# Evaluating Inert Flow Path Components and Entire Flow Path for GC/MS/MS Pesticide Analysis

Application Note

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## Abstract

Flow path inertness plays a critical role in pesticide analysis accuracy and precision, especially for active analytes such as organophosphate pesticides. The Agilent Inert Flow Path, including Ultra Inert columns and liners, Ultra Inert gold seals, and inert split/splitless inlet, provides excellent surface inertness through the entire flow path, prevents loss of analyte response and peak shape distortion, and thus delivers reliable qualitative and quantitative analysis of pesticides. Other inert supplies, including UltiMetal Plus flexible metal ferrules and capillary flow technology devices, are also highly recommended for pesticide analysis in complicated matrices.


## Introduction

Flow path inertness plays a critical role in accurate, precise and reliable analysis of pesticides, especially for sensitive pesticides at trace levels. Active sites on the flow path surface can cause adsorption and degradation of active compounds, resulting in poor peak shape, loss of response, inaccurate integration, and poor quantitation. It is critical to minimize interaction of active analytes along the GC flow path, starting with the injector, to the column, and finally to the detector. The column and inlet liner contribute more than 90% of the contact surface after a sample is injected into the GC system. Other surfaces that the sample can contact include inlet seal, inlet weldments, ferrules, capillary flow technology (CFT) devices, and detectors. All of these surfaces can cause interaction with active analytes, resulting in poor or inaccurate results. To achieve the best inertness of the entire flow path, it is important to use an inert column and liner in combination with other inert supplies.

Multiresidue analysis of pesticides is always a challenge in GC and GC/MS detection. The required quantitation limits for many pesticides are at low ppb levels, which demands more sophisticated analytical processes. Compared to widely used GC/MS analyses, GC/MS/MS techniques provide much better selectivity, thus significantly improving system detection limits. Pesticide compounds frequently contain functional groups such as hydroxyl (-OH) and amino (R-NH-) groups, imidazoles and benzimidazoles (-N=), carbamates (-O-CO-NH-), urea derivatives (-NH-CO-NH-), and organophosphate (-P=O) groups. These types of molecules are prone to interact with active sites on flow path surfaces, resulting in compound adsorption or degradation. As a result, flow path surface inertness is critical for trace pesticide analysis.

Agilent Ultra Inert columns and Ultra Inert liners have demonstrated excellent surface inertness for trace pesticide analysis [1-4]. In this application note, a representative group of 26 challenging pesticides were selected as probes for surface inertness evaluation. The Ultra Inert gold seal was compared to standard gold seal and Siltek deactivated stainless steel inlet seal. UltiMetal Plus flexible metal ferrules were compared to untreated flexible metal ferrules and Siltite ferrules. The Agilent Inert Flow Path was subsequently compared to a standard flow path.

## Materials and Methods

### Chemicals and reagents

All reagents and solvents were HPLC or analytical grade. Ultra Resi analyzed grade acetone was from J.T. Baker (Phillipsburg, NJ, USA). Acetic acid was from Sigma-Aldrich (St Louis, MO, USA). The pesticide standard mix (1,000 µg/mL) was purchased from Ultra Scientific (North Kingstown, RI, USA). Internal standard (triphenyl phosphate, TPP) was from Alfa Aesar (Ward Hill, MA, USA).

### Solutions and standards

Acetic acid (1%) in acetone was prepared by adding 1 mL of glacial acetic acid to 100 mL of acetone, and was used as the reagent blank. This solution was also used to dilute stock pesticide standards. Internal standard (IS) stock solutions (2 mg/mL) were made in acetone and stored at –20 °C. A 20 µg/mL mixed standard (26 pesticides) solution was made in acetone by appropriate dilution of pesticide stock solutions. A 20 µg/mL triphenyl phosphate solution made in acetone was used as IS spiking solution. Five standard solutions of 10, 50, 100, 200, and 500 ng/mL were prepared in 1% acetic acid in acetone spiked with 500 ng/mL IS.

### Instrumentation

All testing was done on an Agilent 7890A GC equipped with an Agilent 7693B Automatic Liquid Sampler and an Agilent 7000 Triple Quadrupole GC/MS. Table 1 lists the instrument conditions, Table 2 shows the flow path consumable supplies, and Table 3 shows the multiple reaction monitoring (MRM) conditions for 26 target analytes. Retention time locking (RTL) was used to eliminate the need for recalibration of the individual retention times and timed events such as the MRM groups [5]. The total run time for a sample spiked with standard was 20.5 minutes.

Table 1. Instrument conditions for the Agilent GC/MS system used for basic drug compounds.

