Application Note

Forensics

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# Analysis of Drugs of Abuse by GC/MS Using Ultra Inert Universal Sintered Frit Liners


**Author**

Angela Smith Henry, Ph.D.
Agilent Technologies, Inc.



## Abstract

Gas chromatography/mass spectrometry (GC/MS) is a common screening technique for controlled substance analyses. Maintaining an inert flowpath is important in these analyses to prevent loss of peak shape and signal of the more sensitive or active compounds, such as amphetamine or oxycodone. A sintered frit liner offers the same protection from complex nonvolatile matrices as glass wool liners, while avoiding wool breakage that may cause loss of peak response.

## Introduction

Maintaining a clean and inert GC/MS system starts at the inlet with an inert flowpath, specifically with the inlet liner. Using deactivated liners provides a good start for preventing peak degradation in the inlet. Inlet liners with glass wool are used because the wool provides a large surface area to aid in sample vaporization. Wool also provides a barrier to trap nonvolatile residue from sample matrices.[1,2] However, glass wool in liners can re-introduce active sites over the lifetime of the liner that manifest as a decrease in peak response or degradation of peak shape. A sintered frit liner provides the surface area for vaporization, and can reduce the sample loss by preventing sample droplets from reaching the bottom of the inlet before vaporization. Fritted liners also provide the ability to trap nonvolatile residue, while removing the possibility of new active sites from broken glass wool, or the intrusion of glass wool into the head of the column.

GC/MS screening methods are important for laboratories running controlled substance analyses, as new illicit substances continue to enter the marketplace, and a target compound list can stretch into the hundreds. For compounds compatible with GC, GC/MS can be used in full scan mode with electron ionization (EI) mode to complete the controlled substance screening. Some compounds, such as amphetamines, can be very sensitive to inlet parameters, inlet liners, and the solvent for dilution. These amphetamines may exhibit bad peak shape if the inlet liner and parameters are not optimized. Improving amphetamine peak shape and good peak response remain important factors in liner technologies. Powder samples are generally dissolved in a solvent such as methanol, hexane, or toluene, while liquid samples are extracted into GC-amenable solvents, and possibly diluted to avoid overloading the GC column or MS detector. Liners were tested with the Agilent forensic toxicology checkout mixture, which includes compounds from different classes such as amphetamines, opiates, and benzodiazepines, along with other high-concentration mixtures of amphetamines, opiates, and cannabinoids.

## Experimental

### Chemicals and reagents

The Agilent GC/MS forensic toxicology checkout mixture standard (p/n 5190-0471, 5 µg/mL) was used to test the liners. Table 1 lists the compounds found in the mixture with the retention times. HPLC/GC grade toluene and methanol were purchased from MilliporeSigma (Burlington, MA, USA). An internal standard (ISTD) mixture of six deuterated polyaromatic hydrocarbons (PAHs) was purchased, containing 2 mg/mL of 1,4-dichlorobenzene-$d_4$, acenaphthene-$d_{10}$, naphthalene-$d_8$, phenanthrene-$d_{10}$, chrysense-$d_{12}$, and perylene-$d_{12}$ in acetone. The 5 µg/mL checkout mixtures had 1 µL of ISTDs added per 1 mL of the checkout mixture for a final concentration of 2 µg/mL for the ISTDs.

Multiple mixtures were purchased from Cerilliant Corporation (Round Rock, TX, USA) and from Cayman Chemical (Ann Arbor, MI, USA) to test peak shape integrity with the sintered frit liner. The multicomponent opiate mixture contained methadone, codeine, hydrocodone, meperidine, and oxycodone each at 250 µg/mL. The amine mixture contained amphetamine, methylenedioxyethylamphetamine (MDEA), methamphetamine, methylenedioxymethamphetamine (MDMA), Methylenedioxyamphetamine (MDA), and phentermine, each at 250 µg/mL. The cannabinoid mixture contained cannabidiol, cannabinol, and tetrahydrocannabinol ($\Delta^9$-THC) at concentrations of 1.0 mg/mL. GC/MS drug mixture 1 contained varied concentrations of caffeine (40 µg/mL), methadone (30 µg/mL), cocaine (30 µg/mL), codeine (50 µg/mL), 6-monoacetylmorphine (75 µg/mL), and heroin (75 µg/mL). GC/MS drug mixture 3 contained methamphetamine, cocaine, heroin, fentanyl, and alprazolam at concentrations of 1.0 mg/mL.

An acetaminophen caplet was used to simulate a real-world matrix. The acetaminophen caplet was crushed and dissolved in a 25% methanol/75% water mixture. The methanol/water/acetaminophen mixture was then extracted into toluene for GC injection.

### Instrument conditions

An Agilent 7890 GC was connected to an Agilent 5977B Inert Plus GC/MSD with a 9 mm extraction lens. The GC was also equipped with an Agilent 7650A automatic liquid sampler, which has the larger turret to hold up to 50 samples. A 20 m × 0.18 mm × 0.18 µm column was used to increase the system efficiency of the analysis with a split 20:1 injection, as controlled substance samples tend to be at higher concentrations. This method has been used for other forensic drug screening applications.[3] The Agilent universal single taper frit liner (p/n 5190-5105) was chosen as the primary test case. The Inert Plus MSD was run in scan mode with extraction tune (etune.u). Table 2 lists the GC and MSD instrumentation and consumables, and Table 3 lists the method parameters.

**Table 1.** Agilent GC/MS forensic toxicology checkout mixture compounds and retention times in elution order.

| No. | Compound | Retention Time (min) |
|---|---|---|
| 1 | Amphetamine | 1.535 |
| 2 | Phentermine | 1.684 |
| 3 | Methamphetamine | 1.758 |
| 4 | Nicotine | 2.536 |
| 5 | MDA | 3.272 |
| 6 | MDMA | 3.563 |
| 7 | MDEA | 3.818 |
| 8 | Meperidine | 4.803 |
| 9 | Phencyclidine | 5.602 |
| 10 | Methadone | 6.762 |
| 11 | Cocaine | 7.09 |
| 12 | Proadifen | 7.556 |
| 13 | Oxazepam | 7.678 |
| 14 | Codeine | 7.948 |

| No. | Compound | Retention Time (min) |
|---|---|---|
| 15 | Lorazepam | 8.017 |
| 16 | Diazepam | 8.144 |
| 17 | Hydrocodone | 8.213 |
| 18 | THC | 8.276 |
| 19 | Oxycodone | 8.531 |
| 20 | Temazepam | 8.758 |
| 21 | Flunitrazepam | 8.832 |
| 22 | Heroin | 8.896 |
| 23 | Nitrazepam | 9.441 |
| 24 | Clonazepam | 9.748 |
| 25 | Alprazolam | 10.177 |
| 26 | Verapamil | 11.231 |
| 27 | Strychnine | 11.358 |
| 28 | Trazadone | 12.666 |

**Table 2.** GC and MSD instrumentation and consumables.

| Parameter | Value |
|---|---|
| GC | Agilent 7890 GC |
| MS | Agilent 5977B Inert Plus GC/MSD with inert EI source |
| Drawout Plate | 9 mm (p/n G3870-20449) |
| Column | Agilent DB-5ms Ultra Inert, 20 m × 0.18 mm × 0.18 µm (p/n 121-5522) |
| Liner | Agilent universal single taper with sintered frit (p/n 5190-5105) |
| Inlet Septum | Agilent Advanced Green, nonstick 11 mm septum (p/n 5183-4759 for 50 pack) |
| Autosampler | Agilent 7650A automatic liquid sampler |
| Vials | Agilent A-Line certified amber (screw top) vials; 100/pk (p/n 5190-9590) |
| Vial Inserts | Agilent deactivated vial inserts; 100/pk (p/n 5181-8872) |
| Vial Screw Caps | Agilent screw caps, PTFE/silicone/PTFE septa, cap size: 12 mm; 500/pk (p/n 5185-5862) |
| Test Mixture | Agilent GC/MS forensic toxicology checkout mixture standard, 5 µg/mL (p/n 5190-0471) |

