Maintaining Your Agilent GC's Split/Splitless Injection Port

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

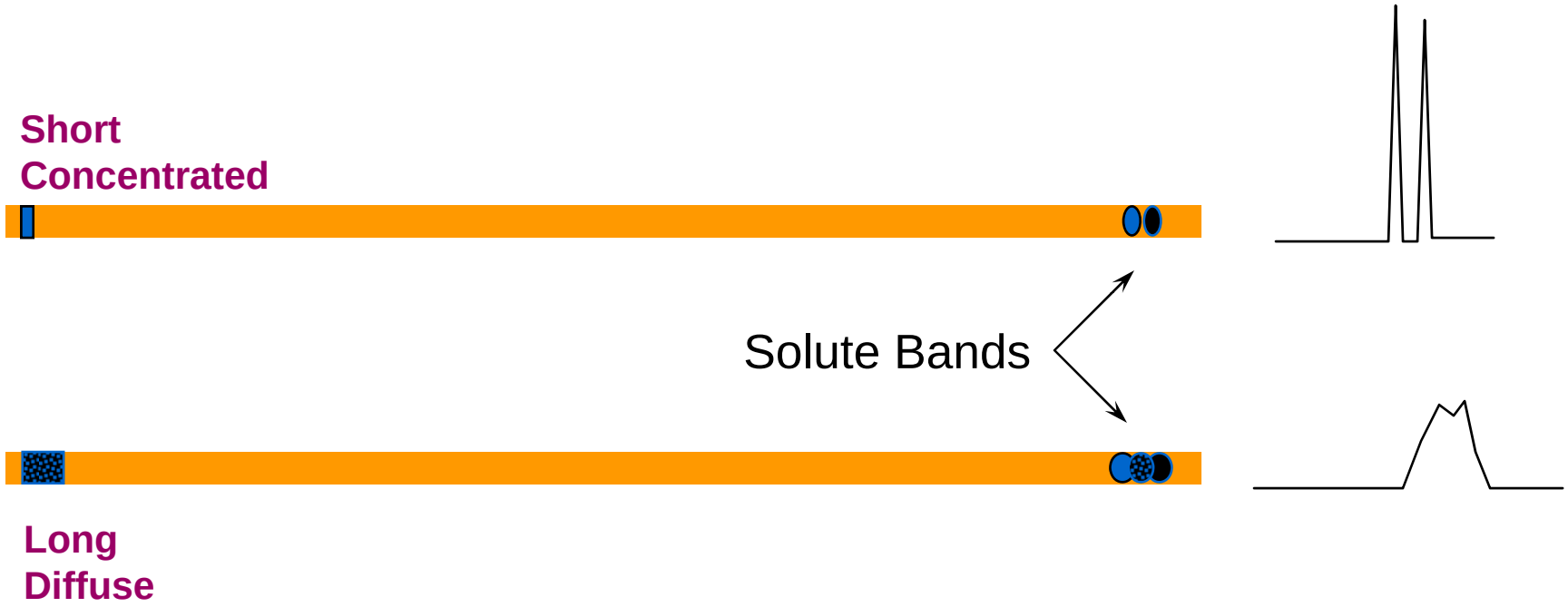
Presentation Expectations

- The #1 inlet used by far is the Split/Splitless inlet, so we will focus on this inlet but allude to other inlets as appropriate.
- Many inlet parts are “consumables,” they have to be replaced for optimal System and Method performance.
- Replacement frequency varies with selection and the samples injected
- Presentation offers set-up and maintenance“tips” to improve performance and inlet robustness.
- Will treat root causes of performance degradation individually

SAMPLE INJECTION
Goals

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

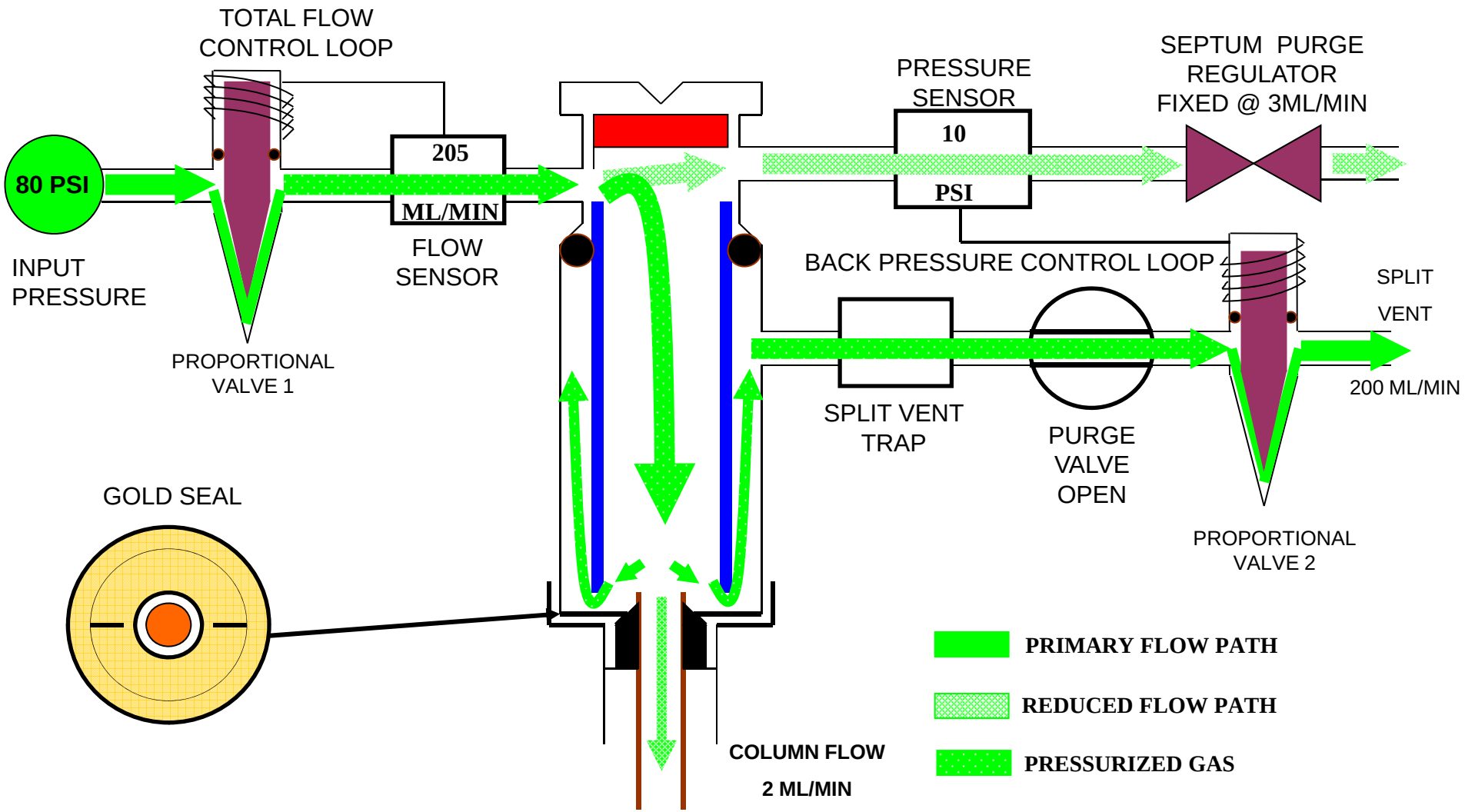
Influence of Injection Efficiency



Same column, same chromatographic conditions

6850/6890 Split Injection

Inlet pressure setting



SPLIT
Flow Considerations

- 1-3 mL/min into the column
- 10-200 mL/min in the inlet (mostly split vent)
- 0.3-6 sec sweep rate
- Results in high efficiency

SPLIT
Split Ratio

- Ratio of split vent and column gas flows
- Approximates the amount of sample splitting
- Split ratio calculations are inaccurate

LOW 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

HIGH SPLIT RATIO

- Limited by flow controller
- Range usually 1:200 to 1:1000

Gold plated inlet seal with cross, p/n 5182-9652

SPLIT RATIO COMPARISON

	High Ratio	Low Ratio
Sample into column	Low	High
Efficiency	High	Low
Discrimination	High	Low
Carrier gas use	High	Low

SPLIT Injection Characteristics

- Little backflash
- Highly Efficient Sample Transfer
- Inlet Discrimination
- Discrimination affected by many variables

Inlet Liners – Volume Considerations

Glass Inlet Liners provide an "inert" space for liquid samples to be uniformly vaporized to a gas and moved to the column.

Liquid-gas phase change involves a significant change in volume.

Gaseous sample volume depends on

- **Injection volume**
- **Solvent type**
- **Column head pressure**
- **Inlet temperature**

- **These aspects should be optimized for your sample volume and application.**

Solvent (1µL, ambient)	Volume (µL at 250°C and 20psig)
n-Hexane	140
Acetone	245
Acetonitrile	350
Methanol	450

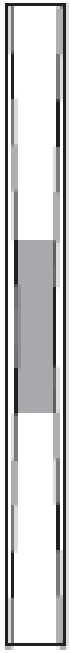
See "A Practical Guide to the Care, Maintenance, and Troubleshooting of Capillary Revised Edition, by Dean Rood, Wiley-VCH, New York, 2001.

SPLIT Liners
Discrimination

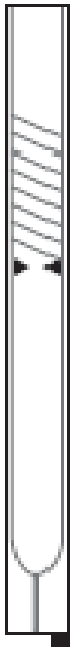
- Liner design influences discrimination
- Greater flow disruption = Less discrimination
- Greater thermal mass = Less discrimination

