Stop the Bleed: Tips and Tricks on GC Column Bleed

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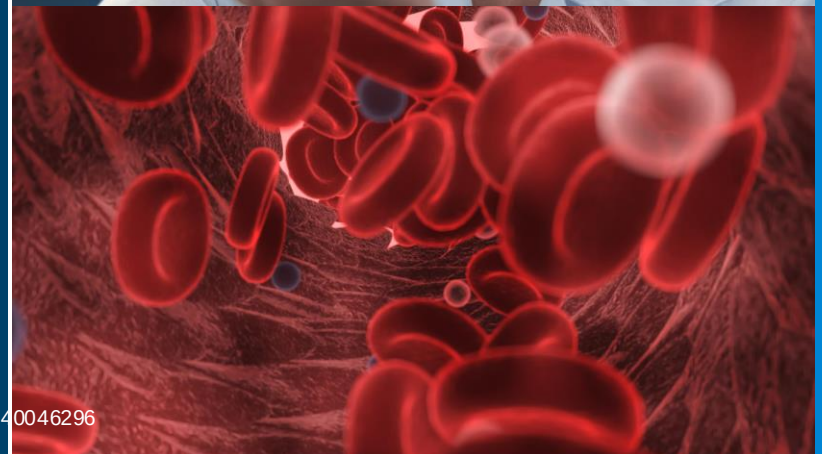
January 27, 2021



Agilent
Trusted Answers

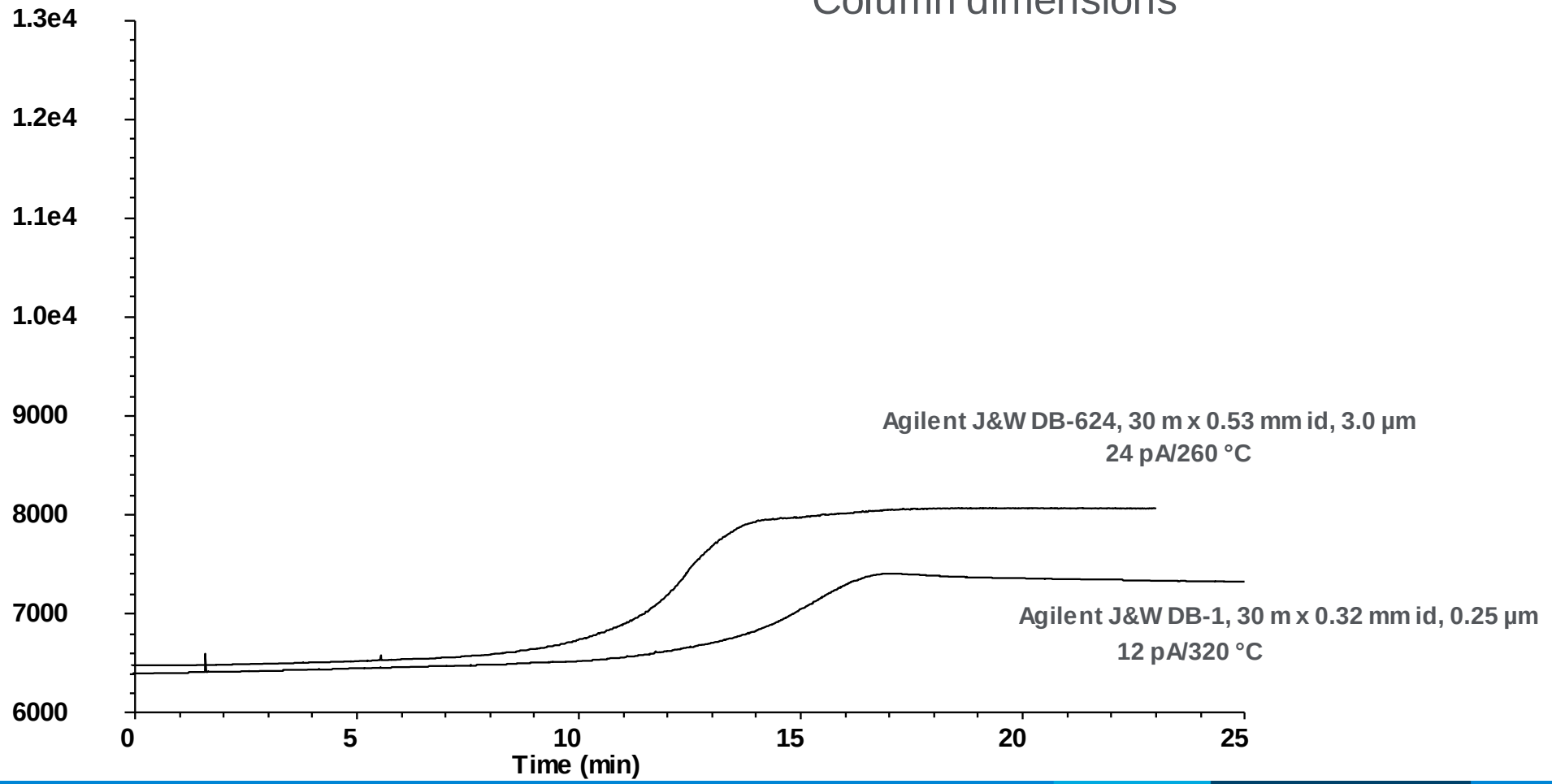
Agenda

- 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



Column Bleed: What Causes It

It's natural

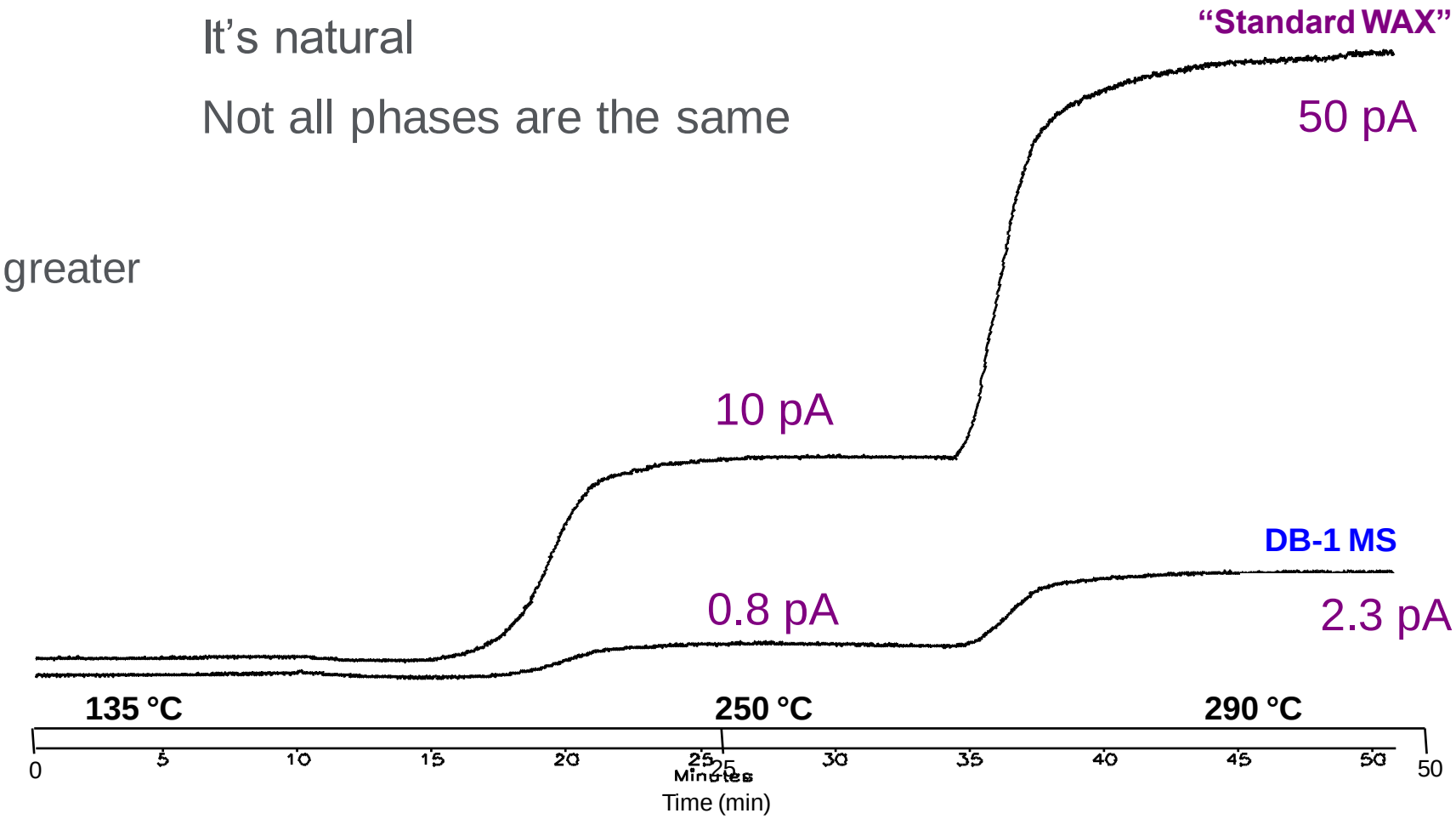
Not all phases are the same

Thermal stability:

- The lower the bleed, the greater the thermal stability

What makes it worse?

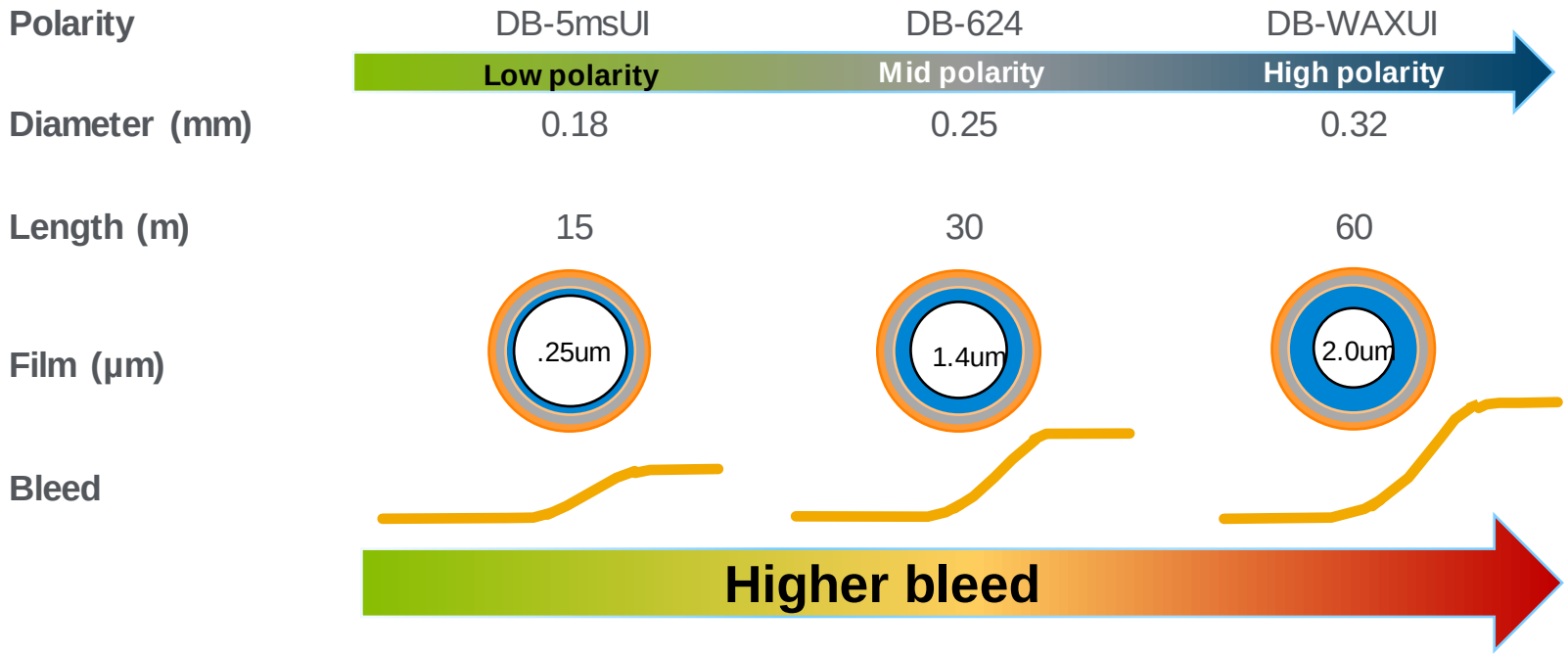
- Oxygen
- High temperatures



What Column Types/Dimensions Produce Higher Bleed?

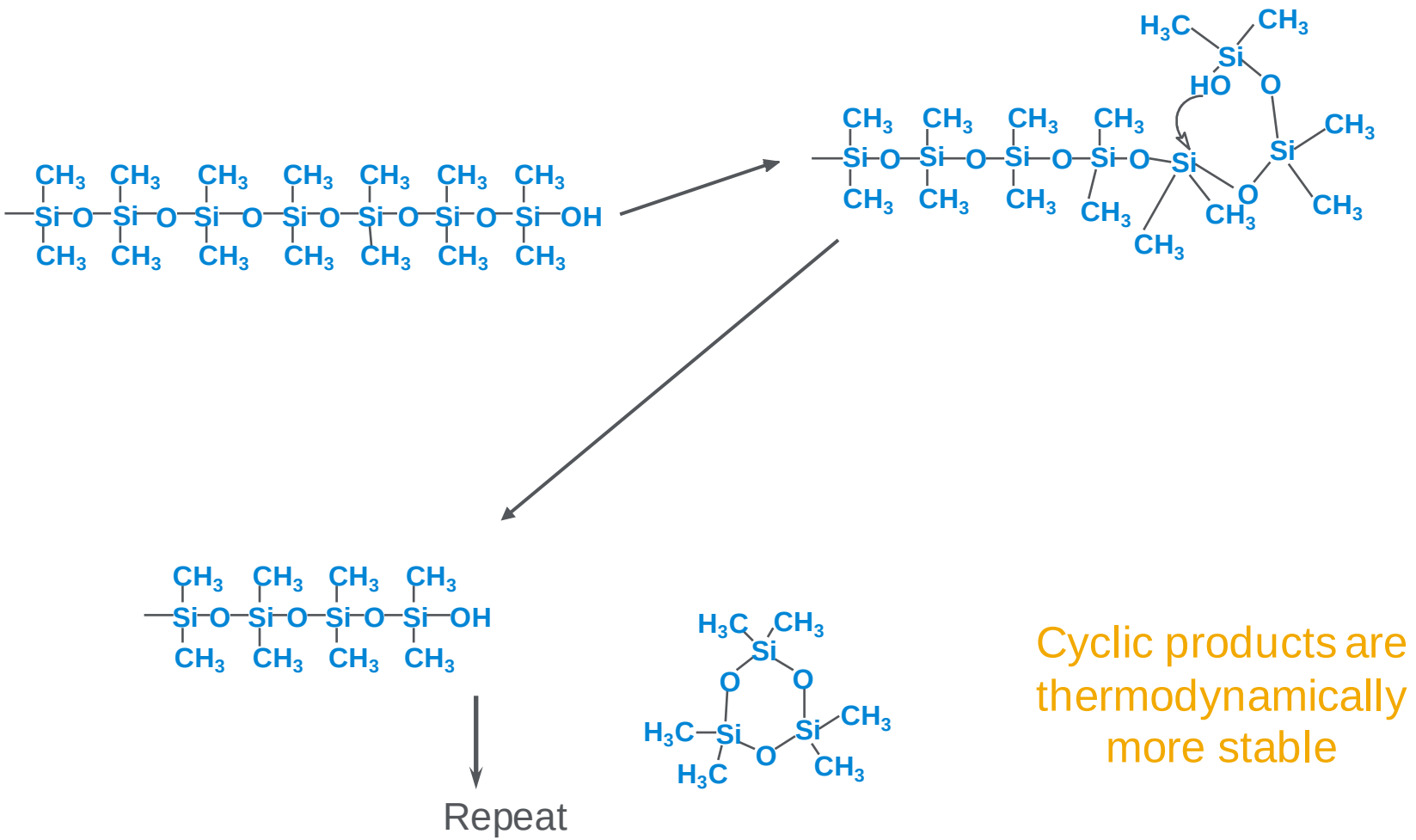
- Polarity: More polar = higher bleed
- Low polarity = More thermally stable
  - Look at temperature limits as a general indicator of thermal stability

- 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?

