Application Note

Forensics

Increased Thermal Stability of the DB-5Q GC Column

Increased signal-to-noise for trace level concentrations of controlled substances

Authors

Vanessa Abercrombie and
Frans Biermans
Agilent Technologies, Inc.

Abstract

The use of gas chromatography/mass spectrometry (GC/MS) for the analysis of controlled substances requires a high level of data accuracy for identification. The Agilent J&W 5Q series of GC columns with increased thermal stability, decreased spectral interference at upper temperature limits, and maintains a constant baseline, allowing for accurate spectral comparison. This study illustrates the improved data accuracy and response due to increased thermal stability of the Agilent J&W DB-5Q for the analysis of controlled substances by GC/MS.

Introduction

The analysis of controlled substances by gas chromatography/mass spectrometry (GC/MS) requires a high level of confidence and thus requires a method that provides optimal data quality. Identification of controlled substances relies heavily on comparison of mass spectra to commercially available spectral libraries, and increased column bleed can cause spectral interference at high temperatures. These interferences can lead to poor library matching probabilities.[1-3] The Agilent J&W 5Q series of gas chromatography columns provide the most thermally stable columns available, which helps to decrease spectral interference due to column bleed and improves data accuracy.

Poor thermal stability will cause an increase in the slope of the baseline as the oven temperature is increased, leading to difficult peak integrations and inaccurate quantitation. Additionally, sloped or elevated baselines can lead to a decreased response and a loss of sensitivity for analytes eluting during the oven ramp or at the method's upper temperature limits. Several commercially available 5ms type columns were compared to the Agilent J&W DB-5Q GC column to examine the impact thermal stability has on response at upper temperature limits.

Experimental

An Agilent 8890 GC system coupled with an Agilent 5977B mass selective detector (GC/MSD) with an inert extractor ion source was used for acquisition of data and MassHunter 10.0 Quantitative and Qualitative Analysis software was used for data analysis.

A representative toxicology mixture, the Agilent GC/MS forensic toxicology checkout mixture (part number 5190-0471) was used to monitor analytical performance. Calibration standards were prepared in methanol (MeOH) at concentrations ranging from 0.1 to 3.0 µg/mL. An internal standard mix was prepared at 10 µg/mL from individual stocks of methamphetamine-d3, diazepam-d5, and hydrocodone-d6 all purchased from Millipore Sigma (St. Louis, MO).

The acquisition method was locked using retention time locking, for the analyte phencyclidine at a retention time of 11.0 minutes, using ions 200, 242, 91 for peak confirmation.

Table 1. GC parameters for the Agilent 8890 GC.

Parameter	Value
8890	
Inlet	300 °C, Split 10:1
Injection Volume	0.5 mL
Inlet Liner	Ultra Inert, split, low pressure drop (part number 5190-2295)
Gas Saver	On, 20 mL/min after 3 min
Septum Purge Flow	3 mL/min
Oven	95 °C (1.5 min), ramp 12 °C/min to 275 °C, ramp 30 °C/min to 300 °C (11 min)
Column	
Carrier Gas	Helium, 1.3 mL/min, constant flow
Column	DB-5Q, 30 m × 0.25 mm, 0.25 µm (p/n 122-5532Q) 5ms Type G 5ms Type X 5ms Type Z
Inlet Connection	Split/splitless inlet (S/SL)
Outlet Connection	MSD

Table 2. MS parameters for the Agilent 5977B GC/MSD.

Parameter	Value
Model	5977B
Source	XTR
Mode	Scan (40 to 400 amu)
Solvent Delay	2.5 min
Source Temperature	300 °C
Quad Temperature	175 °C
Gain	1.0

Table 3. Agilent GC/MS forensic toxicology checkout mixture compounds and retention times in elution order, and identification ions, with quantitation ions in bold.

