Making a Grand Entrance: Techniques for Efficient Sample Introduction, Inlet Types, and Maintenance

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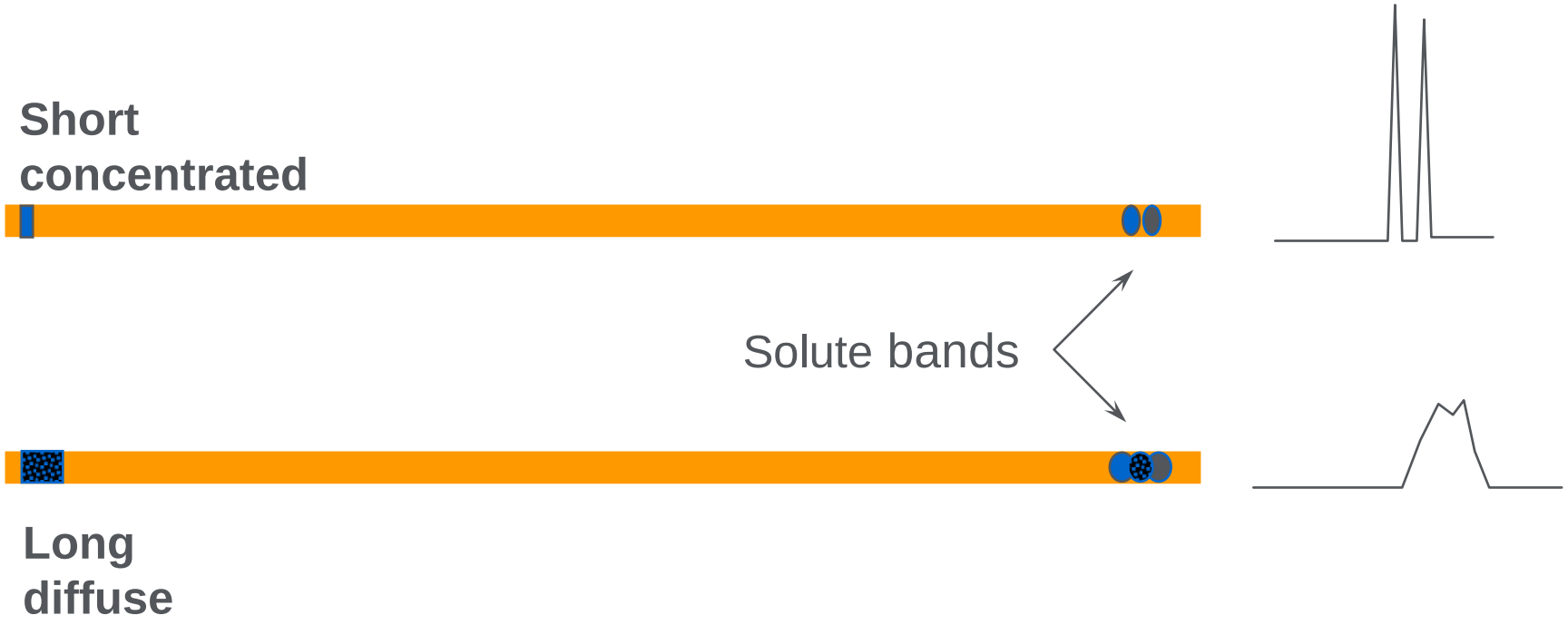
March 19, 2019

Sample Injection Goals

- Introduce sample into the column
- Reproducible
- Minimize efficiency losses
- Representative of sample

Influence of Injection Efficiency



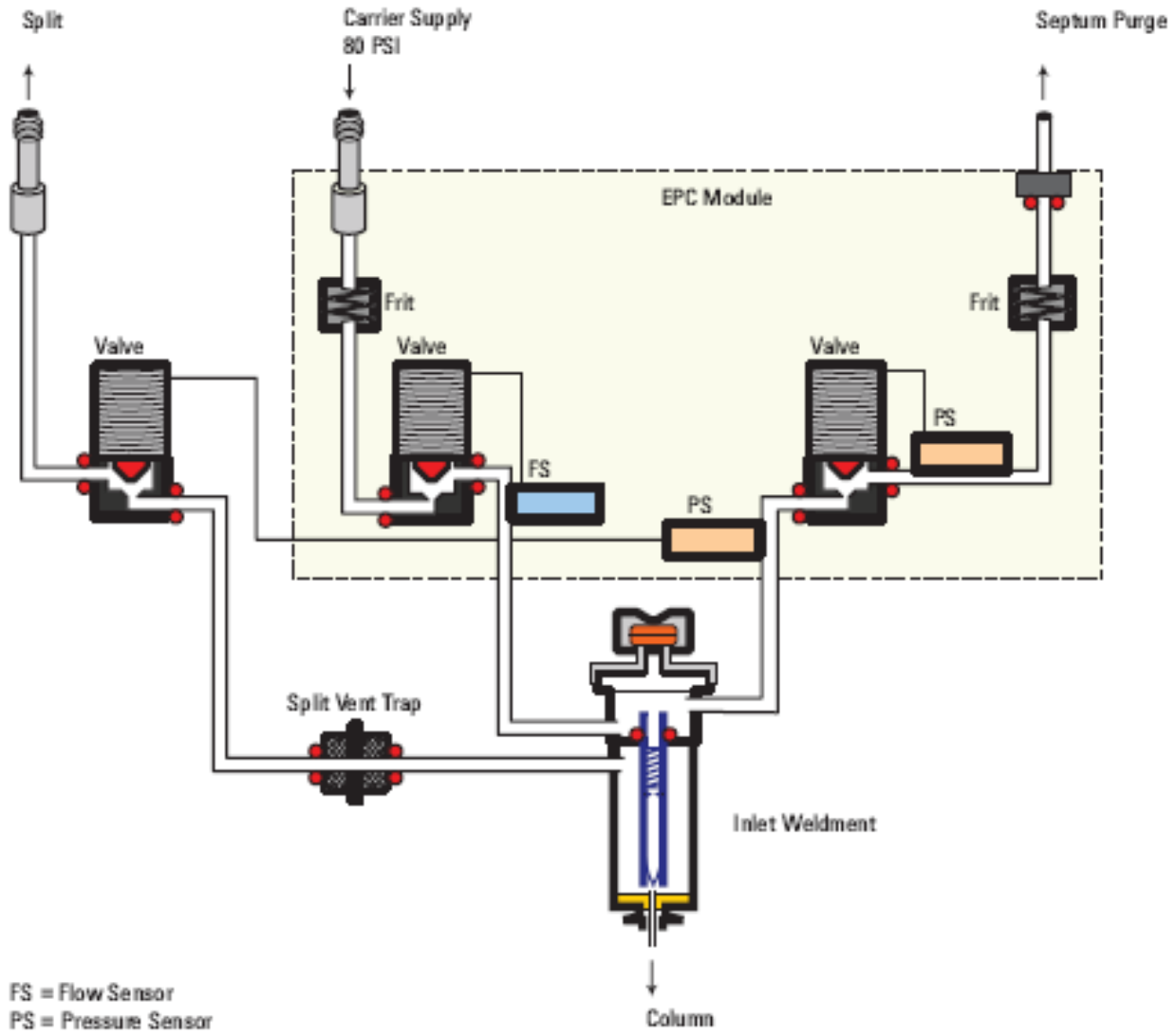
Same column, same chromatographic conditions

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 decomposition	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 and Trap/Headspace	All Very little All
Multimode	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 less problematic (compared to splitless) – 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/consumables
  - Shorter inlet residence time

Glass wool is a compromise

- Exhibits some activity (even if deactivated) – high surface area
- Greatly improves fluidic performance – mixing of the vaporized sample is important for uniform splitting
- Thermal mass/high surface area aides vaporization

Split Liners: Recommended Liner

Split/splitless liner with glass wool, low pressure drop

Split injections have higher carrier gas flow through liner

- Faster transfer onto column
- Split liners have a smaller outer diameter than splitless liners to accommodate high flow to split vent

If potential exists for sample discrimination between low and high boiling components

- Use a liner with wool

Agilent Ultra Inert liners enable excellent peak shapes for tricky analytes

- 5190-2295 is recommended liner; single taper, low pressure drop

Touchless packaging ensures contamination-free installation

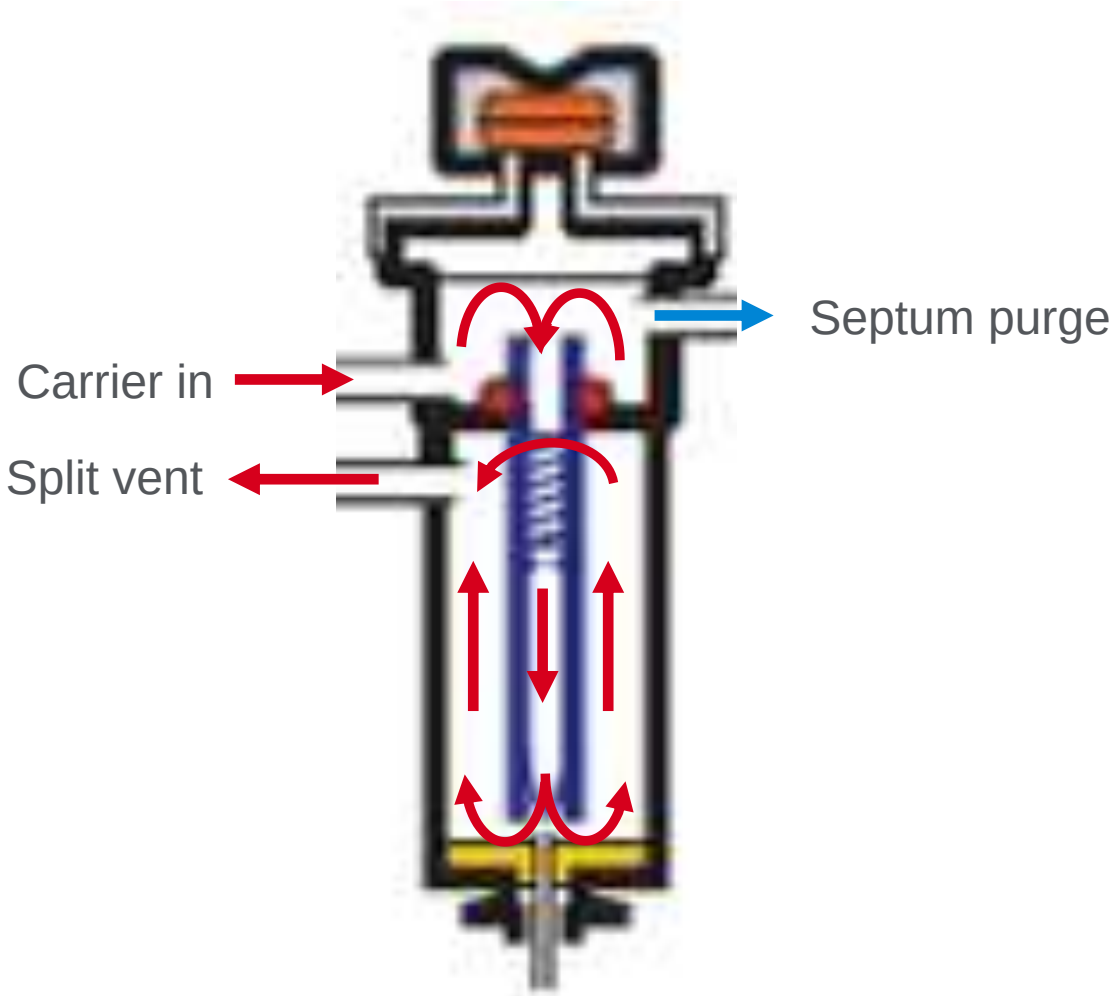
Preinstalled O-ring

Glass wool plug in upper position wipes needle, avoids sample discrimination improves P&A, and collects septum particulate

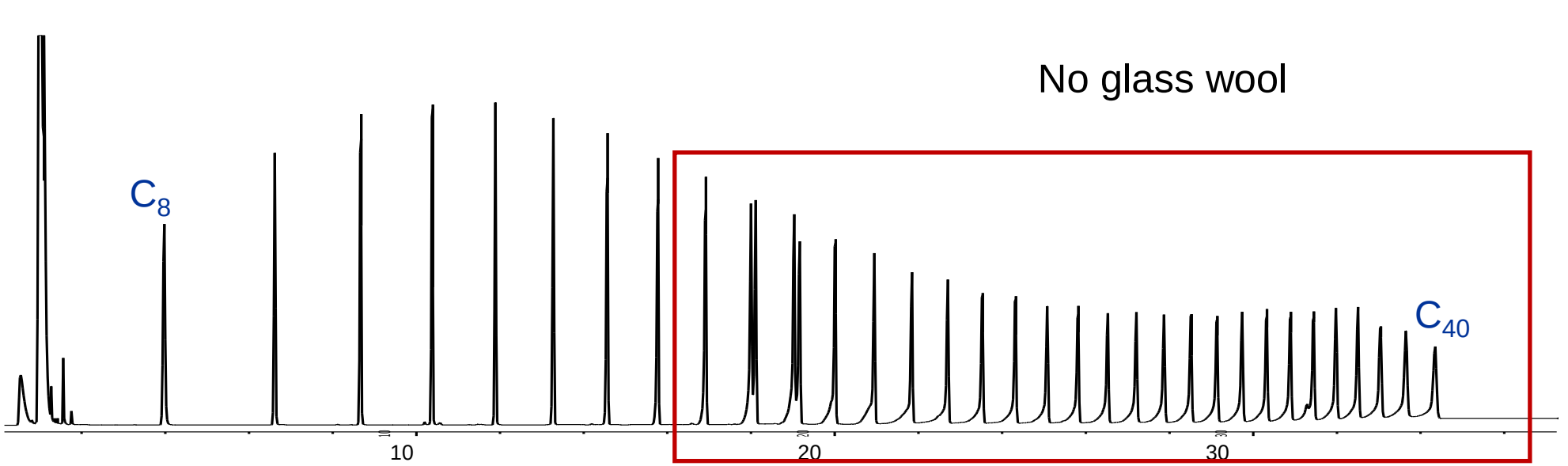
Low pressure drop bead promotes better carrier gas flow for split injections

