Analysis of Drugs by GC/MS using the Ultra Inert Inlet Liners with Wool

Application Note

Forensic Toxicology

Authors

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Abstract

With efficient deactivation on glass wool, the Ultra Inert liners with wool provide excellent inertness, homogeneous sample mixing and evaporation, non-volatile residue trapping, and column and detector protection for drugs of abuse screening.

Introduction

GC inlet liners are the centerpiece of the inlet system where the sample is vaporized, mixed with the carrier gas, and introduced to the capillary column. Inlet liners with wool are widely used because the wool promotes homogenous sample mixing and better quantitation. Wool provides a large surface area which aids the vaporization of liquid samples. It also acts as a trap to collect non-volatile residue in the sample, thus protecting the GC column from the negative impact of sample matrix. Wool liners also reduce sample loss by preventing sample droplets from reaching the bottom of the inlet before vaporization. Agilent MS certified liners with glass wool provide excellent performance for general application purposes. However, for specific applications of active compounds analysis, liners with superior inertness are required to achieve the most reliable results.

GC/MS screening methods are important in forensic toxicology laboratories. With the continuing emergence of new drugs and toxins, the list of target compounds to be screened can number in the hundreds. For those compounds that are compatible with GC, using GC/MS in full-scan mode with electron impact ionization (EI) is well suited for the task [1]. Samples for screening usually require minimal sample preparation, or even no clean-up, to preserve target analytes. However, heavy-matrix samples, such as plasma or urine extracts, deteriorate the performance of the analytical column and detector, resulting in short column life and frequent MS source maintenance. Therefore, it is beneficial to use inlet liners with wool to protect the entire GC/MS system.

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However, if liners with wool are poorly deactivated, they can cause the adsorption or decomposition of target analytes for forensic drugs. As shown in Figure 1, those drugs usually contain hetero atoms, which strongly interact with the free silanol groups (Si-OH) in glass [2]. The resulting compound adsorption and decomposition causes chromatographic problems such as broad or distorted peaks, tailing peaks, ghosting phenomena, and low responses. Liners with glass wool magnify these negative effects due to the large surface area of glass wool and difficulty of complete deactivation. A properly and efficiently deactivated inlet liner with glass wool is imperative for satisfactory chromatography with accurate and reproducible responses for these forensic toxicology applications.

Agilent's Ultra Inert liner deactivation process significantly improves the efficacy and robustness of glass wool deactivation. The surface area is deactivated thoroughly. For the first time, liners with glass wool can analyze basic drugs using GC/MS.

The liners with wool were evaluated using Agilent Forensic Toxicology analyzer checkout standards, including 28 popular and difficult basic drug compounds. These compounds cover the retention range from early to late eluting compounds, and contain different categories of drugs including amphetamins, alkaloids, and benzodiazpines. Figure 1 shows the chemical structures for some of the analytes. All liner tests were conducted using a GC/MS system with simultaneous collection of scan and SIM data. A 5 µg/mL standard was used for chromatographic evaluation. A 500 ng/mL standard (10× dilution) was used to assess the repeatability of injections over 50 injections.

Experimental

Chemicals and Reagents

The Agilent GC/MS Forensic Toxicology analyzer checkout mixture standard (p/n 5190-0471) was used to evaluate the performance of Ultra Inert liners with glass wool. HPLC grade Toluene and Methanol was purchased from Honeywell B&J (Muskegon, MI, USA), and Acetonitrile (AcN) was purchased from Sigma-Aldrich (St Louis, MO, USA). An Internal Standard (IS) was purchased from AccuStandard (New Haven, CT, USA), containing 0.5 mg/mL of Acenaphthene-D_{10}, Phenanthrene-D_{10}, Triphenylphosphate, Chrysene-D_{12}, and Perylene-D_{12} in Acetone.