Peak	Compound	RT	Ions			
1	d-Amphetamine	3.716	**44**	91		
2	Phentermine	3.846	**58**	91	134	
3	Methamphetamine	3.846	**58**	91		
*	Methamphetamine-d3	4.025	**94**	138	44	77
4	Nicotine	5.633	**84**	133	161	
5	MDA	6.941	**136**	77		
6	MDMA	7.900	**58**	135		
7	MDEA	8.078	**72**	135		
8	Meperidien	9.736	**71**	172	91	247
9	Phencyclidine	11.043	**200**	242	91	186
10	Methadone	13.025	**72**	294		
11	Cocaine	13.545	**82**	182		
12	Proadifen	14.293	**86**	99		
13	Oxazepam	14.528	**205**	233	267	
*	Diazepam-d5	14.934	**261**	287		
15	Lorazepam	14.959	**239**	274		
14	Codeine	14.975	**299**	162		
*	Hydrocodone-d6	15.007	**305**	209	245	
16	Diazepam	15.284	**256**	283		
17	Hydrocodone	15.389	**299**	242	185	
18	THC	15.470	**299**	314	231	271
19	Oxycodone	15.852	**315**	230		
20	Tempazepam	16.145	**271**	300	256	
21	Flunitrazepam	16.258	**312**	285	266	
22	Heroin	16.348	**327**	369	268	
23	Nitrazepam	17.241	**253**	234		
24	Clonazepam	17.794	**314**	280		
25	Alprazolam	18.565	**204**	273	279	308
26	Verapamil	20.564	**303**	151		
27	Strychnine	20.832	**334**	120		
28	Trazodone	23.342	**205**			

* Indicates internal standard

Results and discussion

The testing evaluated the Agilent J&W DB-5Q GC column for the analysis of controlled substances by GC/MS. The increased thermal stability of the DB-5Q is seen in the decreased baseline at higher temperatures, as seen in Figure 1, and also provides a decrease in the slope during the oven ramping program.

An increase in the slope of the baseline will also decrease the signal-to-noise ratio (S/N) of an analyte but will also make integration more difficult and lead to less accurate quantitative results. Figure 2 demonstrates the comparison of the DB-5Q and the commercially available 5ms type G column, and the impact to chromatography during the oven ramp. The S/N for the analytes on the oven ramp slope are a factor of ten or greater for the DB-5Q in comparison to the 5ms type G column.