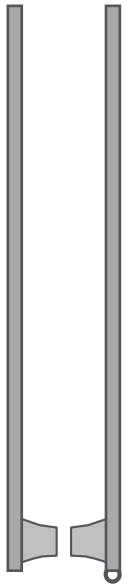
Split flow path

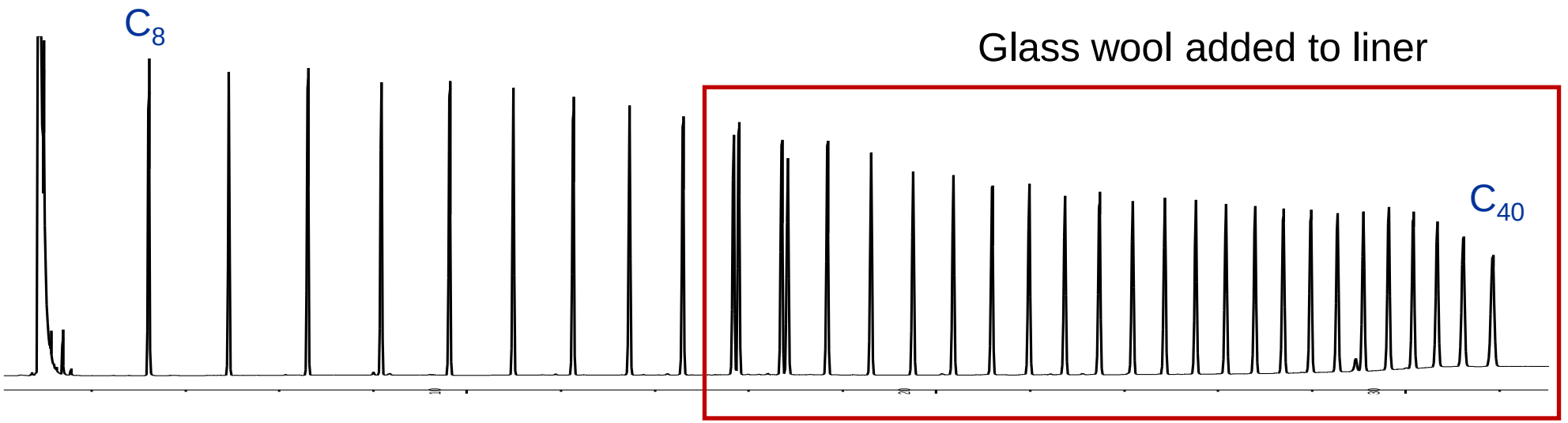
Smaller liner od with ***split*** liners accommodates the higher flow to the split vent

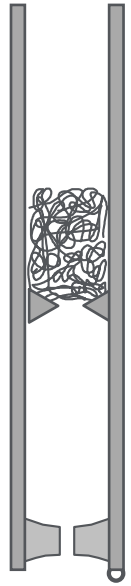


What Does Mass Discrimination Look like?









Split Injections – Maximizing Sensitivity

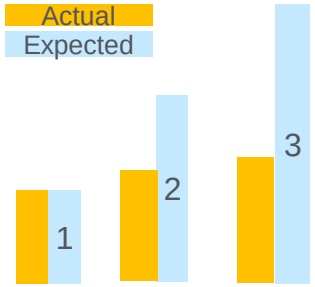
Increase injection volume

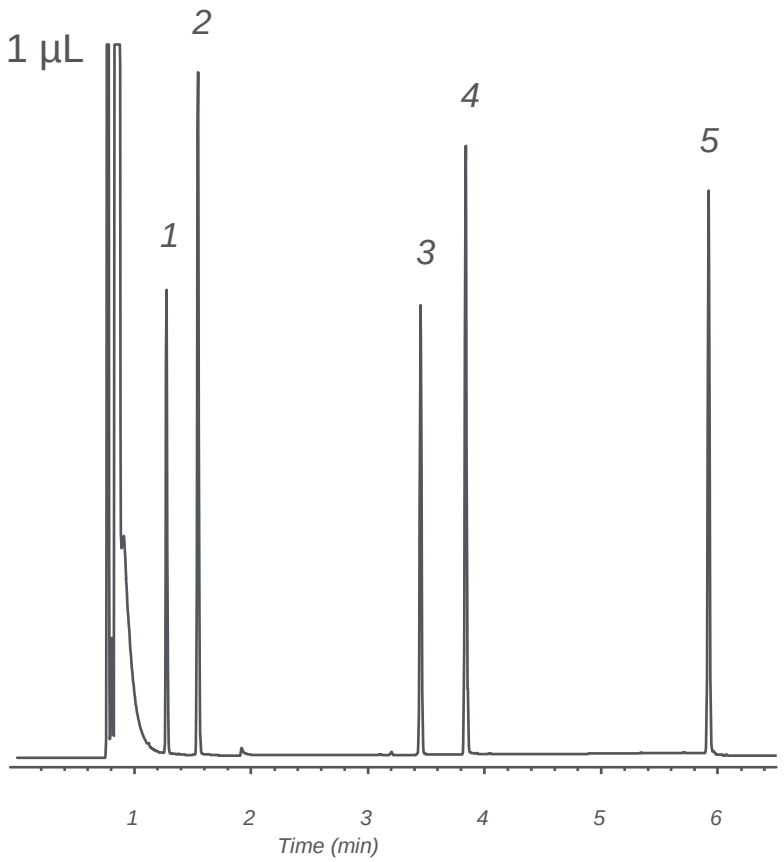
- Liner dependent (use the pressure-volume calculator)
- 2 µL maximum

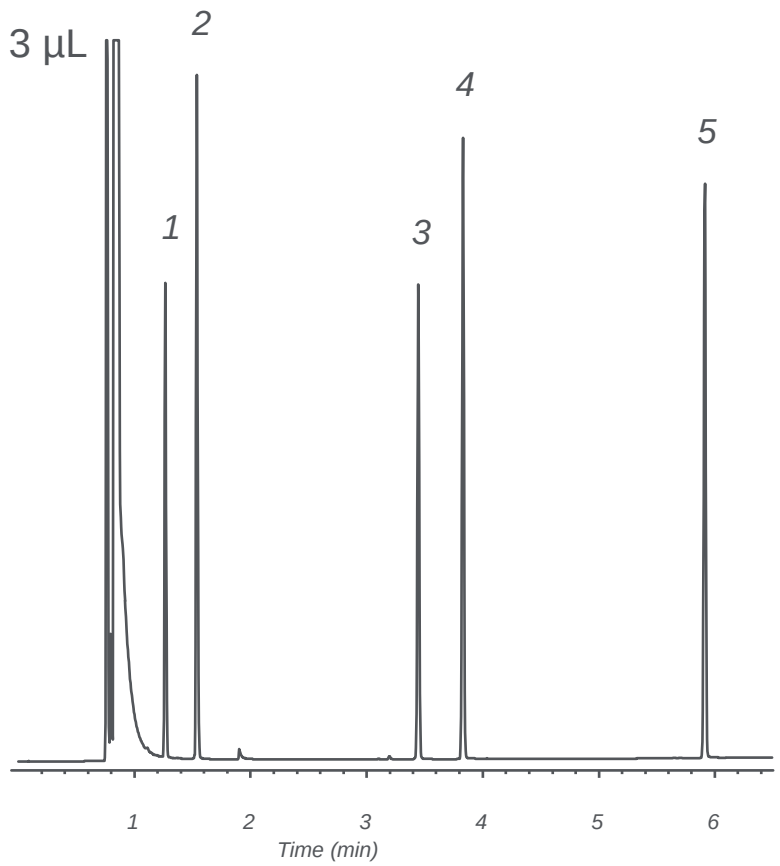
Reduce split ratio

- Go from 50:1 to 10:1
- 10:1 practical lower limit for liquid injections (for 250 – 320 µm id columns)
- 1:1 possible for gas injections with larger diameter columns and correct liner
- Keep total inlet flow at 20 mL/min or higher

Split Injector: Injection Volumes







Agilent J&W DB-1, 15 m x 0.25 mm id, 0.25 µm
60 °C for 1 min, 60-180 °C at 20 °C/min; helium at 30 cm/s
1. n-heptane 2. toluene 3. n-decane 4. n-butylbenzene 5. n-tridecane

Minimum Recommended Split Ratio

mm id	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 s (>200 mL/min)

- Poor mixing will cause discrimination

No glass wool

Solvents with high expansion volume

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 temporarily closed)
- The dilute sample is injected, the sample is volatilized, and majority of analytes transfer to the 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 (less discrimination)
- Longer residence time in inlet will give more time for active compounds to interact with active sites (effectively making splitless less inert)

Splitless Injections – Considerations

Dirty samples can be an issue – better if you have backflushing capabilities

Analyte boiling range – wide

Early eluters need bp difference versus solvent

Solvent properties

- Wide boiling point range
  - But consider BP of earliest eluting analyte
- Wide polarity range (but narrower than split)
  - Water and methanol are the worst choices
- Longer sample residence time (actives)
  - Lower inlet temperatures can be used
    - Better for labile compounds

Splitless Injections – Inertness

Less inert than cool on-column (cool on-column is most inert)

- 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 for actives

Splitless Liners

Single taper with or without wool

Splitless has lower flows through liner

- Splitless liners are typically wider for a more snug fit
  - Ensures all available flow funnels through the liner, not around
- You can do split injections with a split liner as long as split ratio is not too high
  - Poor reproducibility, not enough room for high flows to the vent

Agilent Ultra Inert liners enable excellent peak shapes for tricky analytes

- 5190-2293 is recommended splitless liner; single taper, with wool

In low carrier gas flow splitless analysis, a **bottom taper** helps focus analytes onto head of column

Small plug of **glass wool** near bottom of liner filters matrix

Splitless Injections – Discrimination

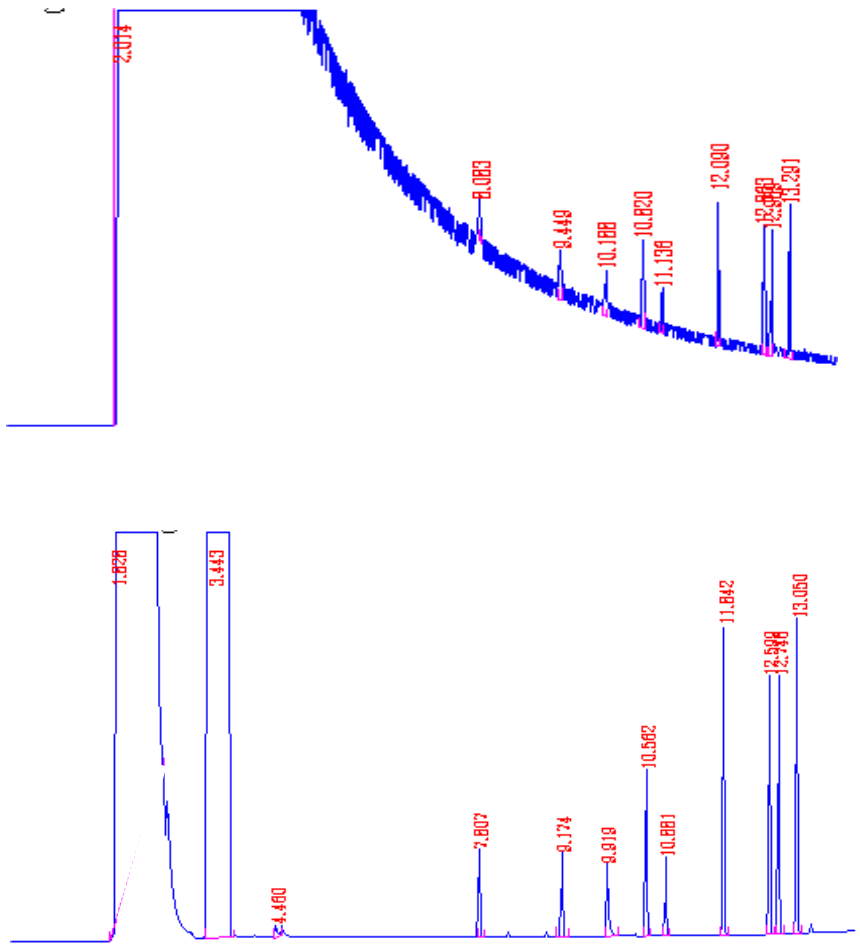
Improper purge time

- Short purge times cause loss of late eluters (not enough time to vaporize)
- Long purge times cause solvent tail interference with early eluters

Improper initial oven temperature (solvent effect – refocusing)

- Too high of a temperature prevents solvent effect and a loss of early eluters

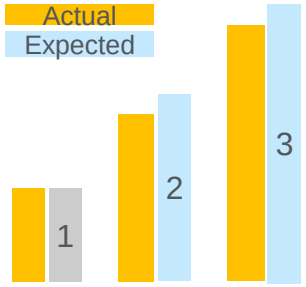
Splitless Injections – Splitless Time (Purge Time On)

