Navigating Troubled Waters: A Deep Dive into Water Injection by GC

Agilent
Trusted Answers

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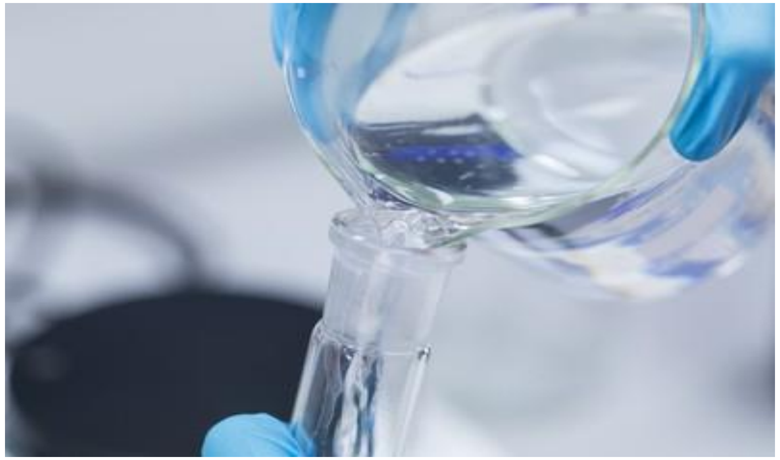
February 27th 2024

Water Injections in GC



Why Inject Water?

Convenient

- Aqueous samples (wastewater, drinking water, and so on)
- Biological samples

Necessary

- Purge and trap

Potential Problems with Water

Injector issues

- Large expansion volume
- Backflash

Detector issues

- Extinguishing FID flame
- Decreasing sensitivity of ECD

Column issues

- Strange peak shape/splitting
- Polarity miss-match
- Phase washout

Potential Problems with Water: Real and Perceived

Solvent – stationary phase mismatch (Real)

- Poor wettability of many stationary phases by water
- Water beads-up on phase
- Think oil and water

Damage to stationary phase (Perceived)

- Change in retention times
- Change in selectivity
- Increase in bleed

What Is Normal Column Bleed?

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

What Is Bleed?

This thermodynamic equilibrium process occurs to some degree in all columns.

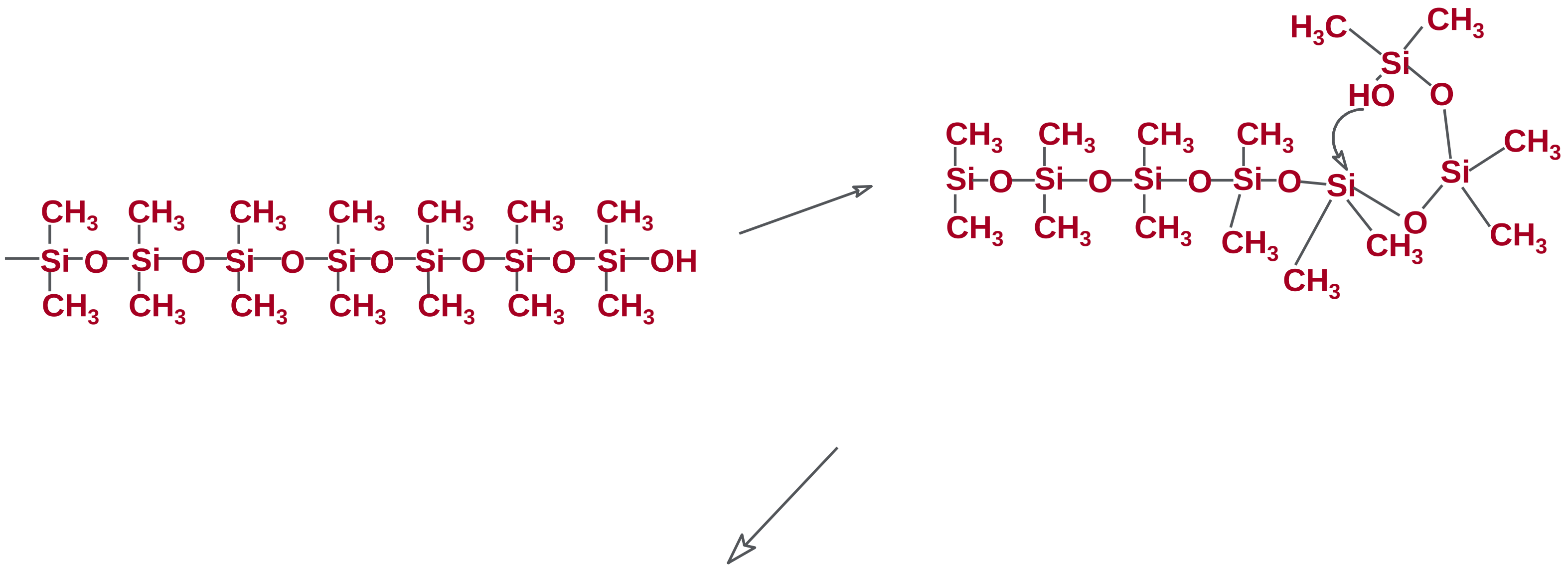
Polysiloxane backbone releases low molecular weight, cyclic fragments.

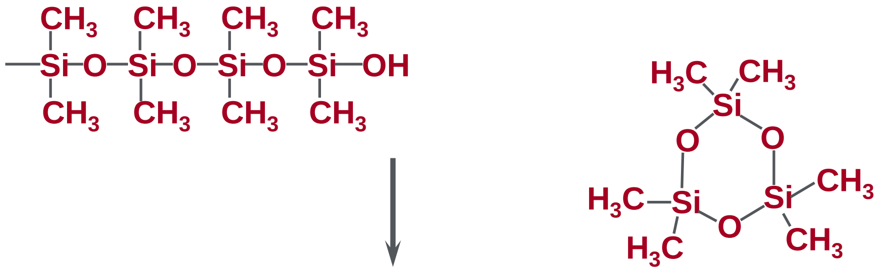
It occurs at low levels in a low temperature, O_2-free, clean system.

It is increased at higher temperatures, and with oxygen exposure, or chemical attacks.

Bleed: Why Does It Happen?

A “back biting” mechanism of product formation

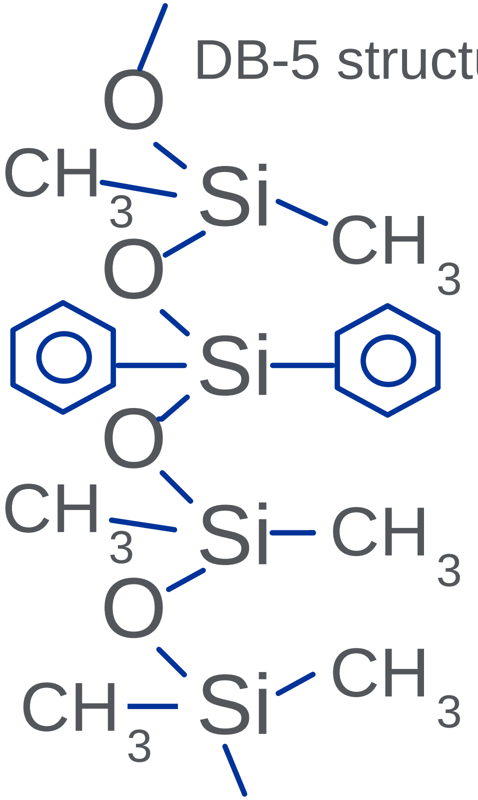




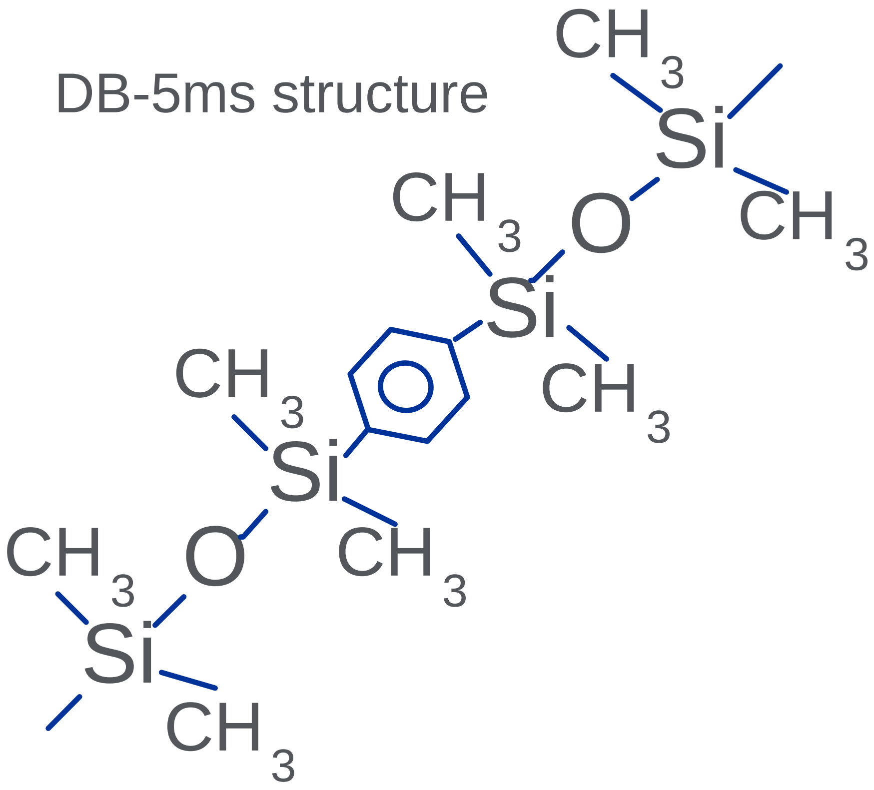
Cyclic products are more thermodynamically stable

