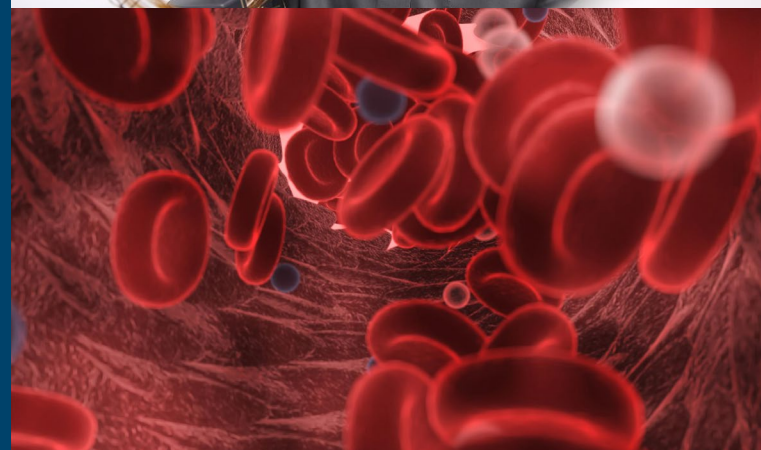
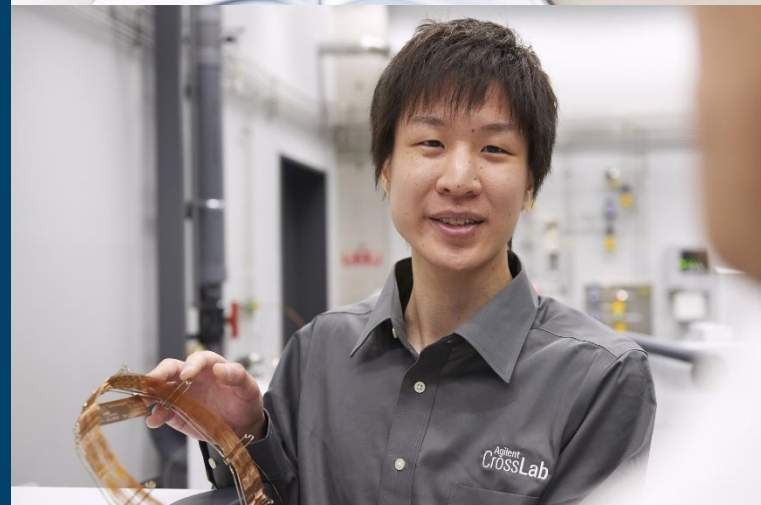


# Beware of GC Column Bleed

Tips and tricks for troubleshooting bleed problems

Alexander Ucci  
Online Application Engineer  
23 August 2022



# Agenda

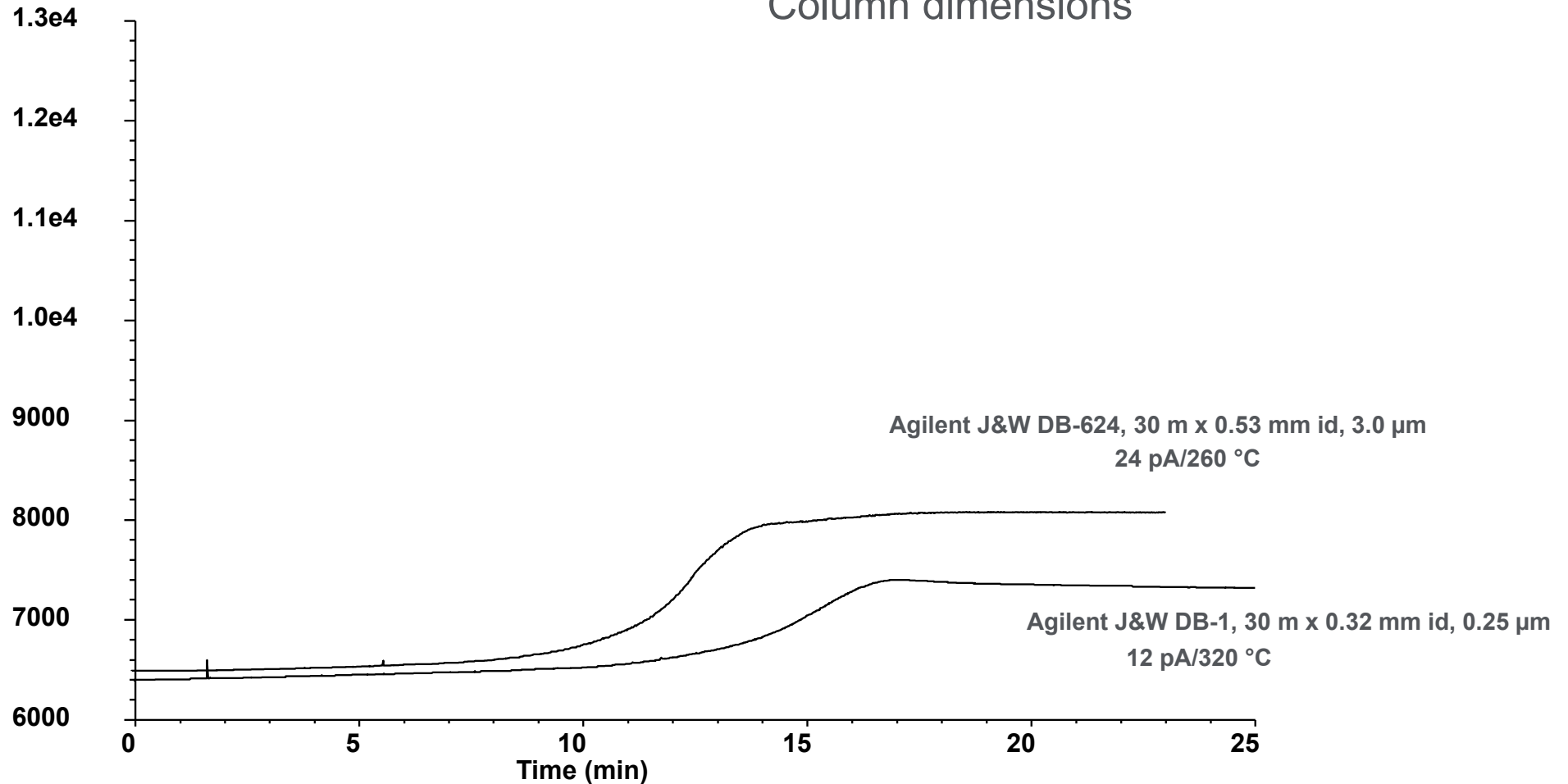
- What is column bleed?
- What is a bleed problem or an abnormal bleed?
- Preventive measures
- Low-bleed phases and column options



# What is Normal Column Bleed?

Normal background signal is generated by the elution of normal degradation products from the column stationary phase. Column bleed is influenced by:

- Phase type
- Temperature
- Column dimensions



# What Causes Column Bleed?

It's natural

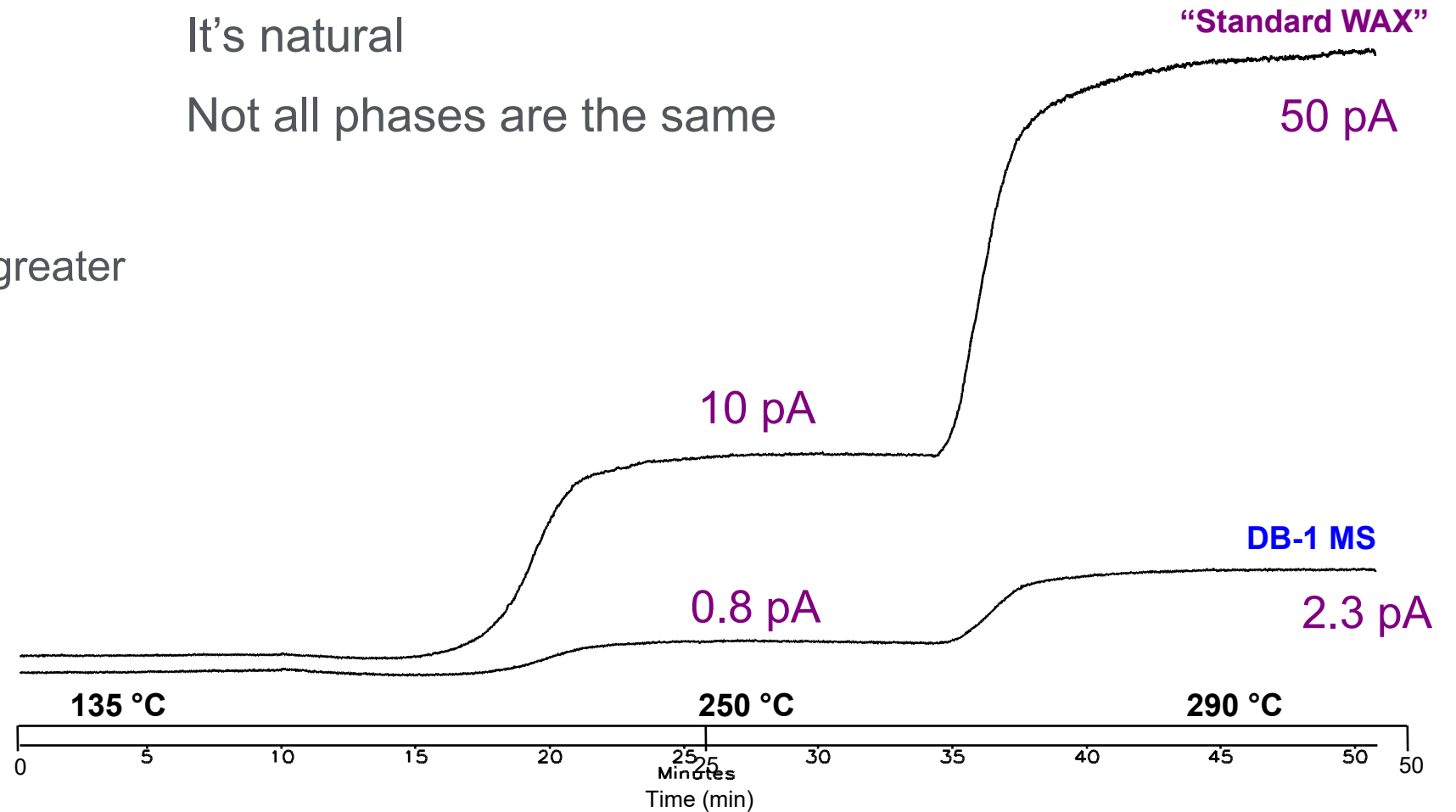
Not all phases are the same

Thermal stability:

- The lower the bleed, the greater the thermal stability

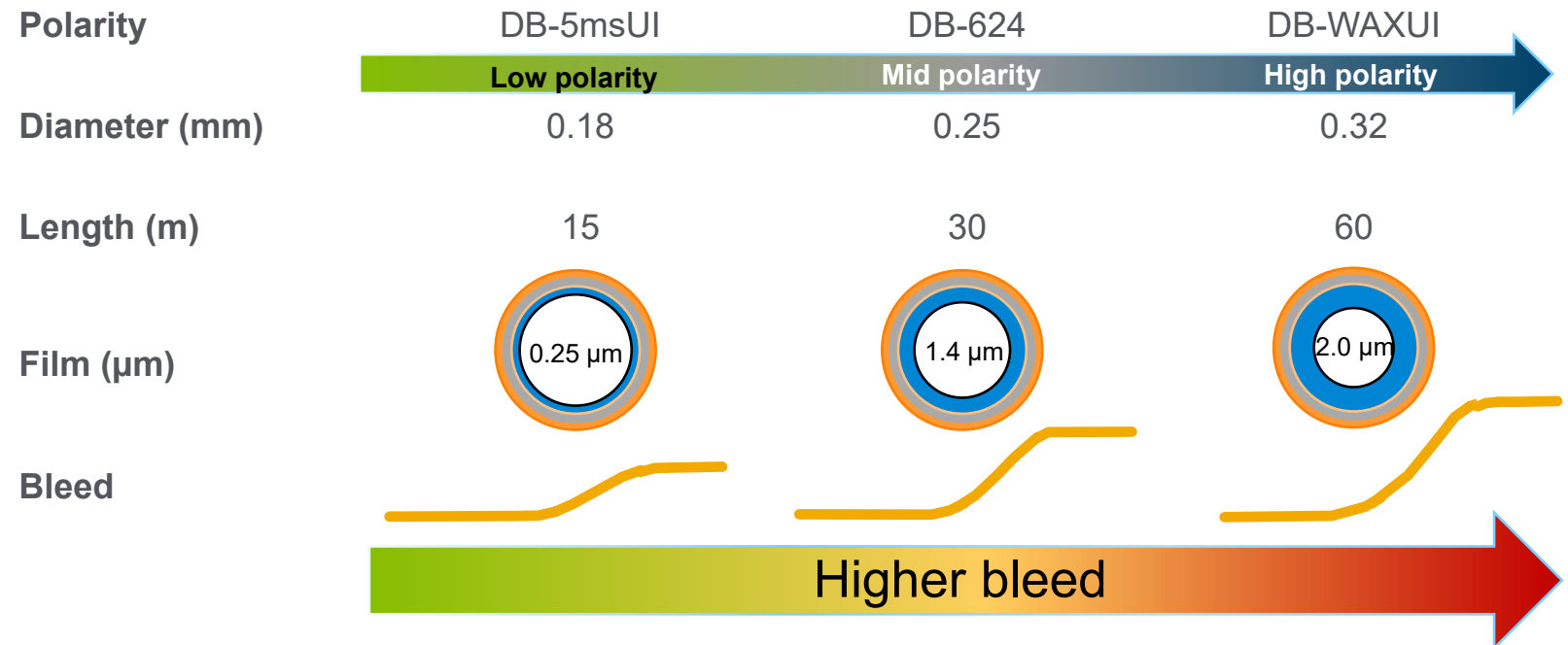
What makes it worse?

- Oxygen
- High temperatures



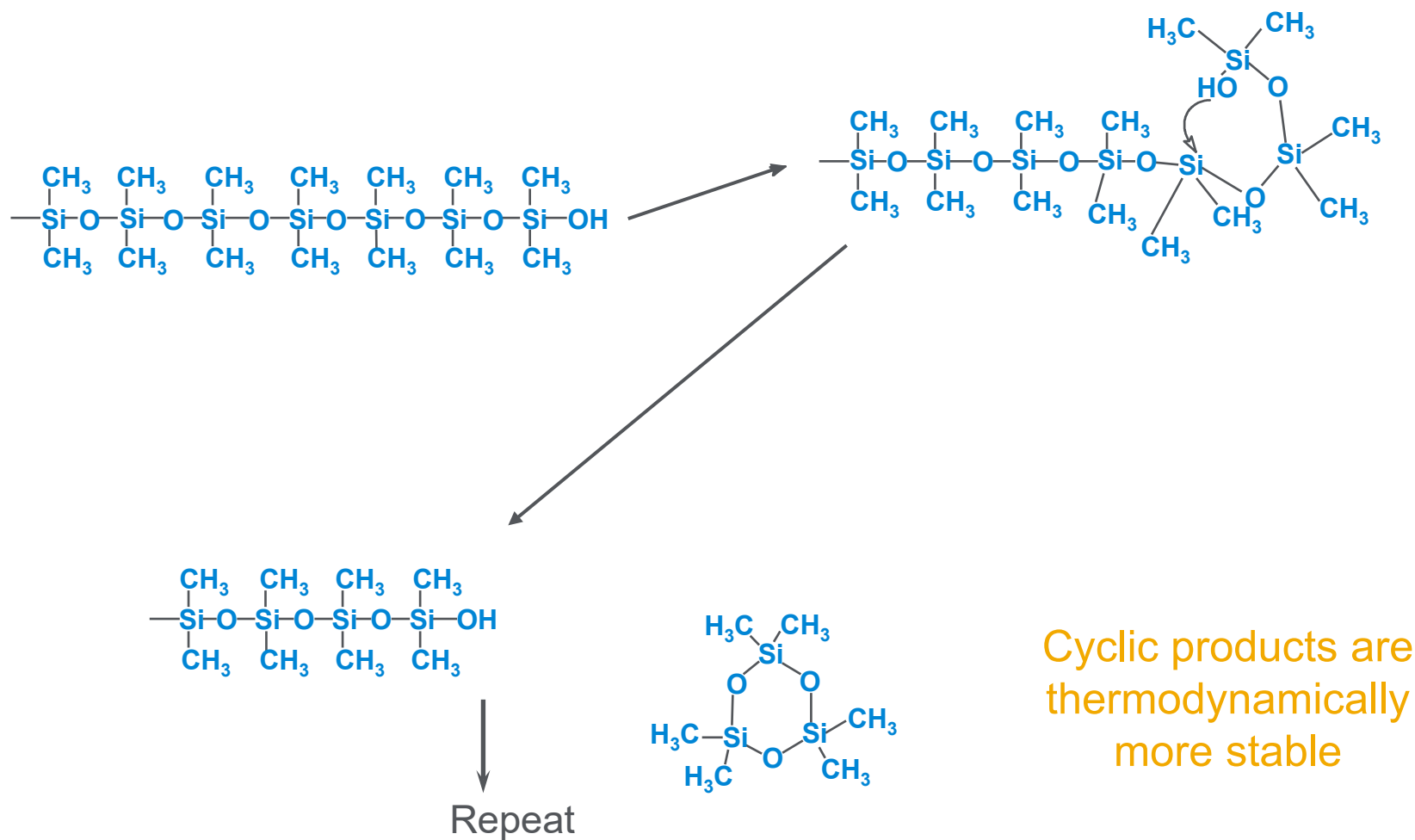
# What Column Types/Dimensions Produce Higher Bleed?

