Technical Overview

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How Does Bleed Impact GC/MS Data and How Can It Be Controlled?

Introduction

Understanding column bleed

Column bleed remains one of the most commonly observed yet misunderstood aspects of mass spectrometry (MS) data. Bleed elevates chromatographic baselines and creates spectral interference, lowering the quality of and confidence in your data. To understand bleed, it must first be considered where the bleed originates. While columns come in a wide variety of phase types, the most commonly used columns today, such as the 1, 5, and 624, are categorized as wall-coated open tubular (WCOT) columns. These columns are composed of fused silica tubing (with varying internal diameters, measured in mm), internally coated with a thin film of stationary phase (with varying thicknesses, measured in µm), as shown in Figure 1. This coated stationary phase interacts with analytes and creates the selectivity and retention characteristics of the chromatography; but, these same characteristics are the origins of column bleed.

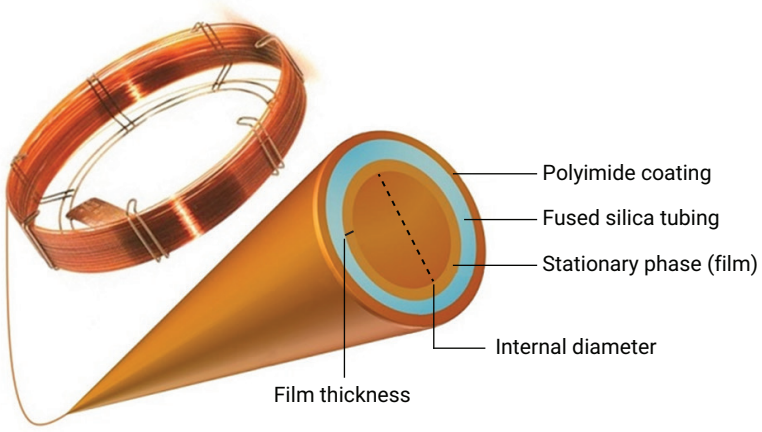

Figure 1. Diagram of a WCOT-fused silica GC column, and typical column attributes.

Column phases can be made from a wide variety of polarities of liquid polymers with a polysiloxane backbone. When heat is applied, the terminal end of the stationary phase polymer starts to bend back and attack itself, which is called "backbiting." Ring structures, which are thermodynamically stable, become liberated and increase the noise and raise the baseline, as shown in Figure 2. The process is then repeated. The higher the temperature provided, the faster the reaction is driven, which is why there is an increase in baseline at temperatures above 300 °C.

An increase in the rise of a baseline can be problematic for low signal-to-noise analytes, as peak integration can negatively impact data quality. Stable, flat baselines optimize the accuracy and repeatability of peak integration. When working with sensitive detectors, such as MS, an increase in the sensitivity of the detector also increases the background noise and bleed detection, causing excess column bleed to be detrimental to the sensitivity of trace-level compounds.

