Evaluation of the Ultra Inert Liner Deactivation for Active Compounds Analysis by GC

Technical Overview

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Abstract

Endrin and DDT breakdown and active semivolatiles tests were used for the Ultra Inert liner deactivation performance evaluation. The results indicate that the Ultra Inert deactivated liners provide superior inertness for analysis of active compounds.

Introduction

The analysis of active compounds by gas chromatography (GC) is challenging in areas such as pesticides, food, environmental and drug analysis. In order to achieve reliable and solid results for these active compounds, it is critical to minimize the interaction of active analytes along the GC flow path starting with the injector, to the column and finally to the detector. The inlet liner plays a key role in influencing the inertness of the entire flow path. Active sites on the liner can selectivity latch onto active analytes, leading to the degradation or adsorption of these analytes, resulting in poor linearity for calibration curves and loss of sensitivity. Therefore, it is critical to deactivate the inlet liner completely to make it fully inert.

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Many pesticides are labile, and intentionally designed to decompose easily so that accumulation in the environment is minimized. Endrin and DDT are two well-known compounds that can degrade excessively during gas chromatographic analysis if the inertness of GC flow path is not well controlled, Endrin decomposes to Endrin Aldehyde (EA) and Endrin Ketone (EK), and DDT degrades to DDE and DDD. Most breakdown reactions occur on hot inlet surfaces. The degradation increases when liner deactivation degrades with continuous use, and fragments of septa and non-volatile residue from dirty samples slowly accumulate in the inlet. These create additional active surfaces and cause the breakdown reactions of Endrin and DDT to increase. Therefore, Endrin and DDT breakdown is a very good probe to evaluate not only the efficiency of liner deactivation, but also the stability of liner deactivation treatment over the time of multiple injections. The calculation of Endrin and DDT breakdown is listed below.

$$\%\ \text{Endrin breakdown} = \frac{(\text{Peak area}_{EA} + \text{Peak area}_{EK})}{(\text{Peak area}_{EA} + \text{Peak area}_{EK} + \text{Peak area}_{Endrin})} \times 100$$

$$\%\ \text{DDT breakdown} = \frac{(\text{Peak area}_{DDE} + \text{Peak area}_{DDD})}{(\text{Peak area}_{DDE} + \text{Peak area}_{DDD} + \text{Peak area}_{DDT})} \times 100$$

USEPA Method 8270 has been used widely to determine the concentration of semi-volatile organic compounds in the environment. This method has been used to analyze a mix of acids, bases and neutrals that must be measured concurrently. This test is a challenge for GC instrument due to the interaction of the analytes with GC flow path, where the inlet liner can be a significant contributor for the system activities. The active sites on liner surface can cause the unwanted adsorption of these compounds, and lead to the loss of system responses. The most active compounds, such as the nitrophenols, showed lower response factors (RFs) at the low concentrations, and this causes poor linearity of the calibration curve and low sensitivity of the analytes. Among those active analytes, 2,4-dinitrophenol, usually showed RFs below EPA method requirements and failed the run. A test mix used for evaluating the new liner deactivation, including potentially troublesome compounds: N-nitrosodimethylamine, aniline, 2,4-dinitrophenol, 4-nitrophenol, 4,6-dinitro-2-methylphenol, 4-aminobiphenyl, pentachlorophenol, benzidine, 3,3-dichlorobenzidine, benzo[b]fluoranthene, benzo[k]fluoranthene. This test mix was used previously for the USEPA 8270 method improvements on the Agilent GC/MSD system. [1] A calibration curve from 2 – 80 µg/mL was used for linearity evaluation.

Experimental

Chemicals and Reagents

Endrin and DDT stock standards were purchased from AccuStandard (New Haven, CT, USA). 8270 customized standard and internal standards were obtained from Ultra Scientific (North Kingstown, RI, USA). Ultra Resi-analyzed grade isooctane and methylene chloride were from J. T. Baker (Phillipsburg, NJ, USA).