- Polarity: More polar = higher bleed
- Low polarity = more thermally stable
  - Look at temperature limits as a general indicator of thermal stability
- The more total mass of polymer in the column the higher the bleed (within a given phase)
  - Larger diameters
  - Longer columns
  - Thicker films



# What is Column Bleed?

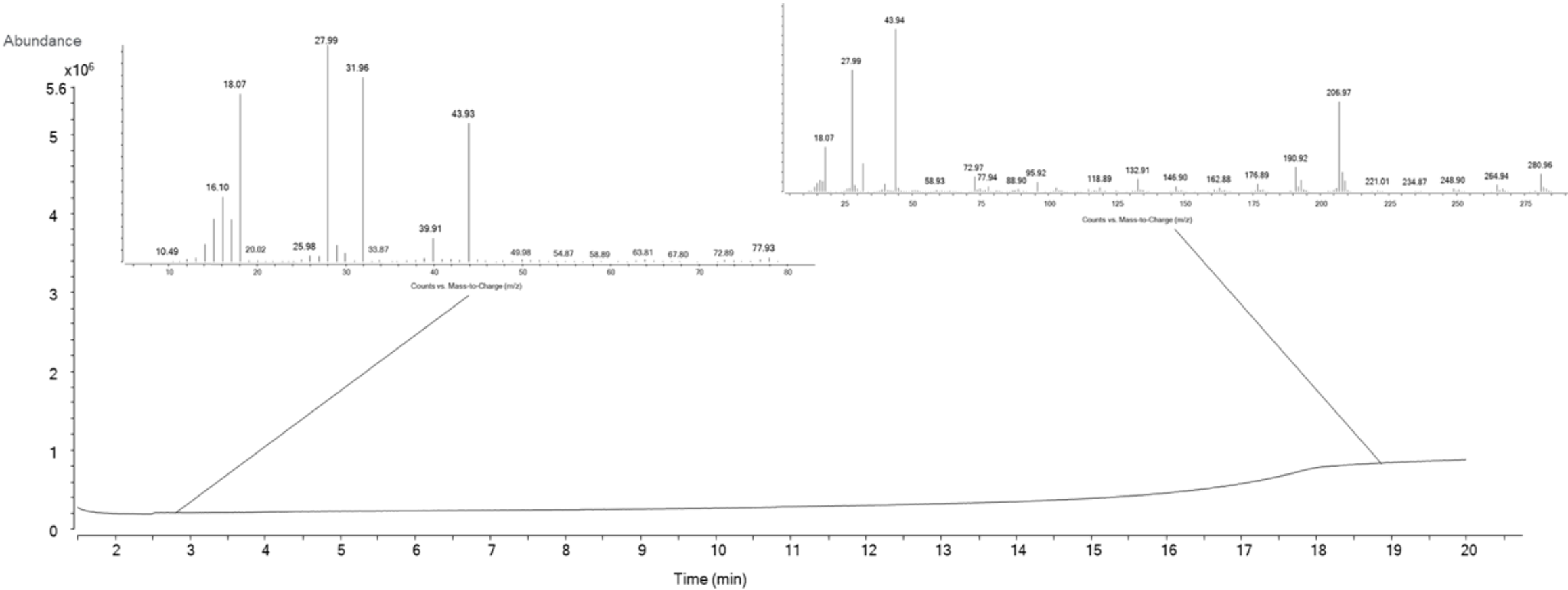
“Back biting” mechanism of product formation



Cyclic products are thermodynamically more stable

# Mass Spectrum of Phenylmethylpolysiloxane Column Bleed

## Normal background (HP-5ms UI)



# Column Performance Testing

Catalog: 19091S-433UI

Serial:



Stationary Phase: HP-5MS UI

Description: 30m x 0.250mm x 0.25µm

Temperature Limits: -60°C to 325°C (350°C Pgm)

## Performance Results

Theoretical Plates/Meter:

n-DECANE 3208

Retention Index:

n-PROPYLBENZENE 953.110

1-HEPTANOL 967.660

Resolution:

1-OCTENE, n-OCTANE 2.97

## Compound Identification

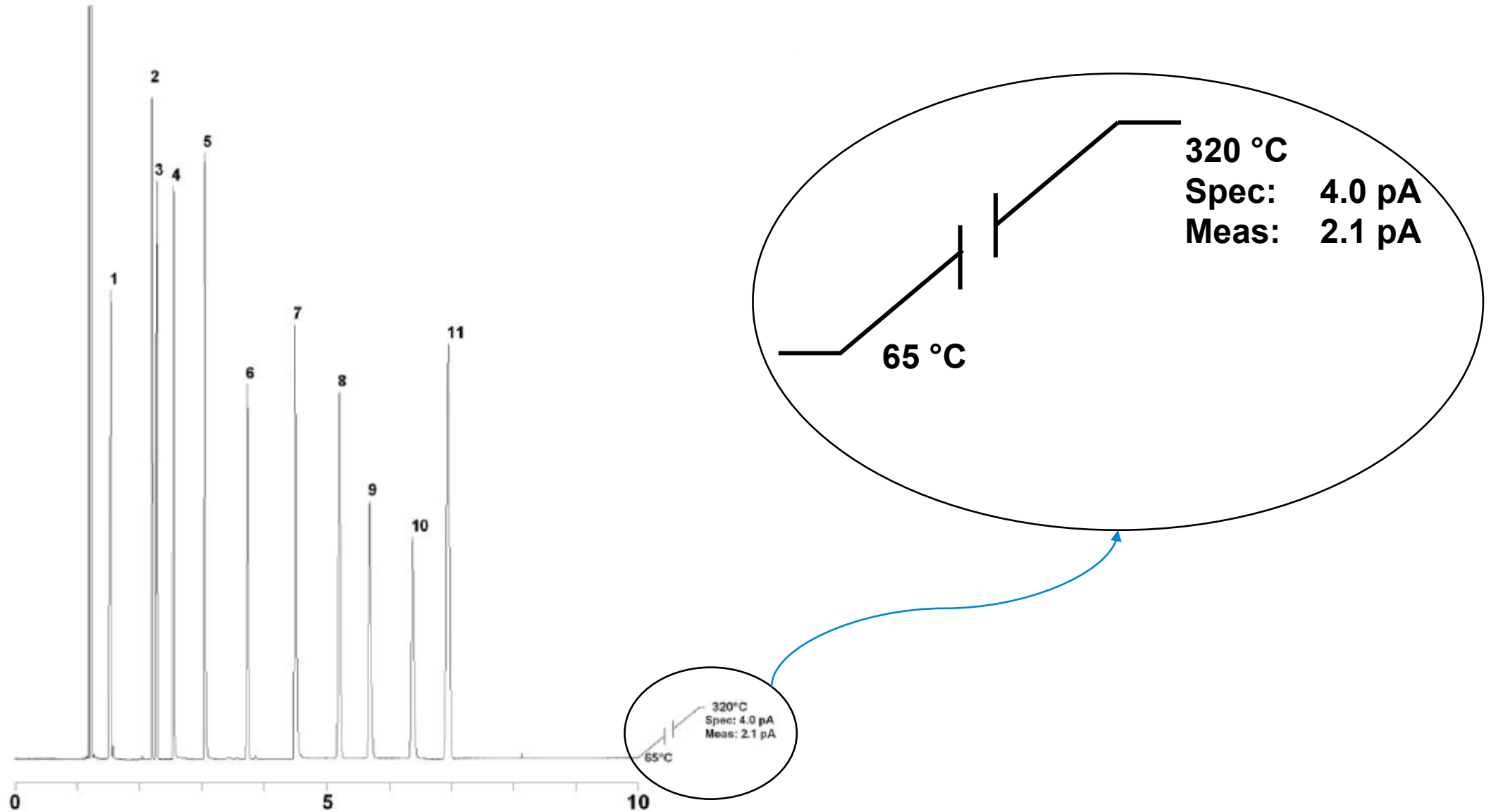
Compound Identification	Retent. Time	Part. Ratio	1/2-Width
1. PROPIONIC ACID	1.543	0.30	0.027
2. 1-OCTENE	2.203	0.86	0.015
3. n-OCTANE	2.282	0.92	0.016
4. 1,3-PROPANEDIOL	2.552	1.15	0.020
5. 4-METHYLPYRIDINE	3.051	1.57	0.021
6. n-NONANE	3.738	2.15	0.027
7. TRIMETHYLPHOSPHATE	4.482	2.78	0.033
8. n-PROPYLBENZENE	5.193	3.38	0.038
9. 1-HEPTANOL	5.682	3.79	0.041
10. 3-OCTANONE	6.368	4.37	0.047
11. n-DECANE	6.940	4.85	0.053

## Test Conditions

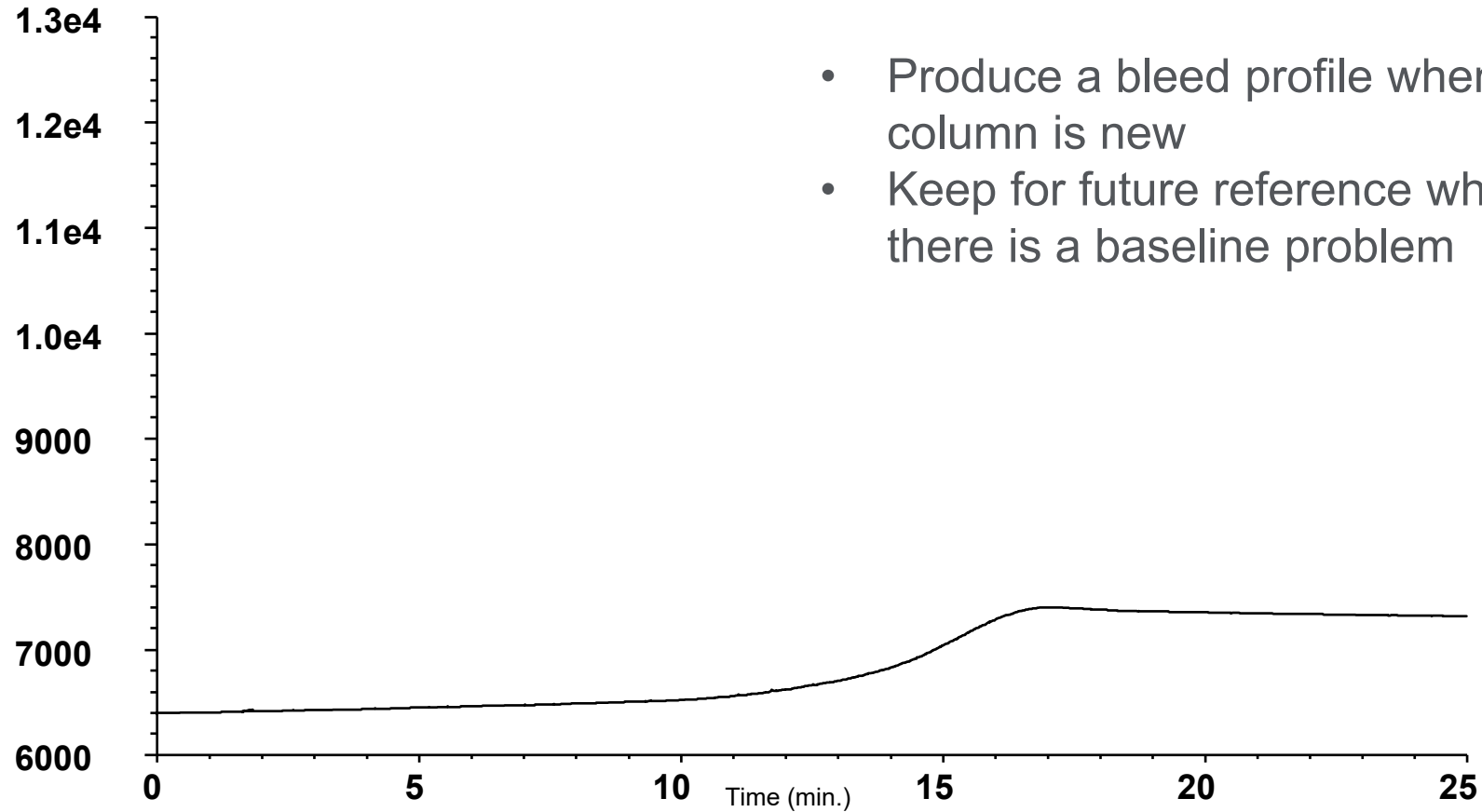
Inlet: Split (250°C)      Detector: FID (325°C)  
 Carrier Gas: Hydrogen      Flow: 42.1 cm/sec (1.2 ml/min)  
 Holdup Compound: Pentane (1.187-min)  
 Temperature Program: Isothermal at 65°C



# Measuring Bleed



# Generating a Bleed Profile



- Produce a bleed profile when the column is new
- Keep for future reference when there is a baseline problem

\*Agilent J&W DB-1 30 m x .32 mm id, 0.25  $\mu$ m  
Temperature program // 40 °C, hold 1 min // 20 °C/min to 320 °C, hold 10 min

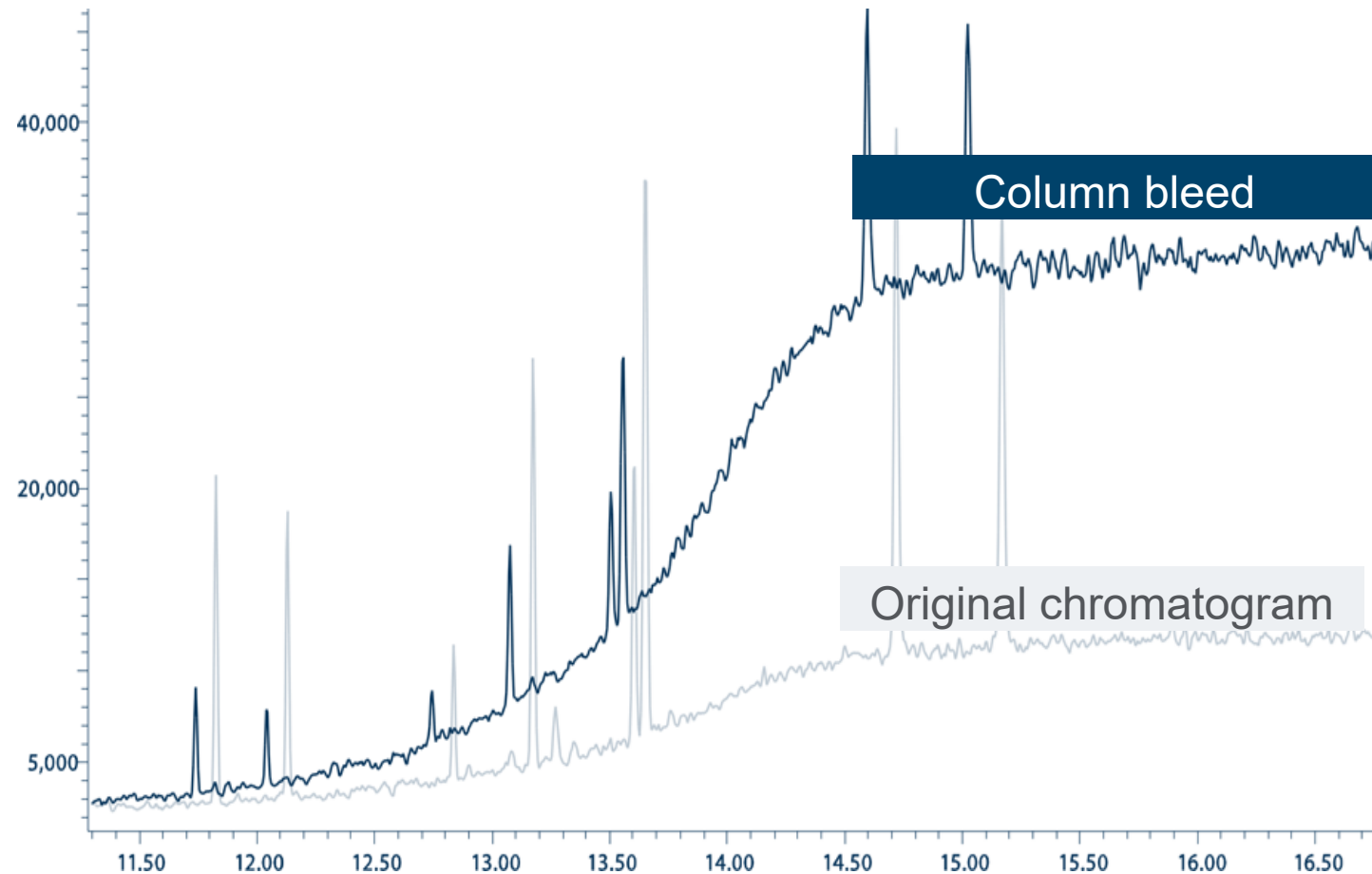
# What is a Bleed Problem?