Low Bleed Phase Structures

DB-5 structure



DB-5ms structure

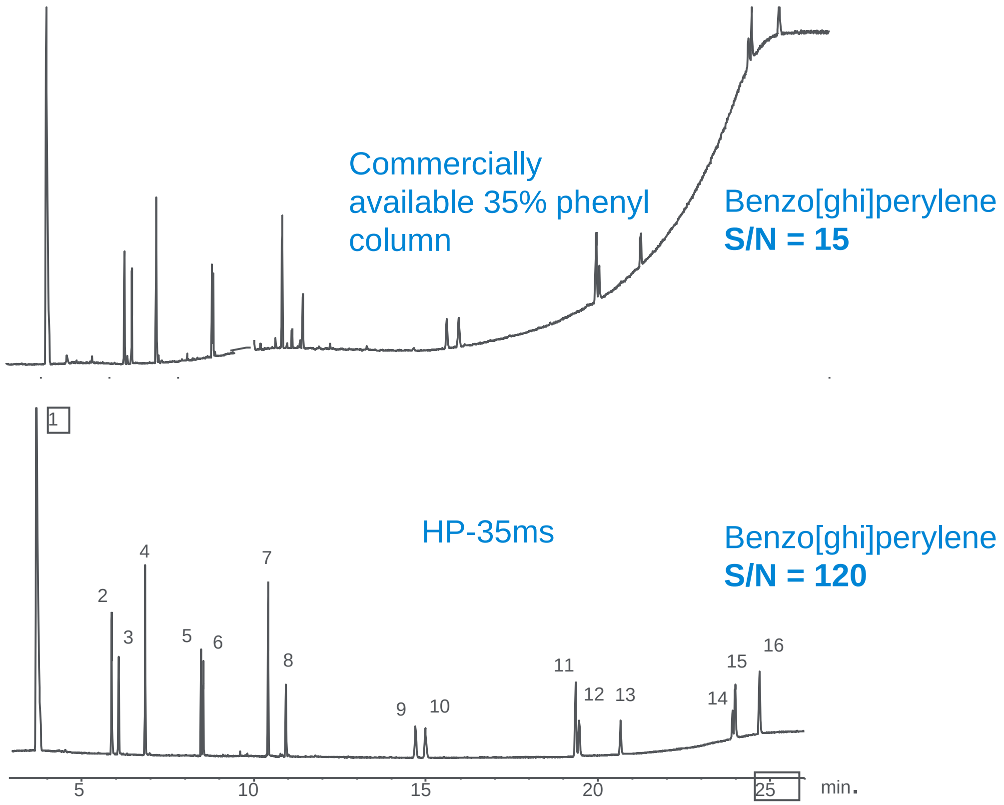


DB-5, HP-5, and HP-5ms
5% phenyl

DB-5ms
1. Increased stability
2. Different selectivity
3. Optimized to match DB-5

Benefits of Low Bleed Phases

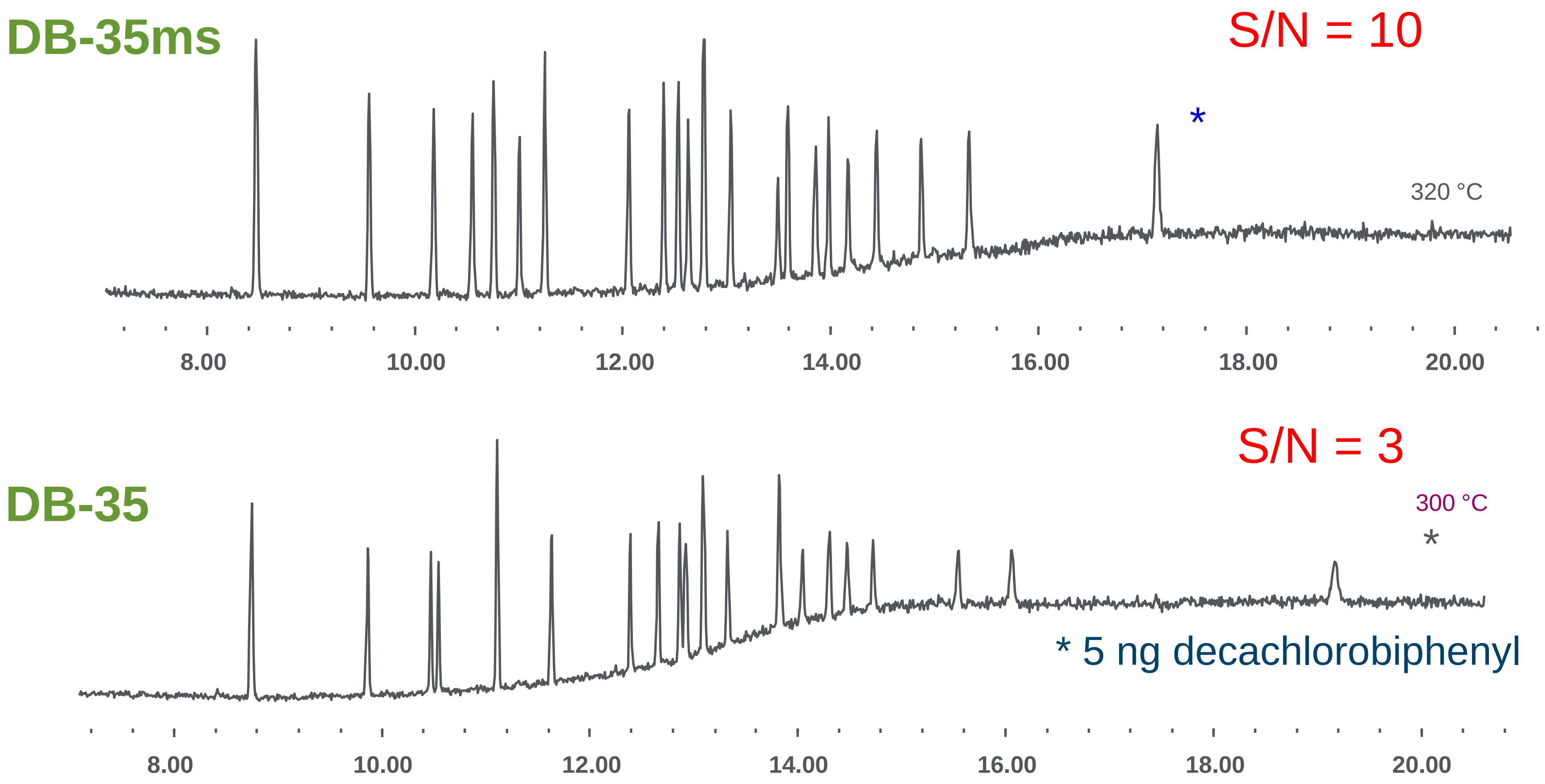
PAH sensitivity using DB-35ms



1. Naphthalene
2. Acenaphthylene
3. Acenaphthene
4. Fluorene
5. Phenanthrene
6. Anthracene
7. Fluoranthene
8. Pyrene
9. Benz[a]anthracene
10. Chrysene
11. Benzo[b]fluoranthene
12. Benzo[k]fluoranthene
13. Benzo[a]pyrene
14. Indeno[1,2,3,-c,d]anthracene
15. Dibenz[a,h]anthracene
16. Benzo[g,h,i]perylene

Columns: 30 m x 0.32 mm x 0.35 µm
Carrier: H_2, constant flow, 5 psi at 100 °C
Injector: 275 °C, splitless, 1 µL, 0.5 to 5 ppm
Oven: 100 to 250 °C (5 min) at 15 °C/min, then to 320 °C (10 min) at 7.5 °C/min
Detector: FID, 320 °C

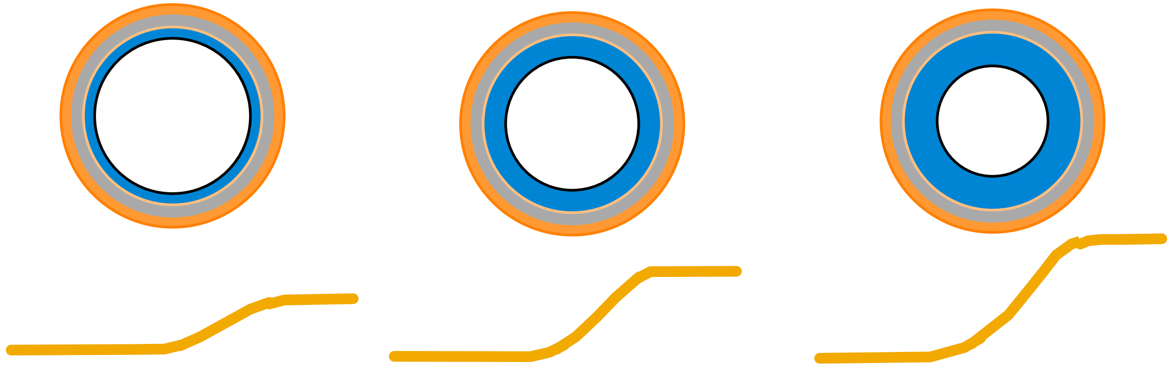
Low Bleed Stationary Phases
DB-35ms versus DB-35



CLP pesticides analysis

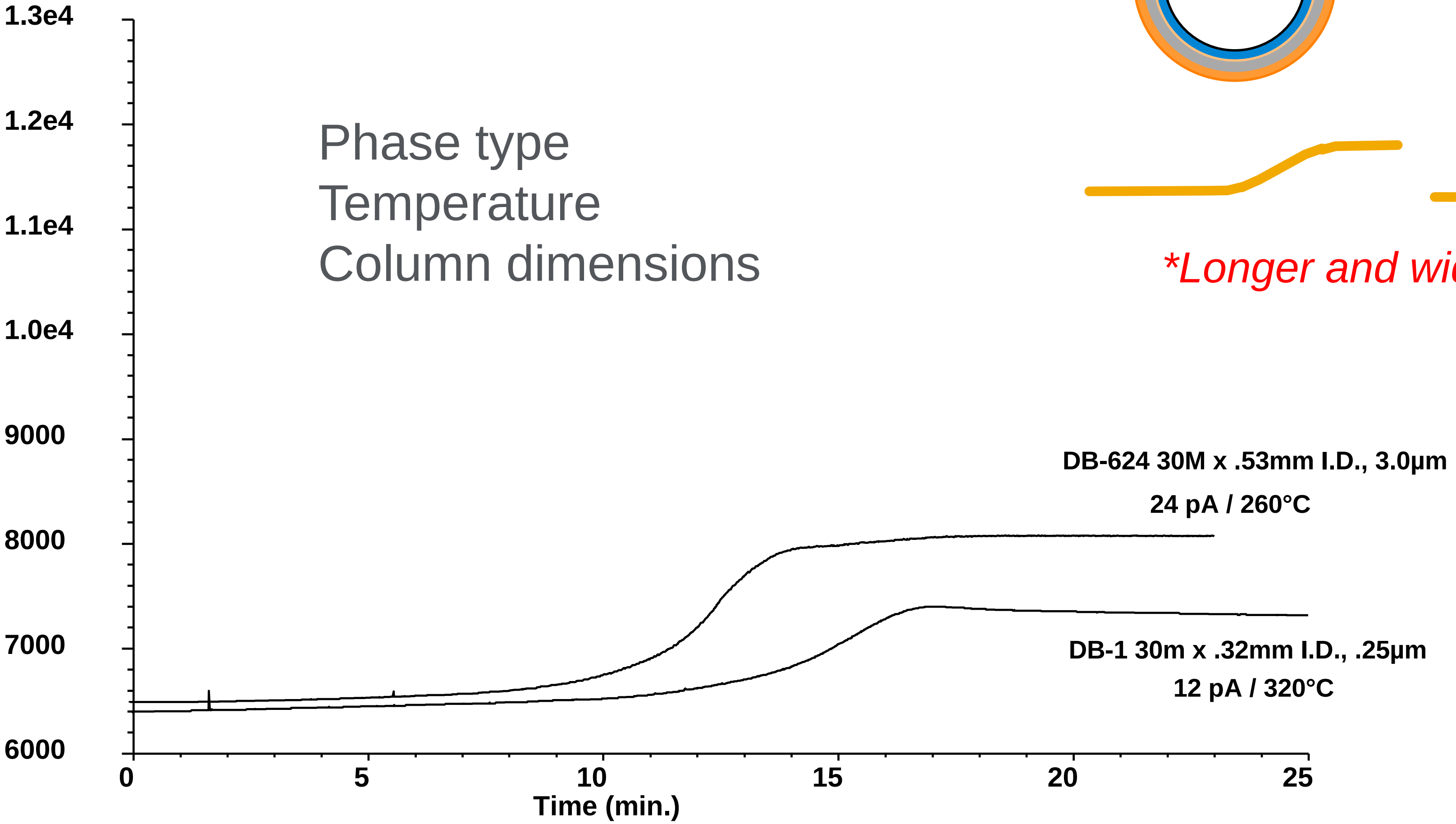
Influences on Column Bleed

More stationary phase = More degradation products



Phase type
Temperature
Column dimensions

Longer and wider columns also bleed more.