Solutions and Standards

The 100/200 µg/mL Endrin and DDT stock solution was diluted 2000 times with isooctane to 50/100 ng/mL Endrin and DDT testing solution. This testing solution was stored at 4 °C in the refrigerator and used for no more than three days. Isooctane was also used for solvent blank injection and syringe rinse solvent.

The 8270 custom standard was purchased as 2000 µg/mL mixture in methylene chloride. A series calibration standards were prepared at 2, 5, 20, 40 and 80 µg/mL by appropriate dilution with methylene chloride. The 8270 semivolatile internal standard (IS) mixture at 4000 µg/mL in methylene chloride was spiked into standards with appropriate volume to generate constant 40 µg/mL IS concentration.

Instrumentation

The Endrin and DDT breakdown test was done on an Agilent GC equipped with an Agilent 7683B autosampler and a µECD. Semivolatile test was done on an Agilent GC equipped with a 7683B autosampler and FID. Table 1 and 2 list the instrumental conditions used on each test. Table 3 lists flow path consumable supplies used in these experiments.

Table 1. Instrumental Conditions for Agilent GC/μECD System Used for Endrin and DDT Test

Autosampler	Agilent 7683B, 10 μL syringe (p/n 5181-3354), 1 μL injection volume.
	Preinj solvent A (Isooctane) washes: 4 (for sample run), 0 (for solvent run)
	Sample pumps: 3 (for sample run), 1 (for solvent run)
	Postinj solvent B (Isooctane) washes: 4 (for sample run), 1 (for solvent run)
Carrier gas	Helium at 0.9 mL/min (31cm/s), constant flow
Inlet	Splitless mode; 250 °C, 30 mL/min purge flow at 0.75 min
Analytical column	Agilent HP-5MS UI column, 15 m × 0.25 mm, 0.25 μm, (p/n 19091S-431UI)
Oven profile	For sample run: 120 °C (1 min), 30 °C/min to 220 °C (0 min), 8 °C/min to 280 °C (1 min).
	For solvent run: 250 °C (5 min)
Detector	μECD, 280 °C, Constant column + makeup flow with combined flow of 60 mL/min

Table 2. Instrumental Conditions for Agilent GC/FID System Used for EPA 8270 Semivolatile Active Compounds Test

Autosampler	Agilent 7683B, 5 μL syringe (p/n 5181-5246), 1 μL injection volume.
	Preinj solvent A (Methylene chloride) washes: 1
	Sample pumps: 3
	Postinj solvent B (Methylene chloride) washes: 3
Carrier gas	Helium at 3 mL/min constant flow
Inlet	Splitless mode; 250 °C, 30 mL/min purge flow at 0.75 min
Analytical column	Agilent Ultra 2 column, 25 m × 0.32 mm, 0.52 μm, (p/n 19091B-112)
Oven profile	For sample run: 40 °C (1 min), 15 °C/min to 310 °C (0 min)
Detector	FID, 250 °C, H_2/Air/Makeup N_2: 40/450/45 mL/min

Table 3. Flow Path Supplies

Vials	Amber screw cap (Agilent p/n 5182-0716)
Vial caps	Blue screw cap (Agilent 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) 0.5 mm id, 85/15 Vespel/graphite (p/n 5062-3514)
O-rings	Non-stick liner O-ring (p/n 5188-5365) Non-stick Flip-Top Liner O-ring (p/n 5188-5366)
Inlet liners	Agilent UI single taper splitless liner (p/n 5190-2292)
	Agilent deactivated single taper splitless liner (p/n 5181-3316)
	Agilent inert single taper splitless liner (p/n 5181-3316i)
	Restek Siltek deactivated gooseneck splitless liner

Results and Discussion

The purpose of these tests was to evaluate the Agilent Ultra Inert liner deactivation and compare it with other available liner deactivations. However, there are other factors possibly contributing to the unexpected degradation or adsorption of active compounds, including the inlet gold seal, column, detector and so forth. Therefore, it is critical to minimize other factors' impact on the flow path activity, and also keep the testing conditions consistent for accurate comparison.