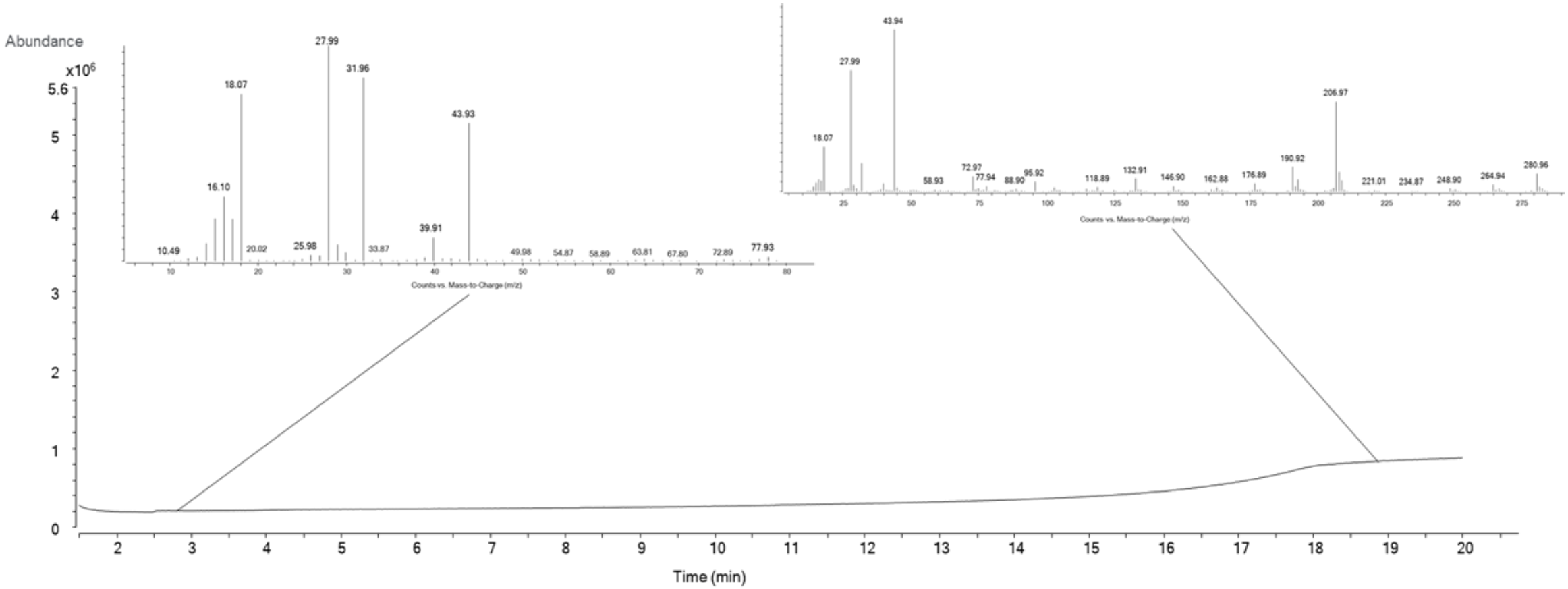
“Back biting” mechanism of product formation



Cyclic products are thermodynamically more stable

Mass Spectrum of Phenylmethylpolysiloxane Column Bleed

Normal background (HP-5ms UI)



Column Performance Testing

Catalog: 19091S-433UI
Serial: F542252H

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:

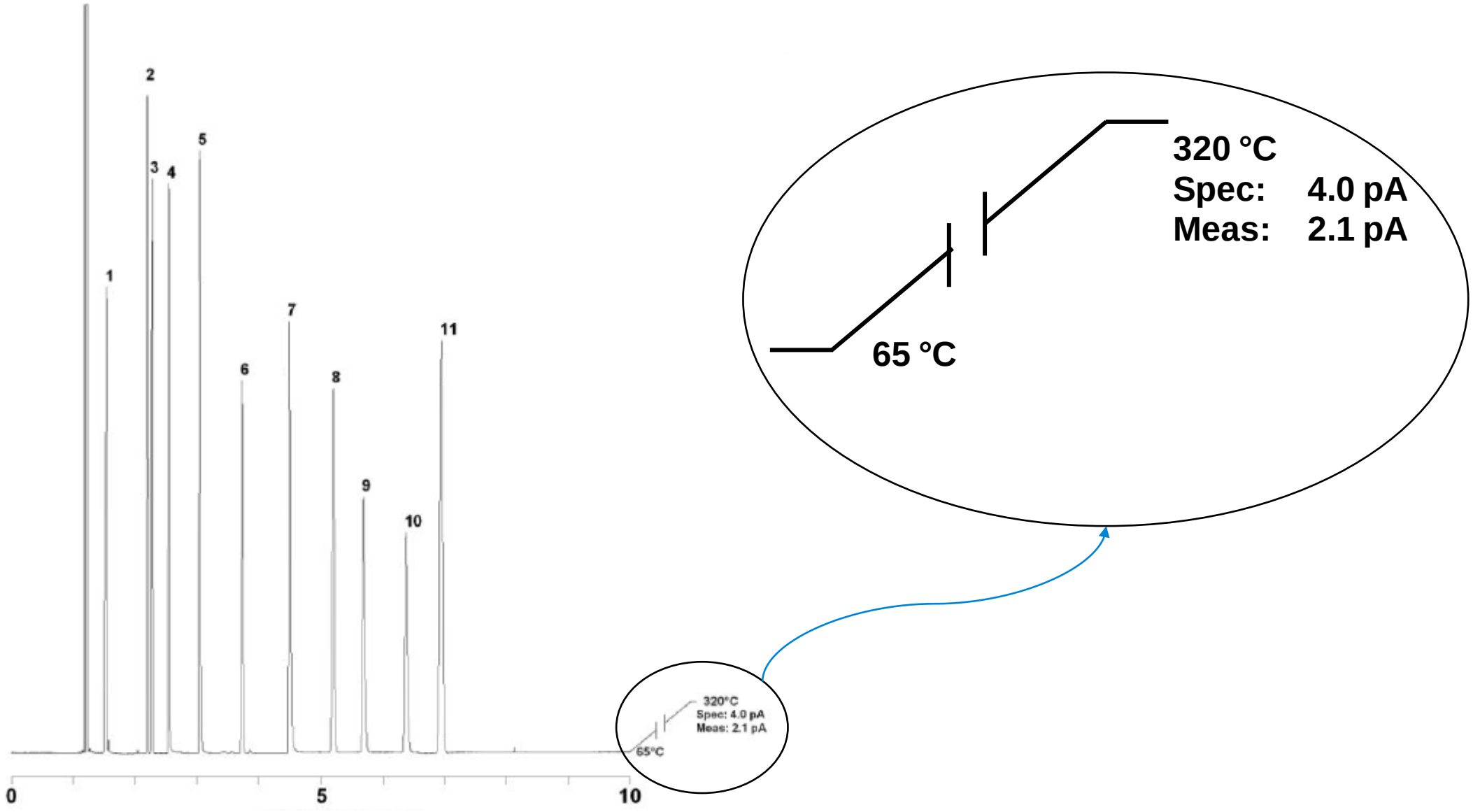
1-OCTENE, n-OCTANE	2.97

Compound Identification	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

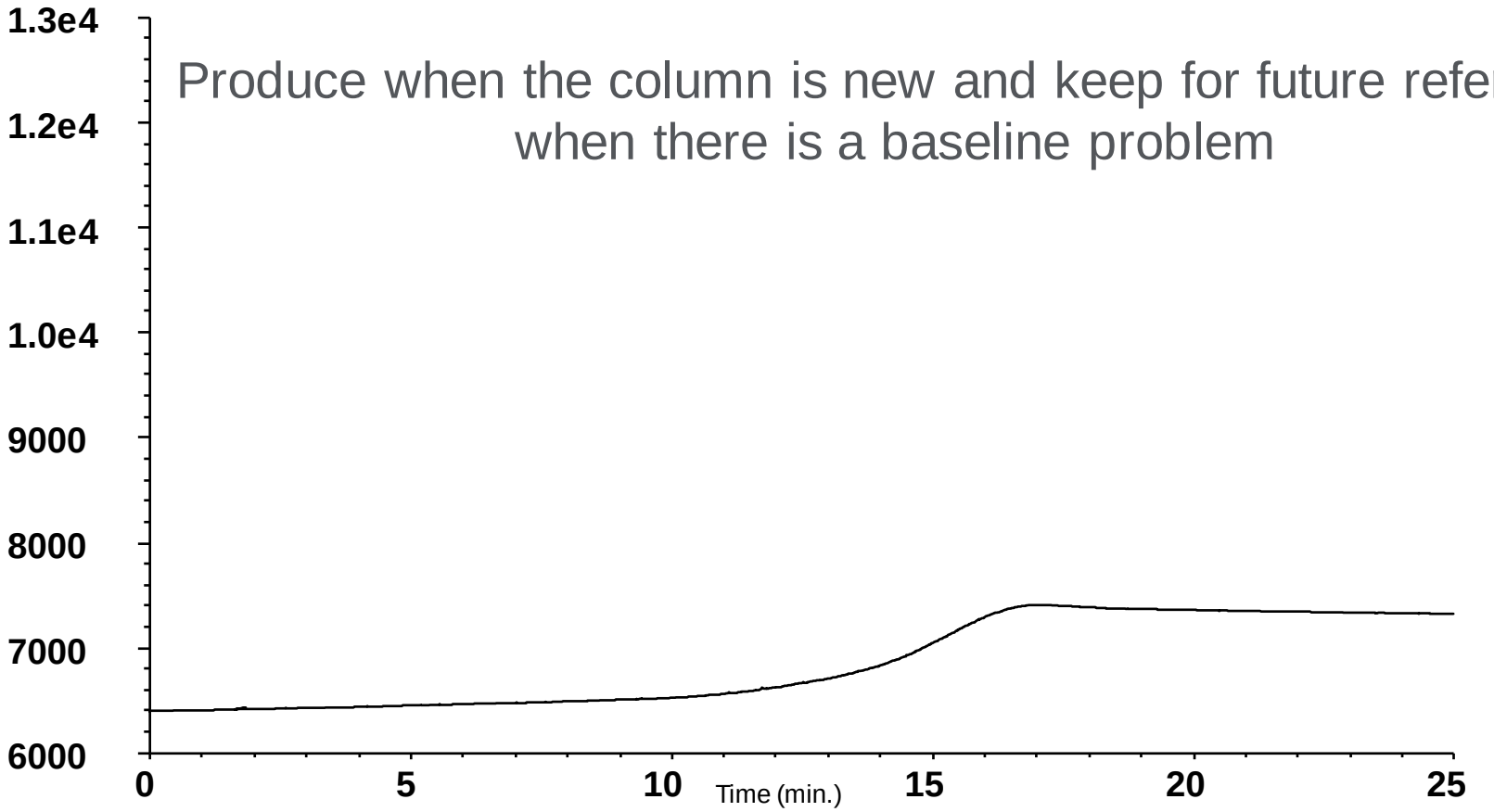
Test Conditions

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



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

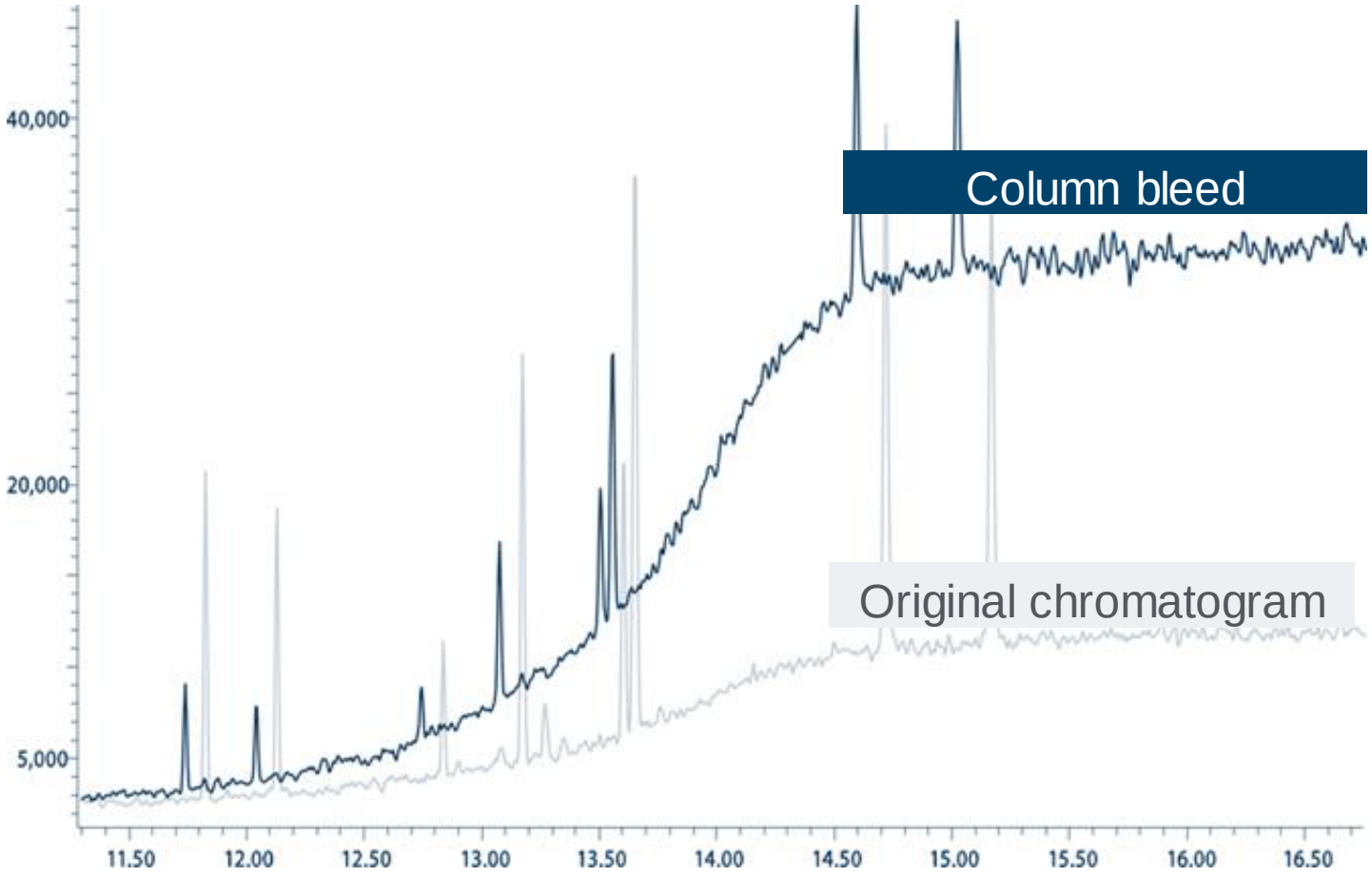
What is a Bleed Problem?