SPLIT LINER

Straight tube



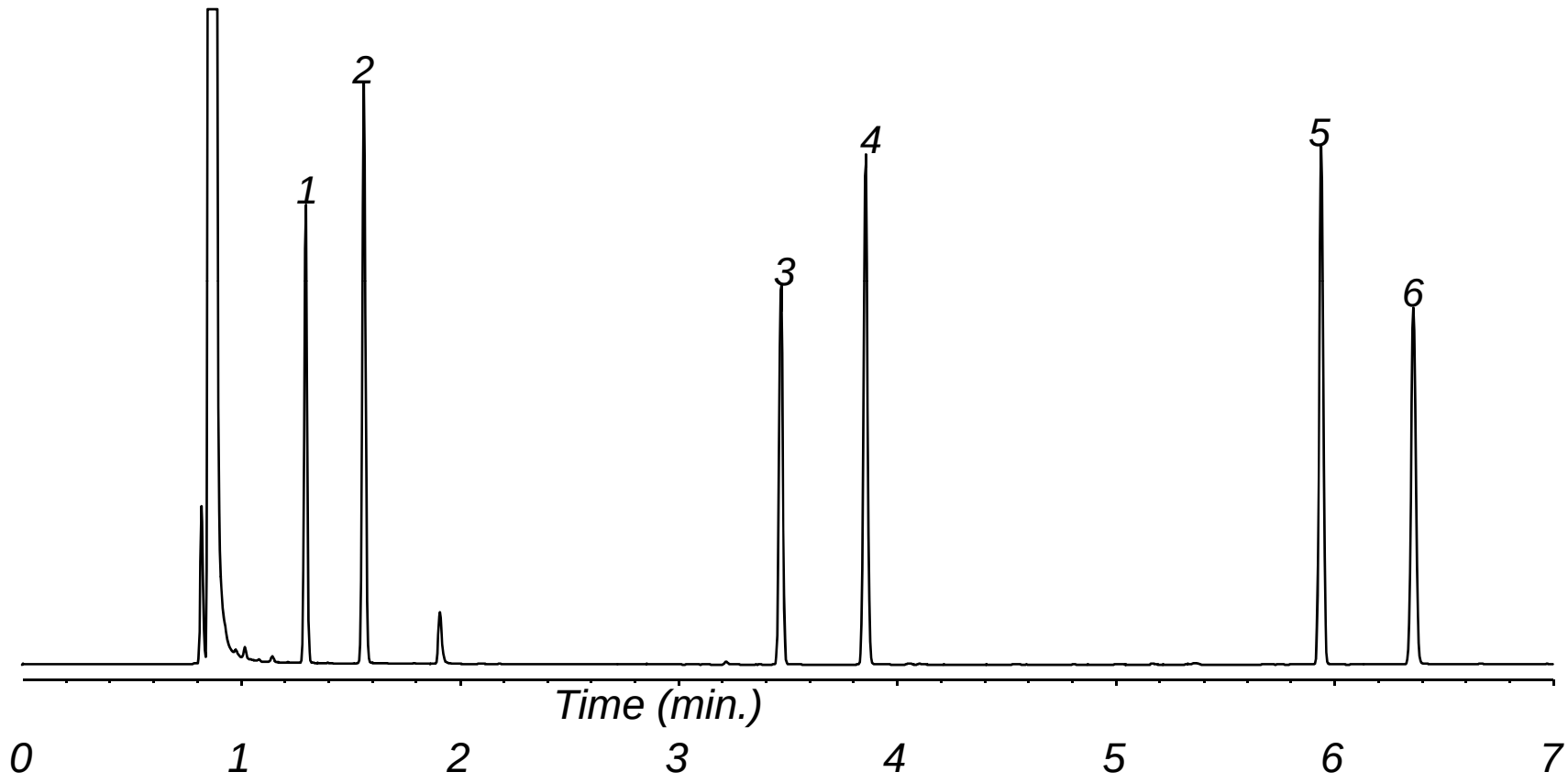
Straight tube with glass wool



Fixed glass wool, low pressure drop

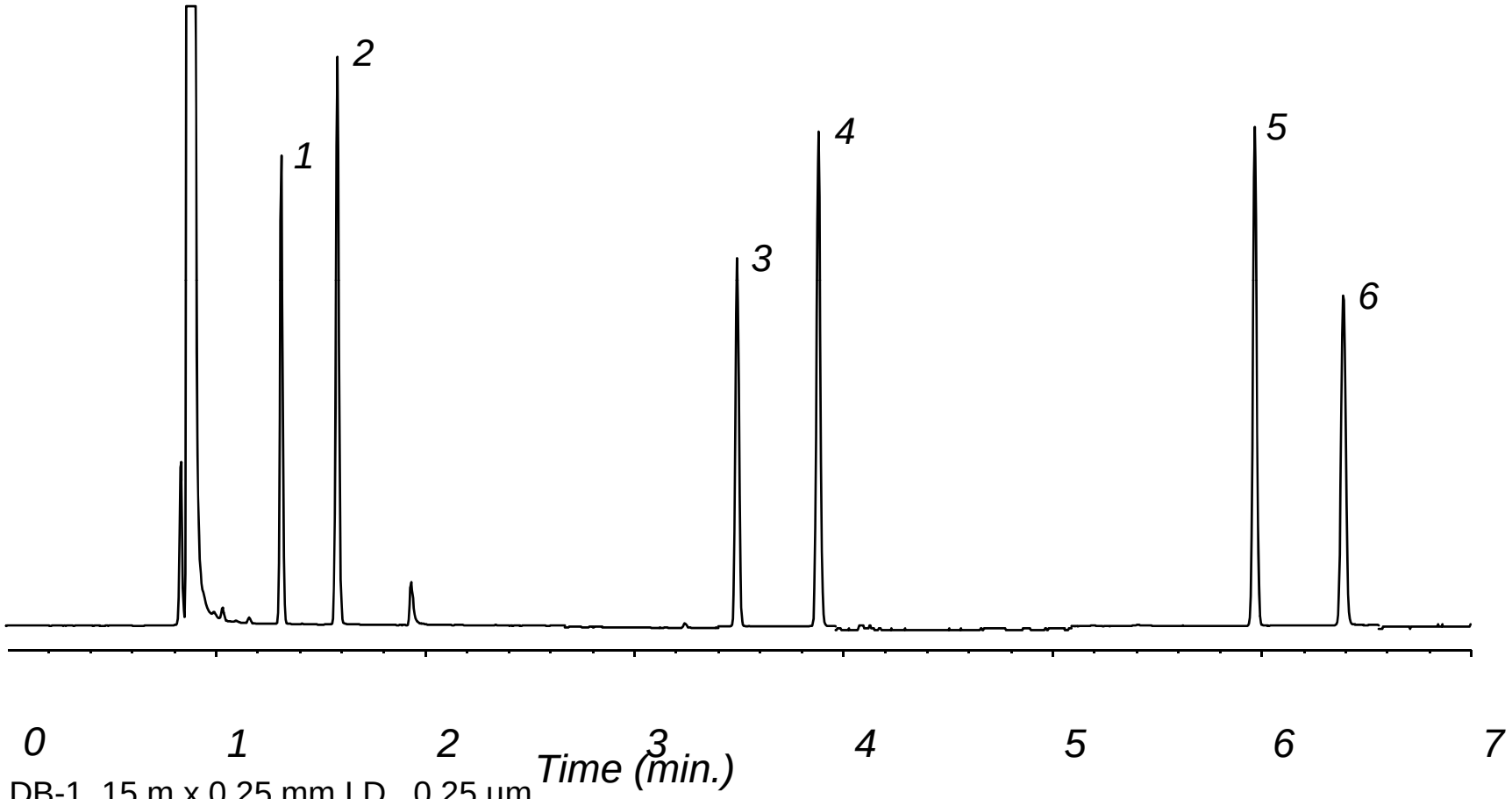
Inverted cup

SPLIT LINER
Packed With Glass Wool



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 6. biphenyl

SPLIT LINER
Inverted Cup



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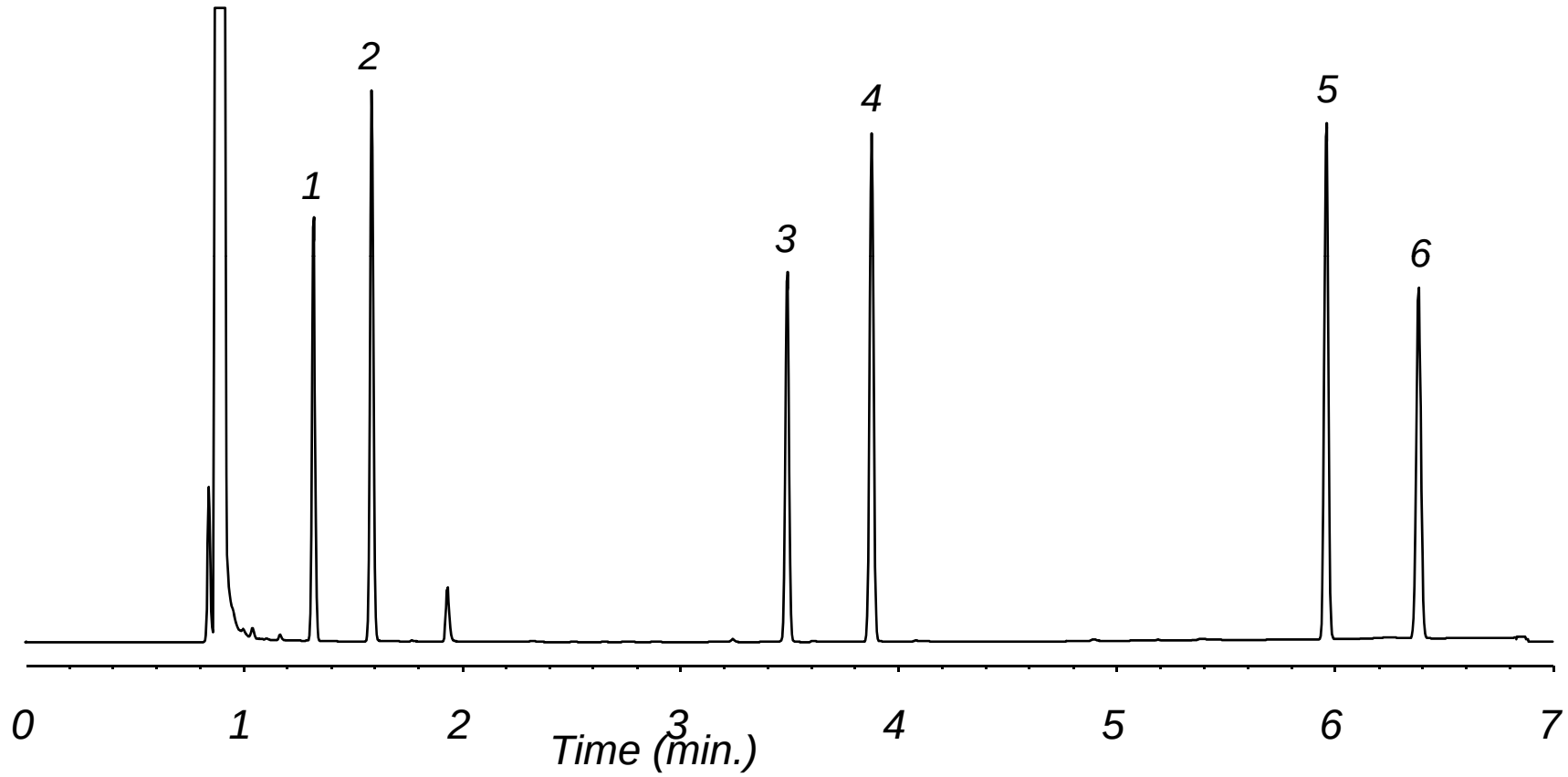
1. n-heptane 2. toluene 3. n-decane 4. n-butylbenzene 5. n-tridecane 6. biphenyl

SPLIT LINER
Straight Tube



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1. n-heptane 2. toluene 3. n-decane 4. n-butylbenzene 5. n-tridecane 6. biphenyl

