Agilent Inert Flow Path Enhancements Improve Drugs of Abuse Testing

Application Note

Forensics & Drug Testing

Author

Ken Lynam

Agilent Technologies, Inc.

Abstract

An evaluation of Siltite versus Agilent UltiMetal Plus Flexible Metal ferrules installed in an Agilent Ultimate Union showed improved benzodiazepine peak shape and response for UltiMetal Plus Flexible Metal ferrules by FID. An Agilent 7890/5975C GC/MS Inert Flow Path equipped with a split/splitless inlet showed peak shape and response improvements for the benzodiazepine class of drugs when compared to a standard flow path split/splitless inlet in both SCAN and SIM modes. Example forensic/toxicology check-out mix chromatograms and overlays are shown.

Introduction

Drugs of abuse are chemically active substances that readily find and adsorb onto active sites anywhere in the flow path of a modern GC/MS system. In gas phase analysis, analytes must survive the passage from injection to detection. This is difficult to achieve when active analytes are compounds of interest, are tracked at low level, and when the flow path has surface activity. As GC/MS detection limits are pushed lower, the need for a suite of inert flow path components to line the flow path that analytes must travel through becomes ever more critical. Activity anywhere in the flow can lead to poor peak shape, poor quantitation, or missed analytes.

Previous efforts to eliminate active sites throughout the flow path have focused on column and liner deactivation strategies [1, 2]. These strategies have been successful to the point where a focus on additional components in the flow path is the next logical step toward achieving a totally inert flow path. Inlet weldments, gold seals, and ferrules used to make gas tight connections are all flow path components that come in contact with analytes of interest [3].

Lessons learned developing surface deactivation strategies for Agilent J&W Ultra Inert columns, liners, and inert MS source component designs serve as an excellent foundation for taking inertness to the next level. These strategies were employed to expand the inert flow path to include split/splitless top and shell weldments, Ultra Inert Gold Seals, and UltiMetal Plus Flexible Metal ferrules. This application note presents a preliminary evaluation of the latest version of the Agilent Inert Flow Path solution for drugs of abuse testing.

Example chromatograms of drugs of abuse analytes injected on both inert flow path and standard flow path equipped split/splitless inlets highlight the impact an inert flow path has on this analyte set. For the sake of comparison, the same Agilent J&W HP-5ms UI GC column was used to generate data using both inlets, serving as a control for direct evaluation of the components comprising the inert and standard inlets.

Experimental

The GC/FID system consisted of an Agilent 7890 GC and an Agilent 7693 Automatic Liquid Sampler. This system was used to evaluate the effect of UltiMetal Plus Flexible Metal ferrules in the flow path on drugs of abuse analytes. A capillary flow technology (CFT) union was used post-column and installed with Siltite or UltiMetal Plus Flexible Metal ferrules. All other conditions were the same. Chromatographic conditions for the GC/FID system are listed in Table 1.

Table 1. Chromatographic conditions for the Agilent 7890 FID system used to evaluate ferrules.

Column:	Agilent J&W HP-5ms UI, 30 m × 0.25 mm, 0.25 µm (p/n 19091S-433UI)
Restrictor:	1.0 m × 0.25 mm id deactivated tubing
Oven:	95 °C 0.5 min hold, 10 °/min to 280 °C, 6 °/min to 300 °C (3.67 min hold)
Gas purifier:	Gas Clean GC/MS, 1/8 in kit (p/n CP17974)
Carrier:	Hydrogen, 74.9 cm/s (3 mL/min) set at 95 °C, EPC constant flow
Inlet:	Split/splitless with standard weldment components
Injection:	Splitless, 1 µL, 280 °C, 50 mL/min purge at 0.35 min
Inlet liner:	Ultra Inert liner with wool (p/n 5190-2293)
Gold seal:	Standard gold seal (p/n 5190-2209, 10/pk)
Syringe:	Blue line, 5 µL (p/n G4513-80206)
CFT:	Ultimate Union (p/n G3182-6580)
Ferrules:	Standard Siltite (p/n 5188-5361), UltiMetal Plus Flexible Metal ferrules (p/n G3188-27501)
Detector:	FID, 320 °C, H_2 40 mL/min, air 400 mL/min, makeup 30 mL/min

The GC/MS system consisted of a 7890 GC coupled to an Agilent 5975C MSD with triple axis detector. A single 7693 Automatic Liquid Autosampler tower was used and switched between front and back split/splitless inlets. The front inlet was set up as the inert inlet, and the rear inlet was set up as a standard inlet. As much as possible, conditions for SCAN and SIM mode experiments were kept the same. The same column was used in both inlets and carefully switched, without trimming, from one inlet to the other. Table 2 shows the chromatographic conditions for the inert flow path GC/MS inlet. Table 3 shows the chromatographic conditions for the standard flow split/splitless GC/MS inlet, Table 4 shows the SIM ion details.