An abnormal elevated baseline at high temperature

It is **not**:

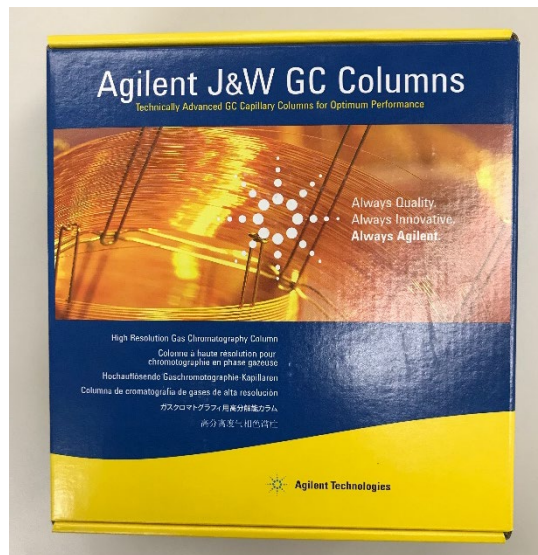
- A high baseline at low temperature
- Wandering or drifting baseline at any temperature
- Discrete peaks

# Troubleshooting Column Bleed

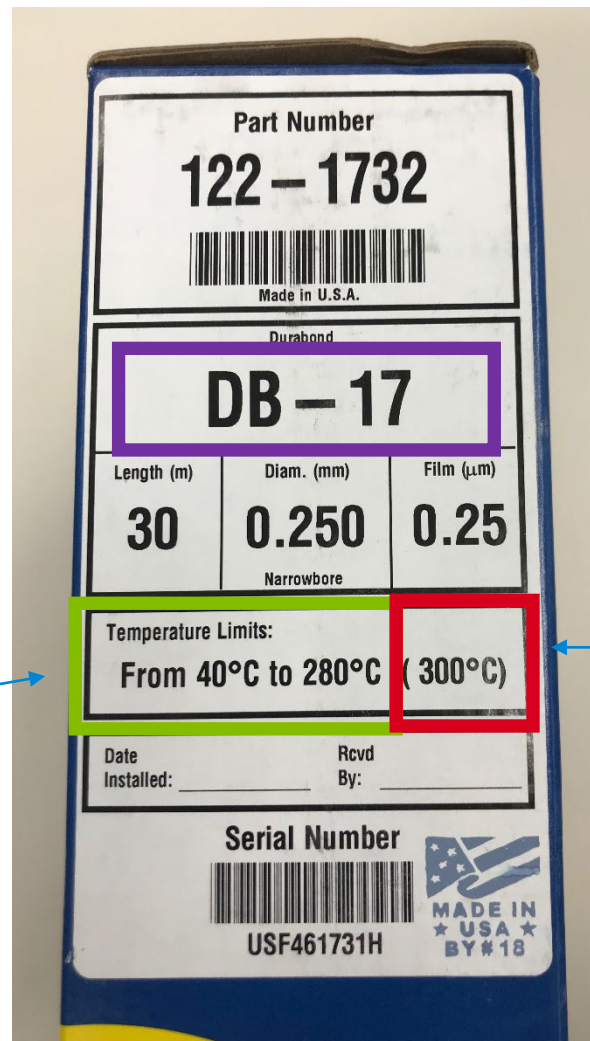


- Have you installed or conditioned the column?
- Are you exceeding the column's upper temperature limit?
- Is your column's film size too thick?
- Could leaks be present in your flow path, or are your carrier gases contaminated with air?
- Do you need to change your split vent trap?

# Pay Attention to the Temperature Limits



Isothermal temperature limits



Programmed temperature limit (<10 min)

# Thermal Damage

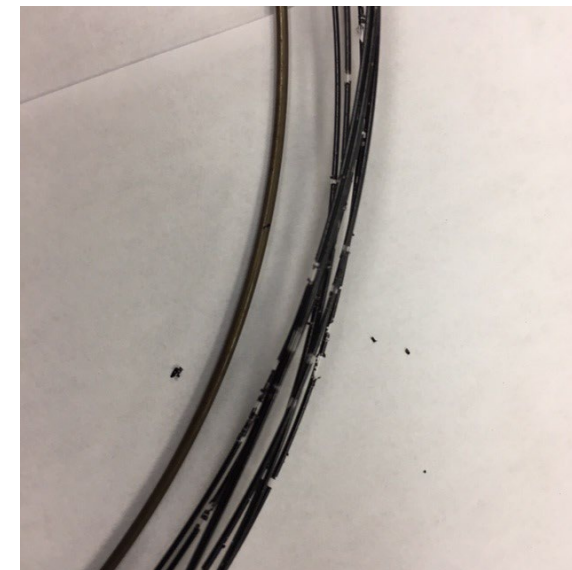
Degradation of the stationary phase increases at higher temperatures

- Rapid degradation of the stationary phase (breakage along the polymer backbone) caused by excessively high temperatures

Isothermal limit = indefinite time

Programmed limit = 5–10 minutes

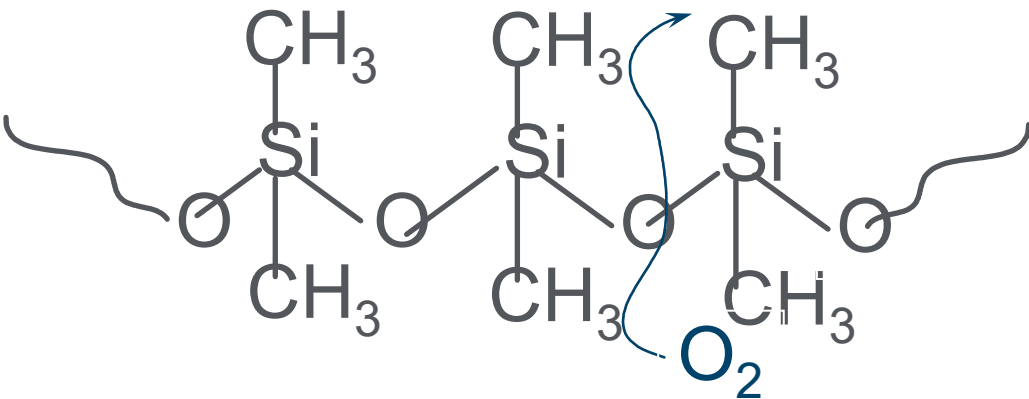
- Temporary "column failure" below lower temperature limit
- If this happens:
  - Disconnect column from detector
  - "Bake out" overnight at isothermal limit
  - Remove 10–15 cm from column end



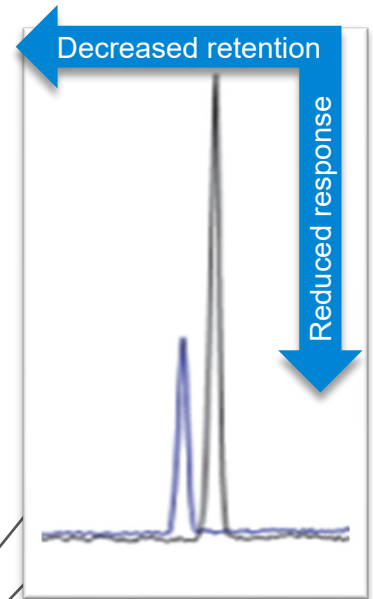
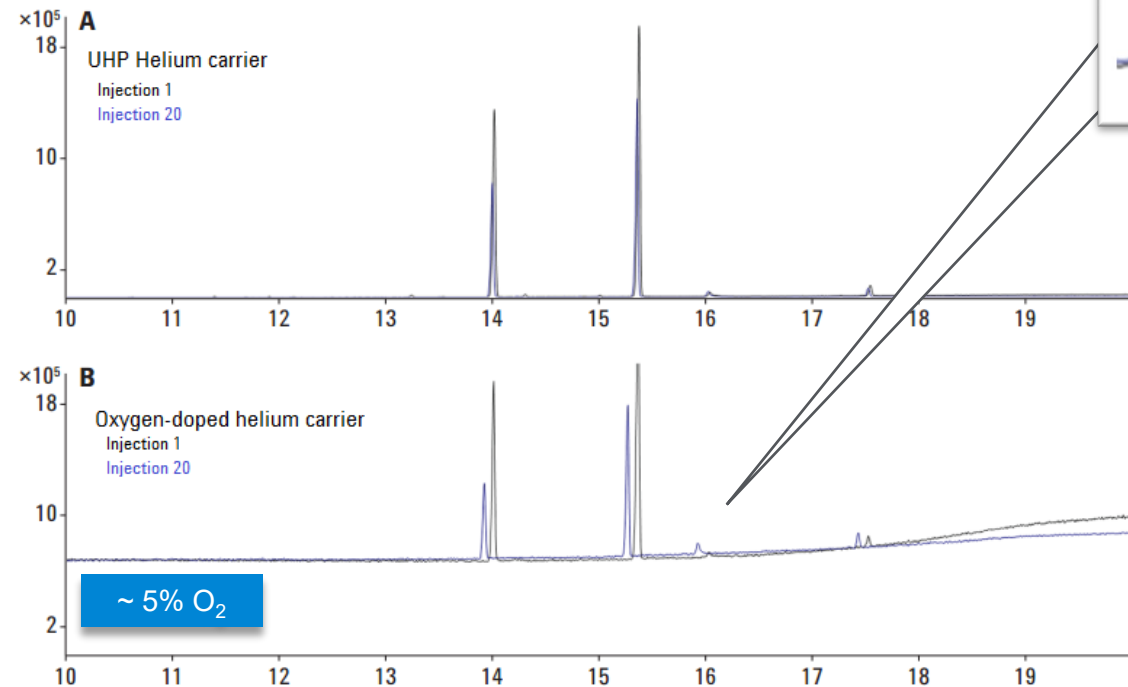
Column continuously exposed to temperatures above its temperature limit

# Oxidation (O<sub>2</sub> Damage)

Oxygen in the carrier gas rapidly degrades the stationary phase. The damage is accelerated at higher temperatures. Damage along the polymer backbone is irreversible (premature filament failure/excessive source maintenance).

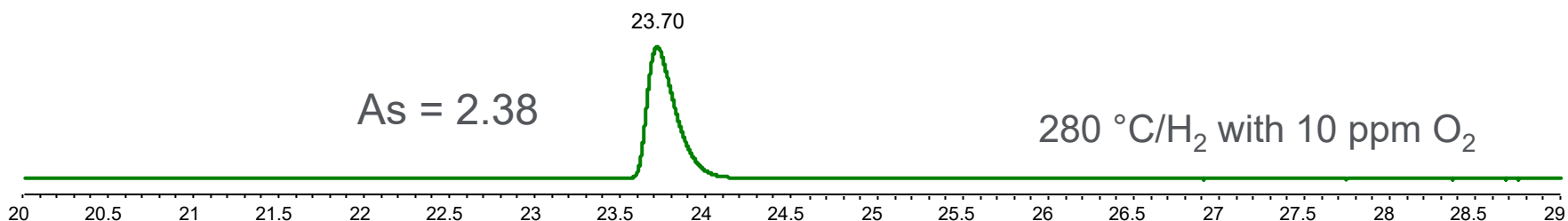
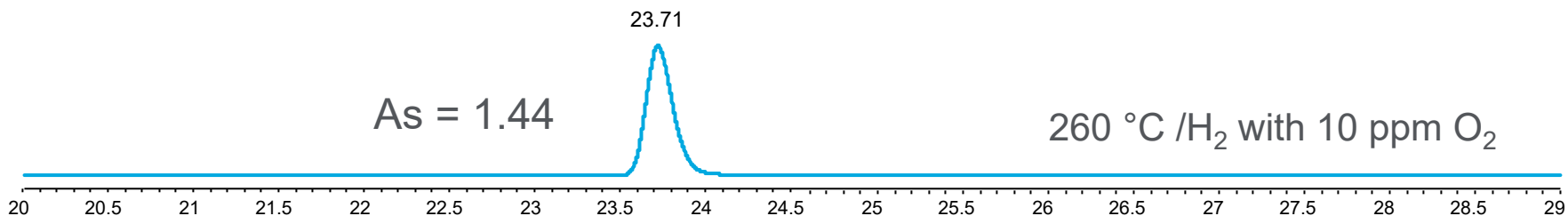
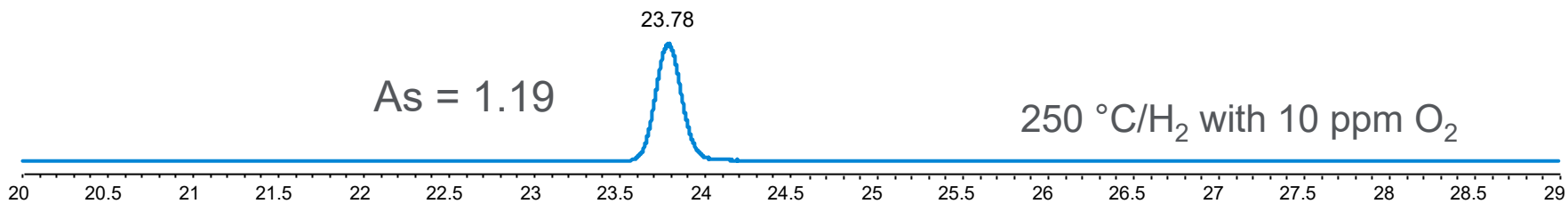
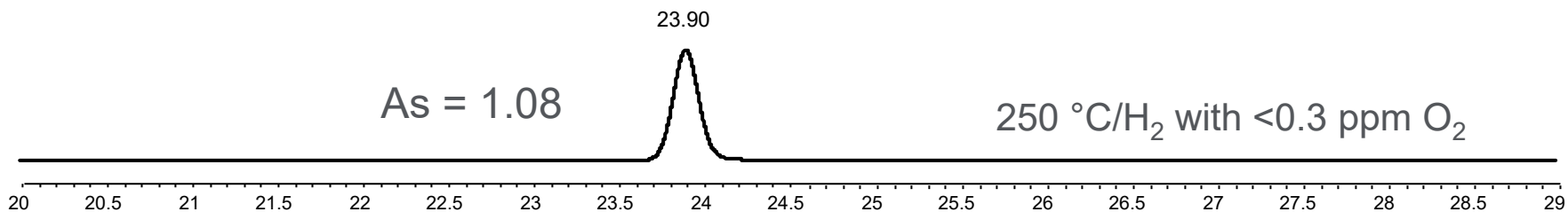


Dimethylpolysiloxane



Higher bleed

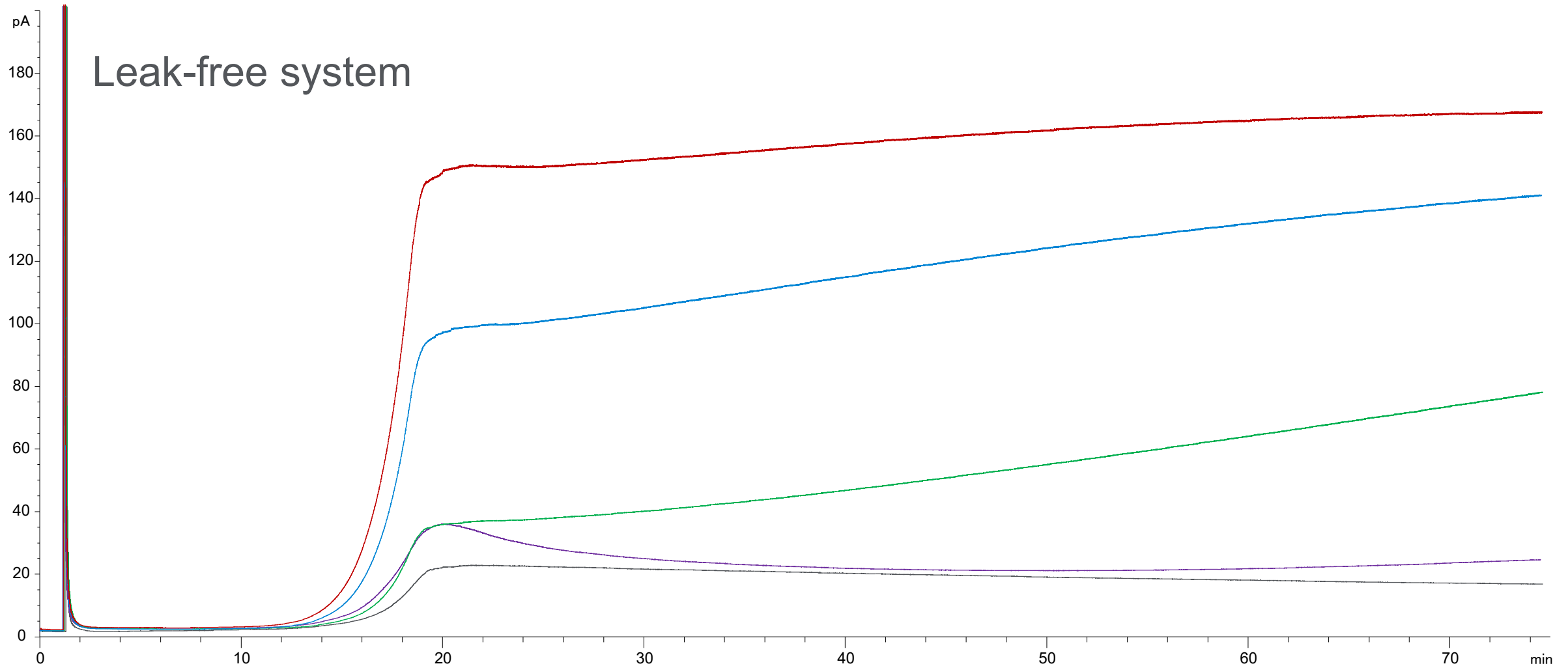
# Effect of Oxygen on Peak Shape of 2-Ethylhexanoic Acid





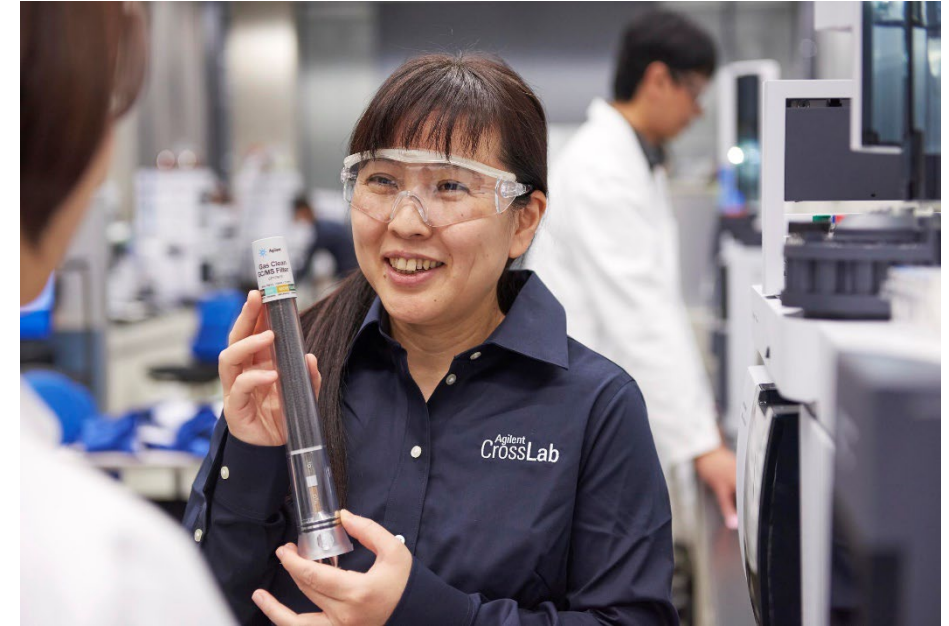
# High Temperature + Leaks = Dead Column

## Air Leaks = Increase in Column Bleed at High Temperatures



# How to Prevent Column Damage by Oxygen

- High-quality carrier gas (four 9s or greater)
- Leak free injector and carrier lines
  - Change septa
  - Maintain gas regulator fittings
- Appropriate impurity traps



Efficient, fast, easy

# Knowing If You Have a Leak Before Using Your GC



p/n CP17973



Remove plugs  
before installation

[www.agilent.com/chem/gasclean](http://www.agilent.com/chem/gasclean)

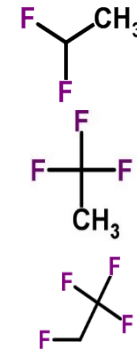
# Use Leak Detector or Electronics Duster to Find Your Leaks

Why use a leak detector?

