Evaluation of Fused Silica Tubing for Active Compound Analysis in an Inert Flow Path

Agilent Ultimate Plus Deactivated Fused Silica Tubing for Guard Columns

Application Note

Food Testing and Agriculture, Environmental

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Abstract

Test probes, including an inert test mixture, acidity test standard, and an endrin/DDT mixture, were used to evaluate the performance of deactivated fused silica tubing. The results indicate that Agilent Ultimate Plus deactivated fused silica tubing provides superior inertness compared to other commercially available deactivated tubing for the analysis of active compounds.

Introduction

Guard columns are widely used in GC and GC/MS applications. The primary purpose of guard columns is to protect the analytical column from contamination. When using a guard column, it also sometimes acts as a retention gap. Both guard columns and retention gaps are typically attached to the front of the analytical column. Retention gaps mainly act as a band-focusing device for liquid samples introduced by on-column and splitless injection techniques. This is done to improve peak shapes for some types of samples, columns, and GC conditions [1]. The benefits of a retention gap are often unintentionally obtained when using a guard column.

The use of a guard column is also an inexpensive technique to extend the lifetime of capillary columns when the guard column is a short piece of uncoated, deactivated fused silica tubing. Since contamination is limited to the front of the column, trimming the guard column periodically to restore performance, instead of the capillary column, preserves the main column. Thus, chromatography, including retention time and resolution, is not affected.

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The chromatographic analysis of active analytes by GC can be challenging unless the GC flow path is inert towards these types of compounds. To ensure accurate and reproducible results, using deactivated fused silica tubing as a guard column to protect the analytical column plays a key role in the inert flow path. High inertness performance to minimize analyte degradation and peak tailing is required.

Agilent Ultimate Plus deactivated fused silica tubing showed excellent performance as a GC restrictor in the analysis of pesticides, semivolatiles, and drugs of abuse checkout mixtures in previous work [3-5]. In this application note, a group of active compounds was analyzed. We used Ultimate Plus deactivated fused silica tubing, and tubing from two different vendors, as guard columns to measure inertness of the tubing under the same GC conditions. Both qualitative (peak shape) and quantitative (tailing factor, T_f values) data were used to assess the activity of the tubing.

Experimental

An Agilent 7890B GC equipped with a microcell electron capture detector (μECD) and a flame ionization detector (FID) was used. Sample introduction was done using an Agilent 7683B Automatic Liquid Sampler with a 5 μL syringe (p/n G4513-80213), and a split/splitless injection port. Table 1 lists the columns and tubing.

Table 1. Columns and tubing.

Column 1:	Agilent J&W HP-5ms Ultra Inert, 15 m × 0.25 mm, 0.25 μm (p/n 19091-431UI)
Column 2:	Agilent J&W HP-5ms Ultra Inert, 30 m × 0.32 mm, 0.25 μm (p/n 19091S-413UI)
Tubing 1:	Agilent Ultimate Plus deactivated FS tubing, 5 m × 0.25 mm (p/n CP802505)
Tubing 2:	Agilent Ultimate Plus deactivated FS tubing, 5 m × 0.32 mm (p/n CP803205)
Tubing 3:	Guard column, 5 m × 0.25 mm, from supplier R
Tubing 4:	Guard column, 5 m × 0.32 mm, from supplier R
Tubing 5:	Guard column, 5 m × 0.25 mm, from supplier P
Tubing 6:	Guard column, 5 m × 0.32 mm, from supplier P

Two guard columns with different serial numbers were purchased from each supplier to demonstrate performance of different manufacturing lots. Two guard columns from each serial number were tested to confirm reproducibility of results. All guard columns were connected to analytical columns using Agilent inert Ultimate Unions, and analyzed in the same manner. The experimental setup is shown in Figure 1.

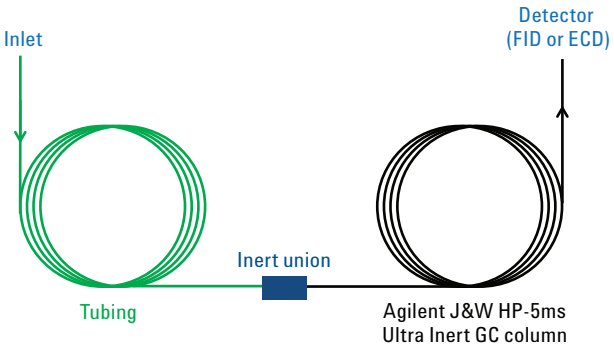

Figure 1. Experimental setup of the GC system for testing deactivated fused silica tubing.