| | |
|---|---|
| GC: | Agilent 7890A GC |
| Autosampler: | Agilent 7693 Automatic Liquid Sampler, 5 µL syringe (p/n 5181-5246), 1 µL injection volume<br>Post-injection solvent A (acetone), 3 washes<br>Sample pumps, 3<br>Post-injection solvent B (acetone), 3 washes |
| Carrier gas: | Helium, constant flow |
| Gas filter: | Gas Clean filter GC-MS, 1/8 in (p/n CP17974) |
| Inlet: | Split/splitless inlet at pulsed splitless mode, 250 °C |
| Injection pulse pressure: | 30 psi until 0.75 minutes |
| Purge flow to split vent: | 30 mL/min at 0.75 minutes |
| Flow rate: | 1.1 mL/min (RT locked) |
| RT locking: | Chlorpyrifos methyl at 9.145 minutes |
| Oven profile: | 60 °C for 1 minute, then to 170 °C at 30 °C/min, to 310 °C at 10 °C/min, hold for 3 minutes |
| Column: | Agilent J&W HP-5ms UI, 30 m × 0.25 mm, 0.25 µm (p/n 19091S-433UI) |
| Connections: | Between column and UltiMetal Plus Ultimate Union (p/n G3182-61580) (for ferrule evaluation only) |
| Restrictor: | Inert fused silica tubing, 0.65 m × 0.15 mm (p/n 160-7625-5) |
| Connections: | Between Purged Ultimate Union and the MSD (for ferrule evaluation only) |
| MSD: | Agilent 7000 Triple Quadrupole GC/MS inert with performance electronics |
| Vacuum pump: | Performance turbo |
| Mode: | MRM |
| Tune file: | Atune.u |
| Transfer line temperature: | 280 °C |
| Source temperature: | 300 °C |
| Quad temperature: | Q1 and Q2, 150 °C |
| Solvent delay: | 3.75 minutes |
| Collision gas flows: | He quench gas at 2.35 mL/min,<br>$N_2$ collision gas at 1.5 mL/min |
| MS resolution: | MS1 and MS2, 1.2 amu |

Table 2. Flow path supplies.

| | |
|---|---|
| Vials: | Amber screw cap (p/n 5182-0716)<br>Vial insert, 150 µL glass insert with polymer feet (p/n 5183-2088) |
| Vial caps: | Blue screw cap (p/n 5182-0717) |
| Vial inserts: | 150 µL glass with polymer feet (p/n 5183-2088) |
| Septum: | Advanced green nonstick, 11 mm (p/n 5183-4759) |
| Ferrules: | 0.4 mm id, 85/15 Vespel/graphite (p/n 5181-3323)<br>UltiMetal Plus Flexible Metal ferrules (p/n G3188-27501) |
| Inlet seal: | Gold-plated inlet seal with washer (p/n 5188-5367)<br>Ultra Inert gold seal with washer (p/n 5190-6144) |
| Inlet liners: | Agilent Ultra Inert deactivated single taper splitless liner with wool (p/n 5190-2293) |
| CFT: | UltiMetal Plus Ultimate Union (p/n G3182-61580)<br>Internal nut, CFT capillary fitting (p/n G2855-20530) |

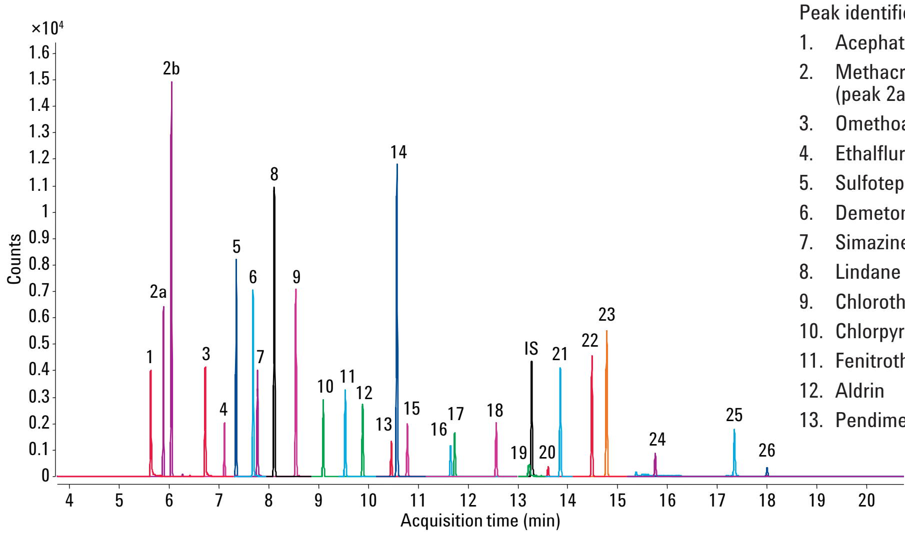
Table 3. Quantifier and qualifier MRM transitions for 26 pesticides.