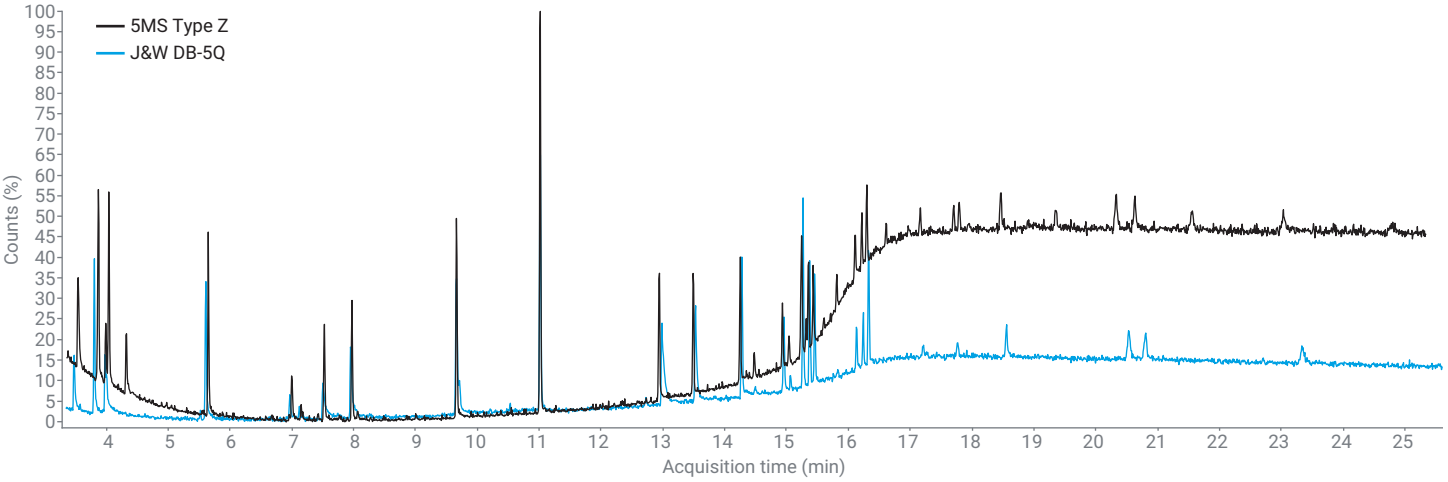

Figure 1. A standard of the Agilent GC/MS forensic toxicology checkout mixture (part number 5190-0471) at 5.0 µg/mL analyzed on an Agilent J&W DB-5Q (blue) and the 5ms type Z (black) column.

J&W DB-5Q

Peak	Compound	Signal-to-Noise
20	Tempazepam	31.0
21	Flunitrazepam	12.56
22	Heroin	9.76

5ms Type G

Peak	Compound	Signal-to-Noise
20	Tempazepam	0.93
21	Flunitrazepam	1.17
22	Heroin	0.57

