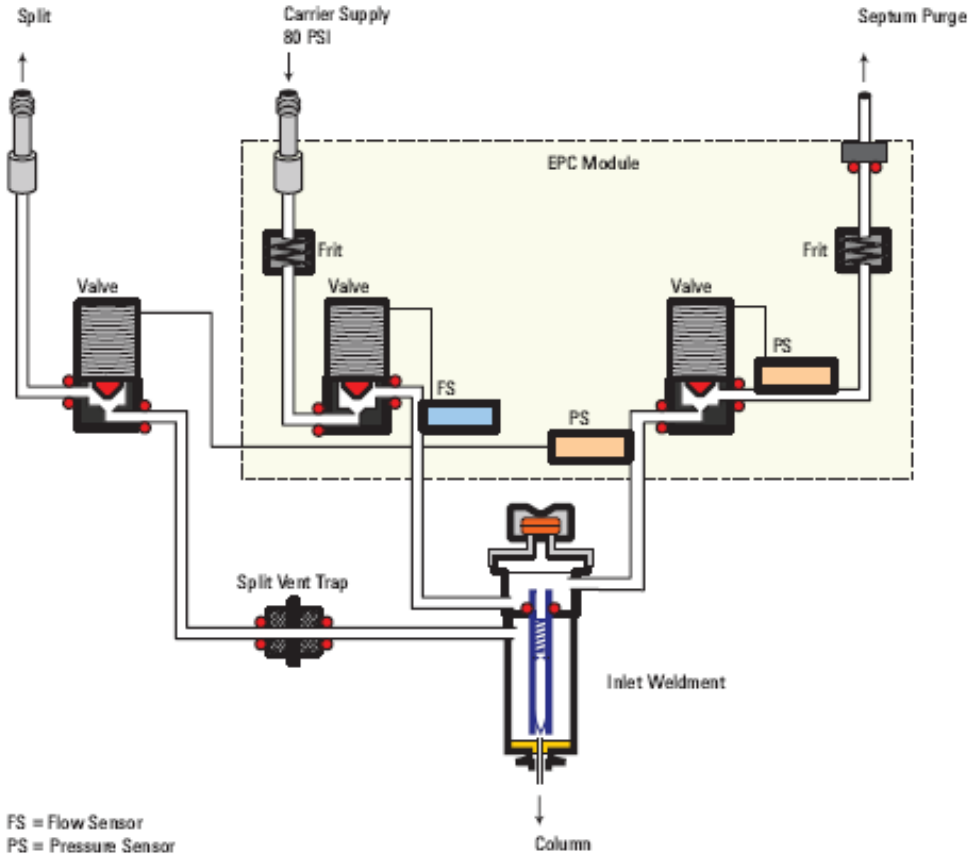
Inlet	Column	Mode	Sample Concentration	Comments	Sample to Column
Split / Splitless	Capillary	Split Purged Split Splitless Purged Splitless	High High Low Low	Most commonly used inlet. Very Flexible	Very Little Very Little All All
Cool-On-Column	Capillary	N/A	Low or labile	Minimal discrimination and decompositoin	All
Packed	Packed Large Capillary	N/A N/A	Any Any	OK if resolution is not critical	All All
Programmed Temperature Vaporizaton	Capillary	Split Pulsed Split Splitless Pulsed Splitless Solvent Vent	High High Low Low Low	Not great for HOT injections. Can concentrate analytes and vent solvent	Very Little Very Little All All Most
Volatiles Interface	Capillary	Direct Split Splitless	Low High Low	Purge & Trap / Headspace	All Very Little All
Multi-Mode	Capillary	Split Pulsed Split Splitless Pulsed Splitless Solvent Vent	High High Low Low Low	Flexibility of standard S/SL inlet and PTV	Very Little Very Little All All Most

Split/Splitless Inlet Schematic and Operation modes

Modes

- Split
- Pulsed Split (useful for small number of applications)

- Splitless
- Pulsed Splitless



Split Injections - Considerations

Dirty Samples are OK - backflushing

Wide Analyte Boiling Range

Solvent Properties

- Wide Boiling Point Range
- Wide Polarity Range

Discrimination can be due to liner or inlet temperature

Most efficient sample transfer = nice sharp peaks

Split Injections - Inertness

More inert than splitless

- Higher velocity through the inlet
- Less exposure to inlet hardware

Glass wool is a compromise

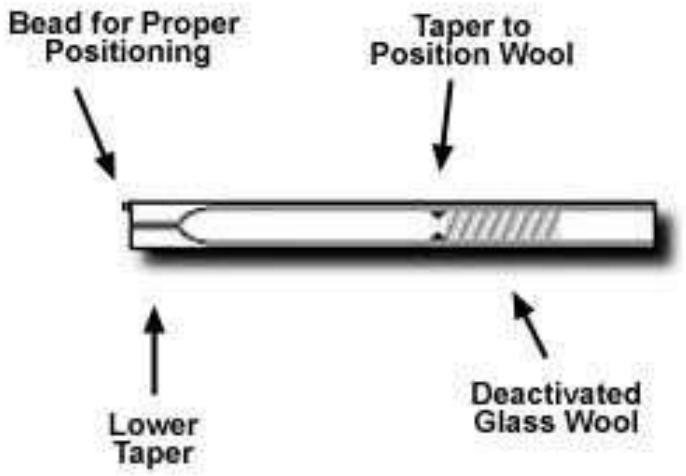
- Exhibits some activity
- Greatly improves fluidic performance – mixing of the vaporized sample is important for uniform splitting

Split Injections - recommended Liners

Agilent p/n 5190-2295

Wiped needle improves

- precision
- peak shape
- discrimination



Split Injections - Maximizing Sensitivity

Increase Injection Volume

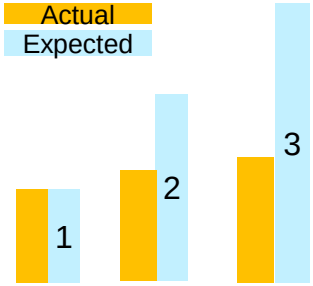
- liner dependent (use the Pressure-Volume Calculator)
- 2 uL maximum

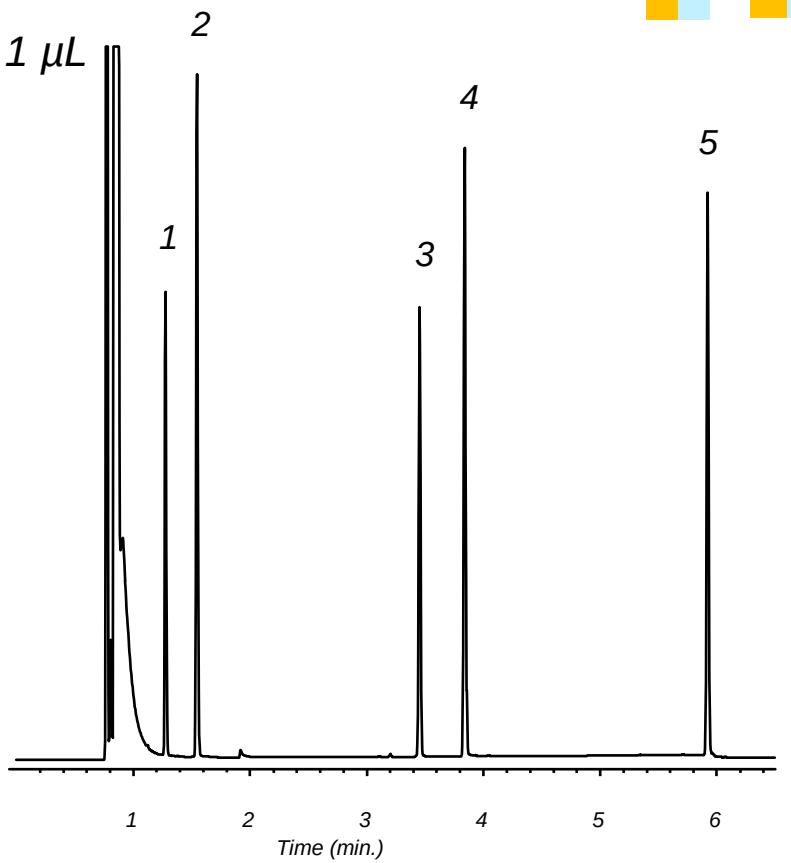
Reduce Split Ratio

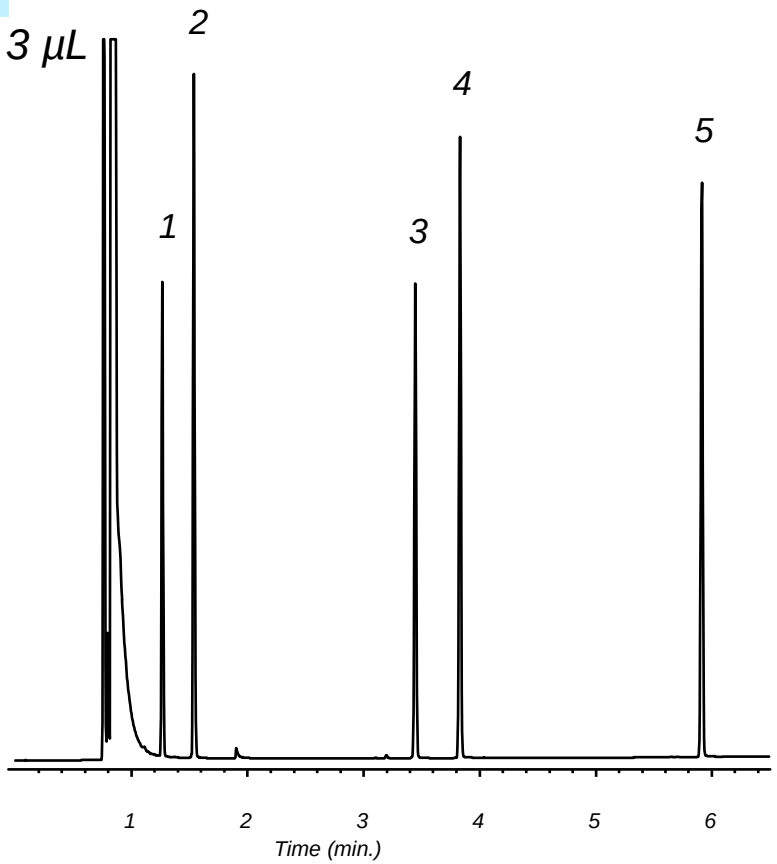
- go from 50:1 to 10:1
- 10:1 practical lower limit for liquid injections (for 250 - 320 um i.d. columns)
- 1:1 possible for gas injections with correct liner
- Keep TOTAL INLET FLOW at 20 mL/min or higher

Split Injector

Injection Volume





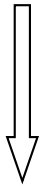


DB-1, 15 m x 0.25 mm I.D., 0.25 µm
60°C for 1 min, 60-180°C at 20°/min; Helium at 30 cm/sec
1. n-heptane 2. toluene 3. n-decane 4. n-butylbenzene 5. n-tridecane

Minimum Recommended Split Ratio

mm I.D.	Lowest ratio
0.10	1:50 - 1:75
0.18 - 0.25	1:10 - 1:20
0.32	1:8 - 1:15
0.53	1:2 - 1:5

Higher flow rates



Split Injections - Troubleshooting

Column pressures <10 psi

- The pressure pulse from evaporating solvent can cause discrimination and poor precision

Liner residence times < 0.5 sec (> 200 ml/min)

- poor mixing will cause discrimination

No glass wool

Solvents with high expansion ratio Backflash

Column position - top to bottom, side to side

Large bore, short columns with a high split ratio

Split Ratio Comparison

	High Ratio	Low Ratio
Sample into Column	Low	High
Efficiency	High	Low
Discrimination	High	Low
Carrier Gas use	High	Low

Splitless Injection Overview

- For trace level analysis.
- Use split/splitless injection port in the splitless mode (split vent closed).
- The dilute sample is injected, the sample is volatilized, and majority of analytes condense on column.
- Later, the split vent is opened and residual solvent is vented.
- Timing, carrier and split vent flows, and oven temperature program are important.
- Sample has longer residence time in the heated inlet giving more opportunity to vaporize high boiling sample components compared to split injection.