The composition of the inert test mixture consisted of the six compounds listed in Table 2, which were analyzed using the chromatographic conditions in Table 3. All standard compounds in the inert test mixture were purchased from Sigma-Aldrich, Corp. (> 98% purity), and the mixture prepared in methanol (Burdick & Jackson High Purity Solvent). The same standard mixture was injected into each guard column at least five times.

Table 2. Inert test mixture in methanol.

1. Pyridine	0.3%
2. 4-Methylpyridine	0.3%
3. Cyclohexanol	0.2%
4. Cyclohexanone	0.2%
5. Trimethyl phosphate	0.4%
6. 1-Heptanol	0.2%

Table 3. Chromatographic conditions for inert test mixture.

Carrier gas:	H_2, constant flow, 44 cm/s
Inlet:	250 °C, Split mode, split ratio 250:1
Oven:	65 °C, isothermal
Analytical column:	Agilent J&W HP-5ms UI, 15 m × 0.25 mm, 0.25 μm (p/n 19091S-431UI)
Guard column:	Agilent Ultimate Plus Deactivated FS tubing, 5 m × 0.25 mm (p/n CP802505) Inert FS tubing, 5 m x 0.25 mm, from suppliers R and P
FID:	300 °C

The acidity test mixture was Phenols EPA Method 604 (0.5 mg/mL, MeOH) standard obtained from Anpel (Shanghai, China). Table 4 shows the chromatographic conditions used for the acidity test.

Table 4. Chromatographic conditions for acidity test mixture.

Carrier gas:	He, constant flow, 2.4 mL/min
Inlet:	275 °C, Split mode, split ratio 50:1
Oven:	80 °C, 4 minutes, to 200 °C, 2 minutes, at 8 °C/min
Analytical column:	Agilent J&W HP-5ms UI, 30 m × 0.32 mm, 0.25 µm (p/n 19091S-413UI)
Guard column:	Agilent Ultimate Plus deactivated FS tubing, 1 m × 0.32 mm (p/n CP803205) Inert FS tubing, 1 m x 0.32 mm, from suppliers R and P
FID:	300 °C
Sample:	0.5 µL, Phenols Method EPA 604 (0.5 mg/mL, MeOH)

Endrin and DDT stock standards and isooctane (> 99.5% purity) were purchased from J&K Chemical (Shanghai, China). The 100/200 µg/mL endrin and DDT stock solution was diluted 2,000 times with isooctane to 50/100 ng/mL endrin and DDT test solution. Table 5 shows the chromatographic conditions used for the endrin and DDT breakdown test. Other supplies in this study are listed in Table 6.

Table 5. Chromatographic conditions for endrin and DDT breakdown test.

Carrier gas:	He, constant flow, 1.7 mL/min
Inlet:	250 °C, Pulsed splitless, 25 psi pulse pressure for 0.75 minutes 30 mL/min purge flow at 0.75 minutes
Oven:	120 °C, hold 1 minute, 30 °C/min to 220 °C, 8 °C/min to 280 °C, hold 2 minutes
Analytical column:	Agilent J&W HP-5ms UI, 30 m × 0.32 mm, 0.25 µm (p/n 19091S-413UI)
Guard column:	Agilent Ultimate Plus Deact FS tubing, 1 m × 0.32 mm, p/n CP803205 Inert FS tubing, 1 m × 0.32 mm, from suppliers R and P
ECD:	280 °C, constant flow plus makeup flow with combined flow of 60 mL/min
Sample:	1 µL, 50/100 ng/mL endrin and DDT

Table 6. Other supplies.

Column nut:	Self-tightening, inlet/detector (p/n 5190-6194)
Ferrules:	UltiMetal Plus Flexible Metal, for 0.32 mm columns (10/pk, p/n G3188-27502), for 0.1 to 0.25 mm columns (10/pk, p/n G3188-27501)
Union:	Ultimate union (p/n G3182-60581)
Liners:	Ultra Inert, universal with wool (p/n 5190-2295), splitless single taper (p/n 5190-2292)
Seal:	Ultra Inert, gold-plated, with washer (p/n 5190-6144)
Internal nut:	CFT capillary fitting (p/n G2855-20530)
Ferrules:	Short graphite:Vespel (15%:85%), 0.32 mm (p/n 5062-3514), 0.1 to 0.25 mm (p/n 5181-3323)
Septa:	Longlife (p/n 5183-4761)

Results and Discussion

The purpose of these experiments was to compare the inertness performance of Agilent Ultimate Plus deactivated fused silica tubing and deactivated tubing from other suppliers, as guard columns. Using a suitable union to attach the fused silica tubing to the analytic column is important. The Agilent deactivated Ultimate Union can provide leak-free, inert column connection. The system was inspected and carefully cleaned if necessary before each test. For consistency, new UI columns, gold seals, liners, and Ultimate Union were used for each tubing test.

