Analyze Drugs of Abuse with Agilent J&W Ultimate Plus Tubing in an Inert Flow Path

Application Note

Forensic Toxicology

Author

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Abstract

Agilent J&W Ultimate Plus deactivated fused silica tubing has great potential to contribute to an inert flow path for fast, sensitive, and reproducible analysis of active compounds, such as drugs of abuse. We evaluated Ultimate Plus tubing versus tubing promoted as having superior performance from another supplier, as GC restrictors for MS in the analysis of 28 active drugs. The improved inertness of Ultimate Plus tubing delivered response improvements for most of the drugs of interest, especially for highly active drugs that are significantly sensitive to an inert flow path.

Introduction

The identification and quantification of drugs of abuse in different types of biological samples such as urine or blood is important to forensic toxicology. Gas chromatography/mass spectrometry (GC/MS) is still regarded as a gold standard for drug confirmatory analysis or for screening tests. This is due to its high sensitivity and selectivity for the determination of drugs and their metabolites at trace levels in complex biological matrixes [1]. Without derivatization prior to GC, drugs of abuse are well known as chemically active compounds and often adsorb onto active sites in the flow path from GC/MS injector to detector. This results in poor peak shape, poor quantification, and peak disappearance of some highly active analytes.

Agilent Technologies

Agilent has successfully minimized active sites throughout the flow path of GC/MS systems, which allows the direct analysis of underivatized drugs with high sensitivity and reproducibility. These efforts focused on improving the inertness of flow path components that directly contact analytes, such as Agilent J&W Ultra Inert GC columns, UI liners, UltiMetal Plus Flexible Metal ferrules, and Ultimate unions [2,3,4,5]. Deactivated fused silica tubing plays a key role in GC/MS systems because it can be used as guard columns or GC restrictors that come into direct contact with the analytes of interest [6,7,8].

This application note presents an evaluation of Agilent J&W Ultimate Plus deactivated fused silica tubing used as a GC restrictor to MS interface for analysis of drugs of abuse. This allows the user to change analytical columns quickly without turning off the high vacuum of the MS. To assess performance, Ultimate Plus tubing was compared to a product from another supplier promoted as having superior performance. Conditions were set up to provide a fair comparison.

Experimental

An Agilent GC/MS toxicology analyzer check mixture standard (p/n 5190-0471) with 28 drugs was used to evaluate the performance of deactivated fused silica tubing from Agilent and another supplier. Analytical grade toluene, methanol, and acetonitrile were purchased from Sigma-Aldrich, Corp.

A 90:5:5 toluene:methanol:acetonitrile solvent mixture was used as a blank. The 5 ng/µL original standard was directly used for injection. In addition, this standard was diluted five times with blank solvent to give a 1 ng/µL solution.

Instrumentation

The experiments were performed on an Agilent 7890A GC system equipped with an Agilent 5975C Series MSD with a triple-axis detector and an Agilent Autosampler 7693A (G4513A). The experimental setup is shown in Figure 1. The analytical column was connected to the deactivated fused silica tubing using an Agilent Purged Ultimate union.

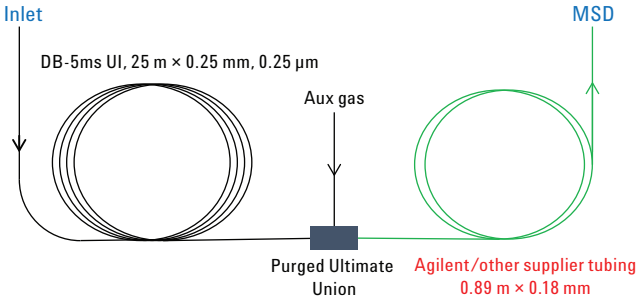

Figure 1. Experimental GC/MSD setup for testing deactivated fused silica tubing.