SPLIT LINER
Peak Area Ratios

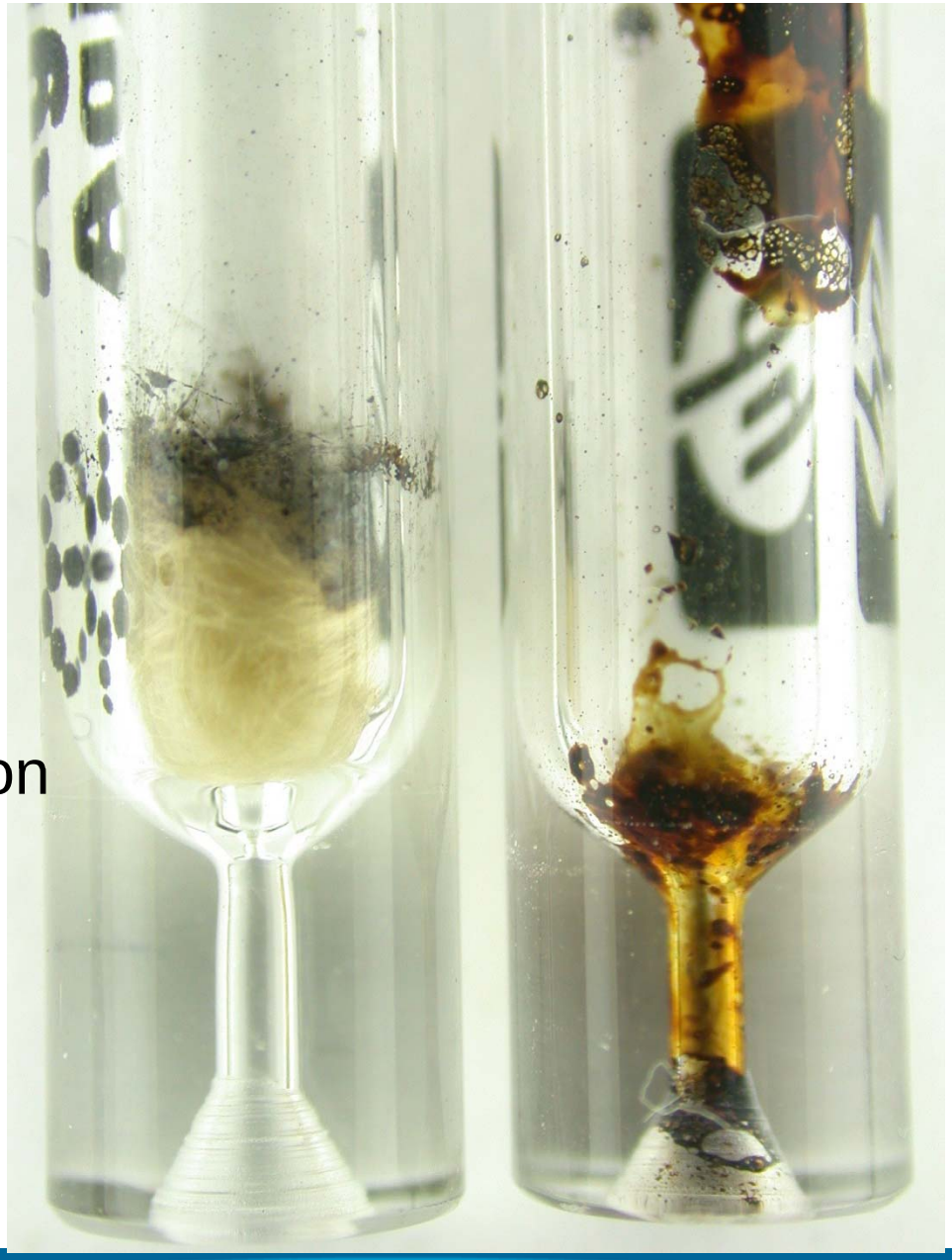
	Peak Area*
Glass Wool	0.76
Inverted Cup	0.82
Straight	0.75