Table 2. Chromatographic conditions for an Agilent 7890/5975C inert flow path inlet.

Column:	Agilent J&W HP-5ms UI, 30 m × 0.25 mm, 0.25 µm (p/n 19091S-433UI)
Oven:	100 °C 4 min hold, 10 °/min to 280 °C, 6 °/min to 300 °C (4.67 min hold)
Gas purifier:	Gas Clean GC/MS, 1/8 in kit (p/n CP17974)
Carrier:	Helium, 52.7 cm/s (2 mL/min) set at 100 °C, EPC constant flow
Inlet:	Split/splitless with inert shell and top weldments
Injection:	Splitless, 1 µL, pulsed, 35 psi pulse to 0.73 min, 0.75 min purge 50 mL/min, gas saver 20 mL/min at 2 min
Inlet liner:	Ultra Inert liner with wool (p/n 5190-2293)
Gold seal:	Ultra Inert Gold Seal (p/n 5190-6144 UI)
Syringe:	Blue line, 5 µL (p/n G4513-80206)
Ferrules:	UltiMetal Plus Flexible Metal ferrules at inlet (p/n G3188-27501), MS (p/n 5188-5361)
Detector:	MSD SCAN Mode, 40 to 450 amu, 300 °C source temp, 150 °C Quad temp, 310 °C transfer line MSD SIM Mode, 300 °C source temp, 150 °C Quad temp, 310 °C transfer line

Table 3. Chromatographic conditions for an Agilent 7890/5975C standard flow path inlet.

Column:	Agilent J&W HP-5ms UI, 30 m × 0.25 mm, 0.25 µm (p/n 19091S-433UI)
Oven:	100 °C 4 min hold, 10 °/min to 280 °C, 6 °/min to 300 °C (4.67 min hold)
Gas purifier:	Gas Clean GC/MS, 1/8 in kit (p/n CP17974)
Carrier:	Helium, 52.7 cm/s (2 mL/min) set at 100 °C, EPC constant flow
Inlet:	Split/splitless with standard shell and top weldments
Injection:	Splitless, pulsed, 35 psi pulse to 0.73 min, 0.75 min purge 50 mL/min, gas saver 20 mL/min at 2 min
Inlet liner:	Standard single taper liner with wool (p/n 5190-3165)
Gold seal:	Standard gold-plated seal with washer (p/n 5190-2209, 10/pk)
Syringe:	Blue line, 5 µL (p/n G4513-80206)
Ferrules:	UltiMetal Plus Flexible Metal ferrules at inlet (p/n G3188-27501), MS (p/n 5188-5361)
Detector:	MSD SCAN Mode, 40 to 450 amu, 300 °C source temp, 150 °C Quad temp, 310 °C transfer line MSD SIM Mode, 300 °C source temp, 150 °C Quad temp, 310 °C transfer line

Table 4. SIM ion details.