An abnormal elevated baseline at high temperature

It is **not**:

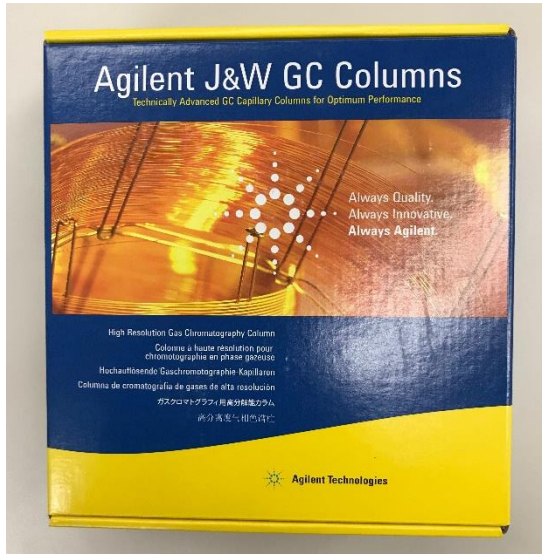
- A high baseline at low temperature
- Wandering or drifting baseline at any temperature
- Discrete peaks

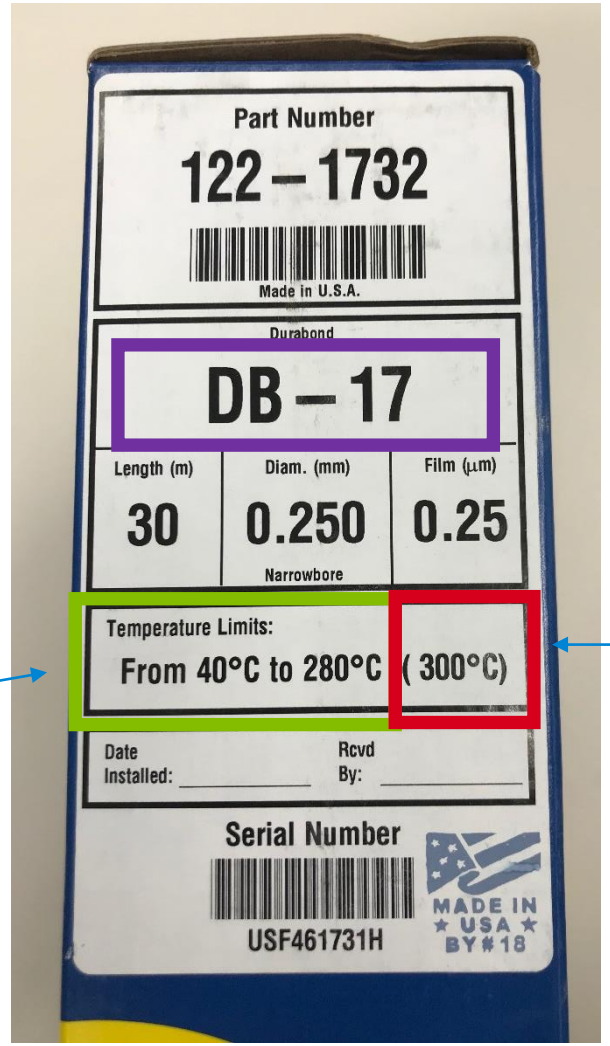
Troubleshooting Column Bleed



- 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)

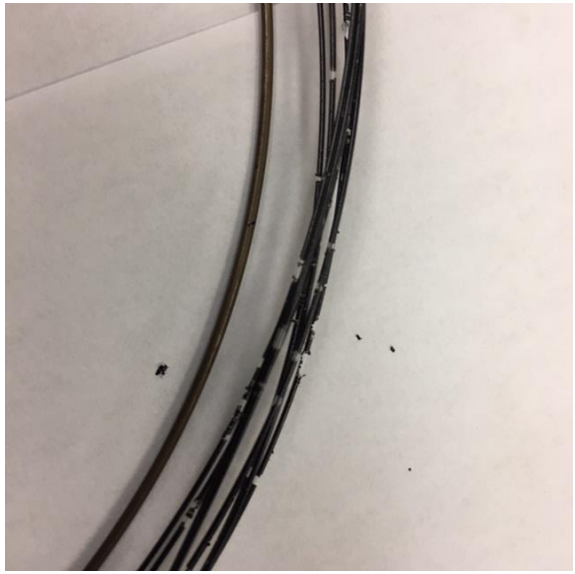
Thermal Damage

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

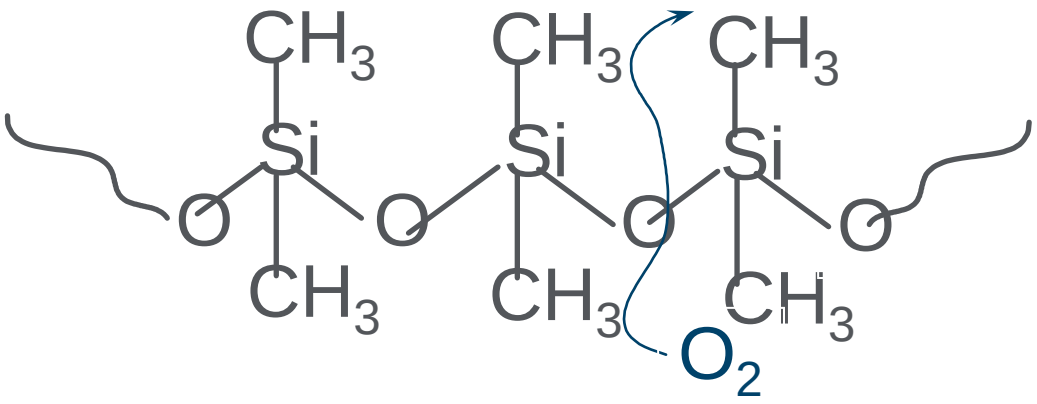
- Temporary "column failure" below lower temperature limit
- If this happens:
  - Disconnect column from detector
  - “Bake out” overnight at isothermal limit
  - Remove 10–15 cm from column end



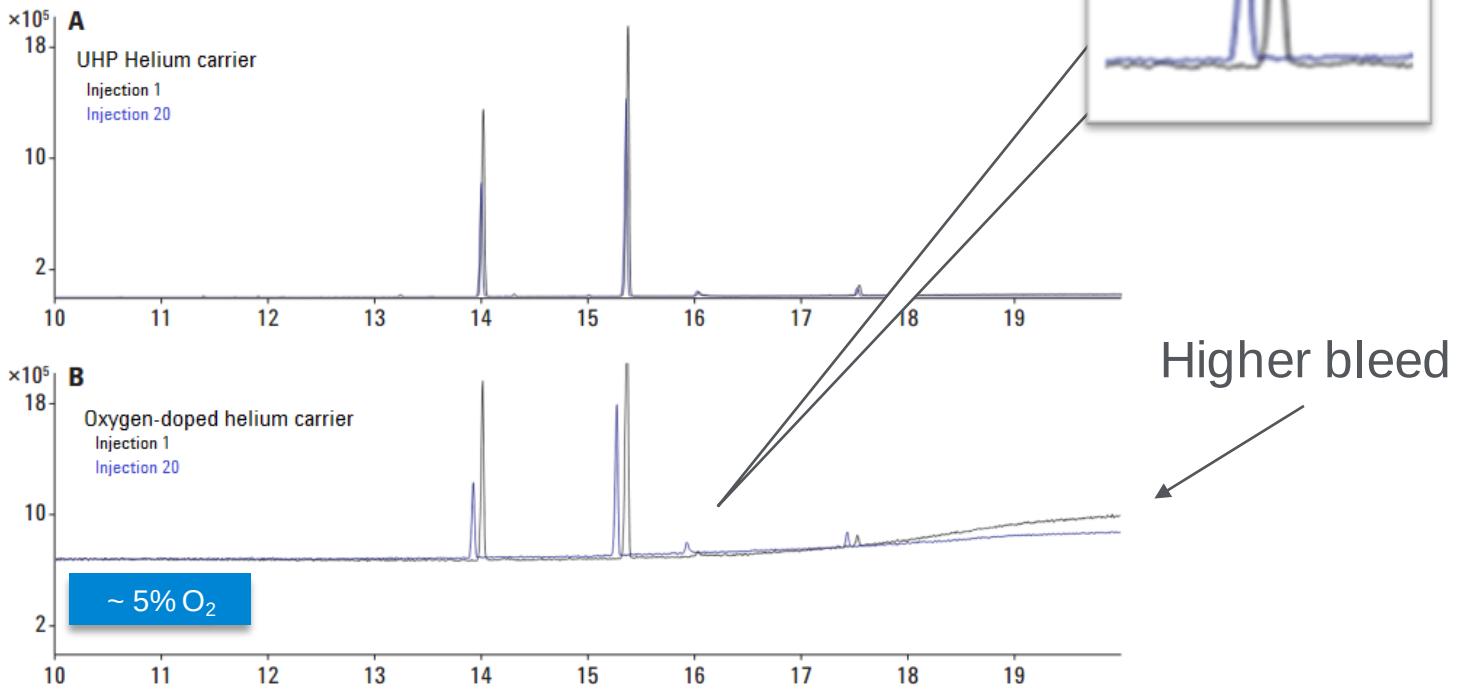
Column continuously exposed to temperatures above its temperature limit

Oxidation (O_2 Damage)

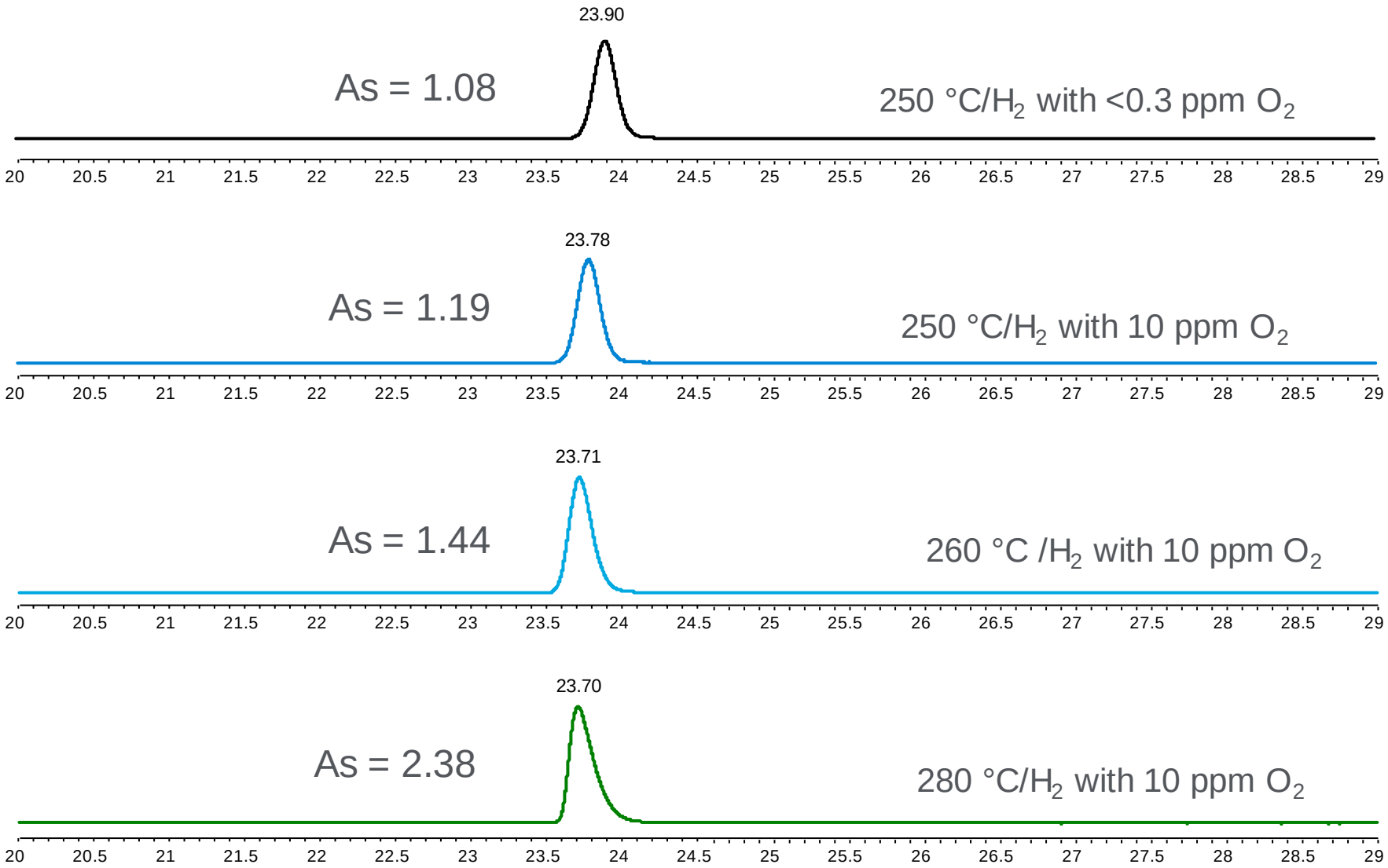
Oxygen in the carrier gas rapidly degrades the stationary phase. The damage is accelerated at higher temperatures. Damage along the polymer backbone is irreversible. (Premature filament failure/excessive source maintenance.)



Dimethylpolysiloxane

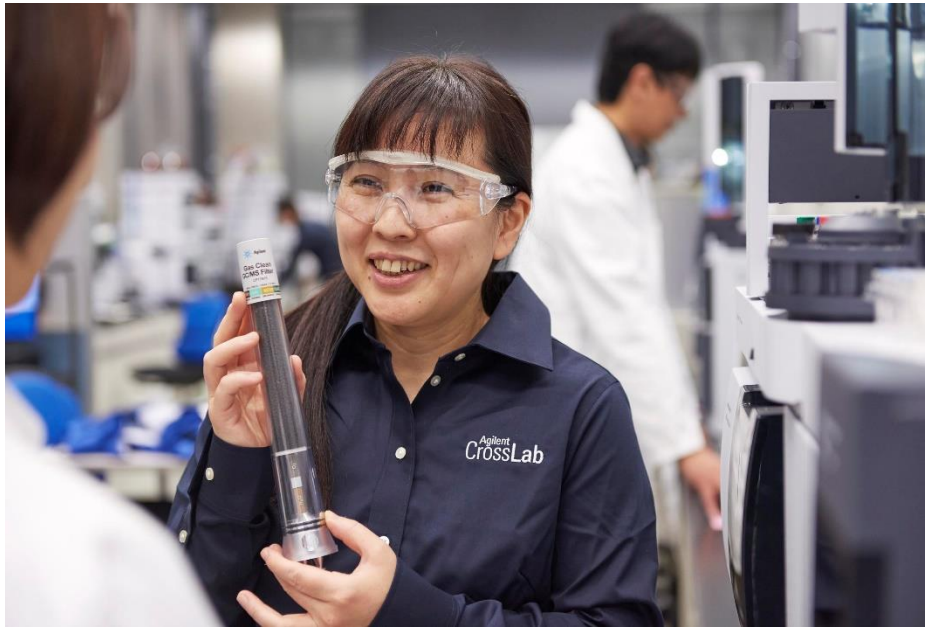


Effect of Oxygen on Peak Shape of 2-Ethylhexanoic Acid



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

Use Leak Detector or Electronics Duster to Find Your Leaks

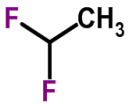
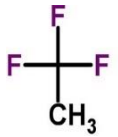
Why use a leak detector?