| Time segment | Start time (min) | Compound name | Precursor ion | Product ion | Dwell (ms) | CE (V) |
|---|---|---|---|---|---|---|
| 1 | 3.75 | Methacrifos | 207.9 | 180.1 | 20 | 5 |
| | | | 124.9 | 47.1 | 20 | 10 |
| | | Acephate | 136 | 42 | 20 | 6 |
| | | | 136 | 94 | 20 | 14 |
| 2 | 6.30 | Ethalfluralin | 275.9 | 202.1 | 20 | 10 |
| | | | 315.9 | 275.9 | 20 | 10 |
| | | Omethoate | 109.9 | 79 | 20 | 15 |
| | | | 156.1 | 79 | 20 | 15 |
| 3 | 7.30 | Sulfotep | 201.8 | 145.9 | 20 | 10 |
| | | | 237.8 | 145.9 | 20 | 10 |
| | | Simazine | 201.1 | 173.1 | 20 | 5 |
| | | | 173 | 172.1 | 20 | 5 |
| | | Demeton-S | 88 | 60 | 20 | 10 |
| | | | 126 | 65 | 20 | 10 |
| 4 | 7.95 | Chlorothalonil | 263.8 | 168 | 20 | 25 |
| | | | 265.8 | 133 | 20 | 53 |
| | | Lindane | 216.9 | 181 | 20 | 5 |
| | | | 181 | 145 | 20 | 15 |
| 5 | 8.85 | Chlorpyrifos methyl* | 287.8 | 93 | 20 | 26 |
| | | | 285.8 | 271 | 20 | 26 |
| 6 | 9.40 | Fenitrothion | 125.1 | 47 | 20 | 15 |
| | | | 277 | 260.1 | 20 | 5 |
| | | Aldrin | 262.9 | 192.9 | 20 | 30 |
| | | | 262.9 | 191 | 20 | 30 |
| 7 | 10.15 | Folpet | 259.8 | 130.1 | 20 | 15 |
| | | | 261.8 | 130.1 | 20 | 15 |
| | | Pendimethalin | 251.8 | 162.2 | 20 | 10 |
| | | | 251.8 | 161.1 | 20 | 15 |
| | | Tolylfluanid | 237.9 | 137 | 20 | 15 |
| | | | 136.9 | 91.1 | 20 | 20 |
| 8 | 11.15 | Dieldrin | 262.9 | 193 | 20 | 35 |
| | | | 277 | 241 | 20 | 5 |
| | | Bupirimate | 272.9 | 193.1 | 20 | 5 |
| | | | 272.9 | 108 | 20 | 15 |
| | | Triazophos | 161.2 | 134.2 | 20 | 5 |
| | | | 161.2 | 106.1 | 20 | 10 |
| 9 | 13.00 | TPP (IS) | 326 | 325 | 20 | 5 |
| | | | 214.9 | 168.1 | 20 | 15 |
| | | Propargite | 149.9 | 135.1 | 20 | 5 |
| | | | 135 | 77.1 | 20 | 5 |
| 10 | 13.50 | Iprodione | 313.8 | 244.9 | 20 | 10 |
| | | | 313.8 | 56 | 20 | 20 |
| | | EPN | 169 | 141.1 | 20 | 5 |
| | | | 169 | 77.1 | 20 | 5 |
| 11 | 14.10 | Mirex | 273.8 | 236.8 | 20 | 15 |
| | | | 271.8 | 234.8 | 20 | 15 |
| | | Phosalone | 182 | 111 | 20 | 15 |
| | | | 182 | 102.1 | 20 | 15 |
| 12 | 15.20 | Coumaphos | 210 | 182 | 20 | 10 |
| | | | 361.9 | 109 | 20 | 15 |
| | | Pyraclostrobin | 132 | 77.1 | 20 | 20 |
| | | | 164 | 132.1 | 20 | 10 |
| | | Deltamethrin | 252.9 | 93 | 20 | 15 |
| | | | 181 | 152.1 | 20 | 25 |

* Chloropyrifos methyl was used for the RT locking.

## Results and Discussion

The purpose of these tests was to use multiresidue pesticides as probes to assess inertness provided by Ultra Inert and UltiMetal Plus parts, then subsequently to compare an inert flow path to a standard flow path. Twenty-six representative and challenging pesticide compounds were selected for the evaluation and comparison (Table 3). These pesticides were from various pesticide groups such as organophosphates, organochlorine, carbamates, and so forth. Figure 1 shows a 500 ng/mL pesticide standard chromatogram using an inert flow path for peak identification of the analytes.




Peak identification

1. Acephate
2. Methacrifos (peak 2a and 2b are isomers)
3. Omethoate
4. Ethalfluralin
5. Sulfotep
6. Demeton-S
7. Simazine
8. Lindane
9. Chlorothalonil
10. Chlorpyrifos-methyl
11. Fenitrothion
12. Aldrin
13. Pendimethalin
14. Tolyfluanid
15. Folpet
16. Dieldrin
17. Bupirimate
18. Triazophos
19. Propargite
20. Iprodione
21. EPN
22. Phosalone
23. Mirex
24. Coumaphos
25. Pyraclostrobin
26. Deltamethrin

IS Internal Standard (TPP)

Figure 1. Standard chromatogram of 500 ng/mL pesticide by GC/MS/MS with MRM for peak identification.

## Ultra Inert gold seal

Ultra Inert gold seals were evaluated and compared to standard gold seals and Siltek treated stainless steel inlet seals. Two batches (three from each batch) of Ultra Inert gold seals were tested and compared to standard gold seals (n = 3) and Siltek treated stainless steel inlet seals (n = 3). These different types of inlet seals were tested alternatively to minimize the impact of instrument and column condition variations. The sequence for each inlet seal test included four injections of pesticide standards at each level. Calibration curves were generated for the range 10 to 500 ng/mL, and the linearity of the calibration curve was compared between the three different types of inlet seals.

When compared to standard gold seals and Siltek treated stainless steel seals, the results on active pesticides demonstrated that the use of an Ultra Inert gold seal supported higher responses and better peak shapes. Figure 2 shows the peaks of active pesticides acephate and omethoate were higher with less tailing when using the Ultra Inert gold seal, than peaks when using standard gold or Siltek inlet seals. In addition, the calibration curve linearity for sensitive analytes was improved (Figure 3), as were the result of increased responses and more accurate peak integration.