Analyte (peak elution order)	SIM T, Q1		RT (min)	Collection window (min)
Amphetamine (1)	**44**,	91	4.05	
Phentermine (2)	**58**,	134	4.72	
Methamphetamine (3)	**58**,	91	5.12	
Nicotine (4)	**84**,	133	7.92	3.2 to 8.30
Methylenedioxyamphetamine (MDA) (5)	**44**,	135	9.88	
Methylenedioxymethamphetamine (MDMA) (6)	**58**,	135	10.61	
Methylenedioxyethylamphetamine (MDEA) (7)	**72**,	135	11.23	8.3 to 12.0
Meperidine (8)	**71**,	247	13.38	
Phencyclidine (9)	**200**,	242	14.94	
Methadone (10)	**72**,	57	17.44	
Cocaine (11)	**182**,	82	17.99	12.0 to 18.3
Prodifen (SKF-525a) (12)	**86**,	99	18.99	
Oxazepam (13)	**239**,	267	19.13	
Triphenyl phosphate (14) ISTD	**326**,	325	19.31	
Codeine (15)	**299**,	162	19.57	
Lorazepam (16)	**239**,	274	19.82	
Diazepam (17)	**256**,	283	20.05	
Hydrocodone (18)	**299**,	242	20.13	
Tetrahydrocannabinol (19)	**231**,	314	20.27	18. 3 to 20.5
Oxycodone (20)	**315**,	230	20.76	
Temazepam (21)	**271**,	273	21.28	
Flunitrazepam (22)	**312**,	286	21.44	
Diacetylmorphine (heroin) (23)	**327**,	369	21.61	
Nitrazepam (24)	**253**,	206	22.6	
Clonazepam (25)	**314**,	286	23.24	
Alprazolam (26)	**279**,	308	24.04	20.5 to 25.0
Strychnine (27)	**334**,	335	25.77	
Verapamil (28)	**303**,	304	25.82	
Trazodone (29)	**205**,	70	27.51	25.0 to end

Table 5 lists the flow path supplies used with the GC/FID and GC/MS systems.

Table 5. Additional Agilent supplies.

Vials:	Amber, silanized, screw top (p/n 5183-2072)
Vial caps:	Blue, screw cap (p/n 5185-5820)
Vial inserts:	Glass/polymer feet, 250 µL (p/n 5181-8872)
Septum:	Advanced Green (p/n 5183-4759)
Gas purifiers:	Gas Clean GC/MS 1/8 in kit (p/n CP17974)
	FID alternative Gas Clean, 4-position, 1/8 in. kit (p/n CP736530)
Inlet liners:	Ultra Inert single taper liner with wool (p/n 5190-3163, 5/pk) Agilent original single taper liner with wool (p/n 5062-3587)
Gold seal:	Gold-plated inlet seal with washer (p/n 5190-2209, 10/pk)
Standard ferrule:	0.4 mm id short, 85/15 Vespel/graphite (p/n 5181-3323, 10/pk)
PCT fitting:	Internal nut (p/n G2855-20530)
Inert ferrule:	UltiMetal Plus Flexible Metal ferrules (p/n 5188-5361, 10/pk)
Magnifier:	20x Magnifier (p/n 430-1020)
Syringe:	Replaceable needle, PTFE plunger, 1 mL (p/n 5190-1539), 0.5 mL (p/n 5190-1525); 5 µL (p/n 5181-1273)

Standard preparation

A 28 component GC/MS forensic/toxicology analyzer checkout mix at a nominal concentration of 5 ng/µL was obtained from Agilent Technologies, Inc. (Santa Clara, CA) (p/n 5190-0471). This mixture was transferred and serially diluted using Class A volumetric glassware and positive displacement syringes. A mix of toluene:methanol:acetonitrile (90:5:5) was prepared to serve as diluent and syringe wash solvent. Ultra Resi-analyzed grade toluene and methanol (J. T. Baker) and high-purity acetonitrile (Burdick and Jackson) were purchased through VWR International. Triphenyl phosphate was prepared for use as an internal standard and added at a nominal final concentration of 0.25 ng/µL.

Results and Discussion

A performance evaluation of UltiMetal Plus Flexible Metal ferrules was done using an FID-equipped 7890 GC by using the same inlet and swapping ferrules, and connecting a post-column ultimate union in line with flexible metal and Siltite ferrules. All other conditions were kept constant to provide as fair a comparison as possible.

Figure 1 shows an example chromatogram at the 1 ng/component on-column level for the forensic/toxicology checkout mix. The setup for this injection included a post-column union connected by UltiMetal Plus Flexible Metal ferrules. Considering that there were amphetamines, opioids, and sensitive benzodiazepines in this mix, the peak shapes and responses were excellent. Some minor peak tailing of nitrazepam and clonazepam was observed.