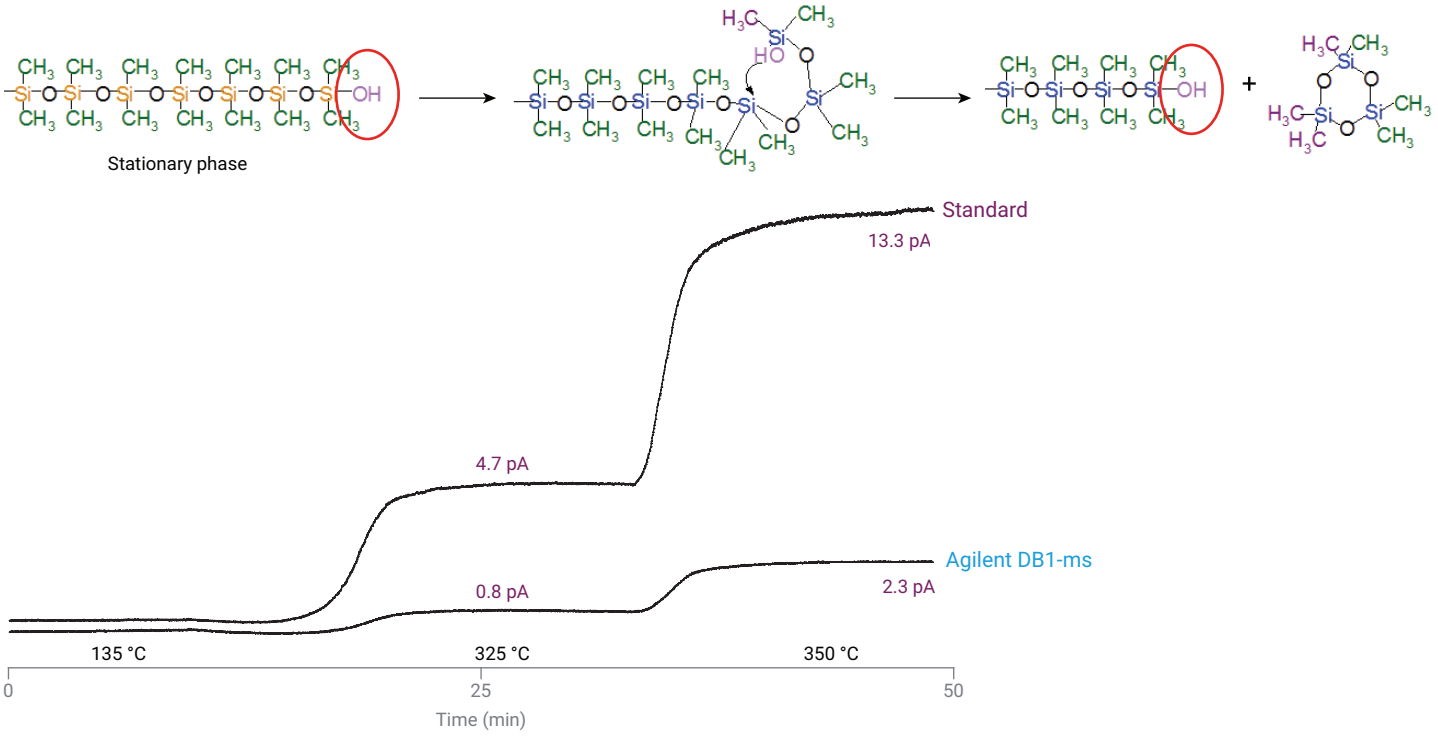

Figure 2. Mechanism of column bleed, along with a comparison of bleed profiles of a "standard" column along with an Agilent J&W DB-1ms column.

As the freed ring structures enter the MS due to backbiting, their masses will also fragment in the ionization source and can generate interfering ions in a mass spectrum. Ions of *m/z* 207 and 281 will most likely be seen in the spectrum, even in column phases with different polarities, as shown in Figure 3. These additional bleed ions can interfere with the spectral integrity of your analytes. Low-bleed columns are thus highly recommended for GC/MS analyses, especially for sensitive GC/TQ and GC/Q-TOF instrumentation.

Working within the thermal limits of your column

Because temperature significantly impacts the amount of column bleed, it is important to observe the thermal programming of your GC column. Every GC column has a maximum allowable operating temperature (MAOT), which will vary based on the type of column phase. This represents the highest temperature to which your column should be exposed to limit premature deterioration. Maximum temperatures are commonly stated with two numbers, for example 325/350 °C. The first, lower number is the MAOT for isothermal analysis. The second, higher number is the MAOT when using thermal gradients or oven programs. Operate your oven programs within the operating limits to optimize column lifetime and limit the impact of bleed.