**Table 3.** GC and MSD instrument conditions.

| Parameter | Value |
|---|---|
| Injection Volume | 1 µL |
| Inlet | Split/splitless inlet 250 °C;<br>Split 20:1;<br>Standard septum purge (3 mL/min) |
| Column Temperature Program | 110 °C, 20 °C/min to 300 °C (hold 4.5 minutes) |
| Carrier Gas and Flow Rate | Helium at 1.5 mL/min, constant flow |
| Transfer Line Temperature | 280 °C |
| Mode | Scan |
| Ion Source Temperature | 250 °C |
| Quadrupole Temperature | 150 °C |
| Mass Range | *m/z* 40 to 500 |
| A/D Samples | 4 |
| Tune | Etune.u |

## Results and discussion

This testing evaluated the Ultra Inert sintered frit liners for screening of controlled substances by GC/MS. The ability of the liners was determined by chromatographic evaluation, liner-to-liner reproducibility, and response repeatability across multiple injections on the same liner.

### Chromatographic performance and amphetamine peak shape

The adsorption or decomposition of basic drug compounds may cause a variety of chromatographic issues such as distorted peak shapes, broad or tailing peaks, or loss in response and sensitivity. Mixtures of individual compound classes, specifically amines, opiates, and cannabinoids were tested for these chromatographic issues before moving onto samples with mixed compound classes. Peak shape issues or loss of sensitivity commonly occur for the early-eluting amine compounds such as amphetamine, methamphetamine, phentermine, and MDA. This method was designed for a high-efficiency column for better results, where the method was optimized for peak shape of the amines.[3] Figure 1 illustrates the optimization of the method for the early-eluting amine peaks. The amphetamine mixture was injected neat, at 250 µg/mL, with the 20:1 split lowering the concentration to 12.5 µg/mL on-column. This high-concentration sample was used, since controlled substance testing tends to have higher concentration samples. The amine compounds are well resolved with well shaped peaks in both the total ion chromatogram (TIC) and extracted ion chromatograms (EICs) (Figure 1A and 1B). The amines mixture was also diluted with toluene to a final analyte concentration of 10 µg/mL. An injected sample of 10 µg/mL results in 0.5 µg/mL on-column, and is used to verify peak shape integrity at lower concentrations and system inertness.

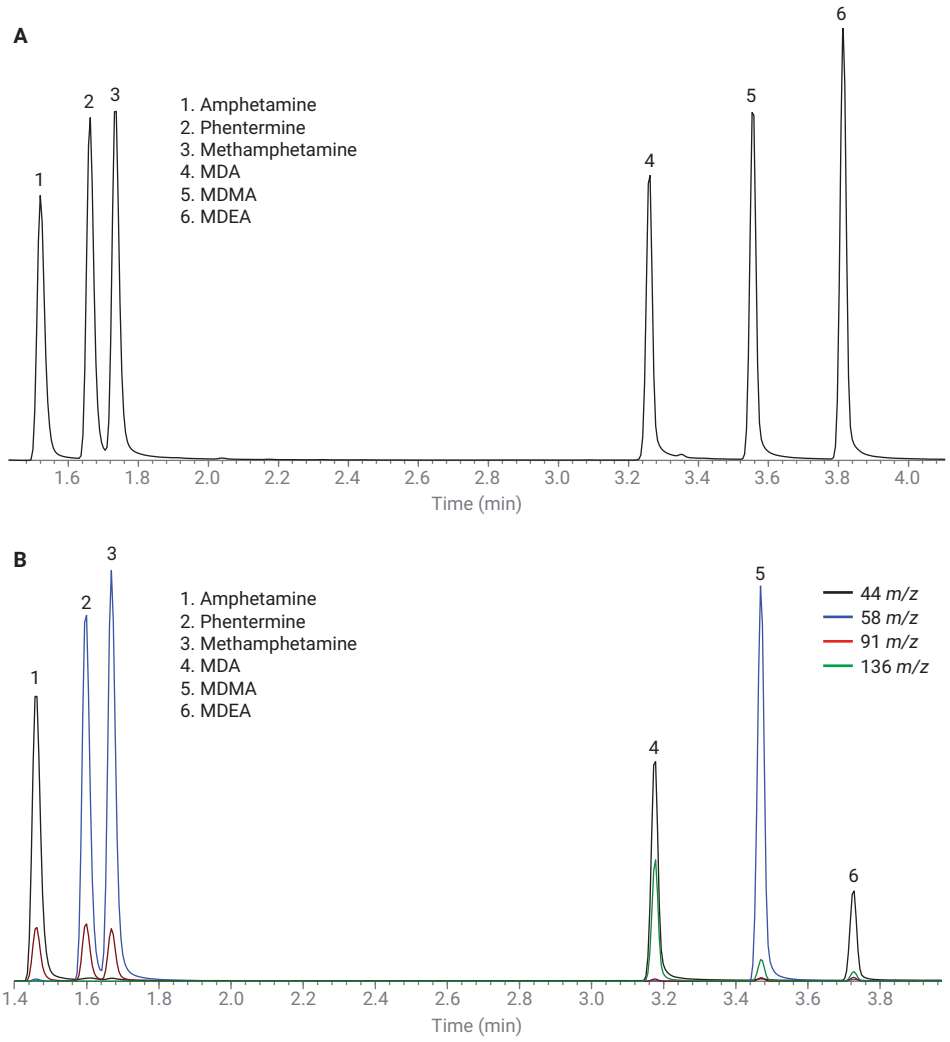


**Figure 1.** A) TIC of amphetamine mixture at 250 µg/mL (split 20:1 for on-column concentration of 12.5 µg/mL); B) extracted ion chromatograms (EICs) of amphetamine mixture at 250 µg/mL.

As shown in Figure 2, the EICs for the amine compounds at 10 ppm retain similar peak shape and responses as the higher concentration results, indicating that the method, liner, and flowpath are optimized and inert.

Peak shapes and resolution of other compound classes were also evaluated with additional standard mixtures, including opiates and cannabinoids. The opiates mixture was tested at neat concentration (250 µg/mL injected), and with a 10 µg/mL sample, diluted in toluene. Hydrocodone and oxycodone can be sensitive indicators for activity in the flowpath. If the column is degrading or the inlet and liner have active sites or are becoming dirty, the peak heights will diminish, and for oxycodone, a significantly broad, tailing peak will develop. Viewing Figure 3A, the high-concentration standard (250 µg/mL) shows excellent separation and response for these opiates. There is minor tailing across all compounds. However, injection of the 10 µg/mL sample (Figure 3B) illustrates less tailing, indicating that the tails are related to the concentration on-column rather than flowpath activity. The cannabinoids standard was diluted to 10 µg/mL in toluene. Similar to the other compound class mixtures, the cannabinoids are well resolved from each other and have excellent peak shape, as observed in Figure 4.