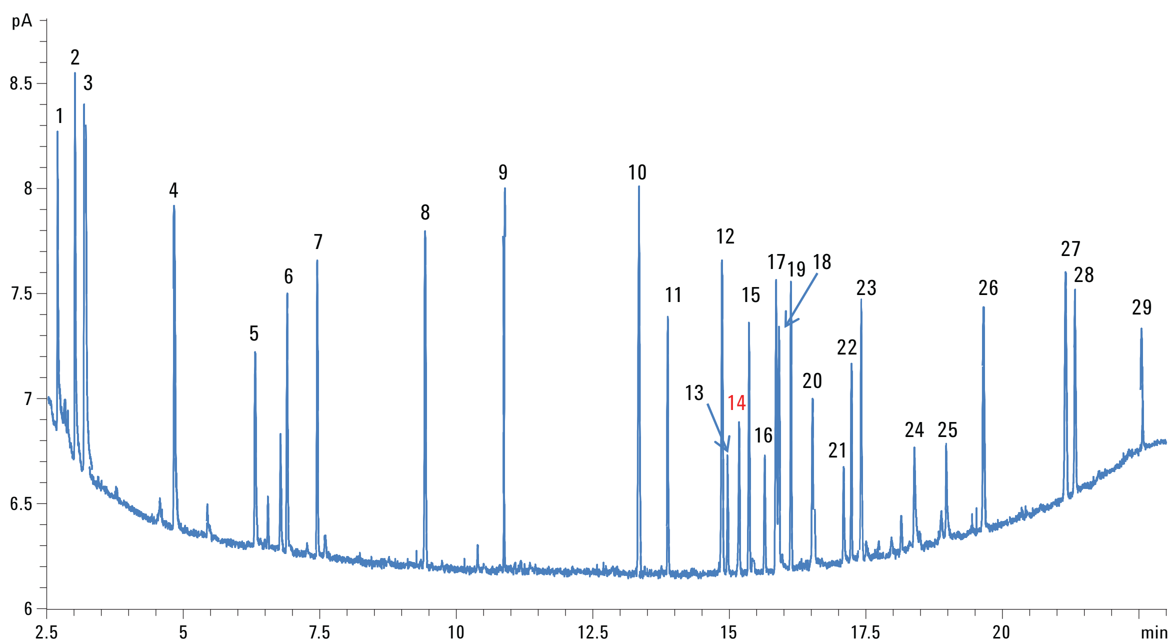

Figure 1. Example FID test chromatogram of forensic/toxicology checkout standard 1 ng/component on an Agilent J&W HP-5ms UI GC column (triphenyl phosphate internal standard added separately). (See Table 1 for GC conditions and Table 4 for peak IDs.)

Figure 2 is an overlay of the FID signals obtained when UltiMetal Plus Flexible Metal ferrules (blue) were installed in the union and Siltite ferrules in (red). The set up was the same for both injections except for changing the ferrules in the union. Peak tailing for nitrazepam and clonazepam was more severe when the Siltite ferrules were installed. Less signal for alprazolam and a dramatic decrease in signal were observed for temazepam with the Siltite ferrules. This suggested that the UltiMetal Plus Flexible Metal ferrules were a better choice when working with sensitive benzodiazepines in drugs of abuse testing.