Splitless Injections - Considerations

Dirty samples are OK - backflushing

Analyte Boiling Range - Wide (but narrower than split)

- early eluters need bp difference vs solvent

Solvent Properties

- Wide Boiling Point Range
  - but consider bp of earliest eluting analyte
- Wide Polarity Range (but narrower than split)
  - Water and Methanol worst choices

Greater Sample Residence Time

Lower Inlet Temperatures can be used

Better for Labile Compounds

Splitless Injections - Inertness

Less inert than COC

- liner and inlet interaction

Less inert than Split

- longer residence time in inlet and on glass wool
- used for trace analysis, so there's a greater chance of analyte loss

Splitless Injections - Discrimination

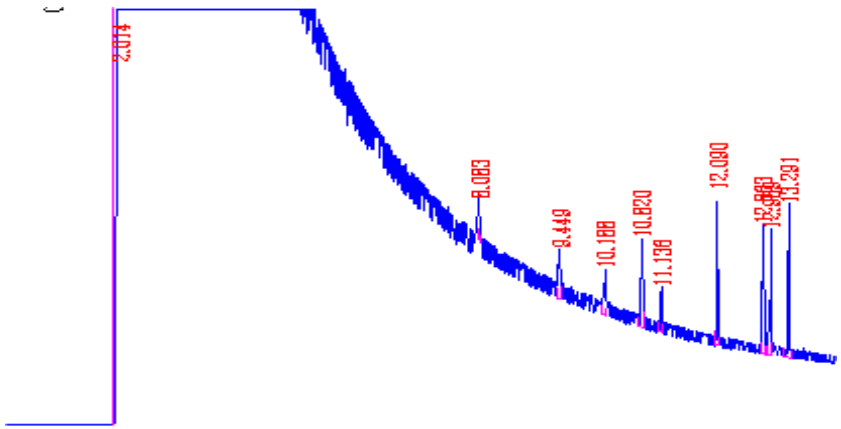
Improper purge time

- short purge times cause loss of late eluters
- long purge times cause solvent tail interference with early eluters

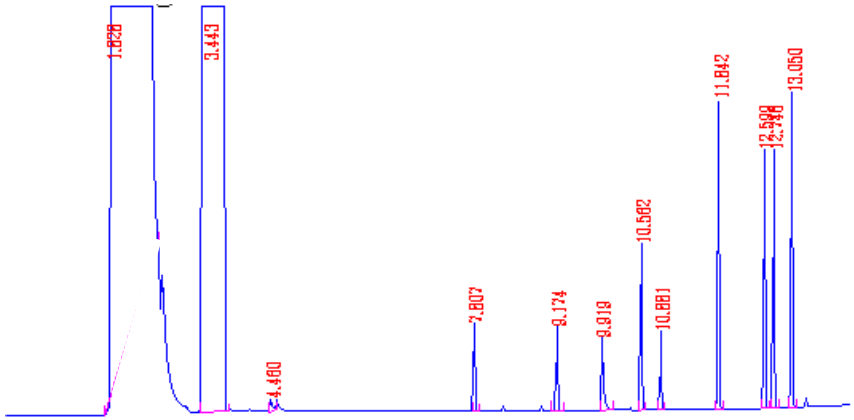
Improper initial oven temp

- too high of a temp prevents solvent effect and a loss of early eluters
- too low of a temp extends run time

Splitless Injections – Splitless Time (purge time on)



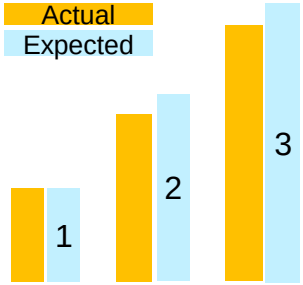
Purge time too long results in large solvent tail

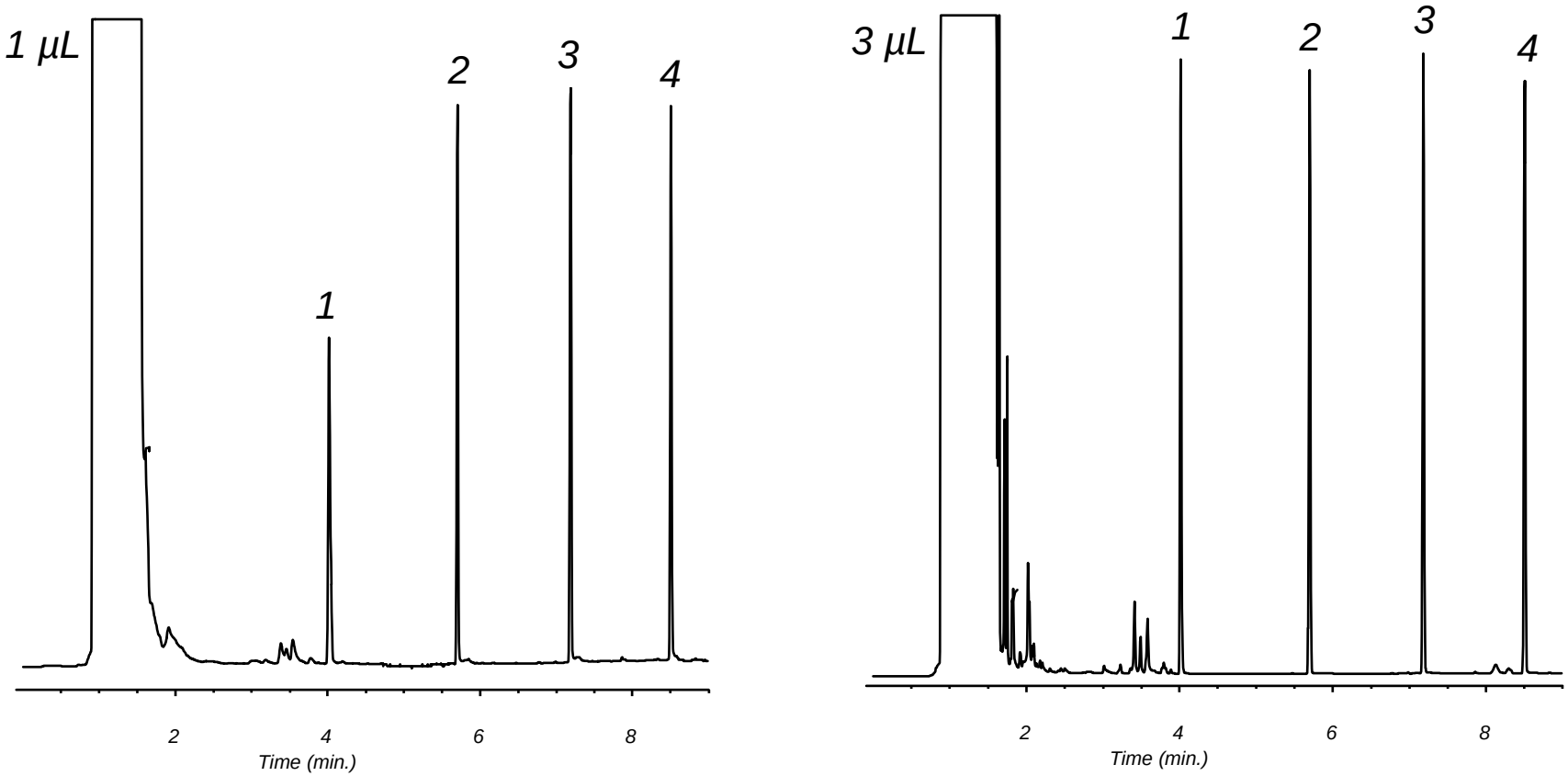


0.75 min purge time clips solvent tail

Splitless Injector

Injection Volume

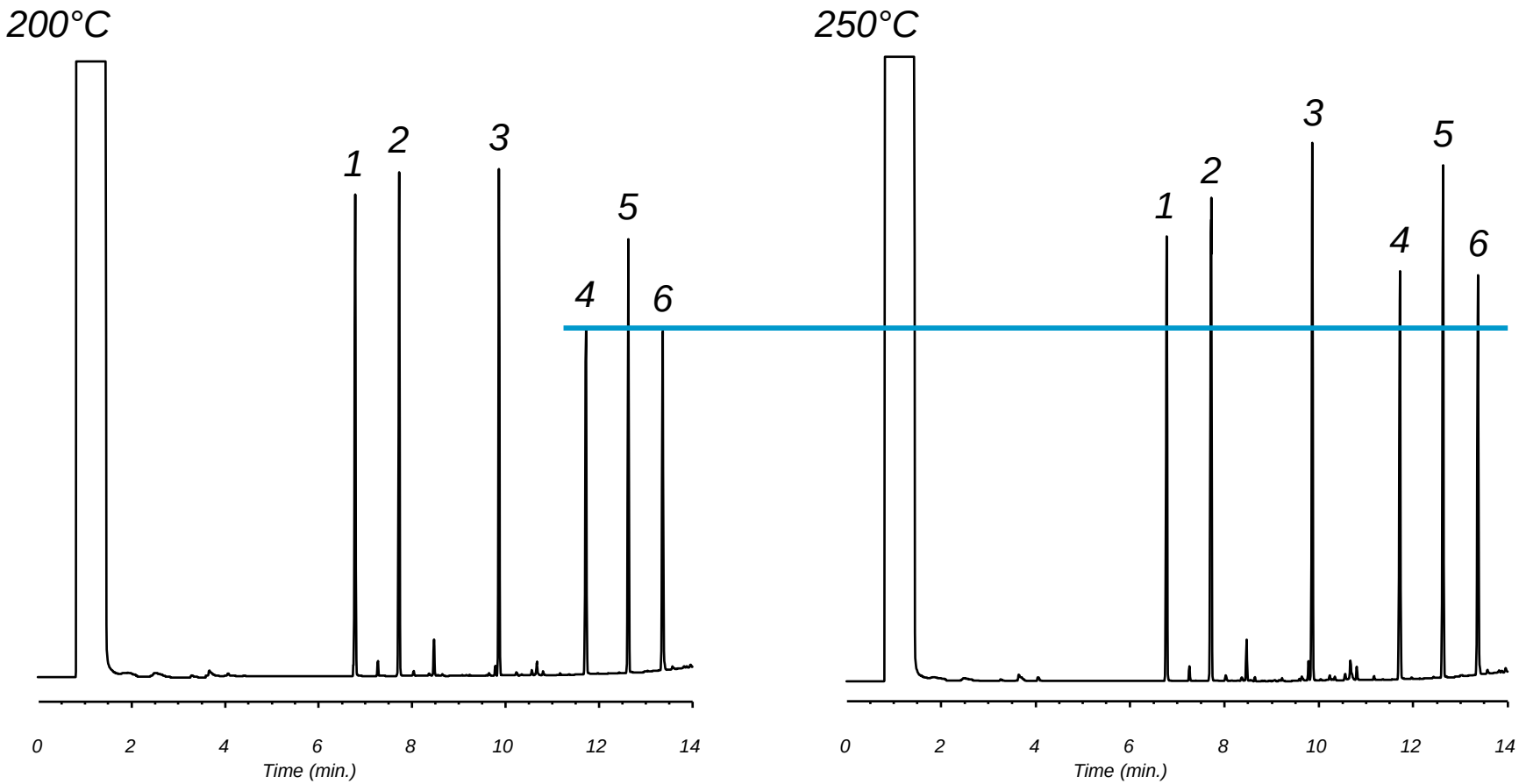




DB-1, 15 m x 0.25 mm I.D., 0.25 µm
60°C for 1 min, 60-180°C at 20°/min; Helium at 30 cm/sec
1. n-decane 2. n-dodecane 3. n-tetradecane 4. n-hexadecane

Splitless Injector

Injector Temperature



DB-1, 15 m x 0.25 mm I.D., 0.25 µm

50°C for 0.5 min, 50-325°C at 20°/min; Helium at 30 cm/sec

Phthalates: 1. dimethyl 2. diethyl 3. dibutyl 4. benzylbutyl 5.bis(2-ethylhexyl) 6. dioctyl

Splitless Injector
Sample Re-focusing

Sample re-focusing improves efficiency

Use low column temperature to refocus solvent
- called the *solvent effect*

Use cold trapping

Splitless Sample Re-focussing

- Sample refocusing
  - Also known as the “solvent effect”
  - Condenses sample as a thin film on the head of the column
  - Initial oven temperature must be at least 10 °C below the solvent B.P.
  - Increases separation efficiency and resolution and better peak shape
    - Especially for low boiling analytes

- “Cold trapping” is a version of sample re-focusing for high boiling analytes
  - Occurs when the starting oven temperature is 150 °C below the boiling point of analytes of interest
  - Condenses the analytes on the head of the column
  - Results in better peak shapes

- Solvent effect and cold trapping can occur in same sample
  - When looking at analytes with a wide distribution of B.P.s