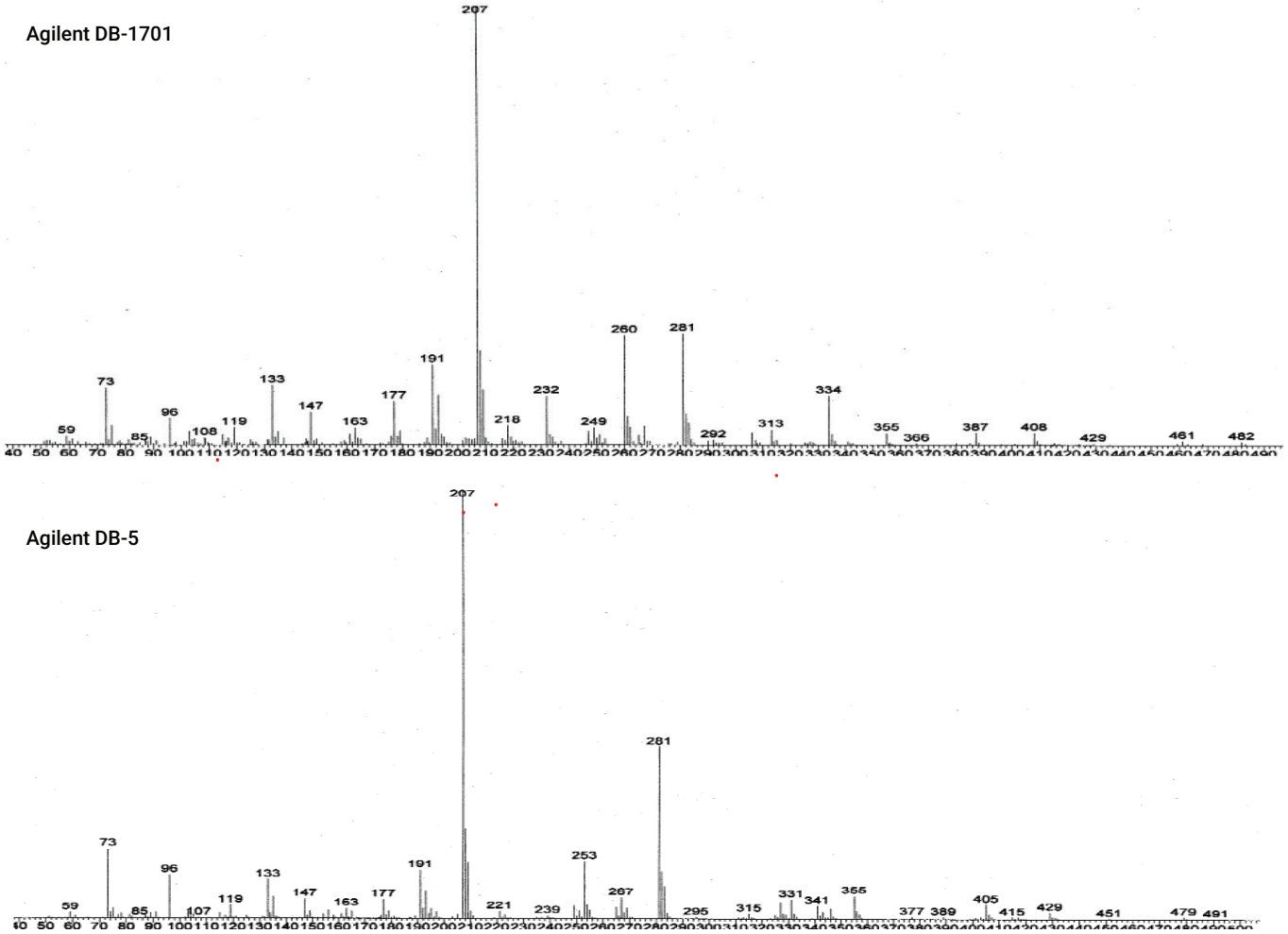

Figure 3. Examples of bleed spectra from two GC column phases with different polarities.[1]

Choose columns designed for gas chromatography/mass spectrometry

In 1991, Agilent introduced the first column designed for low-bleed MS applications—the Agilent J&W DB-5ms. This column quickly became an industry standard for GC/MS applications. Today, GC/MS sensitivity has greatly increased, and continues to advance with higher sensitivity and accurate-mass capabilities. The Agilent J&W DB-5Q and HP-5Q GC columns have ultralow-bleed polymers to meet the sensitivity and spectral requirements of modern, tandem, and time-of-flight mass spectrometers. When compared to conventional GC/MS columns, J&W HP-5Q columns have significantly lower bleed profiles, resulting in more stable baselines for accurate integration, especially at low signal-to-noise ratios. The HP-5Q columns also significantly reduce interfering bleed ions, maintaining analyte spectral fidelity.

Experimental

An Agilent 8890 GC coupled with a flame ionization detector (FID), and an Agilent 5977 Series B GC/MSD were used to collect data. Data acquisition was performed using Agilent OpenLab ChemStation and MassHunter acquisition software. Additionally, an Agilent Intuvo 9000 GC coupled with an FID was used to collect data. J&W DB-5Q and J&W HP-5Q GC columns were compared to various conventional, commercially available 5ms GC columns.

Tables 1 to 8 outline the parameters for each of the methods.

Table 1. Agilent 8890 GC parameters for method A.

Agilent 8890 GC	
Inlet	300 °C, split mode, split 20:1
Injection Volume	0.5 mL
Inlet Liner	Agilent inlet liner, Ultra Inert, split, low pressure drop (p/n 5190-2295)
Gas Saver	On, 20 mL/min after 3 min
Septum Purge Flow	3 mL/min
Oven	90 °C (0.5 min), ramp 20 °C/min to 330 °C (10 min), ramp 10 °C/min to 340 °C (10 min), ramp 10 °C/min to 350 °C (30 min)
Column	
Carrier Gas	Helium, 2.0 mL/min, constant flow
Columns	– Agilent J&W DB-5Q, 30 m × 0.25 mm, 0.25 µm (p/n 122-5532Q) – Agilent J&W HP-5Q, 30 m × 0.25 mm, 0.25 µm (p/n 19091S-433Q) – Conventional 5ms column X, 30 m × 0.25 mm, 0.25 µm – Conventional 5ms column Y, 30 m × 0.25 mm, 0.25 µm
Inlet Connection	Split/splitless inlet
Outlet Connection	FID

Table 2. FID parameters for method A.

Setting	Conditions
Temperature	325 °C
H_2 Flow	30 mL/min
Air Flow	400 mL/min
Make Up Gas	N_2
Make Up Gas Flow	25 mL/min, column + make up = constant

Table 3. Agilent 8890 GC parameters for method B.

Agilent 8890 GC	
Inlet	300 °C, splitless mode
Injection Volume	0.5 mL
Inlet Liner	Agilent inlet liner, Ultra Inert, split, low pressure drop (p/n 5190-2295)
Injection Pressure	12.37 psi
Purge Flow to Split Vent	100 mL/min at 1.0 min
Septum Purge Flow	3 mL/min
Oven	70 °C (1.0 min), ramp 20 °C/min to 200 °C, ramp 10 °C/min to 330 °C (10 min), ramp 10 °C/min to 340 °C (10 min)
Column	
Carrier Gas	Helium, 1.3 mL/min, constant flow
Columns	– Agilent J&W DB-5Q, 30 m × 0.25 mm, 0.25 µm (p/n 122-5532Q) – Conventional 5ms column X, 30 m × 0.25 mm, 0.25 µm – Conventional 5ms column Y, 30 m × 0.25 mm, 0.25 µm
Inlet Connection	Split/splitless inlet
Outlet Connection	MSD

Table 4. MSD parameters for method B.

Parameter	Value
Model	Agilent 5977B GC/MSD
Source	XTR
Mode	Scan (40 to 500 amu)
Solvent Delay	4.0 min
Source Temperature	300 °C
Quadrupole Temperature	175 °C
Gain	1.0

Table 5. Agilent 8890 GC parameters for method C.