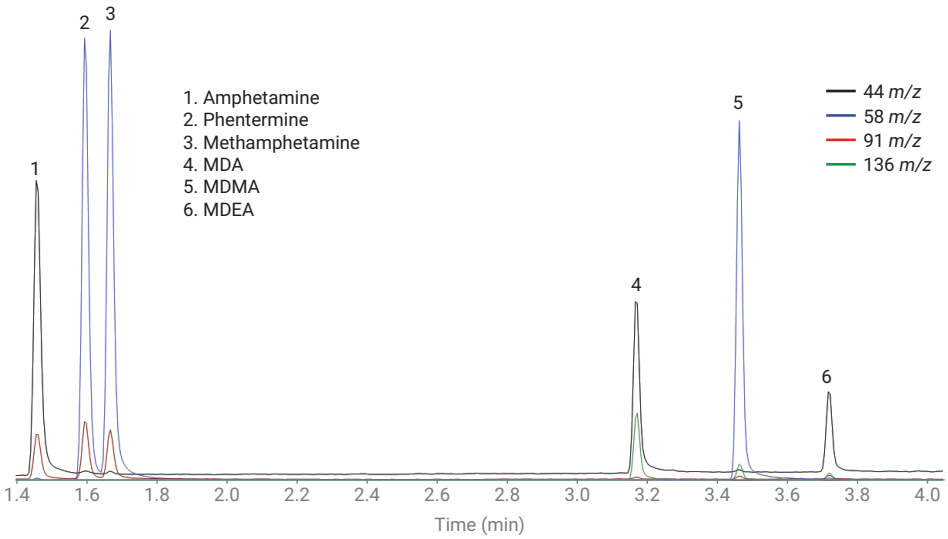


**Figure 2.** EICs of amphetamine mixture at 10 µg/mL (split 20:1 for on-column concentration of 0.5 µg/mL).

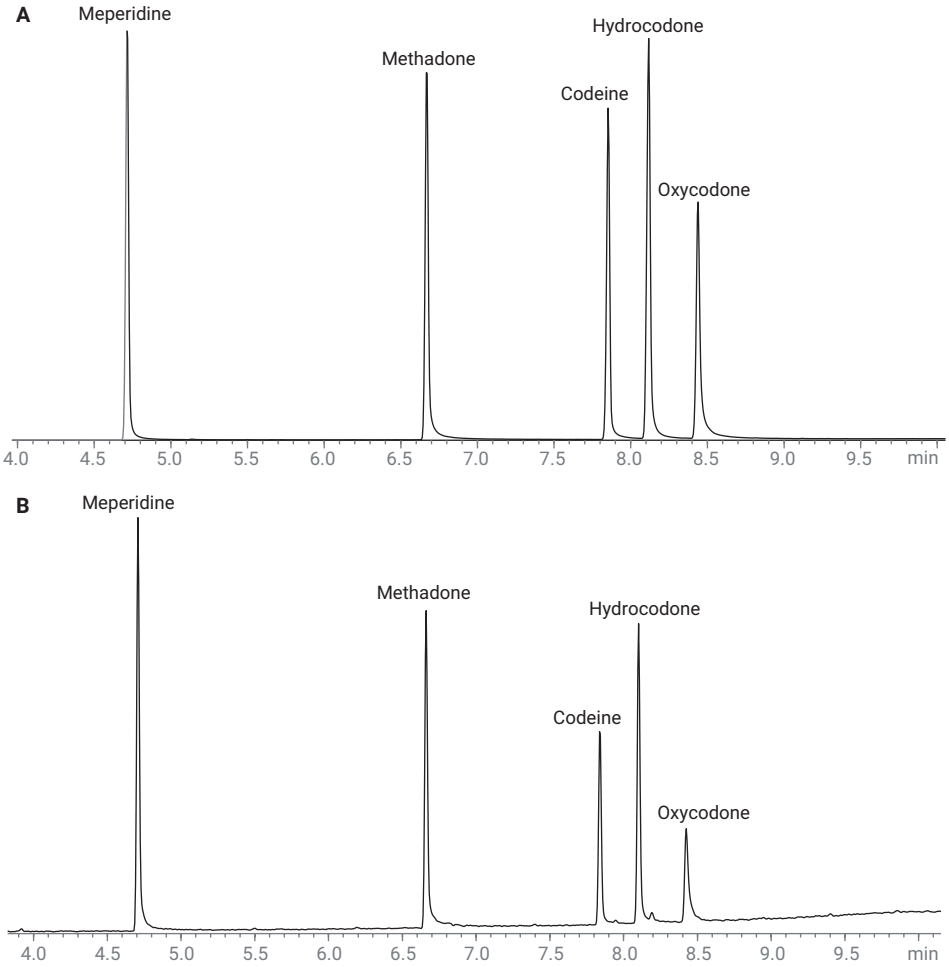


**Figure 3.** TICs of opiates mixture at A) 250 µg/mL, which is split 20:1 for on-column concentration of 12.5 µg/mL and at B) 10 µg/mL, split for on-column concentration of 0.5 µg/mL.

Two drug mixtures (GC/MS drug mixtures 1 and 3) were used to test the interactions of different compound classes with each other, the sintered frit liner, and the high-efficiency column. GC/MS drug mixture 1 was run at its neat concentration and diluted by a factor of 4 to test sub-1 µg/mL concentrations on-column. Figure 5 shows the chromatogram of the diluted sample. The neat concentration sample is not shown, as the chromatogram exhibits very similar peak shapes. All compounds in drug mix 1 have good peaks shapes and resolution from each other.

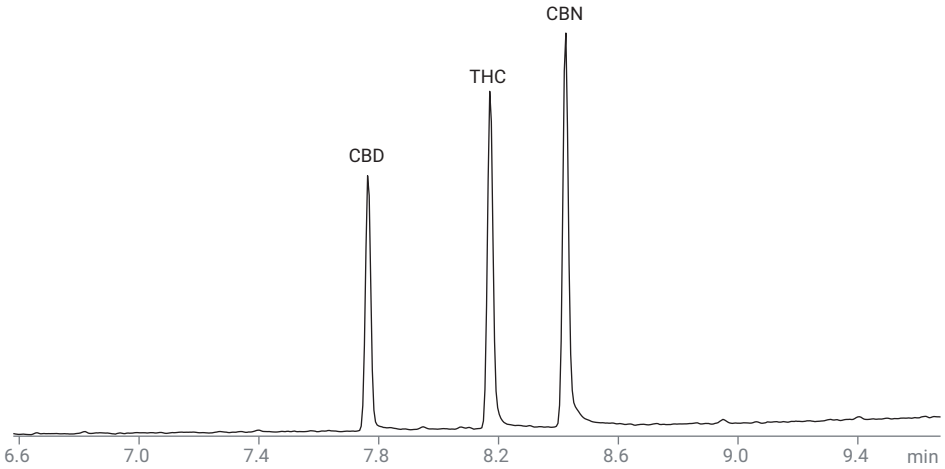


**Figure 4.** TIC of cannabinoids mixture at 10 µg/mL injected, which was split for on-column concentration of 0.5 µg/mL.