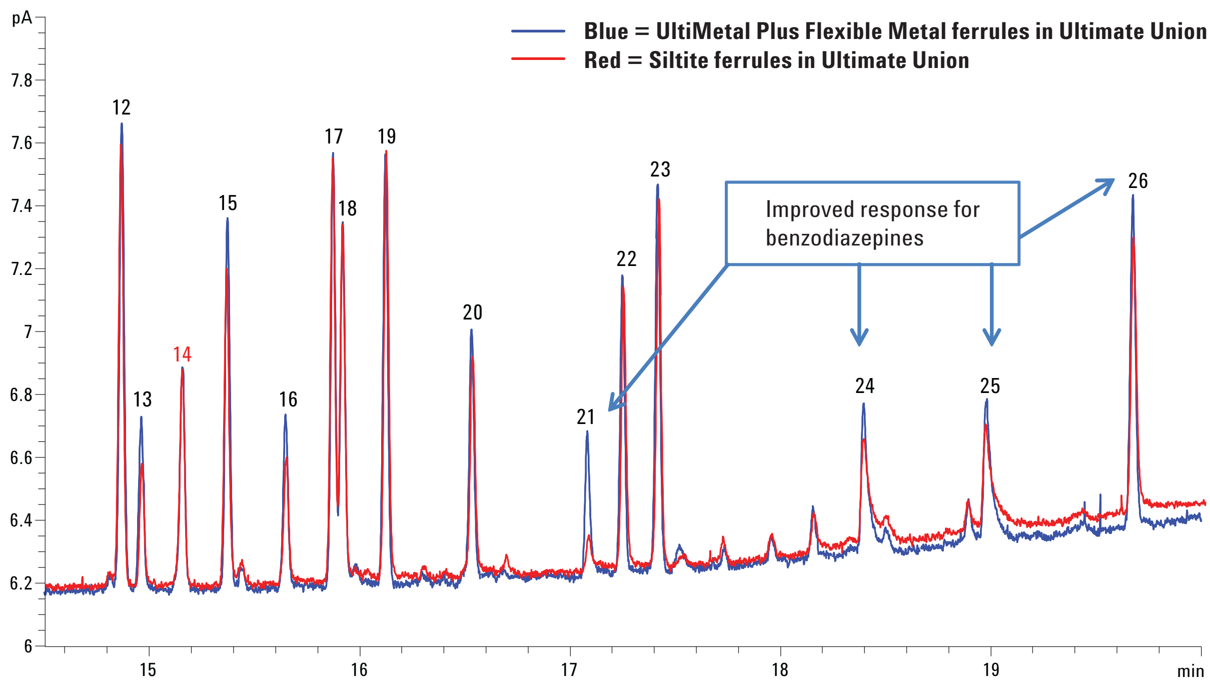

Figure 2. Example FID overlay of 1 ng/component forensic/toxicology mix injected on flow paths with Agilent UltiMetal Plus Flexible Metal ferrules and Siltite ferrules installed in a post-column ultimate union. (Refer to Table 1 for GC/FID conditions and Table 4 for peak IDs.)

Figure 3 displays a total ion chromatogram of the forensic/toxicology checkout mix at the 1 ng/component on-column level on an inert-flow-path equipped 7890/5975C GC/MS. Excellent peak shapes were evident for all components in the mix. Switching between the standard and inert flow paths was accomplished by venting the system, carefully removing the column nut at the inlet, and transferring it directly to the other inlet. This was done to use the column, the inlet connector, and MSD system as controlled variables to provide as fair a comparison as possible.