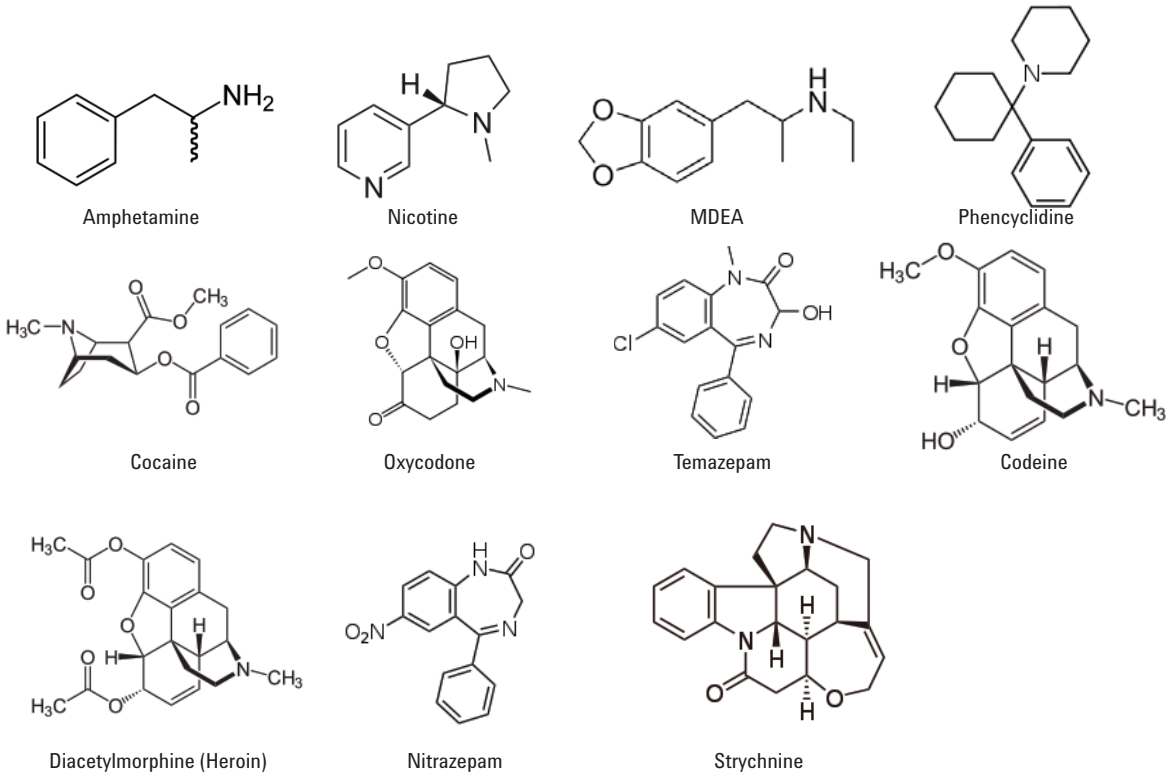

Figure 1. Chemical structure of selected basic drugs.

Solutions and Standards

The original checkout standard sample was made in a 90/5/5 Toluene/MeOH/AcN solution. A 90/5/5 Toluene/MeOH/AcN blank solvent mixture was prepared by combining 90 mL of Toluene, 5 mL of MeOH and 5 mL of AcN, and was used as reagent blank. The 5 µg/mL original standards were directly used for injection, and were diluted 10 times with blank solvent to 500 ng/mL solution. 4 µL of IS stock solution was spiked to 1 mL of standard solution, when necessary, to generate a concentration of 2 µg/mL for IS in the sample.

Instrumentation

All testing was done on an Agilent 7890A GC system equipped with a 7683B autosampler and a 5975C MSD.
Table 1 lists the instrument conditions. Table 2 lists flow path consumable supplies. Table 3 list the Selected Ion Monitoring (SIM) conditions for 28 target analytes.