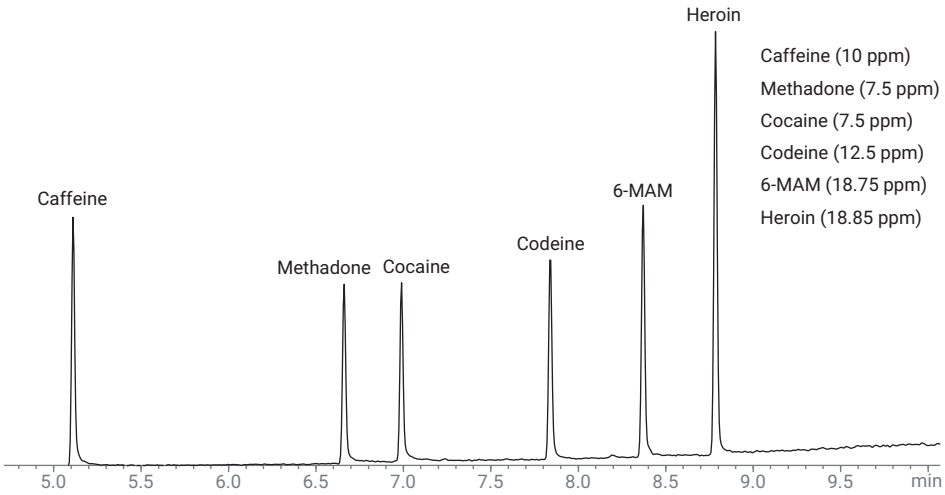


**Figure 5.** TIC of GC/MS drug mixture 1 at concentrations of: caffeine at 10 µg/mL, methadone and cocaine at 7.5 µg/mL, codeine at 12.5 µg/mL, and 6-monoacetylmorphine (6-MAM) and heroin at 18.85 µg/mL injected. The injection was split 20:1, as described in method parameters, to test low concentrations in liner and on-column.

GC/MS drug mixture 3 contained a mixture of methamphetamine, cocaine, heroin, fentanyl, and alprazolam to test drug analytes of current concern in controlled substance analyses. The mixture was diluted to 100 and 10 µg/mL with toluene. The TICs for these concentrations are shown in Figure 6 (A and B), where there is excellent resolution between the compounds. In the 100 µg/mL TIC (Figure 6A), the responses for all compounds are strong, but minor tailing is observed with all peaks. However, injection of the 10 µg/mL sample (Figure 6B) illustrates less tailing, indicating that the tails are related to the concentration on-column, or possibly source temperature, rather than flowpath activity.

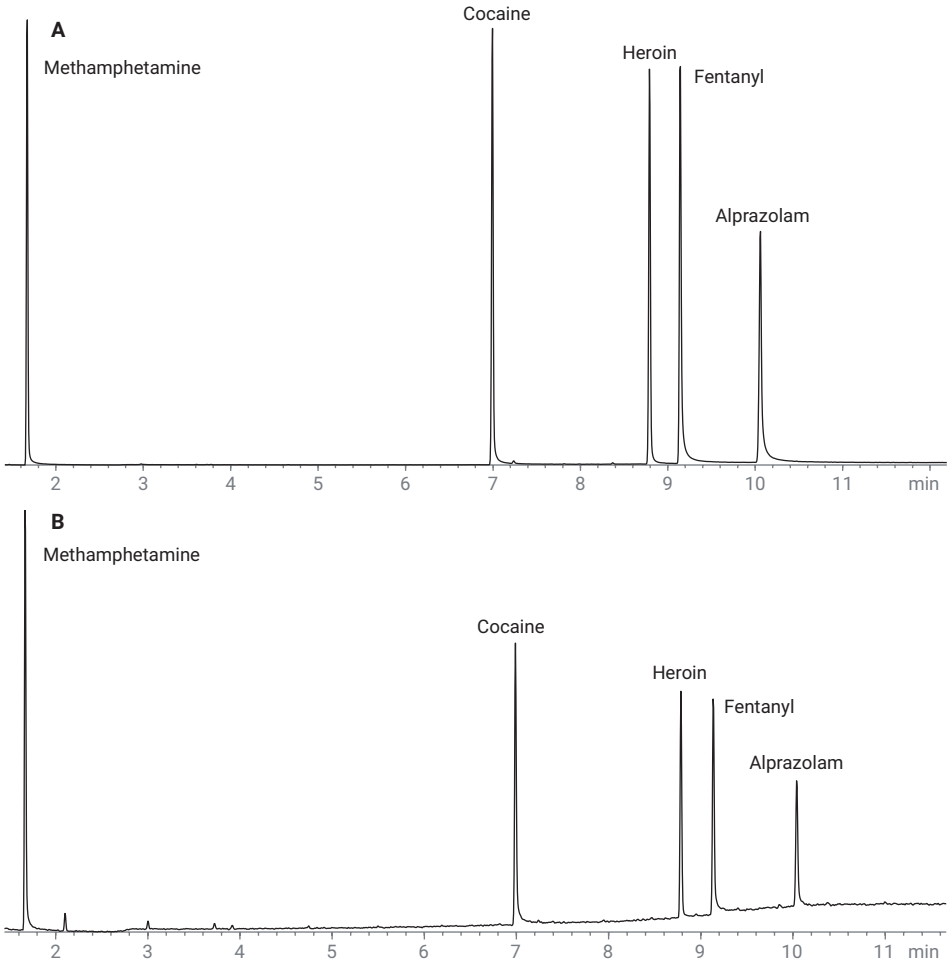


**Figure 6.** TICs of GC/MS drug mixture 3 at concentrations of A) 100 µg/mL injected, with 5 µg/mL on-column, and B) 10 µg/mL injected, with 0.5 µg/mL on-column.

After the method was optimized with focus on the amine compounds, the GC/MS forensic toxicology checkout mixture, with internal standards, was reviewed for compound resolution and peak shape integrity. Figure 7 displays the TIC for the checkout mixture (5 µg/mL) and internal standards (2 µg/mL), where most compounds are resolved from each other in the TIC. In several cases, the internal standards elute very close to target analytes, but are nearly baseline resolved in the TIC and are fully baseline resolved in the EICs. The most notable example is methamphetamine and naphthalene-$d_8$, which elute very closely and are not baseline resolved in the TIC. However, the characteristic ions for methamphetamine and naphthalene-$d_8$ are different, and baseline resolution can be found in the EICs. Another set of features that can indicate issues with flowpath inertness are the peak shapes of benzodiazepines and serotonin reuptake inhibitors. If the system is not inert or clean enough, the peaks for these compound classes may show a loss in response, broadening, or increase in tailing. However, the peaks in the TIC (Figure 7) do not show significant tailing or broadening, which indicates that the system, and specifically the Ultra Inert frit liner, is inert.