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

Agilent G3388B leak detector

link

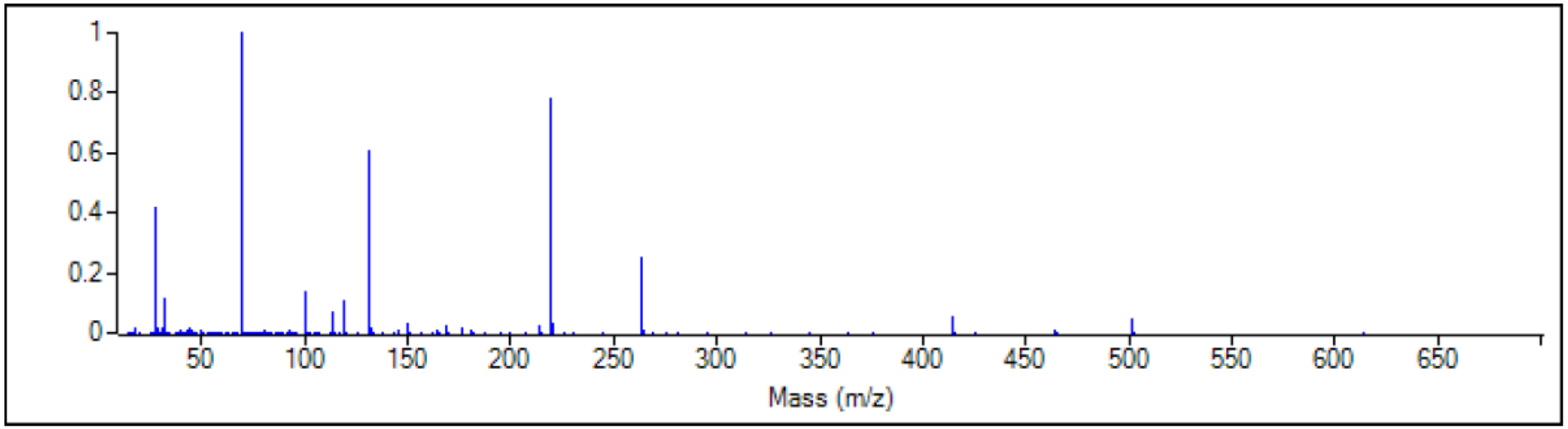
Typical Electronic Duster Components and Ions

	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

Example Tune Report with Leak



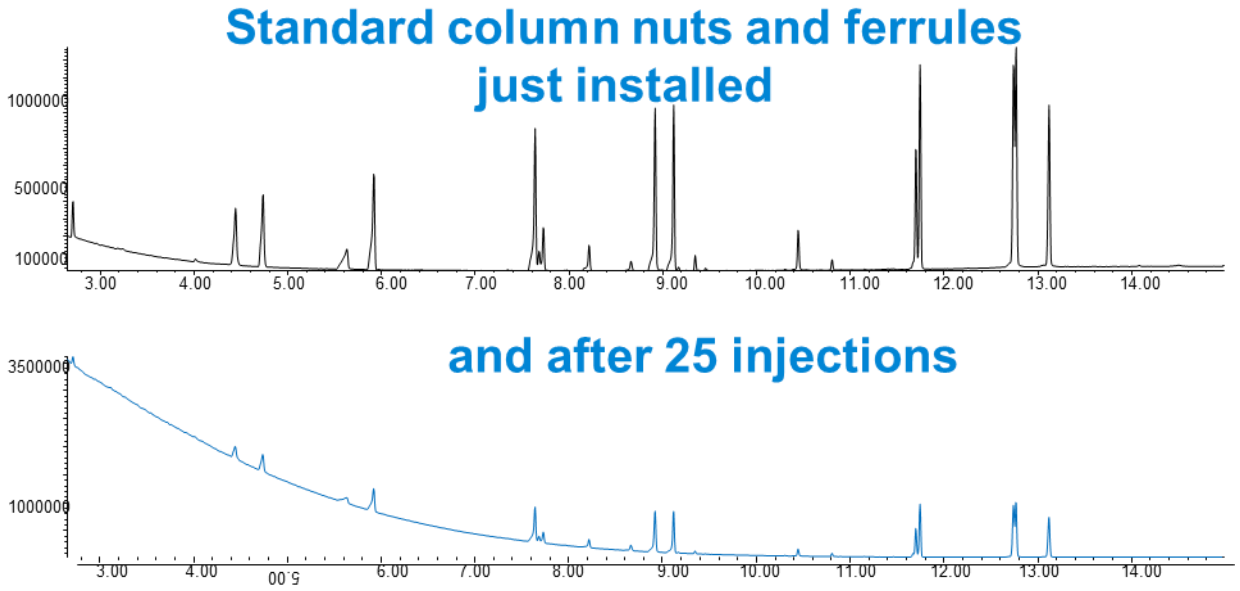
Target m/z	Actual m/z	Abund	Rel Abund	Iso m/z	Iso Abund	Iso Ratio
69.00	69.00	498,432	100.0%	70.00	6,216	1.2%
219.00	219.00	391,232	78.5%	220.00	18,216	4.7%
502.00	502.00	23,680	4.8%	503.00	2,467	10.4%

Air/Water Check: H20 ~1.8% N2 ~42.1% O2 ~11.4% CO2 ~1.3% N2/H20 ~2325.0%

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

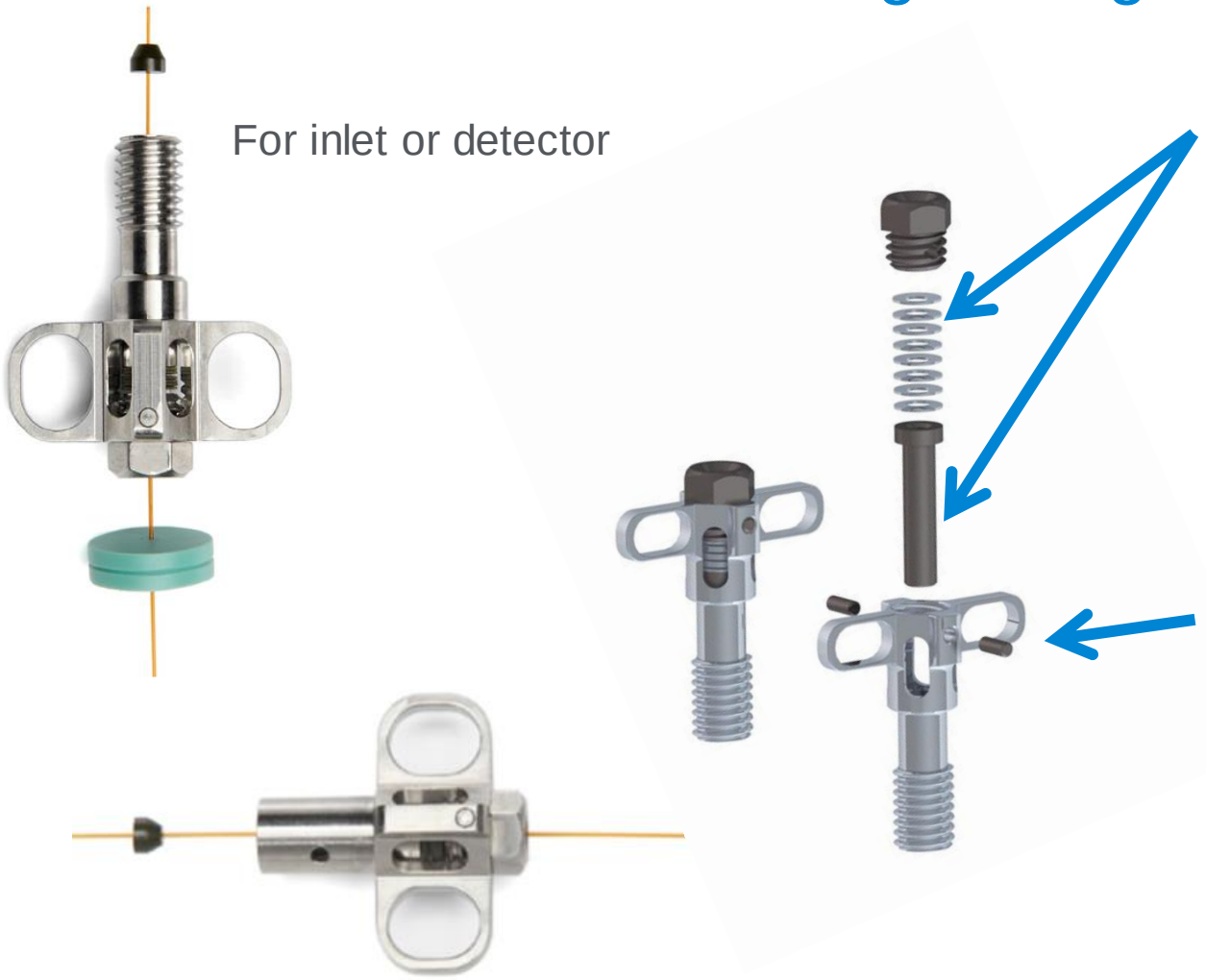
Graphite/Polyimide Blend Capillary Ferrules

- 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



For inlet or detector

For mass spectrometry transfer line

- 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

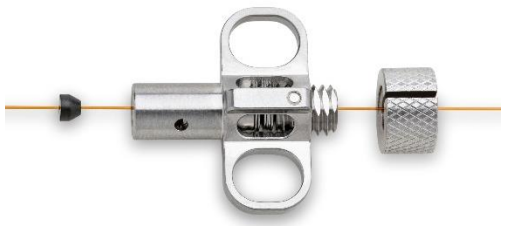
For GC inlet or detector

Innovating for the next generation

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

For mass spectrometry transfer line

Innovating for the next generation



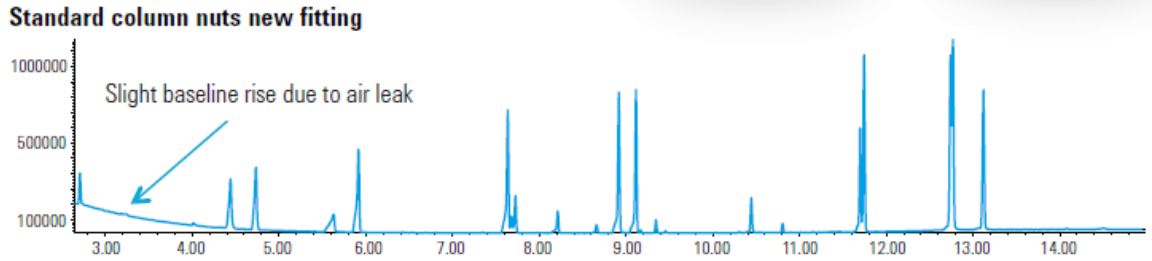
Self Tightening Nuts: No Leaks, No Downtime, No Frustration

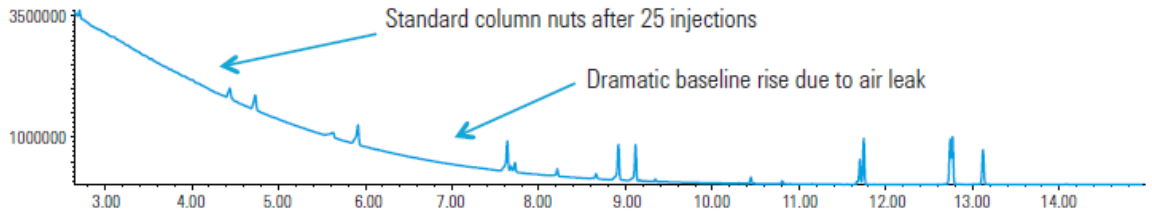
- Spring-driven piston continuously presses against ferrule
- Automatically retightens when ferrule shrinks
- Wing design for finger tightening
- No tools needed

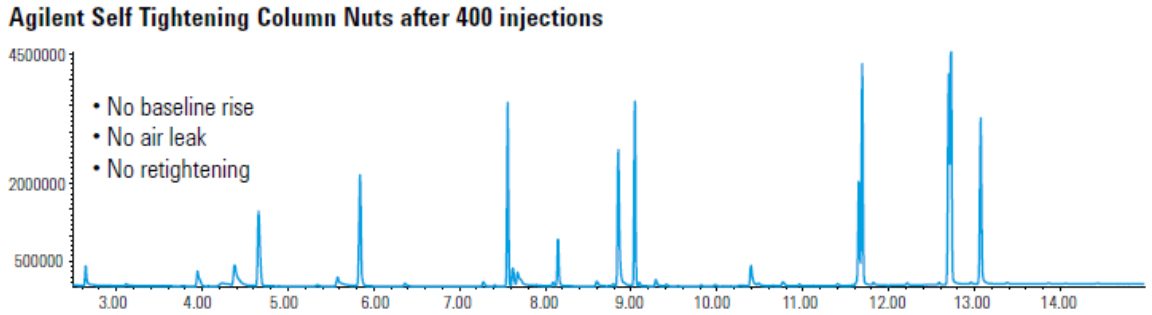
Part Number	Description
G3440-81013	Column Nut, Collared Self-Tightening MSD
G3440-81011	Column nut, Collared Self Tightening Inlet/Detect
G3440-81012	Collar for Self Tigthening Nut