We purposely used FID and µECD as detectors to eliminate any activity contributed from the mass spectrometer. We also selected the Ultra Inert column to minimize the column activity. Different liner configurations have different effects on the liner activities. Although, by using direct connect liner, the inlet gold seal activity contribution can be eliminated, it does increase the operation difficulties, and has limited applications. As splitless injection has been used almost universally, the splitless injection mode and single taper splitless liner configuration were used for this evaluation and parallel comparison. A new gold seal was used for each liner test. It was recommended that a Cool On Column (COC) should be used to test the column performance [1] because the on-column injection eliminates any inlet activity. The COC test was not performed in the study. Firstly, splitless injection is much more practical in applications. Furthermore, the activity comparison can be made as long as all of tests were performed at same conditions.

Endrin and DDT Breakdown Test

As described above, Endrin and DDT breakdown test was used for liner deactivation evaluation. A 50/100 ppb Endrin and DDT solution made in isooctane was used for multiple injections test. Before a new liner was installed, the inlet was inspected and carefully cleaned if necessary. A new gold seal and washer were installed and a new liner O-ring was used. An isooctane blank was normally injected as the very first run on the tested liner. A standard sample was run after to collect the initial data for the Endrin and DDT breakdown. Nine solvent injections were run after the standard run. This cycle was repeated nine more times until 100 injections were made. One more standard was then run, and this was the data for the 101st injection. A fast solvent run method was used for solvent run to save the time. The Endrin and DDT standard runs data were collected; and these data were used to generate the breakdown profile. In addition to the Ultra Inert single taper deactivated liner, the Agilent proprietary deactivated liners (p/n 5181-3316) and Restek Siltek deactivated liners with equivalent liner configuration were tested for the parallel comparison. Multiple Ultra Inert liners (n=16), Agilent Proprietary deactivated single taper liners (n=8) and Restek Siltek Deactivated gooseneck liners (n=8) were tested. Data were collected for comparison.

Figure 1 shows the sample chromatograms of Endrin and DDT standards run on an Agilent Ultra Inert deactivated single taper liner. The first injection delivered 1.2% Endrin breakdown and 2.5% DDT breakdown; and the 101st injection had 12.2% Endrin breakdown and 3.0% DDT breakdown. This data indicated that the Endrin and DDT breakdown were well controlled by the liner inertness provided by the Ultra Inert liner deactivation process.

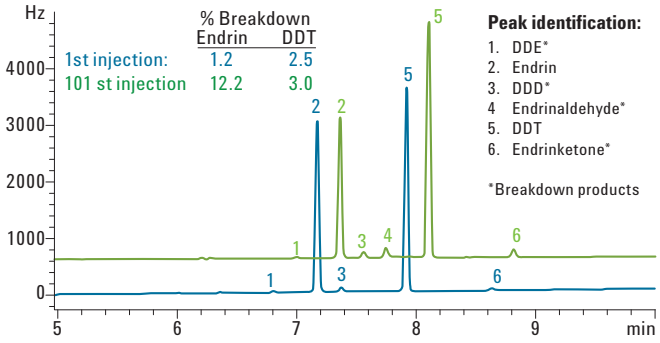

Figure 1. Endrin and DDT breakdown test chromatograms by Agilent Ultra Inert single taper splitless liner (p/n5190-2292)

Figure 2 shows the comparison of Endrin breakdown results over 100 injections for Agilent Ultra Inert deactivated single taper splitless liners, Agilent Proprietary deactivated single taper splitless liners, and Restek Siltek deactivated gooseneck splitless liners. The DDT breakdown is well under control and below 10% for all of tested liners over 100 injections. The results indicate that while all chemistries had similar initial decomposition, the Agilent Proprietary and new Ultra Inert deactivations demonstrated greater stability throughout the test sequence.