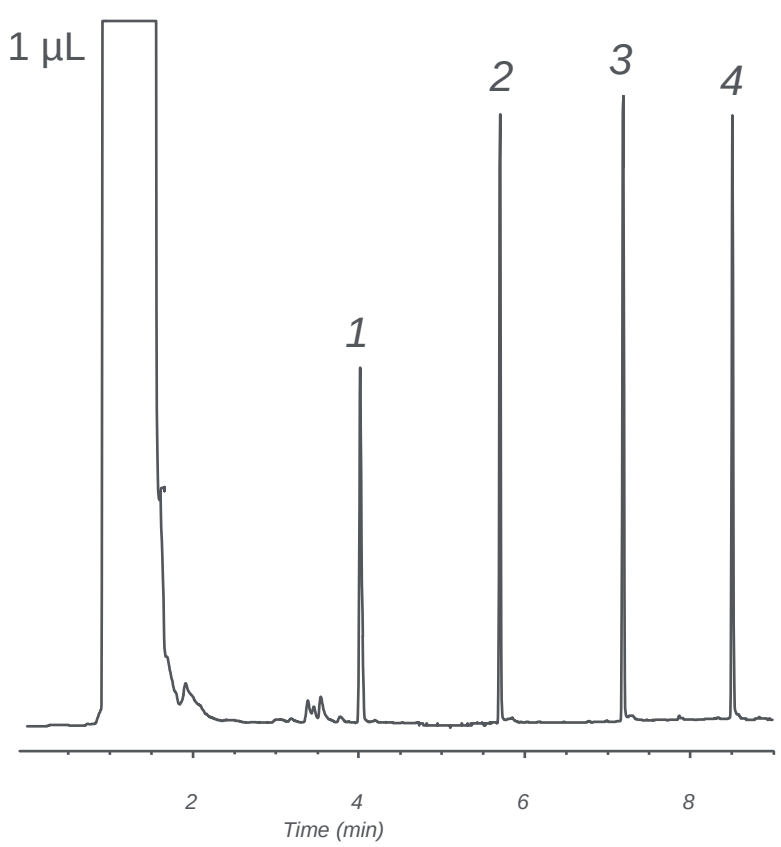


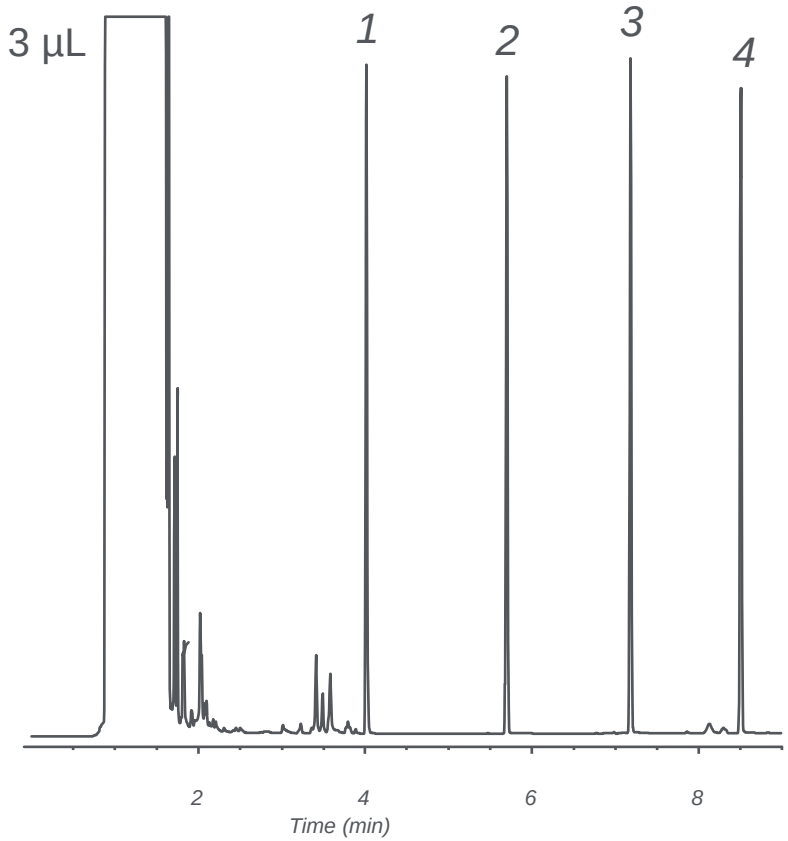
Purge time too long results in large solvent tail

0.75 min purge time clips solvent tail

Splitless Injector: Injection Volume

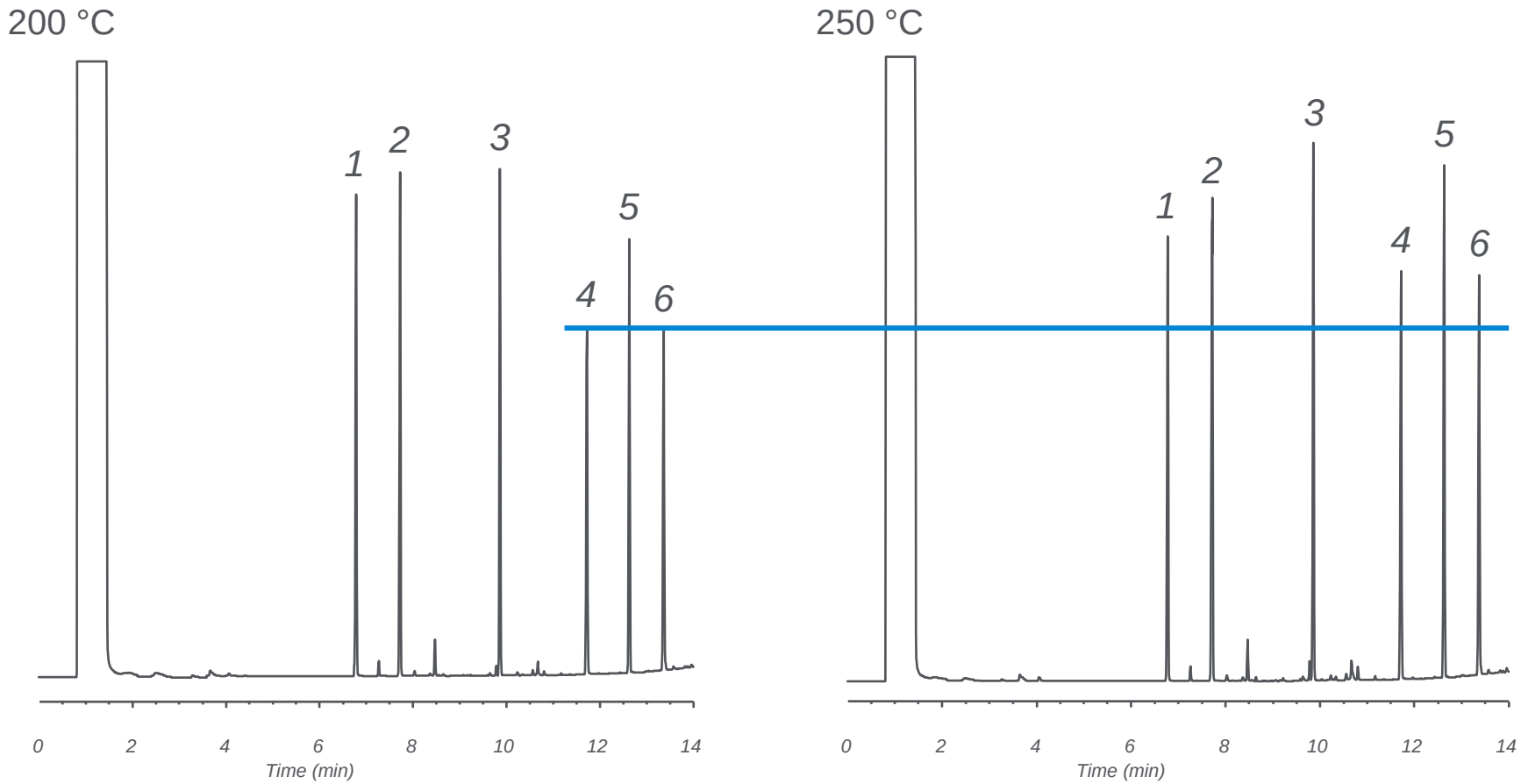






Agilent J&W DB-1, 15 m x 0.25 mm id, 0.25 µm
60 °C for 1 min, 60-180 °C at 20 °C/min; helium at 30 cm/s
1. n-decane 2. n-dodecane 3. n-tetradecane 4. n-hexadecane

Splitless Injector: Injector Temperature



Agilent J&W DB-1, 15 m x 0.25 mm id, 0.25 µm
50 °C for 0.5 min, 50-325 °C at 20 °C/min; helium at 30 cm/s
Phthalates: 1. dimethyl 2. diethyl 3. dibutyl 4. benzylbutyl 5.bis(2-ethylhexyl) 6. dioctyl

Splitless Injector: Sample Refocusing

Sample refocusing improves efficiency

Use low column temperature to refocus solvent (10 – 30 °C less than solvent BP)

- Called the solvent effect

Use cold trapping

Splitless Sample Refocusing

- 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 BP
    - This results in better peak shape
    - Results in smaller injection band than would otherwise occur (improves resolution)
    - Improved peak shape (especially for low boiling analytes)

- “Cold trapping” is a version of sample refocusing 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 BPs

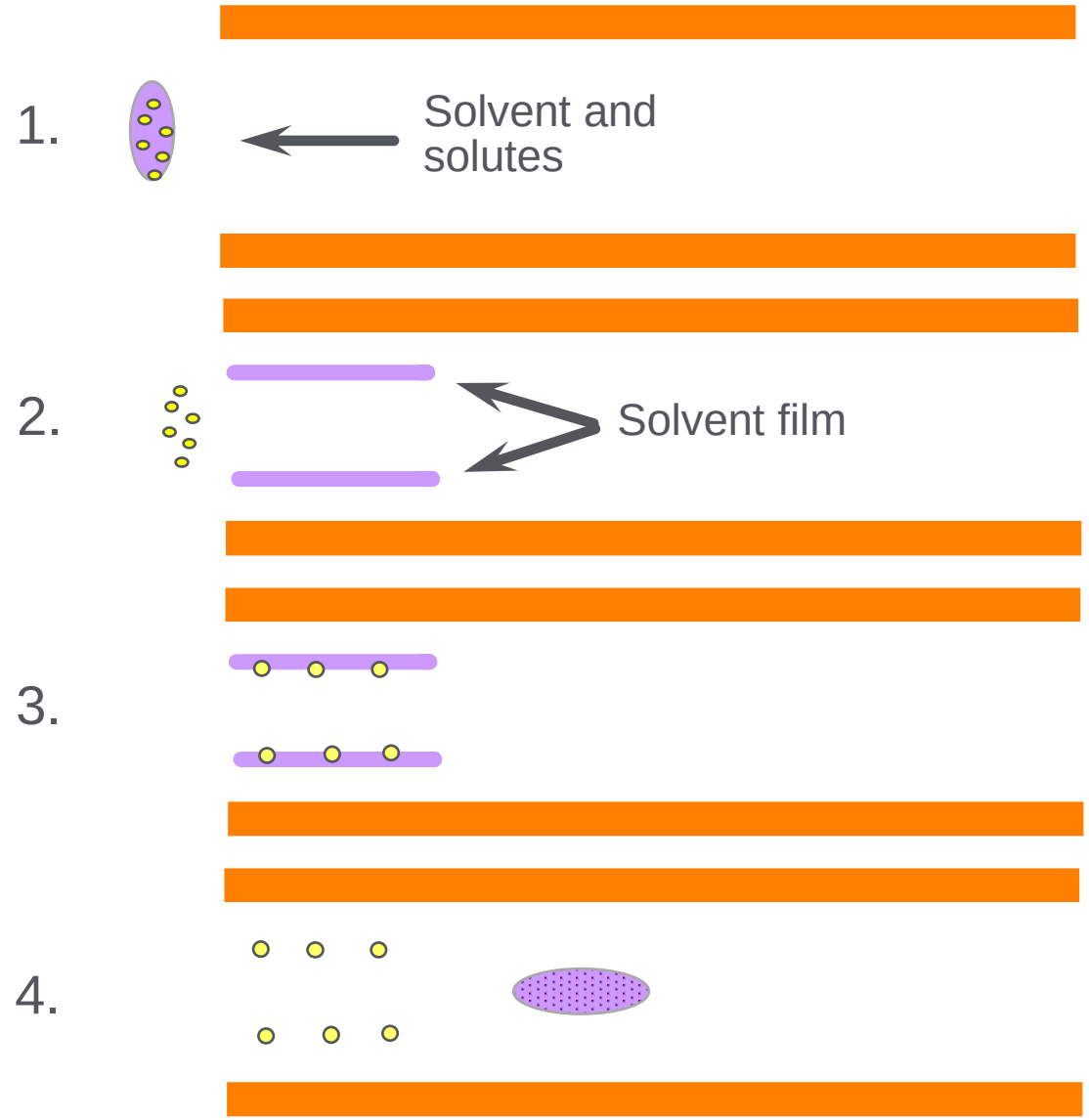
Splitless Injector: Solvent Effect

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

Required to obtain good peak shapes unless cold trapping occurs

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

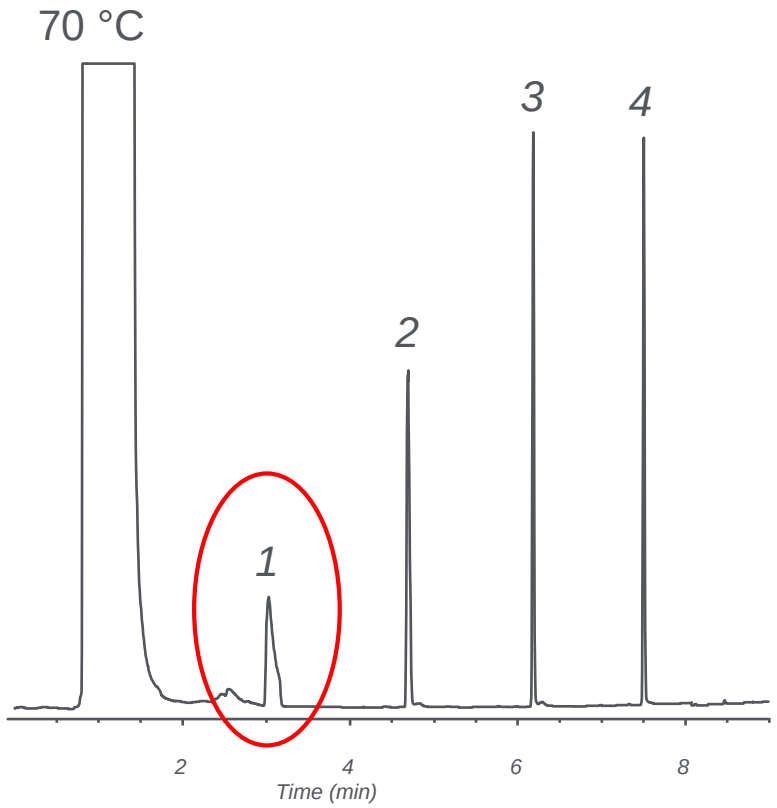
Cold trapping has greater efficiency than solvent effect



