# Analysis of Pesticides in Food by GC/MS/MS using the Ultra Inert Liners with Wool

Application Note

Food


## Authors

Limian Zhao
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19809
USA
David Mao
Agilent Technologies, Inc.
91 Blue Ravine Road
Folsom, CA 95630
USA



## Abstract

With efficient deactivation on glass wool, the Ultra Inert liners with wool provide excellent inertness, homogeneous sample mixing and evaporation, and maximum column and detector protection for reliable pesticides analysis in food by GC/MS/MS.


## Introduction

GC inlet liners are the centerpiece of the inlet system in which the sample is vaporized, mixed with the carrier gas, and introduced to the capillary column. Inlet liners with wool are used widely due to the benefits of glass wool. Glass wool promotes homogenous sample mixing and better quantitation. It provides a large surface area which aids the vaporization of liquid samples. It also traps non-volatile residue, protecting the GC column from the negative impacts of sample matrix. Wool liners also prevent sample from hitting the bottom of the inlet before vaporization.

However, the large surface area of glass wool limits the practical use of wool liners due to high activity resulting from poor deactivation of silanols (-SiOH) on the surface of glass wool. These active sites are detrimental to accurate quantization of many sample types, especially labile analytes such as pesticides and active acidic and basic compounds. Traditional deactivation techniques usually cannot deactivate the glass wool surface area effectively. Active sites left on the wool can cause the degradation or adsorption of sensitive compounds before the analytes get to the column. This complicates quantization resulting from peak tailing or splitting, or for some for particularly sensitive analytes, loss of the sensitivity. As a result, inlet liners with glass wool are usually not recommended for the analysis of active analytes like pesticides.

Pesticides determination in food has gained more and more attention world widely. Multi-residue analysis of pesticides in fruits, vegetables, and other foods is always a challenge for sample preparation as well as instrument detection. The QuEChERS

sample preparation method was introduced for pesticides analysis in food by USDA scientists in 2003. [1] It has been accepted world wide for multi-residue pesticides analysis due to its features, which are quick, easy, cheap, effective, rugged, and safe. The QuEChERS extracts are concurrently analyzed by LC and GC combined with MS to determine a wide range of pesticides residues. However, food extracts processed by QuEChERS method are still complicated containing impurities like high-boiling indigenous compounds. When using GC/MS or GC/MS/MS, the QuEChERS extracts can cause the contamination and deterioration of the analytical column and MS source, resulting in inaccuracy due to the poor peak shape and loss of response for active analytes. It also leads to shorter lifetime for the analytical columns and frequent MS maintenance. Therefore, it is very necessary to use suitable technologies and suppliers to achieve reliable results and to maximize protection to the analytical column and MS source.

Column back-flushing can be beneficial for the analysis of food extracts because it significantly reduces analysis time and, when properly employed, reduces both column head trimming and frequency of MSD source cleaning [2]. Agilent's capillary flow technology (CFT) makes column back-flushing routine, when an analytical column is connected to the capillary flow device and a short restrictor is used to couple to the capillary flow device to the mass spectrometer [3,4]. An inlet liner with glass wool can provide maximum protection from heavy matrices by trapping the non-volatile compounds. The application of liners with glass wool has been restricted for the analysis of pesticides. Usually, pesticide compounds contain reactive functional groups like 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 silanol groups and possibly metal ions on glass and glass wool surfaces, resulting in compound adsorption and degradation. Liners with other configurations have historically been used for the analysis of pesticides in food, such as "Cyclosplitter" liners, single or dual taper liners.

Agilent's Ultra Inert liner deactivation process significantly improves the efficiency and robustness of glass wool deactivation. The large glass wool surface area is deactivated thoroughly. The Ultra Inert deactivated liners with wool can be used for the analysis of pesticides in food. A representative group of 33 difficult pesticides were selected for the liners' evaluation. The pesticides standard was spiked in fruit and vegetables matrix blank samples extracted by QuEChERS AOAC method [1, 5]. The matrix spiked standard was then analyzed by GC/MS/MS under Multiple Reaction Monitoring (MRM) mode. A calibration curve from 5 – 500 ng/mL was used for linearity evaluation, and a 50 ng/mL sample was used for repeatability tests. Liner to liner reproducibility was conducted with the repeated tests of seven liners from three lots. In addition, the similar tests were also conducted with Siltek Cyclosplitter splitless liners and gooseneck splitless liners for comparison, which are widely used for pesticides analysis in food.