Conditions

Column:	Agilent J&W DB-5ms Ultra Inert, 25 m × 0.25 mm, 0.25 µm (p/n 122-5522 UI)
Pre-injection solvent:	A) dichloromethane, 5 washes B) ethanol, 3 washes
Post-injection solvent:	A) dichloromethane, 3 washes B) ethanol, 3 washes
Washes:	Sample, 2; pump, 5
Carrier gas:	He, constant flow, 1.4 mL/min
Inlet:	Pulsed splitless, 80 °C
Purge flow:	30 mL/min at 1 min
Oven profile:	95 °C for 2 min, to 320 °C at 10 °C/min, hold 5.5 min
Aux EPC gas:	He plumbed to Purged Ultimate union
Auxiliary flow:	1.8 mL/min during run
Restrictor:	Inert fused silica tubing (0.89 m × 0.18 mm), Silcotek Silconert 2000-treated fused silica, Agilent J&W Ultimate Plus, cut down from 6 m (p/n CP801806)
Autosampler syringe:	10 µL (p/n 5181-1267)
Injection volume:	1 µL
Tune file:	Atune.u
EMV mode:	Gain factor 25 corresponding to 1,659 V
Transfer line temperature:	300 °C
Source temperature:	250 °C
Quad temperature:	150 °C
Solvent delay:	3 min
Acquisition mode:	Full scan
Scan mass range:	50 to 450 amu

Flow path supplies

Vials:	Amber screw cap (p/n 5182-0716)
Vial caps:	Blue screw cap (p/n 5182-0717)
Vial inserts:	Glass, 150 µL, with polymer feet (p/n 5183-2088)
Septum:	Advanced Green nonstick, 11 mm (p/n 5190-3158)
Flow technology:	Self-tightening column nut for Agilent inlet (p/n 5190-6194), self-tightening column nut for Agilent MS interface transfer line (p/n 5190-5233), Flexible Metal ferrule, 0.1 to 0.25 mm id, for Purged Ultimate union (p/n G3188-27503), ferrule for inlet and MS interface, 0.4 mm id, 85/15 Vespel/graphite (p/n 5181-3323), Ultimate union kit, deactivated (p/n G3182-61580)
Inlet liner:	Agilent Ultra Inert deactivated single-taper splitless liner, no wool, including O-ring (p/n 5190-2292)

Results and Discussion

Figure 2 shows an example chromatogram at 5 ng/component on-column for the drug checkout standard using Agilent 0.89 m × 0.18 mm tubing as the GC/MSD restrictor. The elution order of these substances is shown in Table 1. Good peak shapes and compound responses were found for most of analytes. The most challenging compounds were oxazepam (13), lorazepam (15), and temazepam (20), with significantly low responses compared to other analytes at 5 ng on-column. The presence of active sites on deactivated fused silica tubing used as a GC restrictor can cause significant reduction in responses or even peak disappearance of these compounds, due to the loss of sensitivity caused by their adsorption or decomposition.

Figure 3 is an overlay of total ion current chromatograms of 5 ng/component on-column of the drugs of abuse checkout mixture. Ultimate Plus deactivated fused silica tubing (green chromatogram) or a deactivated fused silica tubing from another supplier (red chromatogram) were alternately installed as GC restrictors to the MS interface. The running conditions were the same for both types of tubing, which allowed a fair comparison to be made. Figure 3 indicates that Ultimate Plus tubing improves inertness performance compared to tubing from another supplier, because of better responses of most of the analytes in the checkout standard.