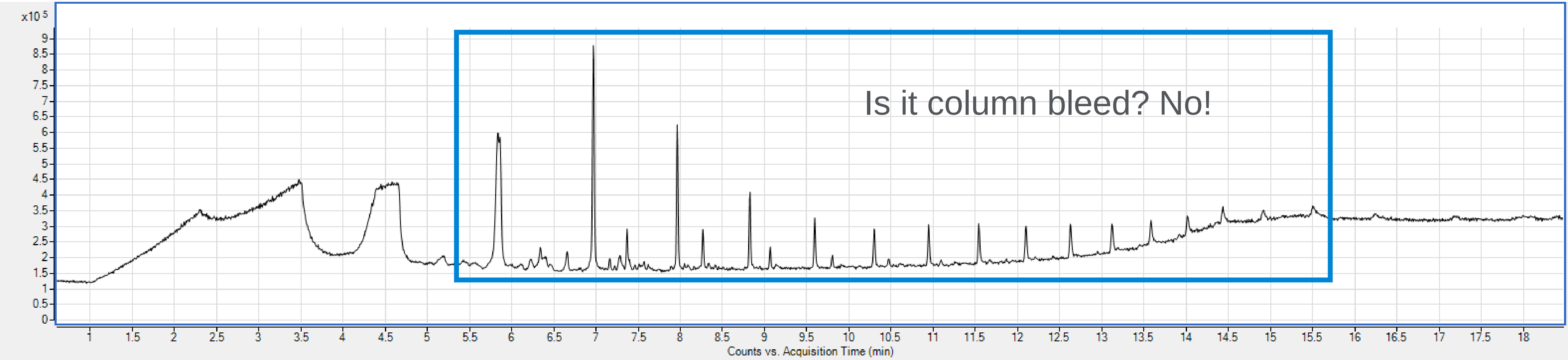
What Is a Bleed Problem?

An abnormal elevated baseline at high temperatures

It is not:

- A high baseline at low temperatures
- A wandering or drifting baseline at any temperature
- Discrete peaks

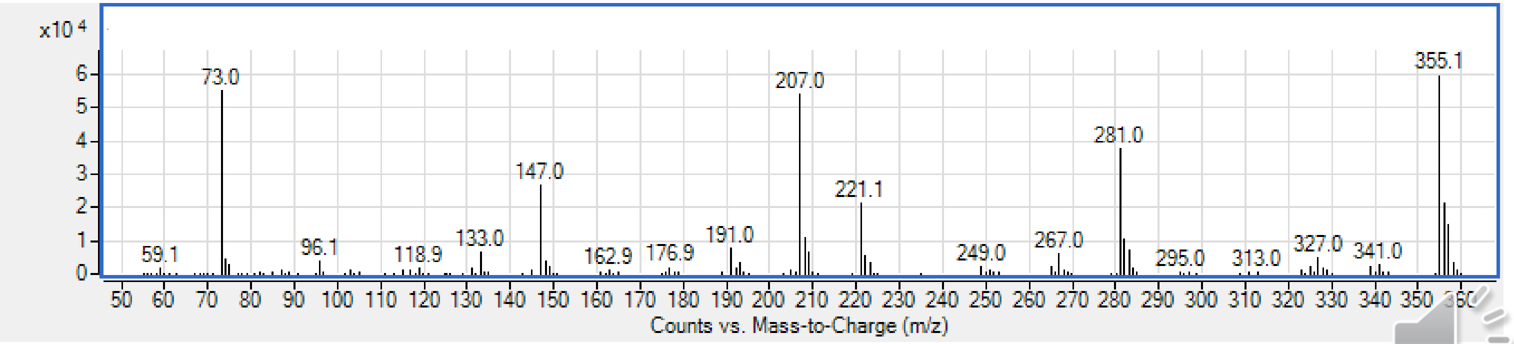
What Are These Repeating Peaks?



Common ions for siloxane molecules:
73
147
207
281
355

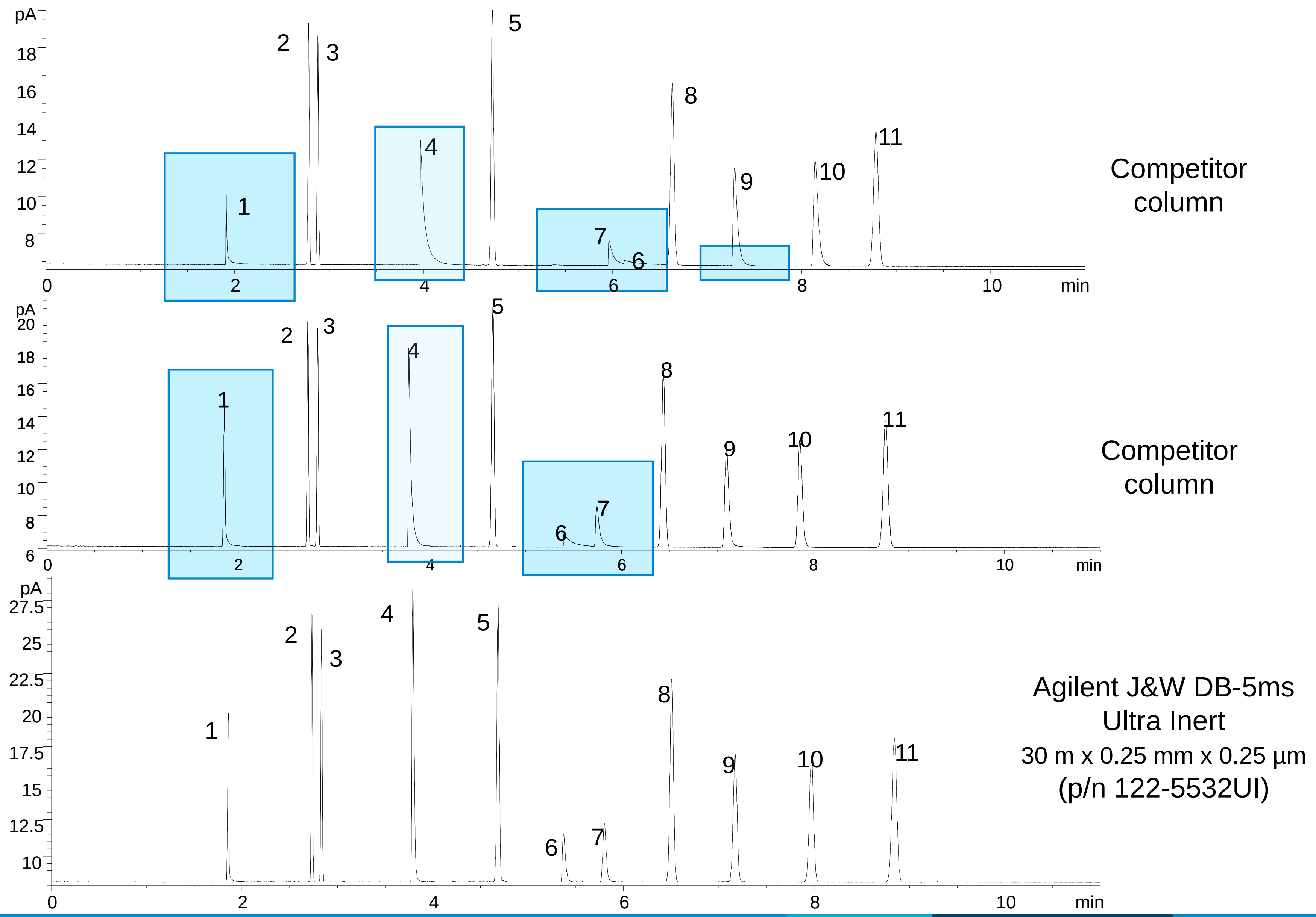
Septa contamination in wash vials or inlet liners can be diagnosed by looking for siloxane polymers in your total ion chromatogram. Each peak in the chromatogram corresponds to a cyclized (ring structure) siloxane molecule. These molecules fragment with very similar patterns.