**Peak #1 Area/Peak #6 Area*

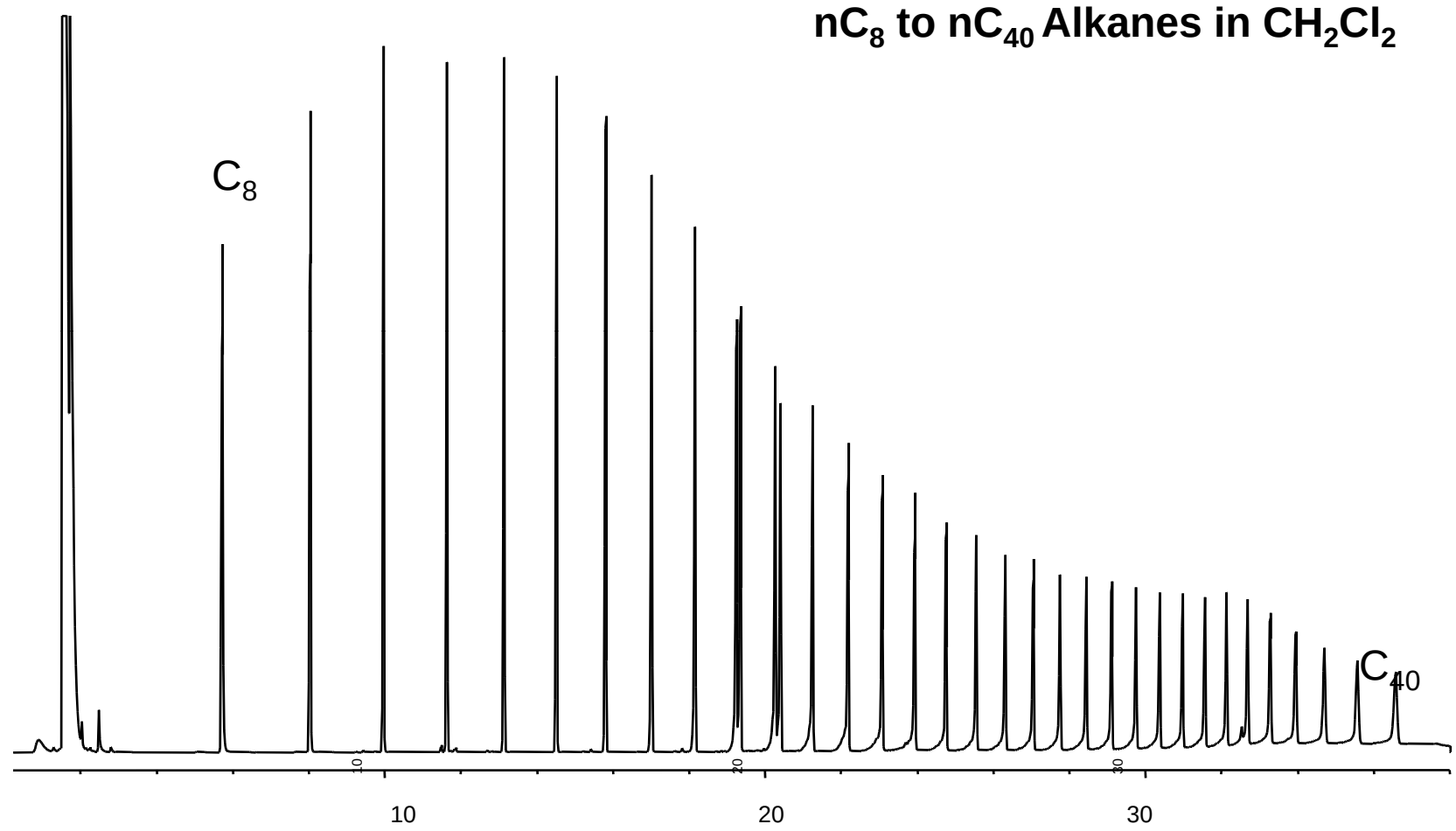
SPLIT

Silylated Glass Wool

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

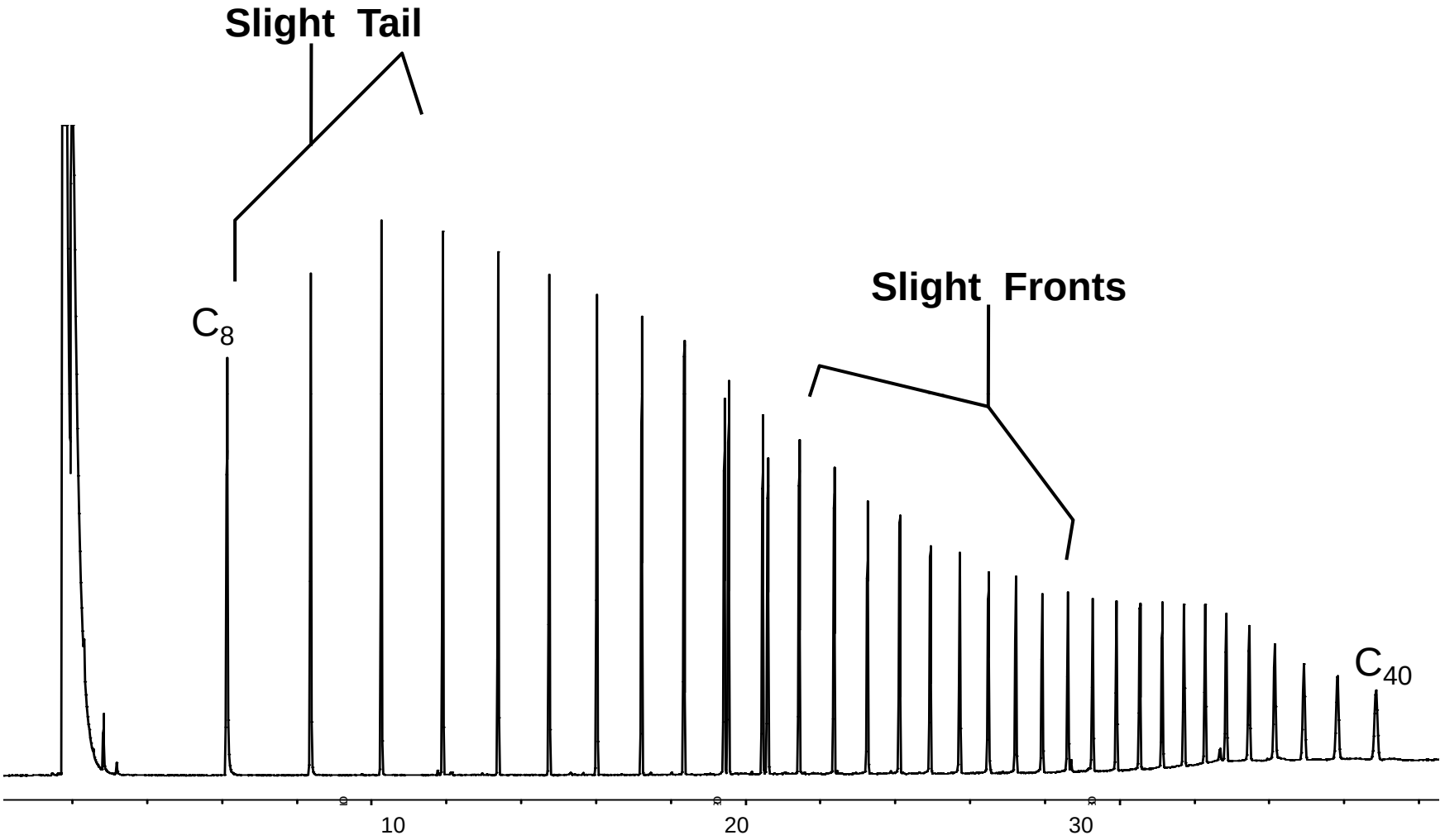


INJECTOR DISCRIMINATION



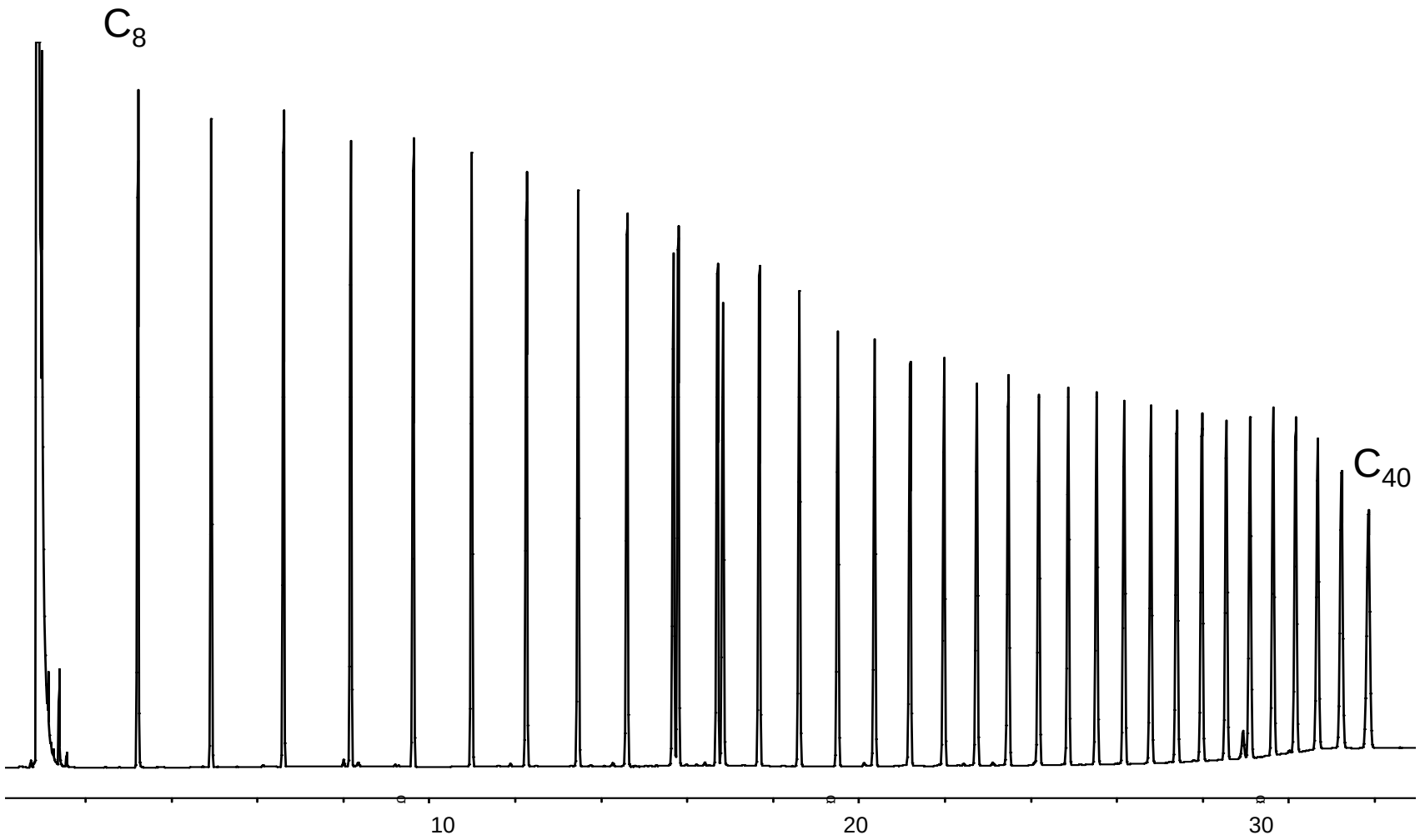
Oven: 35°C for 4 min, 35-320°C at 10°/min, 320°C for 5 min
Carrier Gas: Helium at 5.0 mL/min

ADD 10mg GLASS WOOL TO THE INJECTION PORT LINER



Oven: 35C for 4 min, 35-320°C at 10°/min, 320°C for 5 min
Carrier Gas: Helium at 5.0 mL/min

LARGER PLUG OF GLASS WOOL IN THE LINER



Oven: 35°C for 4 min, 35-320°C at 10°/min, 320°C for 5 min
Carrier Gas: Helium at 9.5 mL/min

GLASS WOOL
Placement in Liner

Near top of liner:

- Wipes syringe needle of sample
- Can improve injector precision
- Helps to prevent backflash

Near bottom of liner:

- Helps in volatilization of high MW components
- Increases mixing

GLASS WOOL
Considerations

- Always use deactivated (silylated) wool
- Borosilicate or quartz material?

GLASS WOOL

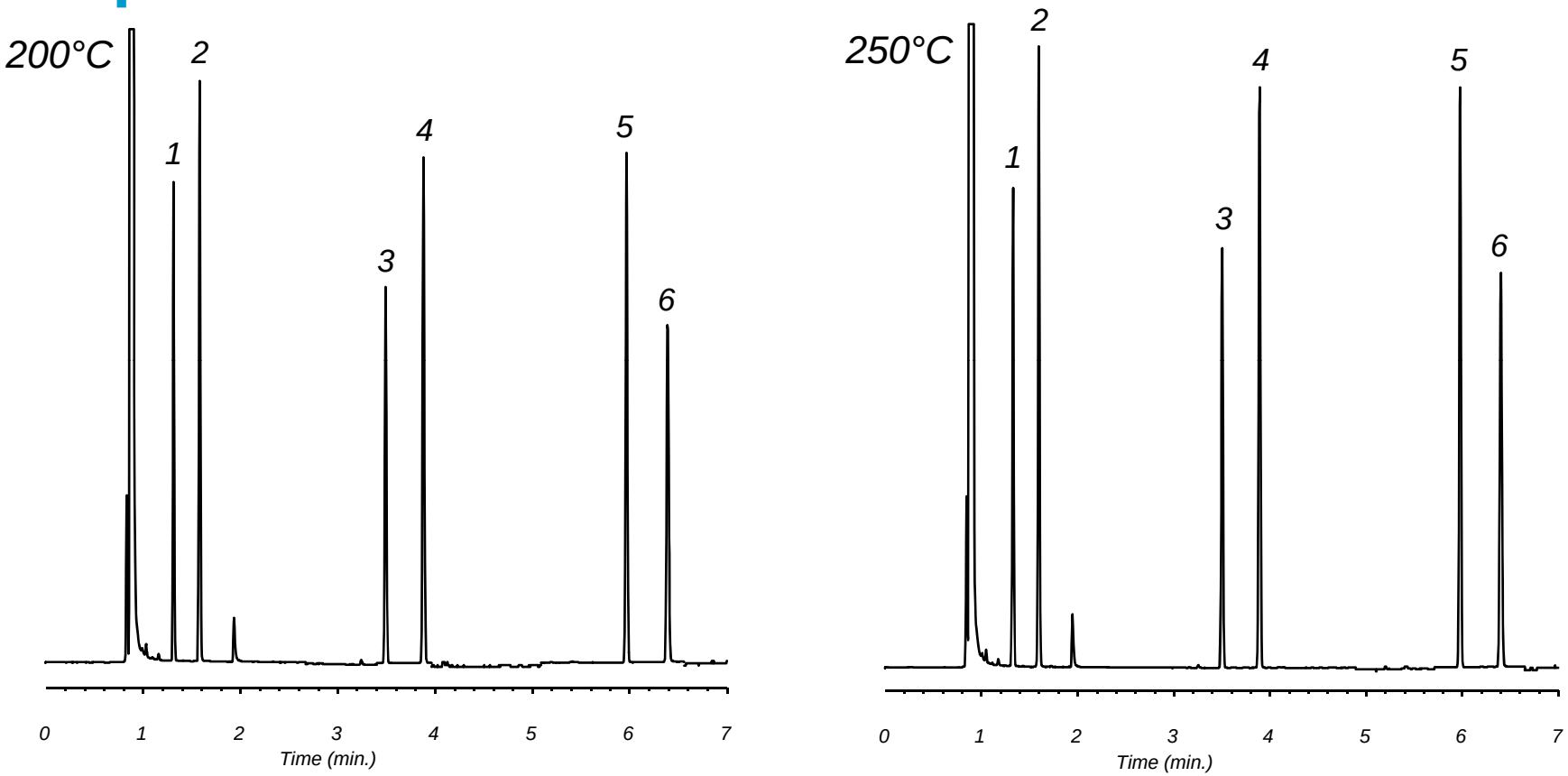
Liner Packing Recommendations

- Amount, size and placement must be consistent for consistent results
- Can be broken upon installation into the liner, exposing active sites
- Liner deactivation with glass wool plug in place is ideal

SPLIT INJECTOR
Temperature

- Hot enough to rapidly vaporize the sample
- May degrade sample or result in injector contamination if too hot
- Typically 200-250°C

SPLIT INJECTOR
Temperature



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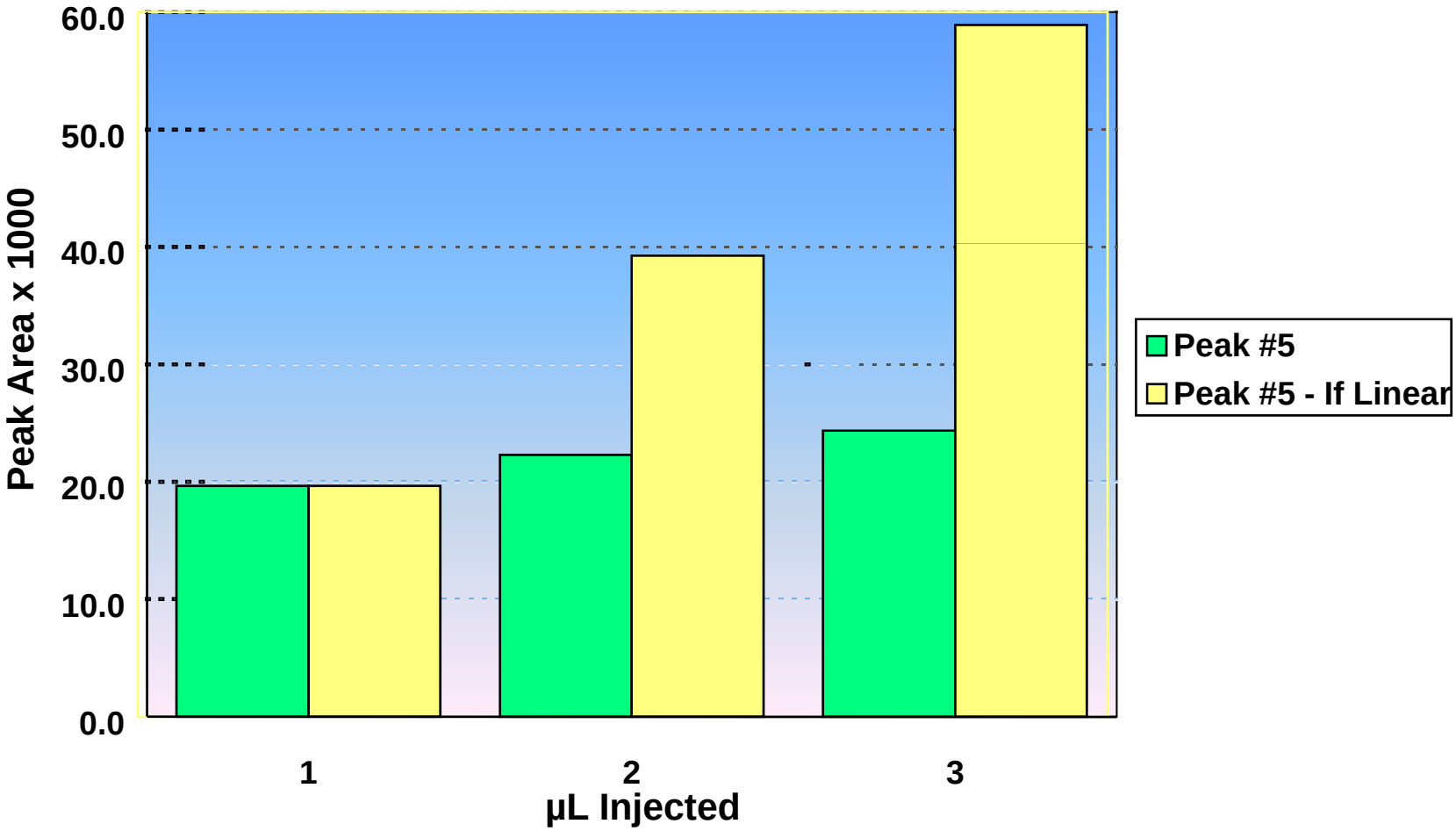
1. n-heptane 2. toluene 3. n-decane 4. n-butylbenzene 5. n-tridecane 6. biphenyl