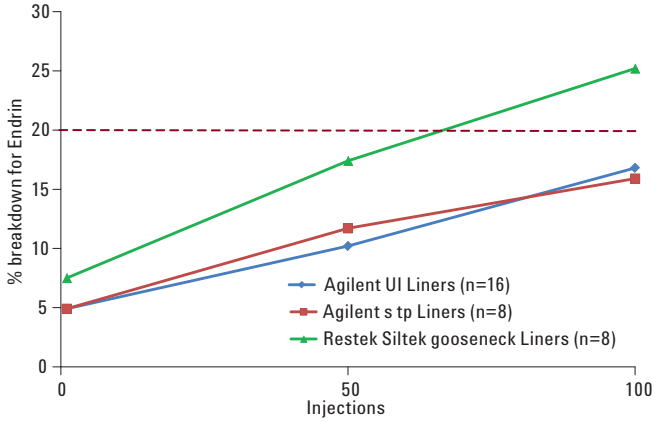

Figure 2. Endrin breakdown profile over 100 injections for Agilent Ultra Inert single taper splitless liner (p/n 5190-2292) (blue), Agilent Proprietary single taper splitless liner (p/n 5181-3316) (red), and Restek Siltek gooseneck splitless liner (green).

Semivolatile Active Compounds Test

In addition to Endrin and DDT breakdown test, an alternative and more sensitive test, EPA 8270 semivolatile active compounds analysis, was conducted. A previously established test mix [1] was used, which includes four phenols, several bases, and several neutral components. These compounds were selected to not only represent 8270 active analytes, but also to be resolved easily and unambiguously detected by GC/FID. Figure 3 shows a sample chromatogram of the test mix with 20 ng on column. The EPA 8270 method does not specify a calibration range, yet traditionally a dynamic range of 20 to 160 ng on column has been widely used in USEPA Contract Lab Program (CLP). However, with the increased sensitivity of newer GC/MS systems, users are moving toward lower and more challenging detection limits. A calibration range of 2 to 80 ng on column was chosen for this analysis. The 2 ng on column quantitation limit gave the satisfied response and peak shape on FID with S/N ratio over 20 for 2,4-DNP, which has the lowest response. In order to evaluate the linearity of GC/FID system over the calibration curve range, the Response Factor (RF) at each calibration level was calculated as below and the overall RF values across the curve were used to calculate the relative standard deviation (RSD).

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

According to USEPA 8270 method requirement [2], the RSD for each target analyte should be less than 20%. Table 4 shows the average RF value across the calibration range and RSD for each testing compound. All of compounds were below the EPA requirement of less than 20% RSD over the 2-80 ng on column range. 3,3-Dichlorobenzidine data is not available due to its complete co-elution with peak of IS 5, Chrysene-d12 (Figure 3).

Table 4. Average RFs and RSD Results for Tested 8270 Semivolatile Active Compounds Using Agilent Ultra Inert Deactivated Single Taper Liners (n=6) (p/n 5190-2292)

Compounds	Average RF over 2 – 80 ng on column	RSD (%)
N-Nitrosodimethylamine	0.563	3.1
Aniline	1.513	1.0
2,4-Dinitrophenol	0.288	15.6
4-Nitrophenol	0.457	3.9
4,6-Dinitro-2-methylphenol	0.382	8.5
4-Aminobiphenyl	0.913	1.1
Pentachlorophenol	0.299	6.1
Benzidine	0.619	4.8
Benzo(b)fluoranthene	0.938	3.3
Benzo(k)fluoranthene	0.897	7.1

- 3,3-Dichlorobenzidine data is not available due to complete co-elution with IS 5.