- High sensitivity
- Recommended for leak detection in gas plumbing and fittings



## Typical Electronic Duster Components and Ions



1,1-  
difluoroethane

*m/z*  
51,65

1,1,1-  
trifluoroethane

*m/z*  
69

1,1,1,2-  
tetrafluoroethane

*m/z*  
69,83

## Use electronics duster

- Hold can upright (don't spray liquid)
- Spray short bursts around possible leak points
- "Live" tune profiling for ions to pinpoint leak

# Agilent CrossLab CS (Cartridge System)

No peaks from leaks

Features:

- Exchangeable cartridge with ADM Flow Meter
- Automatic Notification of Probe Filter Replacement
- Ergonomic and robust design
- Universal 3AA or USB power
- USB connects to web interface for added functionality and firmware updates
- Easy to view OLED Screen
- Kickstand

Leak detector  
cartridge

Handheld



ADM Flowmeter  
cartridge

# The Cost of Leaks

- Cost of gases
- Contamination from exposure
- Reduced consumable lifetime
- Reduced productivity from downtime
- Detector noise and elevated baselines
- Time in troubleshooting

It is critical that every customer checks for leaks. They should have the best tool for the job!

Check valves, fittings, and traps for leaks after every maintenance, and after thermal cycling as these can loosen some types of fittings.



# Assets Available for Launch

- **Agilent.com CrossLab CS Leak Detector**

[www.agilent.com/chem/gas-leak-detector](http://www.agilent.com/chem/gas-leak-detector)

- **Agilent.com – ADM Flow Meter**

<https://www.agilent.com/en/product/gas-purification-gas-management/gas-management/adm-flow-meter>

- **Installation manual**

*Agilent CrossLab CS  
Electronic Leak Detector manual*

Part number: G6693-90000

The installation manual is available on Agilent.com.

- **Innovation minute video**

<https://www.agilent.com/en/video/crosslab-cs-innovation-minute>

- **Technical overview**

*Agilent CrossLab Cartridge System  
(CS) Electronic Leak Detector*

Publication number: 5994-4262EN

The technical overview is available on Agilent.com

- **Brochure**

*GC Troubleshooting in  
the Palm of Your Hand*

Publication number: 5994-3607EN

The brochure is available on Agilent.com

- **Flyer**

*Is a Leak Causing Your  
Inaccurate Results?*

Publication number: 5994-4202EN

The flyer is available on Agilent.com

# Ordering Guide

1 year warranty

- G6693A – CrossLab CS Electronic Leak Detector
- G6694A – Electronic Leak Detector Cartridge
- G6699A - CrossLab CS Bundle: ADM Flow Meter and Electronic Leak Detector
  - The Bundle will include 1 handheld, 2 cartridges, and a **free** carrying case.
- G6694-60005 – Replacement Probe Filter
- G6691-40500 – Carrying Case

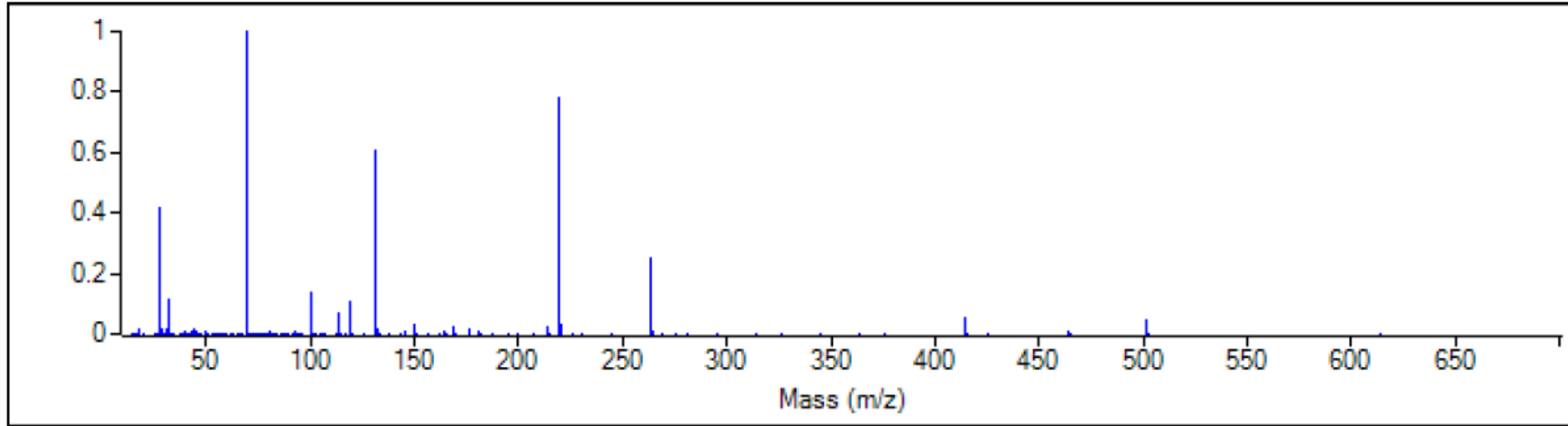


Existing products:

- G6691A – CrossLab CS ADM Flow Meter
- G6692A – ADM Flow Meter Cartridge\*
- Note that the ADM Flow Meter cartridge is ordered annually for calibration. The Electronic Leak Detector does not need to be recalibrated!



# Example Tune Report with Leak



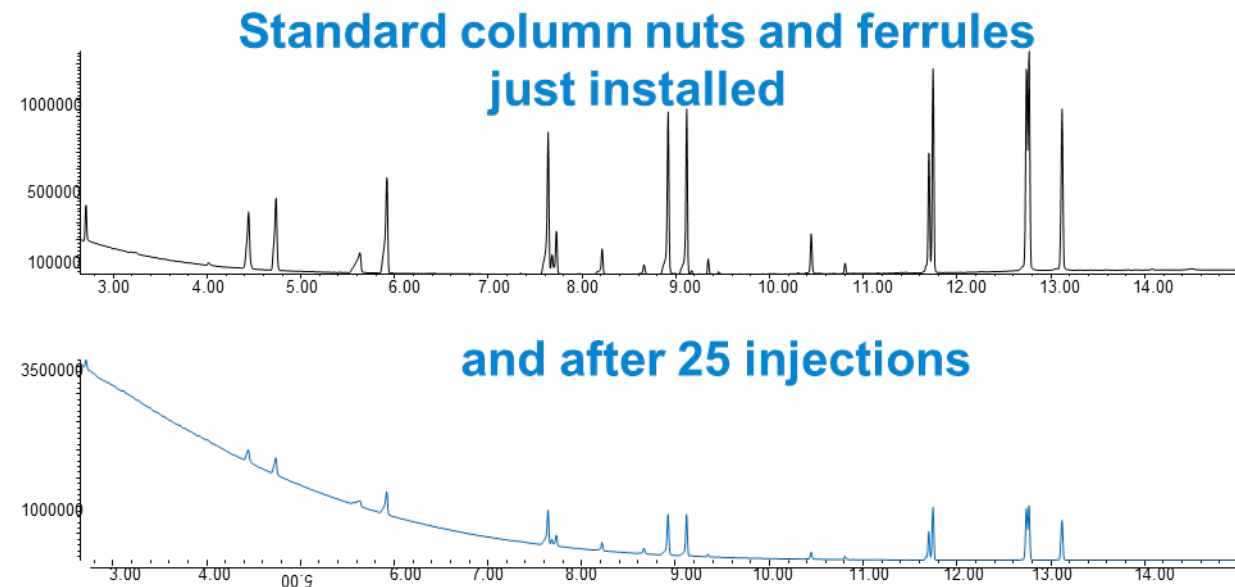
Target m/z	Actual m/z	Abund	Rel Abund	Iso m/z	Iso Abund	Iso Ratio
69.00	69.00	498,432	100.0%	70.00	6,216	1.2%
219.00	219.00	391,232	78.5%	220.00	18,216	4.7%
502.00	502.00	23,680	4.8%	503.00	2,467	10.4%

Air/Water Check: H<sub>2</sub>O ~1.8% N<sub>2</sub> ~42.1% O<sub>2</sub> ~11.4% CO<sub>2</sub> ~1.3% N<sub>2</sub>/H<sub>2</sub>O ~2325.0%

Column(1) Flow: 1.00 Column(2): 1.20 ml/min Interface Temp: 250

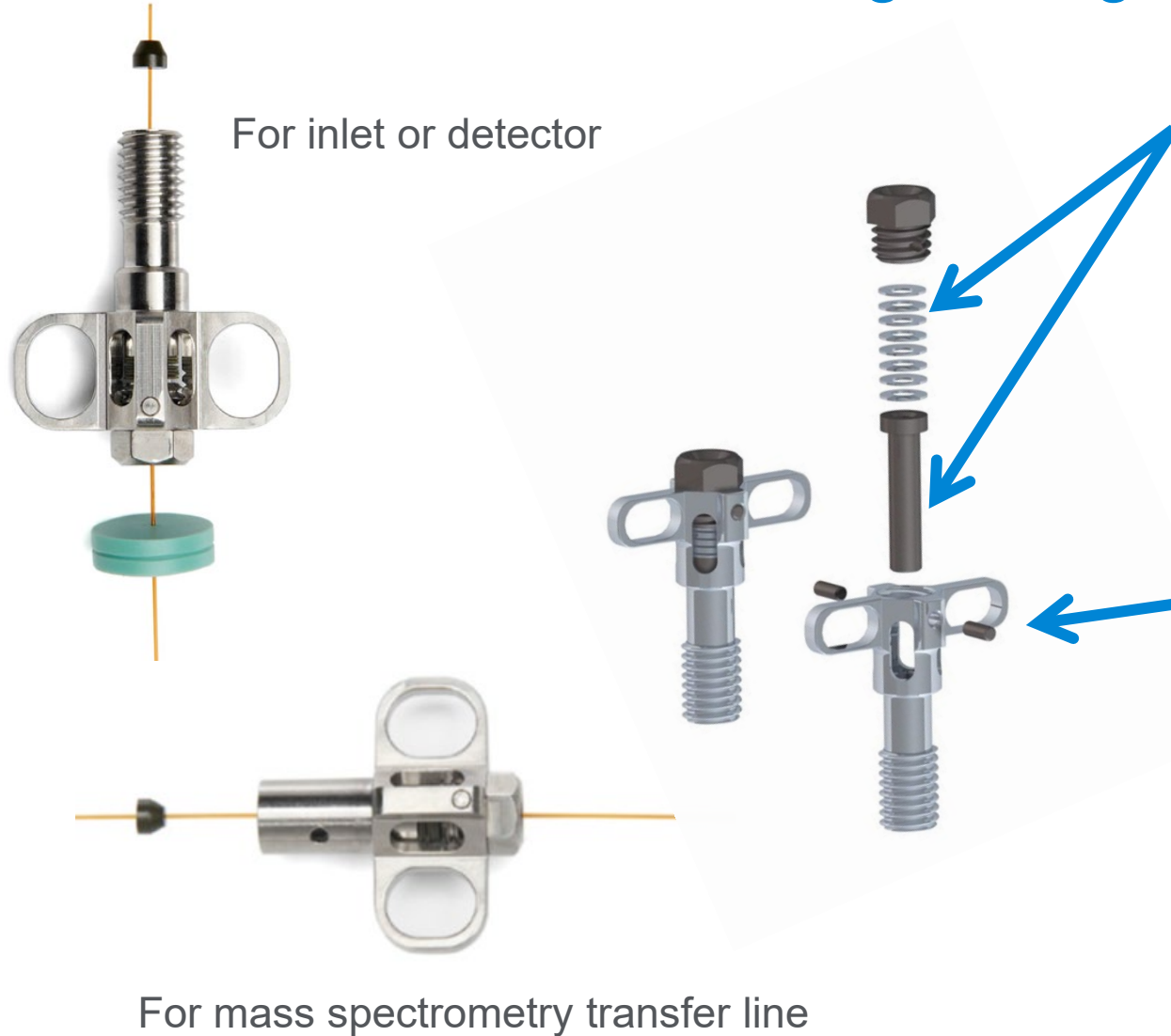
# Graphite/Polyimide Blend Capillary Ferrules

- Unfortunately, a leak occurred following normal temperature program runs
- Studies show that leaking continues with use of the ferrules
  - Not just after the first one or two runs



Frequent retightening of the fitting is needed to maintain a leak-free seal, as well as system performance and productivity.

# Column Installation: Self Tightening Column Nut

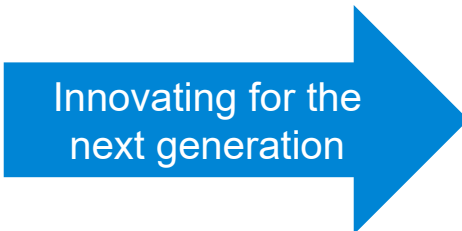
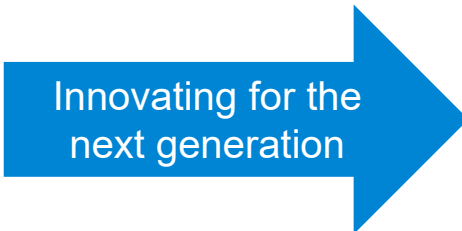


- Spring-driven piston continuously presses against ferrule
- Automatically retightens when ferrule shrinks
- No leaks, no downtime, no frustration
- Wing design for finger tightening
- No tools needed
- No polymer materials for durability
- Compatible with **only** short graphite/Vespel ferrules

# Increasing Ease of Use Through Continued Innovation: Self Tightening Nuts



For GC inlet or detector



- Easier and faster to install
- Collar holds column in place
- Single-hand installation into inlet
- No tools needed



# Self Tightening Nuts: No Leaks, No Downtime, No Frustration



- Spring-driven piston continuously presses against ferrule
- Automatically retightens when ferrule shrinks
- Wing design for finger tightening
- No tools needed

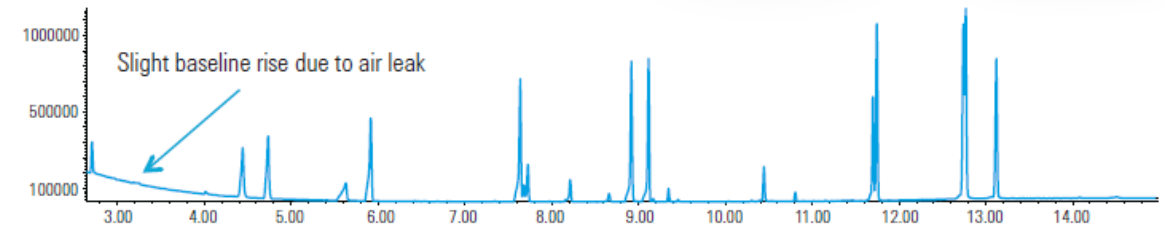
Part Number	Description
G3440-81013	Column Nut, Collared Self-Tightening MSD
G3440-81011	Column nut, Collared Self Tightening Inlet/Detect
G3440-81012	Collar for Self Tightening Nut

<https://www.agilent.com/en/video/gc-supplies-innovation>

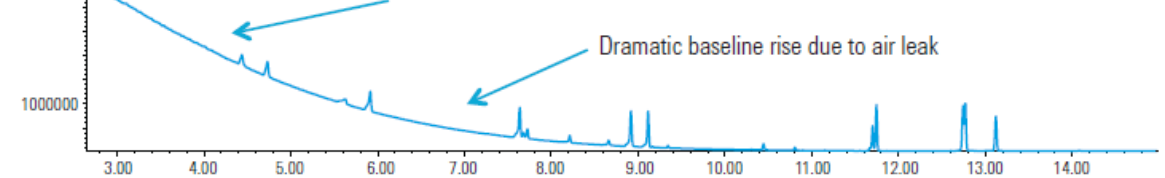
<https://www.agilent.com/en/video/stcn-inlet-detector>

<https://www.agilent.com/en/video/stcn-mass-spec>

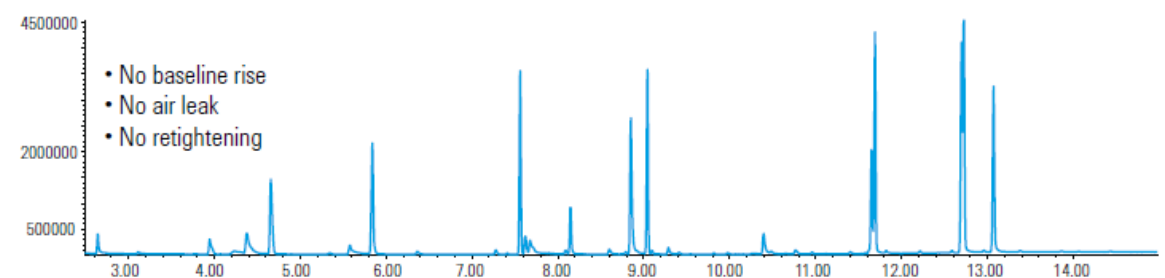
Standard column nuts new fitting



Standard column nuts after 25 injections



Agilent Self Tightening Column Nuts after 400 injections



400 injections

# Chemical Damage

Bonded and crosslinked columns have excellent chemical resistance, except for inorganic acids and bases.