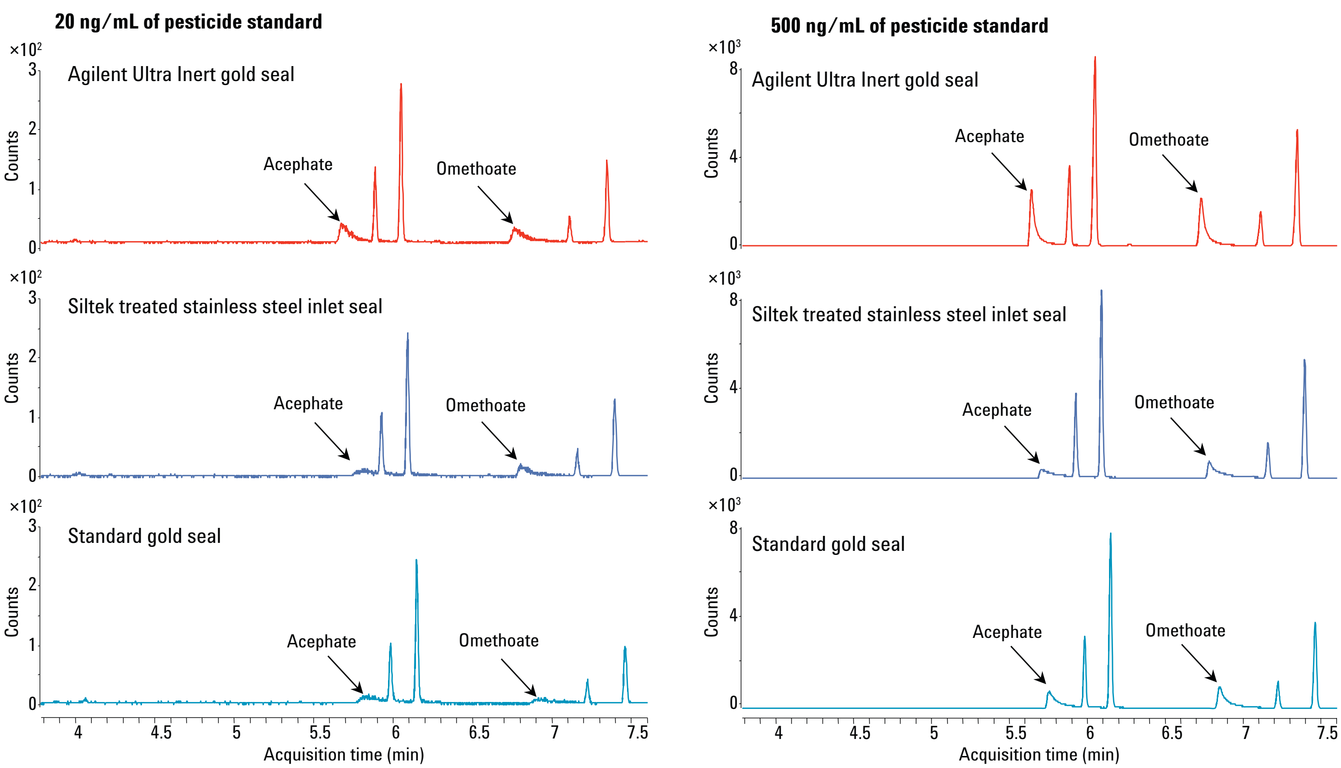



Figure 2. Chromatographic comparison of an Agilent Ultra Inert gold seal, a Siltek stainless steel inlet seal, and a standard gold seal. The original partial chromatograms were enlarged to emphasize the sensitive pesticide comparison.