Agilent 8890 GC	
Inlet	250 °C, split mode, split 100:1
Injection Volume	1.0 mL
Inlet Liner	Agilent inlet liner, Ultra Inert, split, low pressure drop (p/n 5190-2295)
Gas Saver	On, 20 mL/min after 3 min
Septum Purge Flow	3 mL/min
Oven	50 °C, ramp 30 °C/min to 350 °C (150 min), ramp 30 °C/min to 50 °C (10 min)
Column	
Carrier Gas	Helium, 2.0 mL/min, constant flow
Columns	– Agilent J&W HP-5Q, 30 m × 0.25 mm, 0.25 µm (p/n 122-5532Q) – Conventional 5ms column X, 30 m × 0.25 mm, 0.25 µm – Conventional 5ms column Y, 30 m × 0.25 mm, 0.25 µm – Conventional 5ms column Z, 30 m × 0.25 mm, 0.25 µm
Inlet Connection	Split/splitless inlet
Outlet Connection	FID

Table 6. FID parameters for method C.

Setting	Conditions
Temperature	325 °C
H_2 Flow	30 mL/min
Air Flow	400 mL/min
Make Up Gas	N_2
Make Up Gas Flow	25 mL/min, column + make up = constant

Table 7. Agilent Intuvo 9000 GC parameters for method D.

Agilent 9000 GC	
Inlet	300 °C, split mode, split 236:1
Injection Volume	0.5 mL
Inlet Liner	Agilent inlet liner, Ultra Inert, split, low pressure drop (p/n 5190-2295)
Gas Saver	On, 20 mL/min after 3 min
Septum Purge Flow	3 mL/min
Guard Chip	300 °C, isothermal
Bus Temperature	300 °C
Oven	65 °C (11 min), ramp 20 °C/min to 350 °C (30 min)
Column	
Carrier Gas	Helium, 3.27 mL/min, constant flow
Columns	– Agilent J&W DB-5Q, 30 m × 0.25 mm, 0.25 µm (p/n 122-5532Q-INT) – Agilent J&W DB-5ms Ultra Inert Intuvo column, 30 m × 0.25 mm, 0.25 µm (p/n 122-5532UI-INT)
Inlet Connection	Split/splitless inlet
Outlet Connection	FID

Table 8. FID parameters for method D.

Setting	Conditions
Temperature	325 °C
H_2 Flow	30 mL/min
Air Flow	400 mL/min
Make Up Gas	N_2
Make Up Gas Flow	25 mL/min, column + make up = constant
Detector 1 Tail D1	320 °C

Results and discussion

Comparisons of bleed profiles at upper temperature limits

To compare the thermal stability of the HP-5Q columns to conventional 5ms columns, columns were installed on an 8890 GC equipped with dual split/splitless inlets and dual FIDs, and experiments were done in tandem. After confirming that column connections were properly installed, free of leaks, and conditioned, the oven temperature was ramped up to temperatures of 330 and 340 °C and held for 10 minutes each. Then, the oven temperature was ramped to a final temperature of 350 °C (the programed MAOT of the column phases) and held for 30 minutes. As shown in Figure 4, both the DB-5Q and HP-5Q maintained ultralow FID response at the upper temperature limits, both maintaining column bleed levels below 1.0 pA at 340 °C. Also, when operating the columns at 350 °C (the programmed maximum temperature) over a period of 10 hours, both the DB-5Q and HP-5Q maintained bleed levels below 2.0 pA, while the conventional 5ms columns X and Y had bleed levels at 10 and 4 pA respectively, demonstrating the increased thermal stability of the DB-5Q and HP-5Q GC columns.