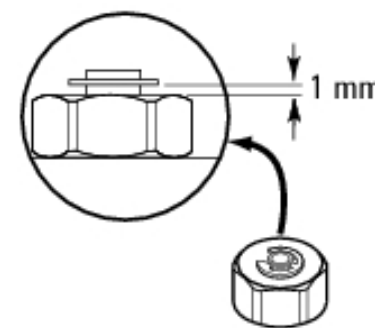
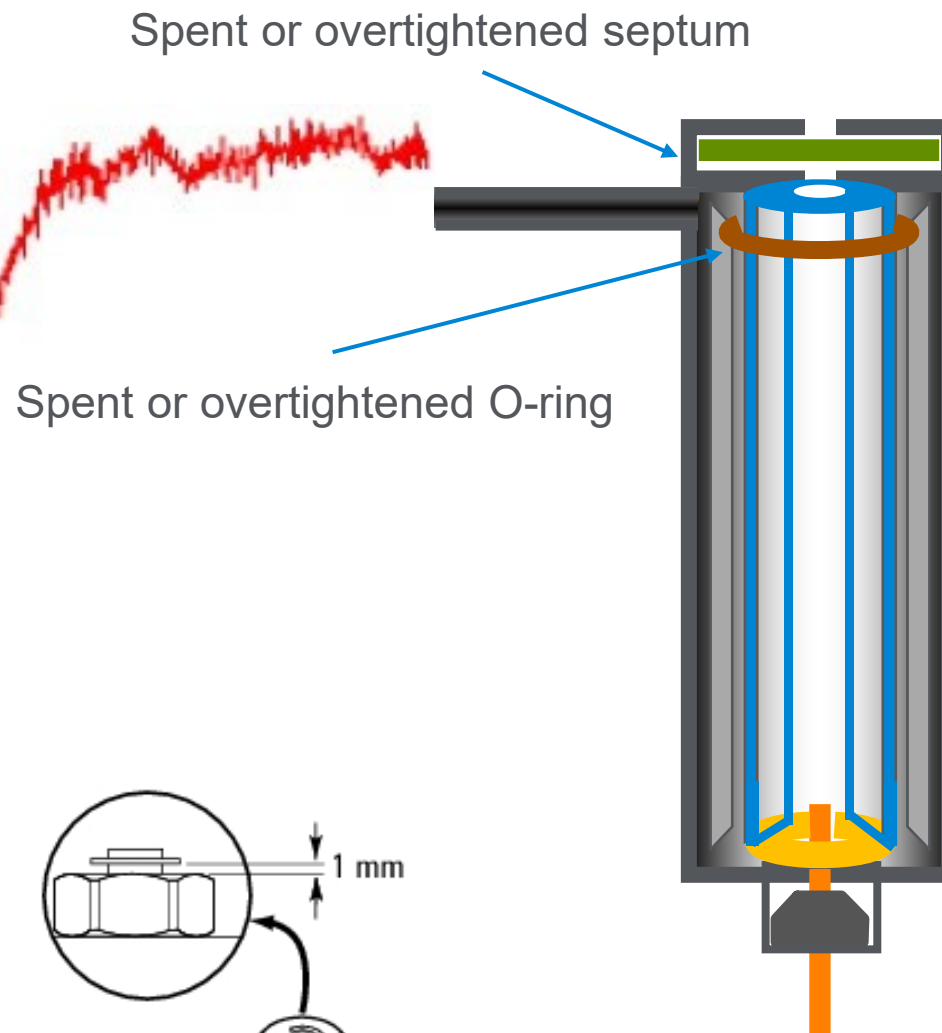
HCl NH<sub>3</sub> KOH NaOH

H<sub>2</sub>SO<sub>4</sub> H<sub>3</sub>PO<sub>4</sub> HF

Chemical damage will be evident through excessive bleed, lack of inertness, or loss of resolution/retention.

# Column Bleed: What It is Not

Peaks are not column bleed!





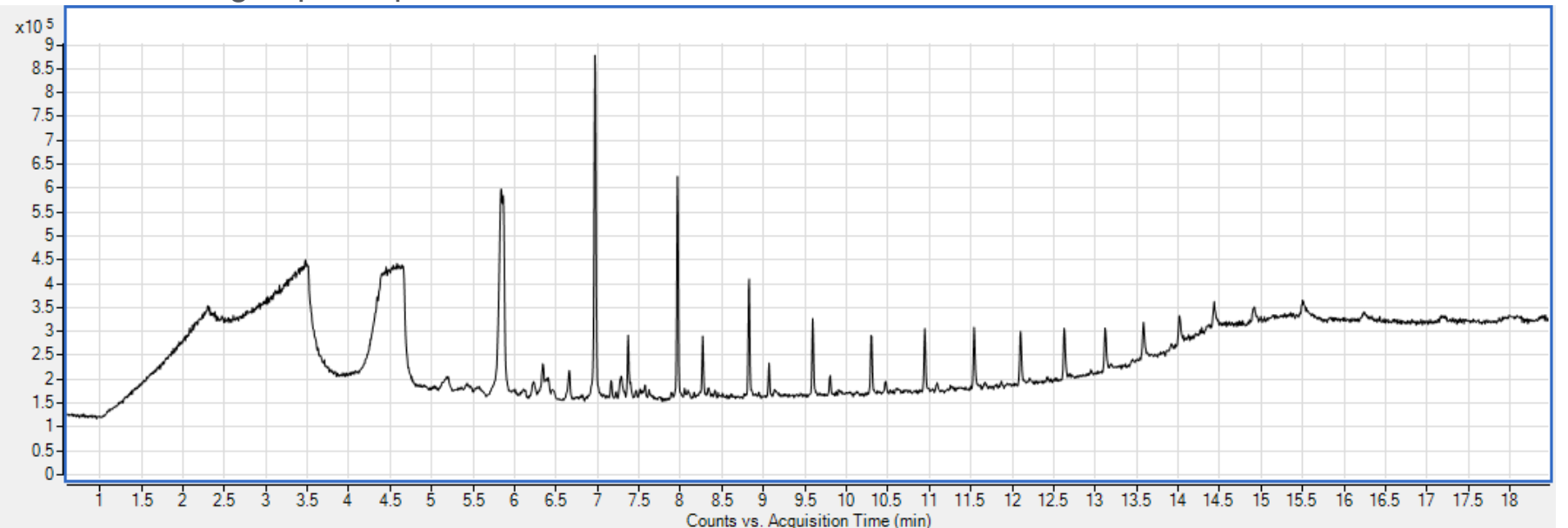
# Septum Maintenance: Septum Coring

- 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
  - This is also very common when making multiple injections from the same vial
  - It is not column bleed even though it looks like it spectrally



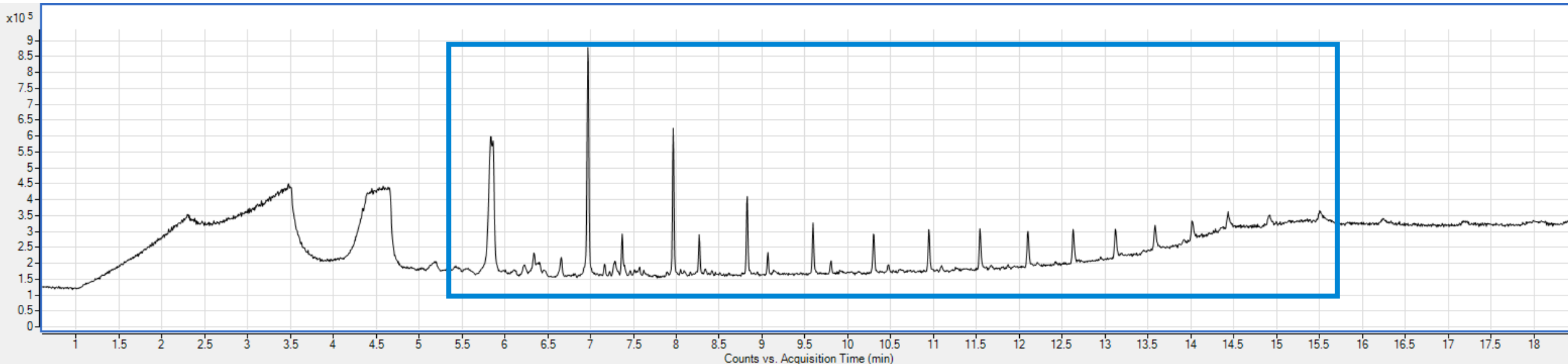
Septum core is placed in a clean liner and a blank injection is performed.

- 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: TIC of an Inlet Septum



Common Ions for  
Siloxane  
Molecules:

73

147

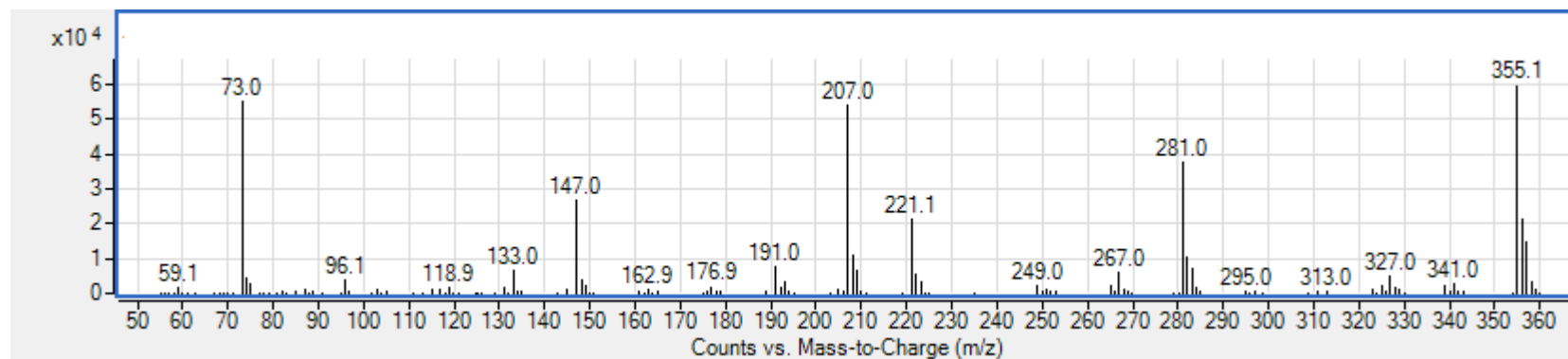
207

281

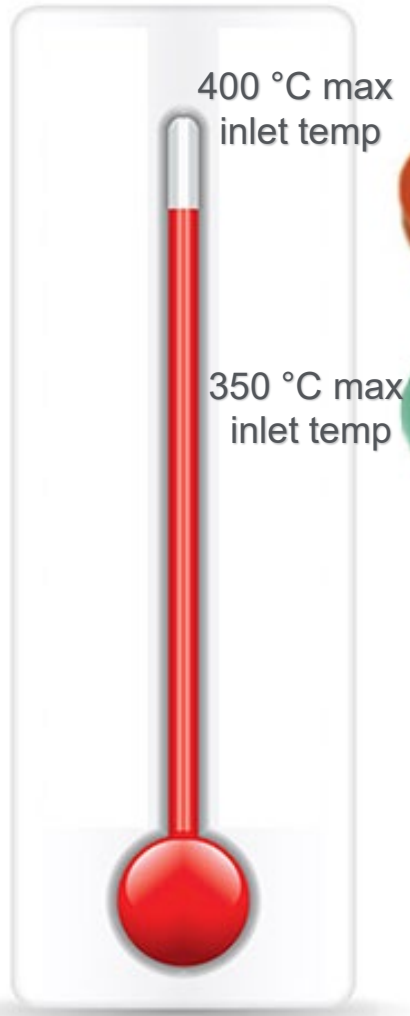
355

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:



# Pick the Right Septa for Your Analysis



## Bleed and temperature optimized (BTO) septa

- Optimized for trace analysis
- Extended temperature range
- Low bleed

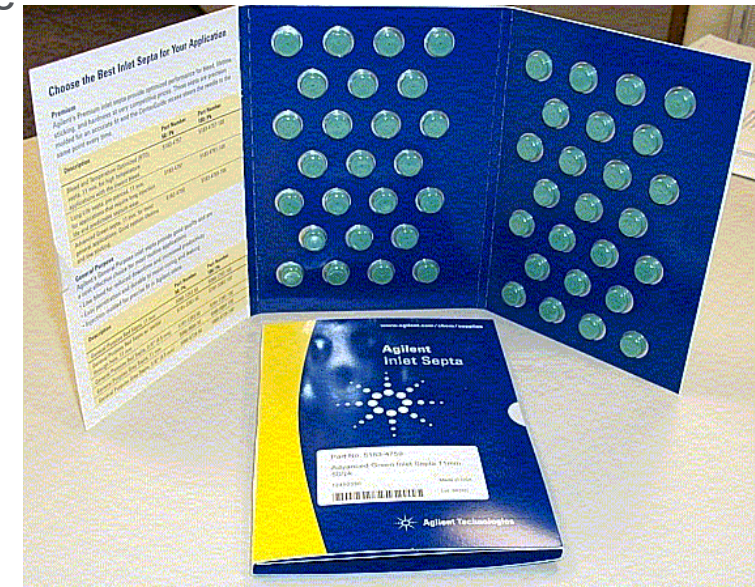


## Long-life septa

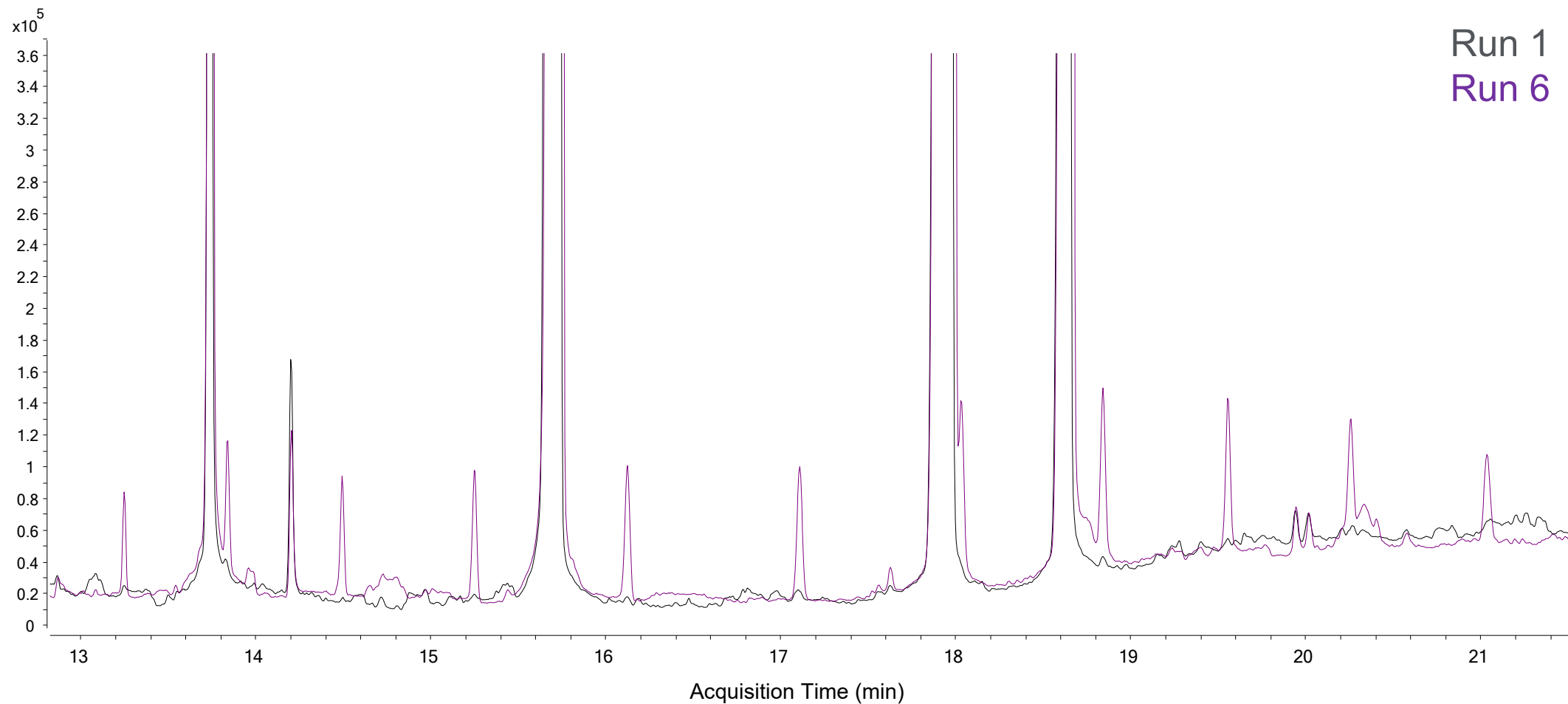
- Optimized for longest lifetime
- Extended puncture lifetime
- Excellent for autosamplers