Splitless Injector

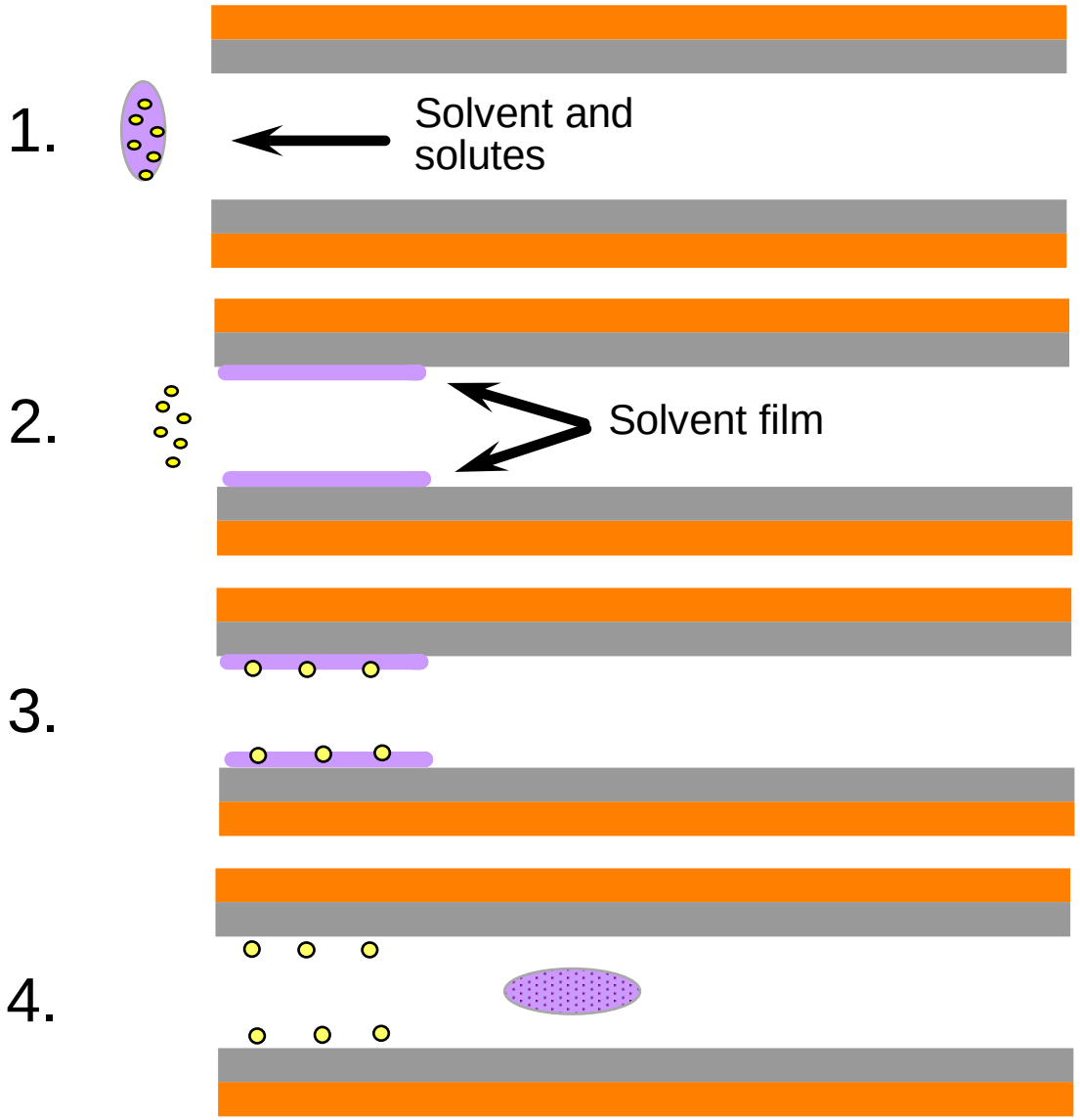
Solvent Effect

Initial column temperature at least ***10°C below*** sample solvent boiling point

Required to obtain good peak shapes unless cold trapping occurs

Rule of thumb, if solute BP >150°C above initial column temperature, the solute will cold trap

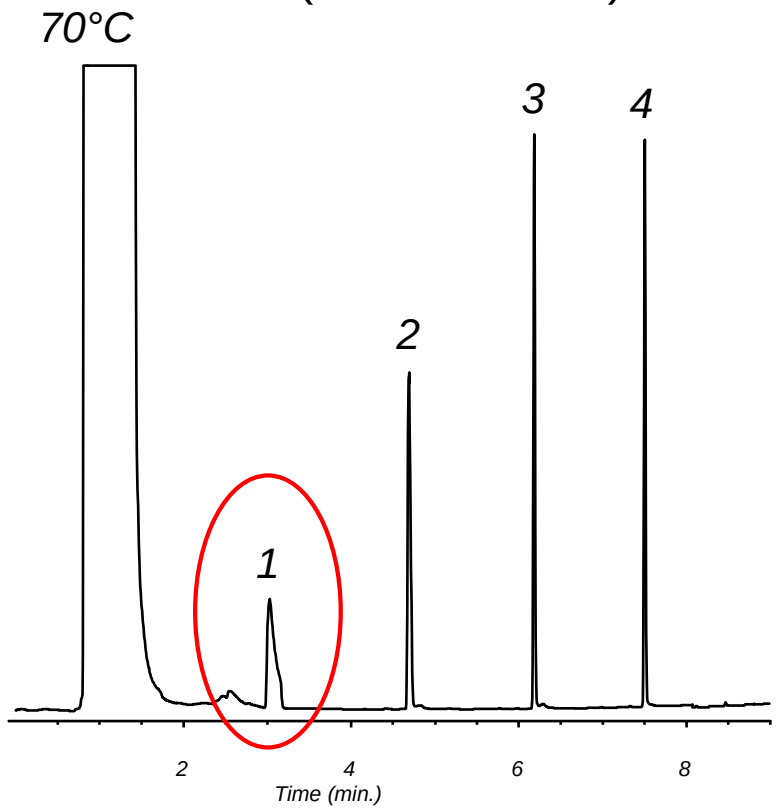
Cold trapping has greater efficiency than solvent effect



Splitless Injector

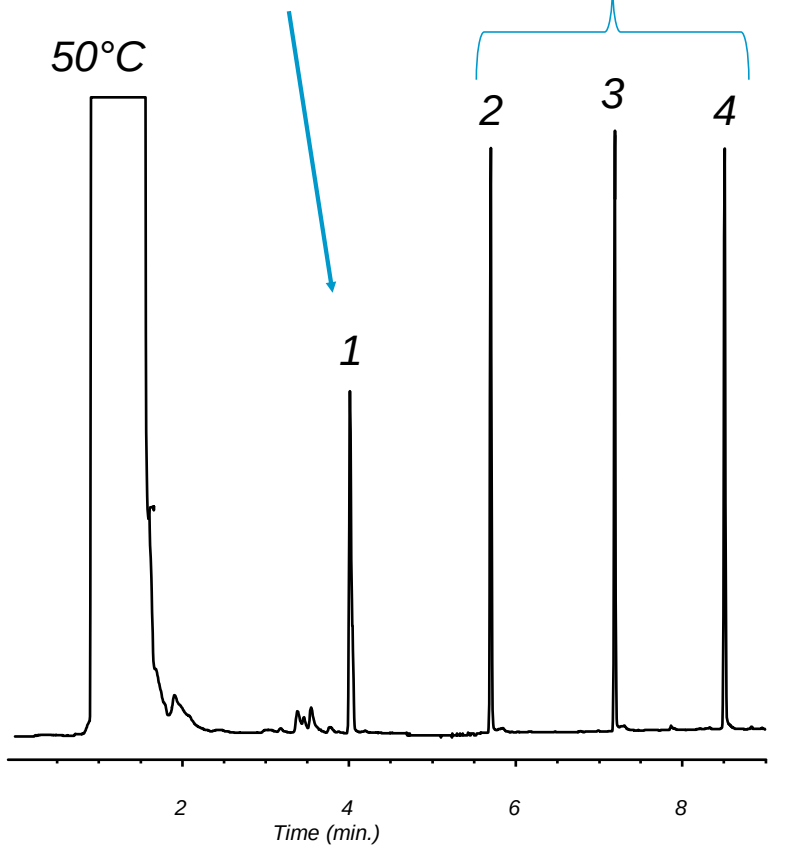
Initial Column Temperature

Hexane Solvent (BP = 68-69°C)