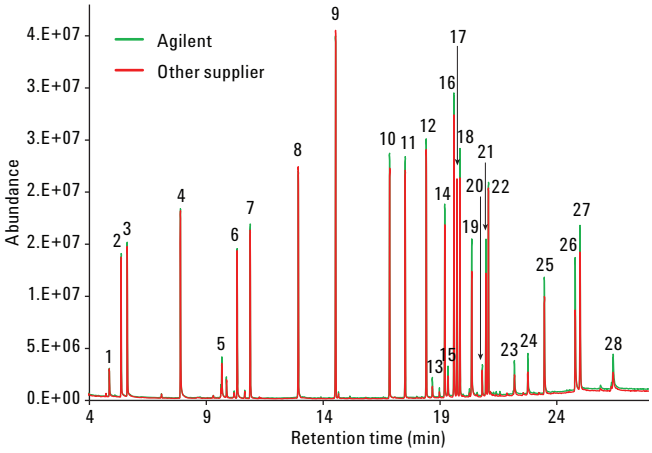

Figure 3. An overlay of total ion current chromatograms of 5 ng/component on-column of a drugs of abuse checkout mixture with Agilent J&W Ultimate Plus deactivated fused silica tubing (green chromatogram) or a deactivated fused silica tubing from another supplier (red chromatogram) used as GC restrictors for MSD (see Table 1 for peak IDs).

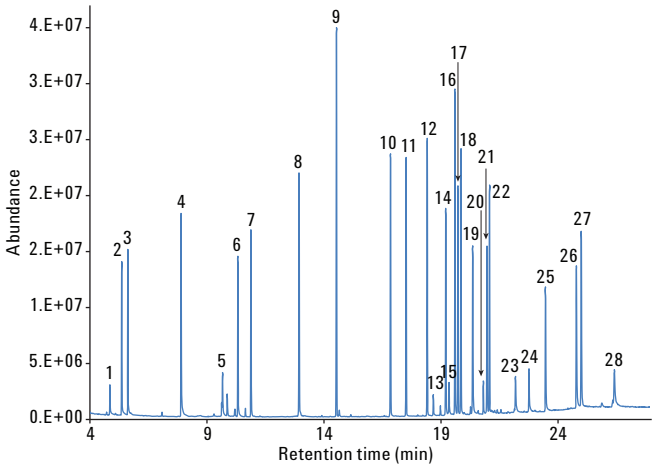

Figure 2. Total ion current chromatograms of 5 ng/component on-column of a drugs of abuse checkout mixture with Agilent J&W Ultimate Plus deactivated fused silica tubing used as a GC restrictor for MSD (see Table 1 for peak IDs).

Table 1. Drugs of abuse (peak numbers on chromatograms).

Peak no.	Compound	Retention time (min)
1	Amphetamine	4.857
2	Phentermine	5.361
3	Methamphetamine	5.624
4	Nicotine	7.9
5	MDA	9.67
6	MDMA	10.326
7	MDEA	10.884
8	Meperidine	12.94
9	Phencyclidine	14.539
10	Methadone	16.846
11	Cocaine	17.515
12	Proadifen (SKF-525a)	18.415
13	Oxazepam	18.678
14	Codeine	19.208
15	Lorazepam	19.348
16	Diazepam	19.602
17	Hydrocodone	19.729
18	Tetrahydrocannabinol	19.855
19	Oxycodone	20.359
20	Temazepam	20.81
21	Flunitrazepam	20.968
22	Diacetylmorphine	21.074
23	Nitrazepam	22.188
24	Clonazepam	22.763
25	Alprazolam	23.467
26	Verapamil	24.788
27	Strychnine	24.996
28	Trazodone	26.408

As seen in Figure 3, responses of compounds are different even though their amounts on-column are the same. More active analytes normally gave lower responses because of their adsorption or decomposition throughout the flow path from injector to detector. Figure 4 shows the extracted ion chromatograms at specific mass channels of the most highly active compounds in this checkout mixture. The peak shapes and responses of these compounds are good indicators of the inertness of flow path components. In this study, the difference in separation performance was attributed to the inertness of tubing because the experimental setup was the same for all other parts of the system. We divided the most challenging compounds as highly active compounds into two groups. Group A consisted of compounds 13, 15, and 20 (Figure 4A), and group B consisted of compounds 23, 24, and 28 (Figure 4B). The response improvements for these highly active compounds (such as oxazepam, lorazepam, temazepam, nitrazepam, and clonazepam) are key differentiators of inertness performance between the tubing because they are evidence of the reduction of numbers of active sites on the surface of the tubing. Figure 4 indicates that a significant improvement in responses of six highly active compounds was obtained when Agilent tubing was used. Ultimate Plus tubing provided better inertness for separation of these highly active drugs. This finding was also observed at a lower level of 1 ng on-column, shown in Figure 5.

