Water Injection: An Expanding Problem

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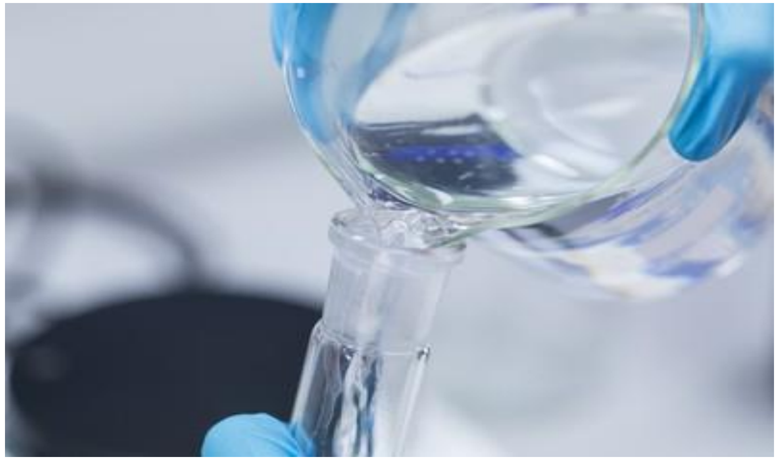
Water Injections in GC
How wet can you get?



Agenda

- ➢ Potential problems with water injections
- ➢ Column bleed explanation and misconceptions
- ➢ Experimental study on the effects of water injections on various columns
- ➢ BackFlash

Why Inject Water?

Convenient

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

Sometimes Necessary

Potential Problems with Water

Injector issues

- Large expansion volume
- Backflash

Detector issues

- Extinguish FID flame
- Decrease sensitivity of ECD

Potential Problems with Water: Real and Perceived

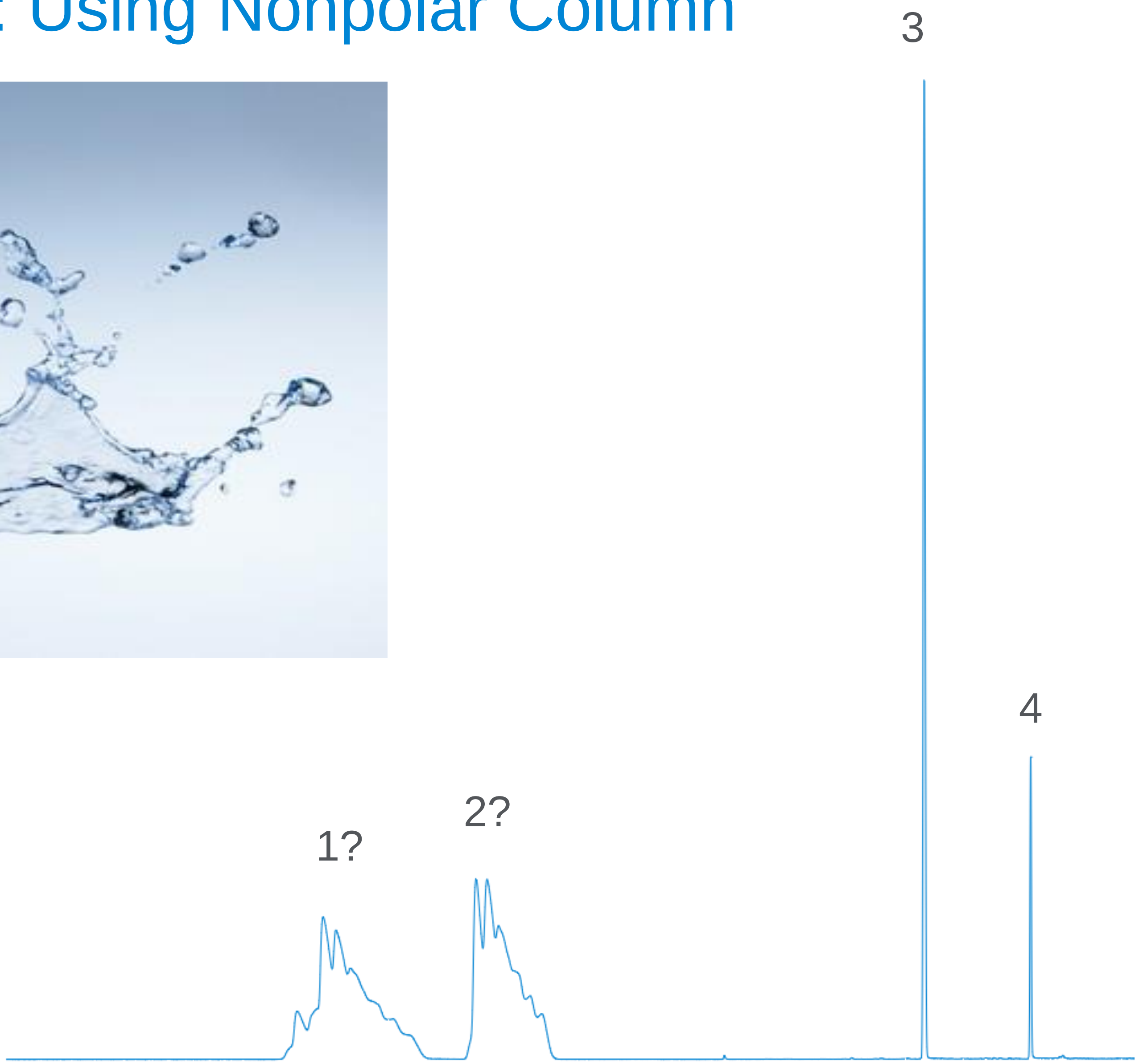
Solvent – Stationary phase mismatch

- Poor wettability of many stationary phases by water
- “Puddles”