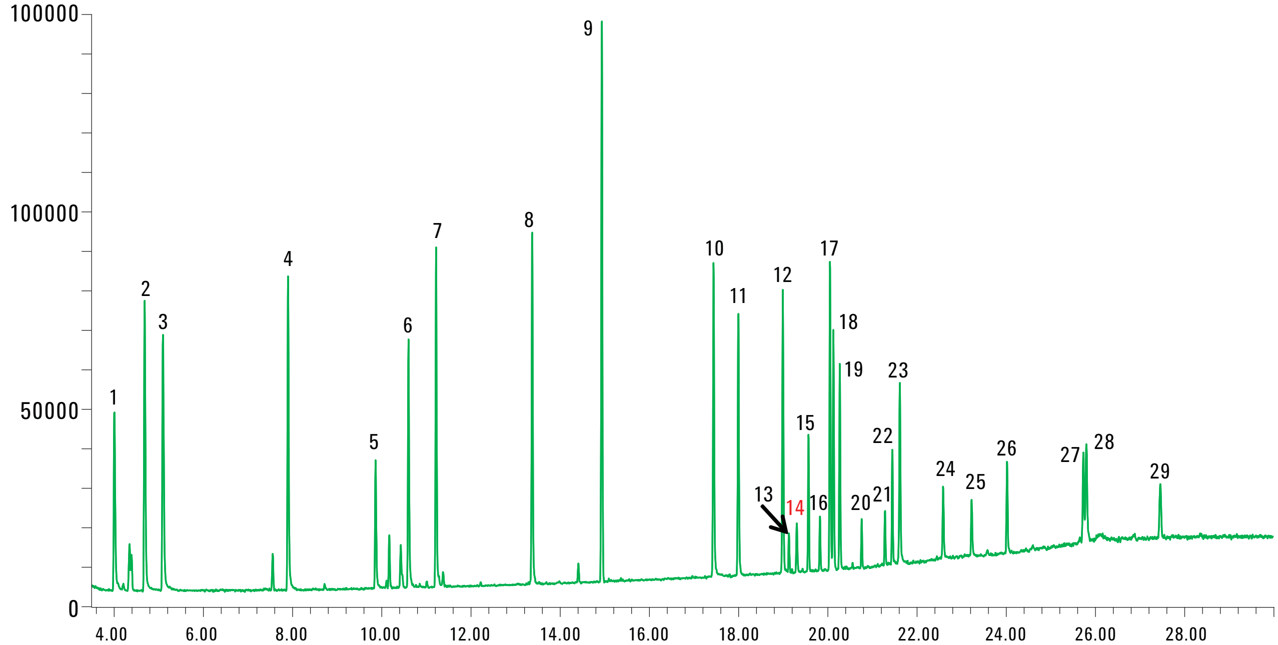

Figure 3. Example TIC at 1 ng/component on-column forensic/toxicology checkout standard on an Agilent J&W HP-5ms UI GC column (triphenyl phosphate internal standard added separately). (Refer to Table 2 for GC/MS SCAN conditions.)

Figure 4 highlights the difference between inert inlet and standard inlet performance for the benzodiazepines region of the total ion chromatogram where they elute. At a 0.5 ng/component loading level in SCAN mode, the temazepam peak disappeared and very poor response was observed for nitrazepam and clonazepam on the standard flow path system. The Inert Flow Path made a difference for these analytes. Temazepam showed a sharp definable peak using the inert flow path and each of the benzodiazepine peaks was sharper, with higher responses. The diacetyl morphine (heroin) peak was also sharper with higher response.

Figure 5 verifies the difference between inert and standard inlet performance for benzodiazepines using a SIM method. Temazepam disappeared completely using the standard flow path at the 0.25 ng/component level. Nitrazepam and clonazepam peaks were also quite broad and disappeared into the noise.

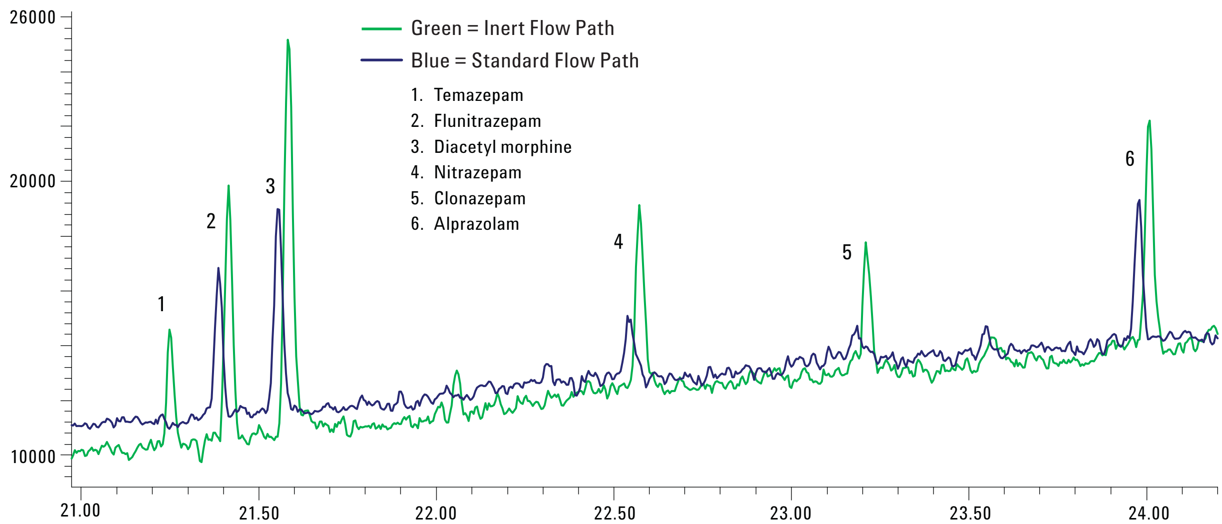

Figure 4. Overlay of a 0.5 ng/component on-column forensic/toxicology checkout standard on an Agilent J&W HP-5ms UI GC column highlighting, the differences between the Inert Flow Path inlet and the standard inlet using a SCAN method. (Refer to Tables 2 and 3 for inert and standard flow path conditions.)