## Experimental

### Chemicals and Reagents

All reagents and solvents were HPLC or analytical grade. Acetonitrile (AcN) was from Honeywell B&J (Muskegon, MI, USA). 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 standards and internal standard (triphenyl phosphate, TPP) were purchased from Sigma-Aldrich (St Louis, MO, USA), Chem Service (West Chester, PA, USA), or Ultra Scientific (North Kingstown, RI, USA).

### Solutions and Standards

A 1% acetic acid in AcN solution was prepared by adding 1 mL of glacial acetic acid to 100 mL of AcN, and was used as reagent blank. This solution was also used as extraction solvent by QuEChERS method, and blank solvent to prepare neat pesticide standards. Standard and internal standard (IS) stock solutions (2 mg/mL) were made in Acetone, individually, and stored at –20 °C. A 20 µg/mL mixed standard (33 pesticides) solution was made in Acetone by proper dilution of individual pesticide stock solutions. A 20 µg/mL triphenyl phosphate (TPP) solution made in AcN was used as internal standard (IS) spiking solution. Six standard solutions of 5, 10, 50, 100, 250, and 500 ng/mL and 50 ng/mL of QC solution were prepared in fruits and vegetables matrix blanks by appropriate dilution of the 20 µg/mL mixed standard solution. Certain volumes of IS solution was then spiked into samples to generate a concentration of 500 ng/mL in the samples.

### Matrix Blank Preparation

Five kinds of fruits and vegetables were selected to prepare matrix blank samples, including white flowers, banana, strawberry, pear and lettuce. The extraction procedure was described in detail previously [5]. The fruits and vegetables were frozen, chopped and then homogenized thoroughly. The homogenized samples were extracted following QuEChERS AOAC method procedure using Agilent BondElut QuEChERS AOAC extraction kit (p/n 5982-5755) and dispersive SPE kit for general fruits and vegetables (p/n 5982-5022). Briefly, 15 g of homogenous sample (flowers excluded) was extracted by 15 mL of acetonitrile w/ 1% acetic acid and separated with aqueous phase by the addition of BondElut QuEChERS AOAC extraction salt packet.

*Table 1. Instrumental Conditions for Agilent GC/MS System Used for Pesticides Test*

| | |
|---|---|
| GC | Agilent 7890A Series |
| Autosampler | Agilent 7693 Autosampler and sample tray, 5 µL syringe (p/n 5181-5246), 1 µL injection volume. Postinj solvent A (Acetone) washes: 3 Sample pumps: 3 Postinj solvent B (Acetonitrile) washes: 3 |
| Carrier gas | Helium, constant pressure |
| Inlet | MMI inlet at pulsed splitless mode: 280 °C, |
| Injection pulse pressure | 36 psi until 1min |
| Purge flow to split vent | 50 mL/min @ 1min |
| Inlet pressure | 18.35 psi (RT locked) during run, and 1.0 psi during back flushing |
| RT locking | Chlorpyrifos methyl @ 8.298 min |
| Oven profile | 100 °C for 2 min, then to 150 °C at 50 °C/min, to 200 °C at 6 °C/min, to 280 °C at 16 °C/min and hold for 6 min (for sample run); 100 °C for 1 min, then to 280 °C at 100 °C/min and hold for 5.2 min (for matrix blank run) |
| Post run | 2 min @ 280 °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 |
| Bleed line | 0.0625-in od × 0.010-in id × 100 cm, 316 SS tubing, on top of the oven |
| Aux pressure | 4 psi during run, 75 psi during back-flushing |
| Analytical column | HP-5MSUI, 15 m × 0.25 mm, 0.25 µm (p/n 19091-431UI) |
| Connections | Between Inlet and 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 7000 Triple Quad 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 | 2.3 min |
| Collision gas flows | He quench gas @ 2.35 mL/min, $N_2$ collision gas at 1.5 mL/min |
| MS resolution | MS1 and MS2 = 1.2 u |

For the flower sample, 5 g of homogenous sample was mixed with 10 mL of water and soaked overnight. This mixture was then extracted following the QuEChERS procedure. After centrifugation, the supernatant was transferred and cleaned up using the general dispersive SPE kit. The mixed sample was centrifuged to separate the supernatant. The five different matrix extracts were then combined and used as the matrix blank for the liner evaluation tests.