Table 1. Instrumental conditions for Agilent GC/MS system used for basic drug compounds test

GC	Agilent 7890A Series
Autosampler	Agilent 7683B, 5 µL syringe (p/n 5181-5246), 1 µL injection volume
	Preinj solvent A (90/5/5 Toluene/MeOH/AcN) washes: 1 Sample pumps: 3 Postinj solvent B (90/5/5 Toluene/MeOH/AcN) washes: 3
Carrier gas	Helium, constant pressure
Inlet	Splitless mode: 280 °C
Purge flow	50 mL/min, switched mode, hold for 0.75 min
Inlet pressure	18.7 psi (RT locked) during run, 1.0 psi during back flush
RT locking	Proadifen (SKF-525a) @ 8.569 min
Oven profile	100 °C for 0.5 min, to 325 °C at 20 °C/min, hold 2.5 min
Post run	2 min at 325 °C
Capillary Flow Technology	Purged Ultimate Union (p/n G3182-61580) used for back flushing the analytical column and inlet
Aux EPC gas	Helium plumbed to Purge Ultimate Union
Aux pressure	4 psi during run, 75 psi during back-flushing
Analytical column	DB-5MSUI, 15 m × 0.25 mm, 0.25 µm (p/n 122-5512UI)
Connections	Inlet to Purged Ultimate Union (p/n G3182-61580)
Restrictor	Inert Fused Silica tubing, 0.65 m × 0.15 mm (p/n 160-7625-5)
Connections	Between Purged Ultimate Union and the MSD
MSD	Agilent 5975C inert with performance electronics
Vacuum pump	Performance turbo
Mode	Scan/SIM
Tune file	Atune.u
EM voltage	Atune voltage
Transfer line temp	300 °C
Source temp	300 °C
Quad temp	150 °C
Solvent delay	1.4 min
Scan mass range	40 – 570 amu

Table 2. Flow Path Supplies

Vials	Amber screw cap (p/n 5182-0716)
Vial caps	Blue screw cap (p/n 5182-0717)
Vial inserts	150 µL glass w/ polymer feet (p/n 5183-2088)
Septum	Advanced Green Non-Stick 11 mm (p/n 5183-4759)
Ferrules	0.4 mm id, 85/15 Vespel/graphite (p/n 5181-3323)
O-rings	Non-stick liner O-ring (p/n 5188-5365)
Capillary Flow Technology	Purged Ultimate Union (p/n G3182-61580) Internal nut (p/n G2855-20530) SilTite metal ferrules, 0.10-0.25 mm id (p/n 5188-5361)
Inlet seal	Gold plated inlet seal with washer (p/n 5188-5367)
Inlet liners	Agilent Ultra Inert deactivated single taper splitless liner with wool (p/n 5190-2293)

Table 3. SIM acquisition conditions used for 28 basic drug compounds by GC/MS

Analytes (Peak no. on chromatogram)	SIM *	RT (min)	Collection window (min)
Amphetamine (1)	**44**, 91	1.77	1.4 – 2.7
Phentermine (2)	**58**, 134	1.96	
Methamphetamine (3)	**58**, 91	2.08	
Nicotine (4)	**84**, 133	3.06	2.7 – 3.6
Methylenedioxyamphetamine (MDA) (5)	**44**, 135	3.92	3.6 – 5.0
Methylenedioxymethamphetamine (MDMA) (6)	**58**, 135	4.27	
Methylenedioxyethylamphetamine (MDEA) (7)	**72**, 135	4.57	
Meperidine (8)	**71**, 247	5.63	5.0 – 7.0
Phencyclidine (9)	**200**, 242	6.49	
Methadone (10)	**72**, 57	7.72	7.0 – 8.9
Cocaine (11)	**182**, 82	8.10	
Prodifen (SKF-525a) (12)**	**86**, 99	8.57	
Oxzepam (13)	**239**, 267	8.73	
Codeine (14)	**299**, 162	9.01	8.9 – 9.5
Lorazepam (15)	**239**, 274	9.08	
Diazepam (16)	**256**, 283	9.22	
Hydrocodone (17)	**299**, 242	9.29	
Tetrahydrocannabiol (18)	**231**, 314	9.36	
Oxycodone (19)	**315**, 230	9.63	9.5 – 10.4
Temazepam (20)	**271**, 273	9.87	
Flunitrazepam (21)	**312**, 286	9.96	
Diacetylmorphine (Heroin) (22)	**327**, 369	10.02	
Nitrazepam (23)	**253**, 206	10.62	10.4 – 11.6
Clonazepam (24)	**314**, 286	10.94	
Alprazolam (25)	**279**, 308	11.32	
Varapamil (26)	**303**, 304	12.03	11.6 – 14.0
Strychnine (27)	**334**, 335	12.18	
Trazodone (28)	**205**, 70	12.96	

* Ions in Bold were quantifiers, and the other ions were qualifiers.