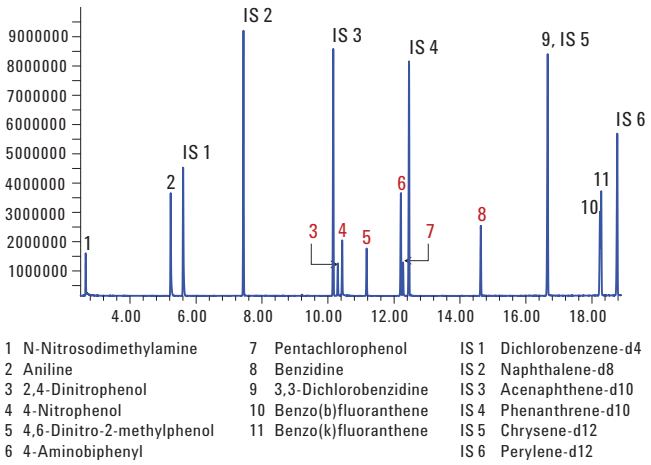

Figure 3. A sample chromatogram of 8270 short test mix with peaks identification by Agilent Ultra Inert single taper splitless liner (p/n 5190-2292).

Six critical compounds were selected for parallel comparison with other equivalent liners. They are 2, 4-dinitrophenol (2,4-DNP), 4-nitrophenol (4-NP), 4,6-dinitro-2-methylphenol (4,6-DN-2-MP), 4-aminobiphenyl (4-ABP), pentachlorophenol (PCP) and benzidine. For the parallel comparison, 5 liner deactivation chemistries but equivalent liner configurations were tested with repeats of n ≥ 4 for each type of liner. Table 5 shows the comparison results for five deactivations. Since 2,4-dinitrophenol (2,4-DNP) is the most active compound, as shown with larger RSD, its' comparison data is shown in detail in Figure 4. The comparison data indicate that, in this EPA 8270 semivolatile active compounds application, the new Ultra Inert deactivation is equivalent to or slightly better than the current Agilent Proprietary, Restek Siltek, and Restek Sky liner deactivations and they all behave better than the SGE deactivation. It should be notified that although they all give similar RSD values across the calibration range, the Ultra Inert deactivation generated higher responses for 2,4-DNP (mean RF of 0.288) than other liners and this number is close to the COC data

Table 5. Average Response Factors and RSD Results Comparison of Five Liner Deactivations of Single Taper Splitless Liner (or Equivalent Configuration)

	2,4-DNP		4-NP		4,6-DN-2-MP		4-ABP		PCP		Benzidine	
	Ave. RF	**RSD**	**Ave. RF**	**RSD**	**Ave. RF**	**RSD**	**Ave. RF**	**RSD**	**Ave. RF**	**RSD**	**Ave. RF**	**RSD**
Ultra Inert Deact. Liners (n = 6)	0.288	15.6	0.457	3.9	0.361	6.3	0.935	7.5	0.299	6.1	0.617	3.3
Restek Siltek Deact. Liners (n = 4)	0.245	16.5	0.449	3.6	0.352	7.2	0.934	7.2	0.298	11.9	0.615	2.7
Restek Sky Liners (n = 4)	0.262	18.2	0.433	5.4	0.352	9.1	0.881	2.4	0.292	5.7	0.495	12.9
Agilent Proprietary Deact. Liners (n = 4)	0.262	15.2	0.475	4.2	0.379	4.9	0.938	7.7	0.325	16.4	0.631	3.1
SGE Deact. Liners (n = 4)	0.252	22.7	0.459	6.6	0.369	8.3	0.921	7.2	0.320	15.0	0.604	5.9

obtained previously.[1] This allows lower detection limits, as was demonstrated with 0.5 ppm of 2,4-DNP test done on GC-MS. As shown in Figure 5, Ultra Inert deactivation liners had higher response for 2,4-DNP at the concentration of 0.5 ppm. It should also be mentioned that the average RFs value of benzidine on Restek Sky liners are about 20% lower than other liner deactivations, and the RSD is 2-3 times higher than other liners. This indicates that Restek's Sky liners are less suitable for basic active compounds like benzidine than other liner deactivations.