## Instrumentation

All testing was done on an Agilent 7890A GC equipped with a 7693B Autosampler and 7000 Series Triple Quad MSD system. An Agilent Ultra Inert GC column, HP-5MSUI, was used to provide a highly inert flow path into the detector. Table 1 list the instrumental conditions used for this test. Table 2 lists flow path consumable supplies used in the experiments, and Table 3 lists the MRM detector conditions for 33 target analytes.

*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. Quantifier and Qualifier MRM Transitions for 33 Pesticides*

| Analytes (peak no. on chromatogram) | Quant MRM (CE) | Quali MRM (CE) | RT (min) |
|---|---|---|---|
| Methamidophos (1) | 141.0 → 95.0 (6) | 95.0 → 79.0 (13) | 2.5 |
| Dichlorvos (2) | 185.0 → 93.0 (15) | 108.9 → 79.0 (5) | 2.7 |
| Acephate (3) | 136.0 → 42.0 (6) | 136.0 → 94.0 (14) | 3.9 |
| Mevinphos(4) | 127.0 → 109.0 (10) | 191.9 → 127.0 (10) | 3.9 |
| σ-Phenylphenol (5) | 169.9 → 115.0 (30) | 169.9 → 141.0 (15) | 4.5 |
| Omenthoate (6) | 156.1 → 79.0 (15) | 156.1 → 110.0 (20) | 5.2 |
| Dimenthoate (7) | 125.0 → 47.0 (15) | 143.0 → 111.0 (10) | 6.6 |
| Altrazine (8) | 214.9 → 58.0 (11) | 200.0 → 94.1 (20) | 6.9 |
| Lindane (9) | 180.8 → 145.0 (12) | 218.8 → 183.0 (20) | 7.0 |
| Diazinon (10) | 304.0 → 178.9 (15) | 178.9 → 121.0 (28) | 7.6 |
| Chlorothalonil (11) | 265.8 → 133.0 (53) | 265.8 → 169.9 (28) | 7.7 |
| Chloropyrifos methyl (12)* | 285.8 → 271.0 (16) | 287.8 → 93.0 (26) | 8.6 |
| Vinclozolin (13) | 211.8 → 172.0 (15) | 211.8 → 145.0 (15) | 8.7 |
| Carbaryl (14) | 143.9 → 116.0 (15) | 143.9 → 89.0 (50) | 8.8 |
| Tolclofos methyl (15) | 264.8 → 250.0 (15) | 264.8 → 93.0 (50) | 8.8 |
| Dichlorfluanid (16) | 223.9 → 123.0 (8) | 223.9 → 77.0 (45) | 9.6 |
| Aldrin (17) | 262.8 → 193.0 (30) | 262.8 → 191.0 (30) | 9.6 |
| Malathion (18) | 173.0 → 99.0 (15) | 157.9 → 125.0 (5) | 9.8 |
| Dichlorobenzophenone (19) | 249.9 → 139.0 (5) | 249.9 → 214.9 (15) | 10.0 |
| Pirimiphos ethyl (20) | 318.0 → 166.0 (12) | 333.1 → 318.0 (5) | 10.7 |
| Toloyfluanid (21) | 237.9 → 137.0 (15) | 237.9 → 91.1 (50) | 11.0 |
| Procymidone (22) | 282.9 → 96.0 (10) | 282.9 → 67.1 (40) | 11.4 |
| Endrin (23) | 262.8 → 193.0 (35) | 262.8 → 191.0 (35) | 12.8 |
| Ethion (24) | 230.8 → 129.0 (25) | 230.8 → 175.0 (35) | 13.4 |
| Endosulfan sulfate (25) | 271.7 → 236.8 (20) | 386.7 → 253.0 (5) | 13.8 |
| DDT (26) | 234.9 → 165.0 (20) | 236.8 → 165.0 (5) | 13.9 |
| TPP (IS) | 325.9 → 169.0 (30) | 325.9 → 233.0 (27) | 14.3 |
| Endrin ketone (27) | 316.7 → 101.0 (20) | 316.7 → 245.0 (20) | 14.5 |
| Iprodione (28) | 313.8 → 56.0 (20) | 186.9 → 123.0 (25) | 14.6 |
| Phosmet (29) | 159.9 → 77.0 (30) | 159.9 → 133.1 (20) | 14.7 |
| Phosalone (30) | 181.9 → 138.0 (5) | 366.9 → 182.0 (5) | 15.3 |
| Permethrin (31) | 183.0 → 168.1 (15) | 183.0 → 153.1 (15) | 16.1 & 16.2 |
| Coumaphos (32) | 361.9 → 109.0 (15) | 361.9 → 81.0 (35) | 16.3 |
| Deltamethrin (33) | 180.9 → 152.0 (26) | 252.8 → 93.0 (20) | 18.2 & 18.5 |