Splitless Injector

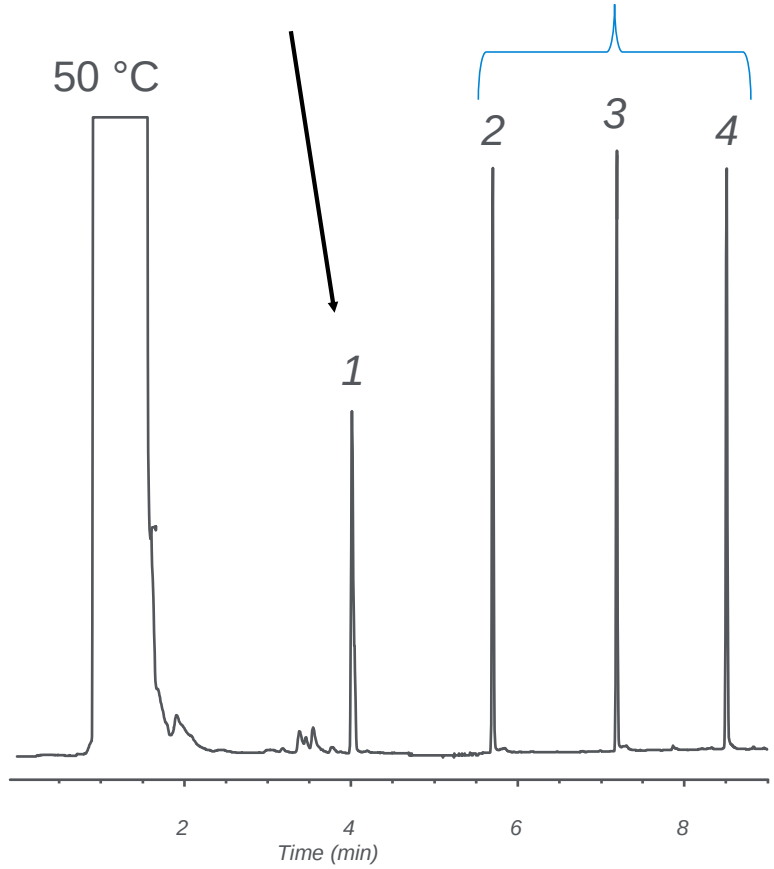
Initial column temperature

Hexane solvent (BP = 68-69 °C)



Solvent effect

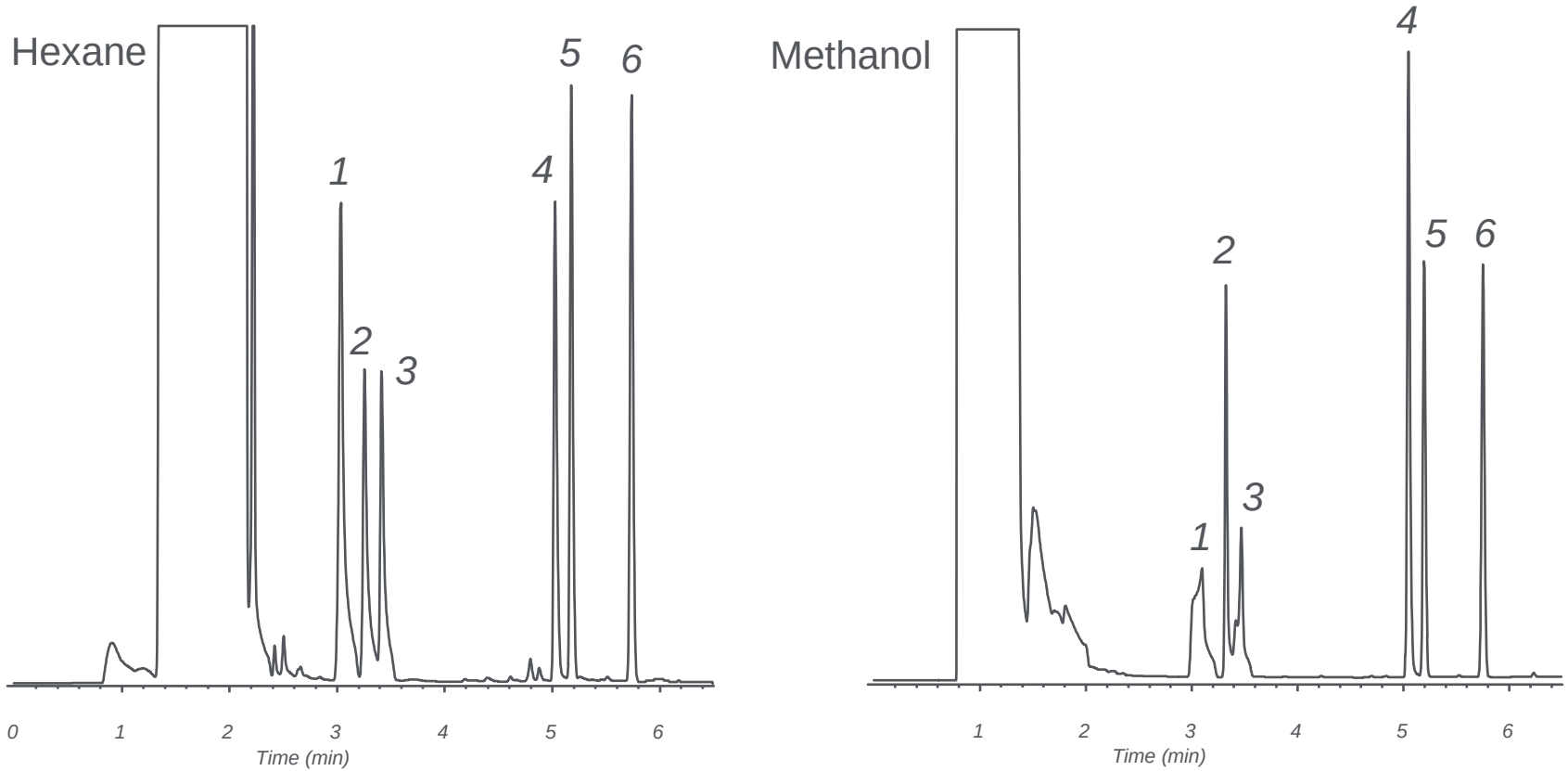
Cold trapping



Agilent J&W DB-1, 15 m x 0.25 mm id, 0.25 µm
50 °C or 70 °C for 0.5 min, to 210 °C at 20 °C/min; helium at 30 cm/s
1. n-decane 2. n-dodecane 3. n-tetradecane 4. n-hexadecane

Splitless Injector

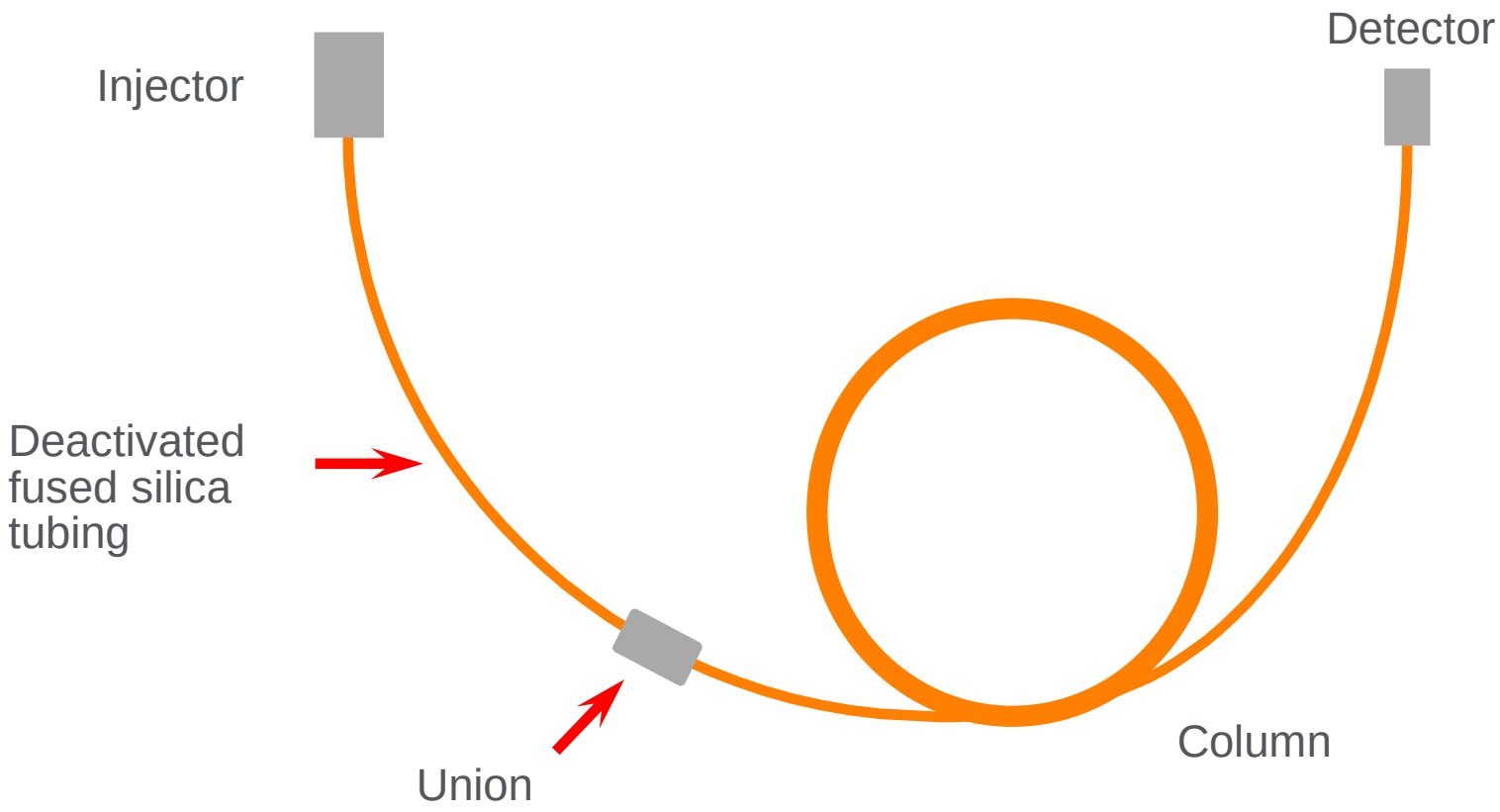
Reverse solvent effect/polarity mismatch



Agilent J&W DB-1, 15 m x 0.25 mm id, 0.25 µm
50 °C for 1 min, 50-210 °C at 20 C°/min; helium at 30 cm/s
1. 1,3-DCP 2. 3-hexanol 3. butyl acetate 4. 1-heptanol 5. 3-octanone 6. 1,2-dichlorobenzene