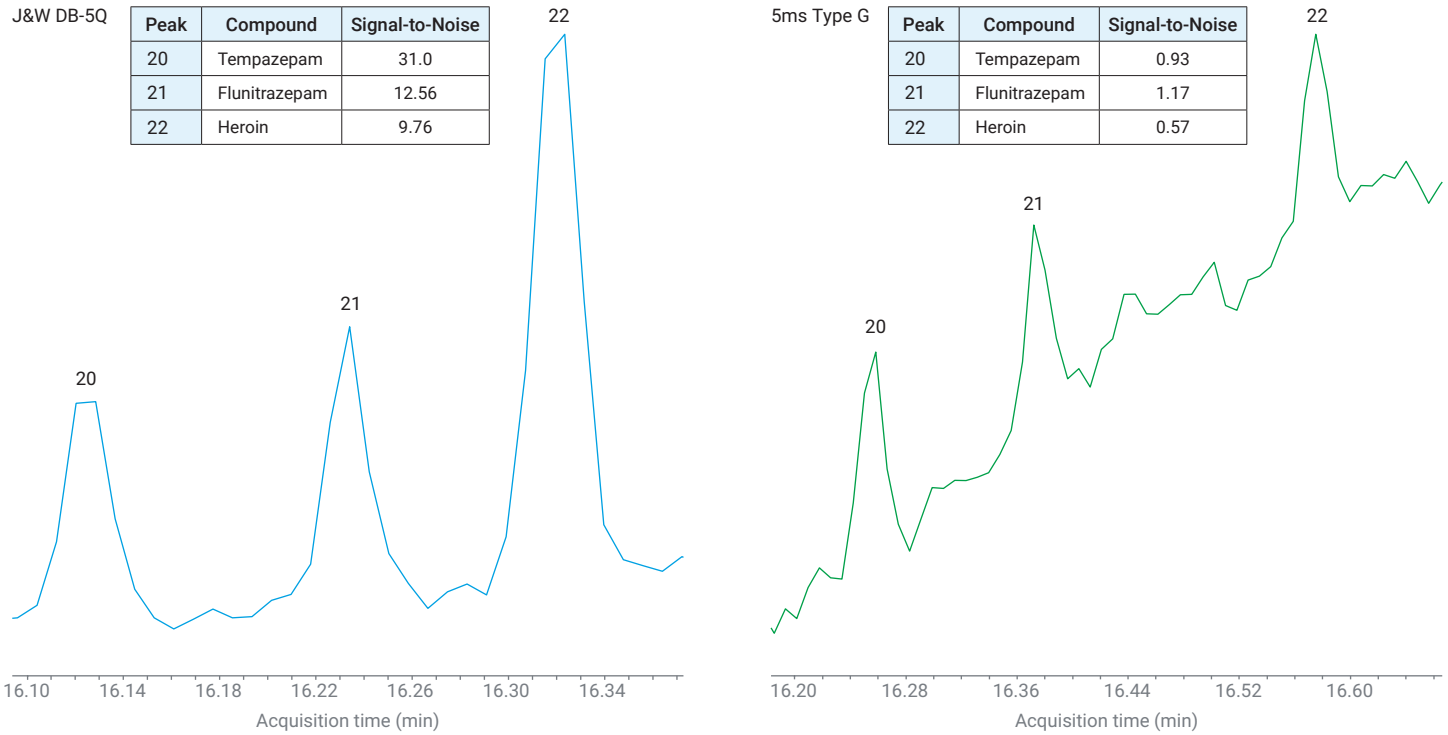

Figure 2. A standard of 3.0 µg/mL of the toxicology standard analyzed on an Agilent J&W DB-5Q (blue) and the 5ms type G (green) column, after 145 matrix injections.

As the baseline can increase at the maximum oven temperature for less thermally stable columns, this will decrease the S/N of the analyte and cause a decrease in sensitivity of the analytical method. Both the DB-5Q and 5ms type G columns were locked, using retention time locking, to ensure the analytes eluted at the same portion of the oven program. The S/N of nitrazepam is ten times greater on the DB-5Q column than it is on the 5ms type G column at the highest calibration point of 3.0 µg/mL even after thermal and matrix stress, as shown in Figure 3. Additionally, when examining the analyte alprazolam at a lower concentration of 1.0 µg/mL after thermal and matrix stress, the DB-5Q has a S/N three times greater than the commercially available 5ms type X GC column, as seen in Figure 4.

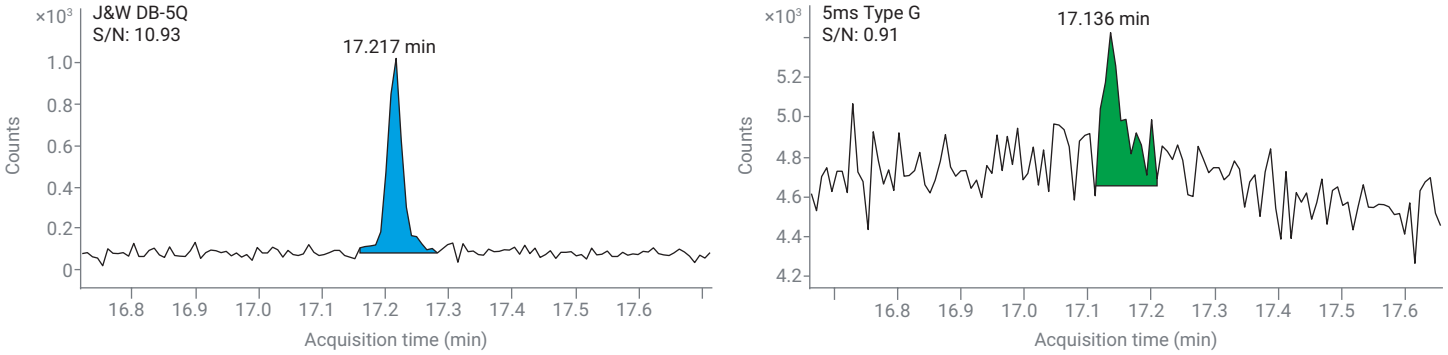

Figure 3. Extracted ion chromatogram (EIC) of nitrazepam (*m/z* 253) at a concentration 3.0 µg/mL after 145 matrix injections collected on an Agilent J&W DB-5Q and 5ms type G column.