SPLIT INJECTOR
Injection Volume



SPLIT INJECTOR
Injection Volume

- Injection volume is not linear

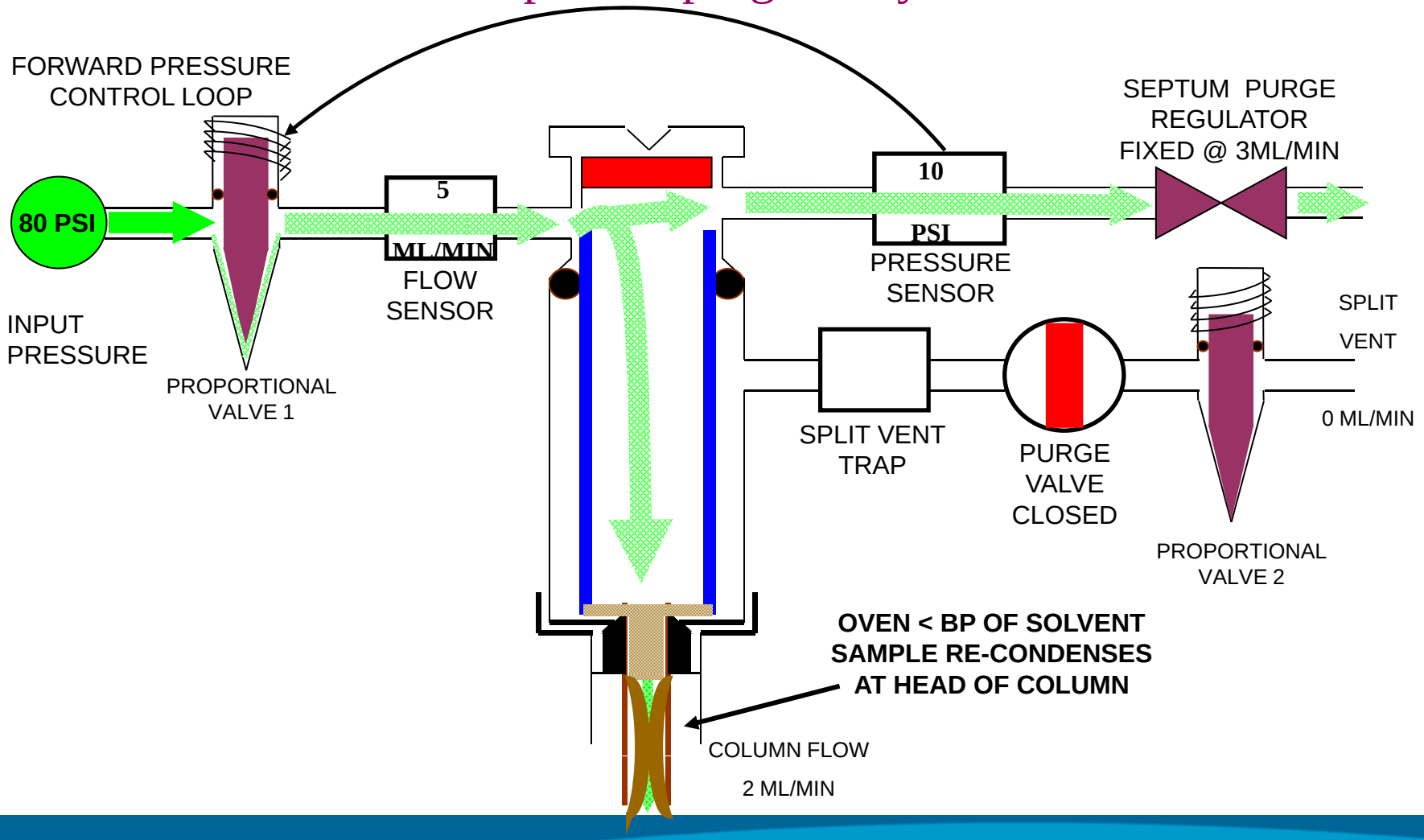
- Inject same volume for all samples and standards for accurate and precise results

SPLIT
Summary

- Concentrated samples
- High efficiency
- All columns
- Liners - be consistent

6850/90 Splitless Injection

Until the end of the splitless purge delay. . .



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

Carrier Gas Flow Considerations At Injection

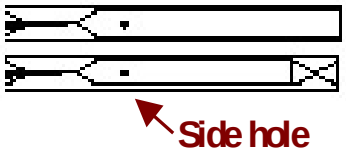
- 1-2 mL/min in the inlet and into column
- 1-2 minutes sweep rate
- Long sample residence time
- Slow sweep rate = Poor efficiency

SPLITLESS INJECTOR
Purge Activation Time

- Purges injector of residual sample
- Reduces solvent front size
- Typically 0.25 - 1.5 minutes
- Not linear in amount injected/unit time

Longer purge time = More sample in column and larger solvent front

Splitless Injection Liners

Liner	Part No.	Comments
	5181-3316	Single taper, deactivated, 900μL volume. Taper isolates sample from metal seal, reducing breakdown of compounds that are active with metals. For trace samples, general application.
	5062-3587	Single taper, deactivated, with glass wool, 900μL volume. Glass wool aides volatilization and protects column. For trace (dirty) samples.
	5181-3315	Double taper, deactivated, 800μL volume. Taper on inlet reduces chance for backflash into carrier gas lines. High efficiency liner for trace, active samples.
	G1544-80730 G1544-80700	Direct connect liners, single and dual taper, deactivated. Capillary column press fits into liner end, eliminating sample exposure to inlet. Ultimate protection for trace, active samples. Side hole permits use with EPC.

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

Splitless Liner Maintenance (contd.)

Advantages of cleaning liners yourself:

- **Reduced cost**

Disadvantages:

- **Time-consuming**
- **Liners with special features (glass wool, cup, etc.) are difficult to clean**
- **Reproducibility of liner is compromised**
- **Removing or inserting glass wool may create significant active sites in glass**

Best advice, keep a supply of new liners on-hand!

Splitless 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

Do liner types really matter?

They do, especially for active compounds like:

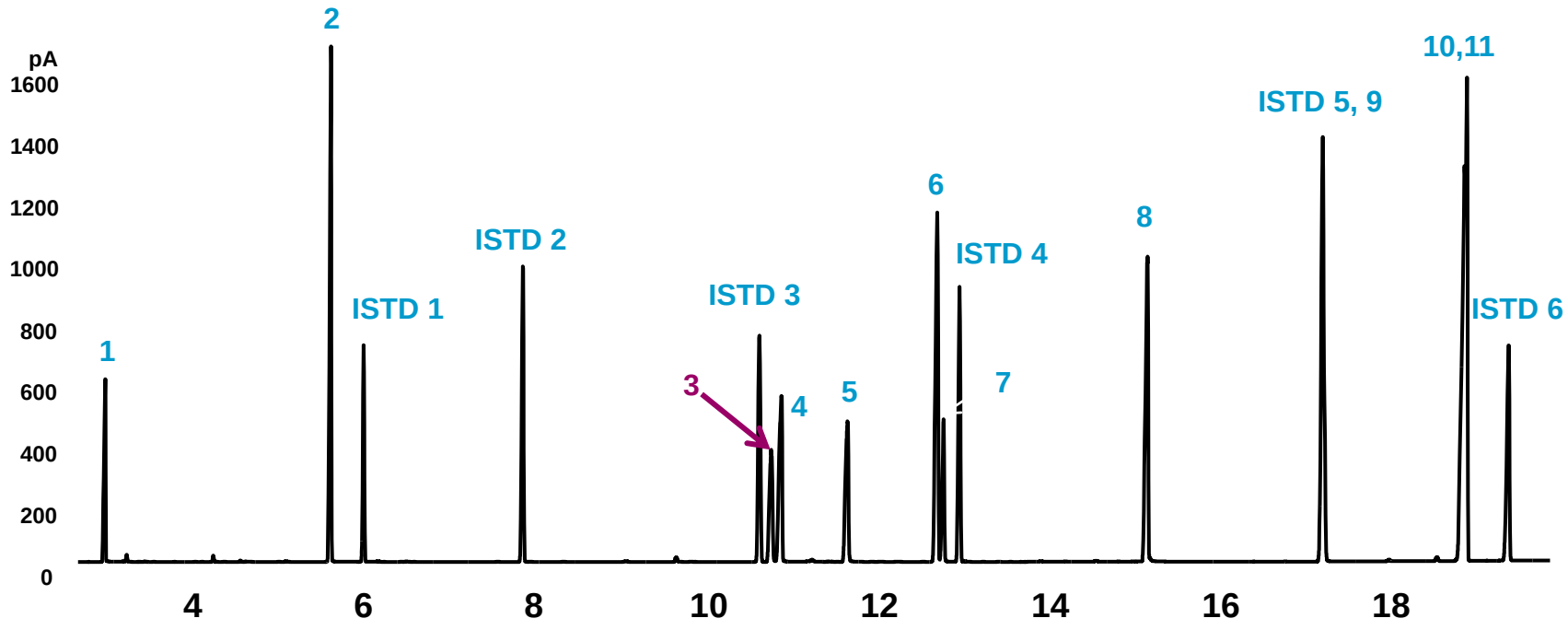
- phenols
- organic acids
- pesticides
- amines
- drugs of abuse, etc.