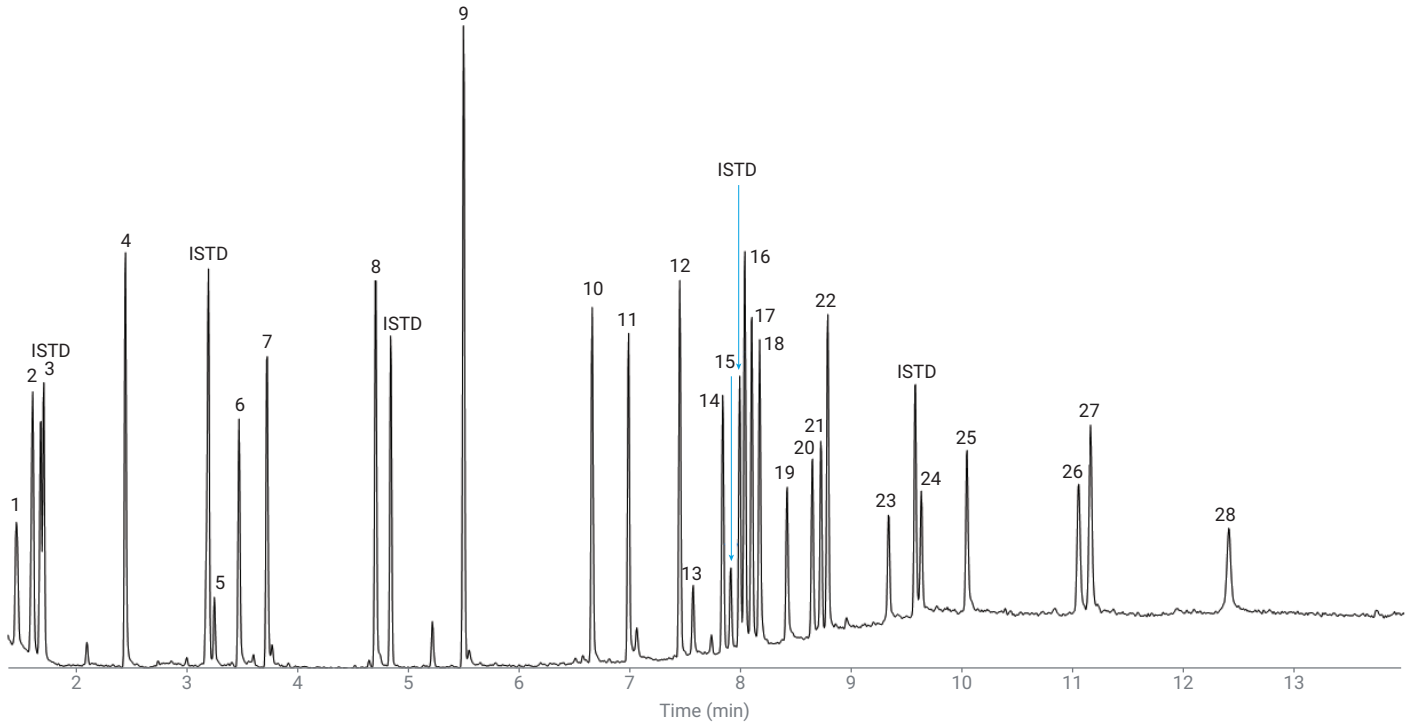


**Figure 7.** TIC of Agilent forensic toxicology checkout mixture standard at 5 µg/mL (injected, 0.25 µg/mL on column) and internal standards at 2 µg/mL (injected, 0.1 µg/mL on-column). Peaks have been enumerated with the corresponding compounds in Table 3. 1,4-Dichlorobenzene-$d_4$ elutes in the solvent delay, and is not included.

### Injection repeatability

Multi-injection repeatability was tested to verify liner and system inertness with multiple injections of a standard. The forensic toxicology checkout mixture (5 µg/mL) was spiked with 2 µg/mL of the deuterated polyaromatic hydrocarbon internal standard mixture to track response factors (RFs) for the target analytes. With the split injection, the target analyte concentration was 0.25 µg/mL on-column, and the ISTD concentration was 0.1 µg/mL, where the lower concentrations can be used to probe the liner consistency and deactivation processes. Ten replicate injections were completed on each liner; the RFs for each run were calculated using the area of the EICs of each compound. The 10 RFs were averaged together per compound to produce an average RF and %RSD value per compound and liner. The first test involved 10 repeated injections of 1 µL of 5 µg/mL checkout mixture, using the same split method parameters found in Table 3. Table 4 shows the %RSD values per liner for 12 drug analytes with 0.25 ng on-column; all compounds can be found in Appendix Table A1. The on-column concentration of 0.25 ng was used because higher concentrations can hide some deviation in responses and generate better repeatability. Lower level concentrations are a more stringent test of the liner and GC/MSD system overall.

In the selected compounds of Table 4, the liners show great injection repeatability, with %RSD values below 15% for each compound and set of 10 replicate injections. Furthermore, all compounds (Appendix Table A1) except clonazepam have %RSD values below 10% RSD, indicating that each liner and the overall system is inert. Benzodiazepines are best analyzed by GC when derivatized;[4,5,6] in this checkout mixture, the benzodiazepines are not derivatized, which may account for the higher %RSD for clonazepam on liner 5.

**Table 4.** Twelve selected basic drug compound %RSD values of 10 replicate injections to show injection repeatability at 0.25 µg/mL on-column. All results are reported in Appendix A, Table A1.

| No. | Compound | %RSD for 10 Replicate Injections | | | | | |
|---|---|---|---|---|---|---|---|
| | | Liner 1 (Lot 2) | Liner 2 (Lot 3) | Liner 3 (Lot 2) | Liner 4 (Lot 3) | Liner 5 (Lot 2) | Liner 6 (Lot 3) |
| 3 | Methamphetamine | 3.69 | 3.72 | 1.65 | 3.39 | 6.26 | 2.03 |
| 6 | MDMA | 4.42 | 3.38 | 2.63 | 3.91 | 7.51 | 3.34 |
| 9 | Phencyclidine | 6.78 | 3.01 | 2.14 | 2.47 | 3.22 | 3.57 |
| 11 | Cocaine | 6.32 | 4.24 | 3.10 | 3.04 | 6.47 | 4.02 |
| 13 | Oxazepam | 9.43 | 4.29 | 6.04 | 4.89 | 5.70 | 4.58 |
| 14 | Codeine | 8.42 | 3.50 | 2.67 | 1.97 | 8.26 | 4.83 |
| 19 | Oxycodone | 7.10 | 3.76 | 2.34 | 4.42 | 2.39 | 4.75 |
| 20 | Temazepam | 3.89 | 2.90 | 2.25 | 3.69 | 3.33 | 3.93 |
| 22 | Heroin | 4.86 | 1.95 | 3.00 | 5.59 | 9.55 | 3.98 |
| 23 | Nitrazepam | 5.03 | 4.48 | 4.69 | 4.14 | 4.17 | 3.92 |
| 24 | Clonazepam | 4.14 | 1.92 | 6.45 | 5.68 | 12.48 | 7.88 |
| 28 | Trazadone | 4.21 | 4.44 | 4.72 | 5.46 | 7.35 | 4.92 |

### Liner to liner reproducibility

Using the data collected for the six Ultra Inert universal sintered frit liners, liner-to-liner reproducibility was calculated to verify inertness and consistency across two lots. Table 5 contains a selected set (12) of the basic drug compounds and the average RFs for each liner; all 28 compounds and their average RFs per liner can be found in Appendix Table A2. The 60 RFs were averaged to calculate an overall average RF and %RSD. Comparing the six liners in Table 5 (and Appendix Table A2), the RFs are generally consistent per compound. This consistency is further confirmed with the overall %RSD values for the 60 total injections, where these values are below 15% for all compounds. Clonazepam has a reproducibility value of 13.37% RSD, while all other compounds are below 10% RSD. The higher %RSD value may be related to the underivatized state of this benzodiazepine. Overall, the result of all %RSD values below 15% indicates that the liners produce highly reproducible data, and that the manufacturing and deactivation processes are consistent.

### Simulated real-world matrix

Acetaminophen extract (in toluene) was generated to simulate a real-world matrix to test compound responses of the 5 µg/mL forensic toxicology checkout mixture after repeated matrix injections. This is because several compounds in the mixture can be sensitive to liner (and system) inertness and cleanliness. A series of injections was made with a repeating section. After a blank injection of toluene, three injections of the checkout mixture were run. This was followed by a repetitive set of acetaminophen extract and checkout mixture samples; 10 injections of acetaminophen extract and one checkout mixture injection were repeated until 100 acetaminophen extract injections were made. The sequence ended with three injections of the checkout mixture, for a total of 15 checkout mixture injections and 116 injections overall.