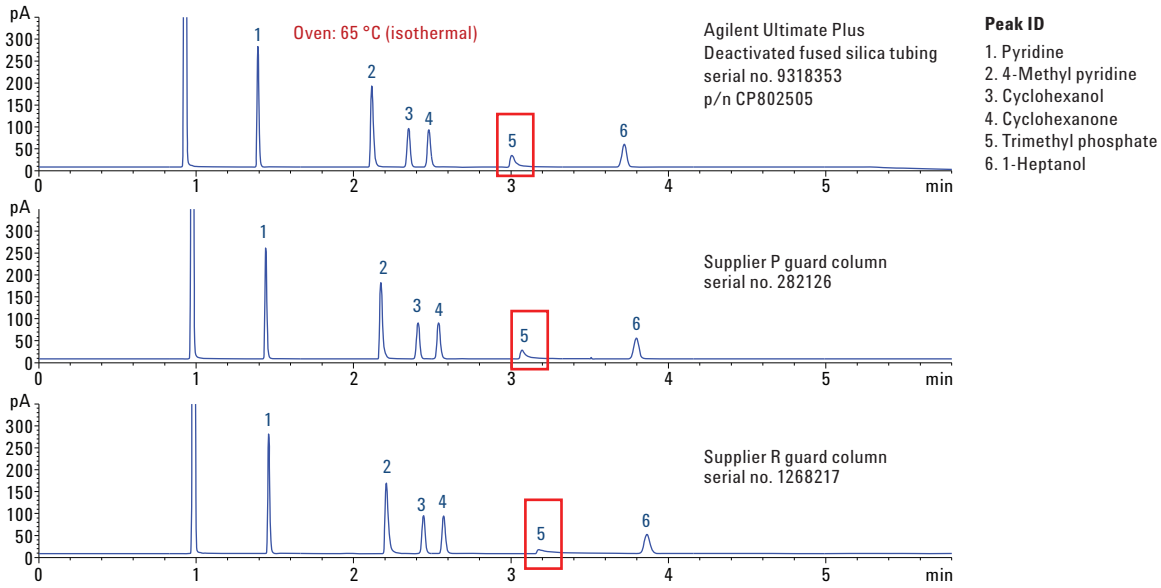

Figure 2. Chromatograms of inert test mixture on different supplier's guard columns. Chromatographic conditions are listed in Table 3.

Inert test mixture

A distinct difference in guard column inertness was observed with compounds in the inert test mixture. This highlighted significant variations in the acidic nature of the active site or surface of deactivated tubing samples, especially for trimethyl phosphate. Visually, there was noticeable tailing and reduced response in the chromatograms for supplier R and P guard columns, as shown in Figure 2. The Ultimate Plus deactivated FS tubing delivered excellent peak shapes. Tailing factors (T_f) can be used to evaluate the inertness performance, because tubing activity leads to poor peak shapes. A measurement of peak tailing was done using the US Pharmacopeia T_f. This is calculated using Equation 1 [5].

$$T_f = \frac{W_{5.0}}{(T_w \times 2)}$$

Equation 1.

Where T_w = distance between peak front and retention time of peak (TR) at 5% of peak height, units are the same as used for $W_{5.0}$, and $W_{5.0}$ = width at 5% of height.

The tailing factors are listed in Table 7. The closer the T_f value is to 1, the more symmetrical the peak. Symmetrical peak shapes, along with the increased peak heights, allow for accurate integration and detection of trace analytes. Therefore, signal-to-noise ratios of peak 5 (trimethyl phosphate) were Agilent tubing 944.7, supplier P 698.8, and supplier R 626.2.

Table 7. Tailing factors (T_f).

	Tailing factor					
Peak no.	**1**	**2**	**3**	**4**	**5**	**6**
Agilent Ultimate Plus tubing	1.25	1.48	1.05	1.19	2.82	0.99
Supplier P tubing	1.33	1.60	1.06	1.19	4.56	1.01
Supplier R tubing	1.30	1.67	1.11	1.16	5.43	1.02

Acidity tests

Acidity tests were performed using the Phenols EPA Method 604 standard. The last four compounds in this mixture proved to be the most troublesome, with 2,4-dinitrophenol being noticeably worse. Figure 3 shows the chromatograms on the column, versus column connected with Ultimate Plus deactivated FS tubing. Similar chromatograms indicated high inertness of the Agilent tubing. Table 8 shows the T_f.