Phenols, for example….in a separation of EPA method 8270 compounds

Cool On-Column-FID Injection of 11 Analyte Test Mix

From "Improvements in the Agilent 6890/5973 GC/MSD System for Use with USEPA Method 8270", Agilent Application Note 5988-3072EN

1	N-Nitrosodimethylamine	7	Pentachlorophenol	ISTD 1	Dichlorobenzene-d4
2	Aniline	8	Benzidine	ISTD 2	Naphthalene-d8
3	2,4-Dinitrophenol	9	3,3-Dichlorobenzidine	ISTD 3	Acenaphthene-d10
4	4-Nitrophenol	10	Benzo(b)fluoranthene	ISTD 4	Phenanthrene-d10
5	4,6-Dinitro-2-methylphenol	11	Benzo(k)fluoranthene	ISTD 5	Chrysene-d12
6	4-Aminobiphenyl			ISTD 6	Perylene-d12



Splitless Inlet Liners Tested

5062-3587 Single-taper, deactivated, with glass wool

5181-3316 Single-taper, deactivated (open top)

5181-3315 Dual-taper, deactivated (closed top)

G1544-80730 Direct Connect, single-taper, deactivated

G1544-80700 Direct Connect, Dual-taper, deactivated

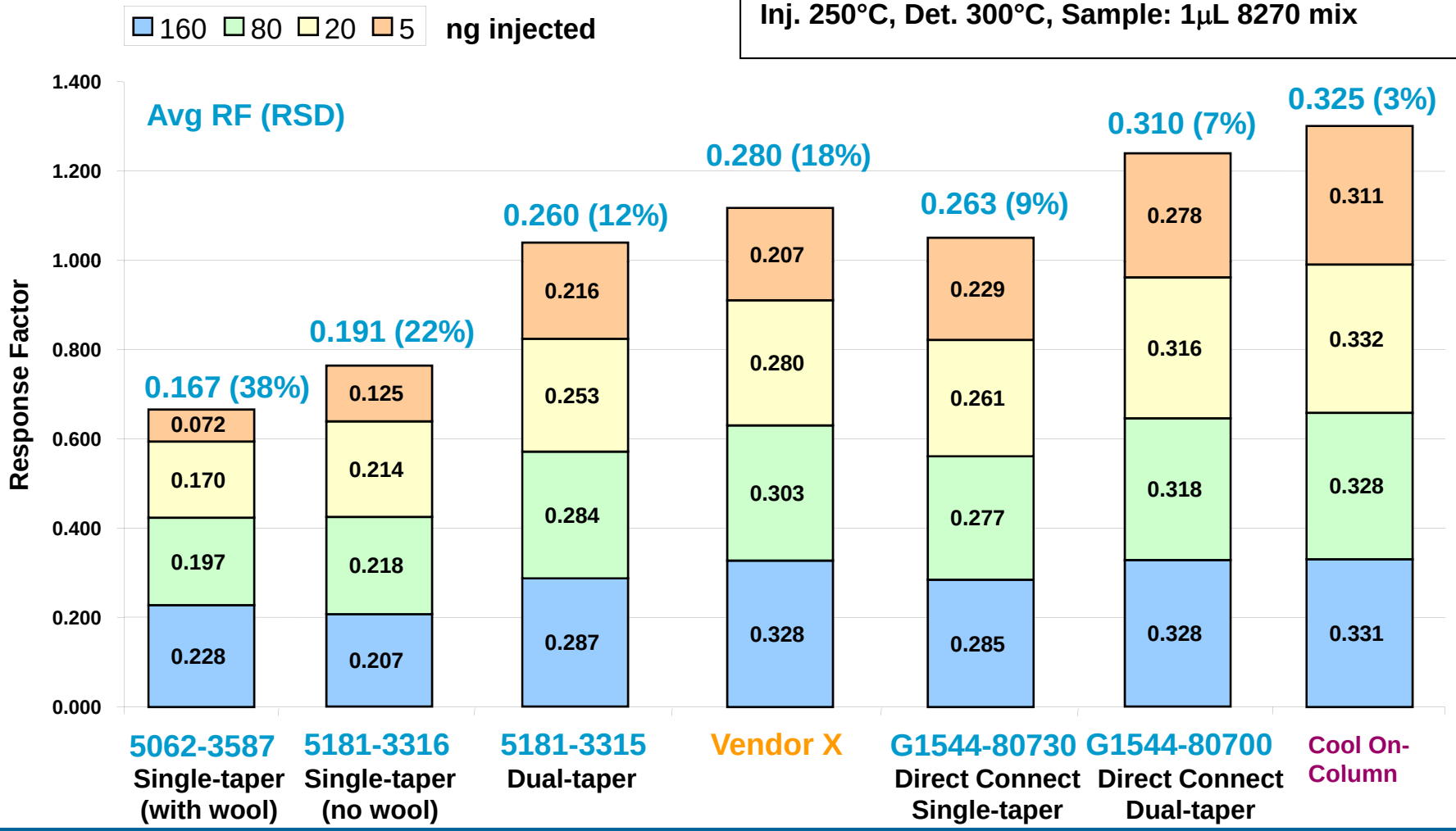
Vendor X Unknown proprietary deactivation

Liner Comparison

Experimental:

Agilent 6890 with FID
Column = HP-5MS 30m x 0.25mm x 0.5µm
Compared COC to various liners
0.75 min Splitless time, 3mL/min column flow
Oven: Temp programmed per 8270 method
Inj. 250°C, Det. 300°C, Sample: 1µL 8270 mix

2,4-Dinitrophenol Response Factors



Splitless Liner Conclusions

Agilent inlet liners can be used with a broad range of samples and analytes and chromatographic response depends heavily on liner type.

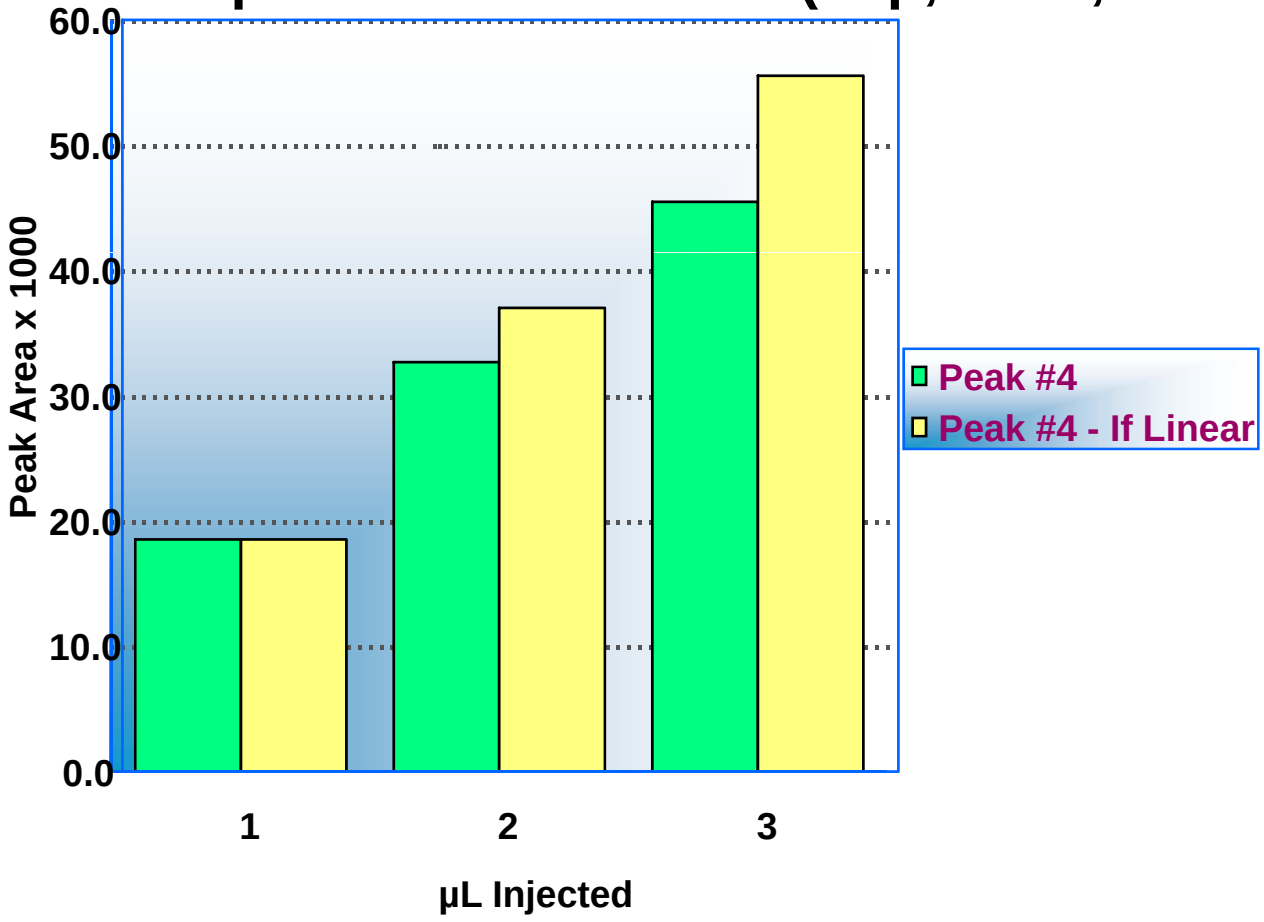
To choose a liner, first consider:

- **Type of inlet in your GC**
- **Concentration and type of sample**
  - **high conc. - use Split**
  - **trace analytes - use Splitless or PTV**
  - **broad range - use Split/Splitless or PTV - general purpose**
  - **heat-sensitive and high boiling point compounds - use On-Column or PTV**

Splitless Liner Conclusions (contd.)

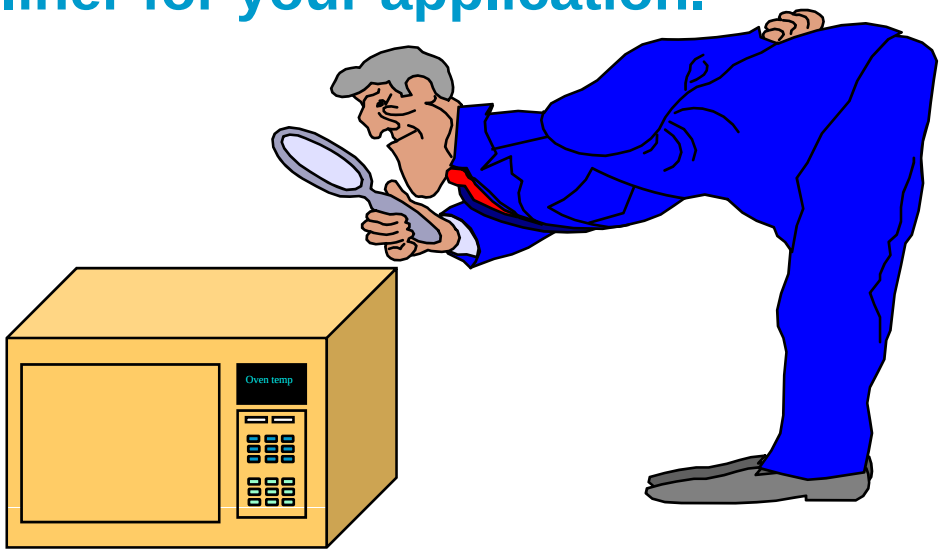
Next, consider…

- **Sample size, solvent, cleanliness, and potential analyte activity - helps to choose special liner features (cup, wool, taper, etc.) and liner volume that are necessary for your application.**



Splitless Liner Conclusions (contd.)

- **Finally, optimize chromatographic conditions for the best separation.**
- **Remember to check liner condition often and replace when necessary to minimize downtime.**
- **Good chromatography starts with the inlet. Choose the correct liner for your application.**



Liner Conclusions (contd.)

Flip Top for Split/Splitless injection ports

- 30 sec liner change out
- No more hunting for that “funny looking” wrench!
- Saves fingers from getting burned. Increases instrument up time

http://www.chem.agilent.com/Scripts/PDS.asp?lPage=12224

Liner Conclusions (contd.)