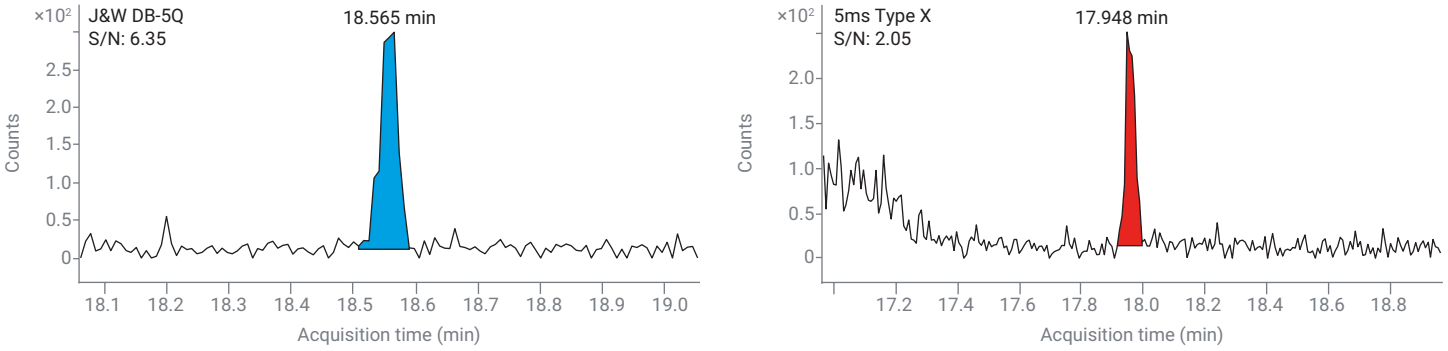

Figure 4. Extracted ion chromatogram (EIC) of alprazolam (*m/z* 204) at a concentration of 1.0 µg/mL after 145 matrix injections collected on an Agilent J&W DB-5Q and 5ms type X column.

Adsorption of basic drug compounds will cause chromatographic issues, such as poor peak symmetry and loss in response, both of these will contribute to the decreased sensitivity of the analytical method. Early eluting basic compounds, such as amines, are especially susceptible to activity, which is why it is important to optimize the method and flow path supplies for optimal peak performance and sensitivity. As shown in Figure 5, the extracted ion chromatograms (EICs) for MDA, MDMA, and MDEA were analyzed on a DB-5Q and a commercially available 5ms type X GC column at a concentration of 1.0 µg/mL. The improved peak shape of the early eluting basic compounds on the DB-5Q helps to improve the analyte response in comparison to the 5ms type X column, indicating that the DB-5Q provides good inertness for low concentrations of difficult analytes even after stressed with a heavy matrix.