Excellent inert flow path gave good performance for all the tubing in Figure 4 and Table 9, but differences were found in Figure 5. Phenanthrene-d_{10} was used as the internal standard. Reduced responses were evident in the chromatograms for the last four compounds on the supplier R guard column. The same response was found for other compounds and ISTD on both Agilent tubing and the supplier R guard column.

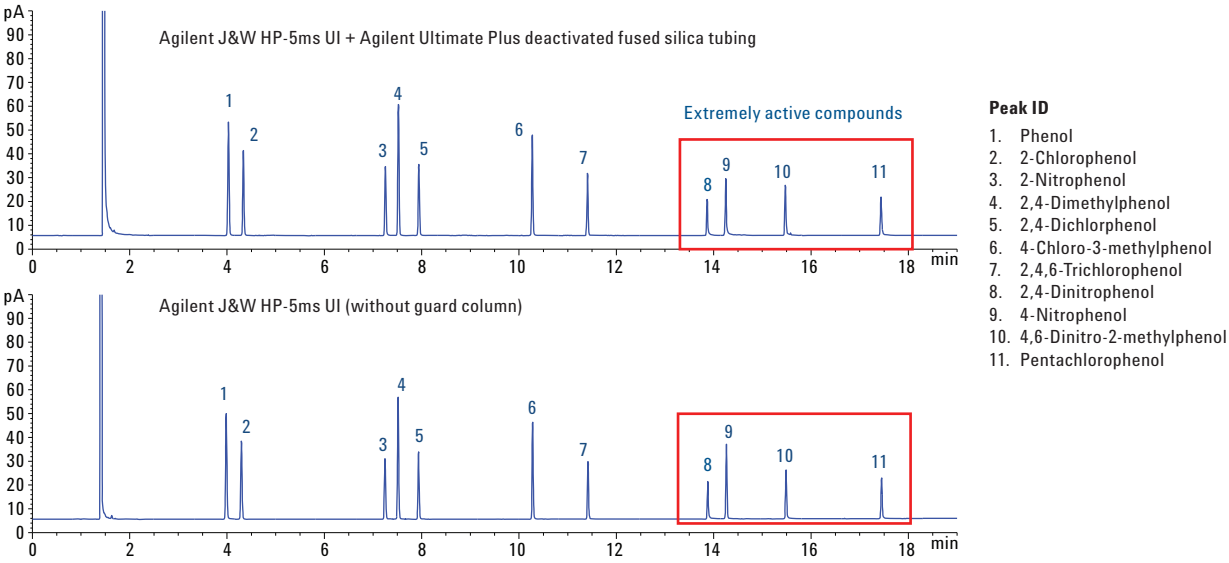

Figure 3. Chromatograms of acidity test mixture at 5 ng/component oncolumn to compare performance of the analytical column with and without the guard column (and union). Chromatographic conditions are listed in Table 4.

Table 8. Tailing factor.

	Tailing factor										
Peak no.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
HP-5ms UI	1.02	1.06	1.12	1.01	1.08	1.00	1.02	1.36	0.98	1.15	1.16
HP-5ms UI + Ultimate Plus tubing	1.02	1.01	1.11	1.04	1.08	1.02	1.07	1.40	1.18	1.21	1.22