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







400 injections

Chemical Damage

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

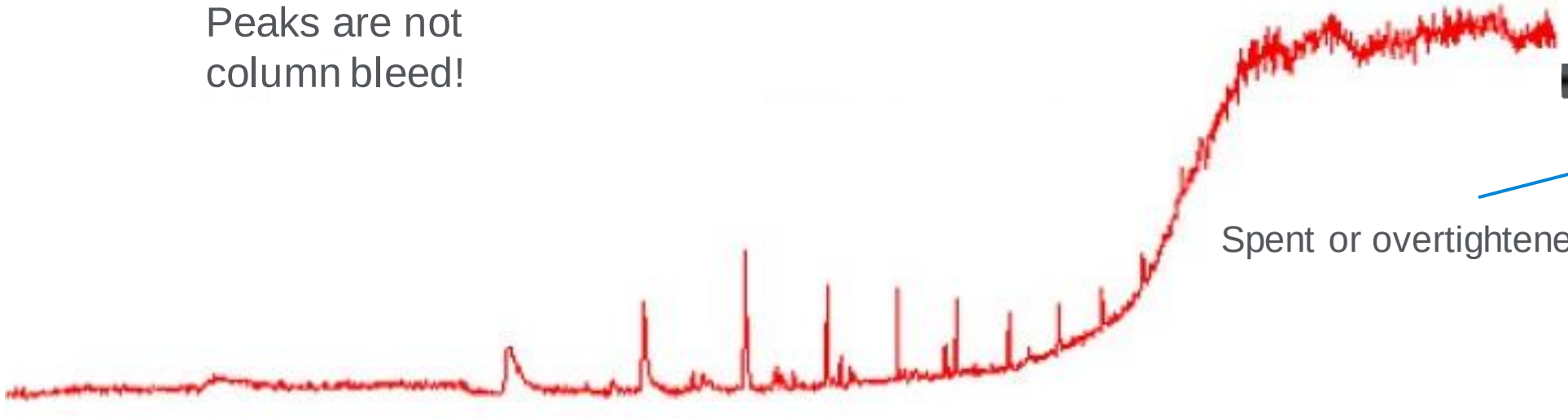
HCl NH_3 KOH NaOH

H_2SO_4 H_3PO_4 HF

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

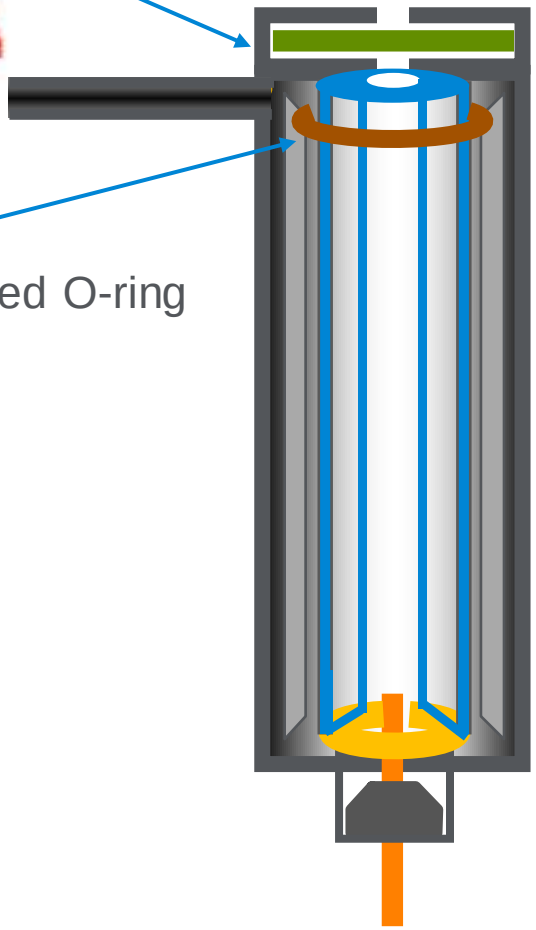
Column Bleed: What It is Not

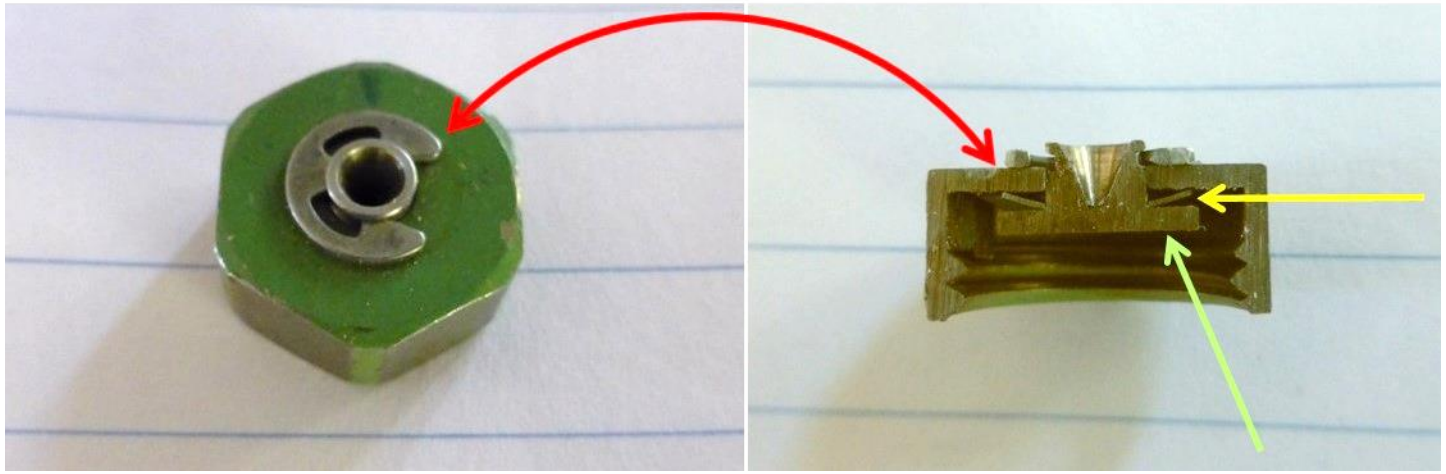
Peaks are not column bleed!

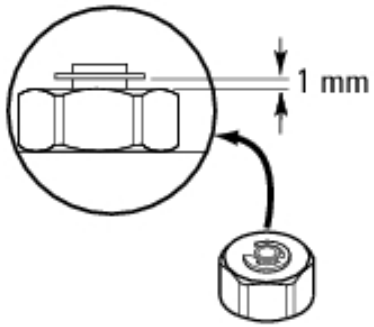


Spent or overtightened septum

Spent or overtightened O-ring





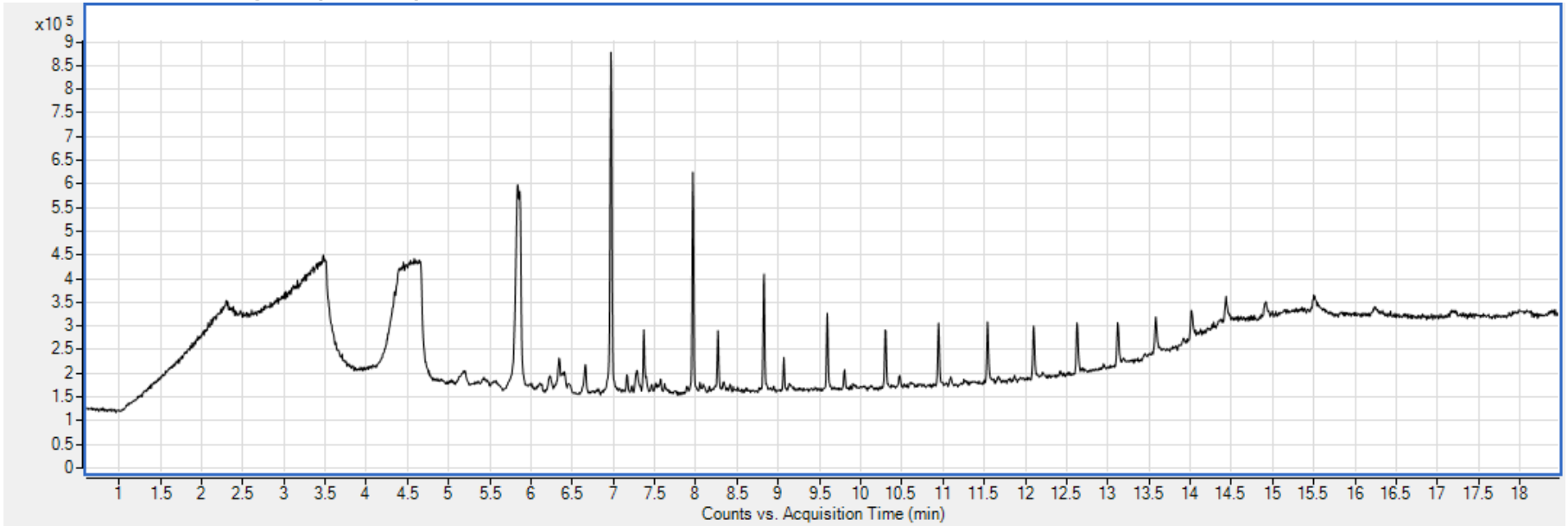


Septum Maintenance: Septum Coring

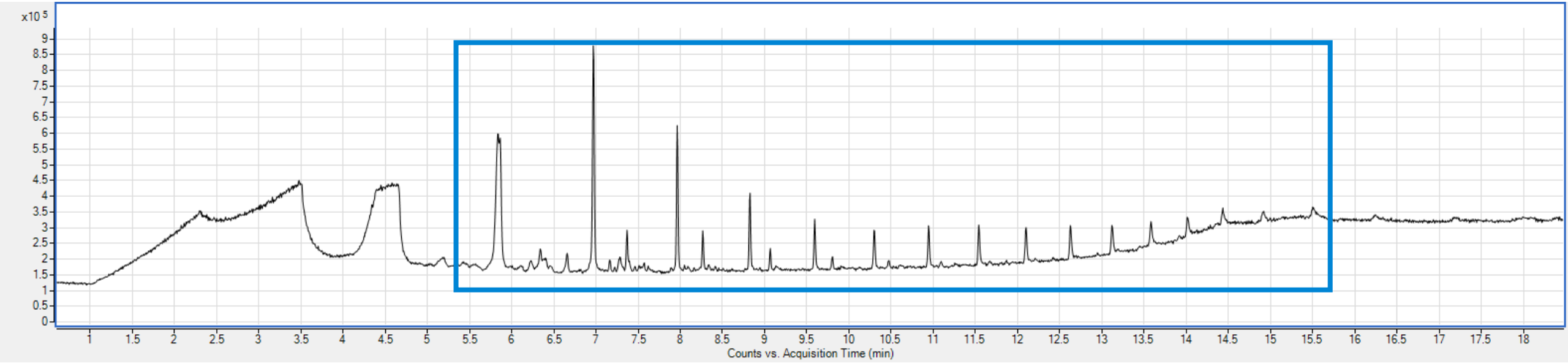
- After many injections, pieces of rubber from the septum may break off and fall into the inlet liner
  - This is called septa coring
  - Replace the inlet septa and liner frequently to prevent septa contamination
  - Use a cone-tipped syringe to reduce the chance of tearing the septum
  - This is also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



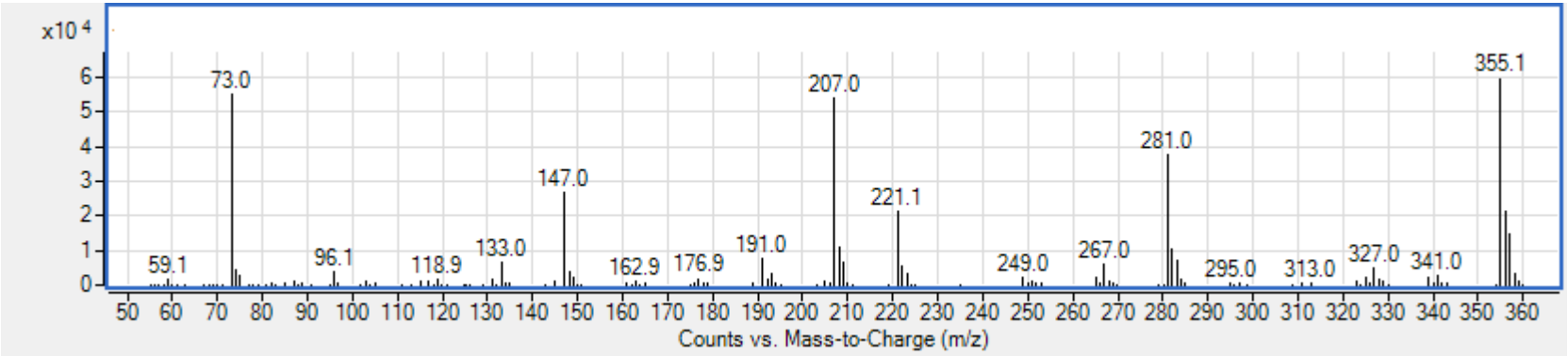
Septum Maintenance: TIC of an Inlet Septum



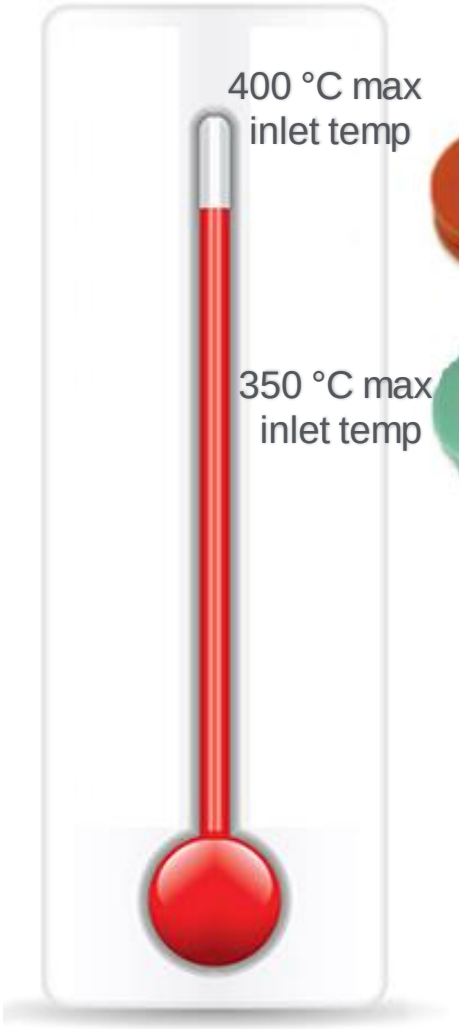
Common Ions for Siloxane Molecules:
73
147
207
281
355