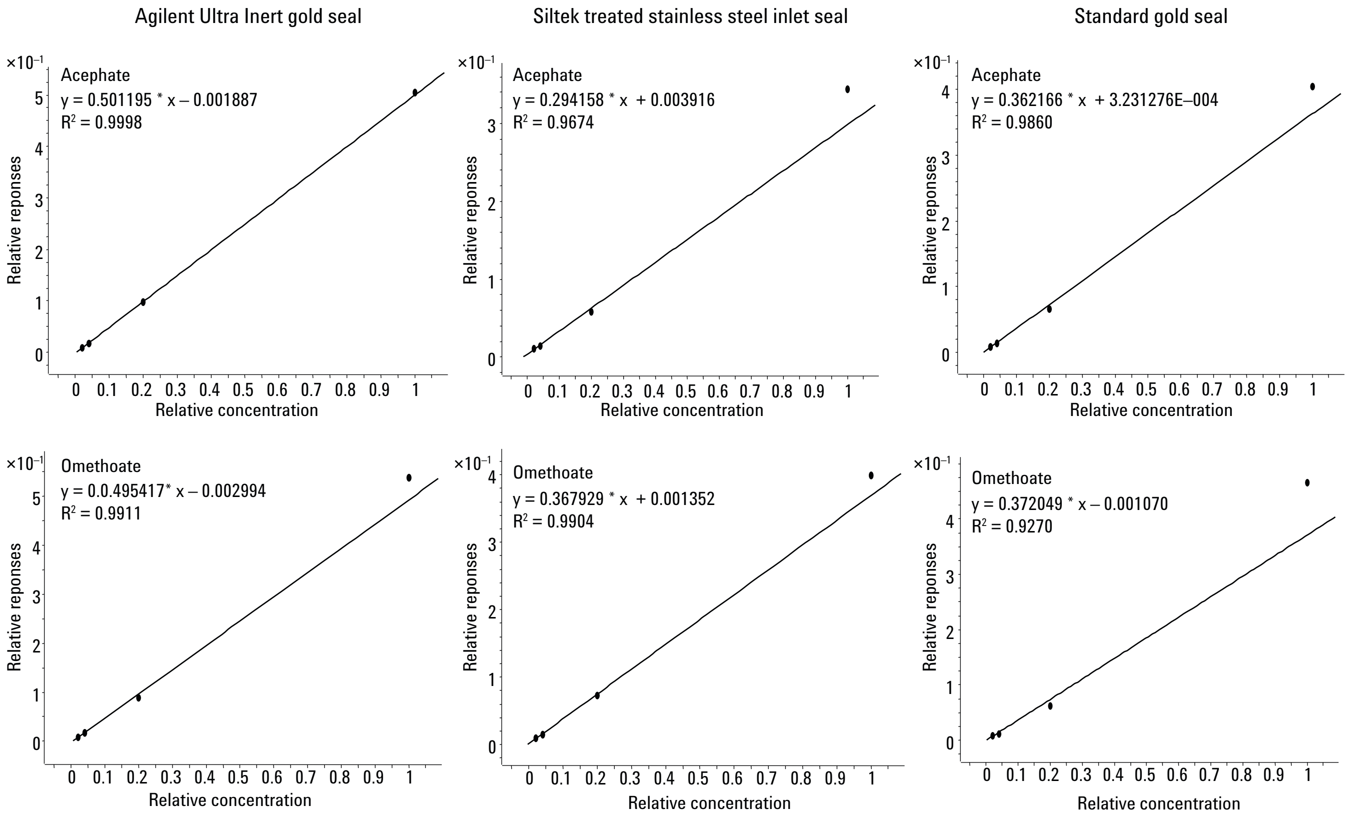



Figure 3. Calibration linearity comparison of an Agilent Ultra Inert gold seal, a Siltek stainless steel inlet seal, and a standard gold seal for sensitive pesticides. Calibration pesticide standards were 10, 20, 100, and 500 ng/mL.

## UltiMetal plus inlet

The inert split/splitless inlet includes a cap inlet shell weldment and an insert weldment treated with the UltiMetal Plus deactivation process. To compare two inlets, an UltiMetal Plus split/splitless inlet was installed on the front inlet. The back inlet was standard split/splitless. The same column and inlet liner were swapped between front and back inlets to minimize contributions from other variables. Due to the very limited surface contact between sample vapor and inlet, it was very difficult to obtain differentiated results with regular operation. The inert inlet was equivalent to the standard inlet for pesticide analysis. To further investigate the potential benefits of the Ultra Inert inlet, a quick exchange test was designed by switching the column back and forth several times between front and back inlets with multiple injections of 10 ng/mL standard each time. An HP-5ms UI column with an Ultra Inert liner without wool was used. The column was intentionally installed incorrectly with a shorter protrusion (2 to 3 mm) above the ferrule. This was to increase the contact of sample vapor with the inlet weldment surface. After three injections of 10 ng/mL pesticide standard, the column was switched to another inlet, without column trimming, and reinstalled with the same of column nut and ferrule. The same liner was also switched to the other inlet for testing. The gold seal was not switched, because the reuse of a gold seal can cause leaks, but gold seals from one batch were used for both inlets. Four test cycles were conducted between front UltiMetal Plus inlet (F) and back standard inlet (B) in the following order: F1 – F3 → B1 – B3 → B4 – B6 → F4 – F6. This order was used to minimize the impact of both column installation variation and column deterioration with injections, to provide a comparison with statistically more meaningful results. A 10 ng/mL standard was used because any differences on analytes responses were more obvious at low detection levels. The average response factors of six injections were then calculated and compared.

Figure 4 shows the 10 ng/mL pesticide standard chromatogram overlay with UltiMetal Plus inlet and standard inlet. The UltiMetal Plus inlet provided higher responses for sensitive organophosphate pesticides, especially acephate and omethoate. Figure 5 shows the profile of average response factors of six injections on each inlet. To make the comparison, the average response factor values with the UltiMetal Plus inlet were normalized using the results with the standard inlet. More than a 20% increase in response was obtained for several active compounds. This specially designed experiment demonstrated the improvements delivered by an UltiMetal Plus inlet for sensitive pesticide analysis at low detection levels.

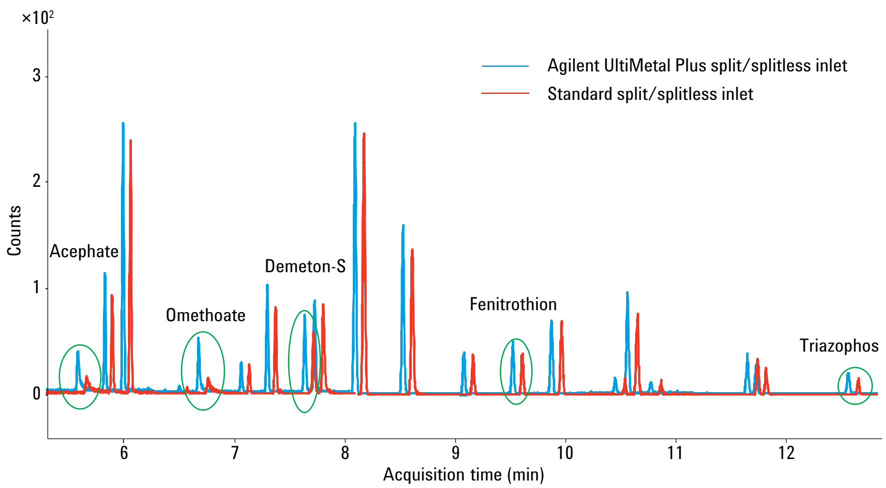



Figure 4. Chromatographic comparison of Agilent UltiMetal Plus split/splitless inlet and standard split/splitless inlet.