** Prodifen was used for the RT locking.

A back-flushing system was used because it shortens analysis times for samples that contain high-boiling matrix interferences, reduces column head trimming, and reduces frequency of MSD source cleaning [3,4]. The instrument configuration is similar to the configuration shown in Figure 1B in the previous setup [4], except no retention gap was used for this application. Retention time locking (RTL) was used to eliminate recalibration of individual retention times and timed events such as SIM groups [5].

Results and Discussion

The purpose of these tests was to evaluate the Ultra Inert deactivated liners with wool for forensic screening analysis of drugs by GC/MS. The Agilent Forensic Toxicology analyzer checkout standard was used for the evaluation (Table 3). The feasibility of using Ultra Inert liners with wool was determined by chromatographic evaluation, liner to liner reproducibility, and multi-injections repeatability. In parallel, liners with wool from multiple sources were tested for comparison.

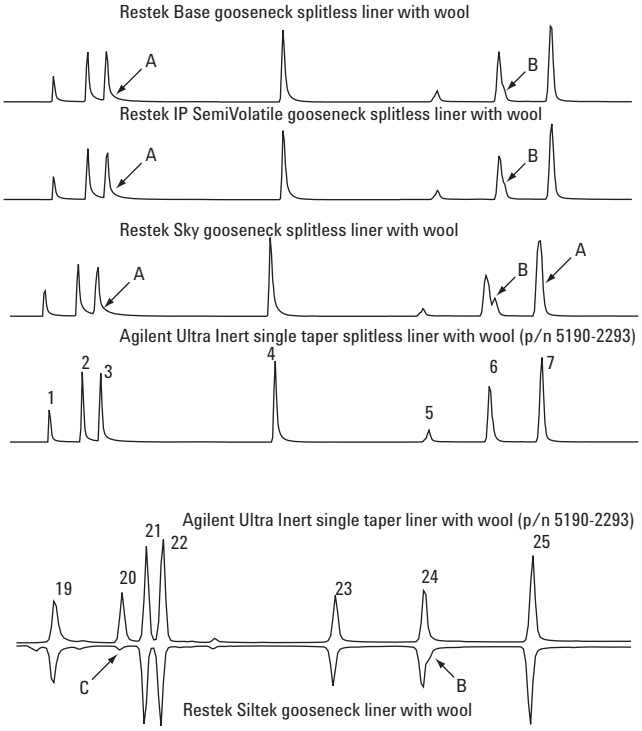

Figure 2. Chromatographic problems for drug compounds shown on GC/MS SIM chromatograms when using other equivalent liners and their comparison with chromatograms obtained by Ultra Inert liners with wool. See Table 3 for peaks identification and Table 1 for instrument conditions. 5 ng checkout standards on column. A) Broad or distorted peak, B) ghosting shoulder, C) poor sensitivity

Chromatographic performance

The adsorption or decomposition of basic drug compounds may cause various chromatographic problems including broad, distorted peaks, peak tailing, ghost peaks, and loss of sensitivity. All of these problems were observed in liners tests using the checkout standard. Peak shape problems usually occurred for early eluting compounds, such as Phentermine, Methamphetamine, MDA, and MDMA. The late eluting compounds, such as Temazepam, can disappear due to the loss of sensitivity. Figure 2 shows problematic chromatograms obtained using similar liners compared to chromatograms obtained using Ultra Inert liners with wool. As seen in Figure 2, with 5 ng on column, other liner deactivations cause chromatographic problems such as broad or distorted peaks and significant loss of response. However, the corresponding chromatograms with Agilent Ultra Inert deactivated liners show better peak shape and typically higher responses. Figure 3 shows a full chromatogram of 5 ng checkout standard on column using Agilent Ultra Inert splitless liner with wool by GC/MS. Figure 3 shows that Ultra Inert liners with wool provide the best chromatogram for all of analytes tested, even though there is small peak tailing or broadening observed for certain compounds. Six replicates of Ultra Inert liners were tested, each providing similar chromatographic performance, indicating excellent liner to liner reproducibility. The satisfactory chromatograms obtained by Ultra Inert liners demonstrate that the Ultra Inert liner deactivation process provides sufficient liner and glass wool inertness to prevent drugs from adsorption and decomposition.