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

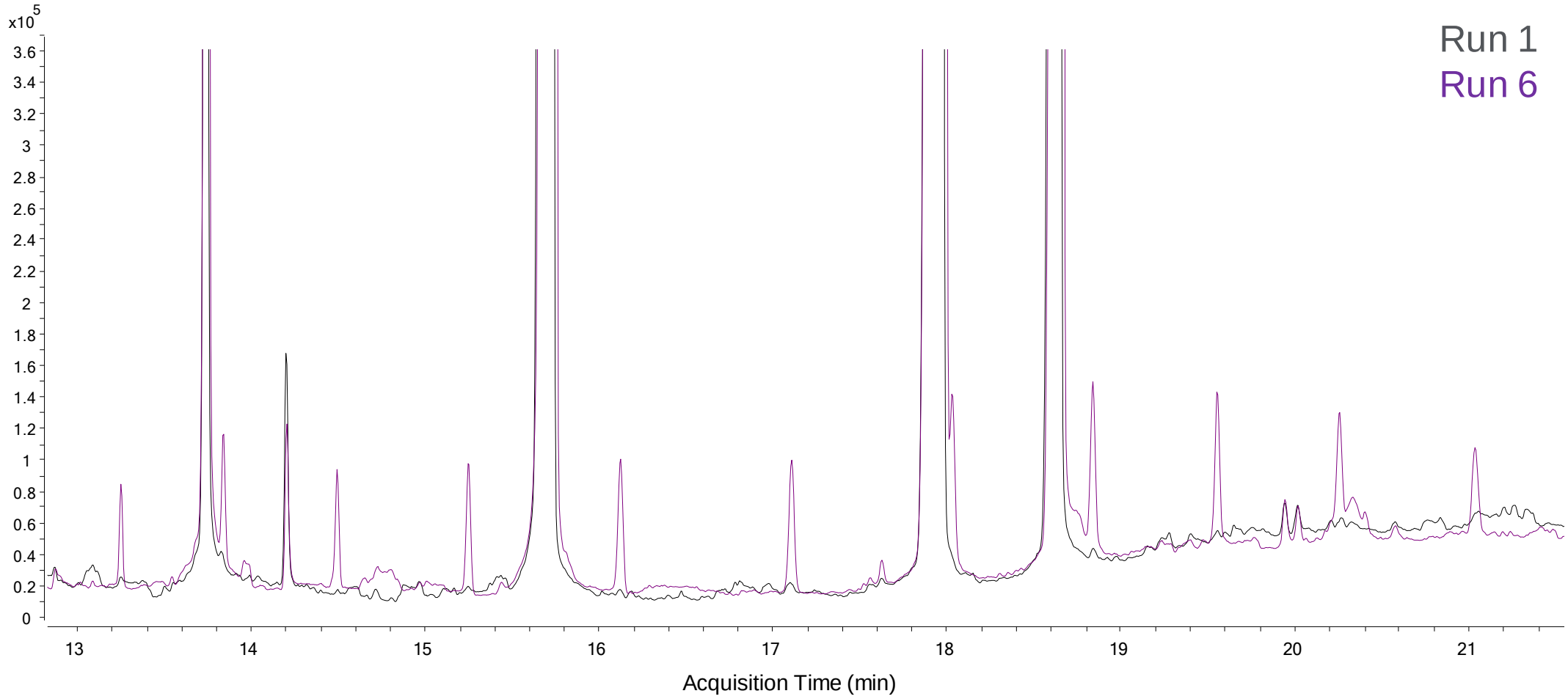
Long-life septa

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

Advanced green septa

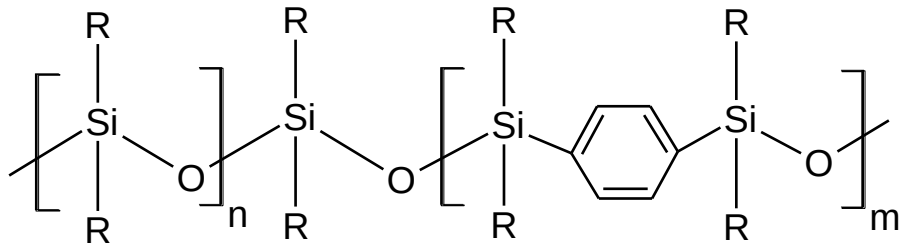
- General purpose septa
- Optimized temperature range/puncture lifetime

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



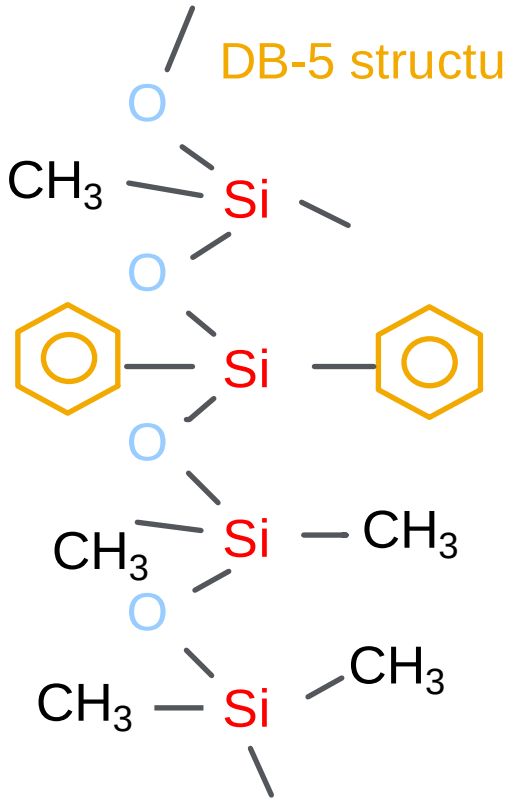
Siarylene backbone

- New phases unrelated to any previously existing polymers
  Examples: DB-XLB

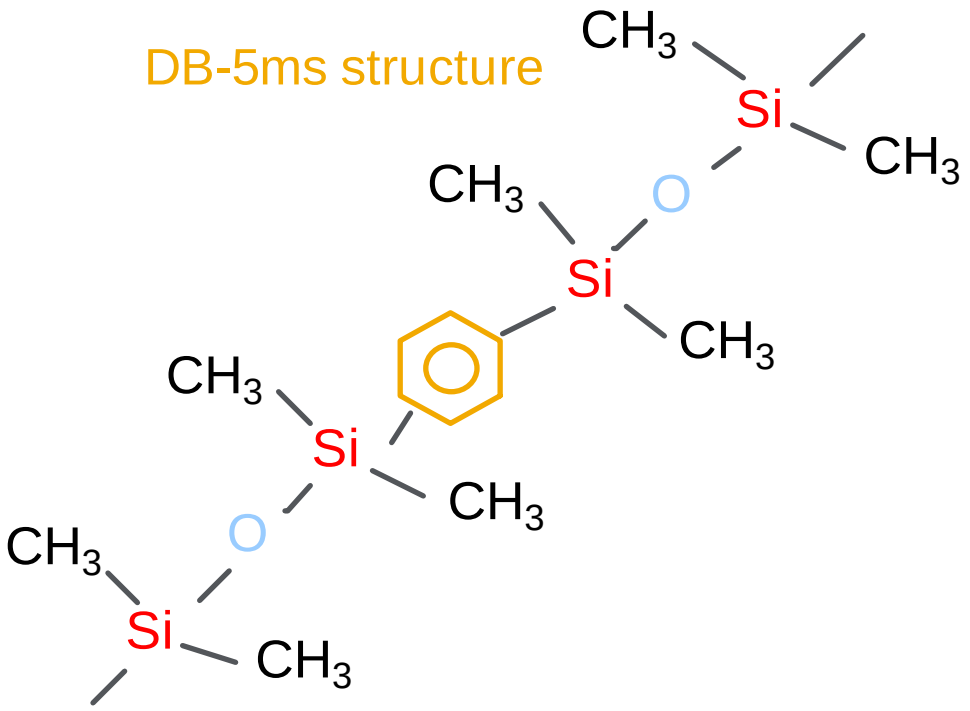
- Optimized manufacturing processes
  Examples: DB-1ms, HP-1ms, HP-5ms, VF-5ms

Agilent J&W DB-5ms Structure

DB-5 structure



DB-5ms structure



DB-5ms:

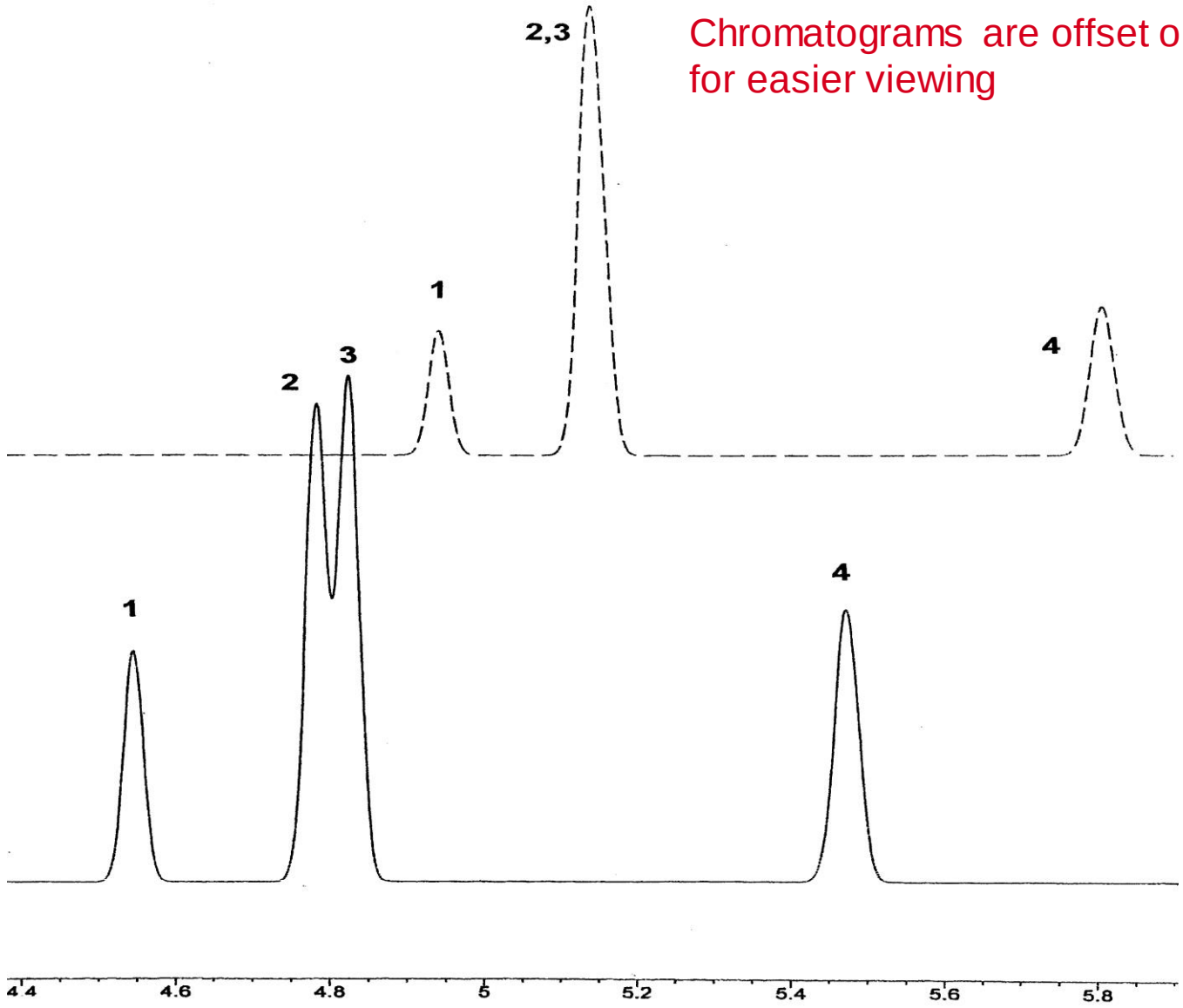
- Increased stability
- Different selectivity
- Optimized to match DB-5 as much as possible