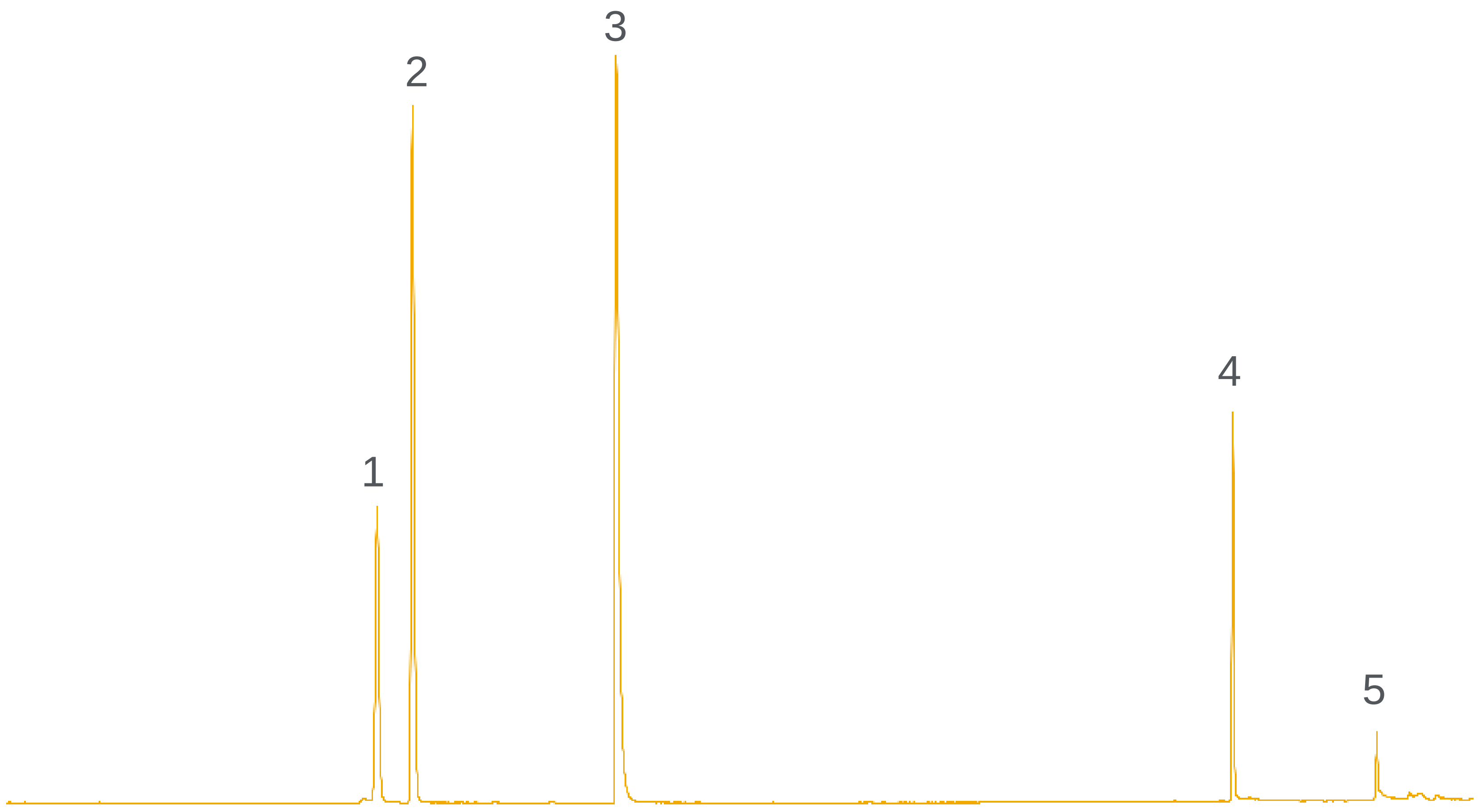
Damage to stationary phase

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

Water Injection: Using Nonpolar Column



Water Injection: Using Nonpolar Column



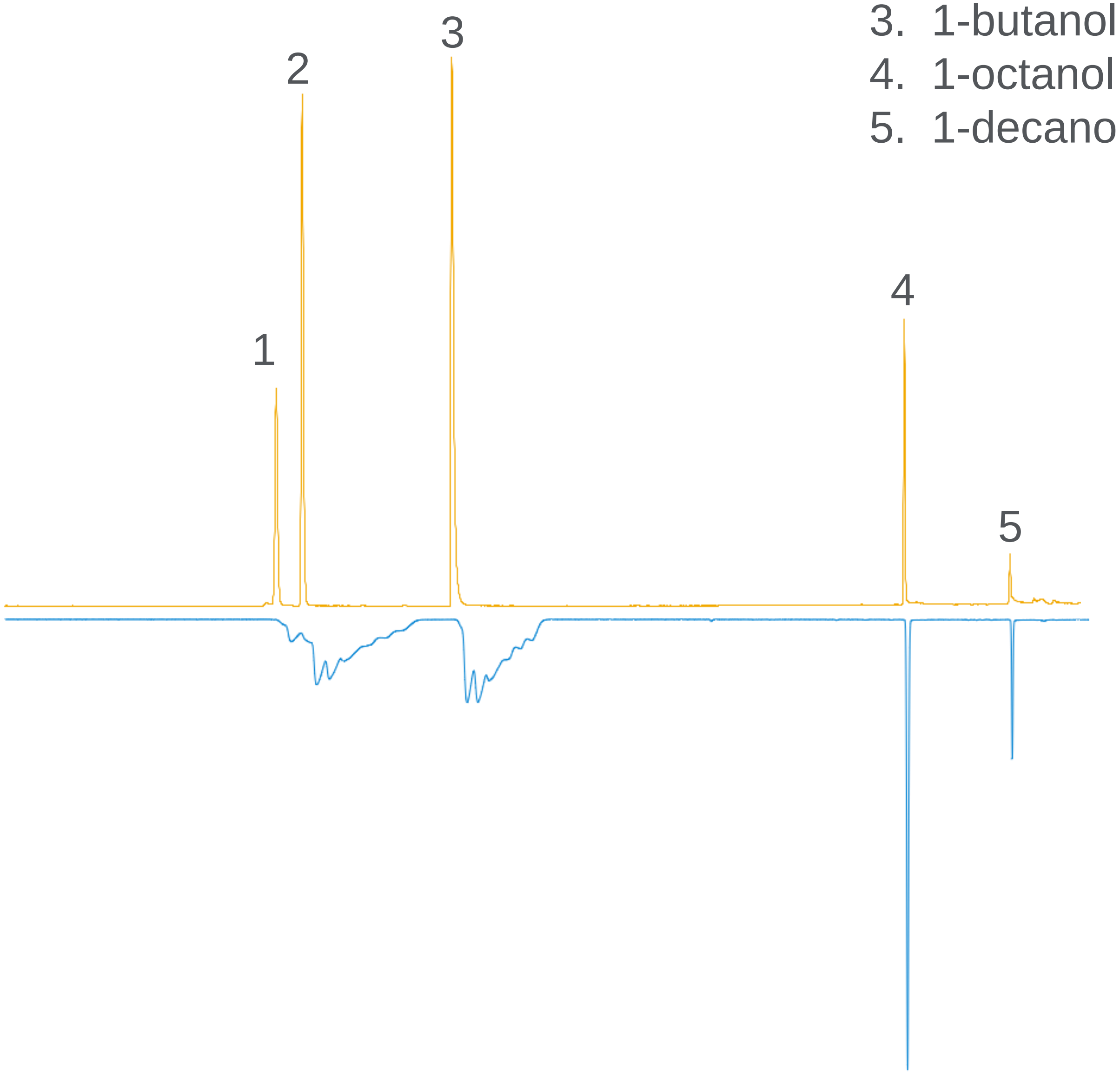
Water Injection: Using Nonpolar Column

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

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

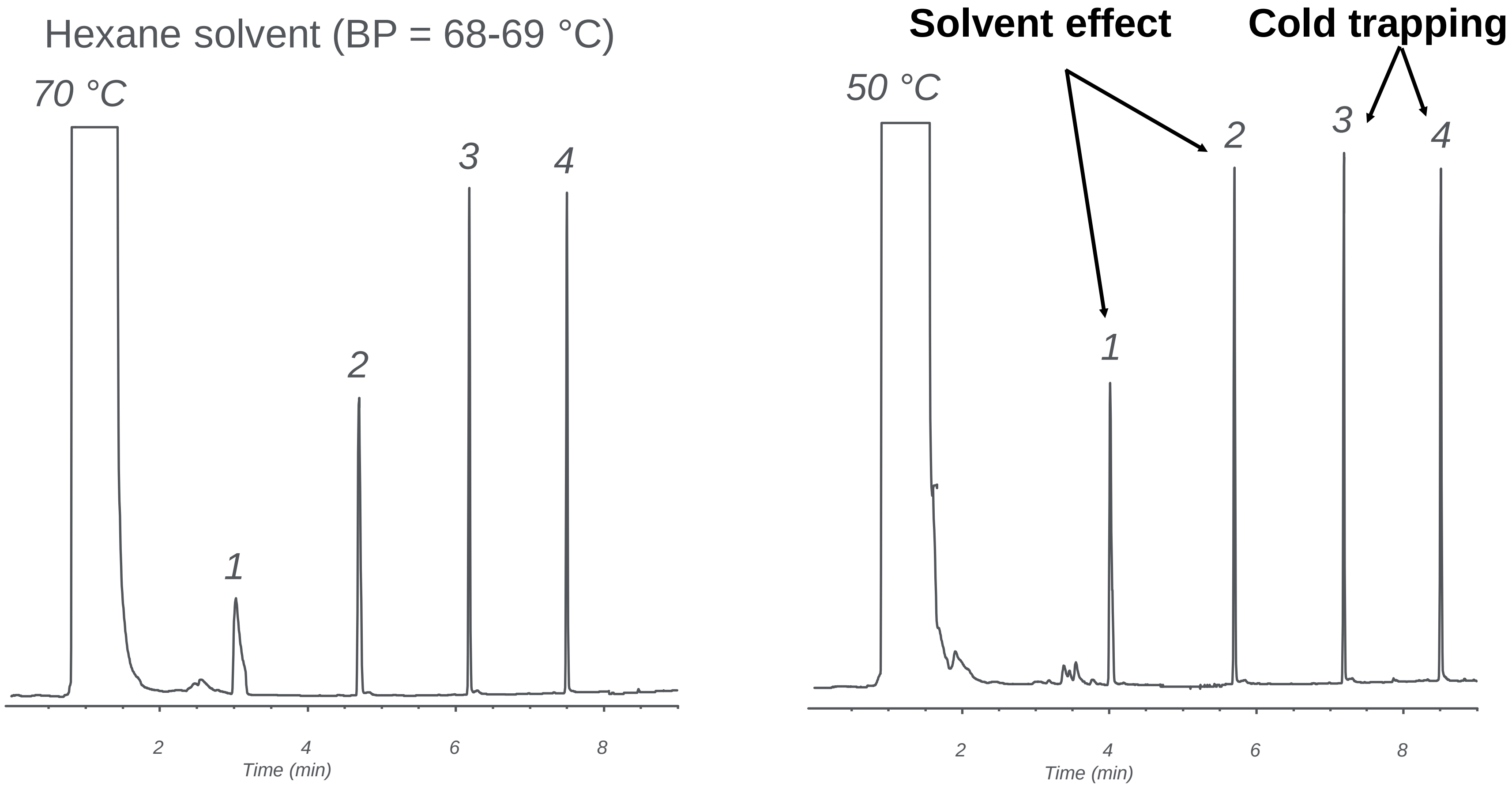
Velocity: 30 cm/s
T prgm: 40 °C for 2 min → 280 at 40 °C/min
hold 2 minutes