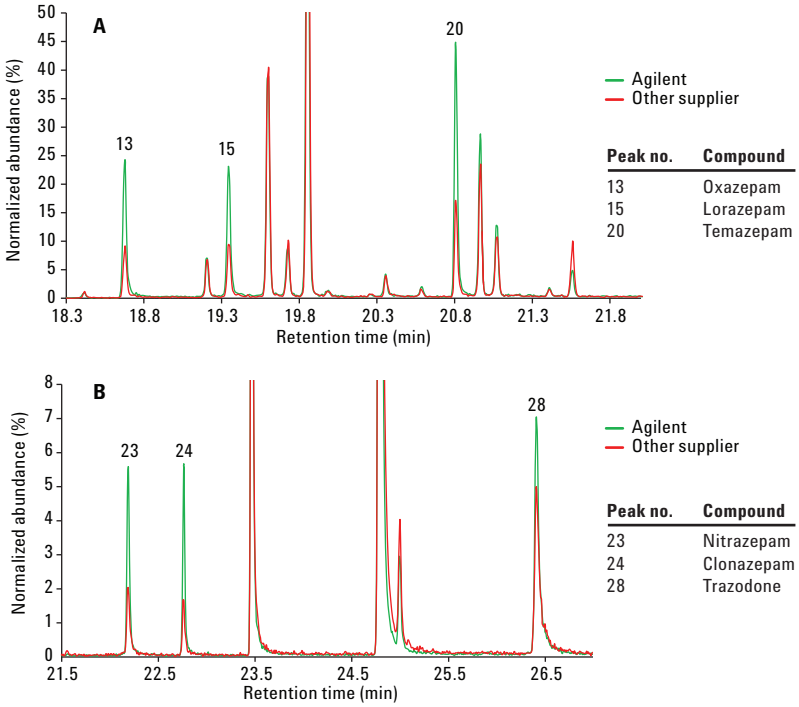

Figure 4. Extracted ion chromatograms of 5 ng/component on-column of a drugs of abuse checkout mixture with Agilent J&W Ultimate Plus deactivated fused silica tubing (green chromatogram) or deactivated fused silica tubing from another supplier (red chromatogram) used as GC restrictors for MSD. A) Group A compounds 13 (*m/z* 205), 15 (*m/z* 239), and 20 (*m/z* 271). B) Group B compounds 23 (*m/z* 280), 24 (*m/z* 280), and 28 (*m/z* 205) (see Table 1 for peak IDs).