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_2 at 40 cm/s

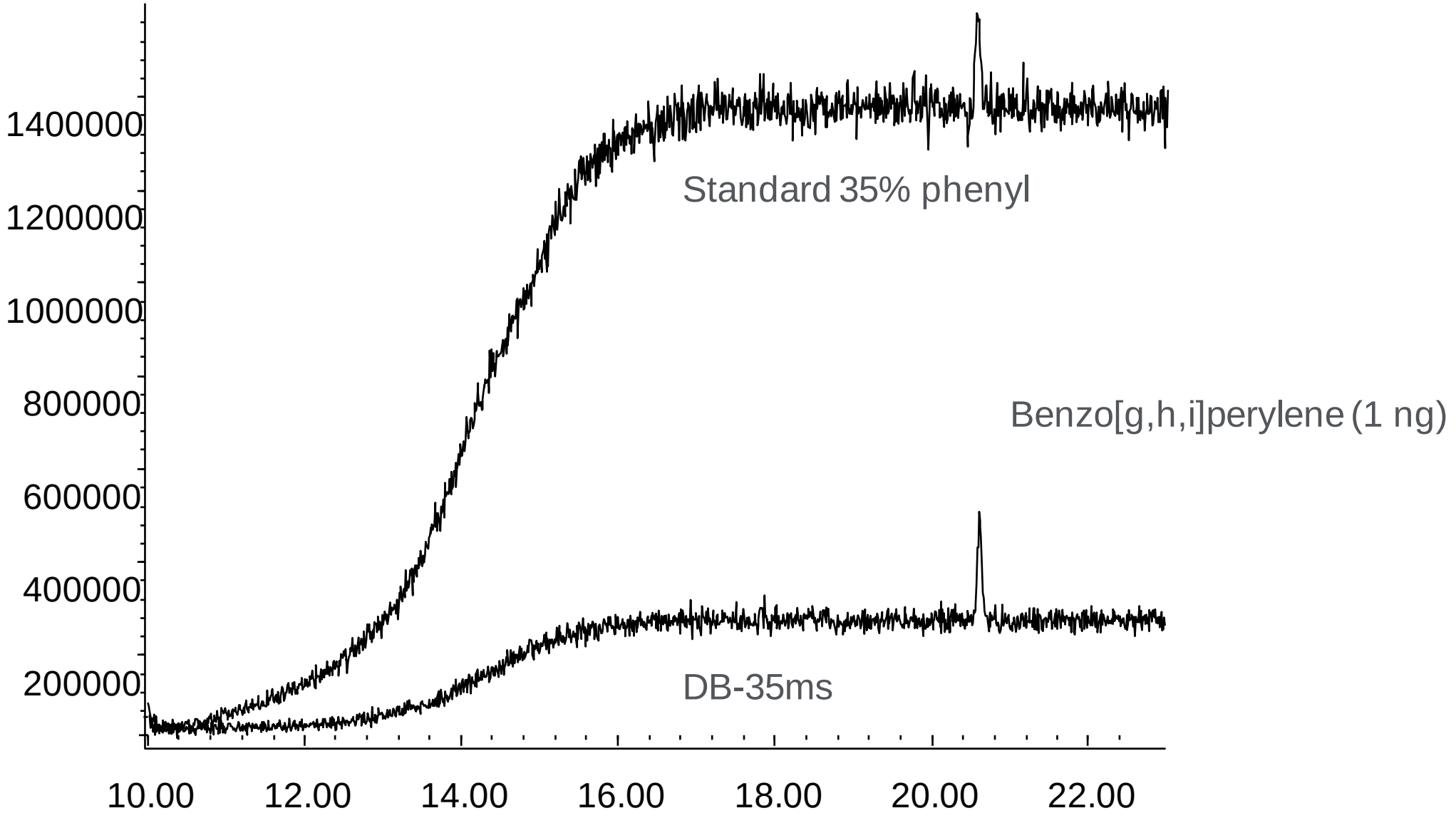
1: Ethylbenzene
2: m-Xylene

3: p-Xylene
4: o-Xylene

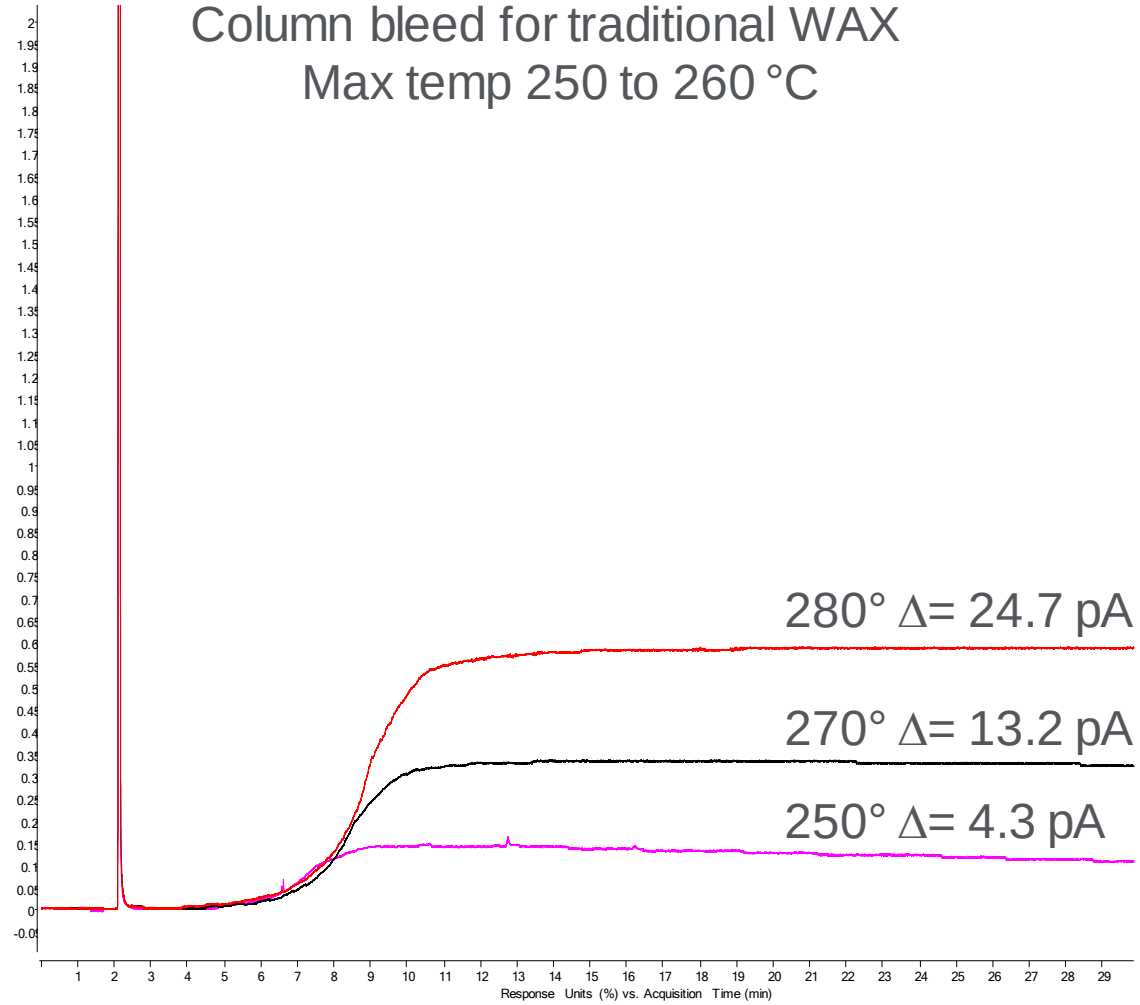


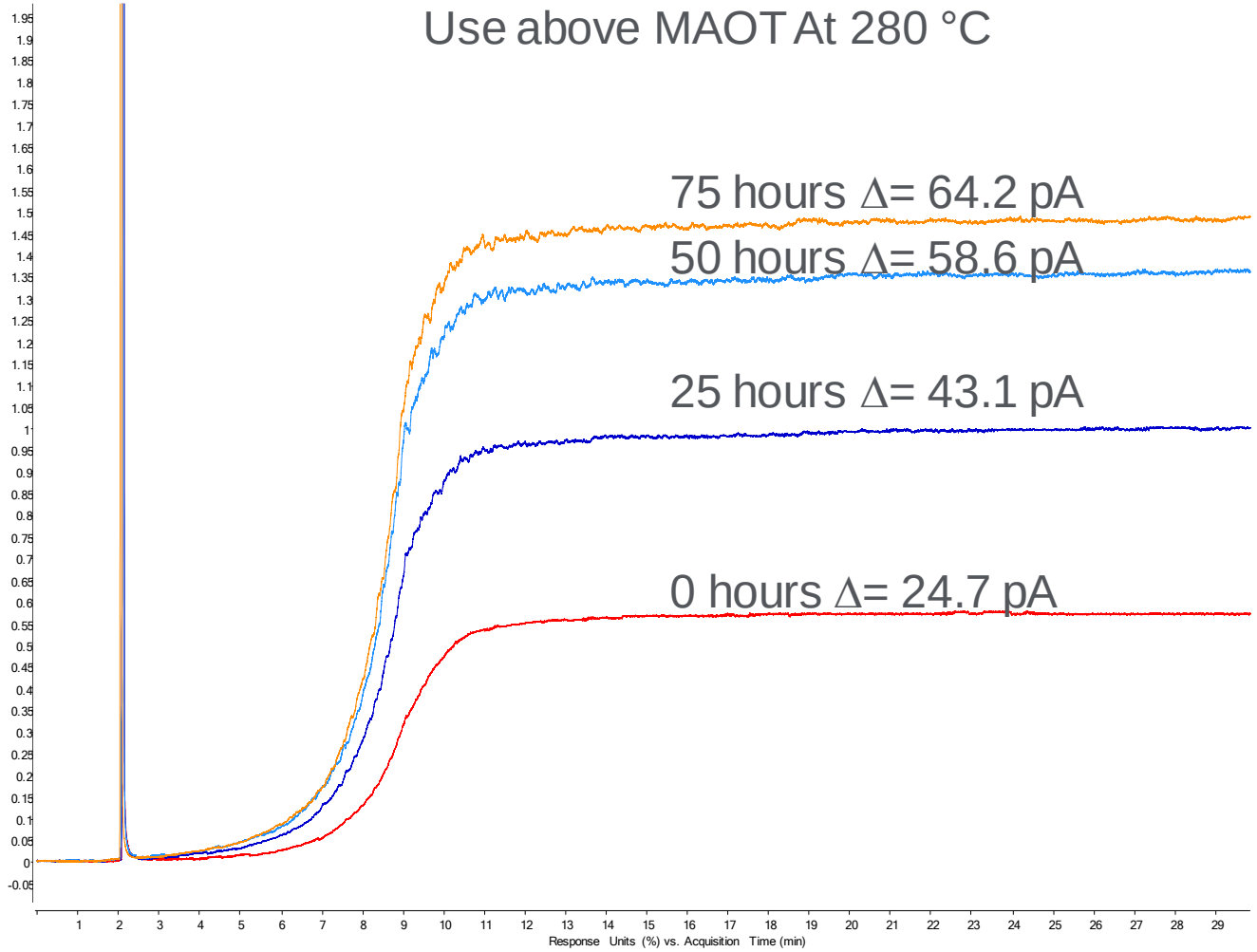
Chromatograms are offset on x-axis for easier viewing

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

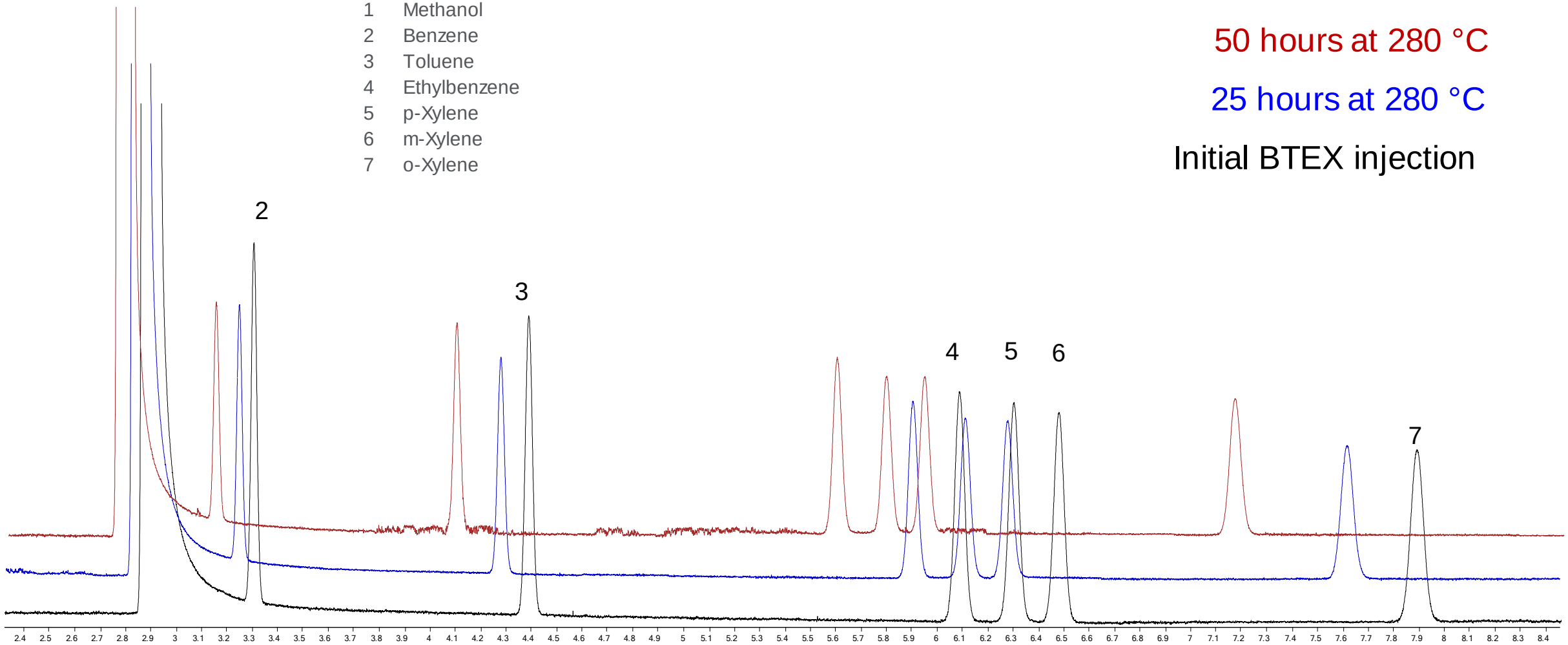
Peak	Compound
1	Methanol
2	Benzene
3	Toluene
4	Ethylbenzene
5	p-Xylene
6	m-Xylene
7	o-Xylene