Example spectrum:



Inertness– DB-5MS Ultra Inert

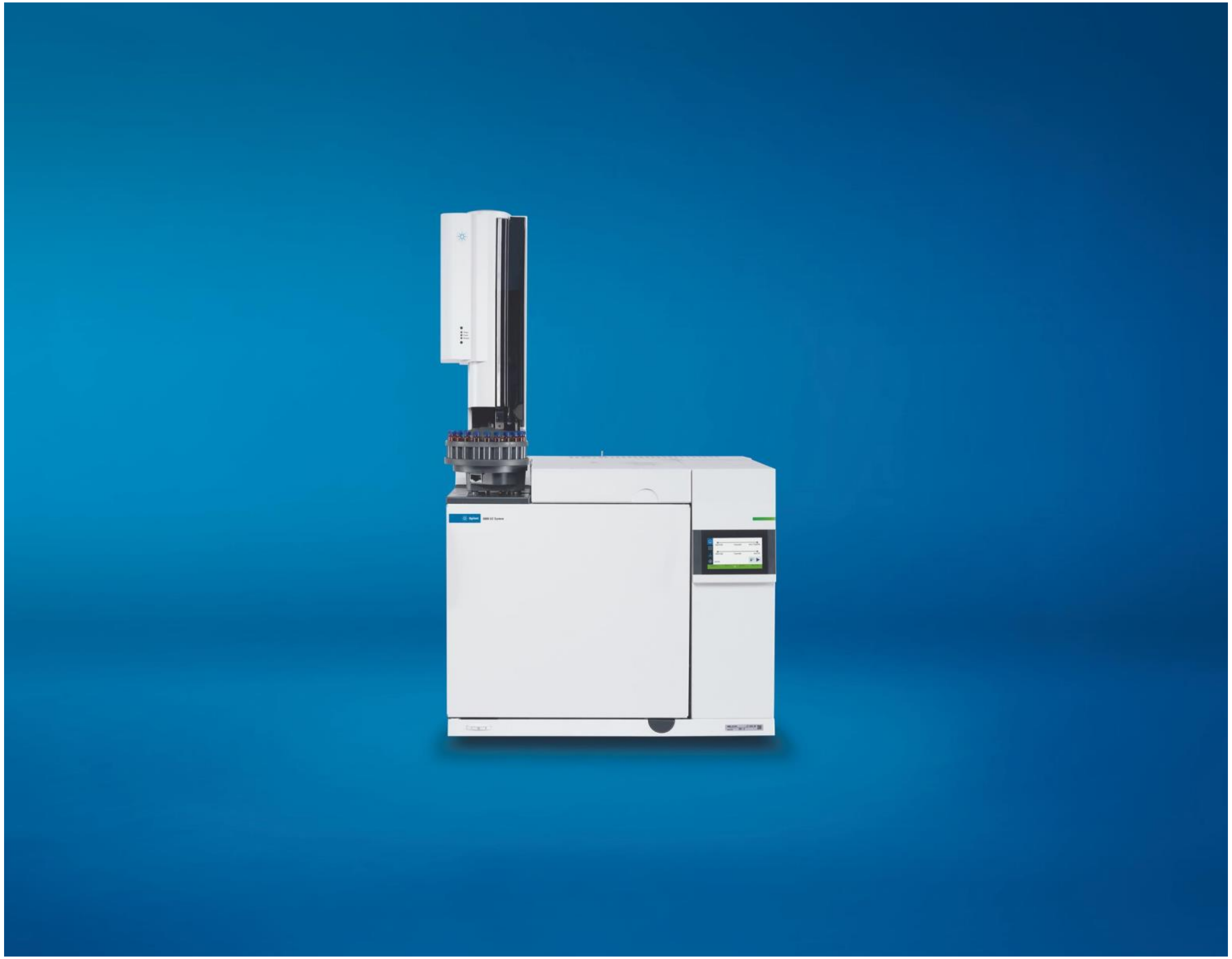
1. **1-Propionic acid**
2. **1-Octene**
3. **n-Octane**
4 **4-Picoline**
5. **n-Nonane**
6. **Trimethyl phosphate**
7. **1,2-Pentanediol**
8. **n-Propylbenzene**
9. **1-Heptanol**
10. **3-Octanone**
11. **n-Decane**



Goals of the Study

Who cares?

- Frequent inquiries
- Water can cause problems
- Quantitative data
- Establish “guidelines”



Stationary Phases

- Dimethylpolysiloxane (DB-1)
- Polyethylene glycol (DB-WAX)
- Cyanopropylphenyl (DB-225)
- Cyclodextrin (CycloSil B)
- Divinylbenzene/ethylene glycol dimethacrylate (HP-PLOT U)
- Columns not compatible with water
  - molsieve, alumina

Experimental Conditions

Instrument:	Agilent GC with auto injector
Injector:	250 °C, 1:5 split
Injection volume:	1 µL
Detector:	FID, 300 °C
Carrier gas:	H_2 at 40 cm/s

Experimental Conditions

Oven: 130, 60, or 200 °C* isothermal

Column dimensions: 30 m x 0.53 mm id x 1.0 µm

1,000 water injections at each temperature on each column

Bleed profile after 250, 500, and 1,000 water injections

Test mix after 250, 500, and 1,000 water injections

*CycloSil B: 30 m x 0.32 mm x 0.25 µm

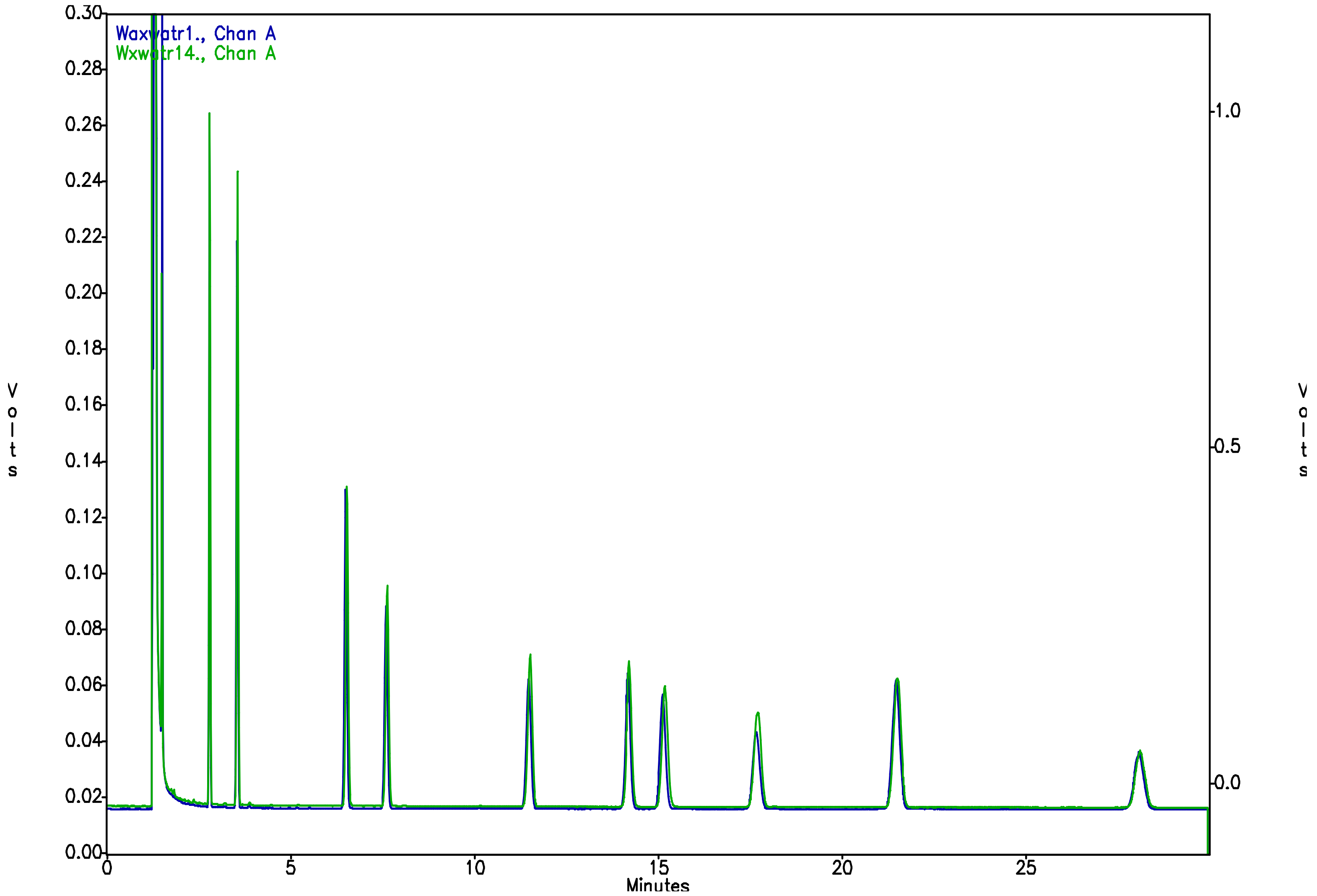
Results DB-1

Parameter	Before Injection	After Injection
Ret. factor (k)	14.6	14.5
Ret. index 1	1349.88	1350.02
Ret. index 2	1427.77	1428.16
Theor. plates	1448	1474
Bleed (pA)	12.8	11.2

Results DB-WAX

Parameter	Before Injection	After Injection
Ret. factor (k)	12.6	12.6
Ret. index 1	1149.54	1149.73
Ret. index 2	1163.44	1163.71
Theor. plates	1277	1261
Bleed (pA)	44.8	32.1

DB-WAX Before and After 2,000 Water Injections



Results DB-225

Parameter	Before Injection	After Injection
Ret. factor (k)	11.5	11.4
Ret. index 1	1622.30	1621.26
Ret. index 2	1711.51	1711.03
Theor. plates	1101	1110
Bleed (pA)	34.5	39.3

Results CycloSil B

Parameter	Before Injection	After Injection
Ret. factor (k)	7.8	7.6
Ret. index	1306.3	1306.0
Resolution	1.9	1.3
Theor. plates	2631	2025
Bleed (pA)	28.4	15.1

Phase wash out

Results HP-PLOT U

Parameter	Before Injection	After Injection
Ret. factor (k)	5.2	5.3
Ret. index	538.0	540.0
Theor. plates	950	982
Bleed (pA)	74.2	35.6

Asymmetry (Skew)

Skew numbers are based on a modified Gaussian-peak model*.

The numbers represent the deviation from an ideal Gaussian, or, perfectly symmetrical, peak.

The smaller the number, the better.

Noticeable tailing starts at ~0.8
Obvious tailing start at ~1.2

*W.W. Yau, Anal. Chem., vol. 49, No. 3 (1977), pp 395-398.

Results DB-1

Asymmetry

Compound	Before Injection	After Injection
Chlorophenol	0.48	0.47
Dimethyl aniline	0.47	0.46
Undecanol	0.51	0.46

Results DB-WAX
Asymmetry

Compound	Before Injection	After Injection
Decanol	0.38	0.47
Dimethyl aniline	0.30	0.32
Dimethyl phenol	0.25	0.27

Results DB-225
Asymmetry

Compound	Before Injection	After Injection
Chlorophenol	0.46	0.47
Dimethylaniline	0.42	0.43
Undecanol	0.63	0.58

Study Summary

For bonded columns, no negative effects of injecting water were observed.

Nonbonded columns and some PLOT columns (for example, Alumina and Molesieve) are not suitable for water injections.

Observe the manufacturer’s recommendations.

Water Injection is Acceptable

If it is acceptable to inject water, why am I having all these problems when I inject it?