1. Amphetamine, 2. Phentermine, 3. Methamphetamine, 4. Nicotine, 5. MDA, 6. MDMA, 7. MDEA, 8. Meperidine, 9. Phencyclidine, 10. Methadone, 11. Cocaine, 12. SKF-525a, 13. Oxazepam, 14. Codeine, 15. Lorazepam, 16. Diazepam, 17. Hydrocodone, 18. Tetrahydrocannabinol, 19. Oxycodone, 20. Temazepam, 21. Flunitrazepam, 22. Heroin, 23. Nitrazepam, 24. Clonazepam, 25. Alprazolam, 26. Verapamil, 27. Strychnine, 28. Trazodone.

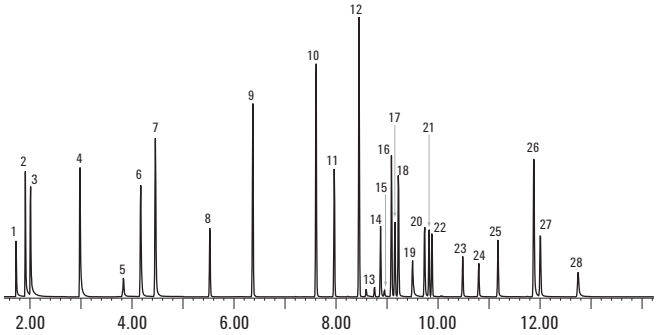

Figure 3. Chromatogram of forensic toxicology analyzer checkout standard (5 ng checkout standards on column) using Agilent Ultra Inert single taper splitless liner with wool (p/n 5190-2293) by GC/MS. See Table 1 for instrument condition. Satisfactory peaks shape achieved for all of analytes

Liner to liner reproducibility

To quantitatively evaluate the liner to liner reproducibility, six Ultra Inert liners from four different lots were tested. 5 µg/mL and 500 ng/mL samples spiked with 2 µg/mL IS were used. Twelve sensitive compounds were selected for evaluation. The Response Factors (RFs) were calculated for each concentration level. The average RF values were evaluation criteria for the liner to liner reproducibility test. See Table 4. The results show excellent liner to liner performance consistency with less than 7% RSD, except for Temazepam with 11.7%, across six liners from four different lots.

*Table 4. Liner to Liner Reproducibility: 12 sensitive basic drug compounds average RF (5 µg/mL and 500 ng/mL) and RSD values for six replicates of UI deactivated liners with wool (p/n 5190-2293) **

Compounds	Liner 1 (Lot 1)	Liner 2 (Lot 1)	Liner 3 (Lot 1)	Liner 4 (Lot 2)	Liner 5 (Lot 3)	Liner 6 (Lot 4)	Mean RF	RSD
Methamphetamine (3)	0.875	0.876	0.882	0.940	0.955	0.904	0.905	3.8
MDMA (6)	0.807	0.789	0.783	0.848	0.874	0.841	0.824	4.4
Phencyclidine (9)	0.494	0.510	0.494	0.488	0.509	0.521	0.503	2.5
Cocaine (11)	0.636	0.645	0.647	0.637	0.660	0.668	0.649	2.0
Oxazepam (13)	0.050	0.055	0.052	0.055	0.062	0.057	0.055	7.6
Codeine (14)	0.096	0.098	0.095	0.090	0.099	0.102	0.097	4.2
Oxycodone (19)	0.073	0.071	0.070	0.076	0.082	0.080	0.075	6.5
Temazepam (20)	0.101	0.121	0.115	0.088	0.096	0.104	0.104	11.7
Heroin (22)	0.097	0.099	0.096	0.095	0.100	0.102	0.098	2.7
Nitrazepam (23)	0.038	0.032	0.037	0.034	0.037	0.036	0.036	6.3
Clonazepam (24)	0.035	0.035	0.034	0.032	0.034	0.033	0.034	3.5
Trazodone (28)	0.061	0.065	0.064	0.058	0.060	0.064	0.062	4.4

$$^{*}\ RF = \frac{\text{Peak Area}_{\text{Analyte}} \times \text{Concentration}_{\text{Internal Standard}}}{\text{Peak Area}_{\text{Internal Standard}} \times \text{Concentration}_{\text{Analyte}}}$$