Solvent Effect

Cold Trapping



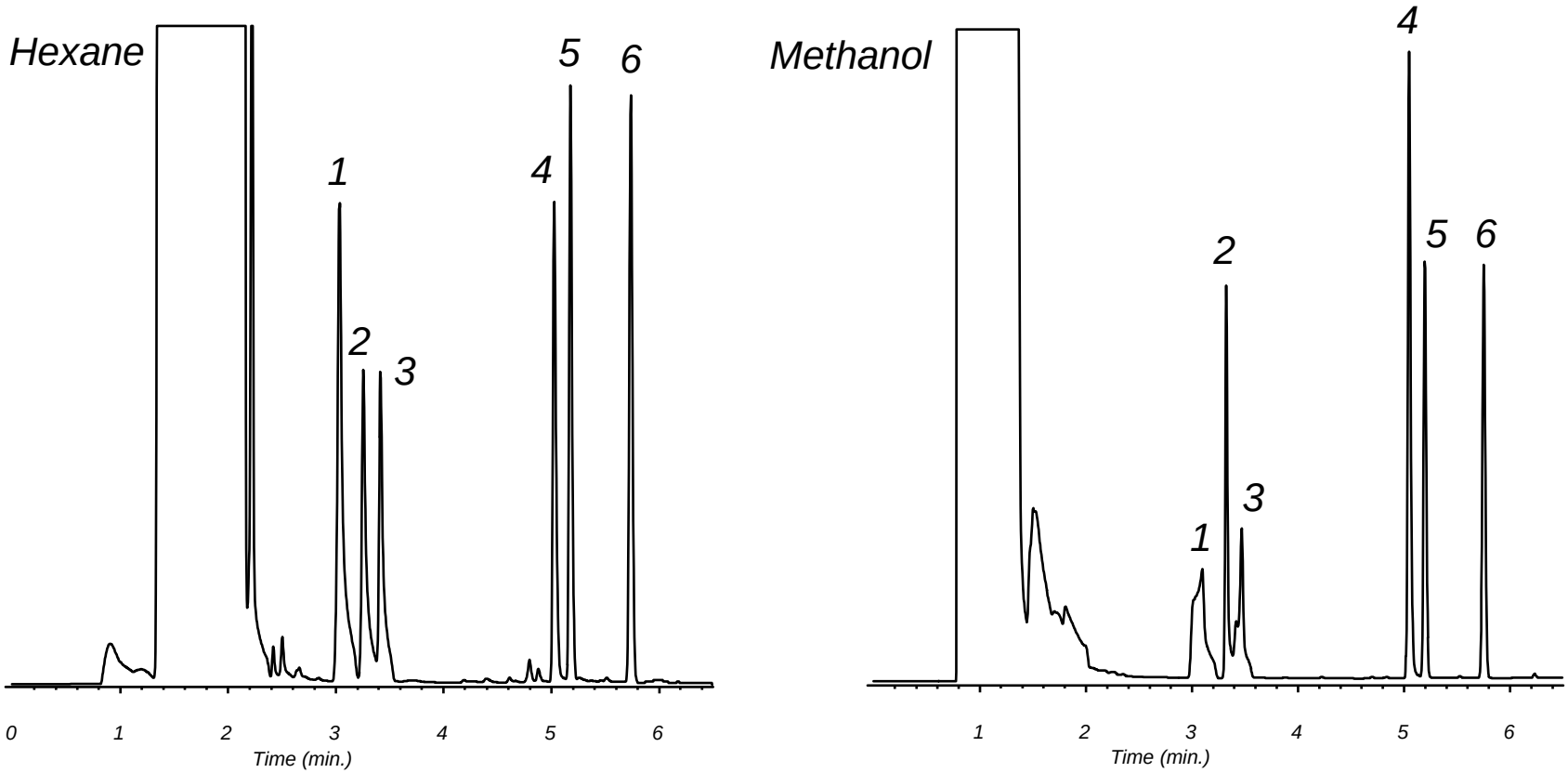
DB-1, 15 m x 0.25 mm I.D., 0.25 µm

50°C or 70°C for 0.5 min, to 210°C at 20°/min; Helium at 30 cm/sec

1. n-decane 2. n-dodecane 3. n-tetradecane 4. n-hexadecane

Splitless Injector

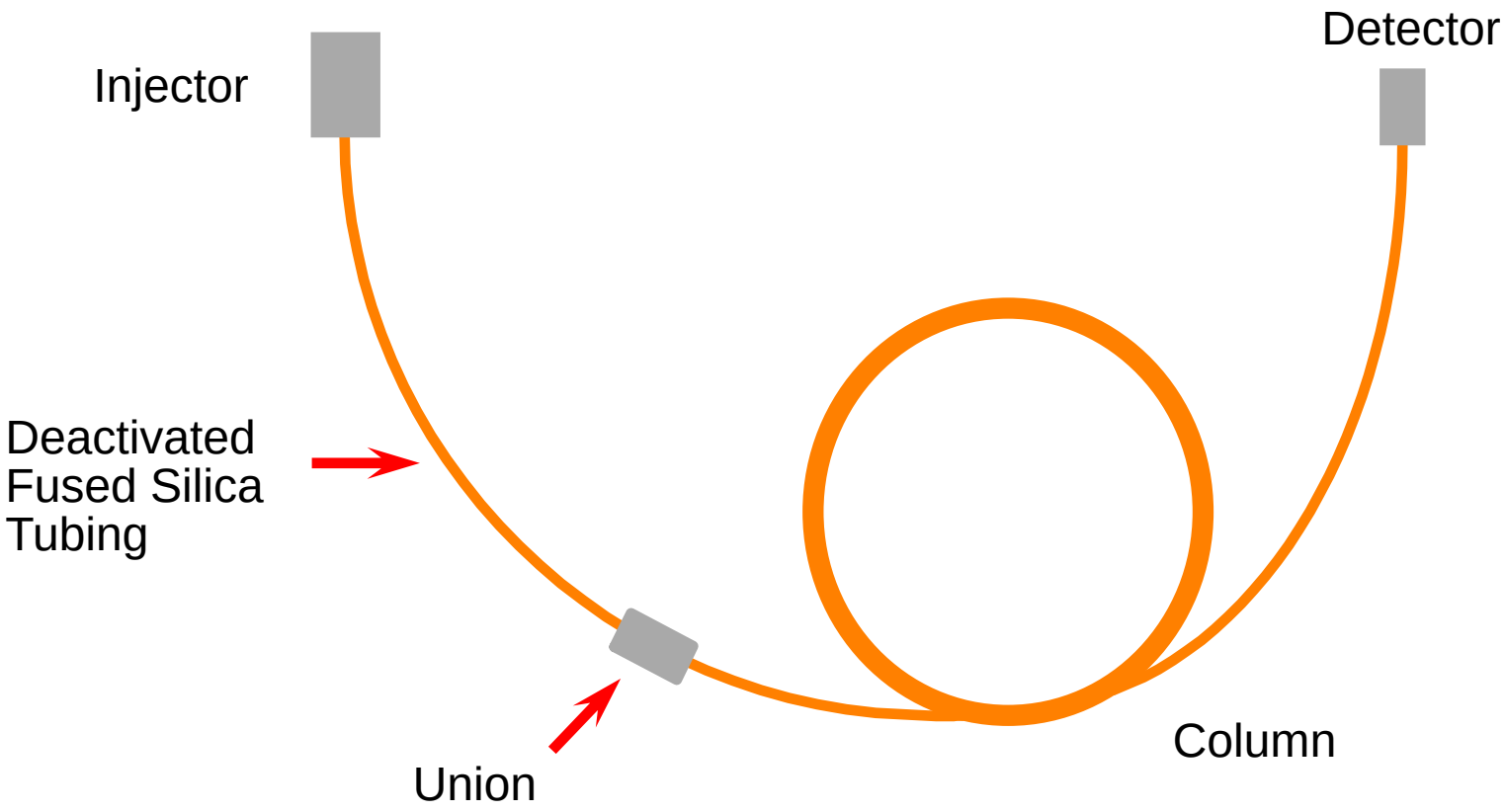
Reverse Solvent Effect/Polarity Miss-Match



DB-1, 15 m x 0.25 mm I.D., 0.25 µm
50°C for 1 min, 50-210°C at 20°/min; Helium at 30 cm/sec
1. 1,3-DCP 2. 3-hexanol 3. butyl acetate 4. 1-heptanol 5. 3-octanone 6. 1,2-dichlorobenzene

Retention Gap

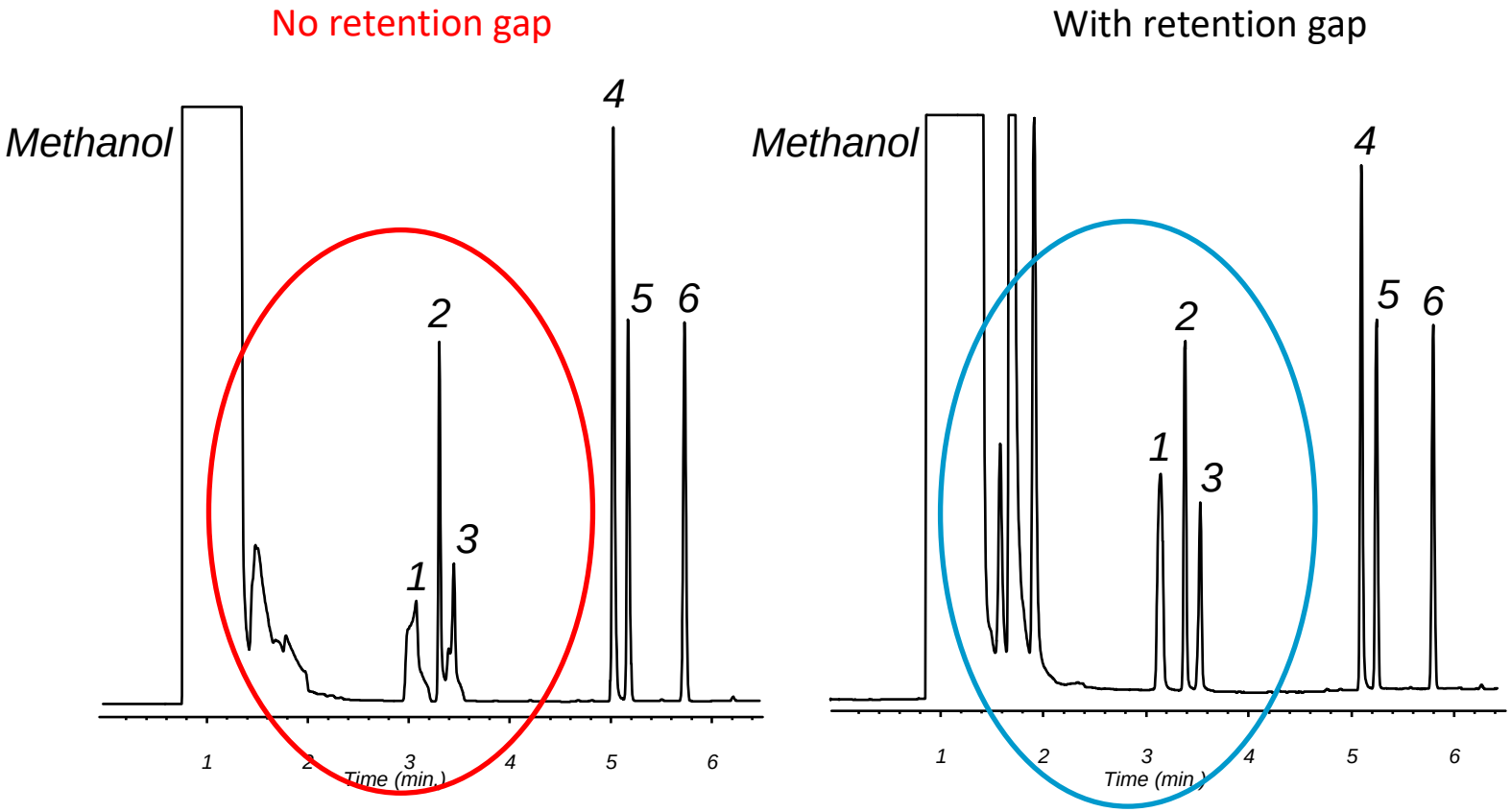
Also Called A Guard Column



Usually 2-10 meters long and same diameter as the column
(or larger if needed)

Splitless Injector

3 m x 0.25 mm I.D. Retention Gap



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EPC for Pulsed Splitless Injection

Pressure Pulse contains sample expansion
and transfers analytes to the column faster.

Pulsed Splitless

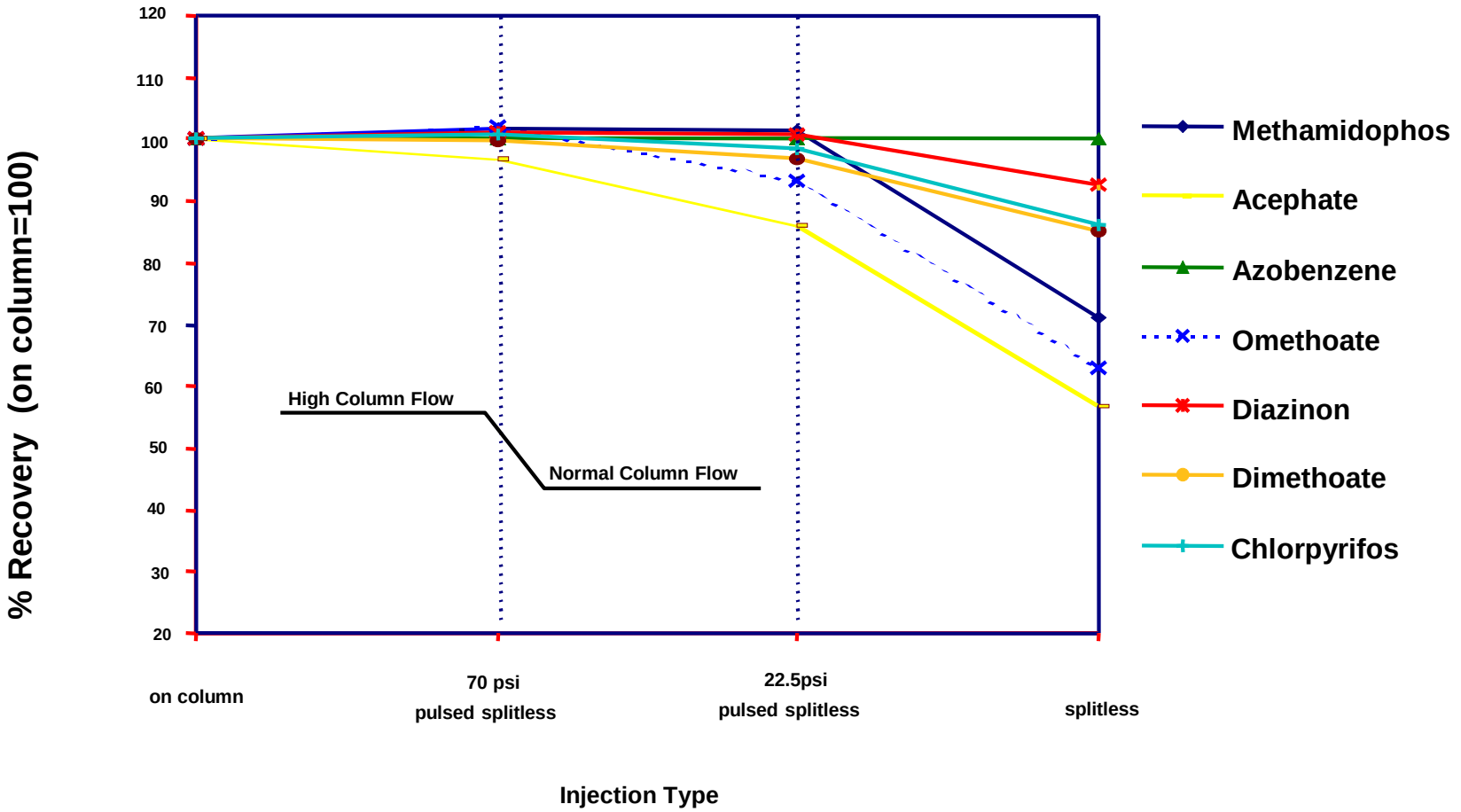
- sample containment more critical than in split injection
- sharper peaks than in traditional splitless injection
- two new parameters to set:
    - pulse pressure and pulse time

Typical starting point

- Pulse pressure = double resting pressure
- Tie pulse time to purge time

Benefits of the Pulsed Splitless Mode

% Recovery of Each Labile Pesticide Relative to Cool On-Column injection



Splitless Injections – Starting Parameters

Injection Volume = 1 uL

- Check the Pressure-Volume Calculator

Initial Oven Temp = 10°C < solvent boiling point

Purge Flow = 20 to 60 mL/min

Purge Time = 0.75 min

- Sweep with 2 liner volumes of carrier gas

No pulse

Try to avoid water and methanol as solvents

Splitless Injections – Troubleshooting Tips

Injecting too much

- column overload = poor peak shape
- inlet overload = poor reproducibility
  - ghost peaks in subsequent blanks are possible

No glass wool

- poor mixing
- dirt on column

Glass wool

- reacts with trace components

Splitless Injections – Troubleshooting Tips

If you think you have an inlet issue related to splitless injections

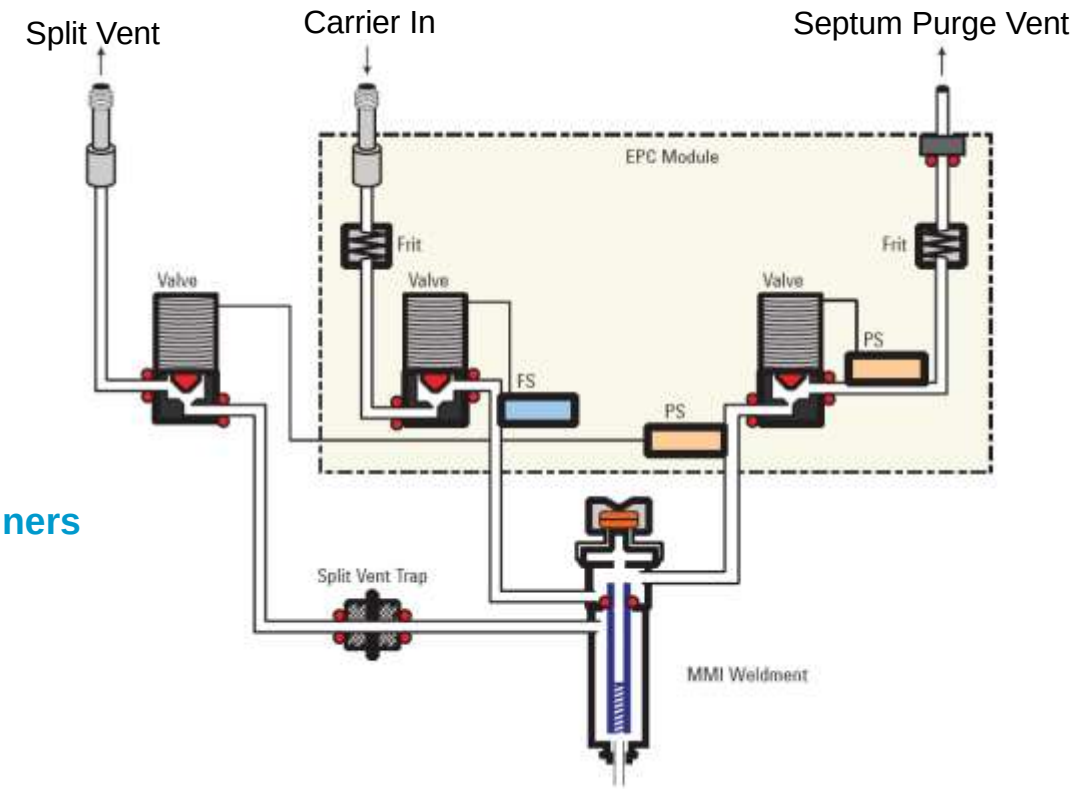
- Run a 10:1 split injection
- Make up a standard at 10x concentration and run a 10:1 split injection

When I changed from split to splitless I didn't see an increase in response!!!