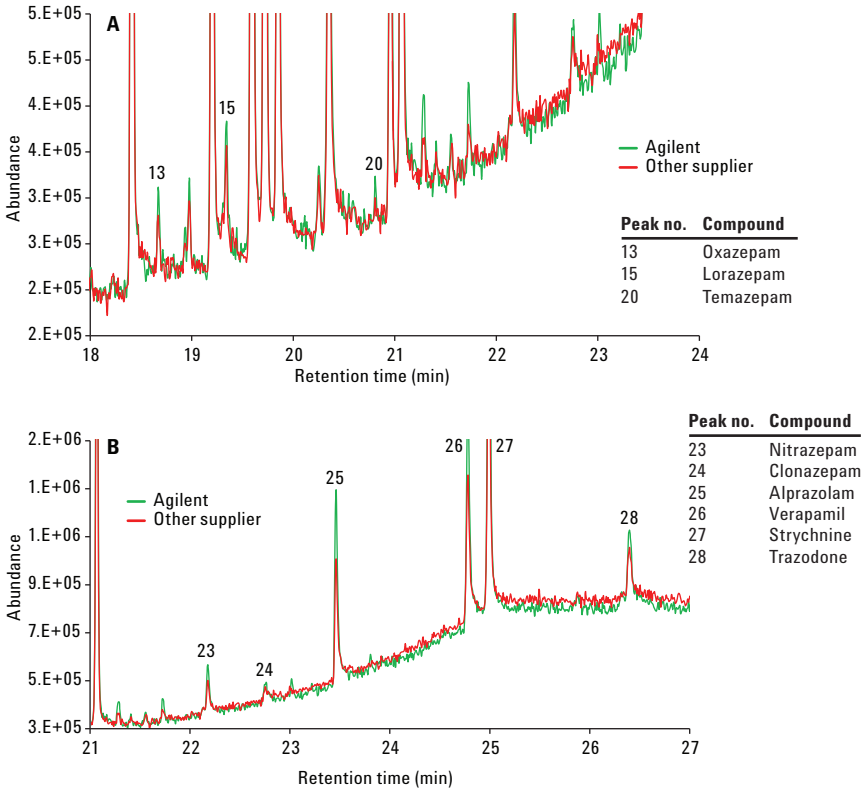

Figure 5. Total ion current chromatograms of 1 ng/component on-column of a drugs of abuse checkout mixture with Agilent J&W Ultimate Plus deactivated fused silica tubing (green chromatogram) or deactivated fused silica tubing from another supplier (red chromatogram) used as GC restrictors for MSD. A) Group A compounds 13, 15, and 20. B) Group B compounds 23, 24, and 28 (see Table 1 for peak IDs).

To compensate for errors due to imperfect injection, such as variation in injection volume, the peak areas of all compounds were normalized to a specific compound. Compound 9 (phencyclidine) was chosen because it was considered as stable and less active compared to the rest of the analytes. Figure 6 shows the normalized results of 28 drugs at 5 ng/component on-column from Agilent or other supplier tubing with GC/MS in full scan mode. An improvement in the responses of most of the analytes was found.