Application note: 5991-9035EN

50 hours at 280 °C

25 hours at 280 °C

Initial BTEX injection



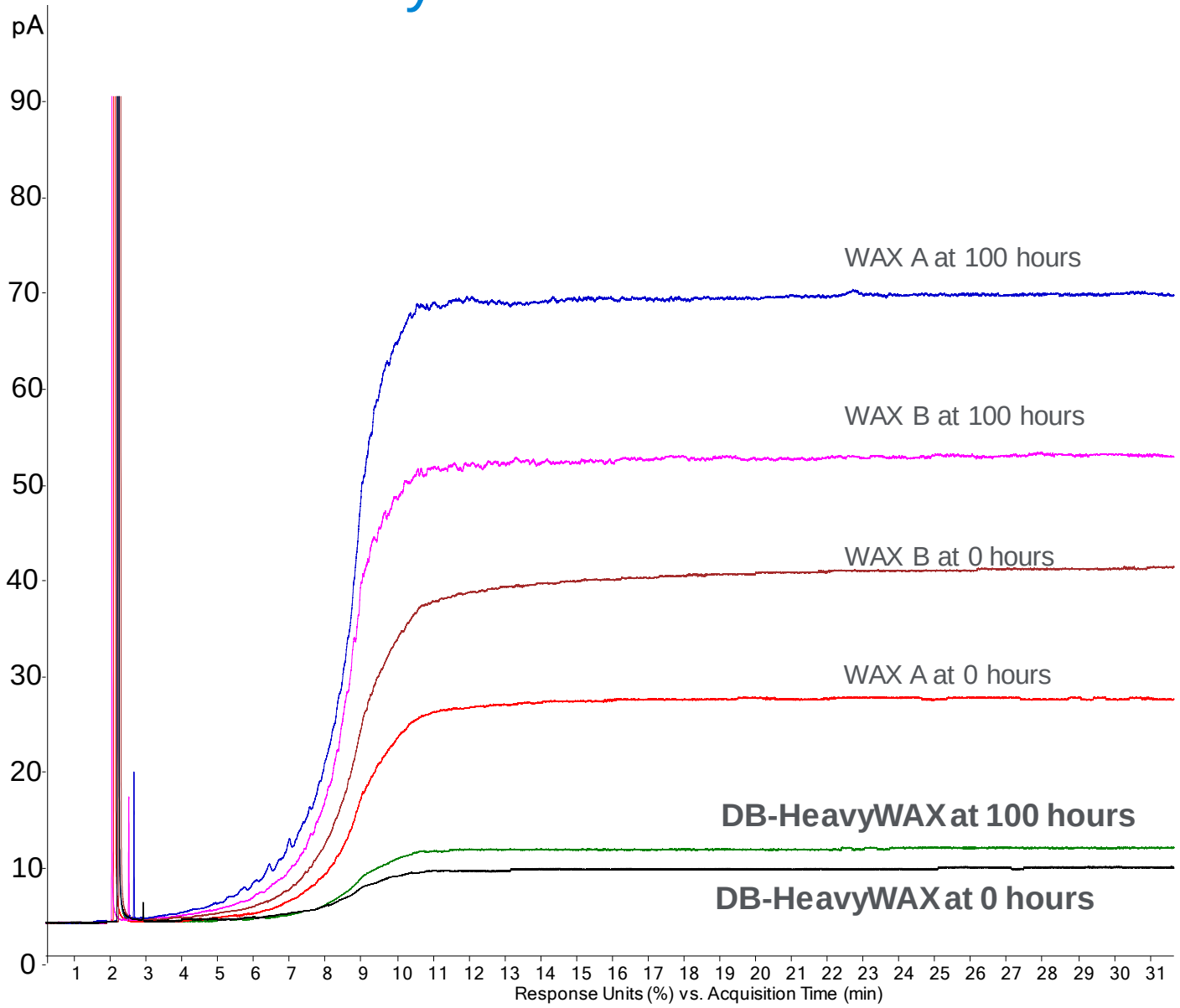
New J&W DB-HeavyWAX

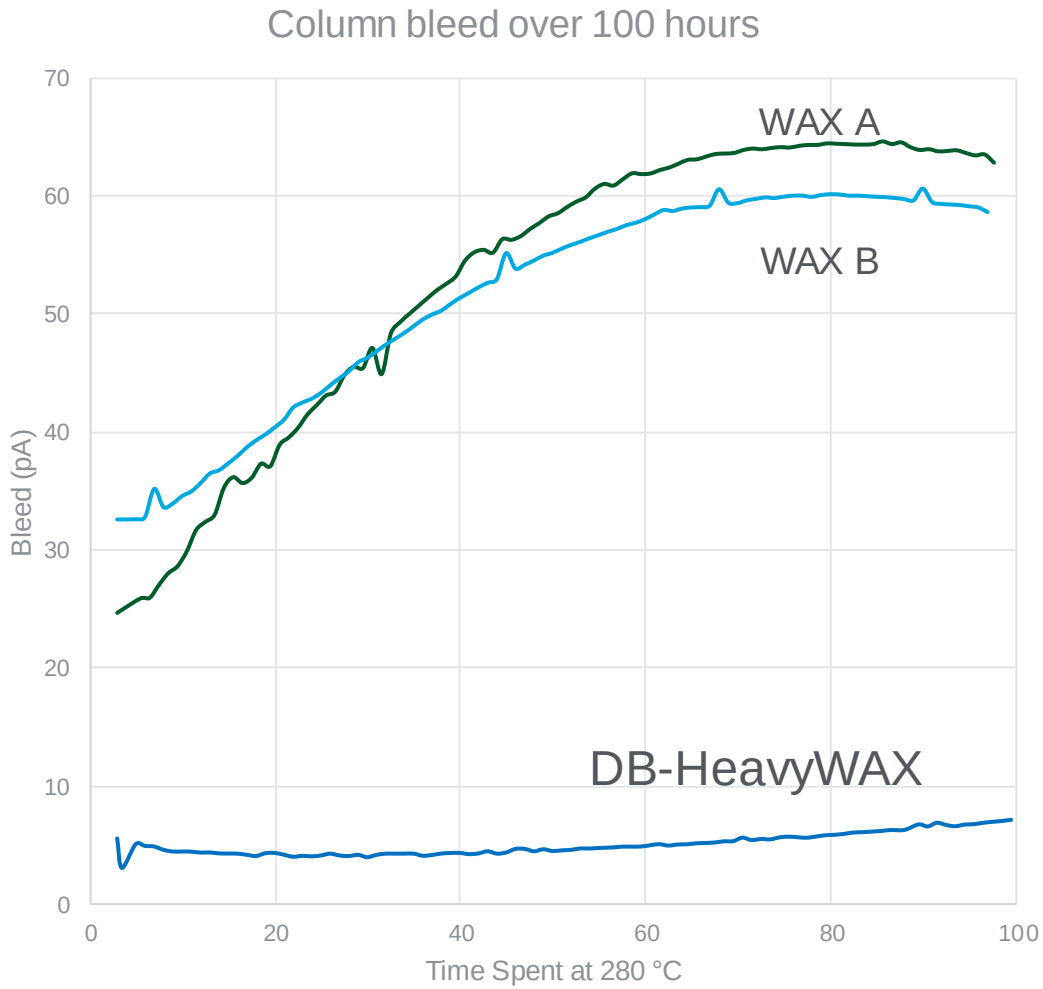
The WAX column you've been waiting for

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

www.agilent.com/chem/db-heavywax

Bleed Summary at 280 °C Over 100 Hours

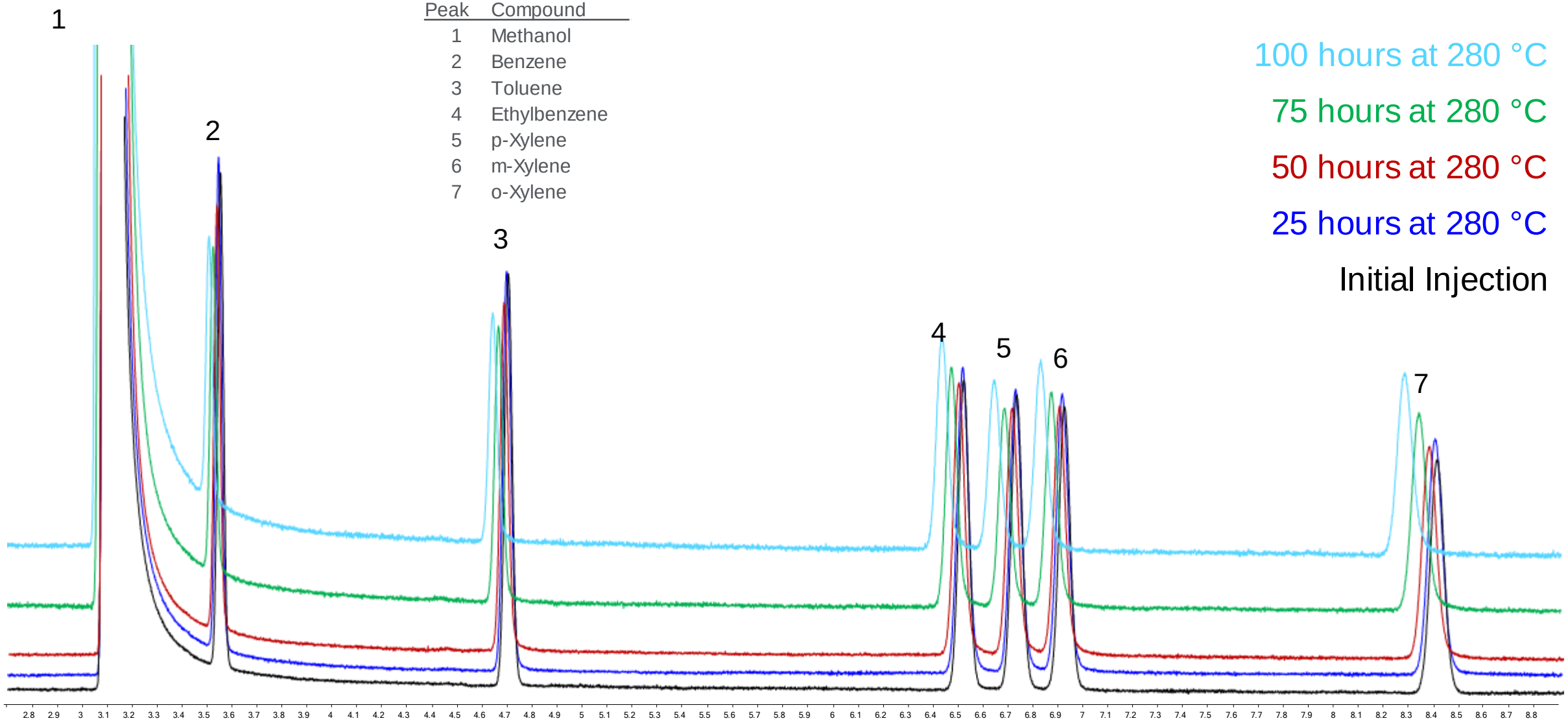




DB-HeavyWAX

Application note: 5991-9035EN

Peak	Compound
1	Methanol
2	Benzene
3	Toluene
4	Ethylbenzene
5	p-Xylene
6	m-Xylene
7	o-Xylene



Benefits of Low Bleed

Pyrolysis gasoline

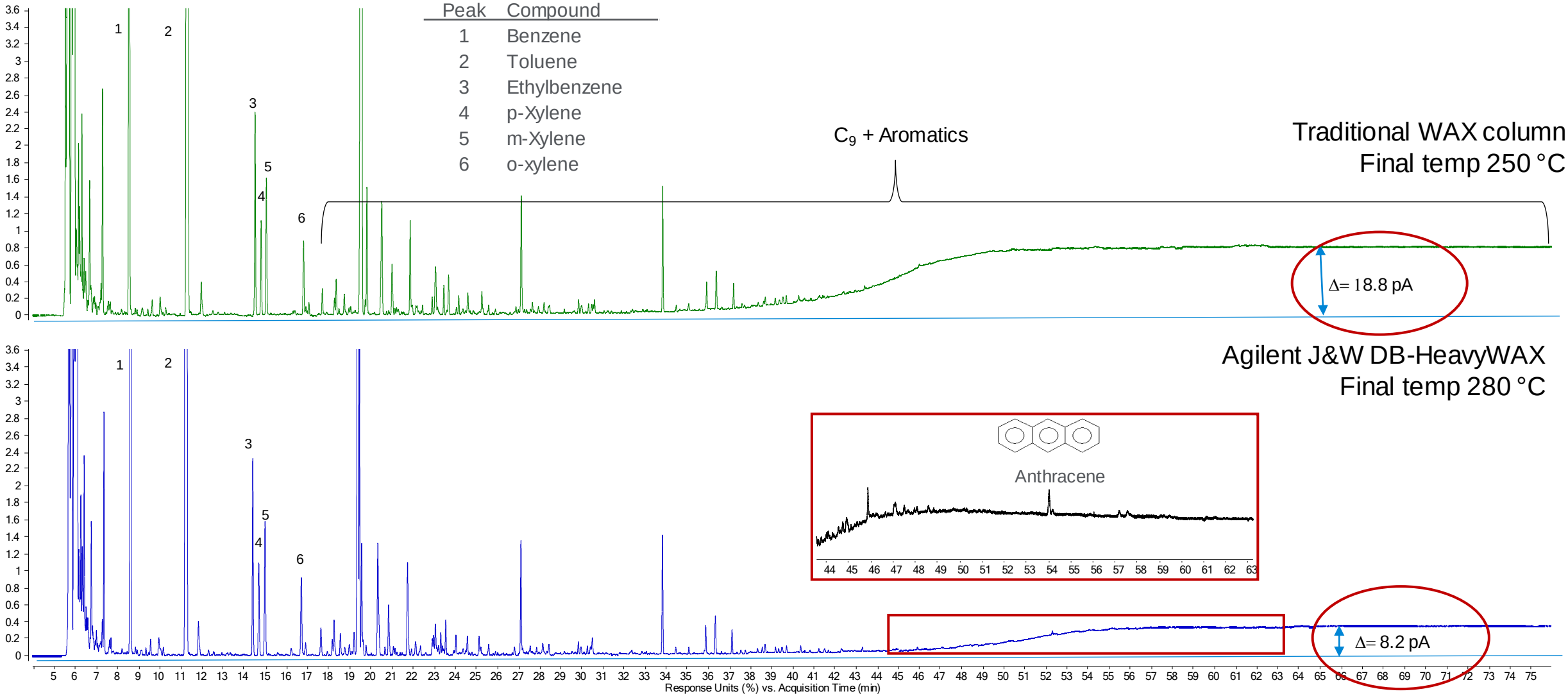
- ASTM D6563
- Heavier aromatic compounds
- Lower bleed at 280 °C than traditional WAX at 250 °C

GC Conditions	
Column	60m 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)

- Increased sensitivity for later eluting compounds
- Increased column lifetime

5991-9115EN

Pyrolysis Gasoline

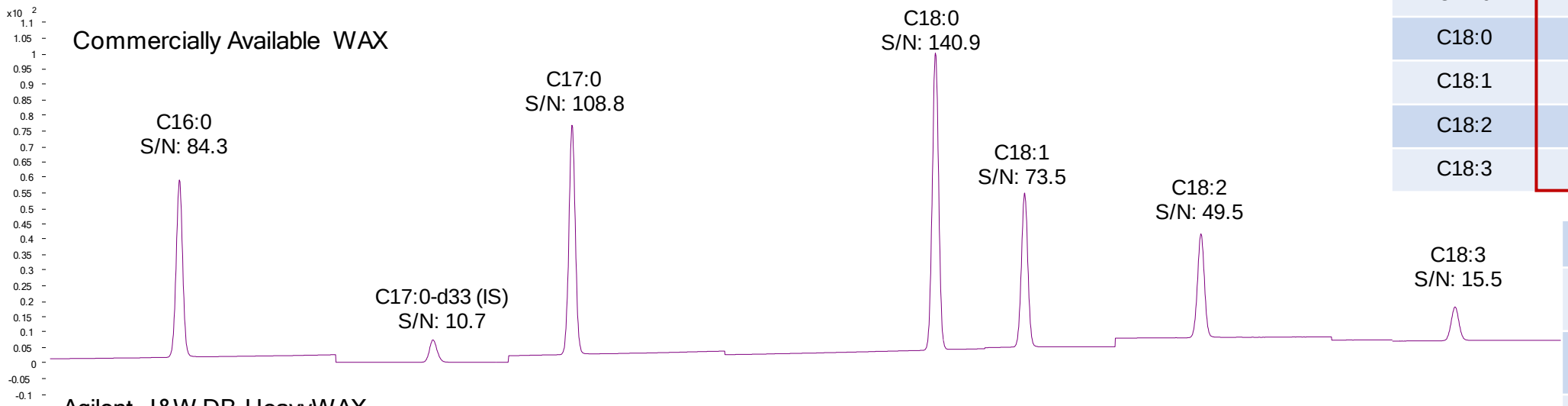


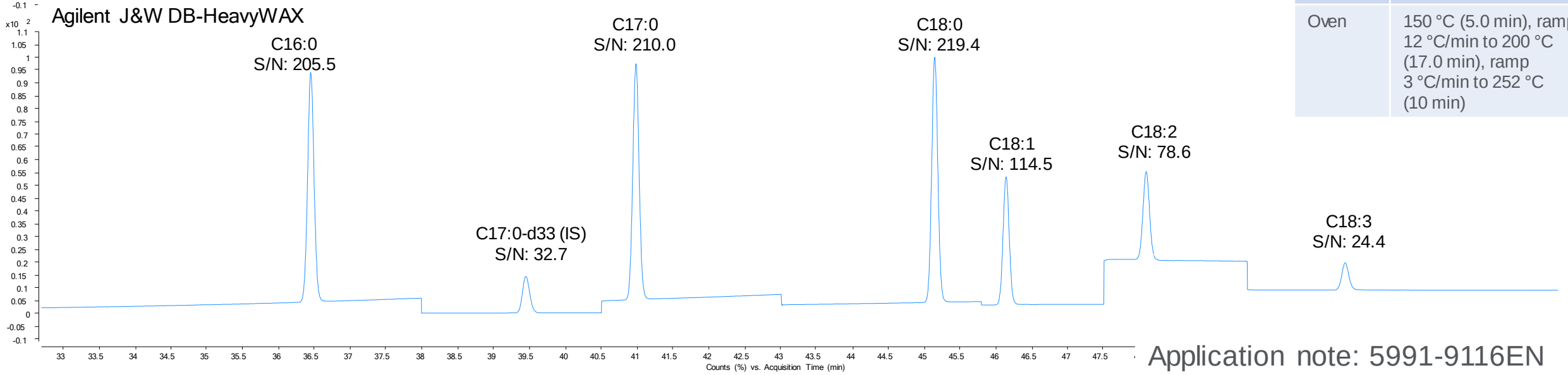
Application note: 5991-9115EN

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)

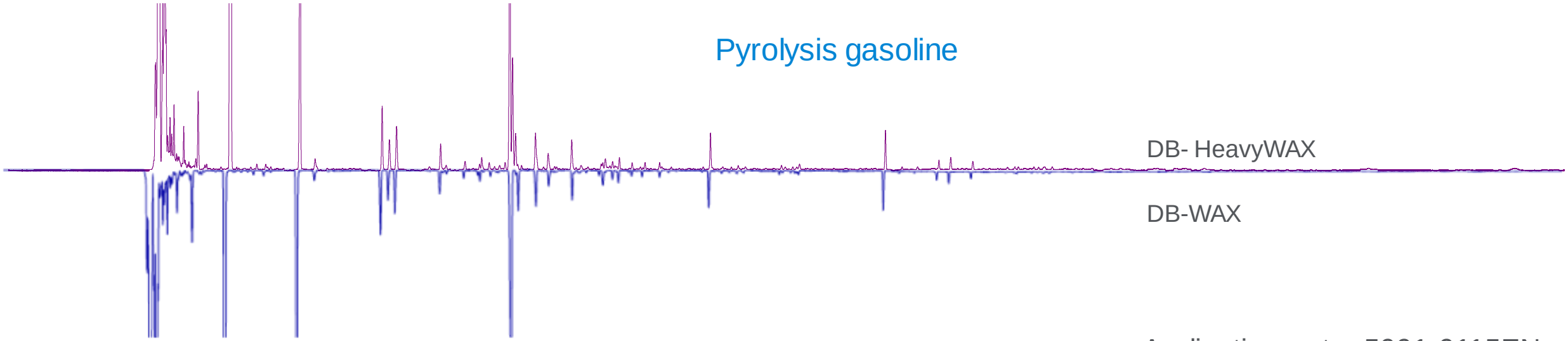
Application note: 5991-9116EN

It Is a WAX

Application note: 991-9078EN

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
- Deceased 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

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

lc-column-support@agilent.com

spp-support@agilent.com

spectro-supplies-support@agilent.com

chem-standards-support@agilent.com