Verify that the purge time is not set to 0 min. Try increasing the purge time.

MMI Inlet

Split/Splitless + PTV



Hot split/splitless (also pulsed)

- similar to the S/SL inlet using the **same liners**
- all previous S/SL discussions apply here

Cold split/splitless (also pulsed)

- Significantly more inert than hot splitless
- Can inject 3-5 uL with no solvent venting
- Better sensitivity than hot splitless because large vapor cloud is not formed which travels outside the liner and portions are lost

LVI-Solvent Vent

- An extension of cold splitless
- Large volume injection for maximum sensitivity

Direct Mode

Uses a Direct Connect Liner – simulates COC * NO purge

MultiMode (MMI) Inlet Features

Hardware

Temperature range of -160C to 450C

Heating @ 15C/sec (900C/min)

Septum/Liner Easily Exchangeable using Turn Top Inlet

Injection Modes: Hot S/SL, Cold S/SL, all in pulsed mode, solvent vent mode, residue removal mode

Support for single stroke injections from 0.1 μL to 250 μL

MultiMode Inlet Solves Many Problems

Performing large volume injection (LVI) of relatively clean samples?

- programmable injection slows solvent evaporation and maximizes analyte transfer into the column/detector
- decrease MDL by injecting more sample

Injecting dirty samples?

- matrix vent, backflush and easy liner changing minimize dirty sample affects

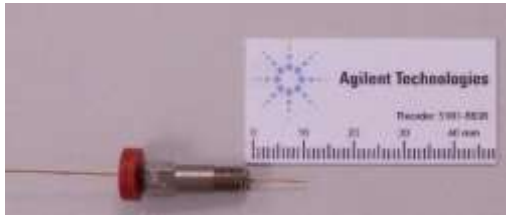
Performing analyses of high molec. wt. and/or thermally labile compounds?

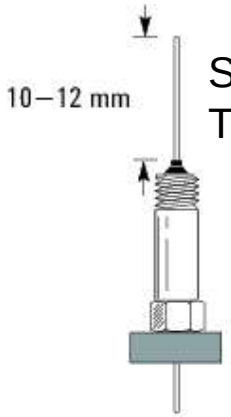
- temperature programming of Multimode inlet elutes analytes at the lowest possible temperature, minimizing breakdown and absorption
- discrimination of high molec. wt. compounds is minimal allowing HT GC

MultiMode GC Inlet - Cold Injections

- No syringe-needle discrimination; Minimal inlet discrimination
- No special syringes, liners or consumables
- Large volume injection (5ul to 250ul) - lower detection limits
- Solvent vent/matrix vent - decrease interference / maintenance
- Flexibility (hot/cold split/splitless, temperature programmed vaporization)
- Cold trapping in liner - improves chromatographic peak shape, resolution
- Capillary column backflush with CFT - decreases cycle time, maintenance

MMI Column Installation





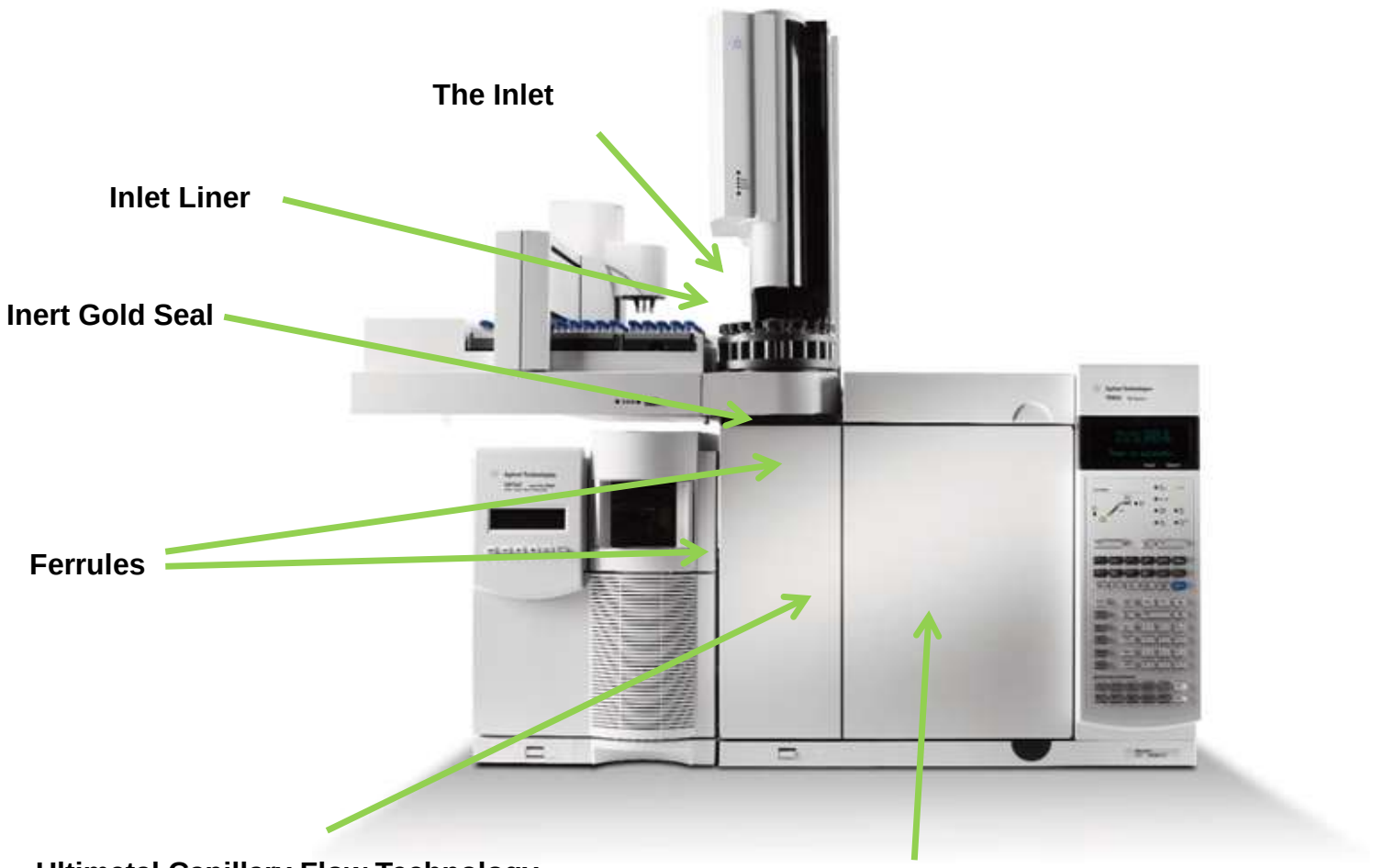
Set to 10-12 mm
Trim the column

- Graphite ferrules are recommended over Vespel

Thread the column into the column adapter – Stabilize the column adapter with a 5/16” wrench

Tighten the column with a 1/4” wrench – continue to hold the column adapter with a 5/16” wrench

GC surfaces that touch the sample



Root Causes of Inlet Performance Degradation, and Consequences

Accumulation of Sample Residues

- Loss of response, tailing on active analytes, split vent trap fouling and inaccurate EPC flow control

Accumulation of Consumables wear particles

- Same as Accumulation of Sample Residues, plus “bleed peaks”

Leak in Septum Nut, Septum

- Damage to O_2 sensitive detectors, irreversible damage to column

Non-Optimized Set-up

- O-ring, Gold Seal, Ferrules, Column Nuts
- Faster inlet performance degradation between maintenance sessions

Inlet Liner Troubleshooting

- Many chromatographic problems are blamed on the column.
- Often, a dirty liner is the culprit.

Symptoms include:

Poor peak shape

Irregular baselines

Poor resolution

Poor response

Splitless

Silylated Glass Wool

- Traps non-volatile materials and mixes sample
- Peak shape and discrimination affected by amount, location and packing density

Splitless Liner Maintenance

- Liners become contaminated with use, collecting non-volatiles, salts, excess reagents, etc., or become damaged/cracked.
- Should inspect and replace liners often.
- Handle with gloves and forceps.
- Insert into or remove liners only from cool injection ports.
- Replacing with a new liner is recommended, to ensure reproducibility

Leak in Septum

Using septa beyond lifetime/temperature recommendations.

•“Use environments” that decrease lifetime include manual injections, wrong syringe tip type, larger gauge syringes, non-Agilent Autosamplers (Agilent’s are precisely aligned).

• Septum Type and Syringe Needle type mating are essential to minimizing leak rate.

Tips to Maximize Septum Life, Minimize Septum Leaks

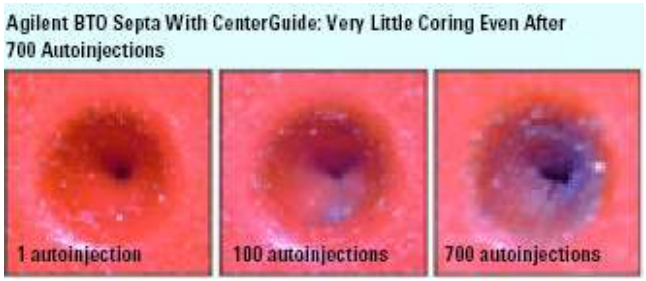
– Use Agilent Gold Standard, 23-26 gauge, HP Point taper syringes. The point style cores septa significantly less when used with CenterGuide Septa. Taper minimizes septum coring/wear.

HP-Point Style

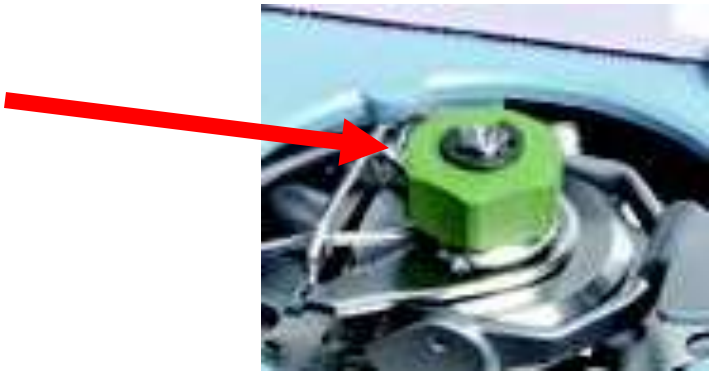
– Use Agilent CenterGuide Septa. The molded hole minimizes septa coring, counter-intuitive, but true.

Solid Septum

CenterGuide Septum



Leaks Due to Septum Nut



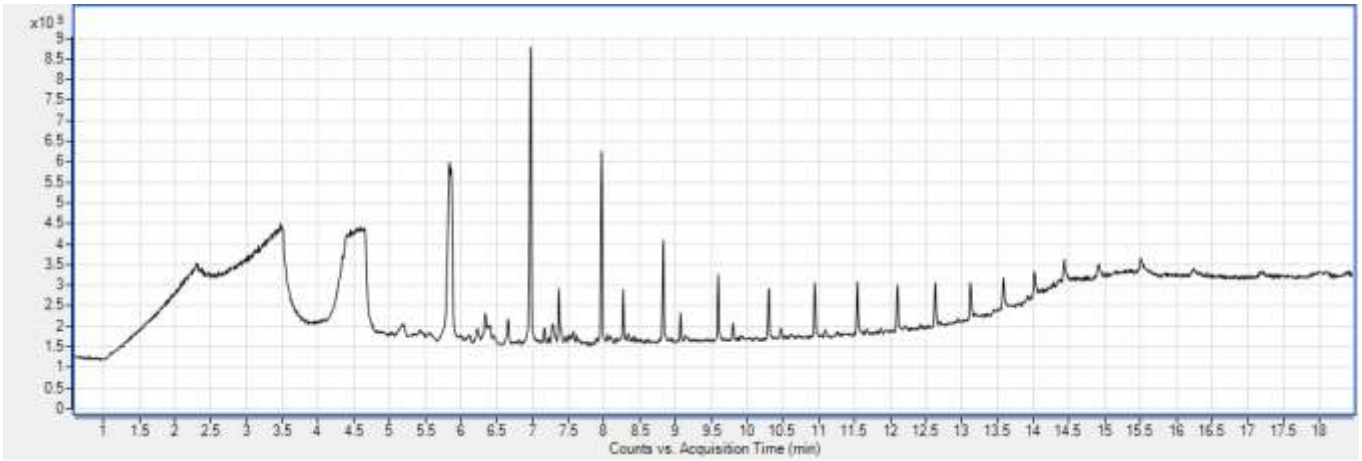
- With repeated use, conical needle guide gets worn, out of round, and needs replacement as septum can begin to “bulge” out, especially with excessive tightening,
- Septa fail faster because needle is not guided with as much precision.
- Under or Over tightening—tighten nut until c-clamp on top stops turning, then ½ to ¾ turn more.
- Non-Agilent septa may be too thin, too thick, or out of round like die-cut septa and may not seal as well.
- “Use Environments” that decrease lifetime, like using non-Agilent Autosamplers (ours are precisely aligned), manual injection, larger gauge syringes
- Replace septum nut annually for peace of mind.