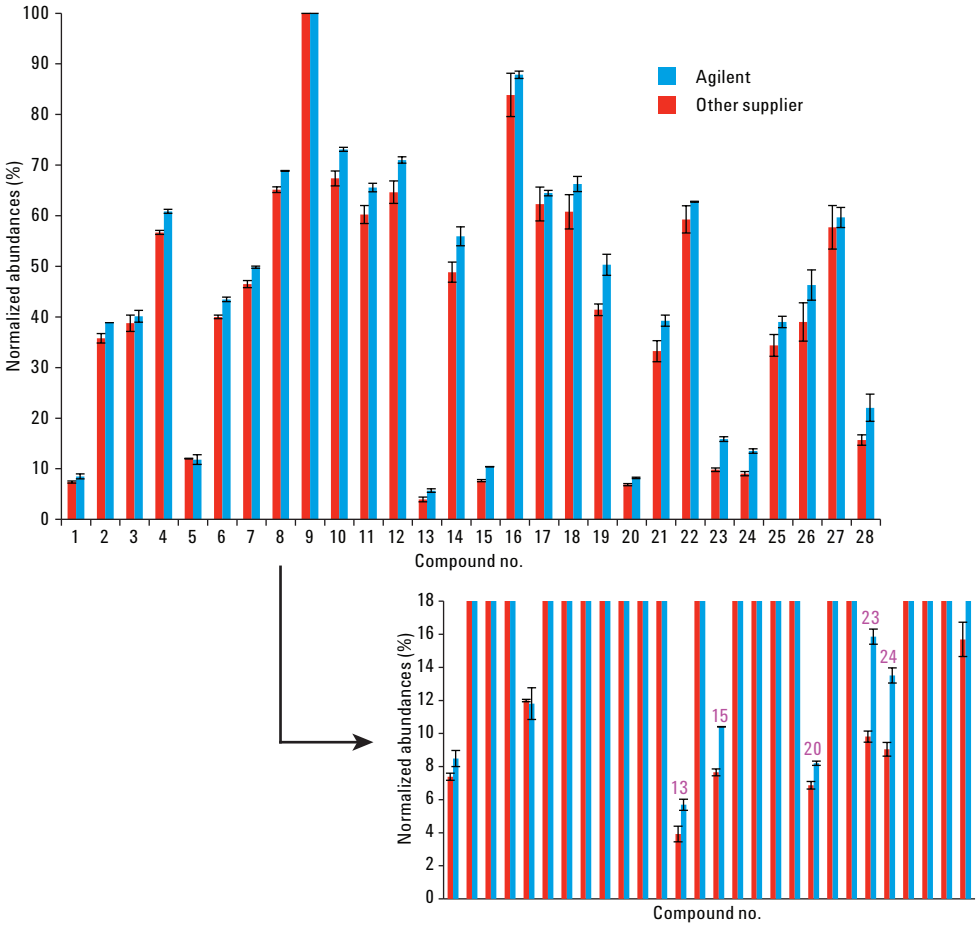

Figure 6. Normalized peak areas of duplicate injections of 5 ng/component on-column of a drugs of abuse checkout mixture with Agilent J&W Ultimate Plus deactivated fused silica tubing (blue bars) or deactivated fused silica tubing from another supplier (red bars) used as GC restrictors for MSD (see Table 1 for peak IDs). Full scan mode was selected for recording the MS signals. Improved responses of active compounds (13, 15, 20, 23, and 24) are highlighted in the insert figure. The peak areas of all compounds were normalized to compound 9, phencyclidine.

Ultimate Plus tubing delivered significant improvements in responses for highly active compounds, including compounds 13, 15, 20, 23, and 24 (see insert in Figure 6). Figure 7 compares differences in the percentage of response of each compound from Agilent and another supplier tubing when the performance of the other supplier tubing was set to 100%, and the performance of Agilent tubing was scaled. Agilent tubing provided better inertness, with clear improvements in response from highly active compounds.

Conclusions

Agilent J&W Ultimate Plus deactivated fused silica tubing delivered response improvements for most compounds in a GC/MS forensic toxicology checkout mixture compared to premium-performance deactivated fused silica tubing from another supplier. The response improvements for highly active compounds (such as oxazepam, lorazepam, temazepam, nitrazepam, and alprazolam) obtained from the Agilent tubing are key indicators of better inertness performance compared to tubing from another supplier. These results are encouraging and suggest that Ultimate Plus deactivated fused silica tubing can play a key role in contributing to an inert flow path for analysis of drugs of abuse at trace levels.

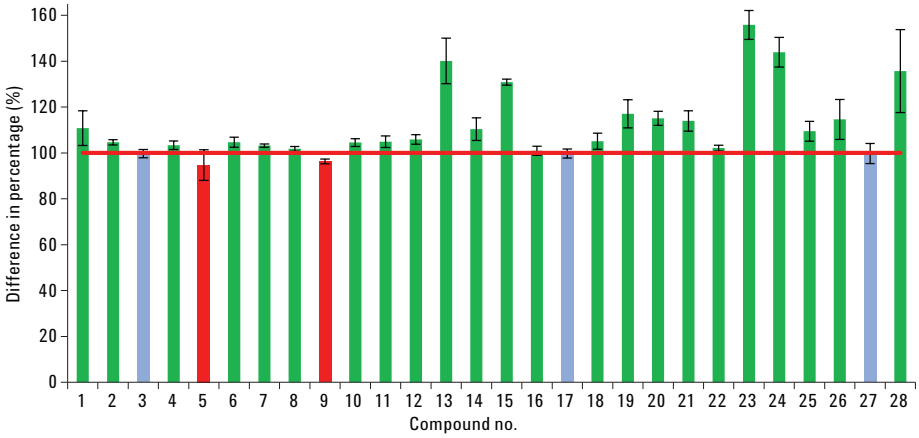

Figure 7. Analyte response comparison for Agilent and other supplier tubing. The results were based on the average response (duplicate injections) of 5 ng/component on-column of a drugs of abuse checkout test mixture using GC/MS in full scan mode. The response of each analyte for tubing from the other supplier was set to 100% (normalized) and the response of each analyte was scaled for Agilent J&W Ultimate Plus tubing.

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