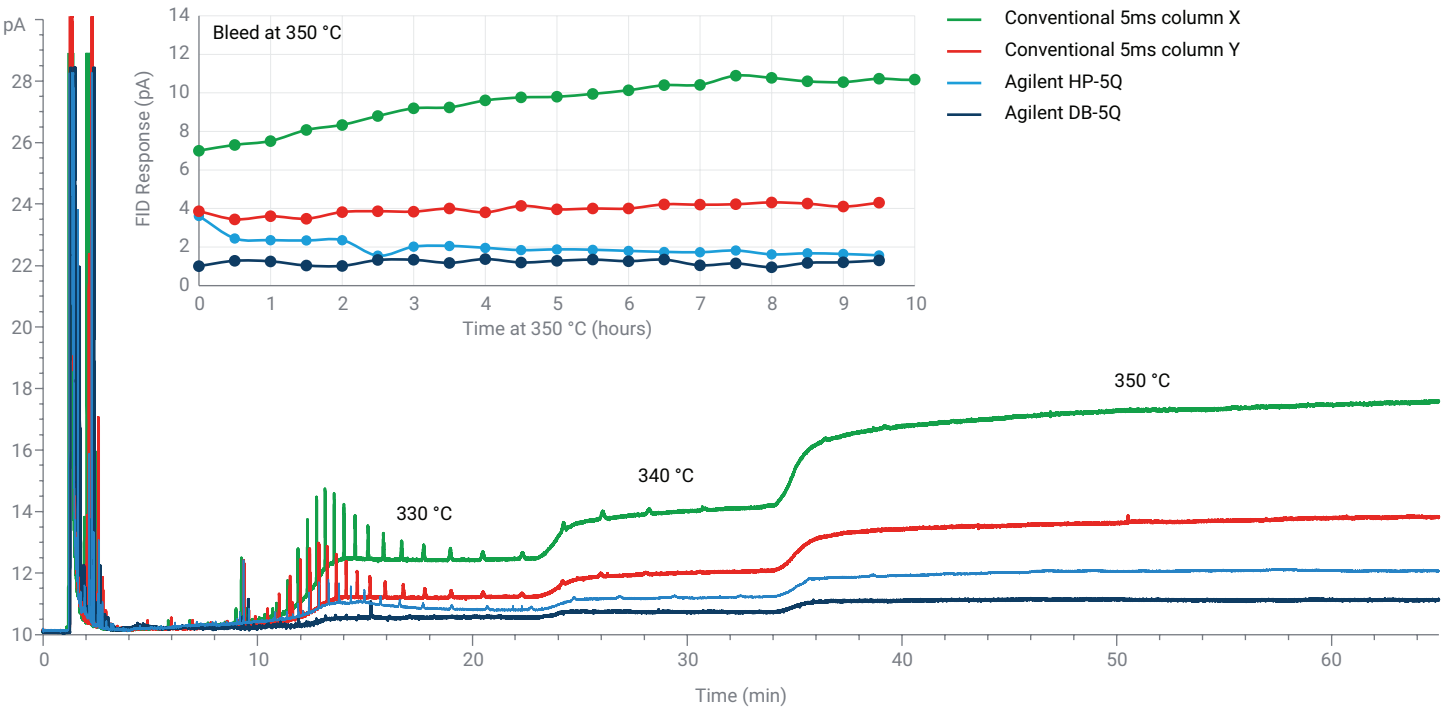

Figure 4. Comparison of bleed profiles for two conventional 5ms columns with Agilent J&W HP-5Q and DB-5Q GC columns collected on an FID using GC method A.

Increased thermal stability leads to less spectral interference

When using a single quadrupole mass spectrometer, spectral interferences due to column bleed can be seen by taking a snapshot of the spectrum at an area in the chromatogram where there are no peaks. Two conventional 5ms columns and a DB-5Q column were installed into a 5977 Series GC/MSD and compared using the 8890 GC with method B. Spectral snapshots were taken at 330 °C, where only increased baselines and no peaks were present, and the mass spectra were compared without scaling. As shown in Figure 5, there was a greater abundance of ions *m/z* 207 and 281 in conventional 5ms columns Y and X compared to the DB-5Q. The decreased spectral interference is evidence of the increased thermal stability of the DB-5Q column phase at the upper temperature limits typically used in sensitive GC/MS trace analysis applications.