Septum maintenance: Septum coring

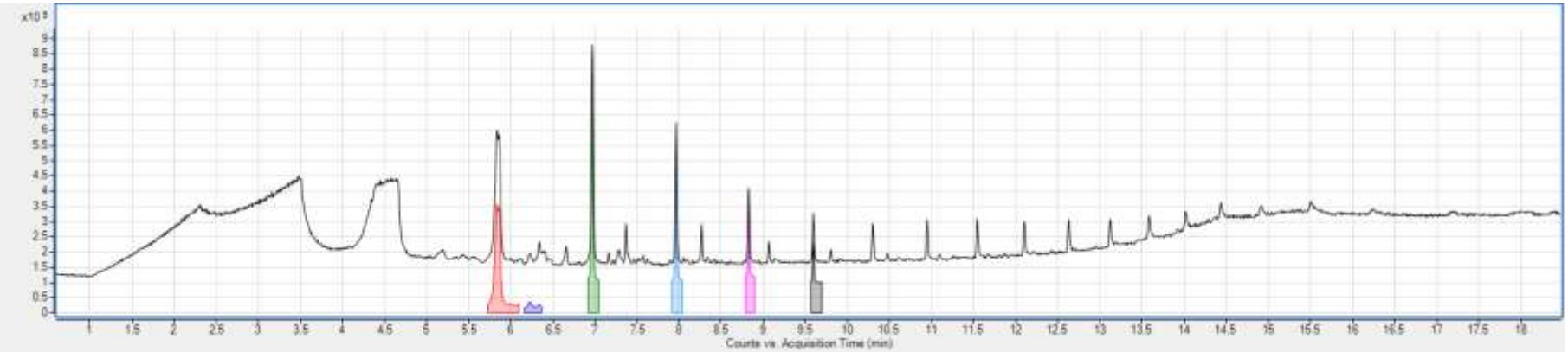
- After many injections, pieces of rubber from the septum may break off and fall into the inlet liner.
  - This is called septa coring
  - Replace the inlet septa and liner frequently to prevent septa contamination
  - Use a cone tipped syringe to reduce the chance of tearing the septum

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



Septum maintenance:
Deconvoluted inlet septa spectrum



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

Main Siloxane Peak Bandit!!!

Multiple injections from same vial

Dissolve silicone into sample and inject

Examples of Non-Optimized Operation

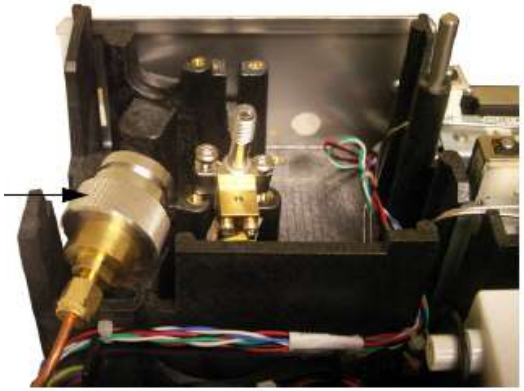
Typical Cause—Re-use and mis-installation.

- Leak from O-ring, Gold Seal, ferrules, column nuts
- O-rings are elastomer compression fittings designed for one use, not perfectly elastic.
- Gold seals are designed for one use, knife edge cuts into gold layer giving leak tight seal w/o shrinkage or potential organic contaminants from polyimide out-gassing/degradation.
- Re-using could result in overlap in seal rings, resulting in a leak.

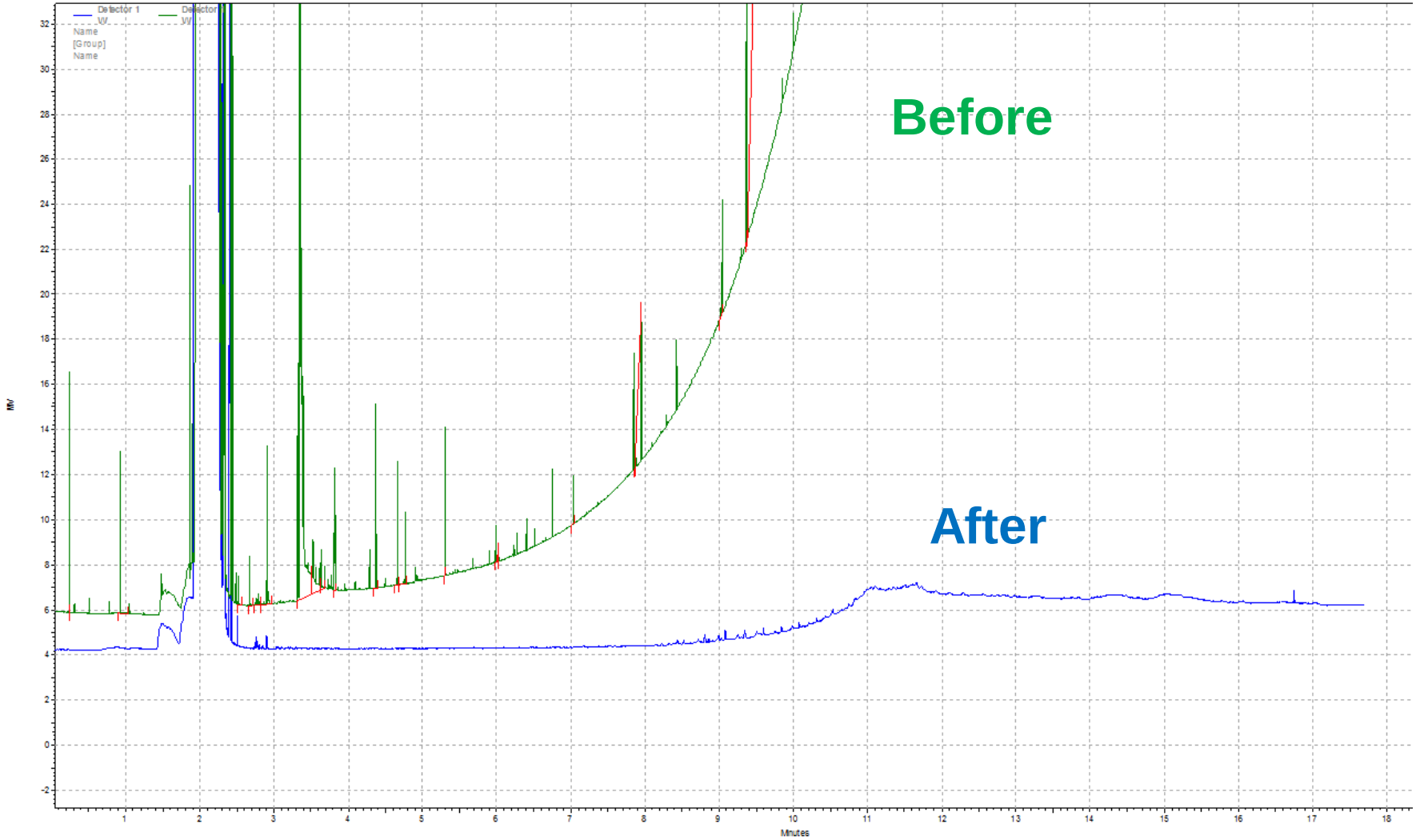
Split Vent Trap

What is it???

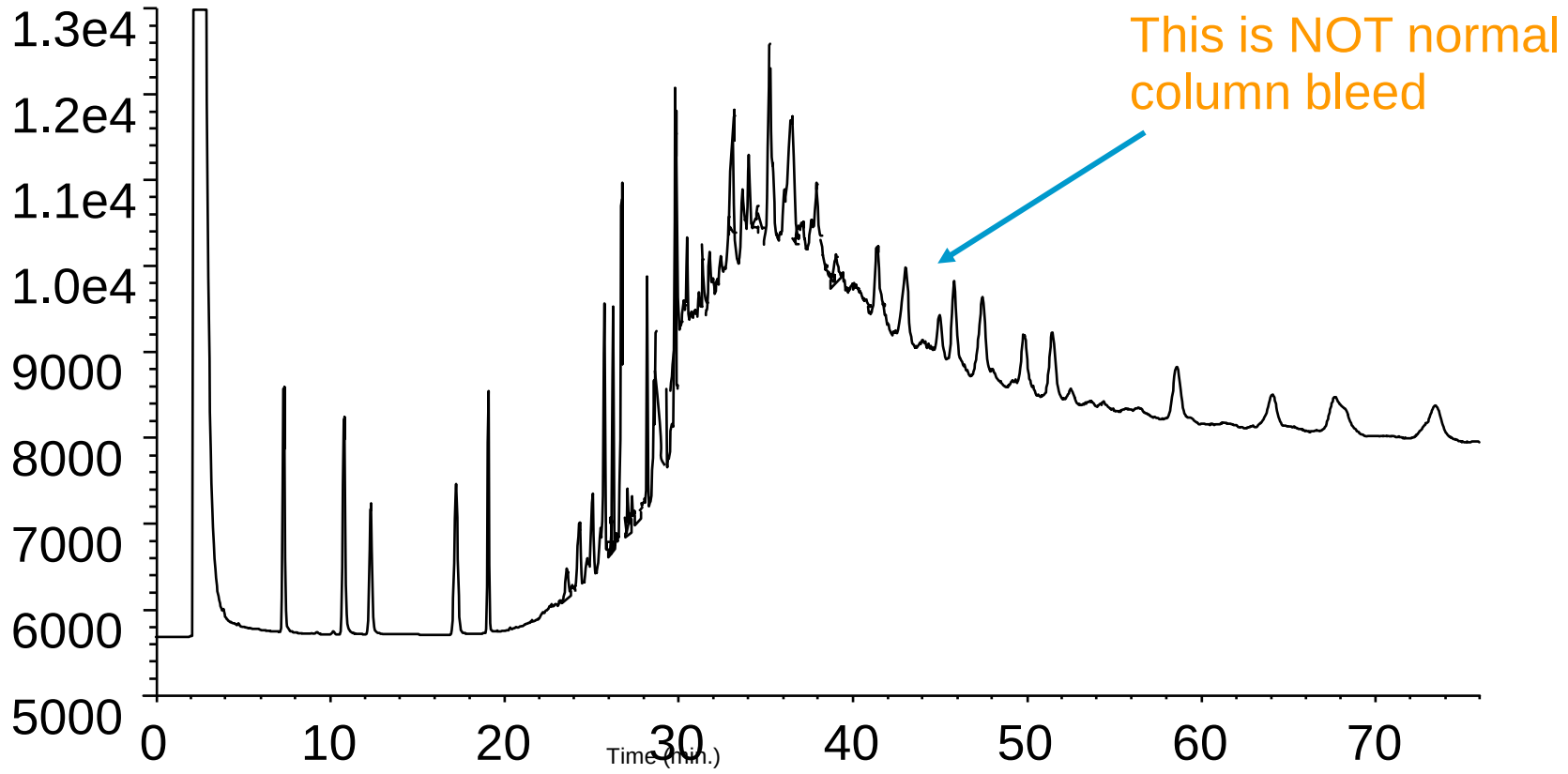
Where is it???



Split Vent Trap Changed (Column Bleed?!?)