## Advanced green septa

- General purpose septa
- Optimized temperature range/puncture lifetime

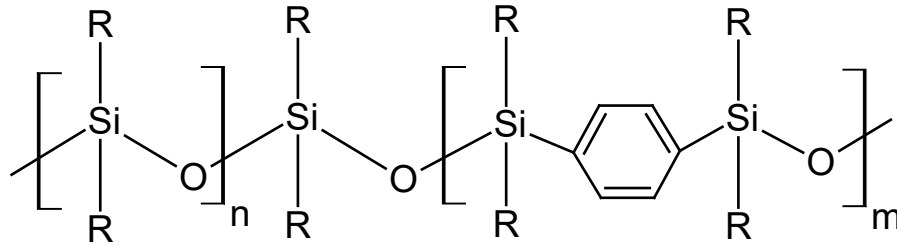


# Multiple Injections From the Same Vial: Siloxanes



# Low Bleed Phases

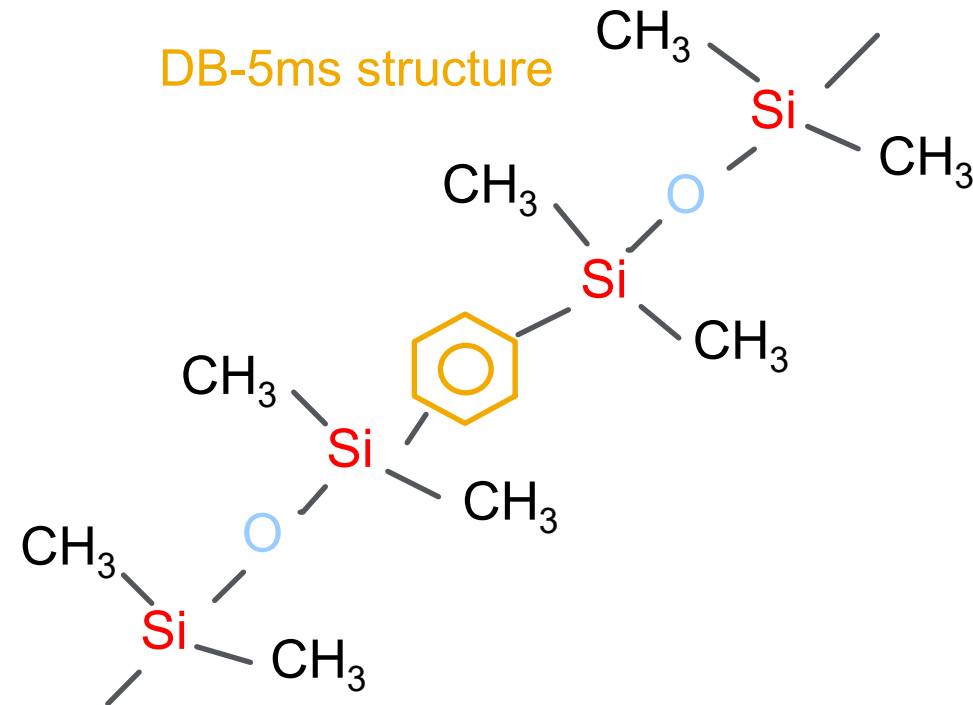
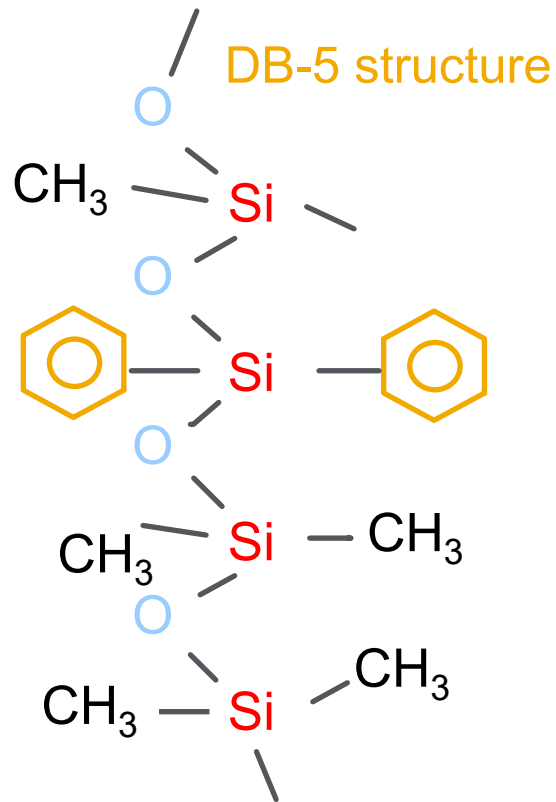
- Phases tailored to “mimic” currently existing polymers  
Examples: DB-5ms, DB-35ms, DB-17ms, VF-1701ms



Siarylene backbone

- New phases unrelated to any previously existing polymers  
Examples: DB-XLB
- Optimized manufacturing processes  
Examples: DB-1ms, HP-1ms, HP-5ms, VF-5ms

# Agilent J&W DB-5ms Structure



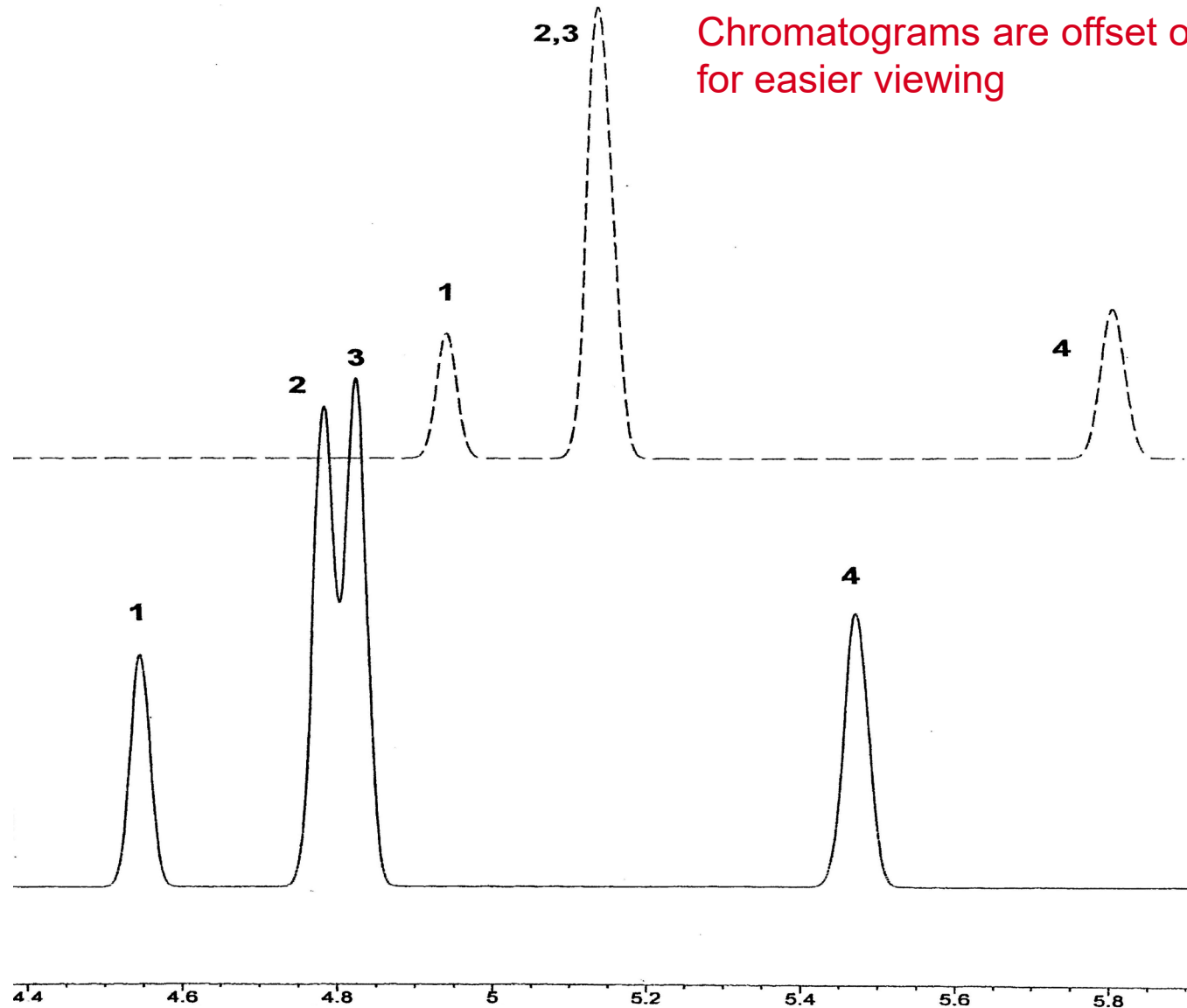
DB-5ms:

- Increased stability
- Different selectivity
- Optimized to match DB-5 as much as possible

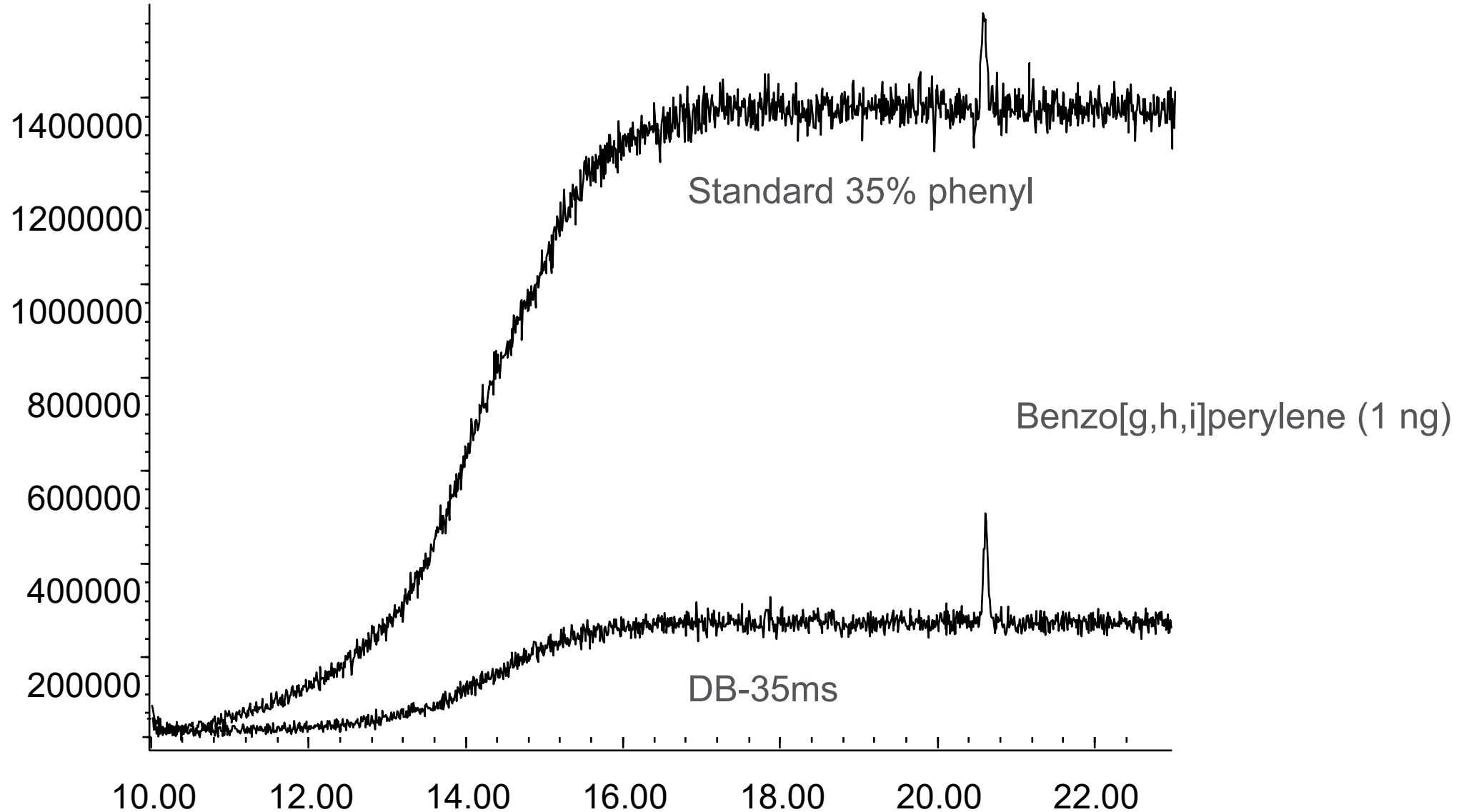
# DB-5ms vs. DB-5 Selectivity

Solid line: Agilent J&W **DB-5ms**  
30 m x 0.25 mm id x 0.25 mm  
Dashed line: Agilent J&W **DB-5**  
30 m x 0.25 mm id x 0.25 mm  
Oven: 60 °C isothermal  
Carrier gas: H<sub>2</sub> at 40 cm/s