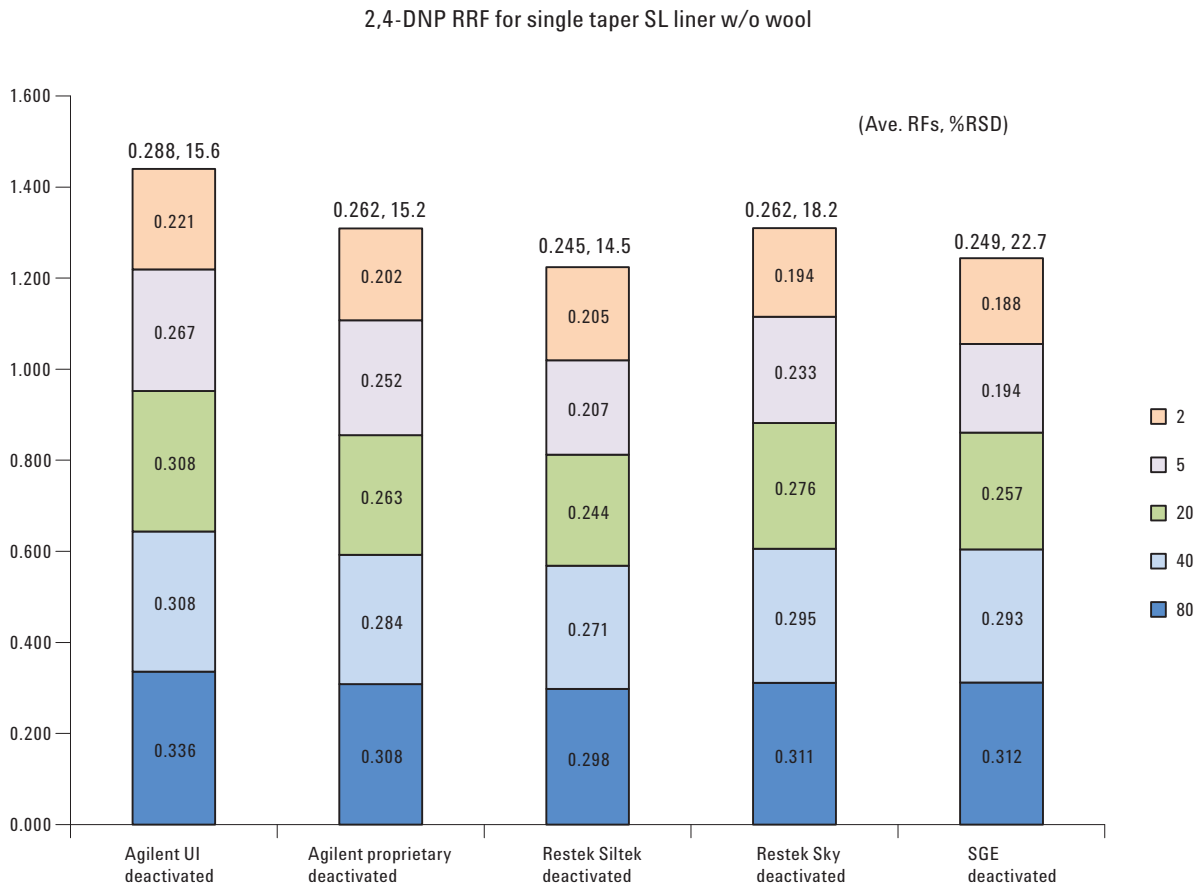

Figure 4. Parallel comparison of splitless single taper liners for Agilent Ultra Inert liners (p/n 5190-2292), Restek Siltek Deact. liners, Restek Sky Deact. liners, Agilent proprietary deact. liners (p/n 5181-3316), and SGE deact. liners using 2,4-Dinitrophenol as a probe.