7890 Turn Top Inlet System

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”

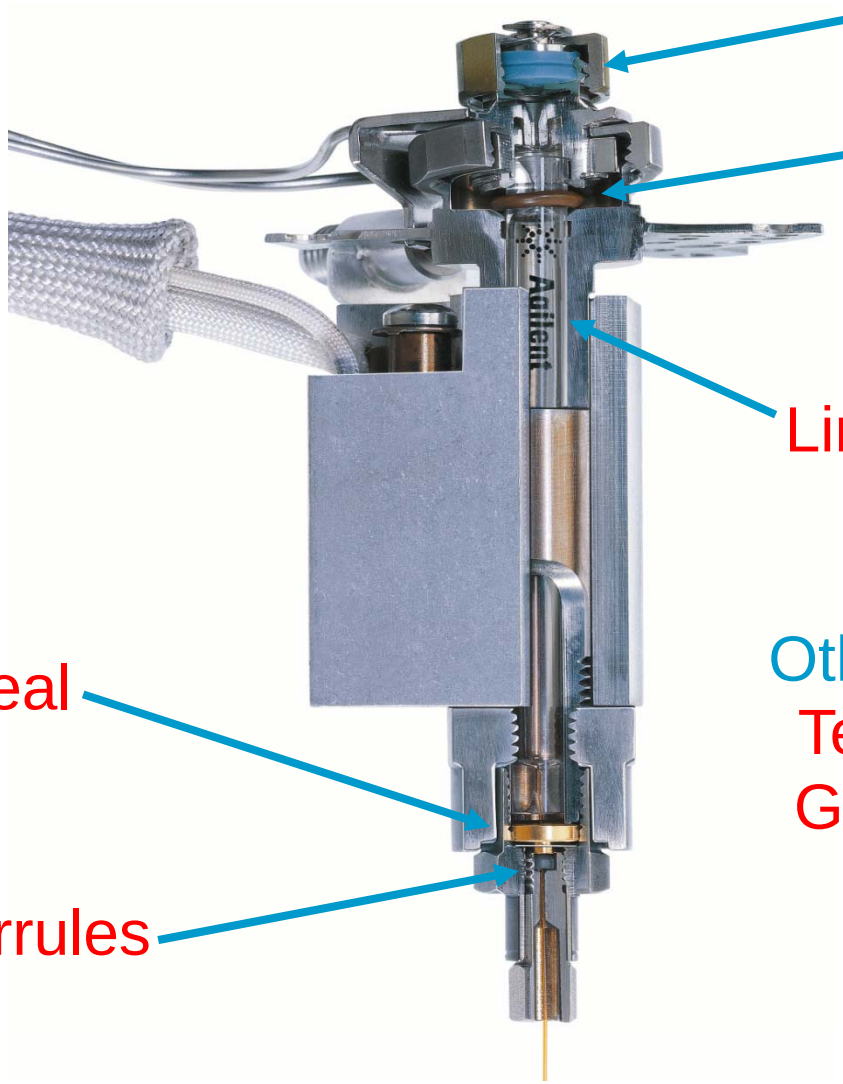
Leak in Septum Nut, Septum

- Damage to O_2 sensitive detectors, irreversible damage to column

Non-Optimized Set-up

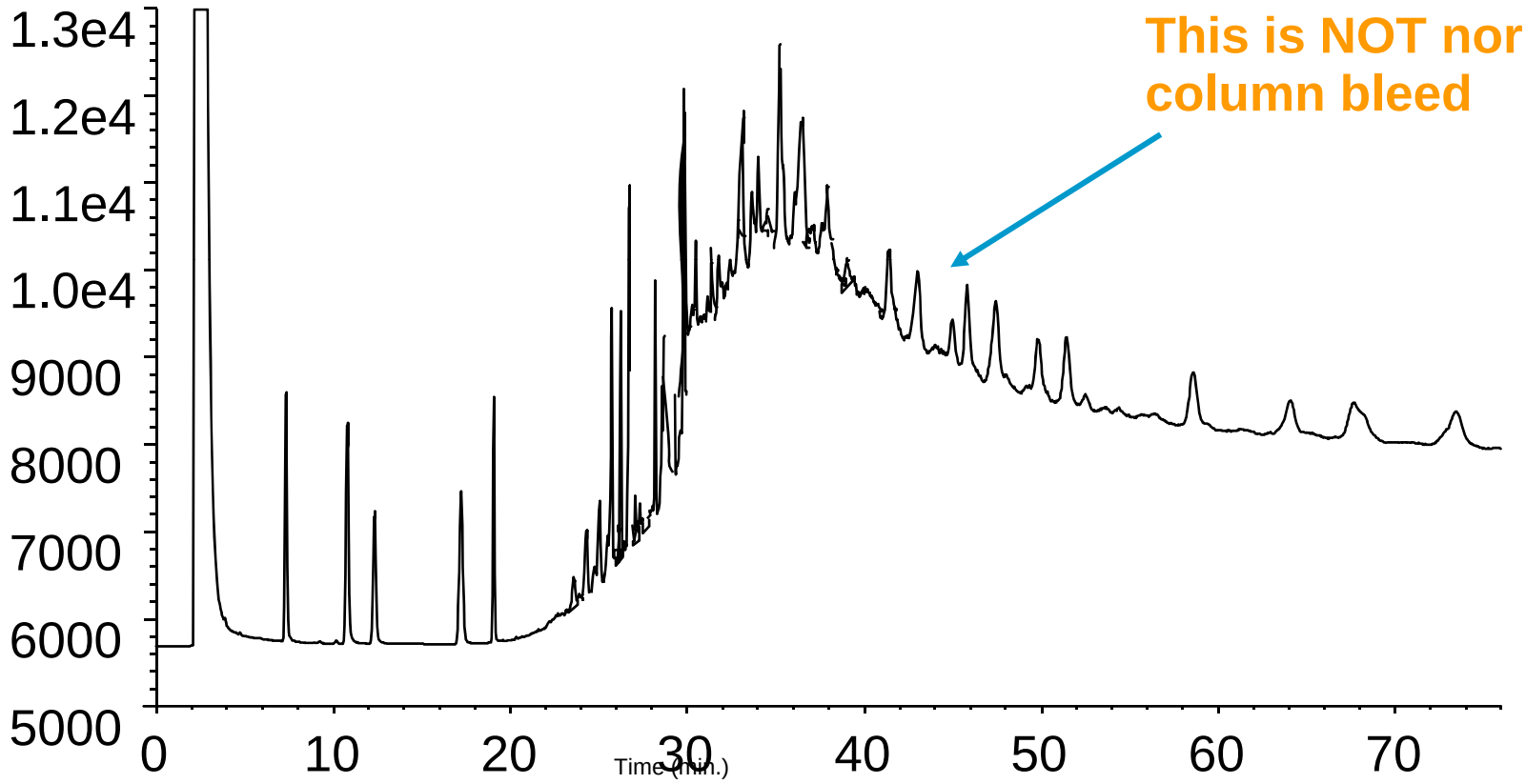
- O-ring, Gold Seal, Ferrules, Column Nuts
- Faster inlet performance degradation between maintenance sessions

Inlet Considerations



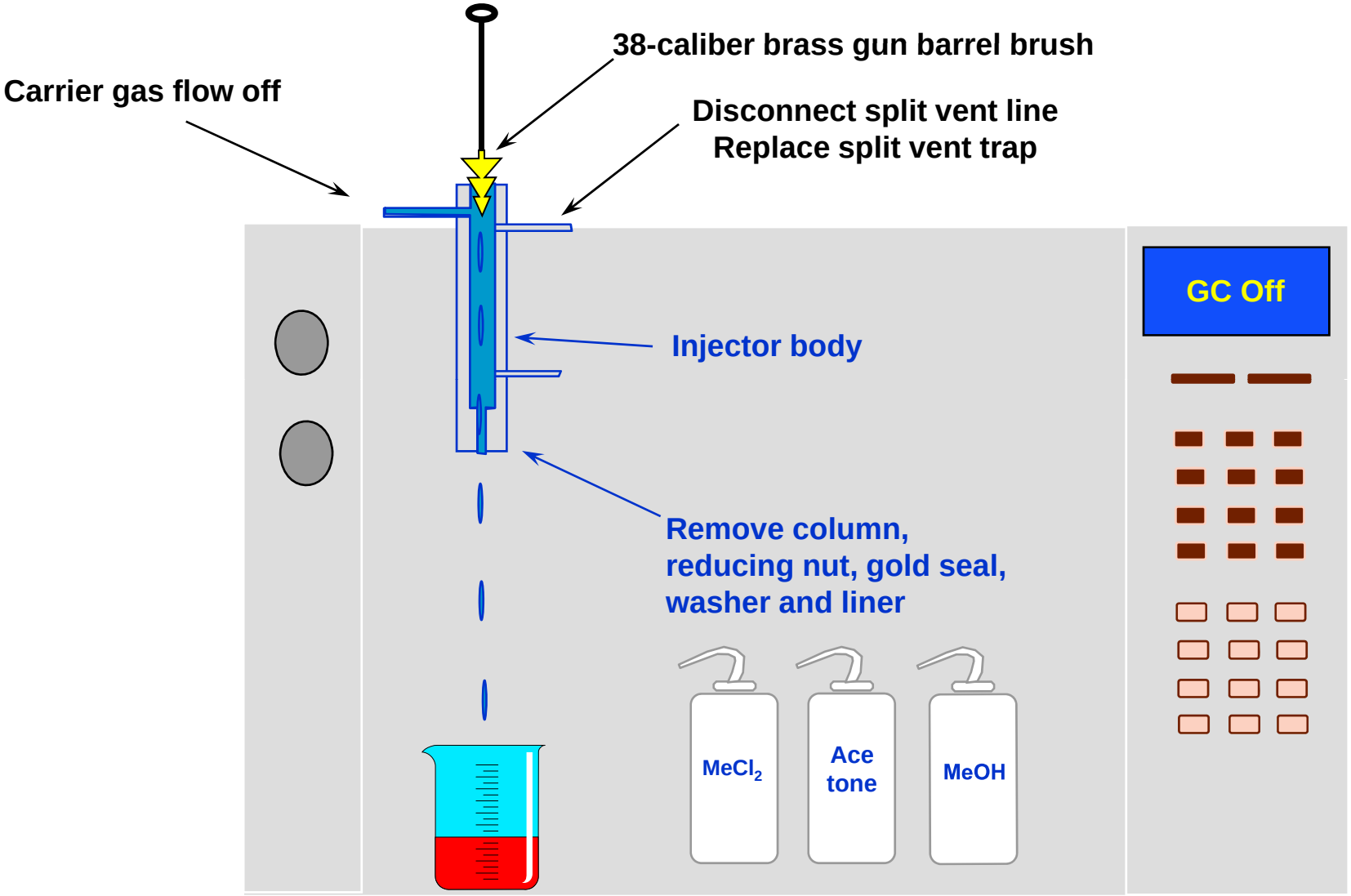
Other Considerations:
- Temperature
- Gas flow rates