1. ethanol (78 ºc)
2. isopropanol (82 ºc)
3. 1-butanol (118 ºc)
4. 1-octanol (194 ºc)
5. 1-decanol (233 ºc)



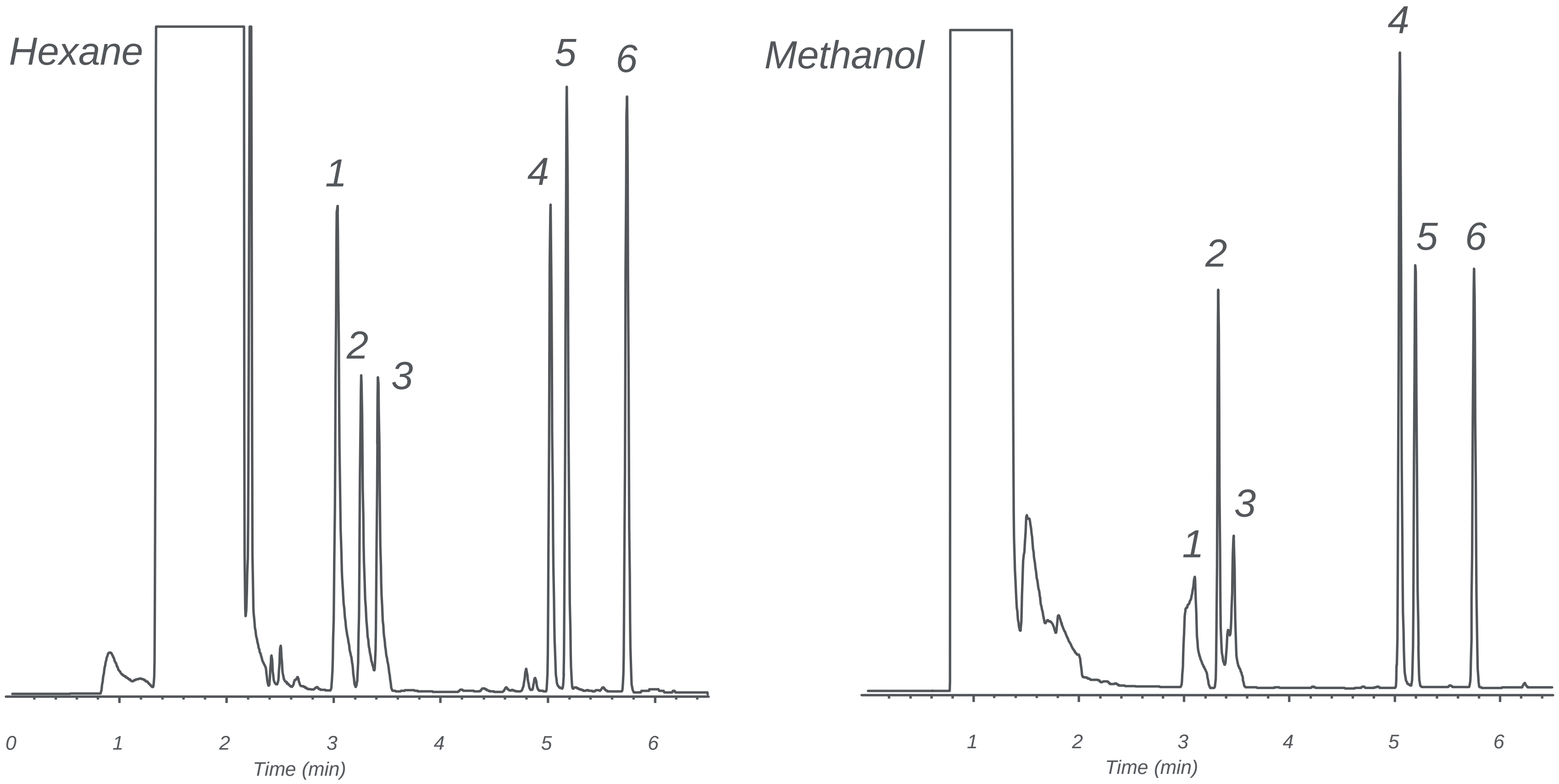
Splitless Injection

Initial column temperature



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 Injection
Polarity mismatch



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

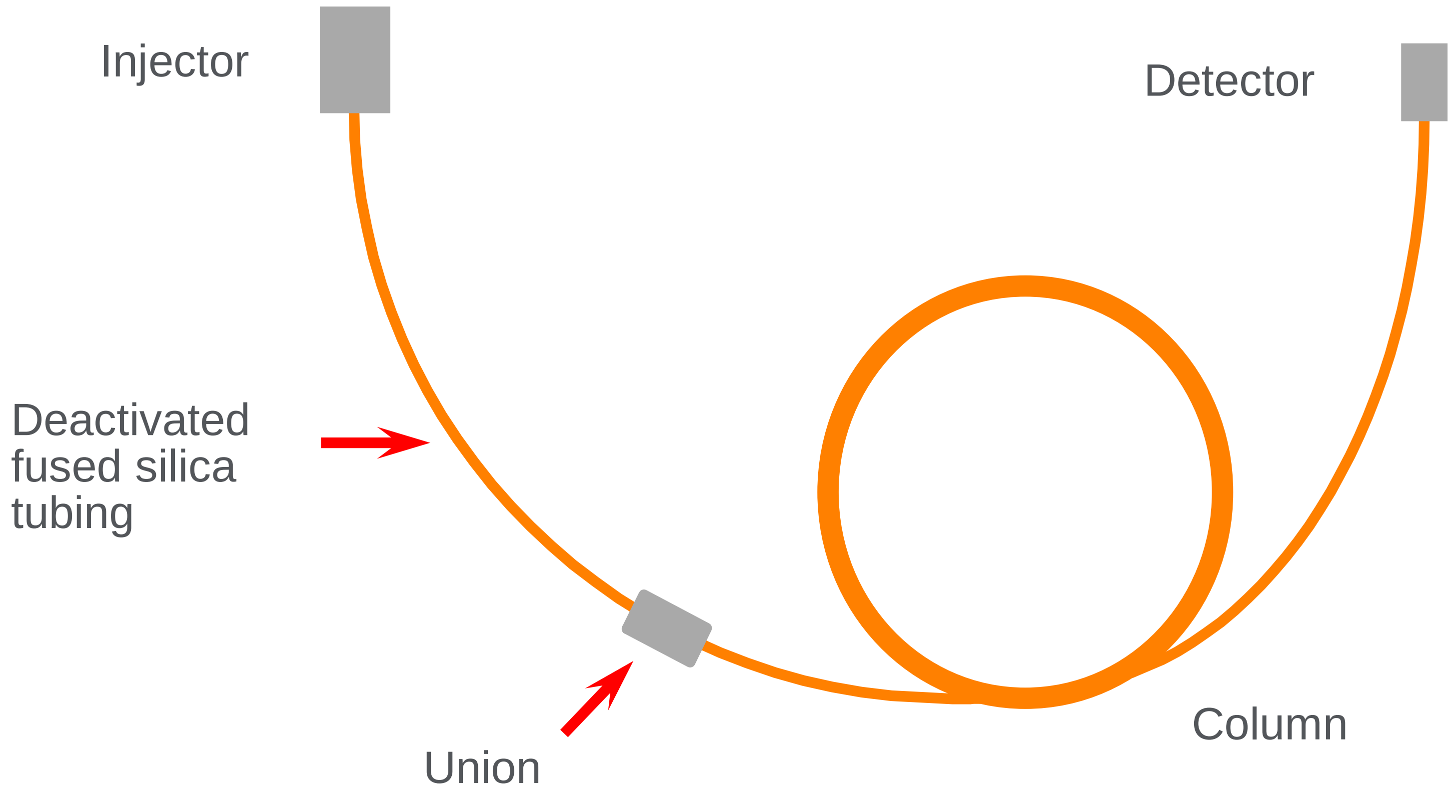
Splitless Injection – Things You Can Do

Polarity mismatch

You could:

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

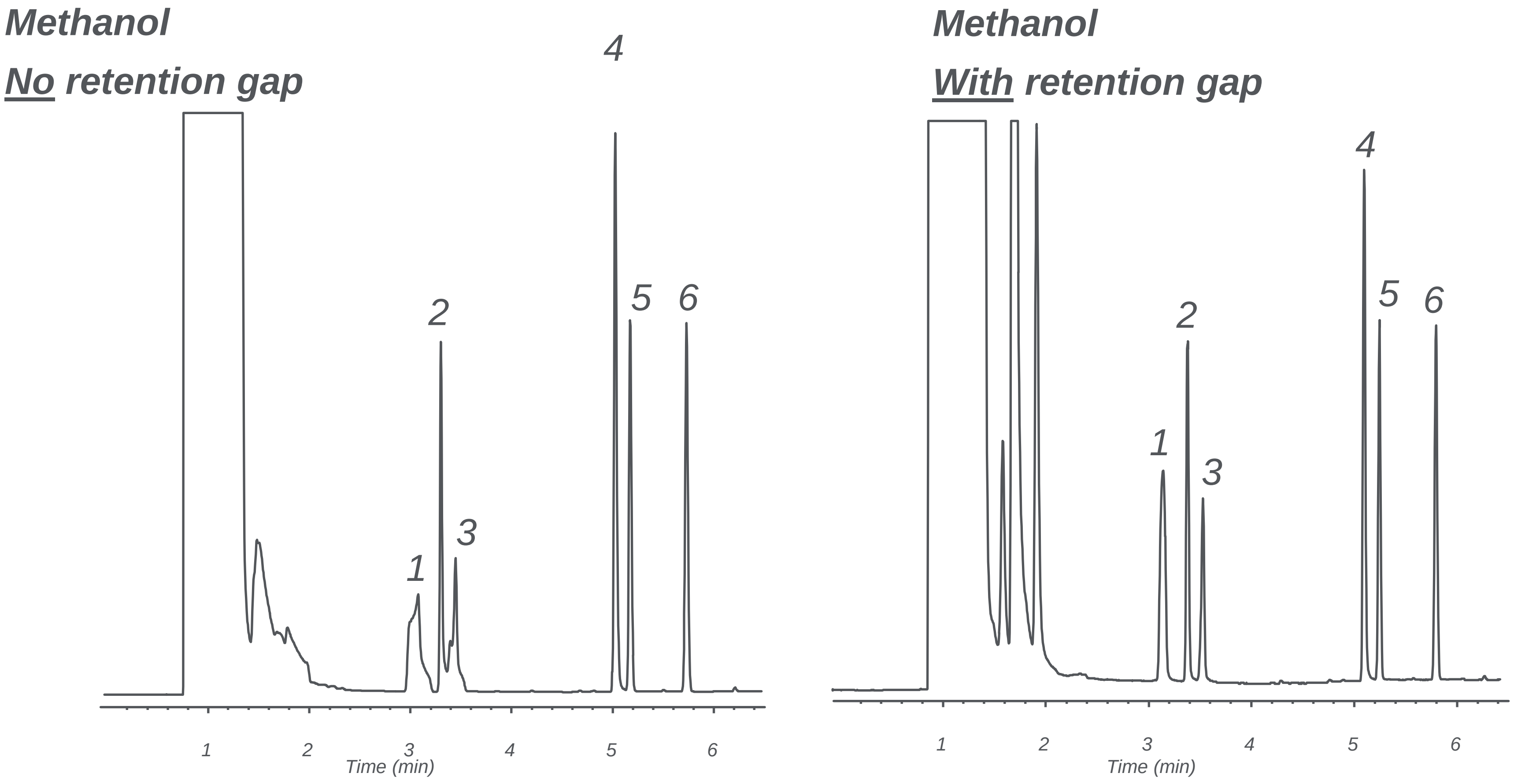
Retention Gap
Also called a guard column



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

Splitless Injection

3 m x 0.25 mm id retention gap



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What is Normal Column Bleed?

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

What is Bleed?

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

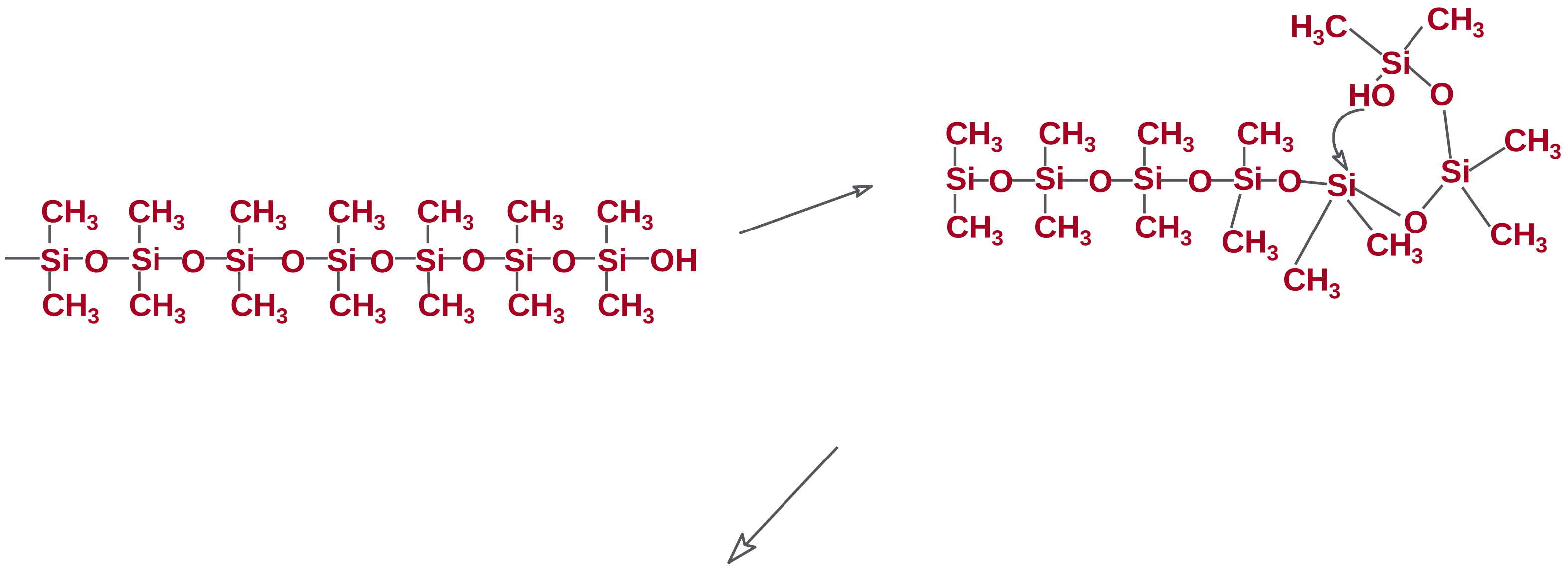
Polysiloxane backbone releases low molecular weight, cyclic fragments.

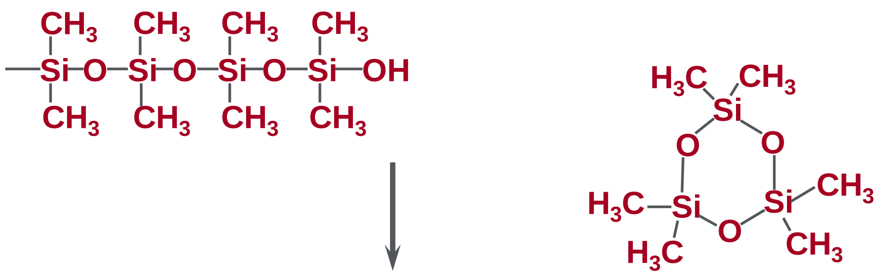
It occurs at low levels in low temperature, O_2-free, clean system.

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

Bleed: Why Does It Happen?