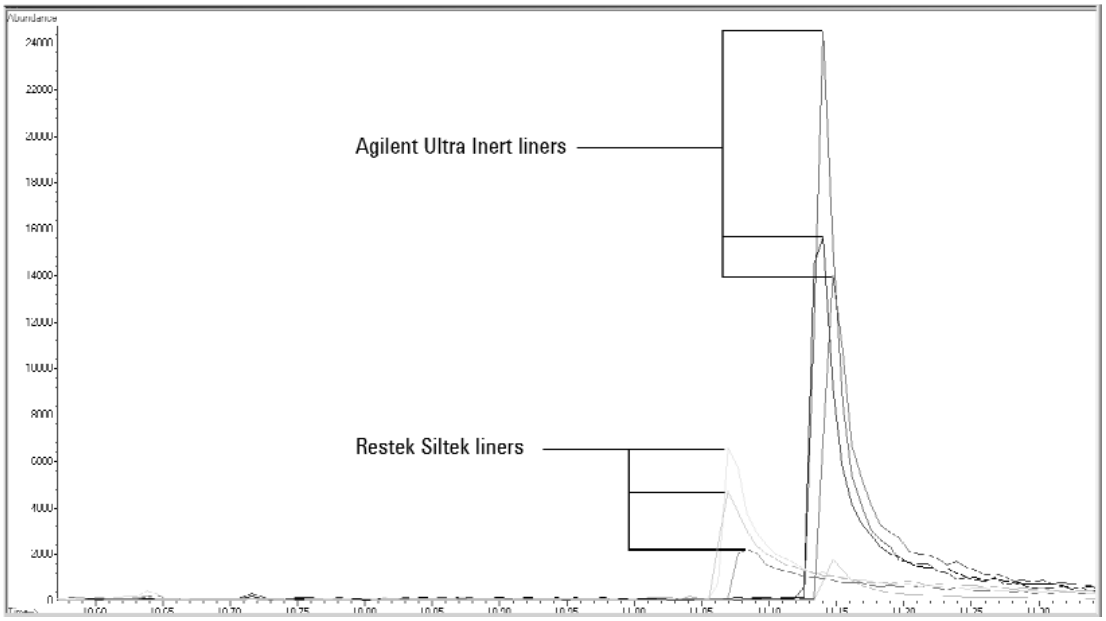

Figure 5. 0.5 ppm 2, 4-Dinitrophenol test by GC/MS SIM mode. Agilent Ultra Inert single taper splitless liners (P/N 5190-2292) give out higher responses for 2, 4-DNP than Restek Siltek liners at lower concentration.

Conclusion

Agilent Ultra Inert deactivated liners were evaluated and compared with equivalent liners including the Agilent Proprietary deactivated liners, Restek Siltek and Sky deactivated liners, using Endrin and DDT breakdown test and EPA 8270 semi-volatile active analytes test as two probes. In the Endrin and DDT breakdown test, the Ultra Inert deactivated liners had <20% Endrin and DDT breakdown over 100 injections at 50 ppb standard level. In 8270 semi-volatile test, the Ultra Inert deactivated liners generated less than 20% RSD for RF values over the calibration range from 2-80 ng on column. For the most active compound, 2,4-Dinitrophenol, the mean RSD over 2-80 ng on column was 15.6% with replicates of six. The Ultra Inert mean RFs value of 0.288 for 2,4-DNP was higher than those obtained by other liners. The results of liner deactivation evaluation and comparison demonstrate that, given the equivalent liner configuration, the new Ultra Inert liner deactivation is well suited for high sensitivity analysis of active analytes.

References

1. M. Szelewski, B. Wilson, P. Perkins, "Improvements in the Agilent 6890/5973 GC/MSD system for Use with USEPA Method 8270." Agilent Technologies publication 5988-3072EN.
2. USEPA 8270D method, http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/8270d.pdf

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