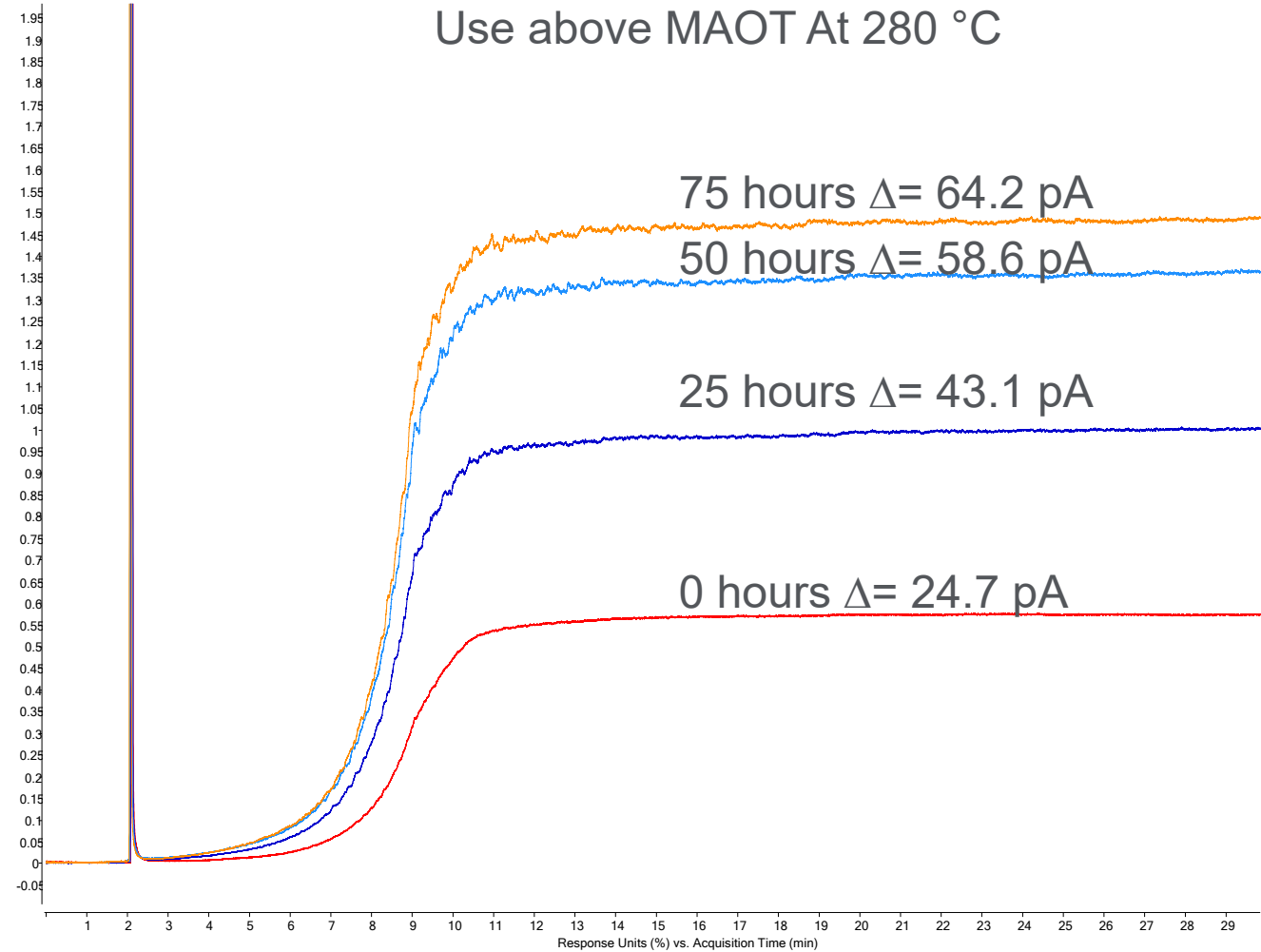
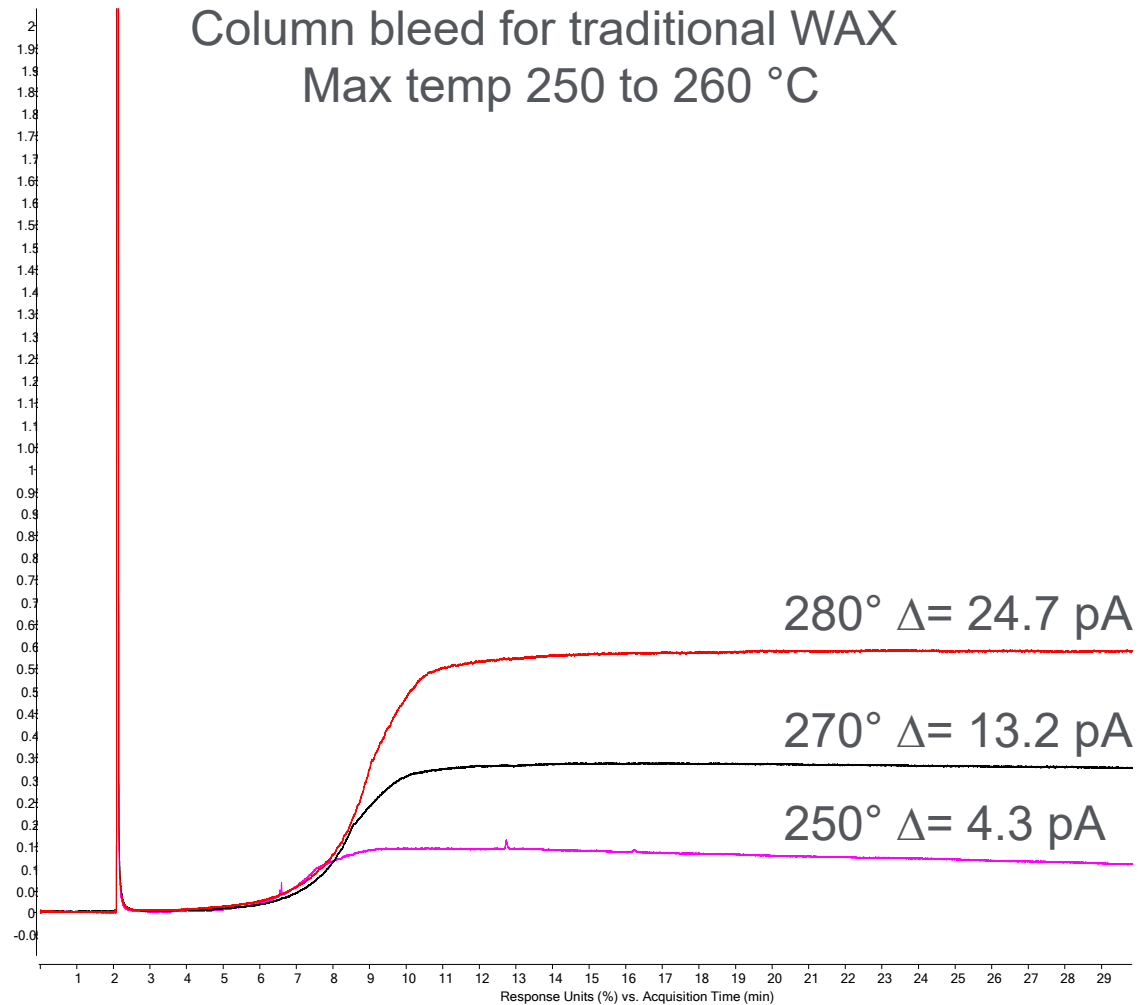
1: Ethylbenzene  
2: m-Xylene  
3: p-Xylene  
4: o-Xylene



# Comparison of Agilent J&W DB-35MS vs Standard DB-35



# Traditional WAX and Going Above the MAOT





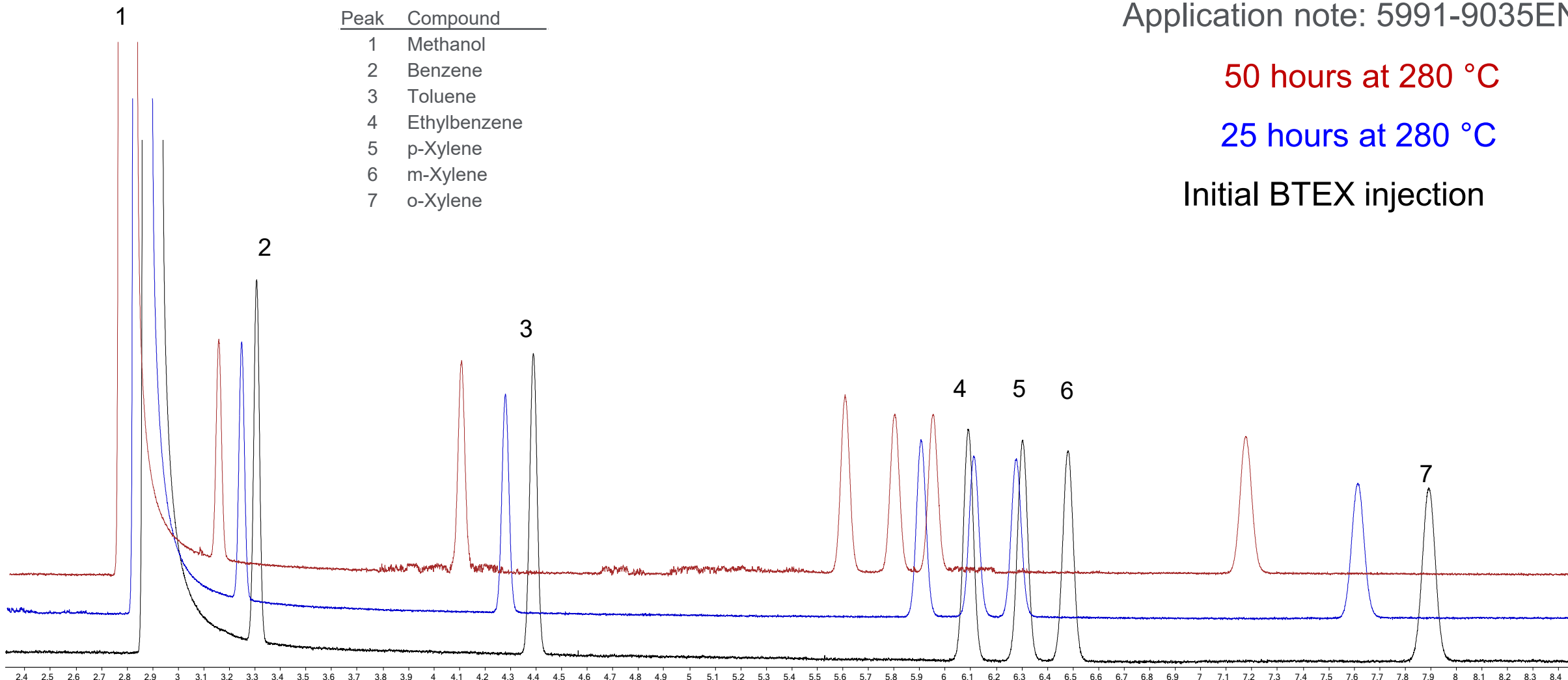
# Traditional WAX: Thermal Stability and Retention Time Shifting

Application note: 5991-9035EN

50 hours at 280 °C

25 hours at 280 °C

Initial BTEX injection



# New J&W DB-HeavyWAX

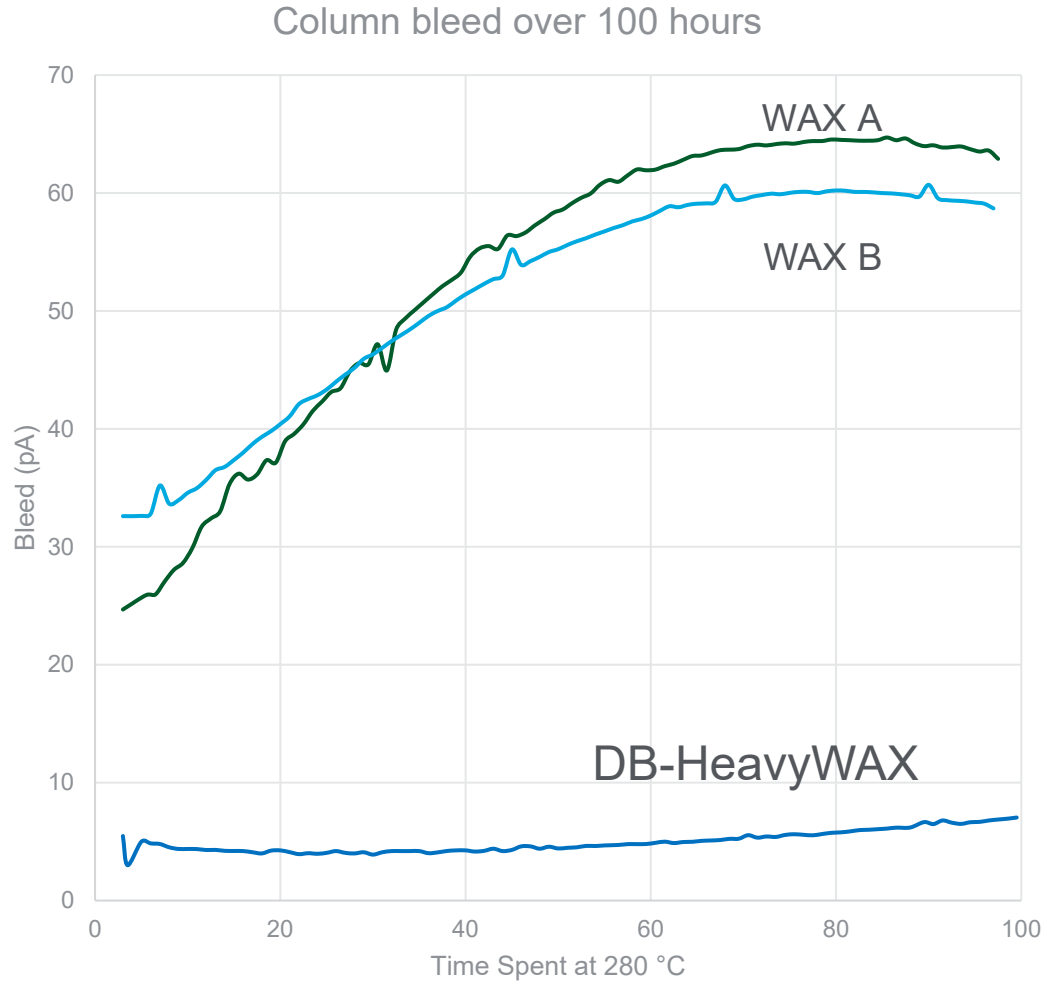
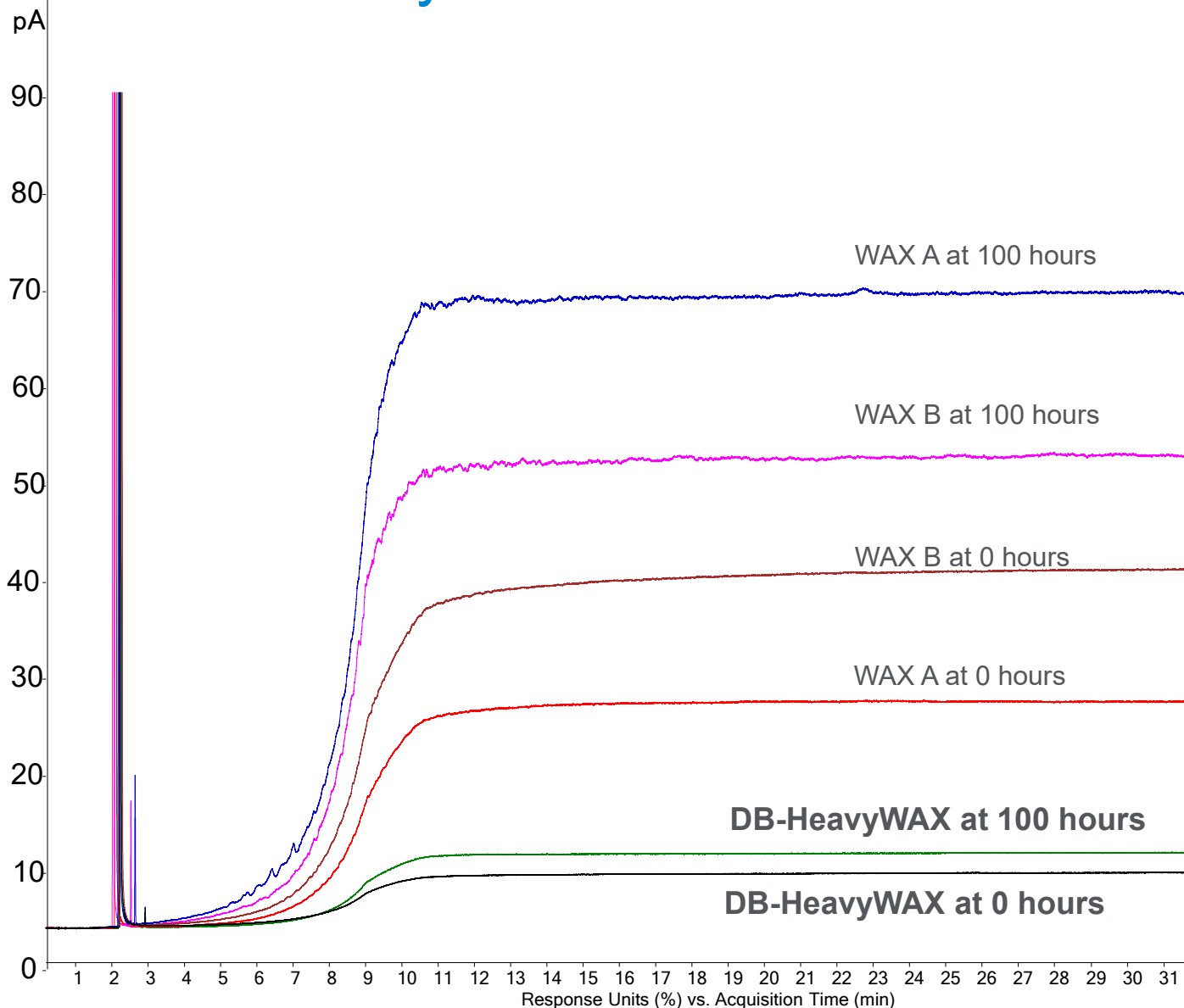
The WAX column you've been waiting for!