“Back biting” mechanism of product formation

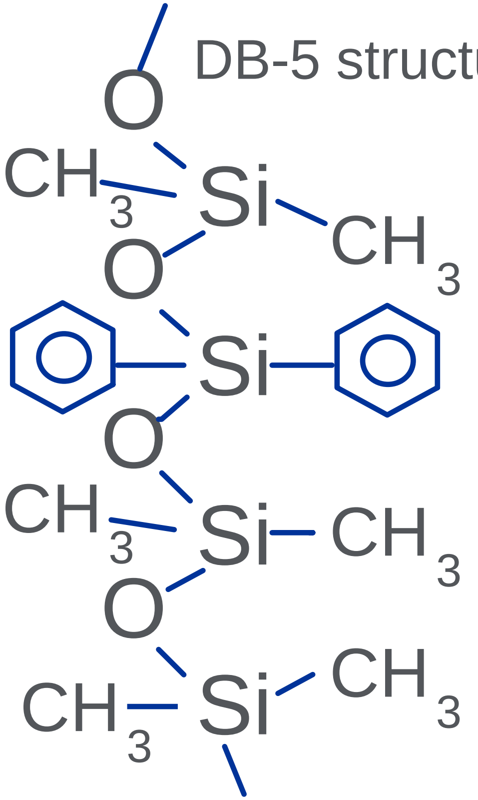




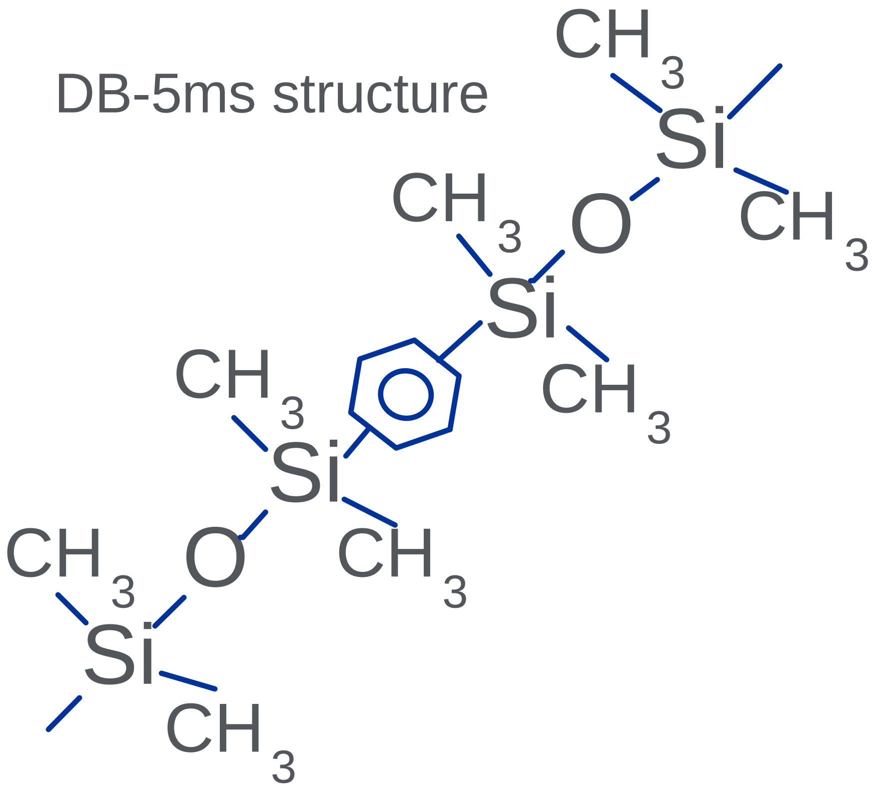
Cyclic products are more thermodynamically stable

Low Bleed Phase Structures

DB-5 structure



DB-5ms structure

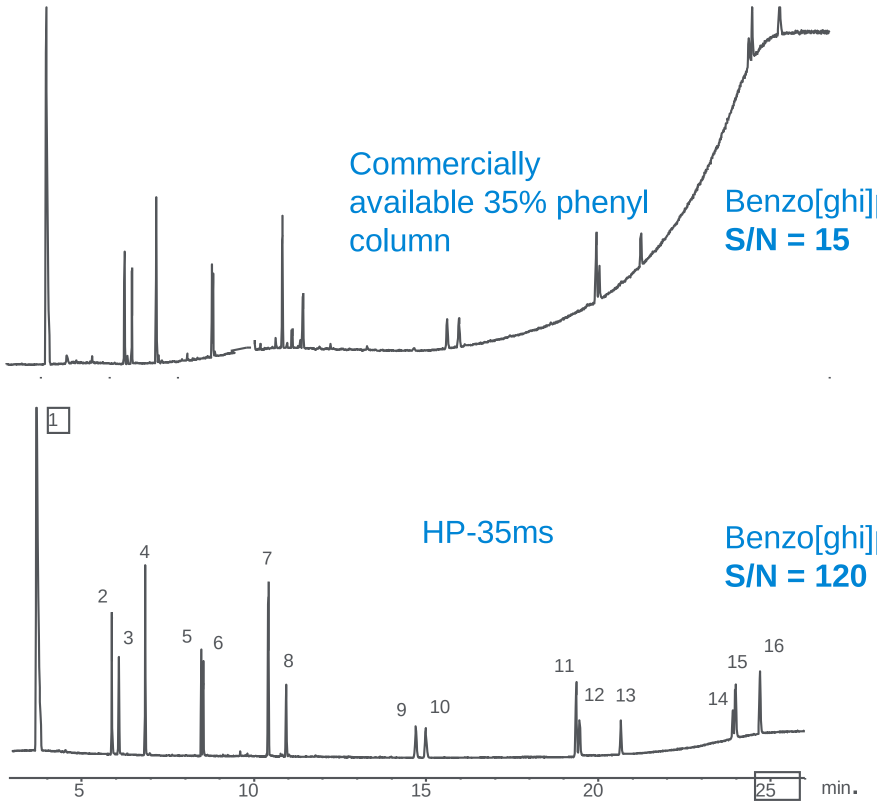


DB-5, HP-5 and HP-5ms
5% phenyl

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

Benefits of Low Bleed Phases

PAH sensitivity using DB-35ms

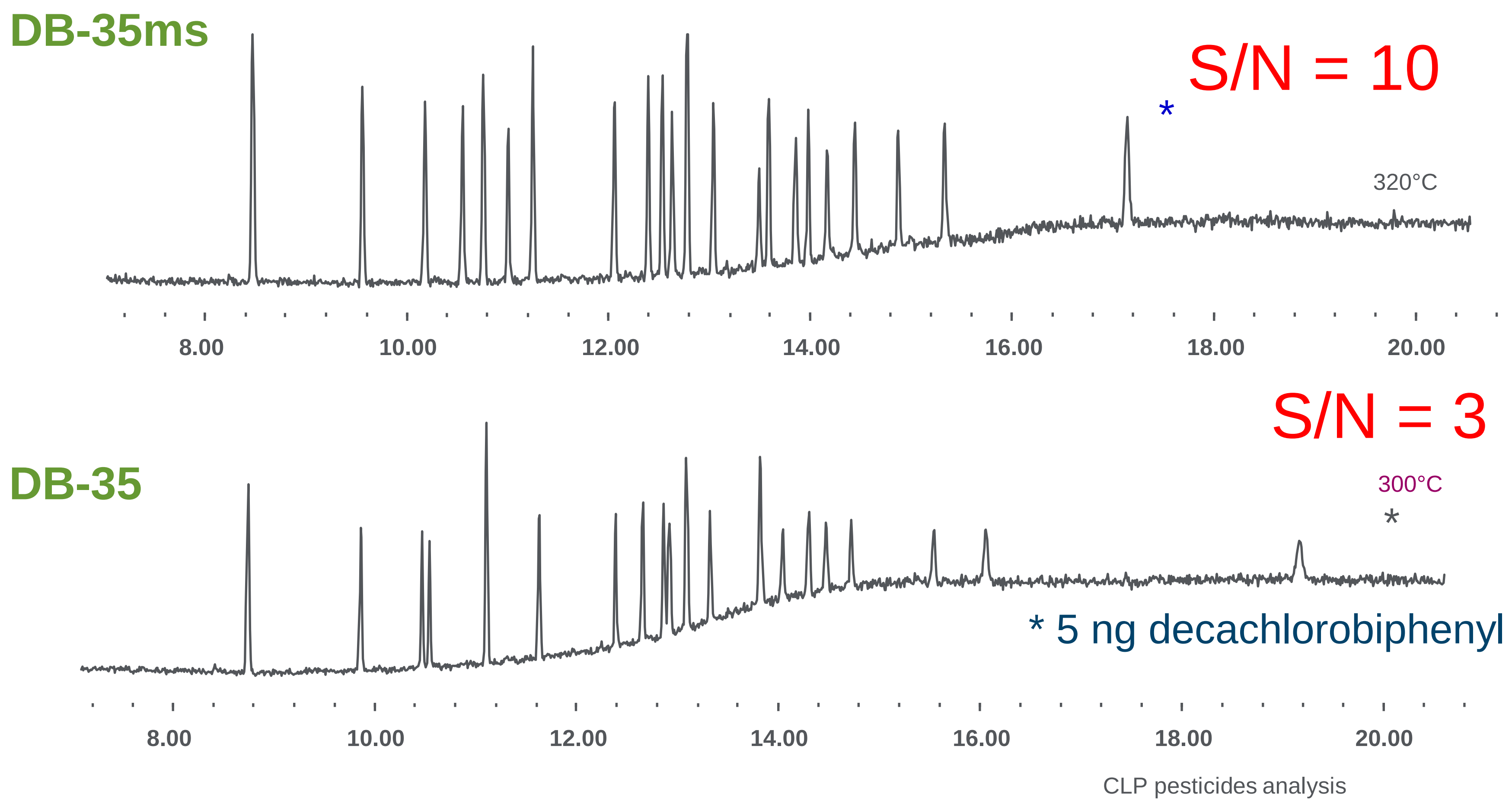


1. Naphthalene
2. Acenaphthylene
3. Acenaphthene
4. Fluorene
5. Phenanthrene
6. Anthracene
7. Fluoranthene
8. Pyrene
9. Benz[a]anthracene
10. Chrysene
11. Benzo[b]fluoranthene
12. Benzo[k]fluoranthene
13. Benzo[a]pyrene
14. Indeno[1,2,3,-c,d]anthracene
15. Dibenz[a,h]anthracene
16. Benzo[g,h,i]perylene