Retention Gap

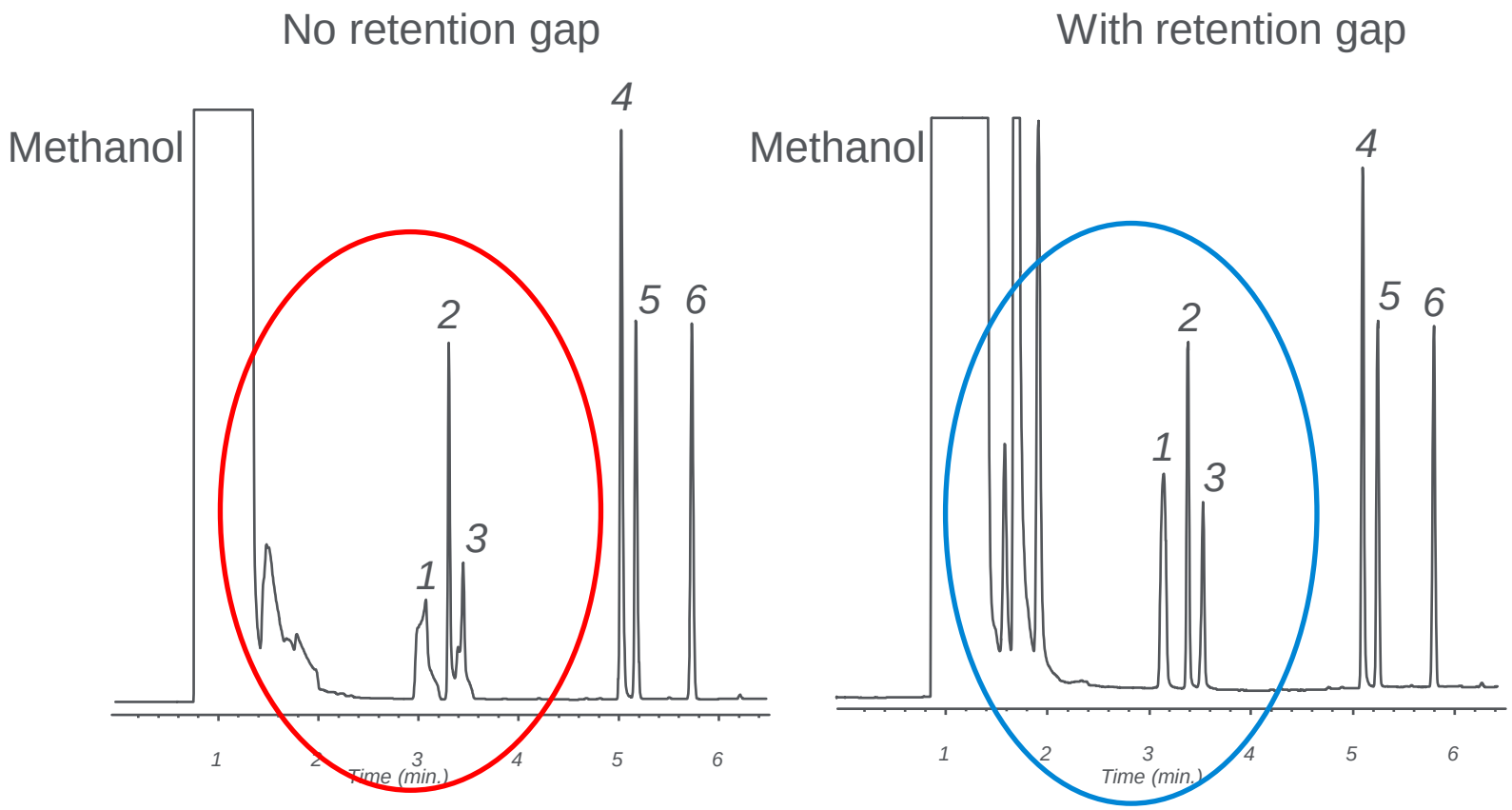
Also called a guard column



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

Splitless Injector

3 m x 0.25 mm id 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

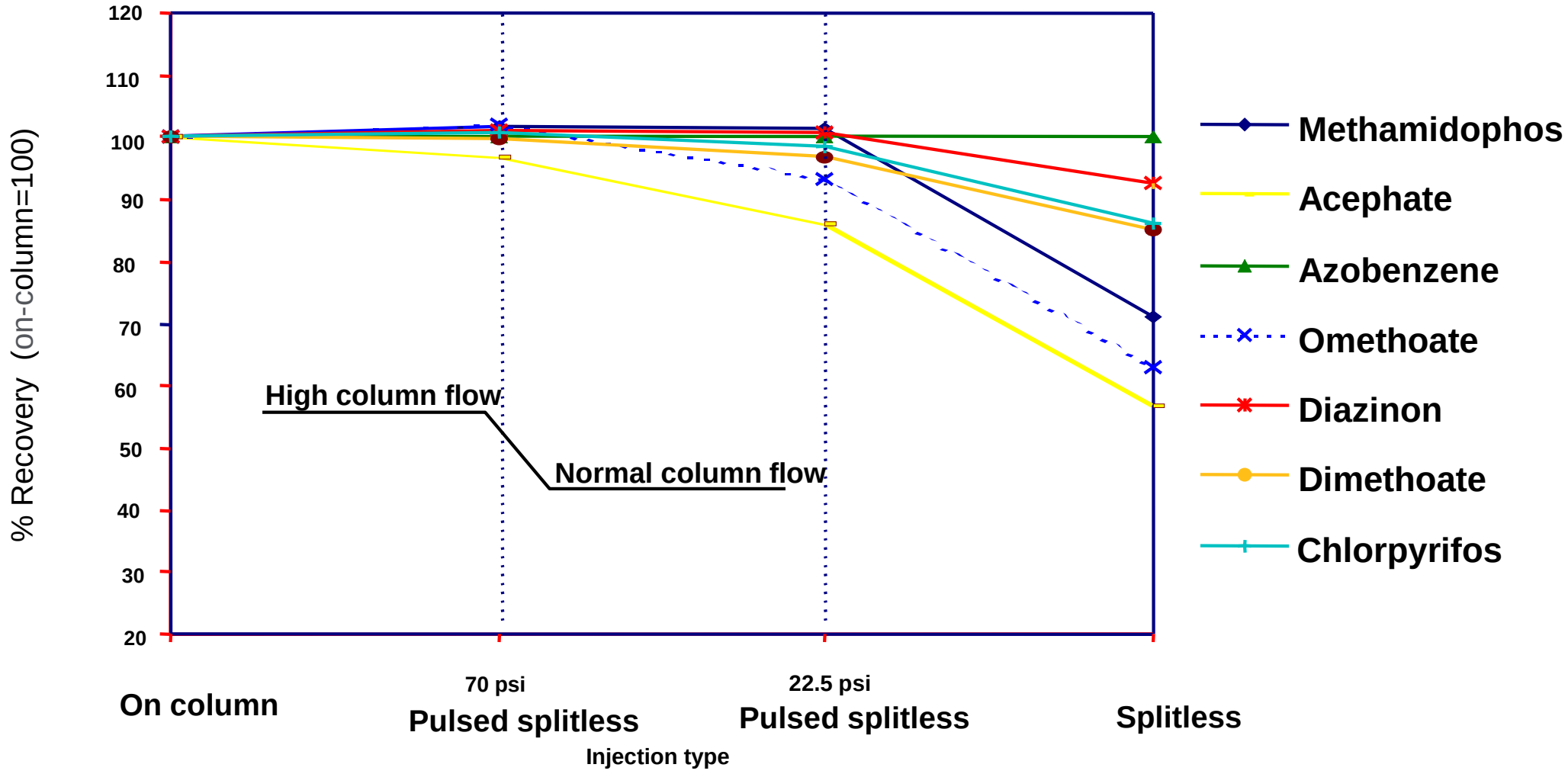
- Sample containment more critical than split injection
- Sharper peaks than in traditional splitless injection
- Two new parameters to set: pulse pressure and pulse time

Typical starting point

- Pulse pressure = double resting pressure
- Tie pulse time to purge time (pulse time slightly longer than 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 µL (if water 0.5 µL)

- 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 two 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 (backflash)
  - Ghost peaks in subsequent blanks are possible

No glass wool

- Poor mixing
- More matrix on column

Glass wool

- Has the potential to react with trace components (high surface area)
- May not be necessary if your samples are reasonably clean

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

What if, when I changed from split to splitless, I don't see an increase in response?

Verify that the purge time is not set to 0 min. Try increasing the purge time ("pseudo" split injection).

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

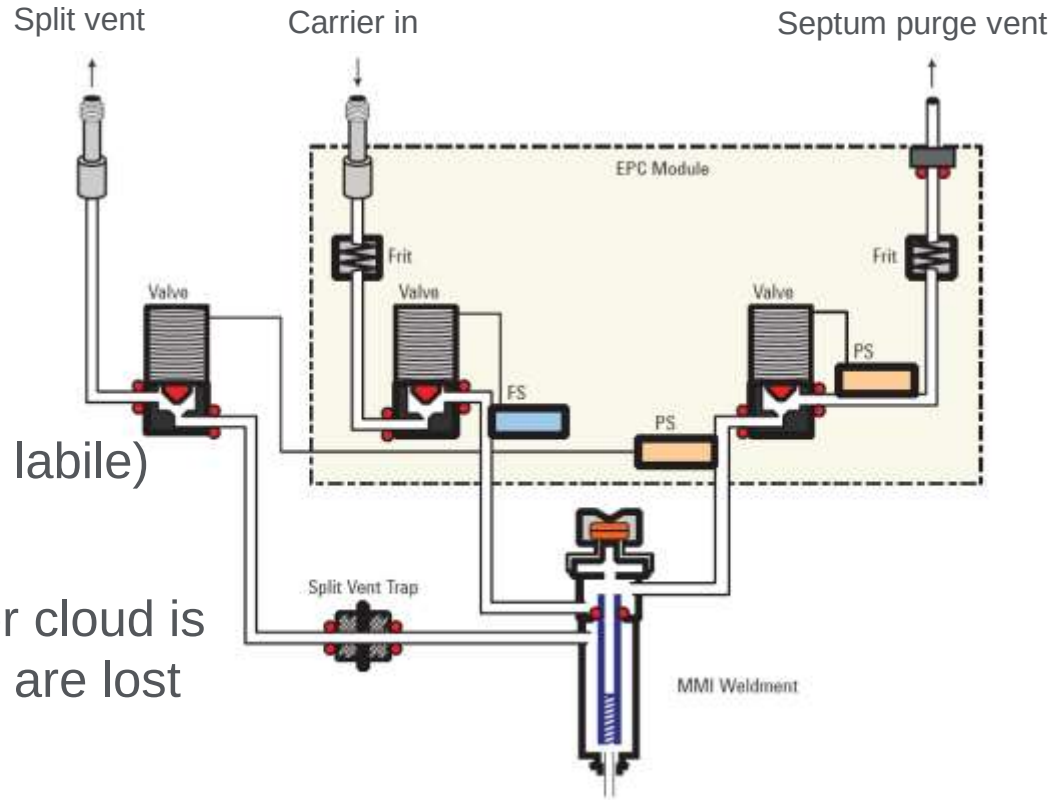
- Significantly more inert than hot splitless (for thermally labile)
- Can inject 3-5 µL with no solvent venting
- Better sensitivity than hot splitless because large vapor cloud is not formed which travels outside the liner and portions are lost

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)



Multi-Mode (MMI) Inlet Features

Temperature range of -160 to 450 ^{o}C

Heating at 15 ^{o}C/s (900 ^{o}C/min)

Septum/liner easily exchangeable using Turn Top inlet

Injection modes: hot S/SL, cold S/SL, pulsed mode, solvent vent mode, residue removal mode

Support for single stroke injections from 0.1 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 effects

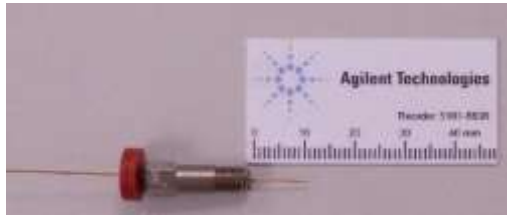
Performing analyses of high mol wt or thermally labile compounds?

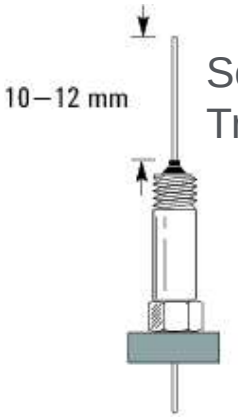
- Temperature programming of multimode inlet elutes analytes at the lowest possible temperature, minimizing breakdown and absorption
- Discrimination of high mol wt compounds is minimal allowing HT GC

MultiMode GC Inlet – Cold Injections

- No syringe-needle discrimination**;** minimal inlet discrimination
- No special syringes, liners, or consumables
- Large volume injection (5 to 250 µL) – lower detection limits
  - **Sensitivity is better, but also introduces that much more matrix**
- 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 12-15.5 mm
Trim the column

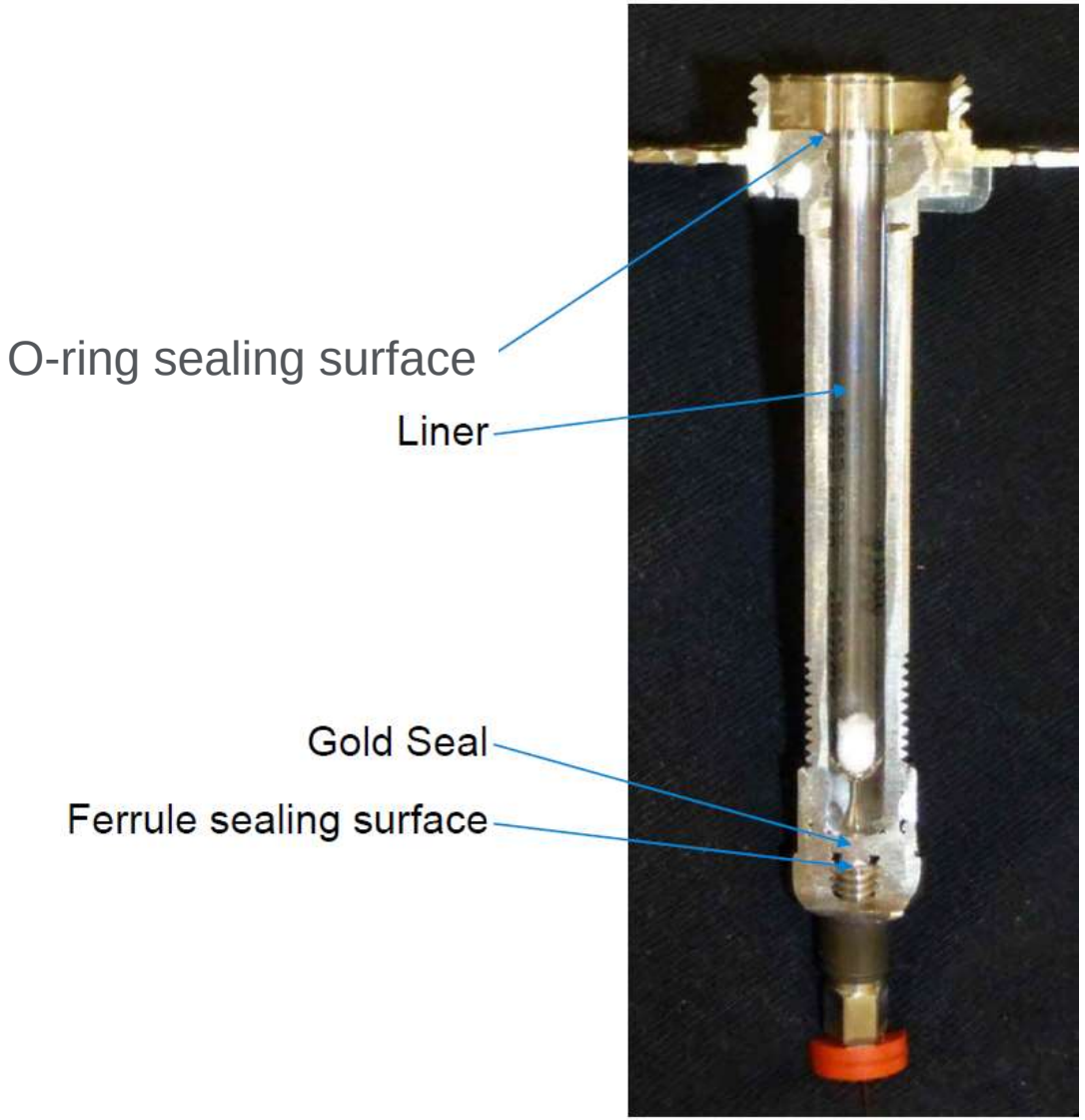
- Graphite ferrules are recommended over Vespel

Thread the column into the column adapter – stabilize the column adapter with a 5/16-inch wrench

Tighten the column nut with a ¼-inch wrench – continue to hold the column adapter with a 5/16-inch wrench

Inlet Degradation and Maintenance

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

Nonoptimized setup

- 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

Evidence of a dirty liner:

- Poor peak shape
- Irregular baselines
- Poor resolution
- Poor response

Dirty Liners

Silylated glass wool

- Traps nonvolatile materials and mixes sample

- Peak shape and discrimination affected by amount, location, and packing density

Liner Maintenance

- Liners become contaminated with use, collecting nonvolatiles, salts, excess reagents, and so on, 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 conditions