Injection repeatability and deactivation stability

Multi-injection repeatability and deactivation stability were tested by continuously injecting 1 µL of 0.5 µg/mL standard samples for 50 injections. Data was collected and RF values were calculated every 10 injections. RSD values were calculated over 50 injections. Table 5 shows the RSD value for all of the basic drug analytes with 0.5 ng on column.

A 0.5 ng on column concentration was used for this repeatability test since low level concentrations show greater deviation contributions than high concentration samples. Higher responses of analytes could hide some deviation impact and generate better repeatability. Twenty-two of 28 analytes have excellent repeatability for 50 injections of standard solution with less than 20% RSD. 5 of 28 analytes have relatively high RSD (between 20 – 25%), but still should be acceptable at the level of 0.5 ng on column. Temazepam is a very difficult compound and extremely sensitive to the liner inertness.

Table 5. Deactivation stability: 50 injections repeatability (%RSD) for Agilent Ultra Inert deactivated liners with wool (p/n 5190-2293) for all of tested basic drug compounds with 0.5 ng of standard on column. (n = 3)

Compound	RSD (%) over 50 injections	Compound	RSD (%) over 50 injections
Amphetamine	0.3	Lorazepam	20.9
Phentermine	1.1	Diazepam	3.7
Methamphetamine	1.5	Hydrocodone	3.7
Nicotine	2.3	Tetrahydrocannabinol	8.5
MDA	3.7	Oxycodone	22.2
MDMA	2.2	Temazepam	59.9
MDEA	2.0	Flunitrazepam	8.7
Meperidine	1.9	Heroin	10.7
Phencyclidine	15.6	Nitrazepam	11.2
Methadone	3.4	Clonazepam	12.0
Cocaine	7.8	Alprazolam	13.1
Prodifen	4.4	Verapamil	15.4
Oxazepam	20.4	Strychnine	11.0
Codeine	20.5	Trazodone	23.6

As shown in Figure 2, when an inefficient deactivated liner was used the response of Temazepam (5 ng on column) can almost disappear. Compared to other similar liners, Agilent Ultra Inert liner with wool generated highest RF for Tempazepam, which is clearly shown in Figure 4. This indicates that Agilent Ultra Inert liners with wool provide the best inertness compared to competitor's equivalent liners.

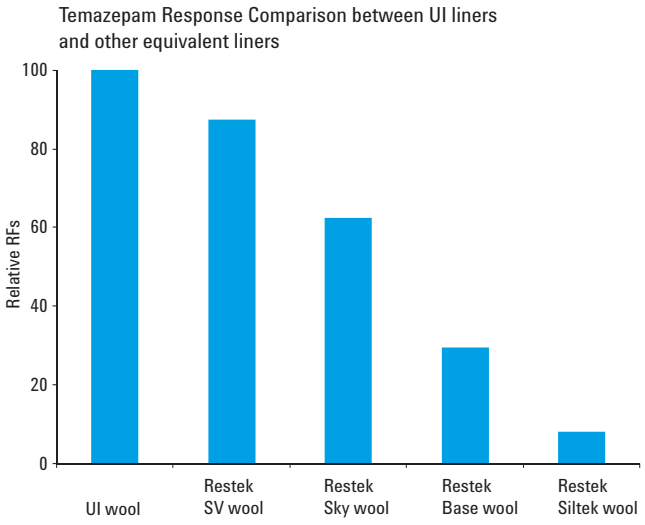

Figure 4. Sensitive compound response (Temazepam) comparison for Ultra Inert liner with wool (p/n 5190-2293) and other equivalent liners. RF calculation was based on the average RF of 0.5 ng and 5 ng standard on column. Ultra Inert liner average RF value was set to 100% and other liners average RF values were scaled.