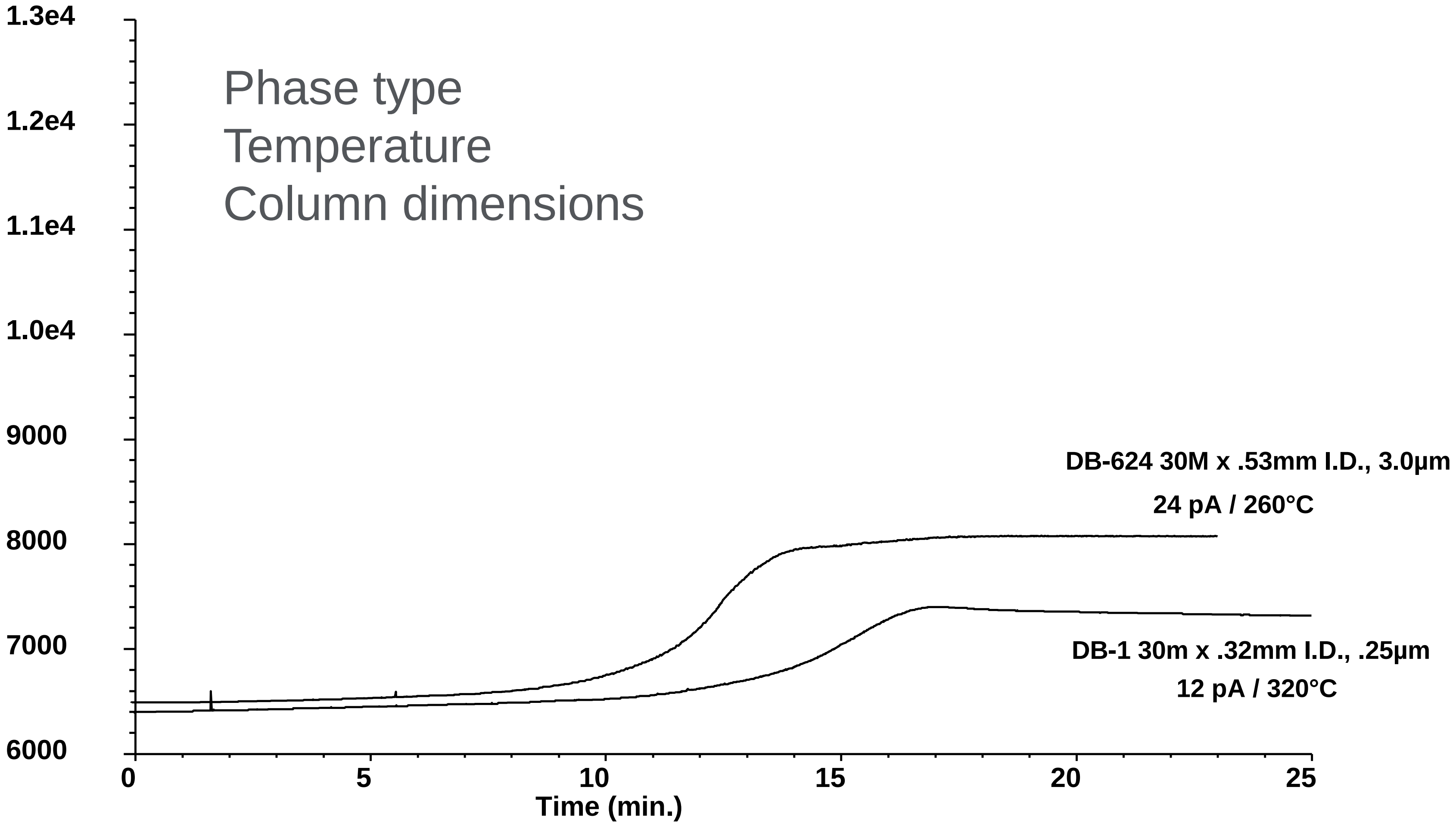
Columns: 30 m x 0.32 mm x 0.35 µm
Carrier: H_2, constant flow, 5 psi at 100 °C
Injector: 275 °C, splitless, 1 µL, 0.5-5ppm
Oven: 100 to 250 °C (5 min) at 15 °C/min, then to 320 °C (10 min) at 7.5 °C/min
Detector: FID, 320 °C

Low Bleed Stationary Phases

DB-35ms versus DB-35



Influences on Column Bleed



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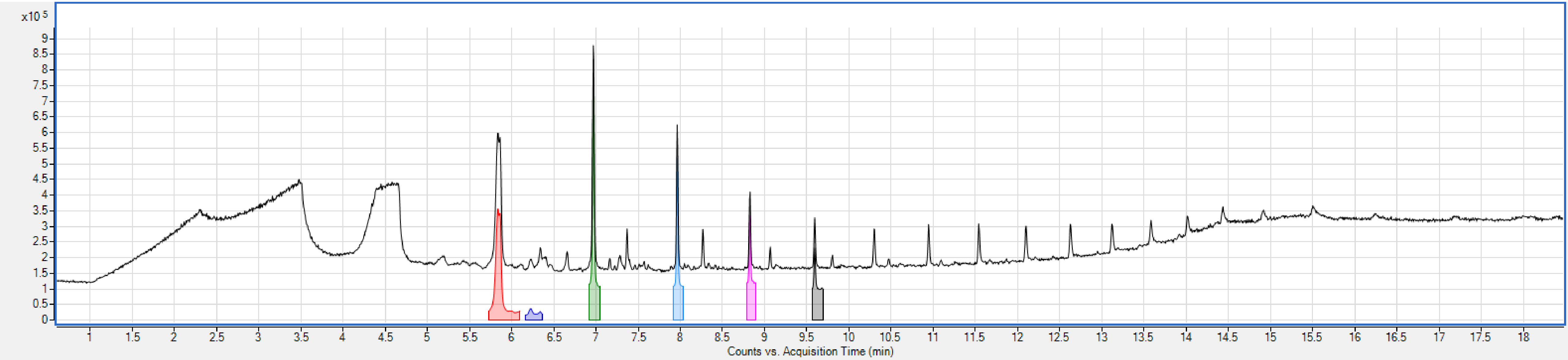
What is a Bleed Problem?

An abnormal elevated baseline at high temperature

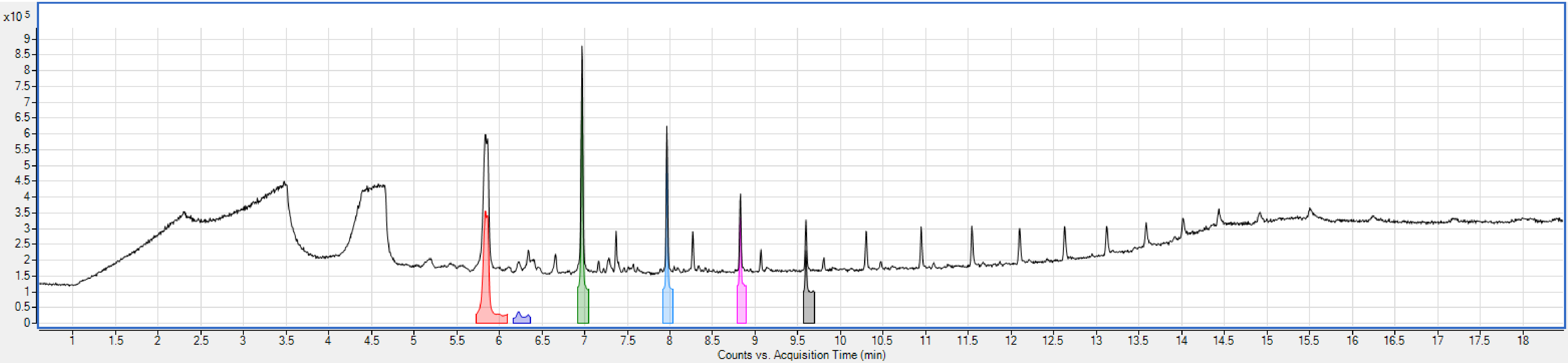
It is <u>not:</u>

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

Septum maintenance: Deconvoluted inlet septa spectrum



Septum maintenance: Deconvoluted inlet septa spectrum



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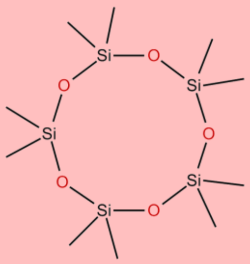
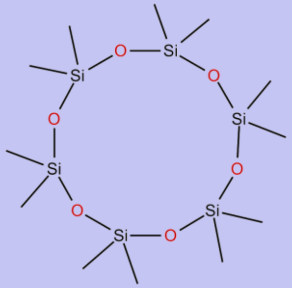