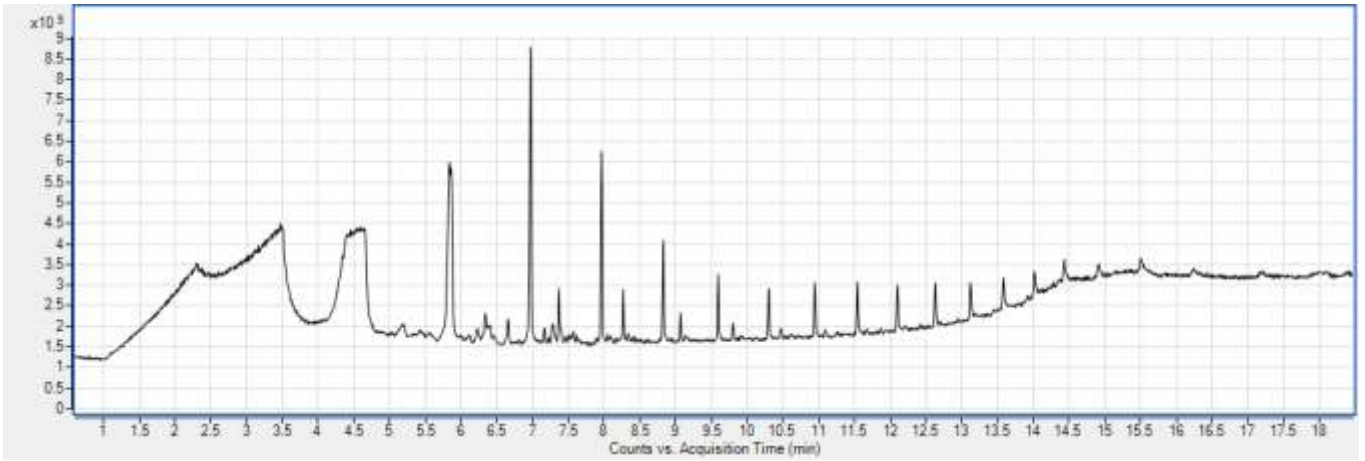
- Use environments that decrease lifetime include manual injections, wrong syringe tip type, larger gauge syringes, non-Agilent autosamplers (Agilent autosamplers are precisely aligned)
- Septum nut too tight
- Septum type and syringe needle type mating are essential to minimizing leak rate
- Typical cost of 1 premium septa ($1.25)
- Typical cost of 1 GC column ($600)
- Proactively change inlet septa

Septum Maintenance: Septum Coring

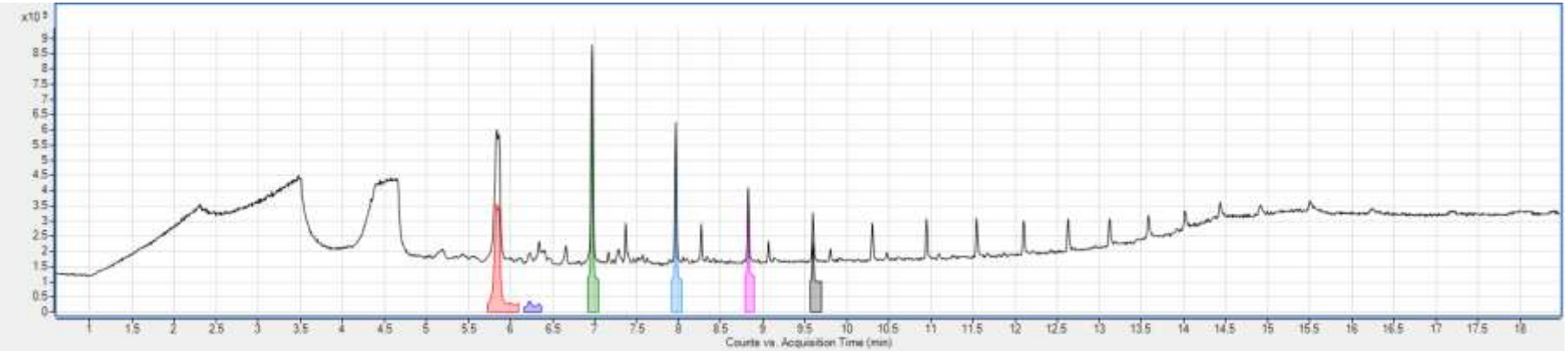
- After many injections, pieces of rubber from the septum may break off and fall into the inlet liner
  - This is called septa coring
  - Replace the inlet septa and liner frequently to prevent septa contamination
  - Use a cone tipped syringe to reduce the chance of tearing the septum

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/min
-Detector: Single quadrupole EI scan, 35 to 500 Da



Septum Maintenance: Deconvoluted Inlet Septa Spectrum



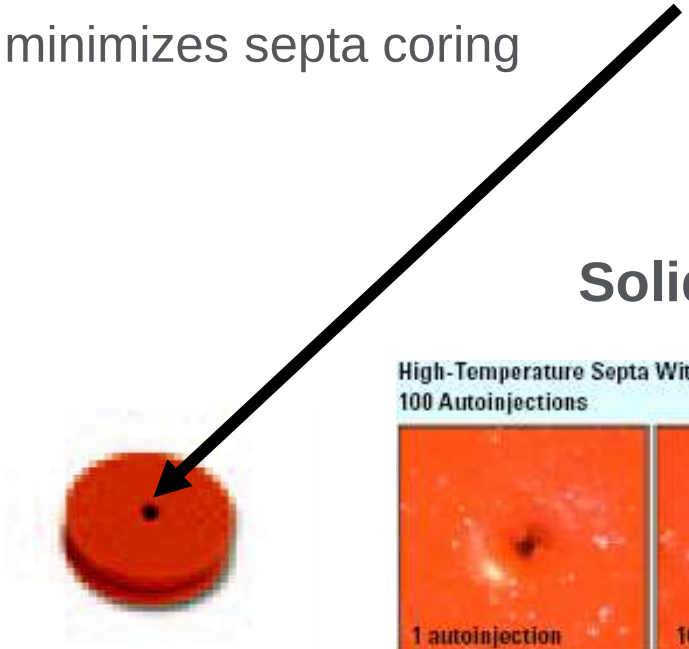
Main Siloxane Peak Bandit

Multiple injections from same vial can dissolve silicone into the sample

Tips to Maximize Septum Life, Minimize Septum Leaks

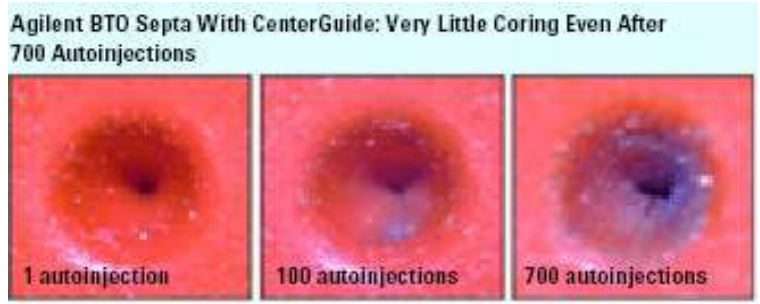
- Use Agilent Gold Standard, 23-26 gauge, HP point taper syringes
  - Point style cores septa significantly less when used with CenterGuide Septa
  - Taper minimizes septum coring/wear
- Use Agilent CenterGuide Septa
  - Molded hole minimizes septa coring

HP-point style



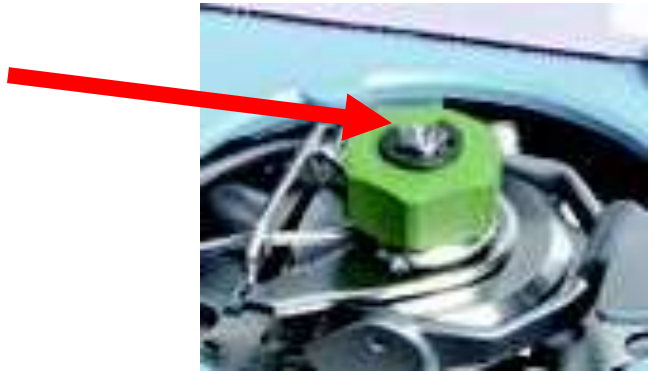
Solid Septum

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



Examples of Non-Optimized Operation

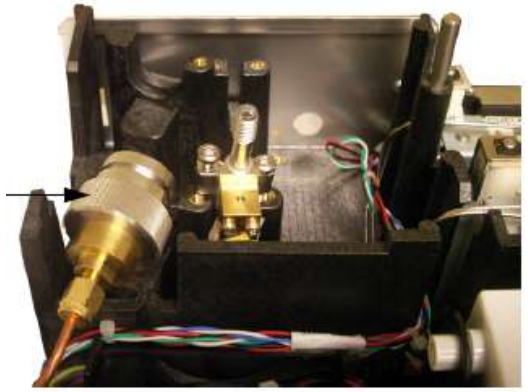
Typical cause: re-use and misinstallation

- 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 without shrinkage or potential organic contaminants from polyimide outgassing/degradation
- Re-using could result in overlap in seal rings, resulting in a leak

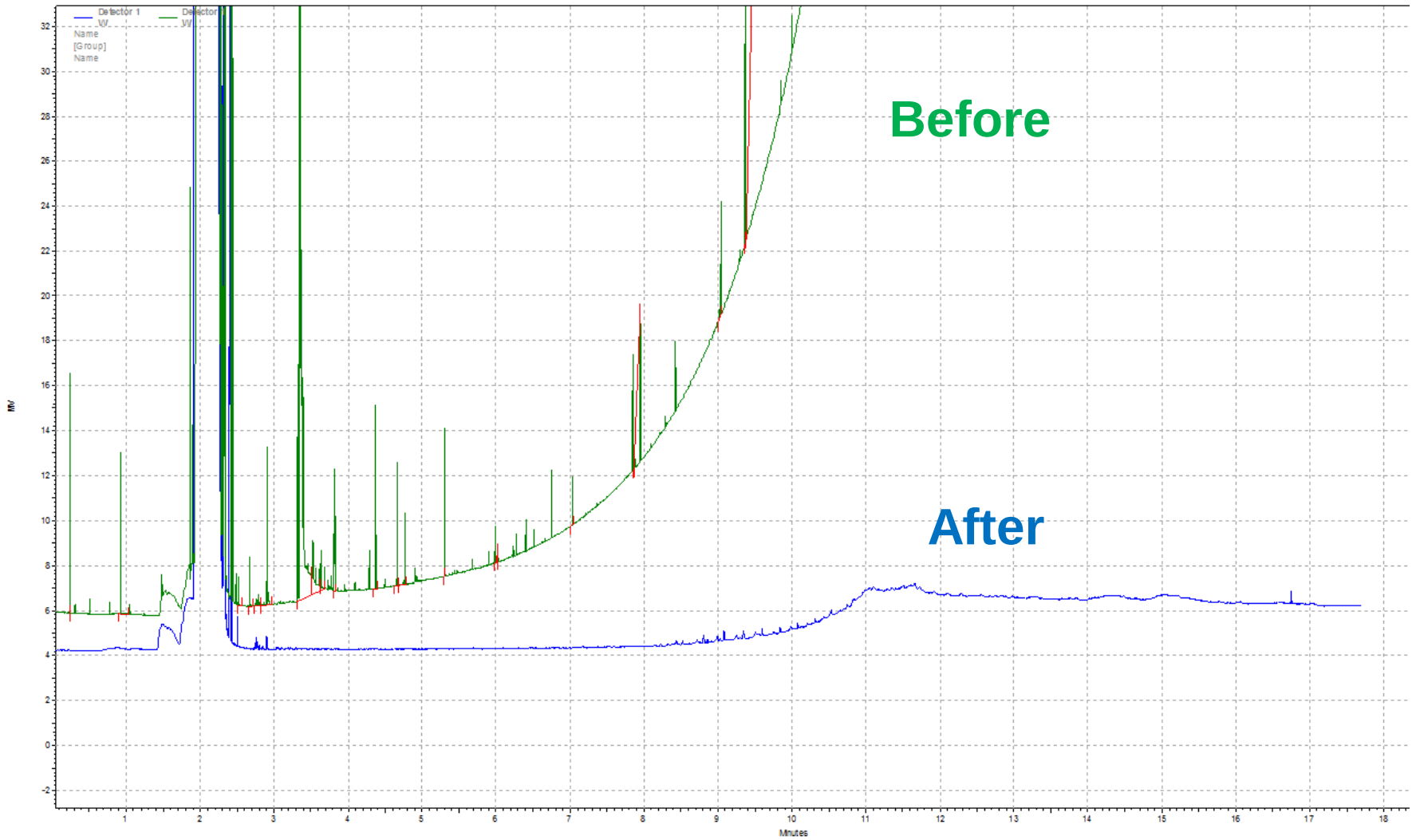
Split Vent Trap

What is it?

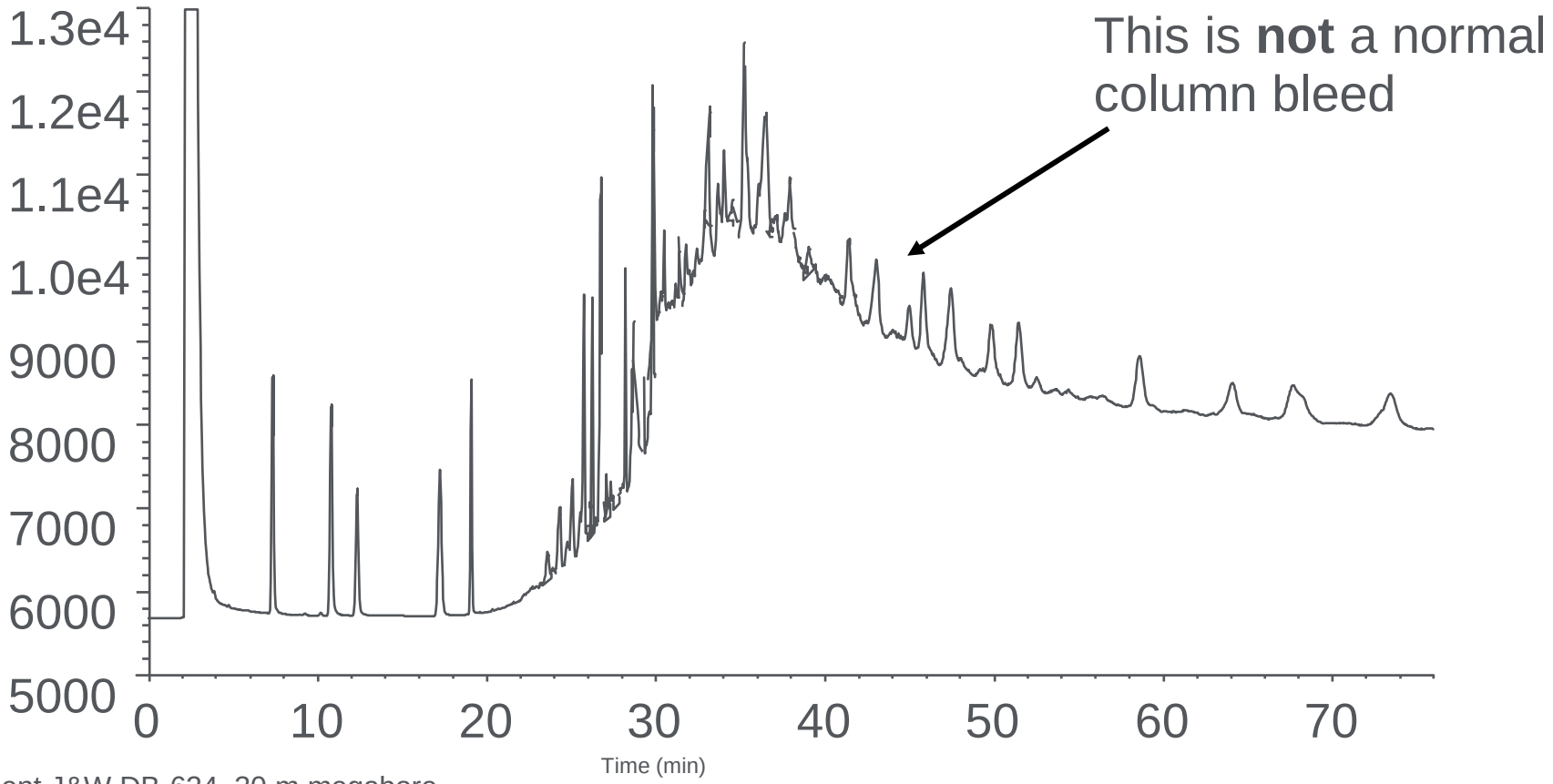
Where is it?