The response of Temazepam decreased with more samples were injected, thus generated high RSD over injections. This phenomenon was observed for all of the liners tested, and the response decrease can be even worse for other liners. When Temazepam is a target analyte and the interested concentration is at ppb level, it is strongly recommend that an Ultra Inert liner with wool should be used for no more than 30 samples.

Real matrix sample analysis

Whole blood extracts prepared for GC/MS analysis were supplied by NMS Labs (Willow Grove, PA). The whole blood was prepared with a single step liquid/liquid extraction into a solvent, evaporated to dryness, and reconstituted in toluene at 1/10th volume. Figure 5 shows the chromatogram of 2 ppm matrix spiked sample using Agilent Ultra Inert liner with wool by GC/MS, which is satisfactory for both early eluted compounds' peak shape and late eluted compounds' sensitivity. There are some minor interference peaks from matrix showing up.

Conclusion

Agilent Ultra Inert liners with wool have shown excellent inertness for the forensic analysis of basic drugs. Ultra Inert liners with wool provide satisfactory chromatography for the selected popular and difficult basic drug compounds. The liner to liner performance shows excellent reproducibility with an average of 5% RSD for these active compound RF values. With efficient and robust deactivation of the wool, Agilent Ultra Inert liners with wool provide excellent inertness for forensic toxicology screening. The benefits provided by liners with wool such as homogeneous sample mixing and evaporation, non-volatile residue trapping, and column and detector protection, are gained without compromise of chromatography or sensitivity of active analytes. Ultra Inert liners with wool are an excellent choice for forensic screening analysis for drugs.

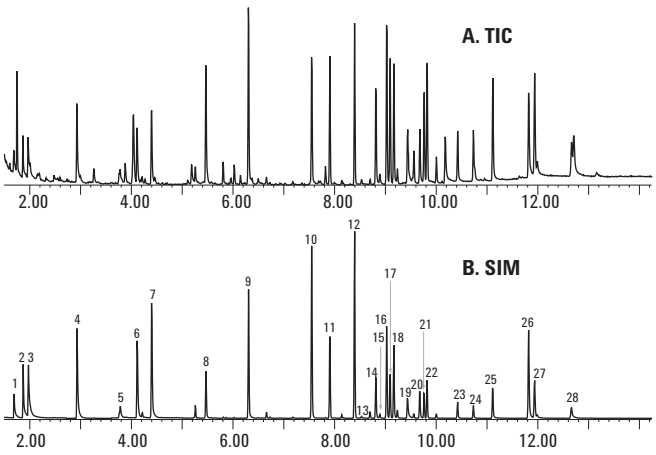

Figure 5 Chromatogram of forensic toxicology analyzer checkout standard (2 ng on column) with whole blood matrix using Agilent Ultra Inert single taper splitless liner with wool (p/n 5190-2293) by GC/MS. Refer to Table 1 for instrument condition, and Table 3 for peaks identification. A) Full scan chromatogram, B) SIM chromatogram. Satisfactory peaks shape and response achieved for all of analytes.

References:

[1] B. Quimby, "Improved Forensic Toxicology Screening Using A GC/MS/NPD System with a 725-Compound DRS Database", Agilent Technologies publication 5989-8582EN.

[2] N. Seyhan and D.C. Ege, "Organic Chemistry", Health and Company, 1984, pp. 124-136.

[3] M. Szelewski and B. Quimby, "New Tools for Rapid Pesticide Analysis in High Matrix Samples", Agilent Technologies publication 5989-1716EN

[4] P. Wylie and C. Meng, "A Method for the Trace Analysis of 175 Pesticides Using the Agilent Triple Quardrupole GC/MS/MS", Agilent Technologies publication 5990-3578EN

[5] V. Giarrocco, B.Quimby, "Retention Time Locking: Concepts and Applications", Agilent Technologies publication 5966-2469EN.

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