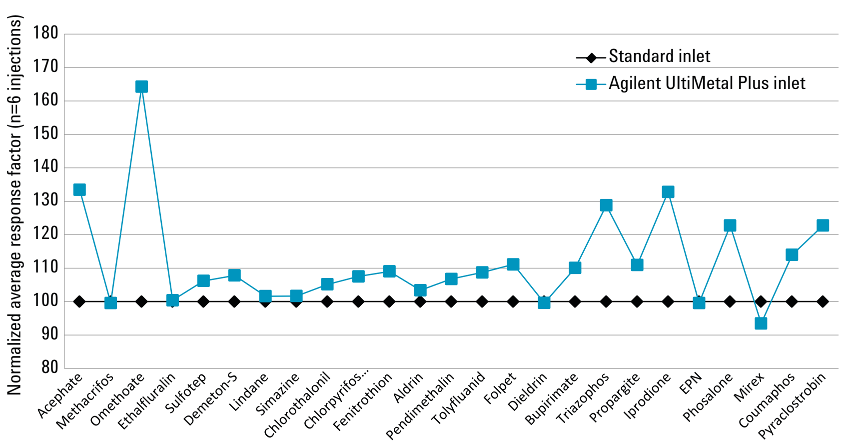



Figure 5. Average response factor comparison of Agilent UltiMetal Plus split/splitless inlet and standard split/splitless inlet. Six injections of 10 ng/mL pesticide standard with alternative injections on both inlets.

## UltiMetal Plus flexible metal ferrules

Agilent UltiMetal flexible metal ferrules are novel metal ferrules designed and recommended for column connections with capillary flow technology (CFT) devices. These new metal ferrules are lighter and less stiff, thus providing flexibility to gently compress around the column, reducing both column breakage and damage to the fitting common with other metal ferrules. The flexible metal ferrules are treated with the UltiMetal Plus deactivation process to improve the inertness of ferrule surface.

Capillary flow technology devices are widely used in pesticides analysis to conduct backflushing after a normal run. As demonstrated elsewhere, backflushing significantly shortened analysis times for samples that contained high boiling matrix interferences, protected the column from quick deterioration, and reduced system maintenance [6]. It is very common to use CFT devices with metal ferrules to connect columns for pesticide analysis in complicated matrices. It is critical to evaluate the inertness of novel metal ferrules using pesticides as probes.

Figure 6 shows the hardware configuration. An HP-5ms UI column was used, as control, to collect column-only data. An UltiMetal Plus Ultimate union was then used to connect the column with 1 m of deactivated fused silica tubing, and a set of two ferrules were tested. The UltiMetal Plus flexible metal ferrules were compared to commonly used Siltite ferrules and also nondeactivated flexible metal ferrules.

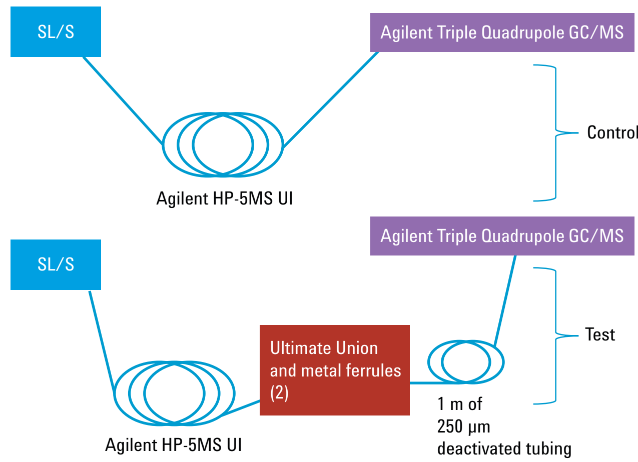



Figure 6. Hardware configuration for metal ferrule evaluation tests.

Figure 7 compares the enlarged chromatograms for sensitive pesticides using UltiMetal Plus flexible metal ferrules, Siltite ferrules, and untreated flexible metal ferrules. It was evident that UltiMetal Plus deactivation treatment improved flexible metal ferrule surface inertness when compared to untreated flexible metal ferrules. UltiMetal Plus flexible metal ferrules were equivalent to Siltite ferrules from the surface inertness aspect. However, Siltite ferrules are made from solid metal that can cause column breakage, damage to the fittings, and possibly more serious problems. Chemical equivalence plus mechanical advantage make UltiMetal Plus flexible metal ferrules highly recommended for column connections to CFT devices for pesticide analysis.

## Inert flow path

Ultra Inert columns and liners have been shown to provide excellent performance for pesticides analysis [1-4]. The inert components described above were therefore combined with the UI column and liner to build an entire inert flow path. To show the benefits of an inert flow path, a standard flow path with corresponding parts was built for comparison.