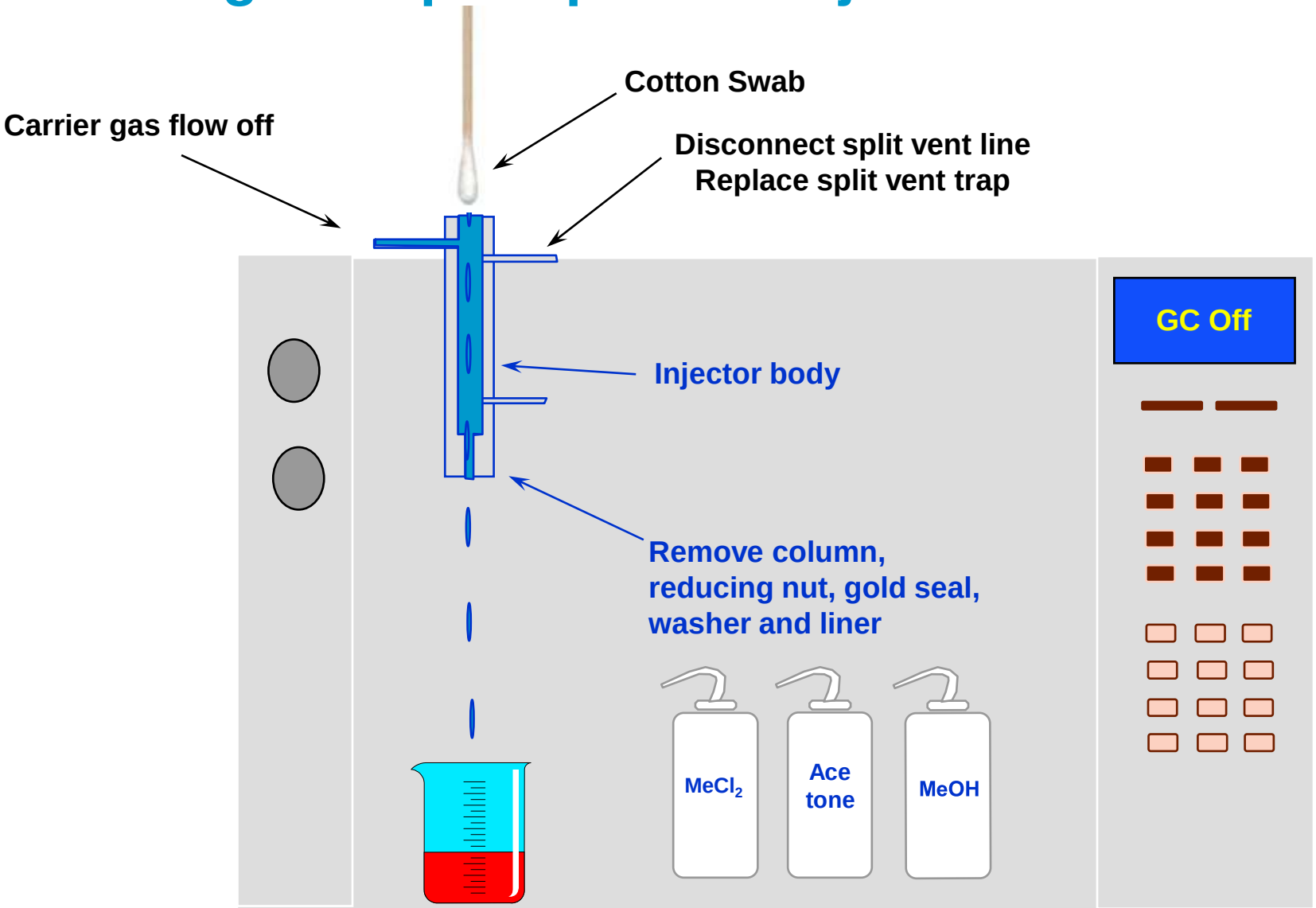


Example Of Gross Contamination

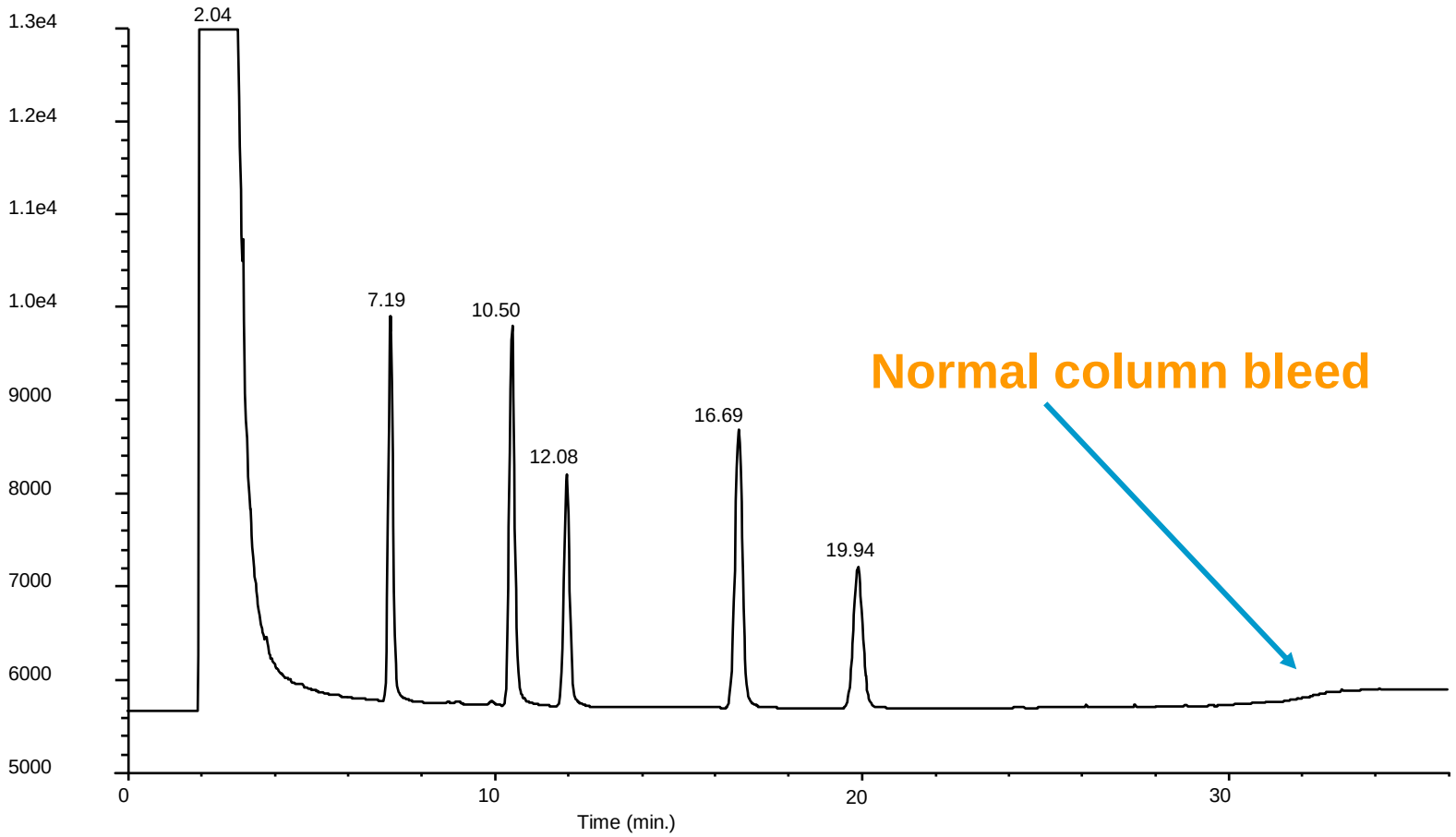


DB-624, 30 meter megabore
Temperature program // 35°C, hold 1.50 min // 30°/min to 65°C, hold 15 min // 20°/min to 260°, hold 50 min

Cleaning the Split/Splitless Injector

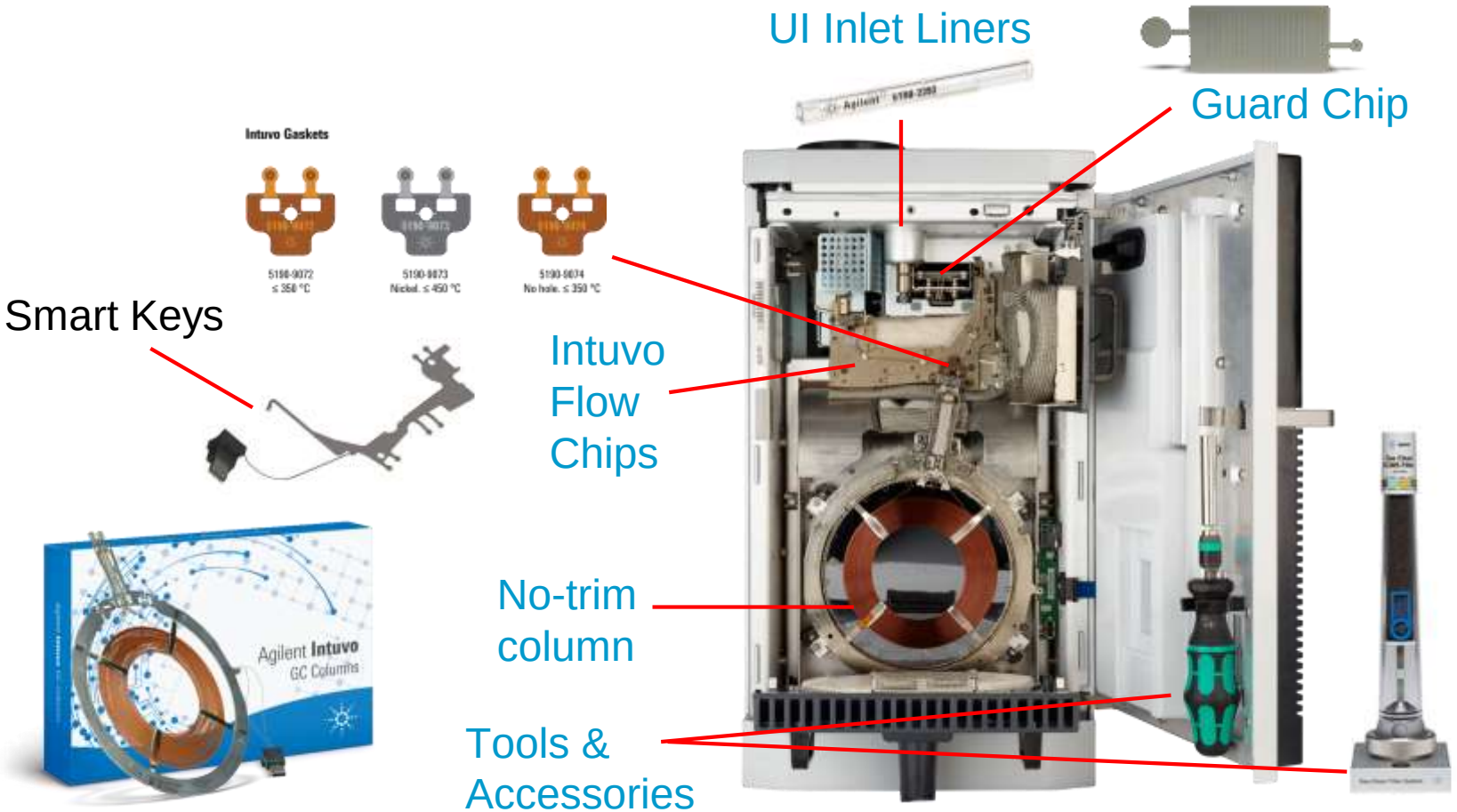


Same Column After Inlet And Column Maintenance



*Temperature program // 35°C, hold for 1.50 min // 30°/min to 65°C, hold 15 min // 20°/min to 260°C for 5 min

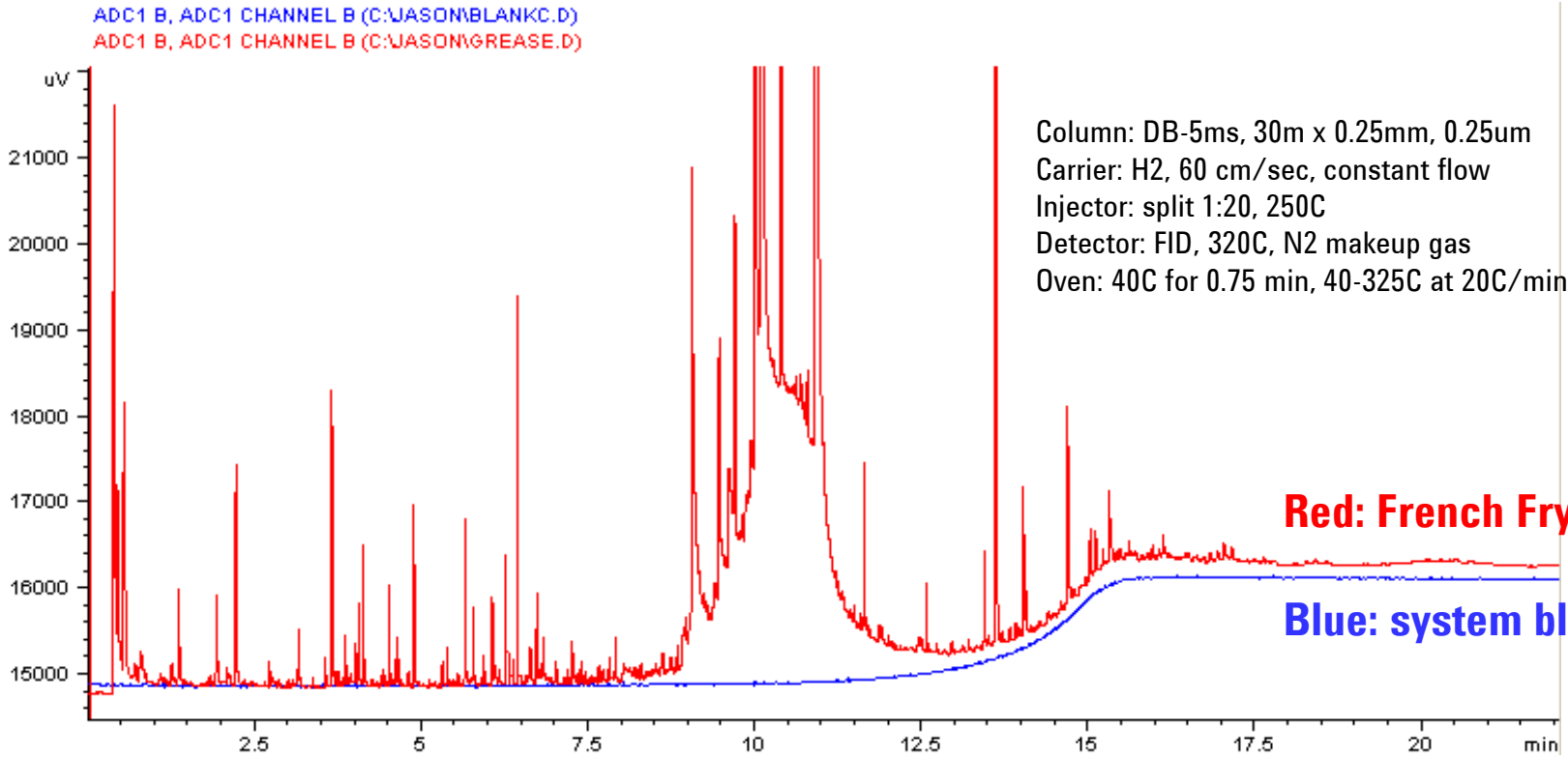
A New Portfolio of GC Consumables



You are Proactively Doing Maintenance

But…..