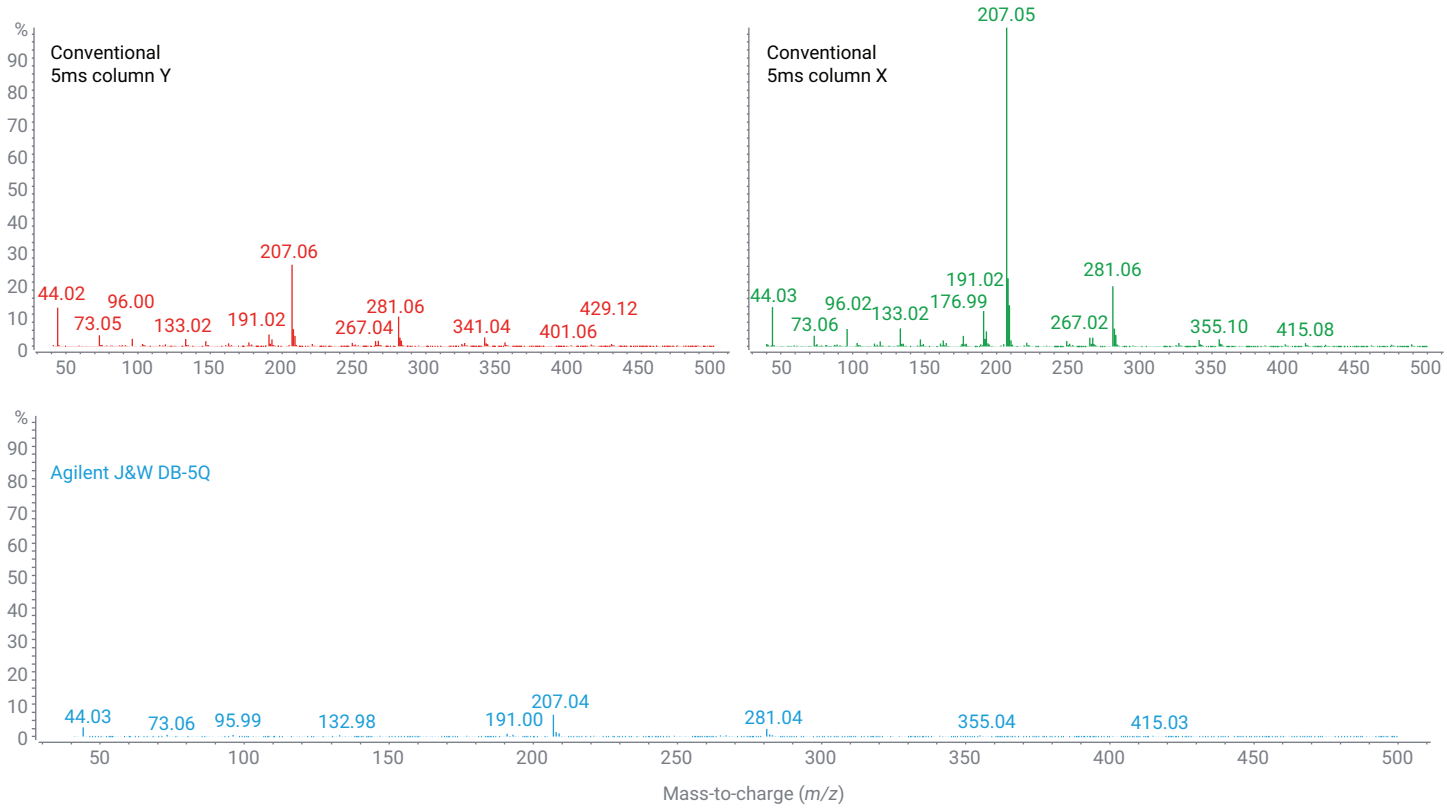

Figure 5. Bleed spectra collected from conventional 5ms columns X and Y, and an Agilent J&W DB-5Q GC column at 330 °C using an Agilent 5977 Series B GC/MSD and 8890 GC, following method B.

Properly condition the GC column

When a column is first installed into your GC inlet and detector, it is important to condition the column. Conditioning prevents excessive baseline noise, extends column lifetime, and provides accurate run-to-run results. Once the column is trimmed and installed, turn on the carrier gas flow and set the oven to a low 40 °C temperature. Purging the moisture and oxygen from your column before increasing the temperature will minimize phase degradation. Once purged and leak-free, bring the column to the maximum operating temperature with the column flow turned on, without exceeding the MAOT for your specific column phase and configuration. Initially, an increase in baseline will occur; but, as the column is held at the maximum temperature needed to perform its analysis, the baseline will drop and become stable and level. At the point where the baseline is level, the column is deemed conditioned. While the amount of time may vary between column phases, generally, the more thermally stable the column phase, the less time it will take to condition the column.

It is not unusual for a nonpolar column, such as a 5% phenyl phase, to take 2 hours to condition. As indicated in Figure 6, the HP-5Q had a level baseline in less than 1 hour when being conditioned at 350 °C, whereas the conventional 5ms column Z required 2 hours to be deemed conditioned. The decreased time required to properly condition the HP-5Q GC column allows a faster return to operating time and helps increase instrument productivity.

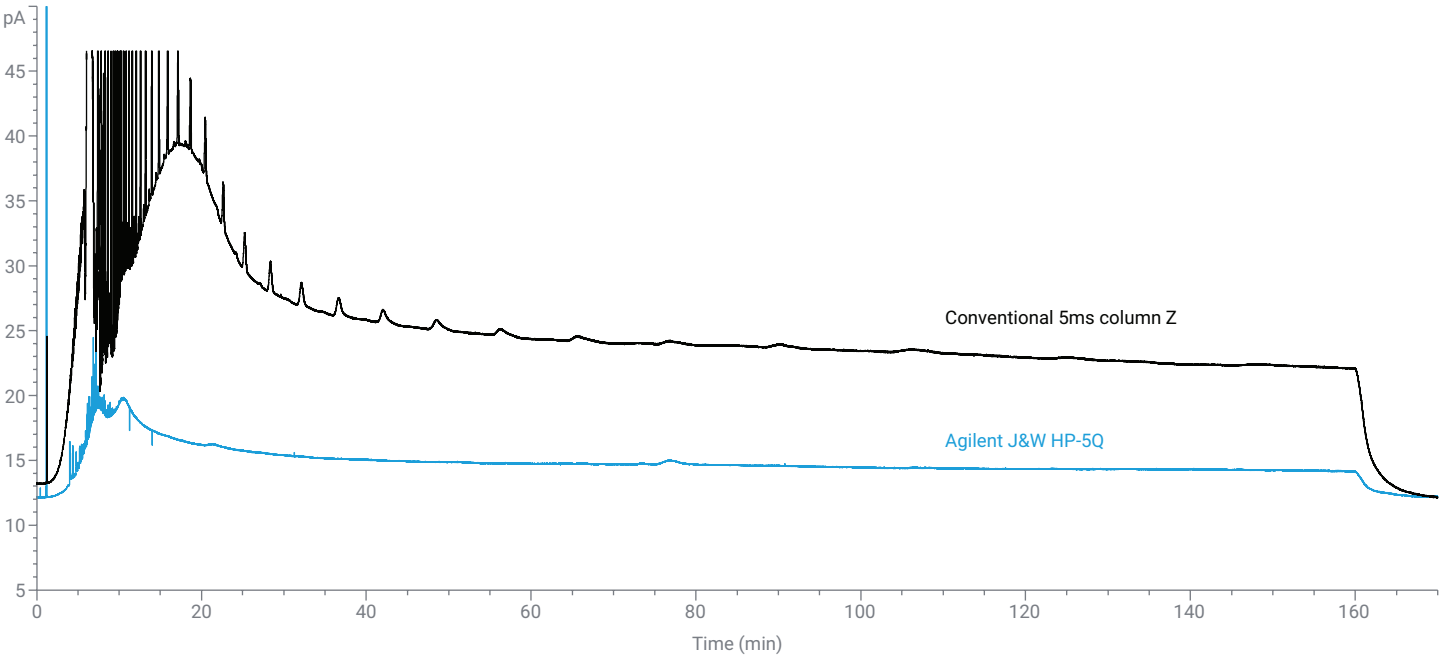

Figure 6. Conditioning profile at 350 °C collected on a conventional 5ms column Z and an Agilent J&W HP-5Q by FID using method C.