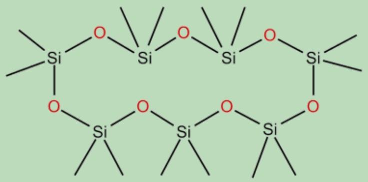
Decamethyl cyclopentasiloxane

12



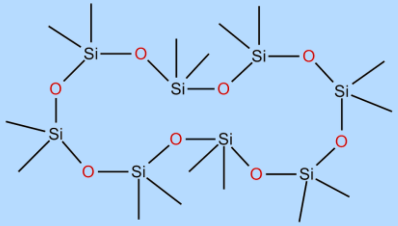
Dodecamethyl cyclohexasiloxane

14



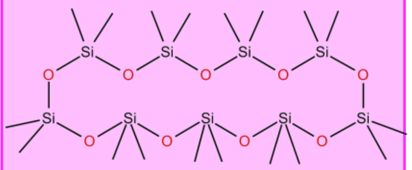
Tetradecamethyl cycloheptasiloxane

16



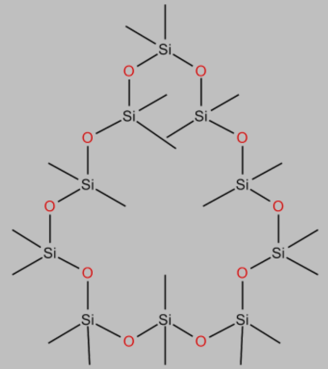
Hexadecamethyl cyclooctasiloxane

18



Octadecamethyl cyclononasiloxane

20



Eicosamethyl cyclodecasiloxane

Goals of 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)

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 °C, 60 °C, 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

*for Cyclosil B and DB-WAX only (possible high temperature problems)

*CycloSil B: 30 m x 0.32 mm x 0.25 µm

Compound Lists

DB-1	DB-WAX	DB-225	CycloSil B	HP-PLOT U
2-chlorophenol	2-octanone	tetradecane	dodecane	methanol
undecane	tetradecane	2-chlorophenol	(R)-linalool	ethanol
2,4-dimethylaniline	1-octanol	Hexadecane	(S)-linalool	pentane
1-undecanol	methyl deconate	naphthalene	naphthalene	ethyl ether
tetradecane	Methylundecanoate	2,4-dimethylaniline	1-nonanol	acetone
acenaphthylene	Naphthalene	1-undecanol	(R) γ-heptalactone	hexane
pentadecane	1-decanol	octadecane	(S) γ-heptalactone	ethyl acetate
	methyl dodecanoate	ethyl dodecanoate	methyl decanoate	
	2,6-dimethylaniline		tetradecane	
	2,6-dimethylphenol			

GREEN: Used for RI
YELLOW: Used for k

Test Mixes

Compound list for chromatograms HP-PLOT U

Peak	Compound
1	methanol
2	ethanol
3	pentane
4	ethyl ether
5	acetone ← Used for RI
6	hexane
7	*ethyl acetate* ← Used for k

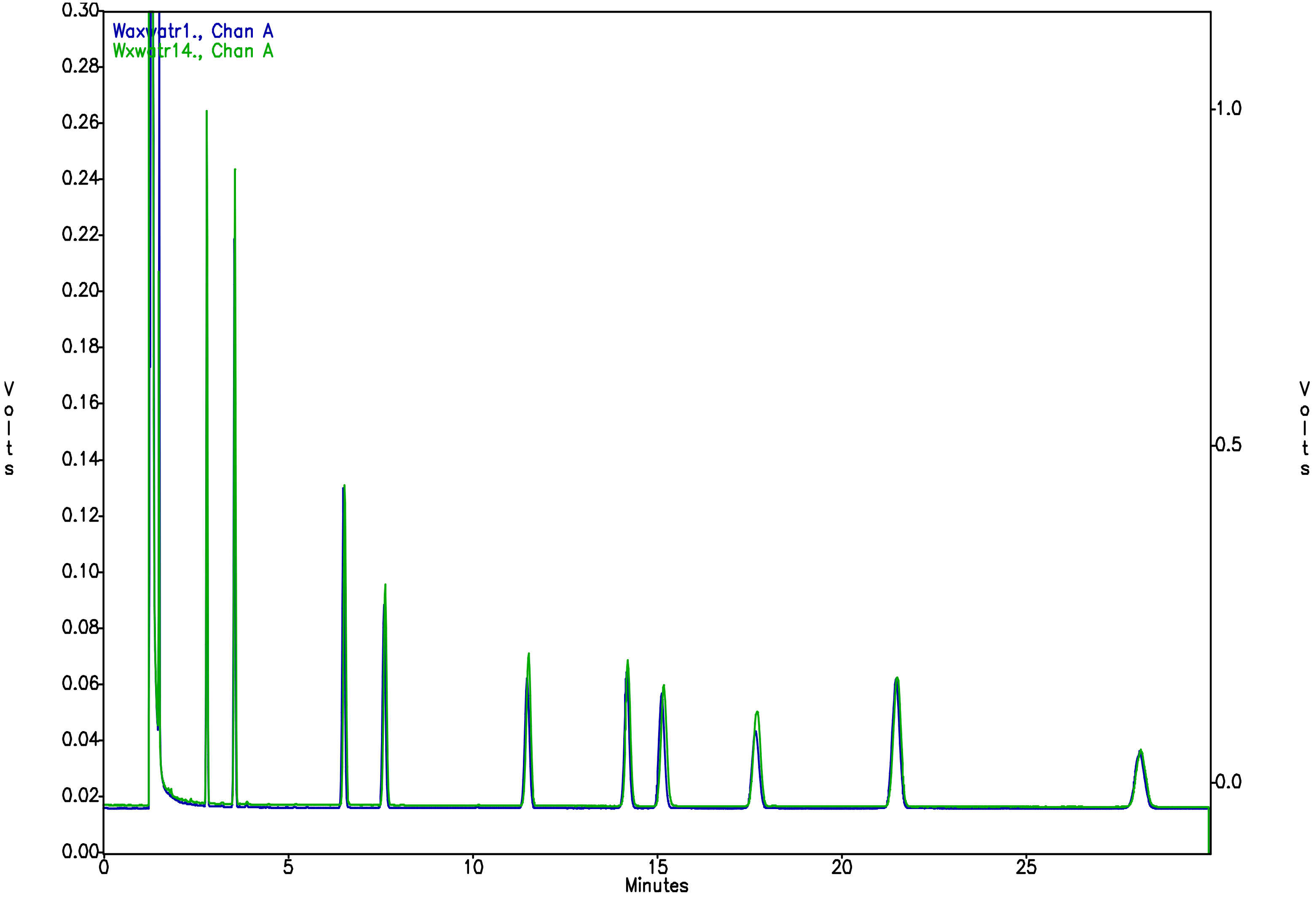
Results DB-1

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, that is, 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

Bonded phases, 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 – loss of resolution, retention, and possibly efficiency.

Amount of washout depends on temperature: at 130 °C and 200 °C it is minimal, at 60 °C it is noticeable.

Solubility of phase material is greater in liquid water (low T, column rinsing) than from water vapor (high T, injections).