Contamination of system by residue on fingers during column installation



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

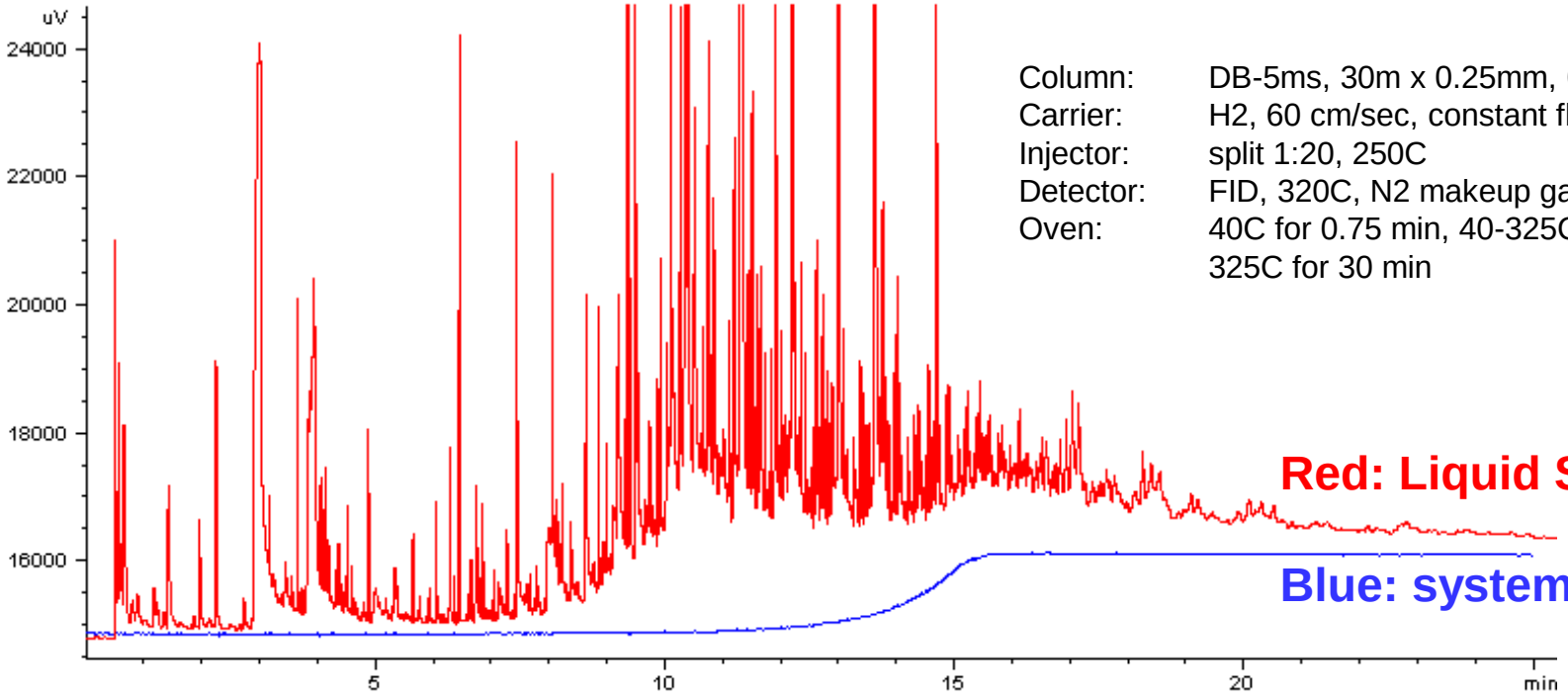
Red: French Fry

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.

Contamination from Liquid Soap



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

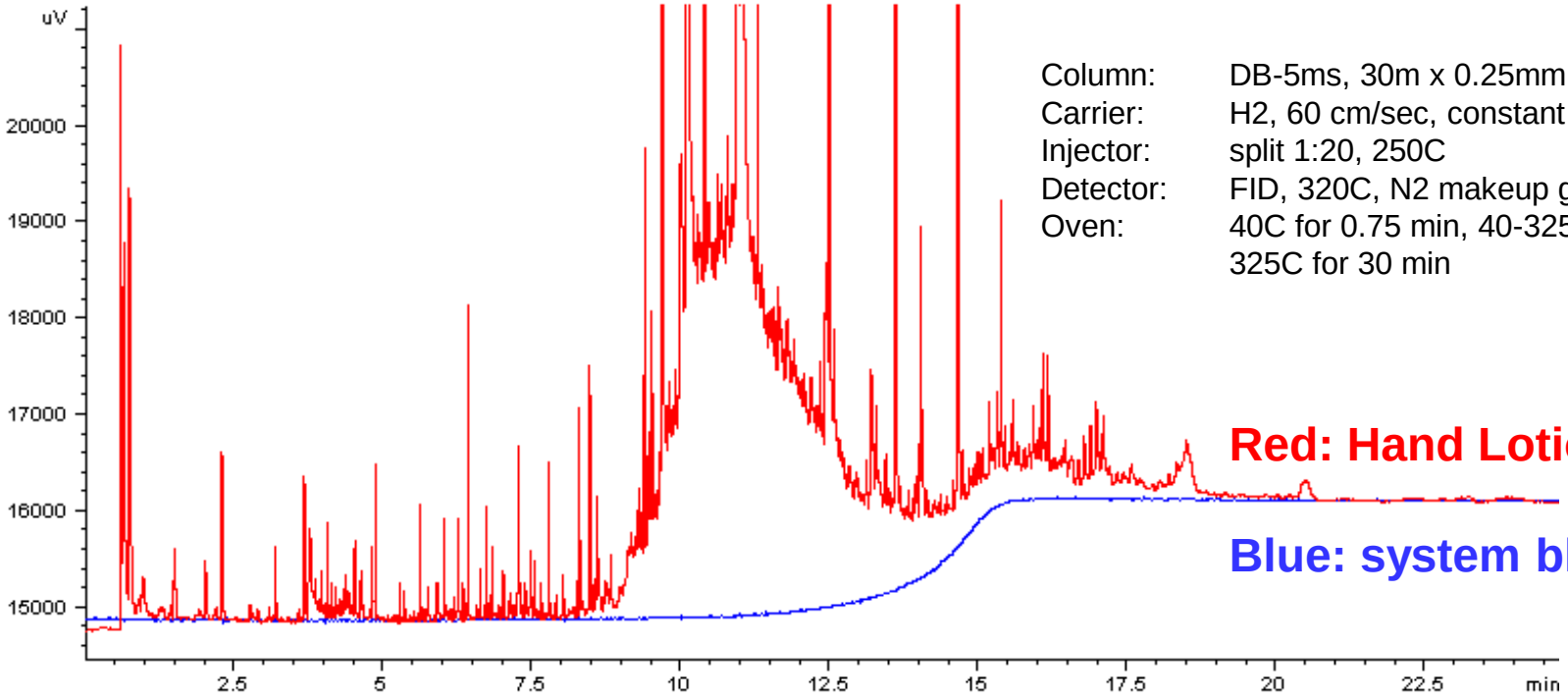
Red: Liquid Soap

Blue: system blank

Procedure:

(1) One very small drop of liquid soap 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 40C.
(6) Started oven temperature program as soon as oven reached 40C.

Contamination from Hand Lotion



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

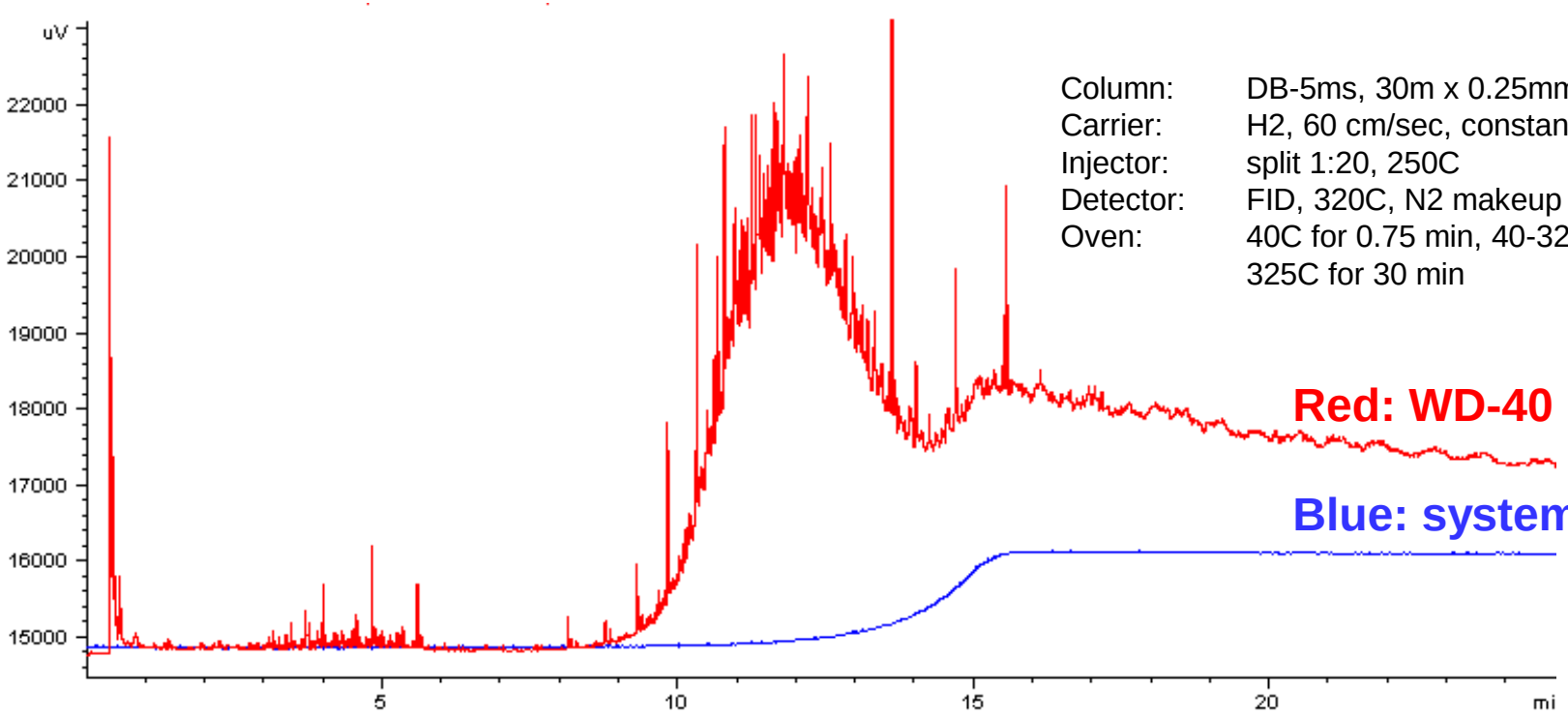
Red: Hand Lotion

Blue: system blank

Procedure:

(1) One very small drop of hand lotion 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 40C.
(6) Started oven temperature program as soon as oven reached 40C.

Contamination from Lubricant



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: WD-40

Blue: system blank

Procedure:

(1) One very small drop of WD-40 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 40C.
(6) Started oven temperature program as soon as oven reached 40C.

When do I Change What?

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

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

Conclusions

- Start off with good inlet parameters!
- Develop a maintenance schedule that fits your application and sample load
- Don’t skimp out on replacing your inlet consumables
- Use the same type of liner for the same type of application
- Trim more than 2 inches from the front of the column

  Back end of the column is clean!!!

- When in doubt….

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