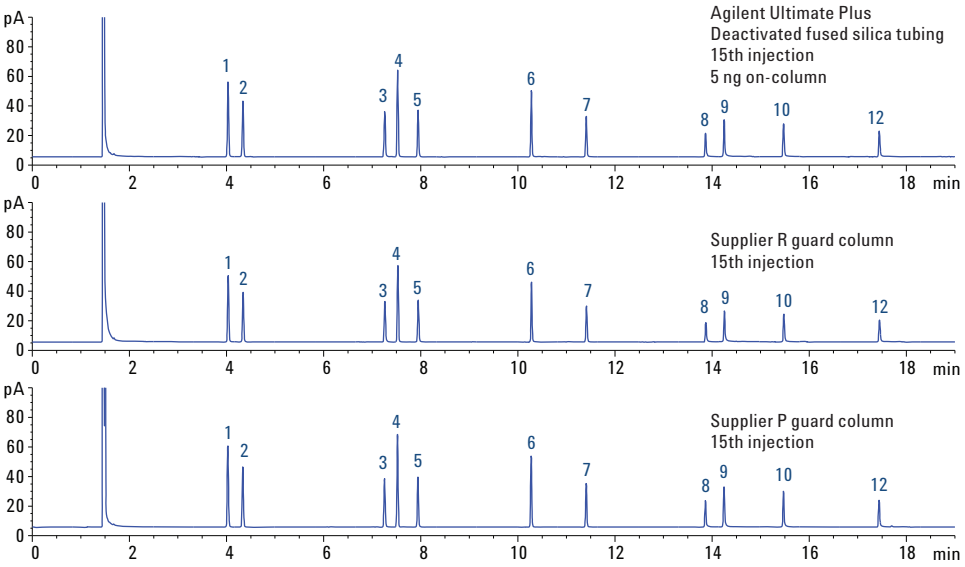

Figure 4. Chromatograms of acidity test mixture on different supplier's guard columns. Chromatographic conditions are listed in Table 4.

Table 9. The last four compounds (most chemically active) RSD values for 5 ng on-column (n = 14).

Compound	Agilent RSD%	Supplier P RSD%	Supplier R RSD%
2,4-Dinitrophenol	6.6	7.0	7.9
4-Nitrophenol	3.04	3.24	3.28
4,6-Dinitro-2-methylphenol	4.2	4.5	4.2
Pentachlorophenol	3.0	3.4	3.7

Endrin/DDT breakdown

Endrin and DDT breakdown is a useful probe to evaluate the inertness of a guard column. According to the US EPA Method 8081B, the percentage breakdown acceptance criteria for endrin and DDT are <15% individually, and their combined percentage does not exceed 30%. Equations 2 and 3 show the calculation of endrin and DDT breakdown, respectively.

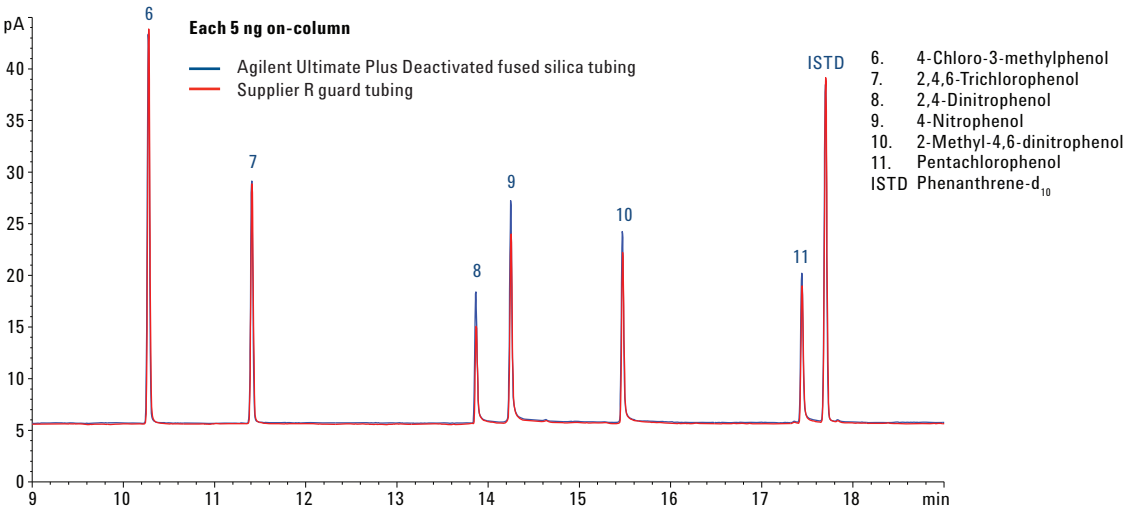

Figure 5. Overlaid chromatograms of acidity test mixture on Agilent and supplier R guard columns. Chromatographic conditions are listed in Table 4.

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

Equation 2.

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

Equation 3.

Figure 6 shows endrin/DDT breakdown test chromatograms using Ultimate Plus deactivated FS tubing. The first injection delivered 1.2% endrin breakdown and 2.2% DDT breakdown, and the 71st injection had 7.3% endrin breakdown and 3.0% DDT breakdown. The results indicated that inertness for the whole flow path was excellent, including the Ultimate Plus deactivated fused silica guard column. Figures 7 and 8 show the comparison of % endrin and % DDT breakdown over 70 injections. DDT breakdown was stable for all tubing, but after 50 injections, the other suppliers' tubing deactivation already exceeded the critical level of 15% endrin breakdown.