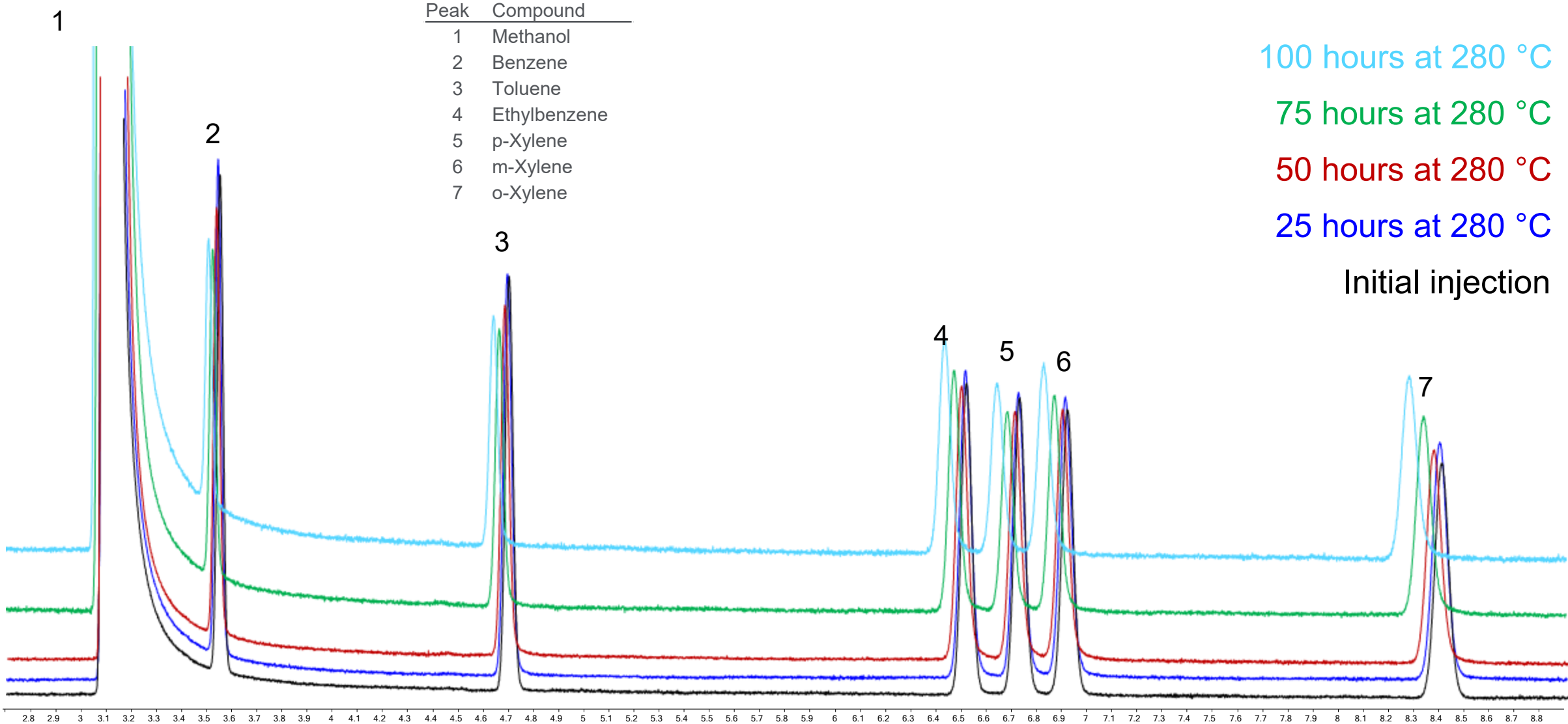
- Increased temperature range
  - 280 °C isothermal
  - 290 °C programmed
- Increased thermal stability
- Lower bleed



[www.agilent.com/chem/db-heavywax](http://www.agilent.com/chem/db-heavywax)

# Bleed Summary at 280 °C Over 100 Hours





100 hours at 280 °C

75 hours at 280 °C

50 hours at 280 °C

25 hours at 280 °C

Initial injection

# Benefits of Low Bleed

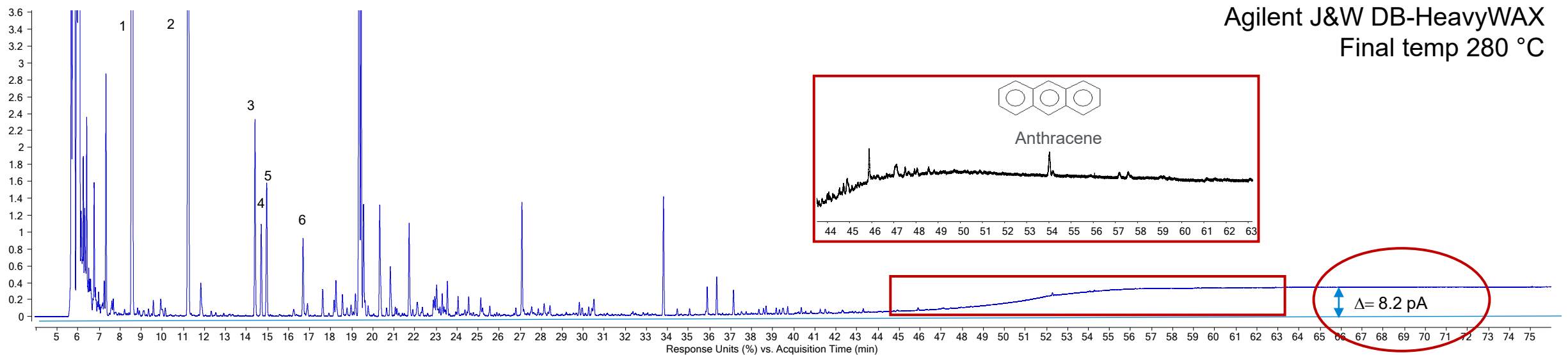
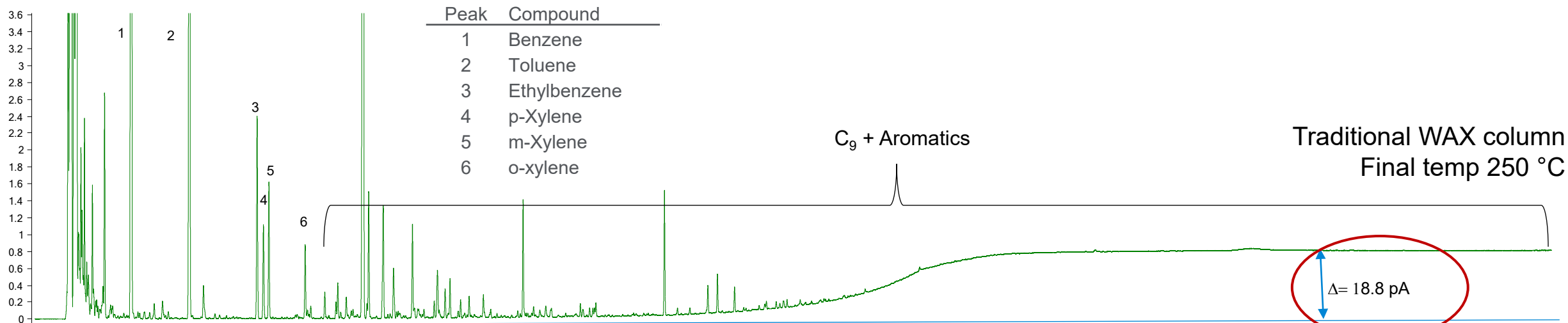
## Pyrolysis gasoline

- ASTM D6563
- Heavier aromatic compounds
- Lower bleed at 280 °C than traditional WAX at 250 °C
- Increased sensitivity for later eluting compounds
- Increased column lifetime

GC Conditions	
Column	60 m x 0.25 mm x 0.25 µm
Carrier	Helium, constant flow, 1.2 mL/min
Oven	70 °C (10.0 min), ramp 5 °C/min to 280 °C (30 min)

5991-9115EN

# Pyrolysis Gasoline

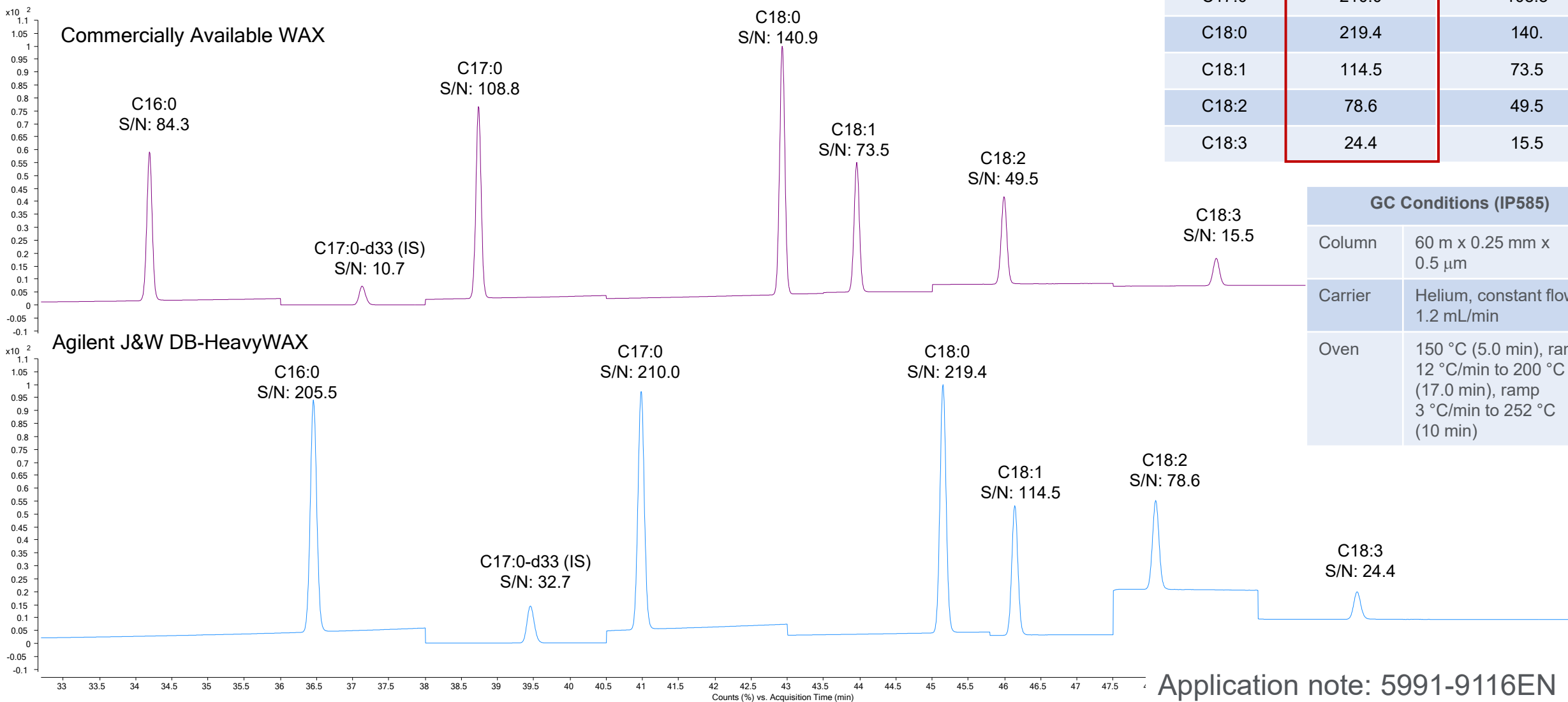


Application note: 5991-9115EN

# Decrease Bleed → Increase Signal to Noise FAMES in jet fuel (IP585)

FAME	DB-HeavyWAX	Commercially Available WAX
C16:0	205.5	84.3
C17:0-d33	32.7	10.7
C17:0	210.0	108.8
C18:0	219.4	140.
C18:1	114.5	73.5
C18:2	78.6	49.5
C18:3	24.4	15.5

GC Conditions (IP585)	
Column	60 m x 0.25 mm x 0.5 μm
Carrier	Helium, constant flow, 1.2 mL/min
Oven	150 °C (5.0 min), ramp 12 °C/min to 200 °C (17.0 min), ramp 3 °C/min to 252 °C (10 min)

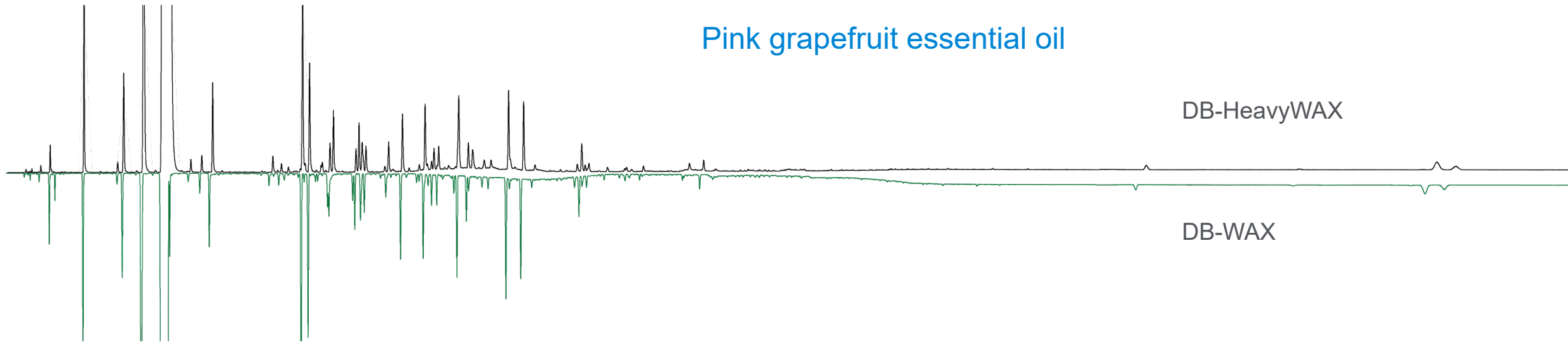


Application note: 5991-9116EN

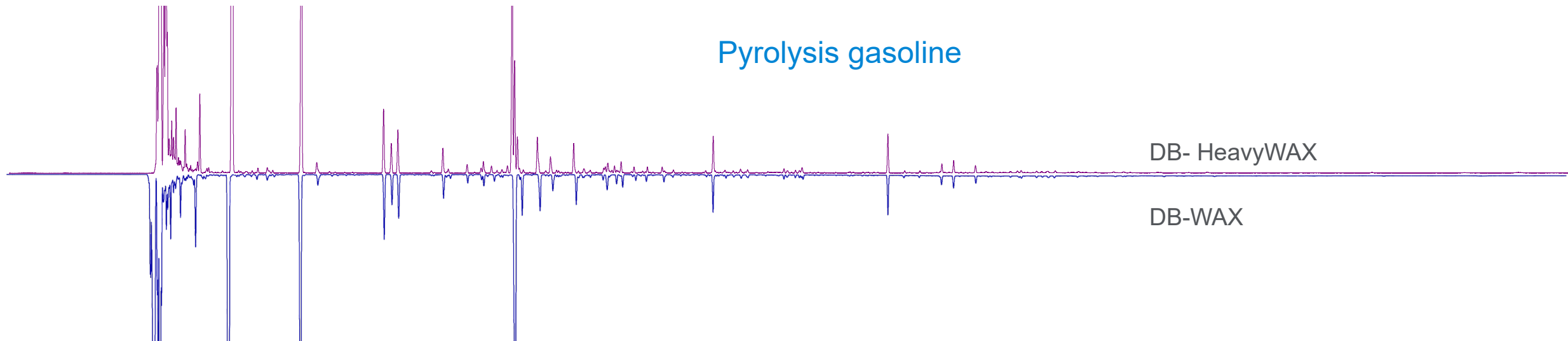
# It Is a WAX

Application note: 5991-9078EN

## Pink grapefruit essential oil



## Pyrolysis gasoline



Application note: 5991-9115EN



# Benefits of the J&W DB-HeavyWAX

- Increased thermal stability
  - Stable retention times
  - Consistent peak order
- Decreased column bleed
  - Greater sensitivity for “heavier” compounds
  - Increase analyte range
  - Decrease analysis time
  - Safely bake out column
    - Up to 290 °C
- Behaves like a WAX because it is a WAX
  - Simpler method translation

## Increased temperature range

- 280 °C isothermal
- 290 °C programmed

Increased thermal stability + decreased column bleed = longer lifetime

# Agilent University

## Why training? What can we help with?

### Agilent University:

- Trained over 38K students FY19
- 98% customer recommended
- 4.6 out of 5 customer satisfaction
- 94% excellent and very good

Labs who want faster and more efficient learning options to help overcome training challenges

Overtasked staff

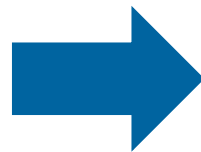
Staff turnover

Pressure to improve quality and productivity

Daily consistency with output and results

Reduce costs associated with lab operations

Flexible and convenient training options when and where you need them:



### Virtual training



Virtual instructor led



eLearning self-paced

### In-person training



Classroom



On-site or virtual on-site

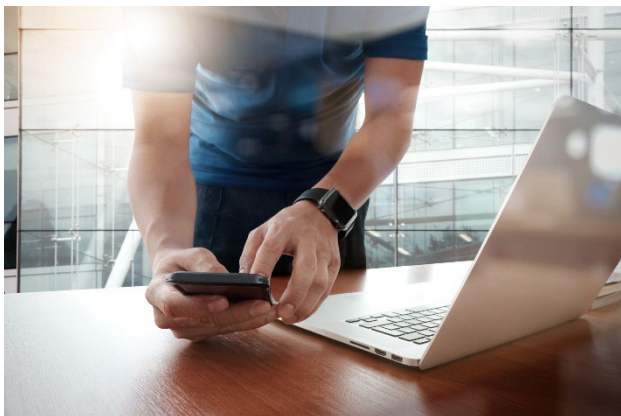
Trust Agilent for answers leveraging up-to-date knowledge and generally accepted practices for all your training needs

# Always Remember

- Column bleed is expected and will never show up as a discrete peak
- Bleed is influenced by column dimensions
- Avoid thermal, chemical, and oxygen damage
- Be careful not to overtighten or overuse GC septa
- Consider a low-bleed column alternative



# 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 USA and Canada 8–5, all time zones**



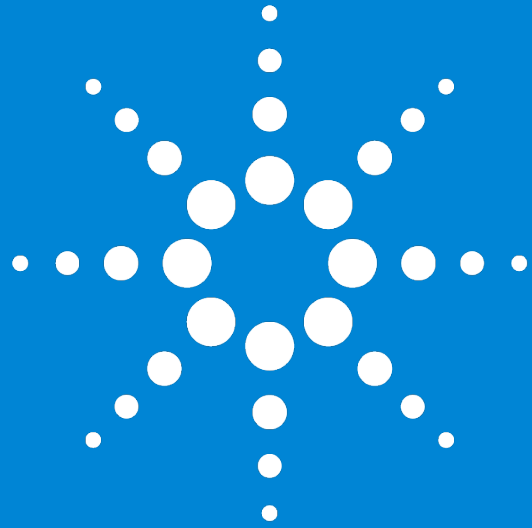
[gc-column-support@agilent.com](mailto:gc-column-support@agilent.com)

[lc-column-support@agilent.com](mailto:lc-column-support@agilent.com)

[spp-support@agilent.com](mailto:spp-support@agilent.com)

[spectro-supplies-support@agilent.com](mailto:spectro-supplies-support@agilent.com)

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# Agilent

Trusted Answers