Example Of Gross Contamination

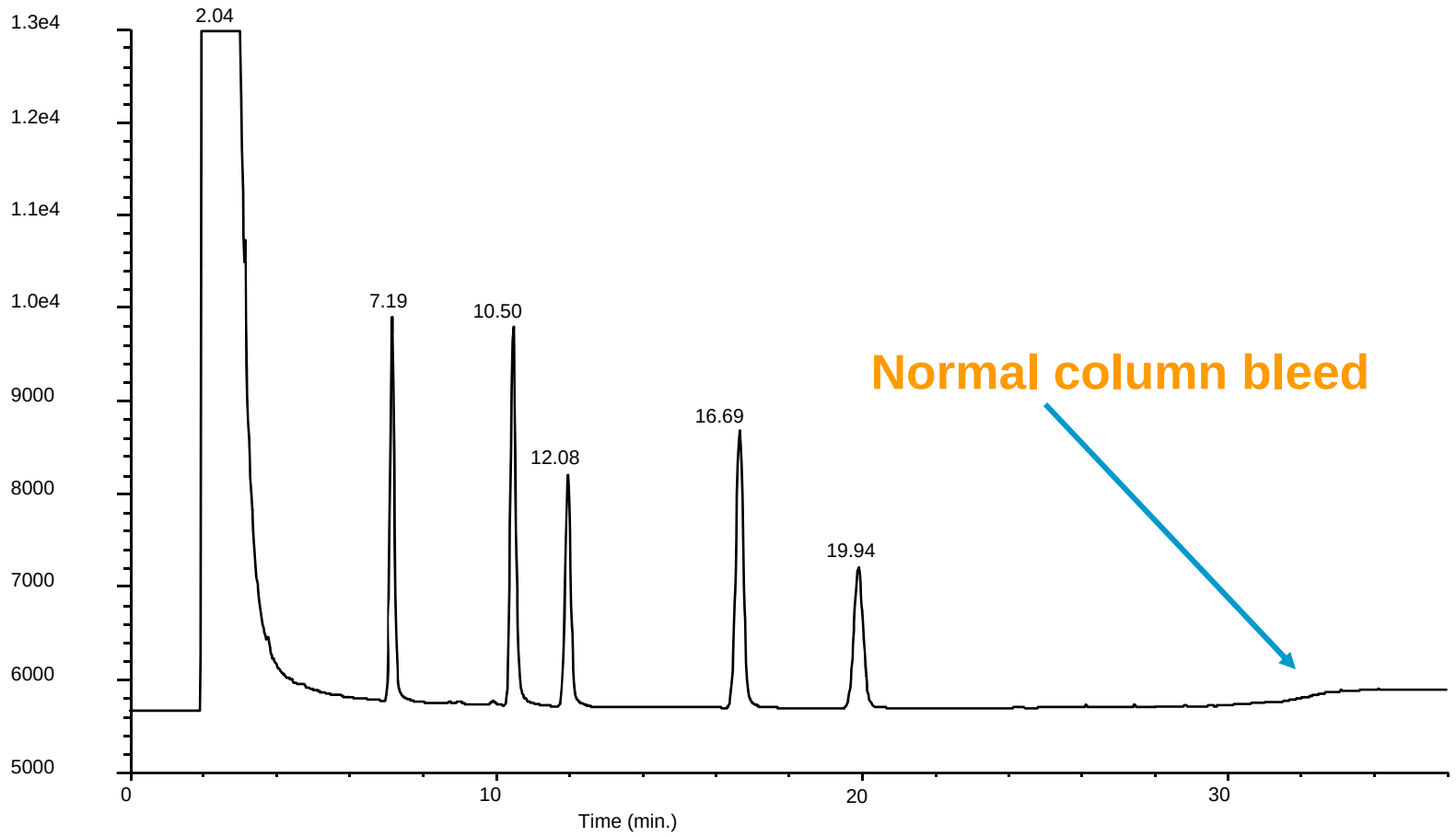


DB-624, 30 meter megabore
Temperature program // 35°C, hold 1.50 min // 30°/min to 65°C, hold 15 min // 20°/min to 260°, hold 50 min

Cleaning the 6890 Split/Splitless Injector



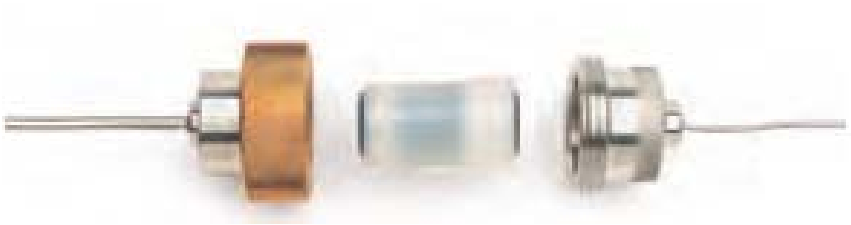
Same Column After Inlet And Column Maintenance



*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

Split Vent Trap

What is it???



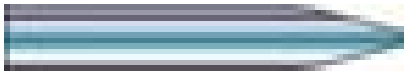
Leak in Septum

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

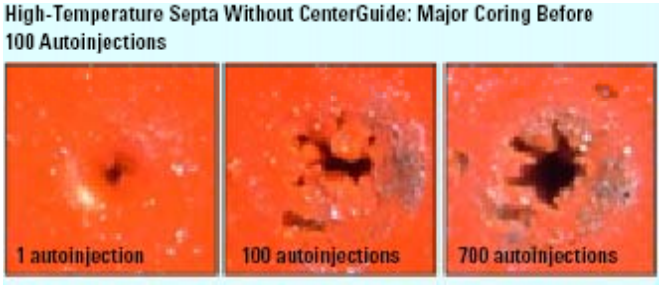
– Use Agilent Gold Standard, 23-26 gauge, HP Point taper syringes. The point style cores septa significantly less when used with CenterGuide Septa. Taper minimizes septum coring/wear.



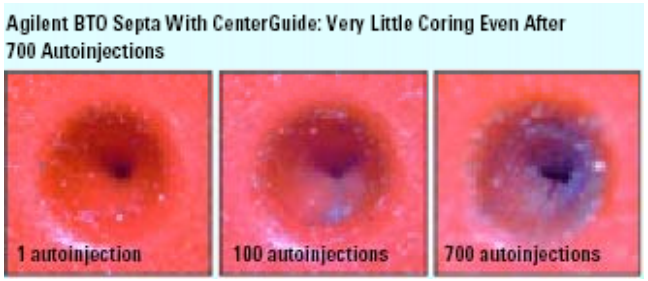
HP-Point Style

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

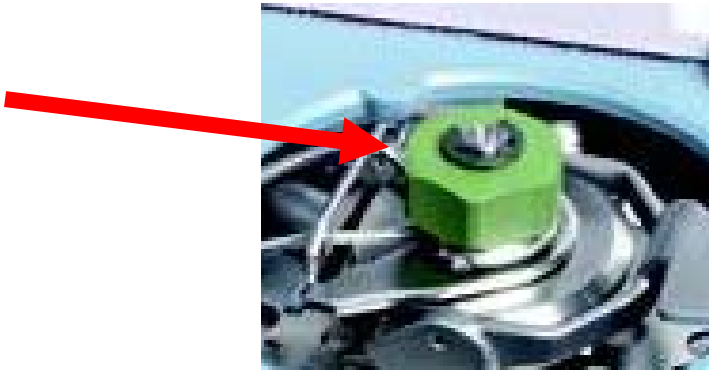
Solid Septum



CenterGuide Septum

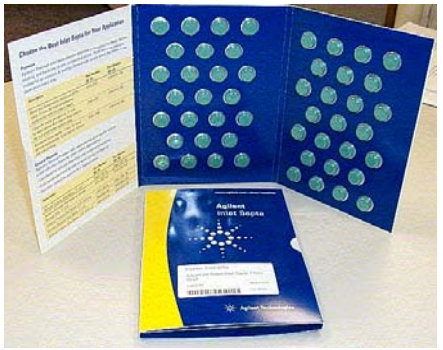


Leaks Due to Septum Nut



- 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.

Other Benefits of Agilent's "Centerguide" Septa



- Packaging eliminates contamination of septa,
  - "first is as good as the last"
- Less Strain on Syringe compared to solid septa
- Bleed/Temperature Optimized, (to 400C, trace analysis), p/n 5183-4757
- Advanced Green, (to 350C, good for general purpose), p/n 5183-4759
- Long Life, (to 350C, more injections before failure), p/n 5183-4761
- Above are 50 packs, 100 packs also available.

Septa vs GC Column Costs

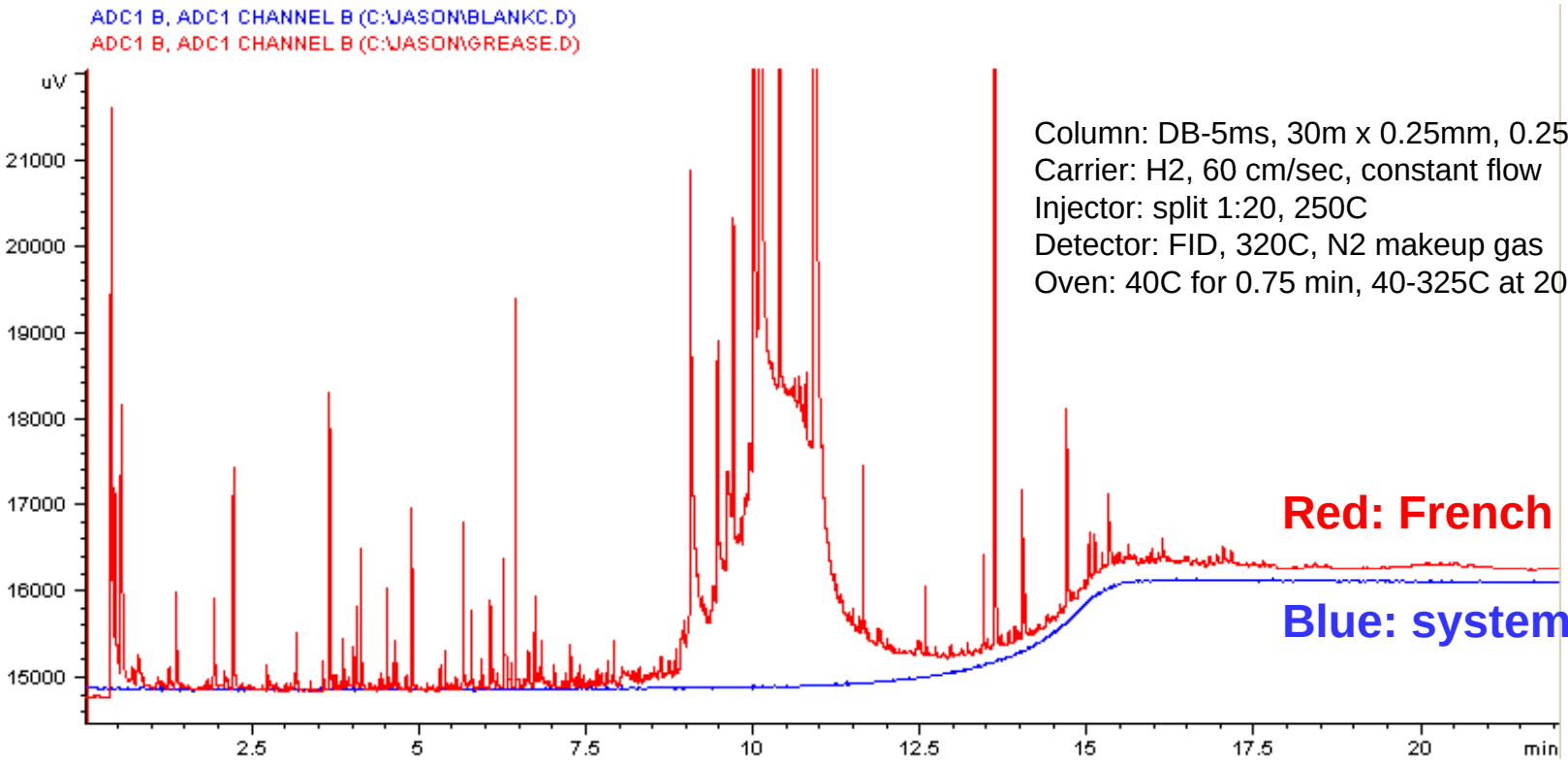
- Typical cost of 1 Premium Septum (list), $1.25
- Typical cost of 1 GC Column, 30 m x 0.25 mm ID, $450.
- No accurate leak rate detector at sub 1 mL/min flow rates.
- “Don’t step over a dollar to pick up a dime!”
- Proactively change inlet septa.

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.

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

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.

Agilent J&W Scientific Technical Support

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* *Select option 3, then 3, then 1.*

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