Problems associated with water injections are often caused by injector-related phenomena: such as **backflash** or **polarity mismatch**

Typical Solvent Expansion Volumes

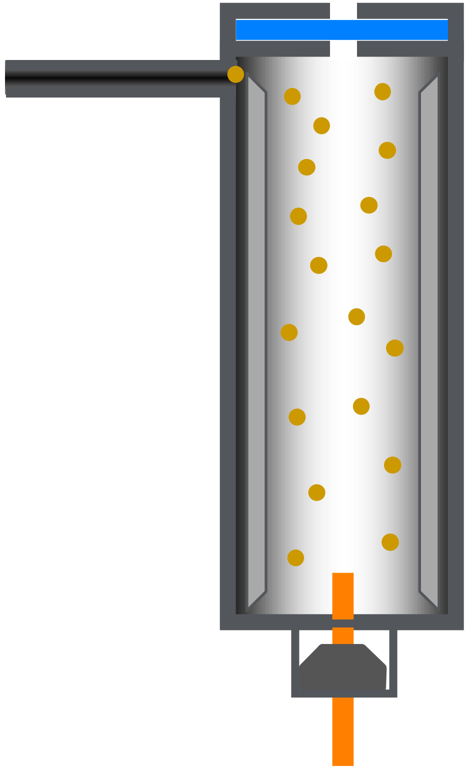
Solvent	Vapor volume (µL) of 1 µL liquid
Water	*1010*
Methanol	450
Carbon disulfide	300
Methylene chloride	285
Acetone	245
n-Hexane	140

GC calculators: https://www.agilent.com/en/support/gas-chromatography/gccalculators

Backflash
Cause

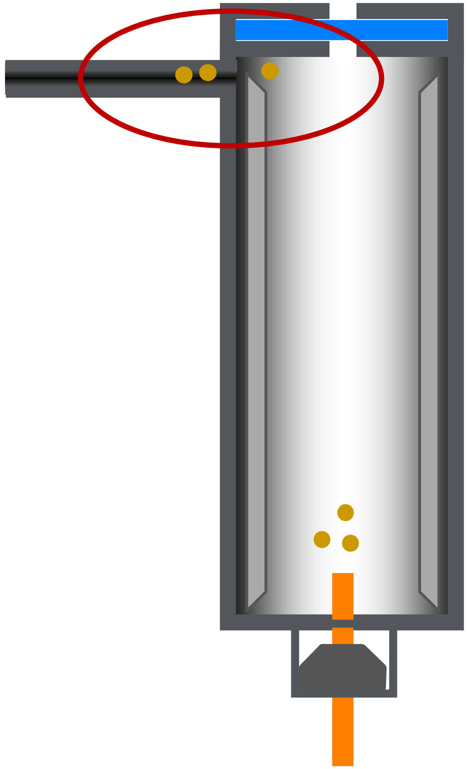
- A vaporized sample expands 100 to 1000 times
- Portions may leave the liner
- It occurs when resulting vapor volume > liner volume

Backflash



The sample expands to overfill the injector.
Some sample condenses on cooler areas
(bottom of the septum, metal body, upstream...etc.).

Backflash



Some sample flows out of the injector.
Lower volatility portions can condense on cooler areas.

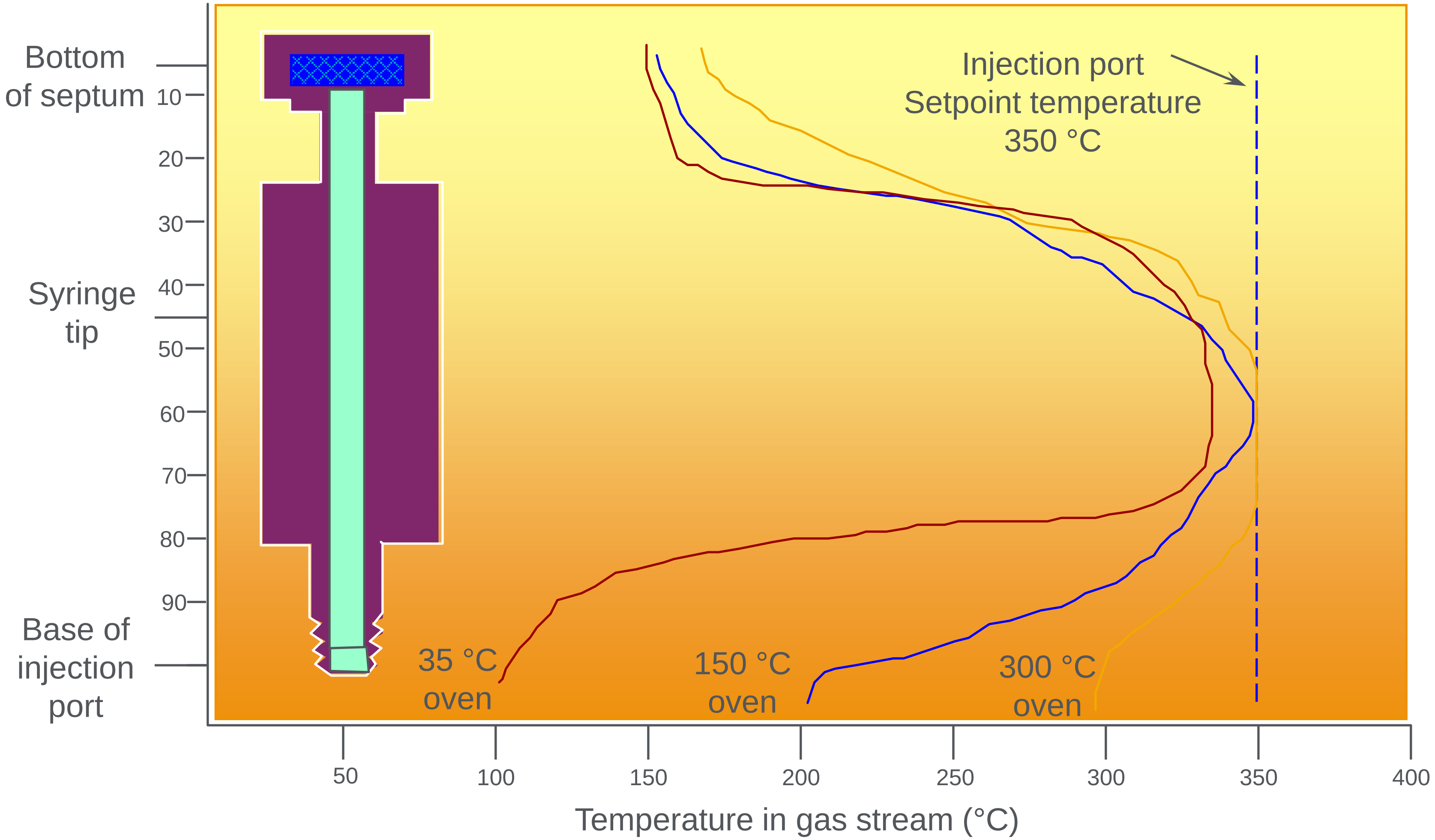
Backflash

As carrier gas flows over the condensed portion, the headspace is brought forward

Subsequent injections can also dislodge condensed sample, as well as depositing more sample

The sample then enters the column

Temperature Profile of a Typical Vaporization Injector vs Oven Temperature



Backflash
Problems

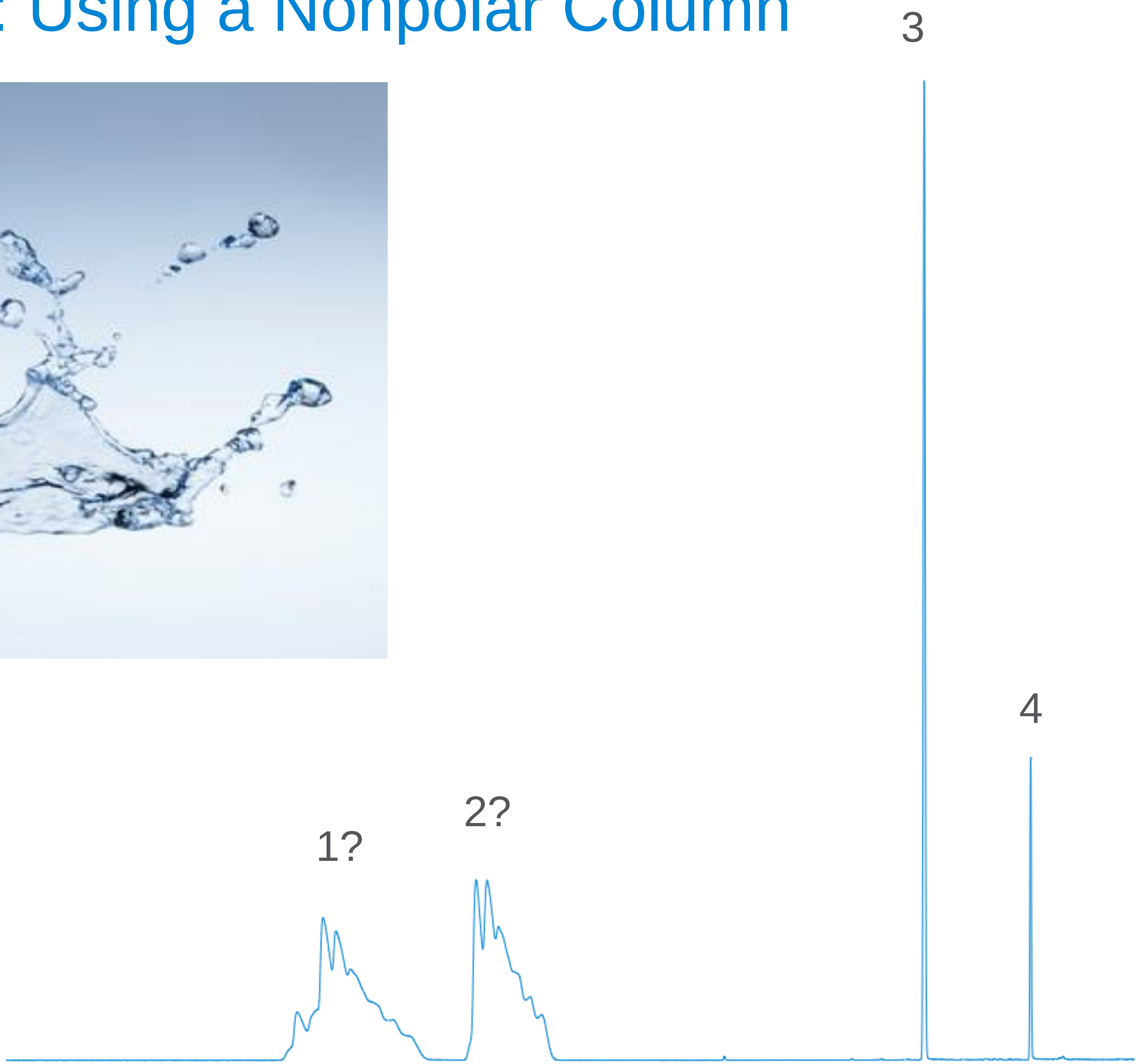
- Loss of sample
- Baseline interferences
- “Ghost” peaks 
- Tailing solvent front or major component
- Carry-over