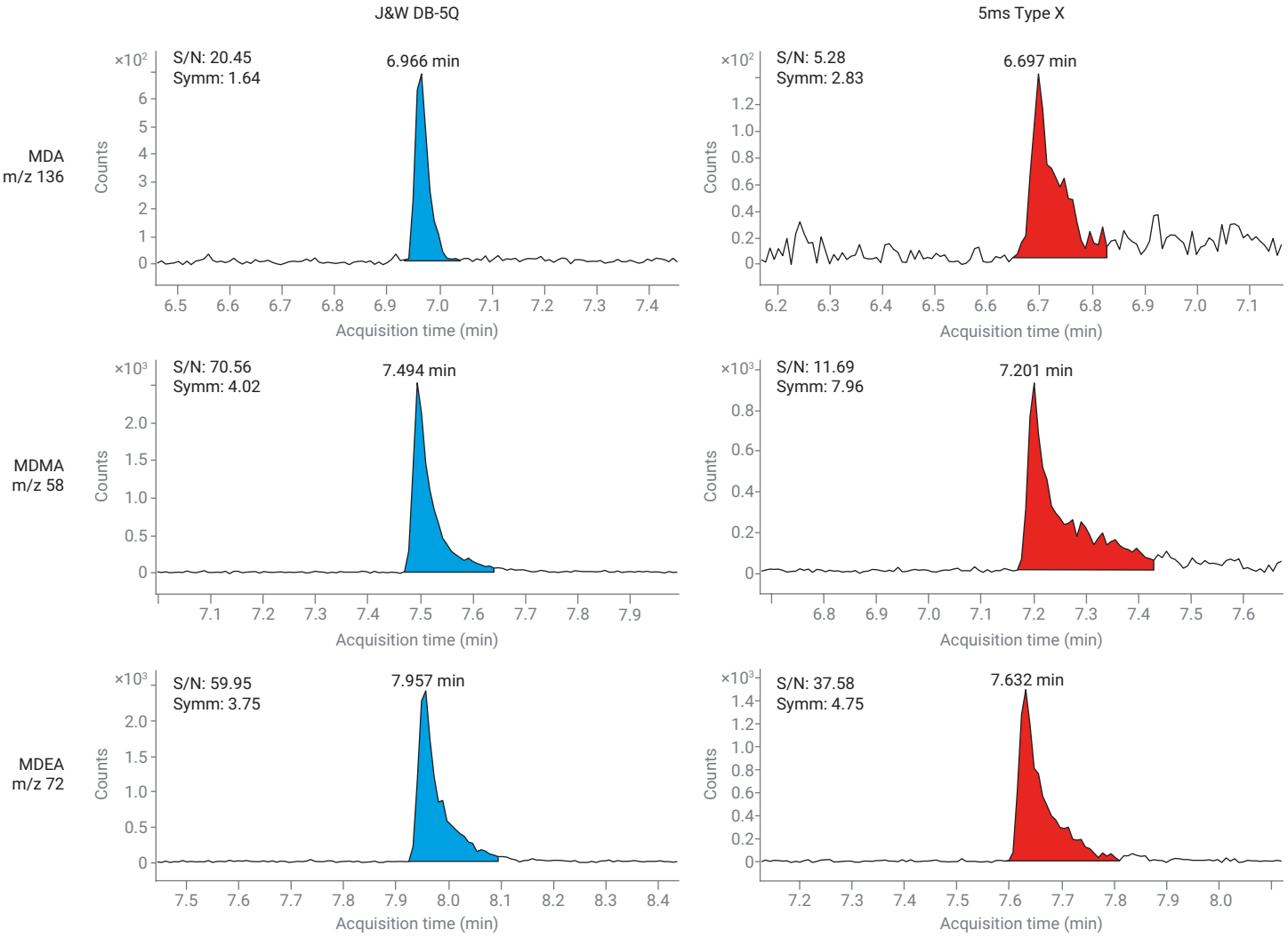

Figure 5. Extracted ion chromatogram (EIC) of amphetamines at a concentration of 1.0 µg/mL after 145 matrix injections collected on an Agilent J&W DB-5Q and 5ms type X column.

Conclusion

The increased thermal stability of the Agilent J&W DB-5Q GC column demonstrated in this application note provides a decreased baseline at higher temperatures, which allows for an increased response for later eluting compounds at trace level concentrations. The baseline of the DB-5Q remains more consistent as the temperature is increased. This consistency allows for more accurate integration and quantitation for analytes eluting during oven ramping, in comparison to other commercially available 5ms-type columns. The robust DB-5Q column phase maintained peak shape for trace level basic compounds even when stressed with a heavy matrix and is an excellent choice for the analysis of controlled substance analysis.

References

1. How Does Bleed Impact My GC/MS Data and How Do I Control It? *Agilent Technologies technical overview*, publication number 5994-7586EN, **2024**.
2. Abercrombie, V.; Biermans, F.; Gerardi, A.; Lake, R. Improved Thermal Stability Leads to Retention Time Stability and Data Accuracy for the Analysis of Pesticides. *Agilent Technologies application note*, publication number 5994-8447EN, **2025**.
3. Smith, A. Analysis of Drugs of Abuse by GC/MS Using Ultra Inert Universal Sintered Frit Liners. *Agilent Technologies application note*, publication number 5994-1012EN, **2019**.

www.agilent.com

Printed in the USA, September 12, 2025
5994-8658EN

Agilent
Trusted Answers