Average RF and %RSD values were calculated from the checkout mixture injections to understand the inertness and cleanliness of the GC/MS system over the 100 acetaminophen extract injections. Several opiates and benzodiazepines can be used to track system maintenance scheduling.

**Table 5.** Twelve selected basic drug compound response factors of 10 replicate injections to show liner-to-liner reproducibility at 0.25 µg/mL on-column, including the overall average RF and %RSD values for the 60 total injections per compound. All 28 compound results are reported in Appendix A, Table A2.

| No. | Compound | Average RF | | | | | | Overall Average RF | % RSD |
|---|---|---|---|---|---|---|---|---|---|
| | | Liner 1 (Lot 2) | Liner 2 (Lot 3) | Liner 3 (Lot 2) | Liner 4 (Lot 3) | Liner 5 (Lot 2) | Liner 6 (Lot 3) | | |
| 3 | Methamphetamine | 0.584 | 0.586 | 0.649 | 0.619 | 0.611 | 0.592 | 0.606 | 5.05 |
| 6 | MDMA | 0.818 | 0.814 | 0.948 | 0.905 | 0.890 | 0.847 | 0.870 | 7.07 |
| 9 | Phencyclidine | 0.361 | 0.354 | 0.346 | 0.348 | 0.358 | 0.361 | 0.354 | 4.07 |
| 11 | Cocaine | 0.223 | 0.234 | 0.200 | 0.230 | 0.233 | 0.241 | 0.227 | 7.44 |
| 13 | Oxazepam | 0.015 | 0.016 | 0.017 | 0.016 | 0.017 | 0.017 | 0.016 | 8.13 |
| 14 | Codeine | 0.051 | 0.051 | 0.042 | 0.051 | 0.050 | 0.052 | 0.050 | 8.91 |
| 19 | Oxycodone | 0.067 | 0.071 | 0.073 | 0.075 | 0.072 | 0.071 | 0.071 | 5.31 |
| 20 | Temazepam | 0.089 | 0.099 | 0.110 | 0.107 | 0.108 | 0.103 | 0.103 | 7.77 |
| 22 | Heroin | 0.081 | 0.090 | 0.099 | 0.093 | 0.095 | 0.087 | 0.091 | 8.24 |
| 23 | Nitrazepam | 0.040 | 0.041 | 0.042 | 0.039 | 0.040 | 0.041 | 0.041 | 4.87 |
| 24 | Clonazepam | 0.066 | 0.072 | 0.039 | 0.034 | 0.033 | 0.030 | 0.032 | 13.37 |
| 28 | Trazadone | 0.082 | 0.091 | 0.102 | 0.091 | 0.090 | 0.087 | 0.091 | 8.36 |

As the GC/MS system becomes dirty or there is an issue with inertness, these compounds, including oxycodone, lorazepam, codeine, temazepam, and trazadone, will decrease in peak area and height. Additionally, oxycodone will develop a significant tail; all of these peak changes will also affect and increase %RSD values over time. Table 6 compiles the average RF and %RSD values for all 28 analytes in the checkout mixture. Reviewing the %RSD values, 24 compounds retain %RSD values below 20%, which is excellent repeatability with the simulated real-world matrix of acetaminophen extract. Three compounds, codeine, lorazepam, and oxycodone, had %RSD values between 20 and 30%, and temazepam had a %RSD of 40.06%.

**Table 6.** Test of inertness stability with average RF and %RSD values from 15 injections of a 5 µg/mL checkout mixture throughout a sequence with 100 acetaminophen extract injections.

| No. | Compound | Average RF | % RSD |
|---|---|---|---|
| 1 | Amphetamine | 0.284 | 12.45 |
| 2 | Phentermine | 0.474 | 15.81 |
| 3 | Methamphetamine | 0.488 | 10.03 |
| 4 | Nicotine | 0.582 | 8.43 |
| 5 | MDA | 0.126 | 13.57 |
| 6 | MDMA | 0.722 | 7.21 |
| 7 | MDEA | 0.871 | 10.65 |
| 8 | Meperidine | 0.297 | 8.69 |
| 9 | Phencyclidine | 0.384 | 8.05 |
| 10 | Methadone | 0.553 | 12.19 |
| 11 | Cocaine | 0.162 | 12.81 |
| 12 | Proadifen | 0.489 | 12.67 |
| 13 | Oxazepam | 0.013 | 17.85 |
| 14 | Codeine | 0.054 | 23.49 |
| 15 | Lorazepam | 0.019 | 22.07 |
| 16 | Diazepam | 0.135 | 6.20 |
| 17 | Hydrocodone | 0.104 | 6.15 |
| 18 | THC | 0.085 | 11.55 |
| 19 | Oxycodone | 0.049 | 20.25 |
| 20 | Temazepam | 0.069 | 40.06 |
| 21 | Flunitrazepam | 0.032 | 12.96 |
| 22 | Heroin | 0.079 | 10.31 |
| 23 | Nitrazepam | 0.020 | 11.76 |
| 24 | Clonazepam | 0.025 | 13.69 |
| 25 | Alprazolam | 0.048 | 13.80 |
| 26 | Verapamil | 0.260 | 16.34 |
| 27 | Strychnine | 0.124 | 13.69 |
| 28 | Trazadone | 0.063 | 11.00 |

Temazepam, and the benzodiazepine class in general, is very sensitive to inlet inertness and cleanliness, accounting for the elevated %RSD values for these analytes over the 100 acetaminophen extract injections. As more matrix injections were made, the temazepam response decreased, as shown in Figure 8A, and plotted in Appendix Figure A1. Figure 8A includes oxycodone, flunitrazepam, and heroin peaks, which all show varying decreases in peak response with increased injections. Figure 8B shows the recovery of temazepam, and other nearby peaks, when the liner is replaced, indicating that the matrix in the liner was causing the response loss. Overall, these results are very good for underivatized benzodiazepines, and excellent results across the board for the rest of the analytes. Comparison of the simulated matrix %RSD values (Table 6) with the overall average %RSD values from the reproducibility study (Table 5) generally shows an increase in %RSDs with the simulated matrix work. This is understandable, as matrix is being deposited into the liner, and can cause undesired interactions with basic drug analytes.