Backflash
Minimizing

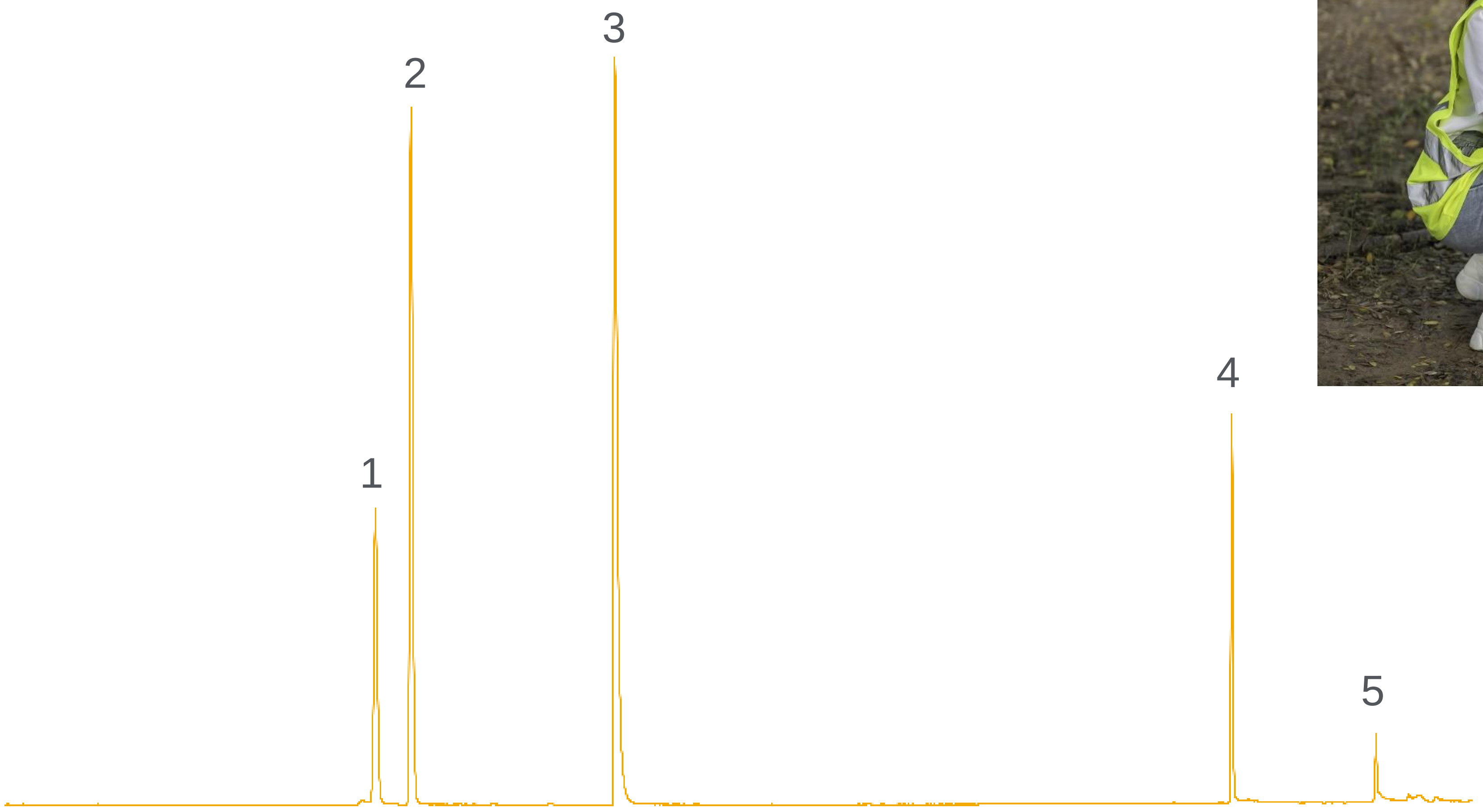
- Large volume liner (possibly tapered)
- Small injection volume (< 0.5 uL)
- Low expansion solvent
- Low injector temperature
- High carrier gas flow rates
- High head pressures (possibly pulsed)
- Rule of thumb keep injection volume <0.5 uL
  - Best to use vapor volume calculator

GC calculators: https://www.agilent.com/en/support/gas-chromatography/gccalculators

Water Injection: Using a Nonpolar Column



Water Injection: Using a Polar Column (i.e. WAX)



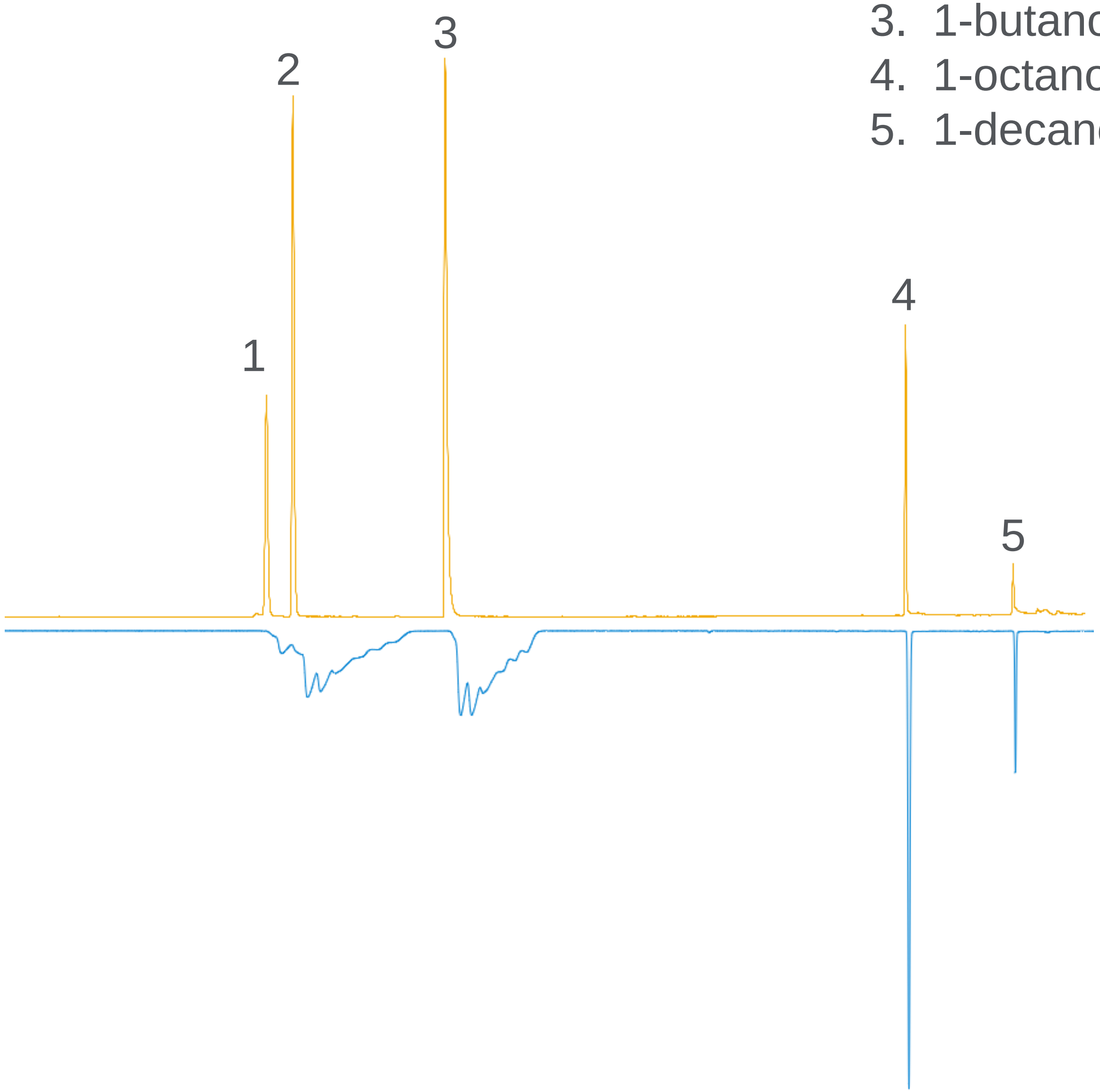
Water Injection: Using a Nonpolar Column

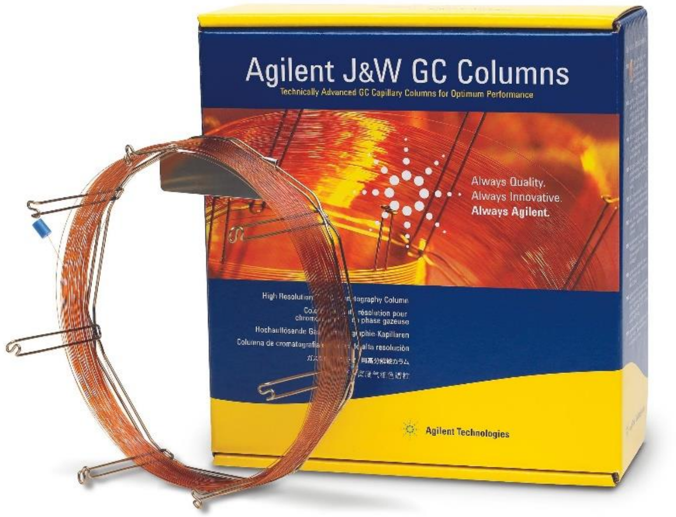
1. Ethanol (78 ºC)
2. Isopropanol (82 ºC)
3. 1-butanol (118 ºC)
4. 1-octanol (194 ºC)
5. 1-decanol (233 ºC)