Study Conclusions

For binded and cross-linked columns it is safe to inject water

Nonbonded columns: Water injections can wash out stationary phase – use with caution

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: **backflash**

Typical Solvent Expansion Volumes

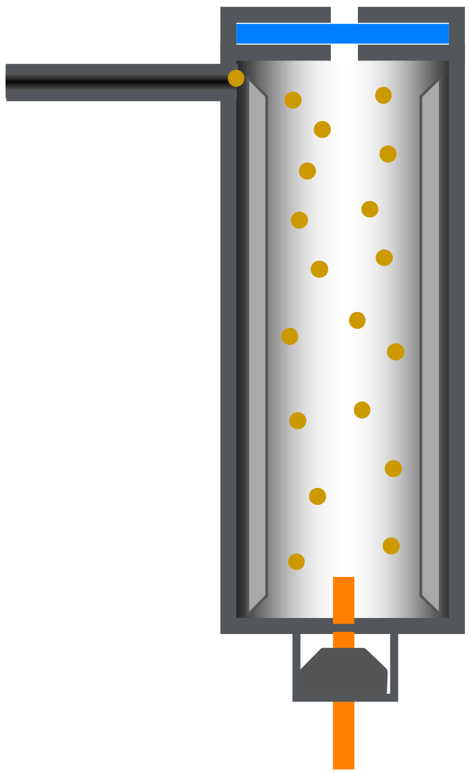
Solvent	Vapor volume (µL) of 1 µL liquid
Water	*1010*
Methanol	450
Carbon disulfide	300
Methylene chloride	285
Acetone	245
n-Hexane	140

Backflash

Cause

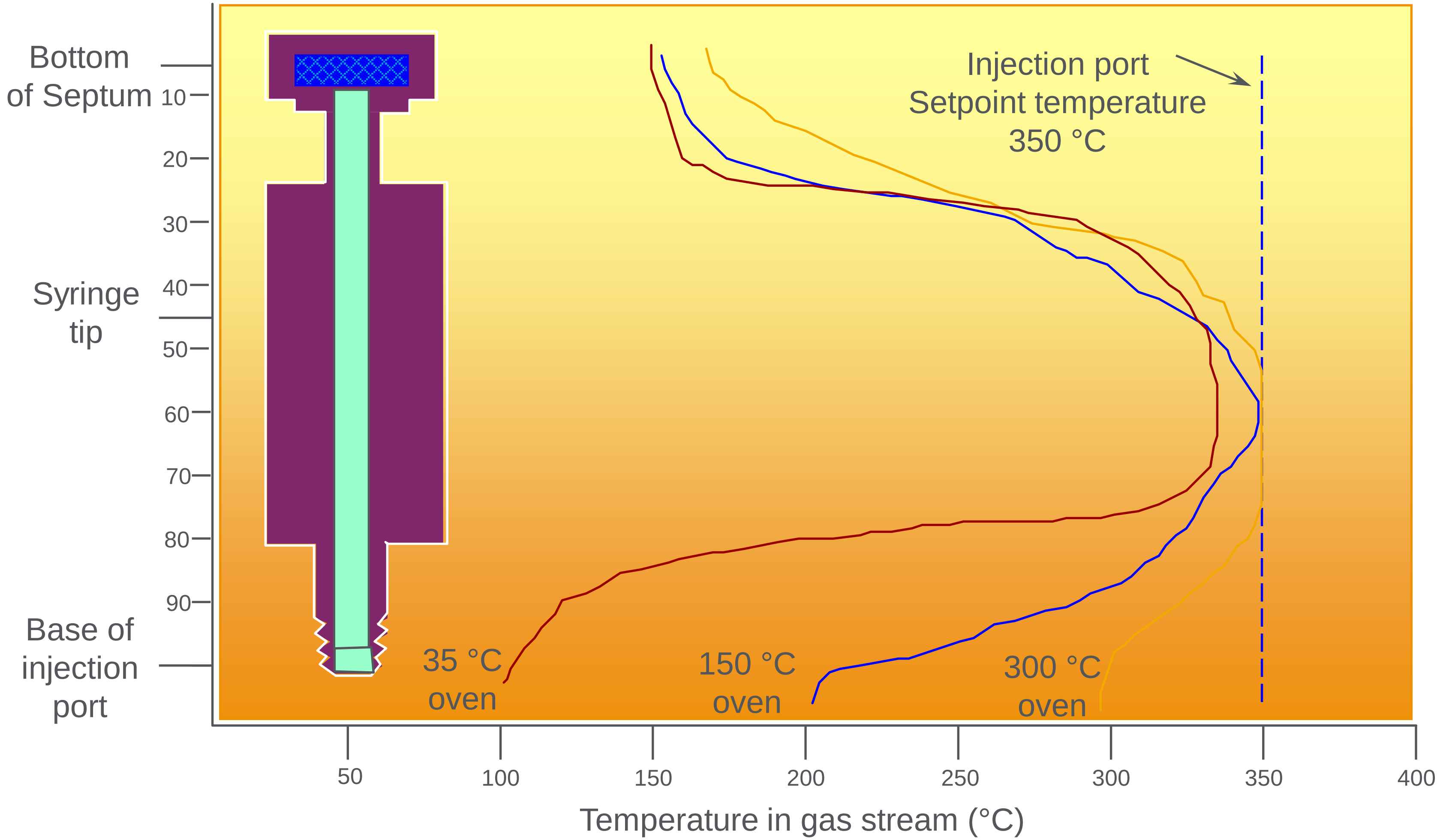
- Vaporized sample expands 100 to 1000 times
- Portions may leave the liner
- Occurs when vapor volume > liner volume

Backflash



Sample expands to overfill injector
Some sample condenses on cooler areas (bottom of septum, metal body, and so on)

Temperature Profile of a Typical Vaporization Injector vs Oven Temperature



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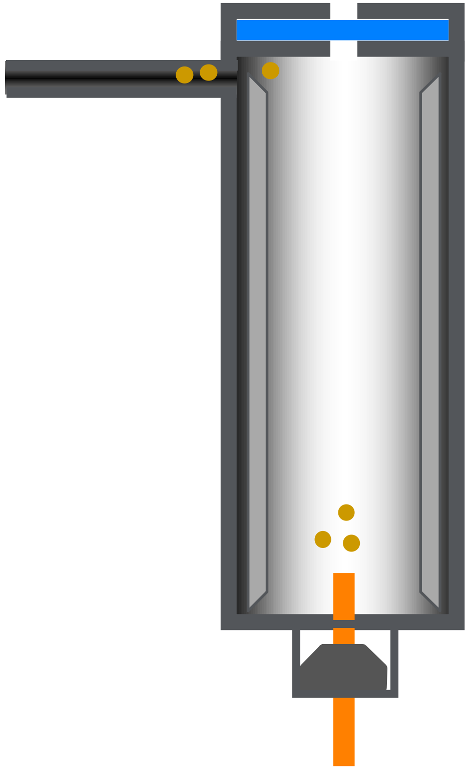
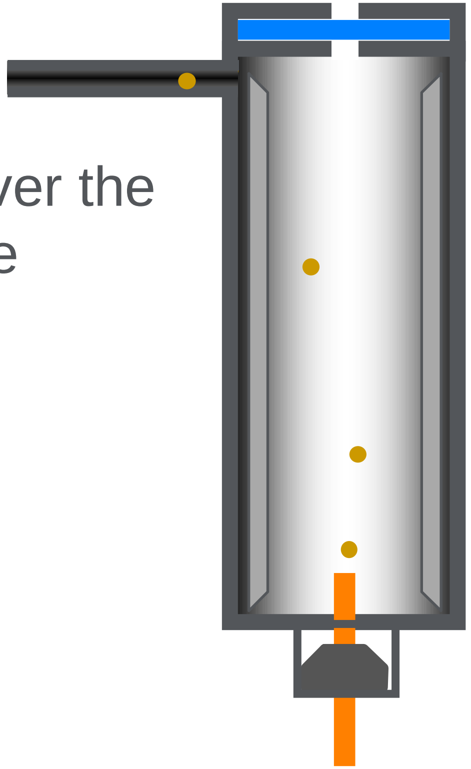
Backflash



Some sample flows out of 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 deposit more sample

Sample then enters the column

Backflash
Problems

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

Backflash
Minimizing

- Large volume liner (possibly tapered)
- Small injection volume
- Low expansion solvent
- Low injector temperature
- High carrier gas flow rates
- High head pressures (possibly pulsed)
  - Smaller column diameters

Use vapor volume calculator!
https://www.agilent.com/en/support/gas-chromatography/gccalculators

Summary

- Water injection can be tricky
  - Polarity miss-match
- For bonded columns and many PLOT columns, no negative effects of injecting water were observed
- Water is not recommended for Non-bonded columns and some PLOT columns, (e.g., Alumina and Molesieve, are not suitable for water injections)
  - Observe manufacturers recommendations
- Backflash is largely responsible for perceived issues related water injections

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 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

Thank you!

Questions?