How column bleed affects baseline stabilization

On most chromatograms, it is easy to see how column bleed can impact the upper temperature limits of a GC oven program, but thermal stability can also have an impact on the beginning of a run by way of baseline stabilization. There are many indicators that a GC acquisition program will use to determine when an instrument is ready to start the next injection, such as *oven temperature setpoint ready and equilibrated*, or *inlet temperature ready*. Once these settings have reached their setpoints, an injection cycle will begin. However, this does not account for if a baseline is determined to have returned to an expected baseline. Traditional air bath ovens, such as with the 8890 GC, can have longer cycle-to-cycle times because it can take longer to reach and equilibrate to a lower oven setpoint after reaching high temperatures. This is why this phenomenon is not seen as frequently with air bath ovens. However, with the fast heating and cooling cycles of the Intuvo 9000 GC, there is a decrease in potential cycle-to-cycle time, which can lead to a GC being "ready" before the column baseline has returned to the expected setpoint, which is demonstrated in Figure 7.

In Figure 7A, an initial injection was performed using a routine test mix, known as the UBER mix, on an Agilent J&W DB-5ms UI. After all compounds had eluted, the oven was ramped up to 350 °C for 30 minutes. After this analysis was complete, the sequence moved to the following line, and, when the GC stated that it was ready, the next injection was performed and data were collected. As shown in Figure 7B, the baseline still decreased even at the start of the run, indicating that the column baseline had not yet returned to normal after being held at high temperatures. This altered baseline caused a distortion of the integration of the compounds, which led to inaccurate quantitation as well as poorly observed peak shape for the early-eluting compounds.

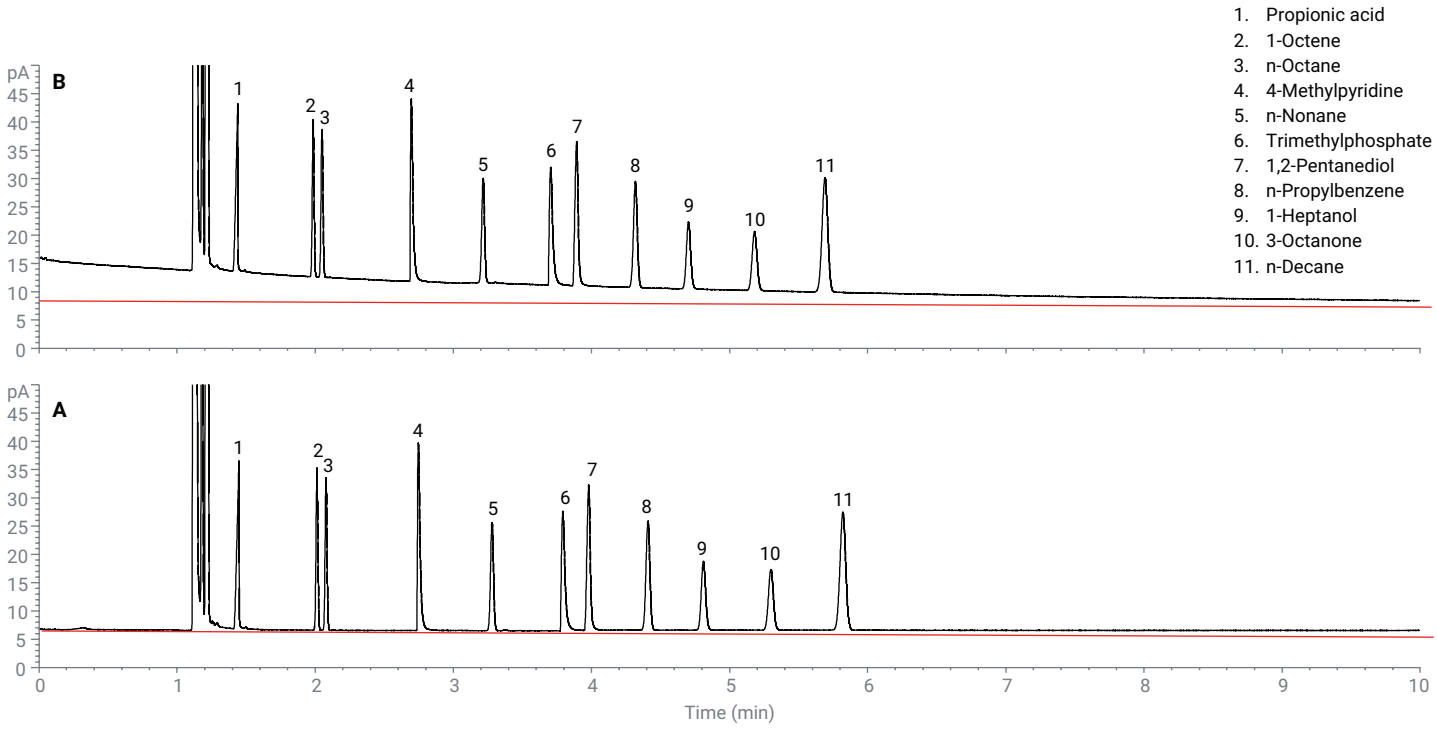

Figure 7. (A) Initial injection of UBER test mix analyzed on an Agilent J&W DB-5ms Intuvo GC column. (B) Subsequent injection of UBER test mix analyzed after cycling the oven to 350 °C.