Intuvo 9000
Inlet: 250 °C
FID: 275 °C
Split: 25:1

Splitless: Purge flow: 50 mL/min
Purge time: 0.5 min

Velocity: 30 cm/s
T prgm: 40 °C for 2 min → 280 at 40 °C/min
Hold 2 minutes

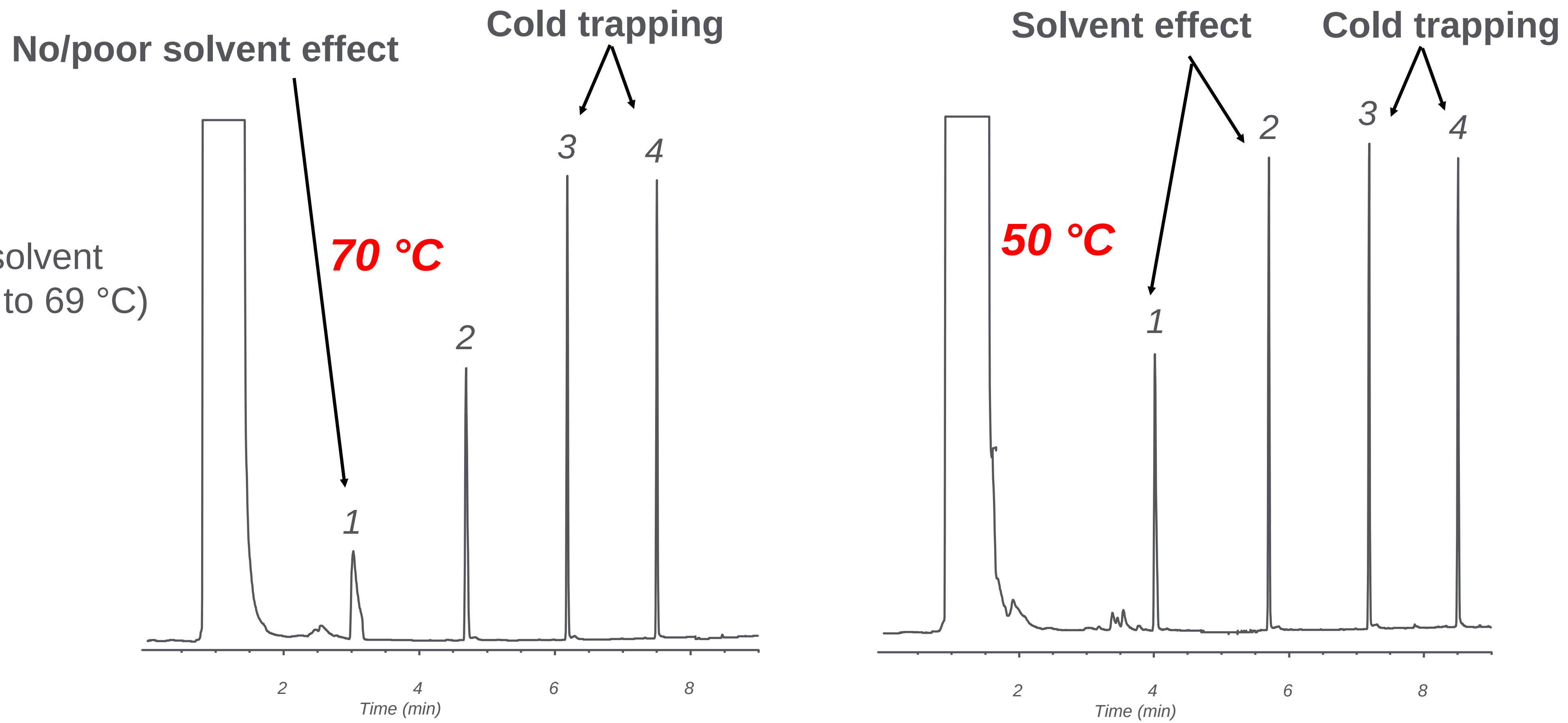




Splitless Injection

Initial column temperature

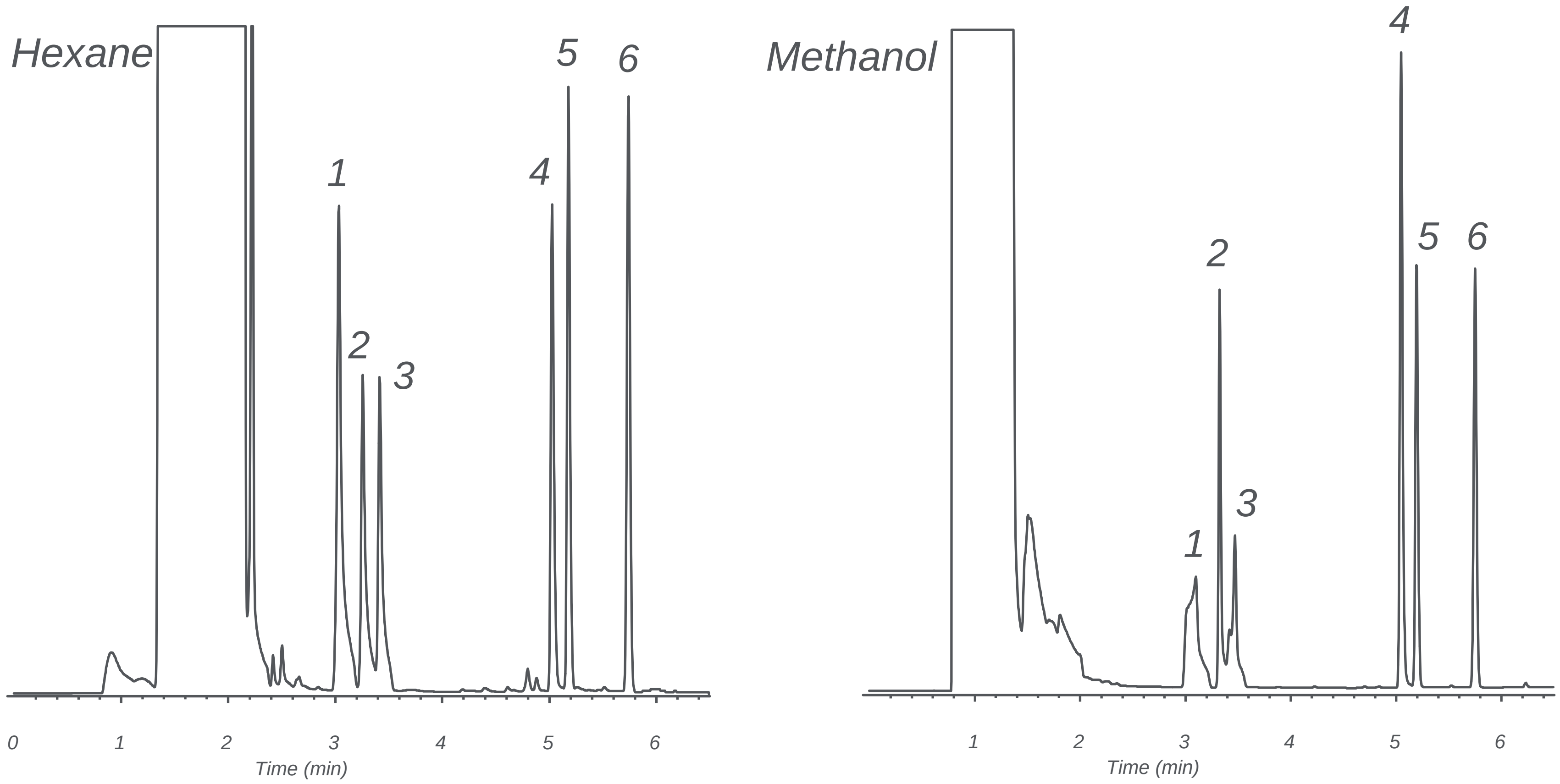
Hexane solvent
(BP = 68 to 69 °C)



DB-1, 15 m x 0.25 mm id, 0.25 µm - SPLITLESS
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 Injection

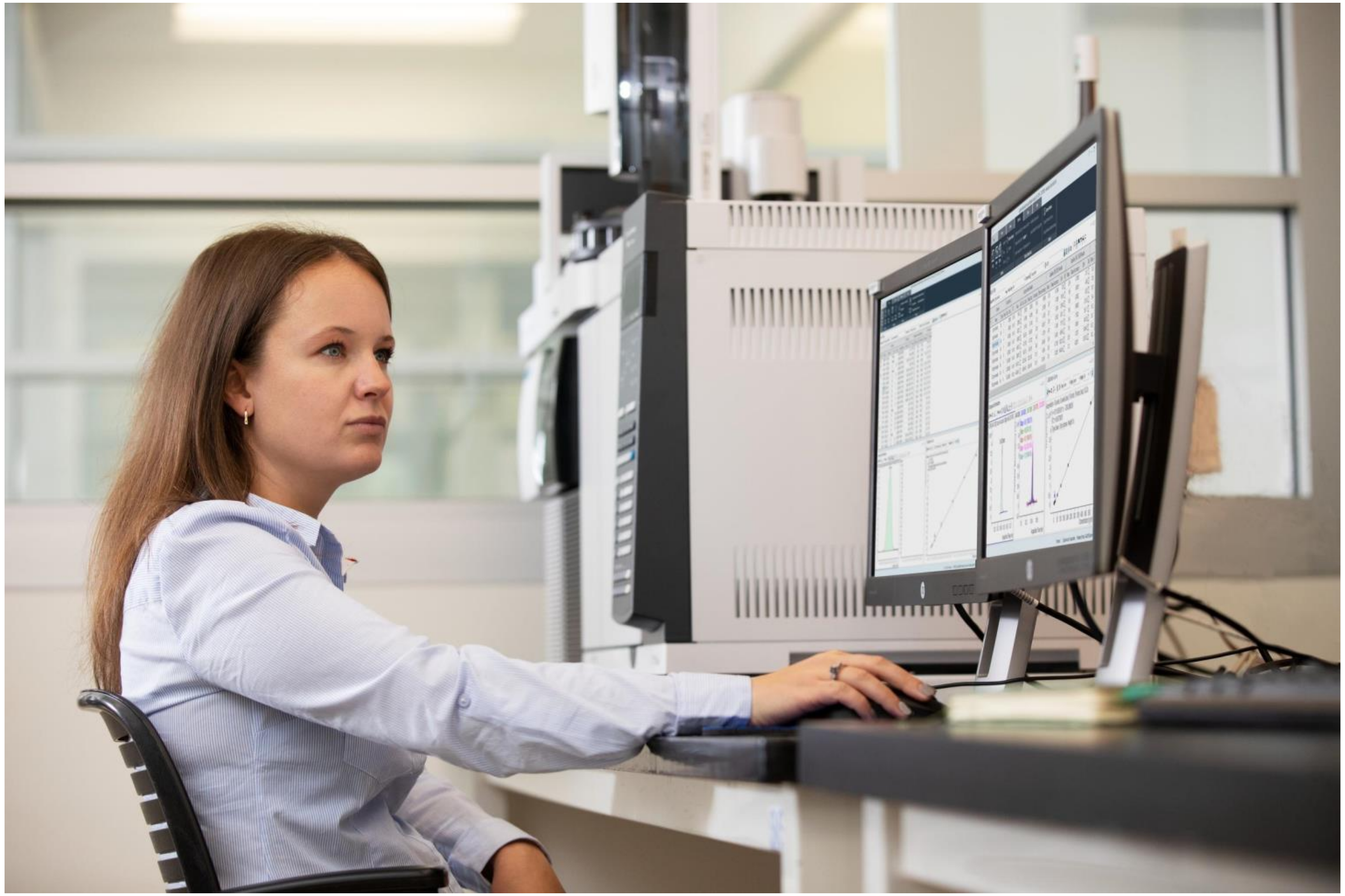
Polarity mismatch



DB-1, 15 m x 0.25 mm id, 0.25 µm
50 °C for 1 min, 50 to 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