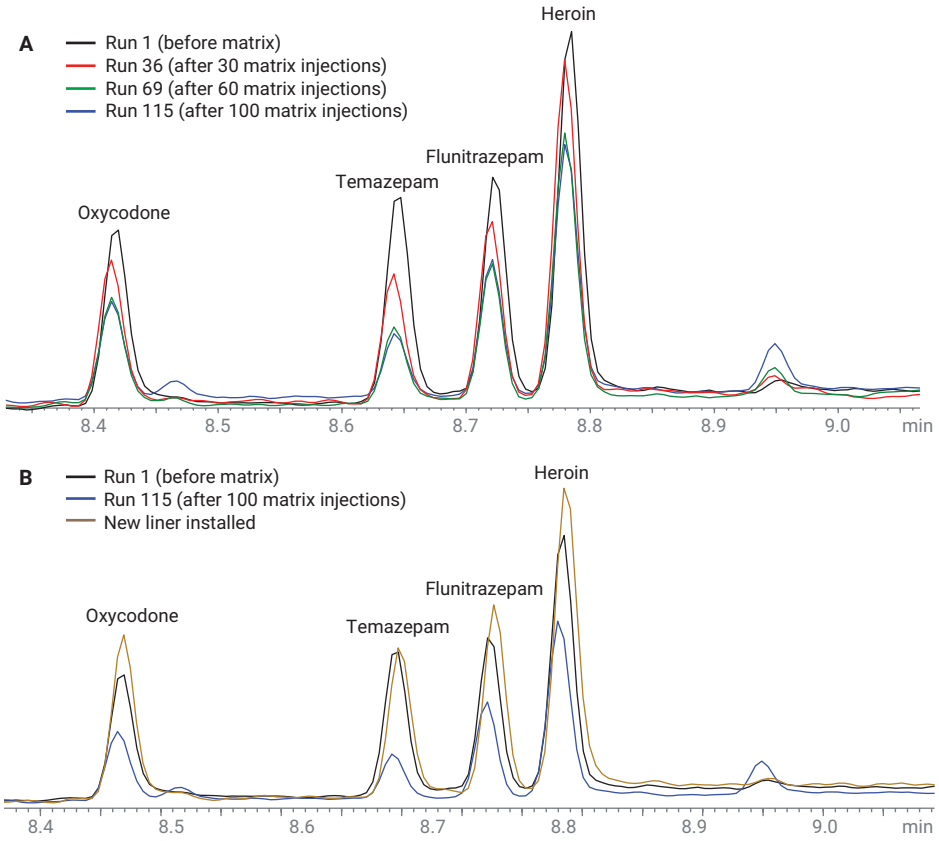


**Figure 8.** A) TIC overlays of the simulated matrix testing for oxycodone, temazepam, flunitrazepam, and heroin comparing the peak responses during the 100-run experiment at run 1 before matrix (black), run 36 after 30 matrix extract injections (red), run 69 after 60 matrix injections (green), and the final run 115 after 100 matrix injections (blue). B) TIC overlays of the simulated matrix testing for oxycodone, temazepam, flunitrazepam, and heroin comparing the peak responses at run 1 before matrix (black), the final run 115 after 100 matrix injections (blue), and the first run after the liner was replaced (gold).

## Conclusion

Agilent Ultra Inert sintered frit liners provide excellent inertness and great promise for the analysis of controlled substance samples. Using the high efficiency column and split injection, the liners show excellent chromatography, peak shape, and resolution for amines, opiates, and benzodiazepine compound classes, along with several other compound classes that were investigated with the forensic toxicology checkout mixture. The liner-to-liner performance indicates high reproducibility across the tested liners, with an average of 8.9% RSD for all 28 analyte RF values. The amine compound class has excellent peak shapes even to low concentrations of 0.25 µg/mL on-column. Injection repeatability and simulated matrix repeatability testing also illustrated the robustness of the liner, where the matrix testing had an average %RSD of 14.2% across the 28 analytes after 100 simulated matrix injections. The Ultra Inert universal single-taper liner with sintered frit provides space for sample evaporation, nonvolatile matrix trapping, and column and detector protection, and is a great choice for split injections in controlled substance analysis.

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# Appendix

**Table A1.** Complete list of basic drug compound %RSD values for six Ultra Inert universal single taper with sintered frit liners (p/n 5190-5105) to show injection repeatability at 0.25 µg/mL on-column.

| No. | Compound | Average RF | | | | | |
|---|---|---|---|---|---|---|---|
| | | Liner 1 (Lot 2) | Liner 2 (Lot 3) | Liner 3 (Lot 2) | Liner 4 (Lot 3) | Liner 5 (Lot 2) | Liner 6 (Lot 3) |
| 1 | Amphetamine | 3.30 | 3.94 | 2.92 | 2.38 | 3.00 | 1.53 |
| 2 | Phentermine | 4.36 | 3.64 | 1.48 | 3.40 | 5.20 | 2.23 |
| 3 | Methamphetamine | 3.69 | 3.72 | 1.65 | 3.39 | 6.26 | 2.03 |
| 4 | Nicotine | 8.80 | 4.39 | 2.43 | 3.96 | 7.76 | 5.93 |
| 5 | MDA | 5.15 | 3.10 | 2.29 | 4.50 | 5.42 | 4.62 |
| 6 | MDMA | 4.42 | 3.38 | 2.63 | 3.91 | 7.51 | 3.34 |
| 7 | MDEA | 6.44 | 4.16 | 2.62 | 1.62 | 2.09 | 3.88 |
| 8 | Meperidine | 6.36 | 4.07 | 1.97 | 2.80 | 2.77 | 4.44 |
| 9 | Phencyclidine | 6.78 | 3.01 | 2.14 | 2.47 | 3.22 | 3.57 |
| 10 | Methadone | 4.18 | 2.49 | 1.54 | 2.92 | 6.22 | 4.42 |
| 11 | Cocaine | 6.32 | 4.24 | 3.10 | 3.04 | 6.47 | 4.02 |
| 12 | Proadifen | 2.97 | 2.53 | 1.18 | 1.64 | 3.40 | 2.32 |
| 13 | Oxazepam | 9.43 | 4.29 | 6.04 | 4.89 | 5.70 | 4.58 |
| 14 | Codeine | 8.42 | 3.50 | 2.67 | 1.97 | 8.26 | 4.83 |
| 15 | Lorazepam | 9.59 | 5.14 | 3.60 | 5.80 | 4.56 | 5.88 |
| 16 | Diazepam | 4.68 | 3.05 | 3.53 | 3.05 | 4.05 | 2.68 |
| 17 | Hydrocodone | 4.47 | 3.93 | 2.41 | 2.76 | 3.00 | 3.79 |
| 18 | THC | 3.02 | 4.75 | 3.60 | 3.16 | 2.76 | 4.08 |
| 19 | Oxycodone | 7.10 | 3.76 | 2.34 | 4.42 | 2.39 | 4.75 |
| 20 | Temazepam | 3.89 | 2.90 | 2.25 | 3.69 | 3.33 | 3.93 |
| 21 | Flunitrazepam | 4.81 | 4.14 | 2.48 | 5.62 | 8.30 | 4.93 |
| 22 | Heroin | 4.86 | 1.95 | 3.00 | 5.59 | 9.55 | 3.98 |
| 23 | Nitrazepam | 5.03 | 4.48 | 4.69 | 4.14 | 4.17 | 3.92 |
| 24 | Clonazepam | 4.14 | 1.92 | 6.45 | 5.68 | 12.48 | 7.88 |
| 25 | Alprazolam | 8.80 | 4.51 | 6.93 | 3.96 | 8.18 | 2.93 |
| 26 | Verapamil | 4.59 | 2.27 | 3.09 | 2.97 | 2.19 | 1.56 |
| 27 | Strychnine | 3.02 | 1.88 | 4.11 | 3.40 | 5.98 | 2.22 |
| 28 | Trazadone | 4.21 | 4.44 | 4.72 | 5.46 | 7.35 | 4.92 |

**Table A2.** Complete list of the forensic toxicology checkout mixture compounds for six Ultra Inert universal single taper with sintered frit liners (p/n 5190-5105) to show liner-to-liner reproducibility with average response factors per liner, the overall average RF for the 60 total injections, and overall %RSD values for 0.25 µg/mL on-column.