Split Vent Trap Changed (Column Bleed)

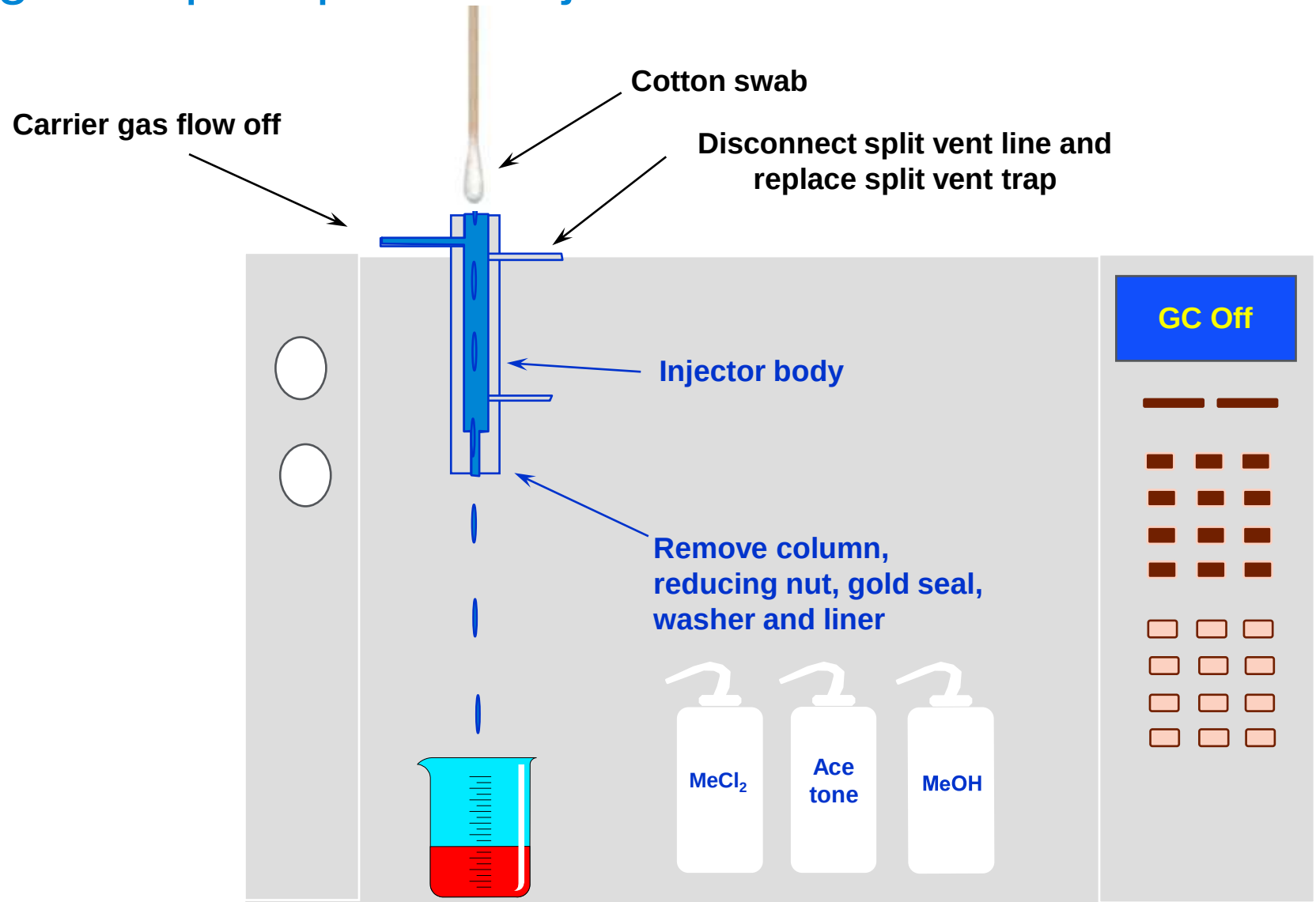


Example Of Gross Contamination

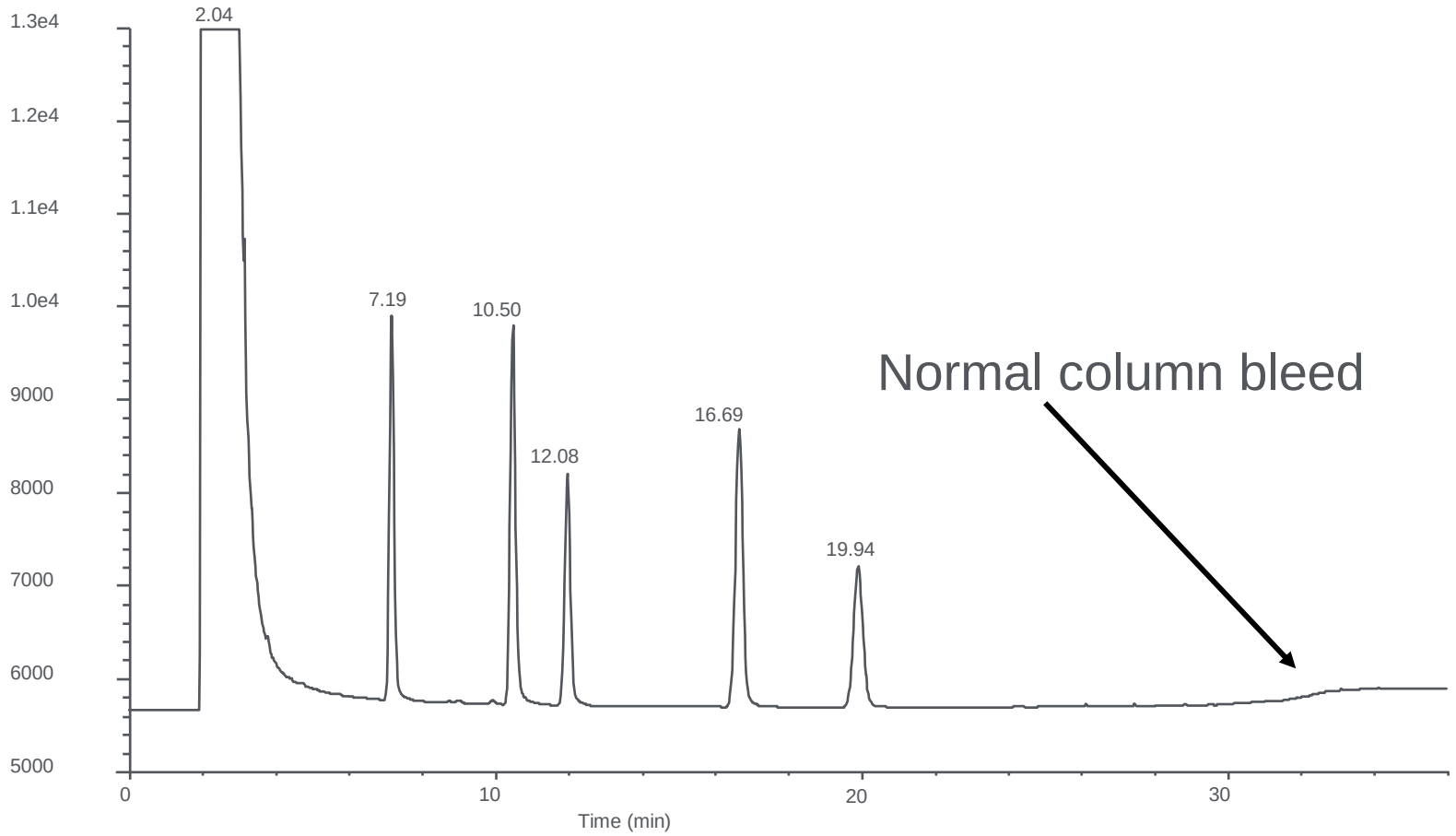


Agilent J&W DB-624, 30 m megabore
Temperature program: 35 °C, hold 1.50 min, 30 °C/min to 65 °C, hold 15 min, 20 °C/min to 260 °C, hold 50 min

Cleaning the Split/Splitless Injector



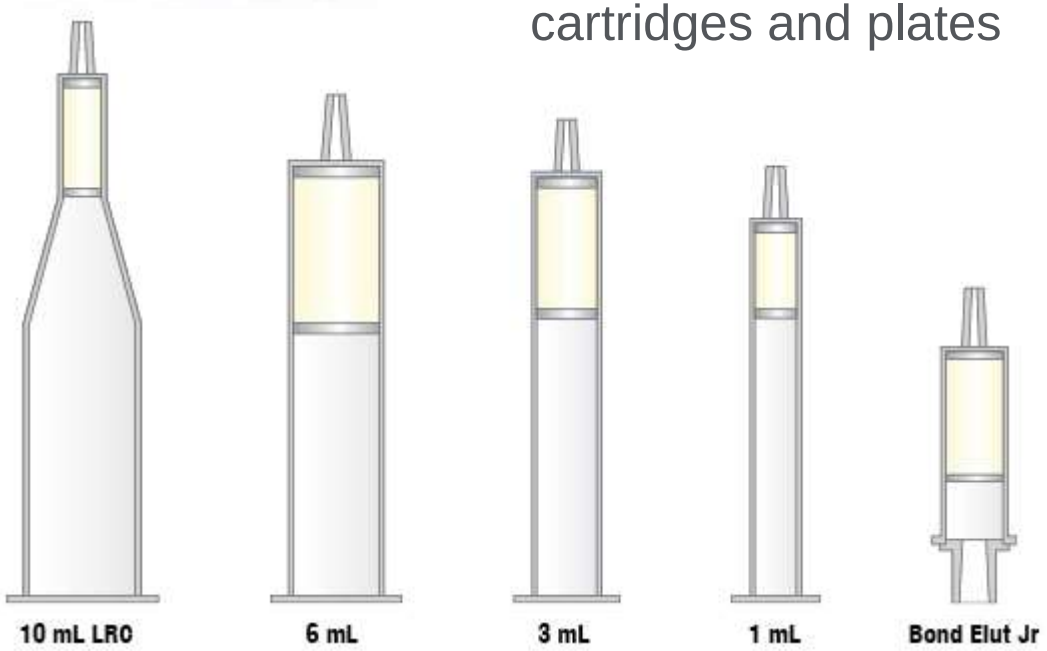
Same Column After Inlet and Column Maintenance



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

Agilent Bond Elut Sample Cleanup Products

Solid Phase Extraction cartridges and plates



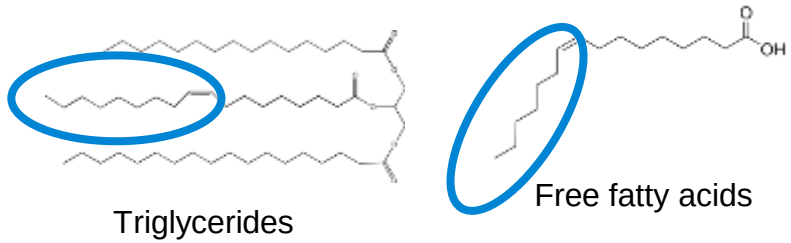
Filtration cartridges and plates

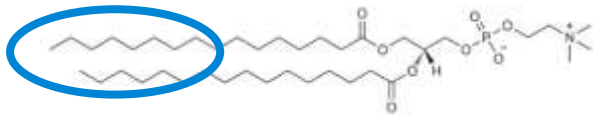
Captiva EMR Lipid

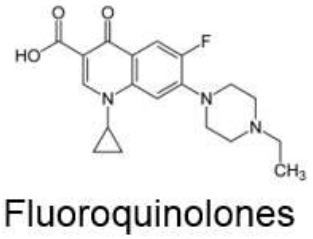
Enhanced Matrix Removal: Agilent Captiva EMR-Lipid

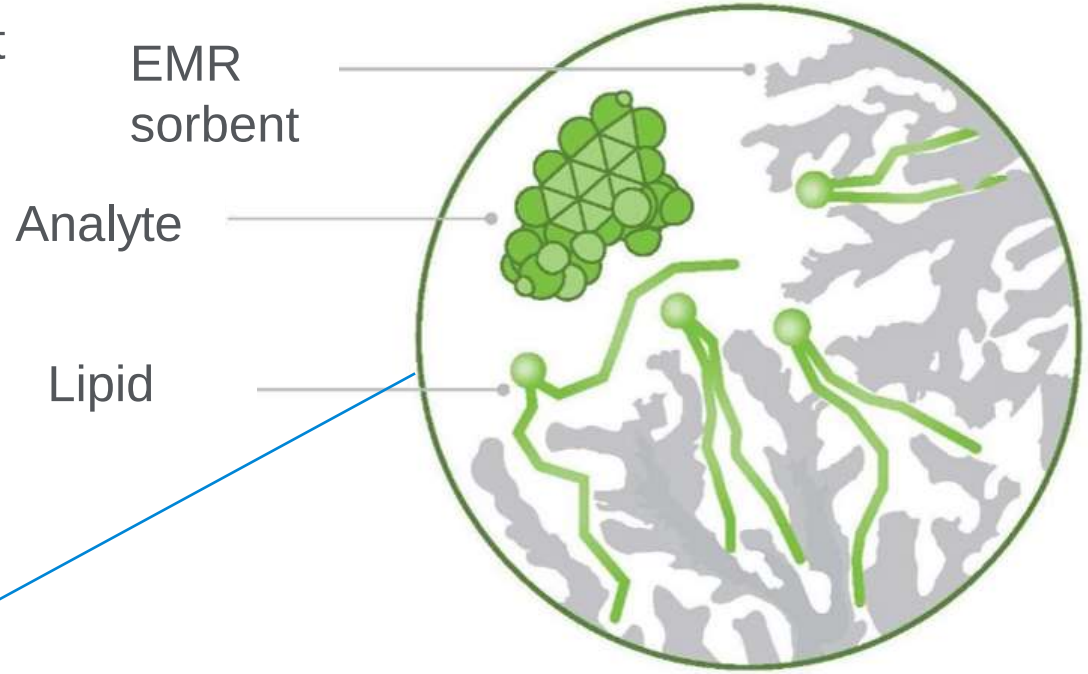
EMR-Lipid sorbent technology effectively traps lipids through two mechanisms:

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









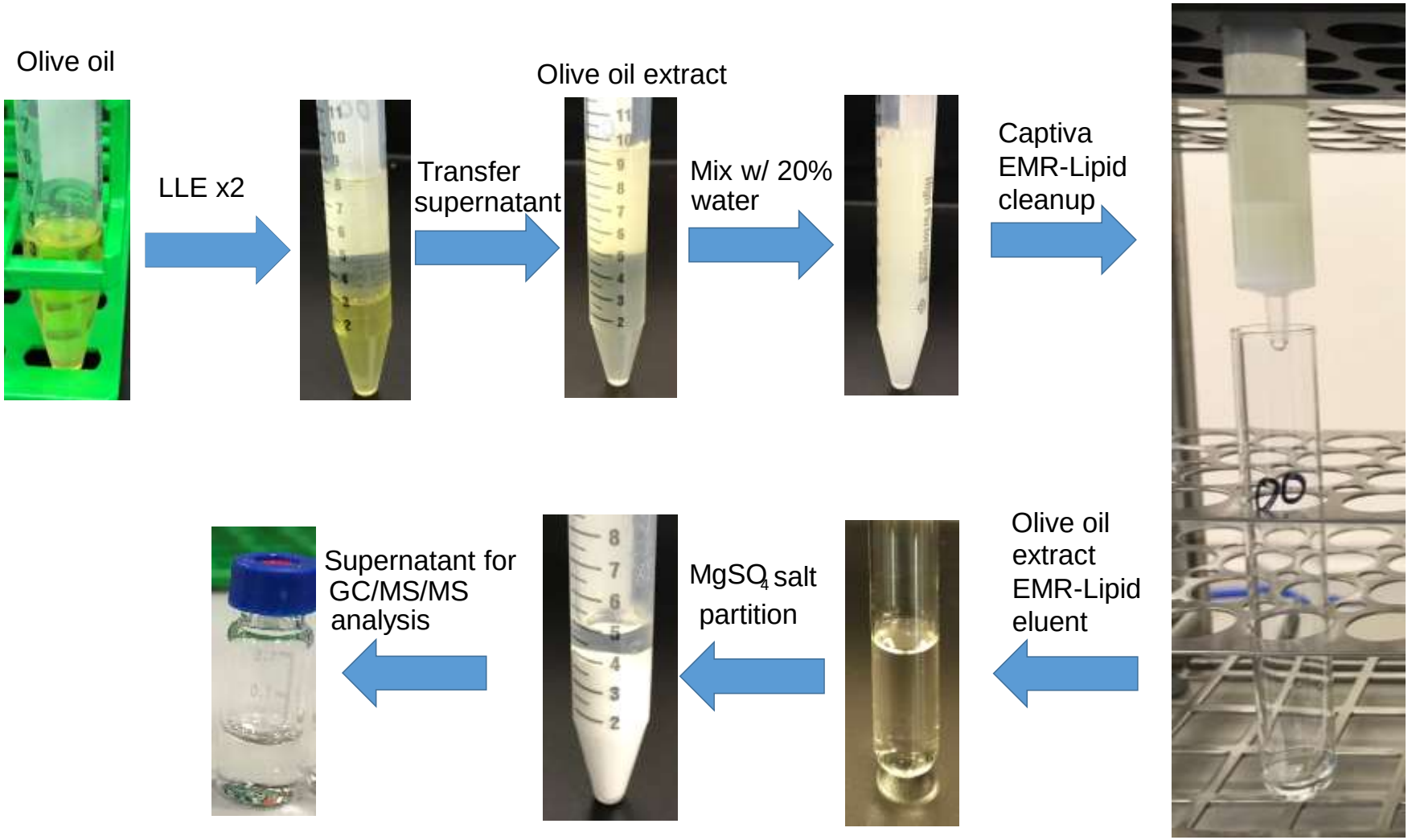
Application Case – Pesticides in Edible Oil by GC/MS/MS

Classification	Pesticides	Classification	Pesticides	Classification	Pesticides
	Dichlorvos		Lindane	**Sulphamide**	Dichlorfluanid
	Trichlorfon		Aldrin		Tolylfluanid
	Sulfotep		Endrin	**Phthalimide**	Captan
	Diazinon	**Organochlorine**	Endosulfan		Folpet
	Chlorpyriphos-methyl		DDT		Captafol
	Phosmet		Oxychlordane	**Dicarbosimide**	Procymidone
Organophosphate	Coumaphos		Mirex	**Pyrimidinol**	Bupirimate
	Malathion	**Pheonl**	2-Phenylphenol	**Dicarboximide**	Iprodione
	Parathion	**Dinitroaniline**	Ethalfluralin		Permethrin
	Dimethoate	**Chloronitrile**	Chlorothalonil		Deltamethrin
	Fenamiphos	**Pyridazinone**	Norflurazon	**Pyrethroid**	Esfenvalerate
	Terbufos sulfone	**Pyridine**	Thiazopyr		Fenvalerate
	Chlorpyriphos		Atrazin		Bifenthrin
Oxazole	Vinclozolin	**Triazine**	Prometryne	**Strobilurin**	Pyraclostrobin
Uracil	Bromacil		Propazine	**Carbamate**	Thiobencarb
				Diphenyl ether	Nitrofen

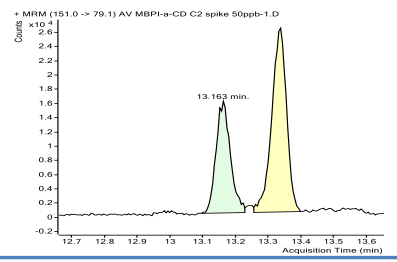
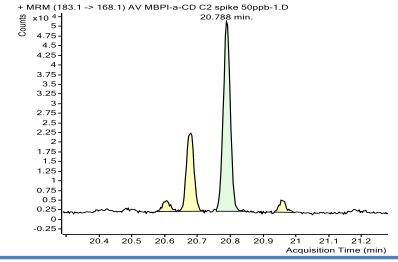
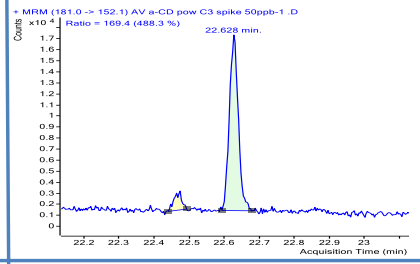
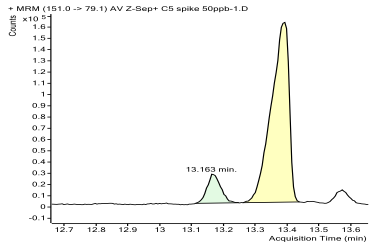
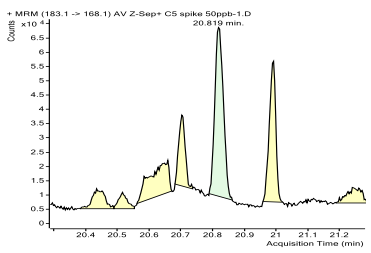
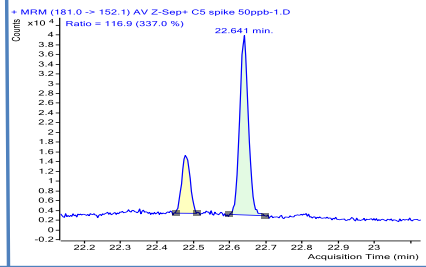
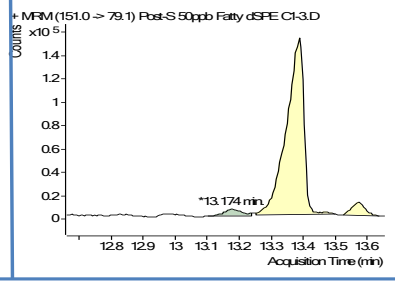
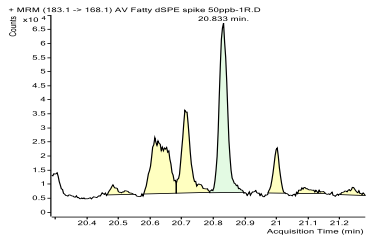
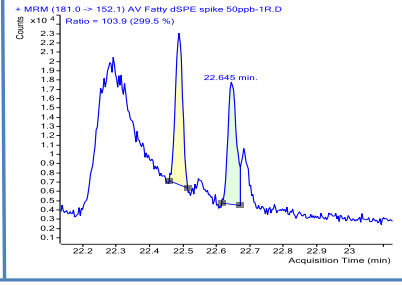
Captiva EMR-Lipid
6 mL cartridge

Pesticides in Edible Oil by GC/MS/MS

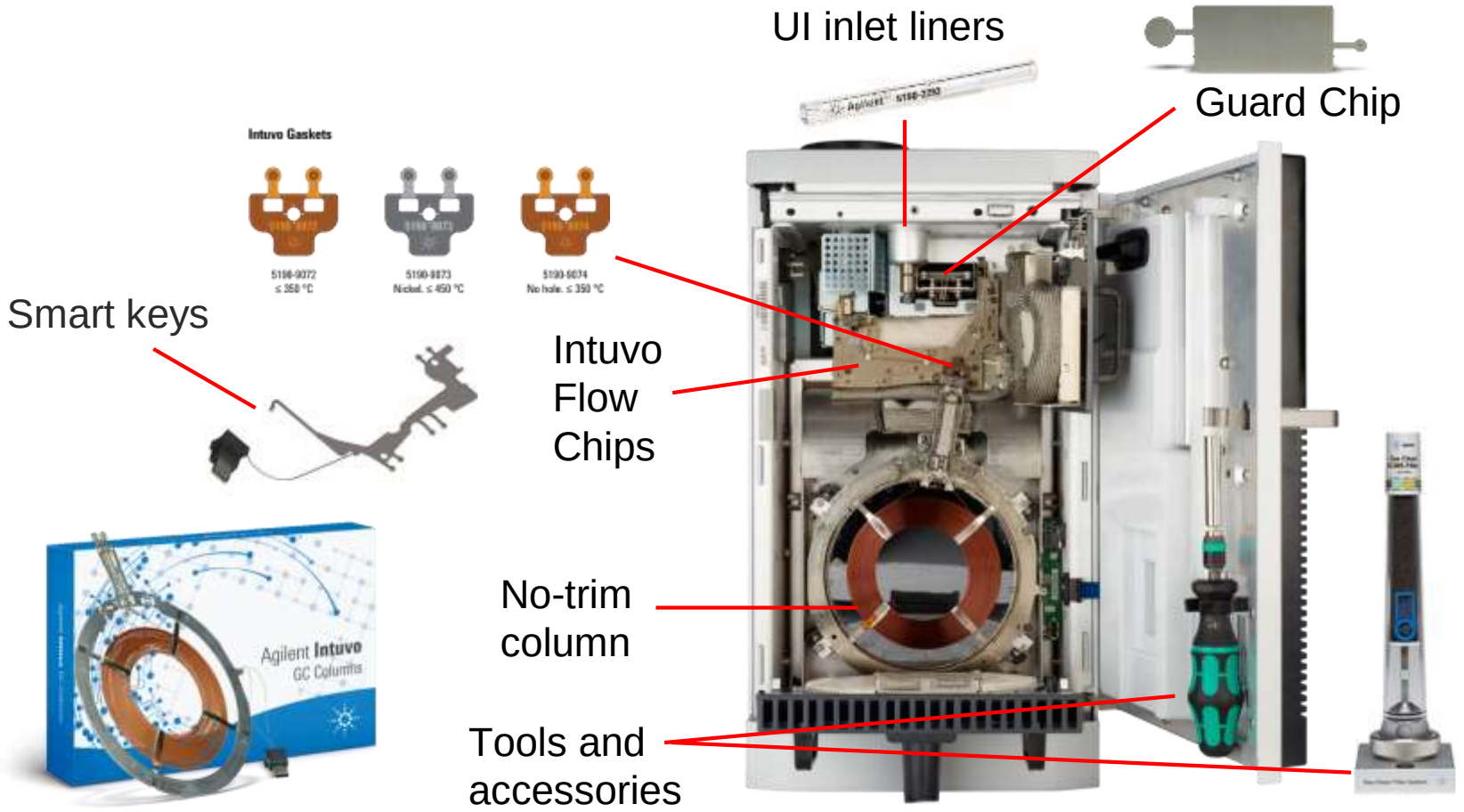
Sample preparation procedure visual



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

Pesticide	Captan	Permethrin	Deltamethrin
EMR-Lipid cleanup			
Zirconia sorbent cleanup			
C18/PSA cleanup			

A New Portfolio of GC Consumables



When Do I Change Each Part?

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 septa	100-200 injections	Depends on septum type and manual/auto injections.

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

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 in from the front of the column (back end of the column is clean)

Contact Agilent Chemistries and Supplies Technical Support

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

Option 1 for GC or GC/MS columns and supplies

Option 2 for LC or LC/MS columns and supplies

Option 3 for Sample preparation, filtration and QuEChERS

Option 4 for Spectroscopy supplies

Available in the U.S. 8-5 all time zones

gc-column-support@Agilent.com

lc-column-support@agilent.com

spp-support@agilent.com

spectro-supplies-support@agilent.com