Splitless Injection – Things You Can Do

Polarity mismatch

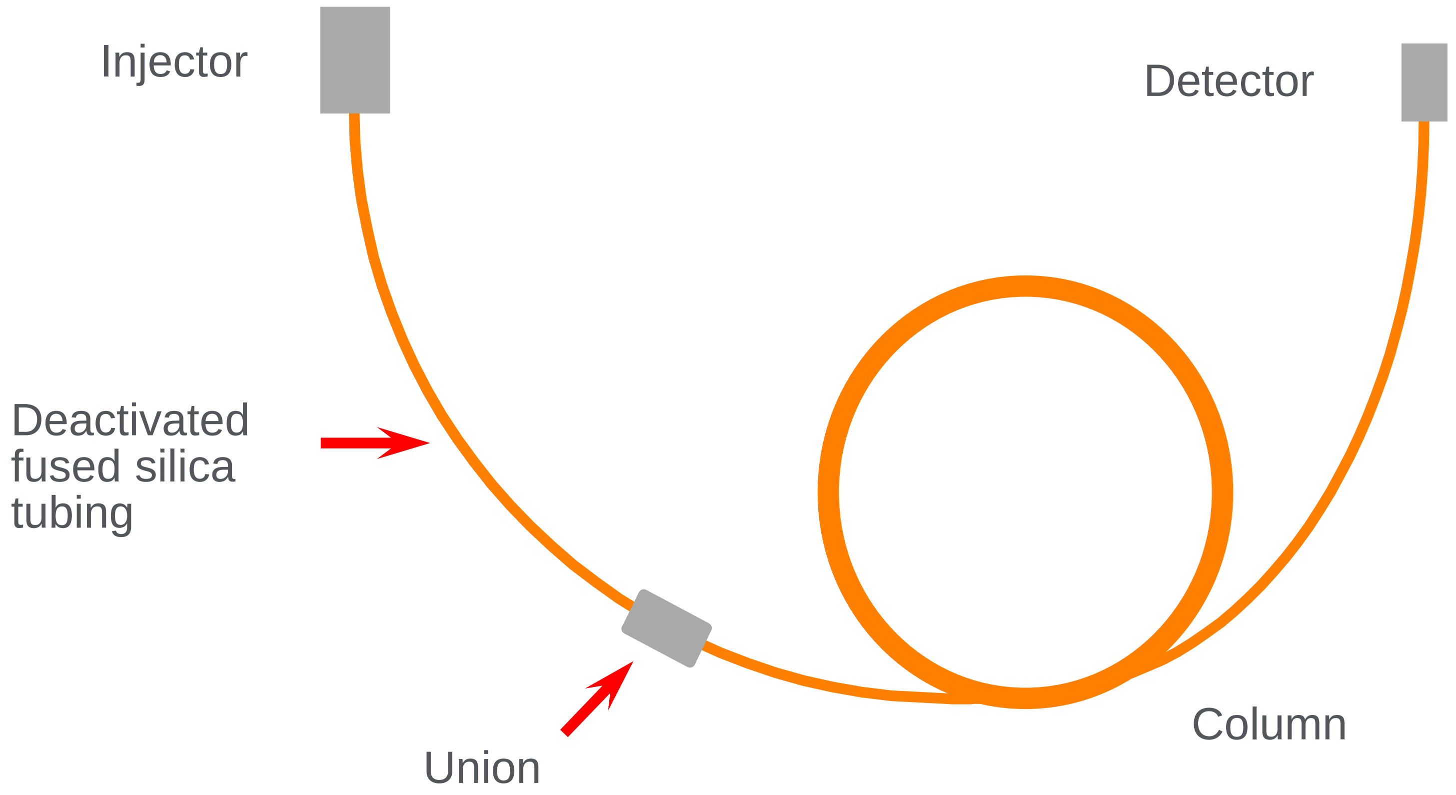


You can:

- Change the polarity of the solvent
- Change the polarity of the stationary phase
- Use a retention gap

Retention Gap

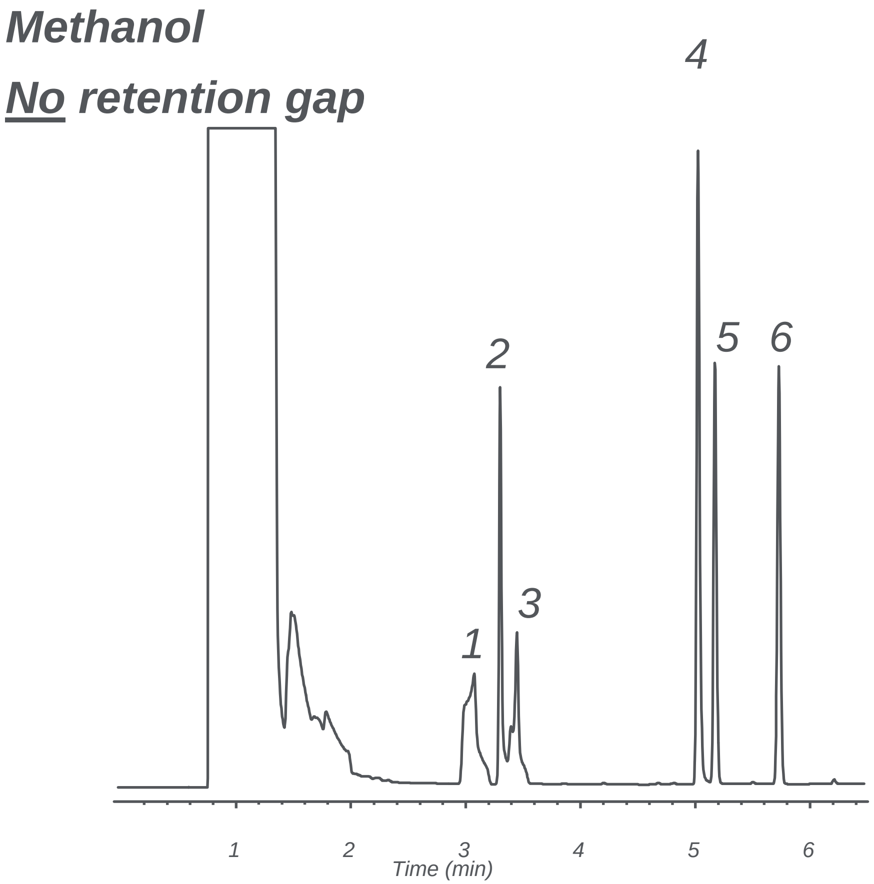
Also called a guard column

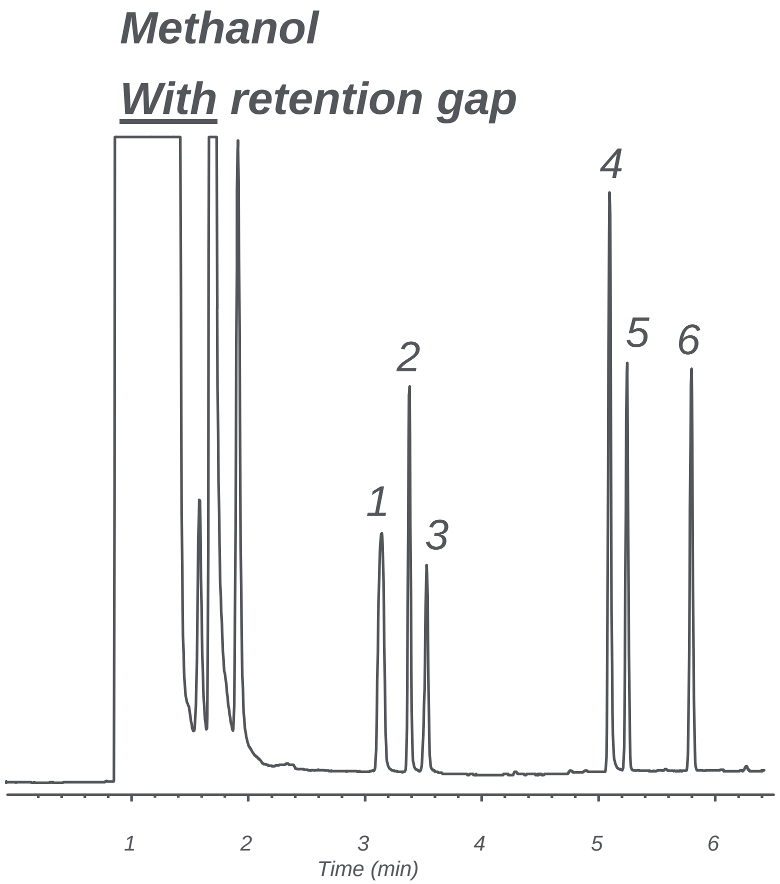


Usually 2 to 10 m long and the same diameter as the column (or larger if needed).

Splitless Injection

3 m x 0.25 mm id retention gap





DB-1, 15 m x 0.25 mm id, 0.25 µm
50 °C for 1 min, 50 to 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

Study Summary

For bonded phases, there is no change in:

- Polarity
- Selectivity
- Retention
- Efficiency
- Activity
- Bleed

For nonbonded phases, like CycloSil B, water injections can wash out part of the nonbonded stationary phase, resulting in loss of resolution, retention, and possibly efficiency.

Summary

- Water injection are OK only for bonded column phases
- Perceived problems are related to:
  - Backflash keep injection volume <0.5uL; use calculator
  - Polarity mismatch
  - Solvent effect
  - Retention gap
- Non-bonded phases should be avoided

Contact Agilent Chemistries and Supplies Technical Support

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

Option 1 for GC and GC/MS columns and supplies

Option 2 for LC and LC/MS columns and supplies

Option 3 for sample preparation, filtration, and QuEChERS

Option 4 for spectroscopy supplies

Option 5 for chemical standards

Available in the U.S. and Canada 8–5, all time zones

gc-column-support@agilent.com

lc-column-support@agilent.com

spp-support@agilent.com

spectro-supplies-support@agilent.com

chem-standards-support@agilent.com

Thank you

Questions?