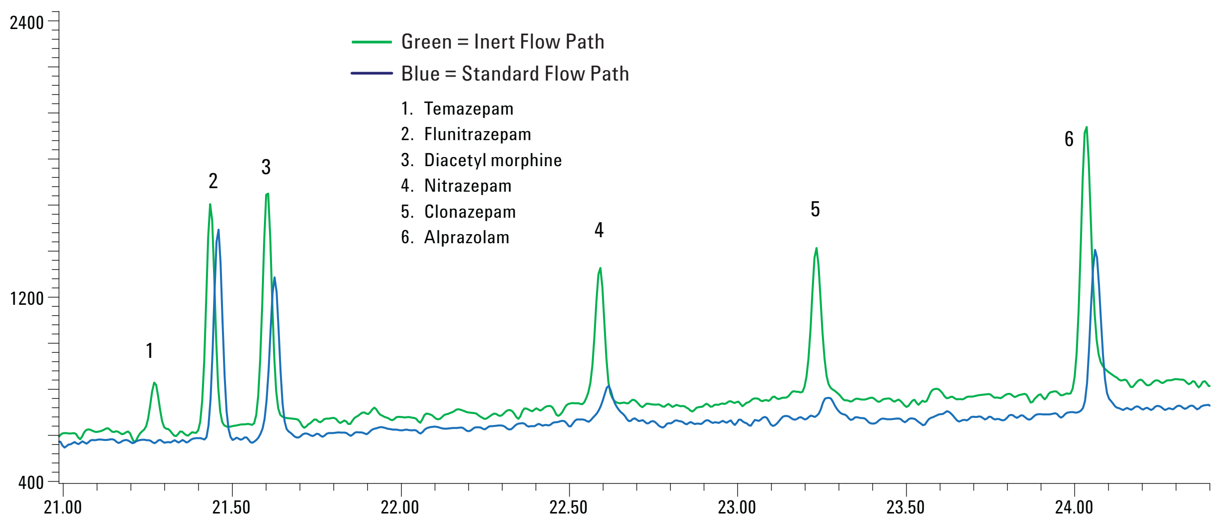

Figure 5. Overlay of 0.25 ng/component on-column forensic/toxicology checkout standard on an Agilent J&W HP-5ms UI GC column, highlighting the differences between the Inert Flow Path inlet and the standard inlet using a SIM method. (Refer to Tables 2 and 3 for inert and standard flow path conditions.)

Conclusions

Agilent UltiMetal Plus Flexible Metal ferrules showed peak shape and response improvements for several analytes in a forensic/toxicology checkout mix when compared to Siltite ferrules. The improvement was observed primarily for the benzodiazepine class of drugs. Temazepam at the 1 ng on-column level by FID was nearly lost in the noise using Siltite ferrules installed post-column in an Ultimate Union, whereas the same setup with UltiMetal Plus Flexible Metal ferrules gave a sharp, well-defined peak. This was a somewhat unexpected yet encouraging result given the limited amount of surface area in contact with analytes on the surface of the ferrules installed in an Ultimate Union. For work with benzodiazepines, Agilent UltiMetal Plus Flexible Metal ferrules are a better option than Siltite.

The IFP inlet on the Agilent 7890/5975C GC/MS system also showed improved performance for the benzodiazepine class of drugs against the standard component inlet. Temazepam was a key differentiator between the inert flow path inlet and the standard one. This was true with both SCAN and SIM experiments. Temazepam, nitrazepam and clonazepam all seemed to disappear much faster into the baseline using a standard flow path inlet than with an Inert Flow Path inlet. These results are encouraging and suggest that better detection and quantitation of benzodiazepines is readily achievable with an inert flow equipped inlet.

References

1. K. Lynam. *Semivolatile Analysis Using an Inertness Performance Tested Agilent J&W DB-5ms Ultra Inert Column*. Application Note, Agilent Technologies, Inc. Publication number 5989-8616EN (2008).
2. L. Zhao, A. Broske, D. Mao, A. Vickers. *Evaluation of the Ultra Inert Liner Deactivation for Active Compounds by GC.* Technical Overview, Agilent Technologies, Inc. Publication number 5990-7380EN (2011)
3. Anon. *Optimize your GC flow path for inertness*. Poster, Agilent Technologies, Inc. Publication number 5990-8902EN (2013).

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

Printed in the USA
February 21, 2013
5991-1859EN

Agilent Technologies