## Inert flow path

- Agilent UltiMetal Plus split/splitless inlet (insert weldment, inert, p/n G3452-60586 and cap inlet shell weldment assembly, inert, p/n G3452-60570)
- Agilent Ultra Inert gold seal with washer (p/n 5190-6144)
- Agilent Ultra Inert single taper splitless liner with wool (p/n 5190-2293)
- Agilent HP-5ms UI column, 30 m × 0.25 mm, 0.25 µm (p/n 19091S-433UI)

## Standard flow path

- Standard split/splitless inlet (insert weldment, p/n G3452-60585 and cap inlet shell weldment, G3452-80570)
- Standard gold seal with washer (p/n 5188-5367)
- Agilent original single taper splitless liner with wool (p/n 5062-3587)
- HP-5 column, 30 m × 0.25 mm, 0.25 µm (p/n 19091J-433)

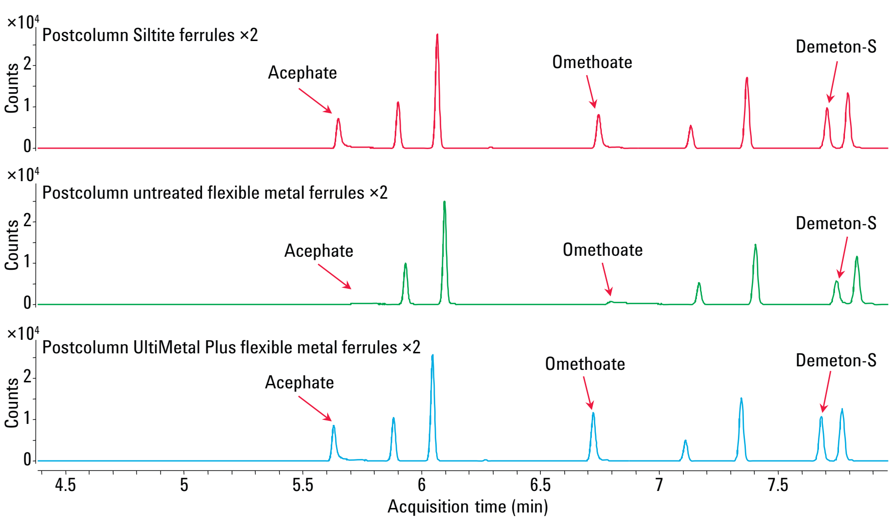



Figure 7. Chromatographic comparison of Agilent UltiMetal flexible metal ferrules, Siltitle ferrules, and untreated flexible metal ferrules, postcolumn. Chromatograms were obtained after injection of 500 ng/mL pesticide standard.

Figure 8 shows the comparison of 10 ng/mL pesticide MRM chromatograms with inert flow path (top) and standard flow path (bottom). Clearly, the use of the inert flow path provided much better performance than the standard flow path, especially for sensitive pesticides such as acephate, omethoate, phosalone, demeton-S, and pyraclostrobin. For these sensitive pesticides, the analyte response and peak shape were significantly improved. More symmetrical and sharper peaks for acephate and omethoate were obtained at 10 ng/mL by the inert flow path, while at the same concentration these two troublesome analytes were almost undetectable when using the standard flow path. Even at 500 ng/mL, acephate and omethoate peaks were still much smaller with significant tailing in the standard flow path.

The improvement of analyte response and peak shape also directly contributed to the linearity of the calibration curve for the sensitive pesticides (Figure 9). The average response factors of 10, 100, and 500 ng/mL standard injections (n = 4 at each level) was calculated to generate a comparison profile as shown in Figure 10. The comparison between inert flow path and standard flow path clearly showed that the improvement on the flow path surface inertness significantly improved the entire system performance for a large variety of pesticides, increasing the quality of data achieved and making the quantitation results more accurate and precise.

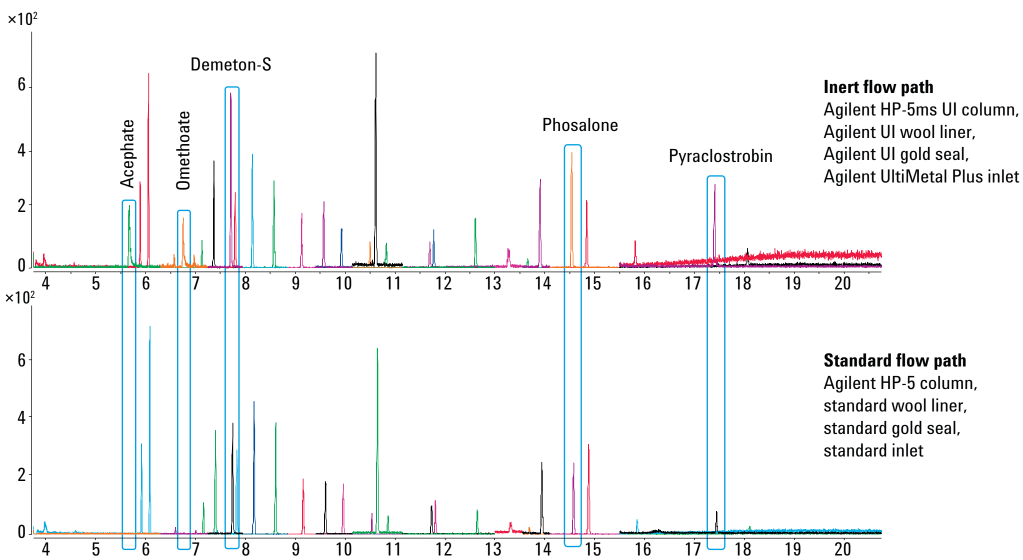



Figure 8. Chromatographic comparison of an Agilent Inert Flow Path and standard flow path. Chromatograms were obtained after injection of 10 ng/mL pesticide standard.