| No. | Compound | Average RF | | | | | | Overall Average RF | Overall % RSD |
|---|---|---|---|---|---|---|---|---|---|
| | | Liner 1 (Lot 1) | Liner 2 (Lot 2) | Liner 3 (Lot 3) | Liner 4 (Lot 3) | Liner 5 (Lot 1) | Liner 6 (Lot 2) | | |
| 1 | Amphetamine | 0.263 | 0.257 | 0.268 | 0.262 | 0.268 | 0.263 | 0.263 | 3.16 |
| 2 | Phentermine | 0.540 | 0.545 | 0.603 | 0.574 | 0.574 | 0.546 | 0.564 | 5.26 |
| 3 | Methamphetamine | 0.584 | 0.586 | 0.649 | 0.619 | 0.611 | 0.592 | 0.606 | 5.05 |
| 4 | Nicotine | 0.737 | 0.750 | 0.876 | 0.808 | 0.780 | 0.725 | 0.779 | 8.66 |
| 5 | MDA | 0.124 | 0.126 | 0.136 | 0.134 | 0.128 | 0.128 | 0.129 | 5.36 |
| 6 | MDMA | 0.818 | 0.814 | 0.948 | 0.905 | 0.890 | 0.847 | 0.870 | 7.07 |
| 7 | MDEA | 1.022 | 1.025 | 1.124 | 1.116 | 1.076 | 1.056 | 1.070 | 5.19 |
| 8 | Meperidine | 0.348 | 0.351 | 0.365 | 0.369 | 0.361 | 0.356 | 0.358 | 4.33 |
| 9 | Phencyclidine | 0.361 | 0.354 | 0.346 | 0.348 | 0.358 | 0.361 | 0.354 | 4.07 |
| 10 | Methadone | 0.620 | 0.645 | 0.557 | 0.645 | 0.656 | 0.656 | 0.630 | 6.74 |
| 11 | Cocaine | 0.223 | 0.234 | 0.200 | 0.230 | 0.233 | 0.241 | 0.227 | 7.44 |
| 12 | Proadifen | 0.509 | 0.526 | 0.498 | 0.541 | 0.542 | 0.539 | 0.526 | 4.03 |
| 13 | Oxazepam | 0.015 | 0.016 | 0.017 | 0.016 | 0.017 | 0.017 | 0.016 | 8.13 |
| 14 | Codeine | 0.051 | 0.051 | 0.042 | 0.051 | 0.050 | 0.052 | 0.050 | 8.91 |
| 15 | Lorazepam | 0.021 | 0.021 | 0.017 | 0.020 | 0.020 | 0.022 | 0.020 | 9.17 |
| 16 | Diazepam | 0.150 | 0.162 | 0.177 | 0.174 | 0.173 | 0.163 | 0.167 | 6.59 |
| 17 | Hydrocodone | 0.118 | 0.126 | 0.127 | 0.134 | 0.131 | 0.125 | 0.127 | 5.09 |
| 18 | THC | 0.098 | 0.101 | 0.100 | 0.106 | 0.105 | 0.100 | 0.102 | 4.52 |
| 19 | Oxycodone | 0.067 | 0.071 | 0.073 | 0.075 | 0.072 | 0.071 | 0.071 | 5.31 |
| 20 | Temazepam | 0.089 | 0.099 | 0.110 | 0.107 | 0.108 | 0.103 | 0.103 | 7.77 |
| 21 | Flunitrazepam | 0.038 | 0.041 | 0.041 | 0.041 | 0.042 | 0.041 | 0.041 | 6.20 |
| 22 | Heroin | 0.081 | 0.090 | 0.099 | 0.093 | 0.095 | 0.087 | 0.091 | 8.24 |
| 23 | Nitrazepam | 0.040 | 0.041 | 0.042 | 0.039 | 0.040 | 0.041 | 0.041 | 4.87 |
| 24 | Clonazepam | 0.066 | 0.072 | 0.039 | 0.034 | 0.033 | 0.030 | 0.032 | 13.37 |
| 25 | Alprazolam | 0.028 | 0.032 | 0.082 | 0.072 | 0.070 | 0.067 | 0.071 | 9.27 |
| 26 | Verapamil | 0.382 | 0.381 | 0.355 | 0.372 | 0.375 | 0.393 | 0.377 | 4.14 |
| 27 | Strychnine | 0.152 | 0.154 | 0.168 | 0.155 | 0.155 | 0.150 | 0.156 | 5.15 |
| 28 | Trazadone | 0.082 | 0.091 | 0.102 | 0.091 | 0.090 | 0.087 | 0.091 | 8.36 |

As described in the simulated real-world matrix section, the forensic toxicology checkout mixture was run after every 10th acetaminophen extract run, with three checkout mixture runs at the beginning and end of the sequence, and RFs calculated for each run. At the end of this matrix study, the liner was replaced with a new Ultra Inert universal single taper with sintered frit liner, and the checkout mixture was run again to determine if the response increased back to normal. To plot these data, the first three RFs were averaged together and used to calculate a normalized RF for each run, including the new liner (listed as run 117). Figure A1 displays a plot of the normalized RFs for the acetaminophen extract experiment.

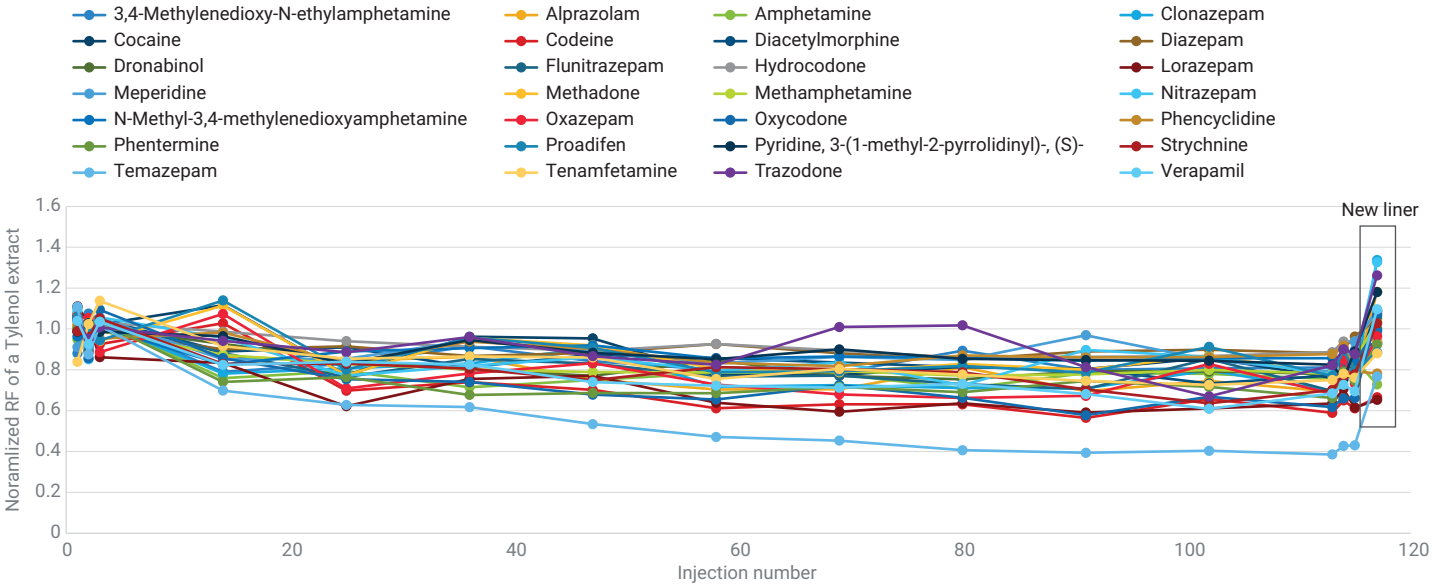


**Figure A1.** Normalized RFs for each forensic toxicology mixture (0.25 µg/mL on column) run for the simulated real-world matrix (acetaminophen extract) testing, where the final data point corresponds to the new liner.

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