In Figure 8, the same test was performed using a DB-5Q Intuvo GC column. Due to the improved thermal stability and ultralow column bleed, there was no increase in the baseline at the beginning of the subsequent injection of test mix. This indicates that the improved thermal stability of the DB-5Q Intuvo column not only maintains a low overall column bleed but also will be ready to provide optimum low bleed even when using the fast cooling option feature of the Intuvo 9000 GC.

Conclusion

While column bleed is a normal phenomenon, there are ways to decrease its impact on the analysis and sensitivity of the detector being used. Improved column technology, with the introduction of the Agilent J&W DB-5Q and HP-5Q GC columns, has provided an increase in thermal stability that dramatically reduces column bleed. The decrease in column bleed at high temperatures allows an increase in sensitivity of mass spectrometers, as the spectral interference from ions due to backbiting of column phase is reduced. This demonstrates that the DB-5Q and HP-5Q GC columns are optimal for use with sensitive detectors such as GC/MS, triple quadrupole GC/MS, and GC/Q-TOF.

References

1. Reese, A.; Vickers, A.; George, C. GC Column Bleed: A Mass PerSPECtive, *Agilent Technologies*, publication number B-0442, **2001**.

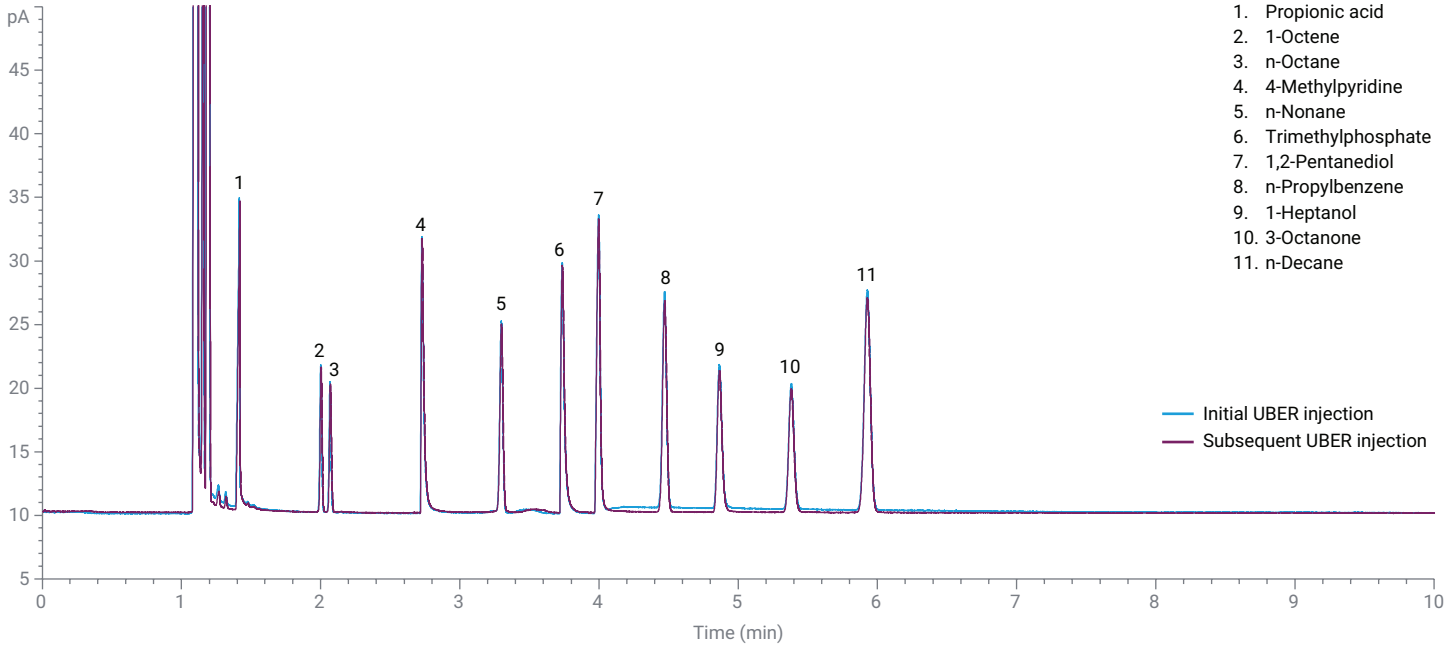

Figure 8. UBER test mix analyzed on an Agilent J&W DB-5Q Intuvo GC column initially and after being held at a maximum temperature of 350 °C for 30 minutes.

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