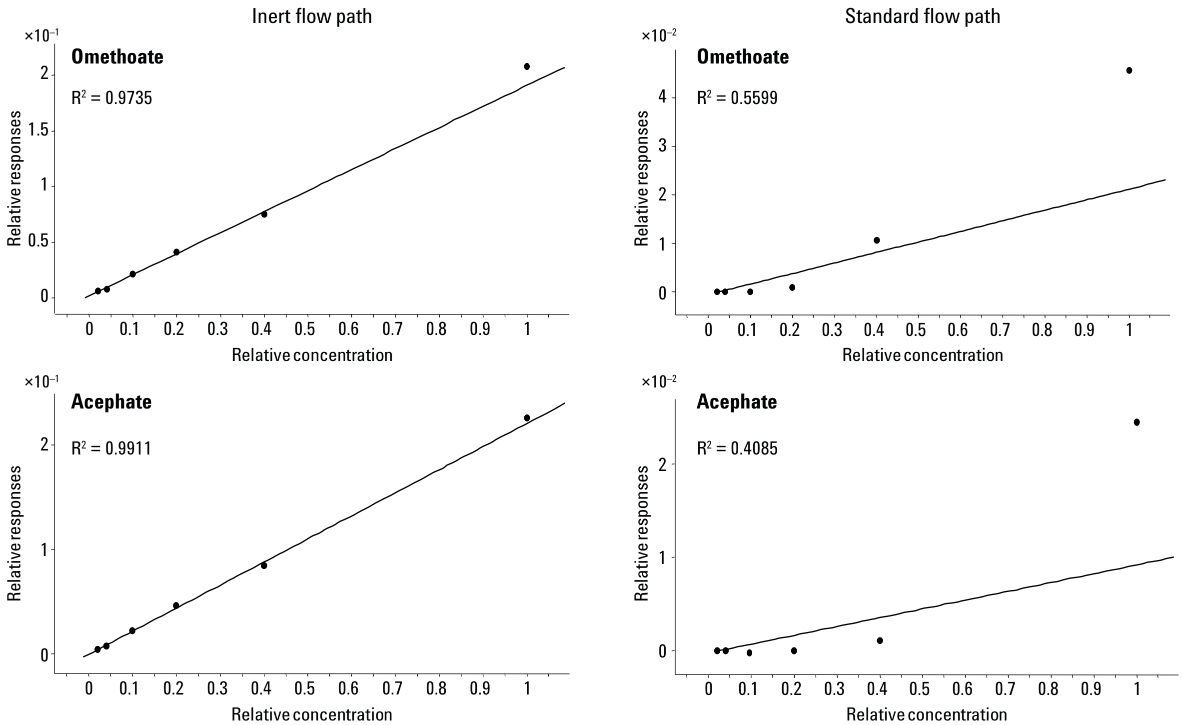



Figure 9. Calibration curve linearity comparison of an Agilent Inert Flow Path and standard flow path. Calibration pesticide standards 10, 20, 50, 100, 200, and 500 ng/mL.

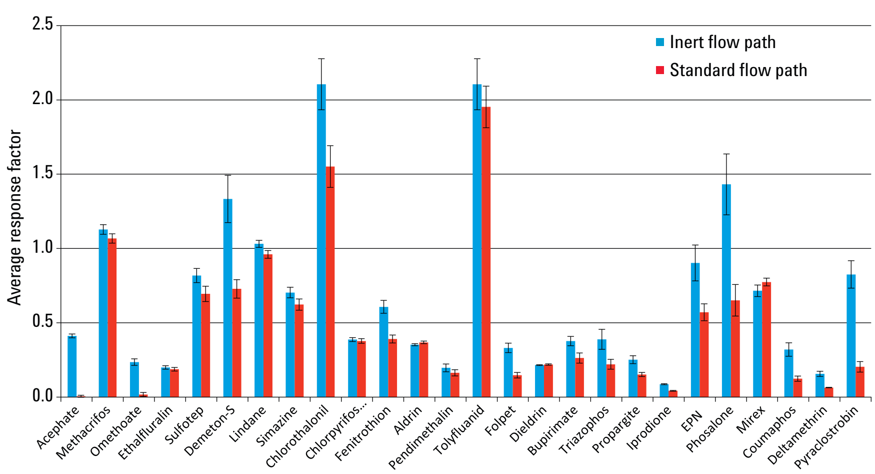



Figure 10. Overall average response factor comparison of an Agilent Inert Flow Path and standard flow path. Results were calculated for the average of response factor of 10, 100, and 500 ng/mL pesticide standards with four injections at each level.

## Conclusion

Agilent Ultra Inert gold seal, UltiMetal Plus Flexible Metal ferrules, and UltiMetal Plus split/splitless inlet weldments were qualified for pesticide analysis. Ultra Inert gold seals provided higher responses and better peak shape for sensitive pesticides than standard gold seals and Siltek treated stainless steel inlet seals. UltiMetal Plus Flexible Metal ferrules had equivalent or slightly better inertness than Siltite ferrules. However, the novel design of flexible metal ferrules prevents column breakage and damage of fittings. With inertness and mechanical advantages, UltiMetal Plus flexible metal ferrules are highly recommended for column connections to CFT devices. UltiMetal Plus inlet weldments supported pesticides analysis with potential benefits of improved weldment surface inertness. When tested under extreme conditions to purposely increase the contact of sample vapor with inlet weldment surface, higher response and better peak shapes were obtained for sensitive pesticides such as organophosphates. The entire Agilent Inert Flow Path was developed and compared to a corresponding standard flow path. When using the Agilent Inert Flow Path, significant improvements were obtained, including higher response, better peak shape, better calibration linearity, and entire flow path consistency. These results show that minimizing flow path active sites is critical in achieving accurate, precise and reliable results for pesticide analysis.

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