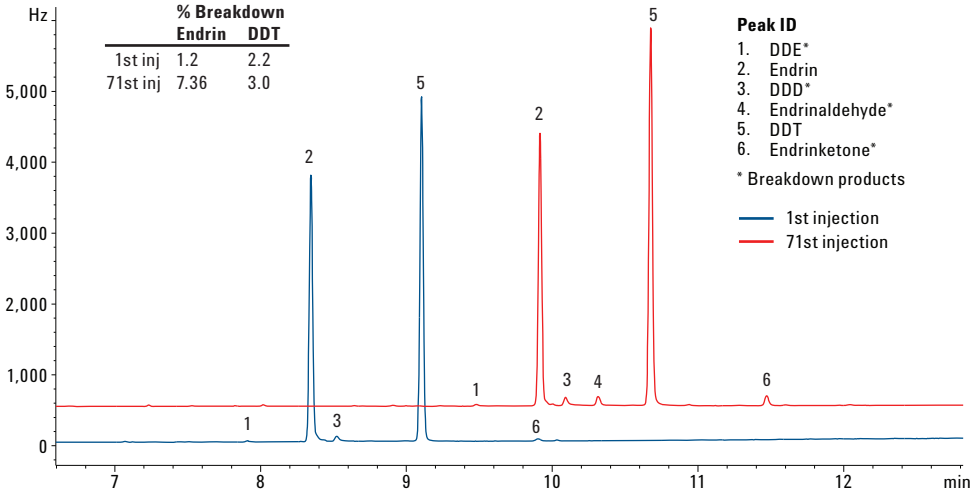

Figure 6. Endrin/DDT breakdown test chromatograms using Agilent Ultimate Plus deactivated fused silica tubing. Chromatographic conditions are listed in Table 5. Blue is the 1st injection and red is the 71st injection.

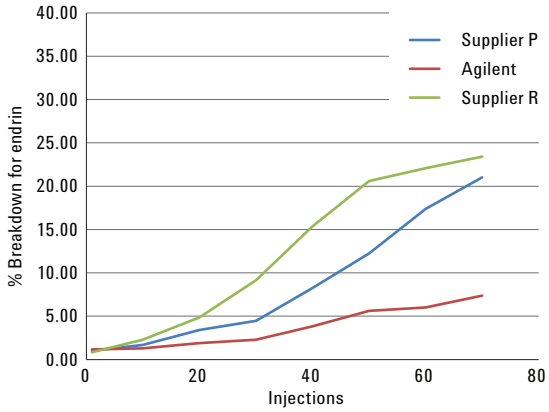

Figure 7. Endrin breakdown profile for Agilent Ultimate Plus deactivated fused silica tubing and other suppliers' tubing.

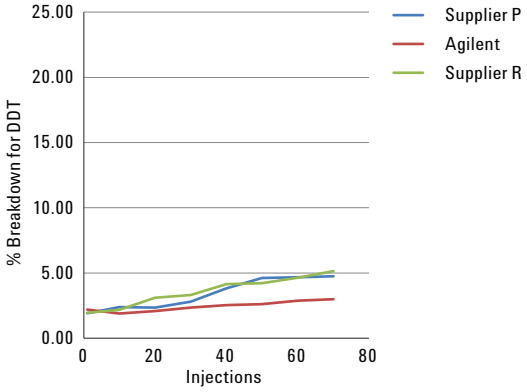

Figure 8. DDT breakdown profile for Agilent Ultimate Plus deactivated fused silica tubing and other suppliers' tubing.

Conclusions

Agilent Ultimate Plus deactivated fused silica tubing was evaluated for use as a guard column and compared with tubing from other suppliers. Using a very active inert test mixture, Phenols EPA Method 604 standards, and endrin and DDT breakdown tests as probes, Ultimate Plus deactivated FS tubing was superior to other vendors' tubing for the analysis of active compounds. The high level of inertness of the Agilent tubing gave better peak shapes for active compounds. This was visually apparent, and verified by tailing factors. A high level of sensitivity means that lower detection limits are achieved. Good repeatability for active compounds, including nitrophenols, ensures reliable results.

Ultimate Plus deactivated fused silica tubing can be used to improve GC flow path performance when used as guard columns, headspace transfer lines, or restrictors for high sensitivity GC and GC/MS analyses of active compounds.

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