*Chloropyrifos methyl was used for the RT locking.

A Back-flushing system was used because it significantly shortens analysis times for samples that contain high-boiling matrix interferences and, as mentioned earlier, reduces system maintenance [2, 4]. The instrument configuration is very close to the configuration shown in Figure 1B in previous setup [4], except no retention gap is used for this application. Retention time locking (RTL) was used to eliminate the need for recalibration of the individual retention times and timed events like MRM groups [6]. The total run time for a sample spiked with standard is 23 min and 2 min for back-flush. For matrix blanks run in the middle, a fast oven gradient totaling 8 min and 2 min back-flush were used to save time.

## Results and Discussion

The purpose of these tests was to evaluate the Ultra Inert deactivated liners with wool for the analysis of pesticides in fruit and vegetable matrices by GC/MS/MS. Inlet liners with wool are beneficial for sample analysis in complicated matrices. With efficient deactivation on glass wool, the Ultra Inert liners with wool demonstrated excellent performance to support pesticides analysis in food matrices. The feasibility of using Ultra Inert splitless liners with wool was determined by chromatographic evaluation, sensitivity and linearity, liner-to-liner reproducibility, and liner deactivation stability for matrix sample injections, and the protection provided to the column and MS source. Restek Siltek Cyclosplitter dual taper splitless liner and Siltek single taper splitless liner without wool were selected for comparison due to their popularity in pesticides analysis.

The 33 representative and difficult pesticide compounds were selected for the evaluation (Table 3). The compounds were from various pesticides groups such as organophosphate pesticides (OPs), organochlorine pesticides (OCs), carbamates, and phenols. These compounds also included many difficult active pesticides such as Methamidophos, Acephate, Omenthoate, Dimenthoate, Carbaryl, Endrin, Tolyfluanid, Chlorothalonil, DDT, Phosmet, and Iprodione. Evaluation and comparison focused on the performance of liners for these active compounds.

## Chromatographic performance

The adsorption or decomposition of pesticides may cause some chromatographic problems, including broad, distorted peaks, peak tailing, loss of peak intensity etc. Early eluting pesticides such as Methamidophos, Acephate, usually show peak tailing when active sites exist. Omenthoate is another sensitive compound that may totally disappear at low levels when the flow path is not inert. The adsorption or degradation increases when liner deactivation degrades with continuous use, and nonvolatile residue from dirty samples slowly accumulate in the inlet liner and column.  As a result, the peak shape and intensity may deteriorate faster as more complicated samples are injected.

With superior selectivity of GC/MS/MS under MRM mode, a "clean" chromatogram with fewer interference peaks can be achieved easily when analyzing samples in complex matrix. Figure 1 shows the GC/MS/MS chromatogram (MRM) for a mixed fruit and vegetable sample spiked with 10 ng/mL of pesticides standard. As seen in Figure 1, the chromatogram shows relatively clean background around the analytes of interest. There are still some interference peaks showing up with certain pesticides' MRM channel. These interference peaks are either completely separated with the real pesticide's peak or with significant lower intensity compared to the real peak; therefore the presence of those interference peaks do not affect the integration and quantitation of target analytes. All analytes except Methamidophos and Acephate show excellent, sharp and symmetric peak shape. Methamidophos and Acephate are more polar compounds with negative Log P value (Methamidophos:  –0.79, and Acephate: –0.89), and are relatively difficult for GC analysis. Acceptable peak shapes were still obtained with slight tailing observed for those two compounds at 10 ppb level. Omenthoate is another difficult pesticide that may not survive at low concentration when active sites exist in the flow path. As seen in Figure 1, the Omenthoate peak (peak no. 6) shows excellent peak shape and intensity at 10 ppb level with S/N ratio over 20.

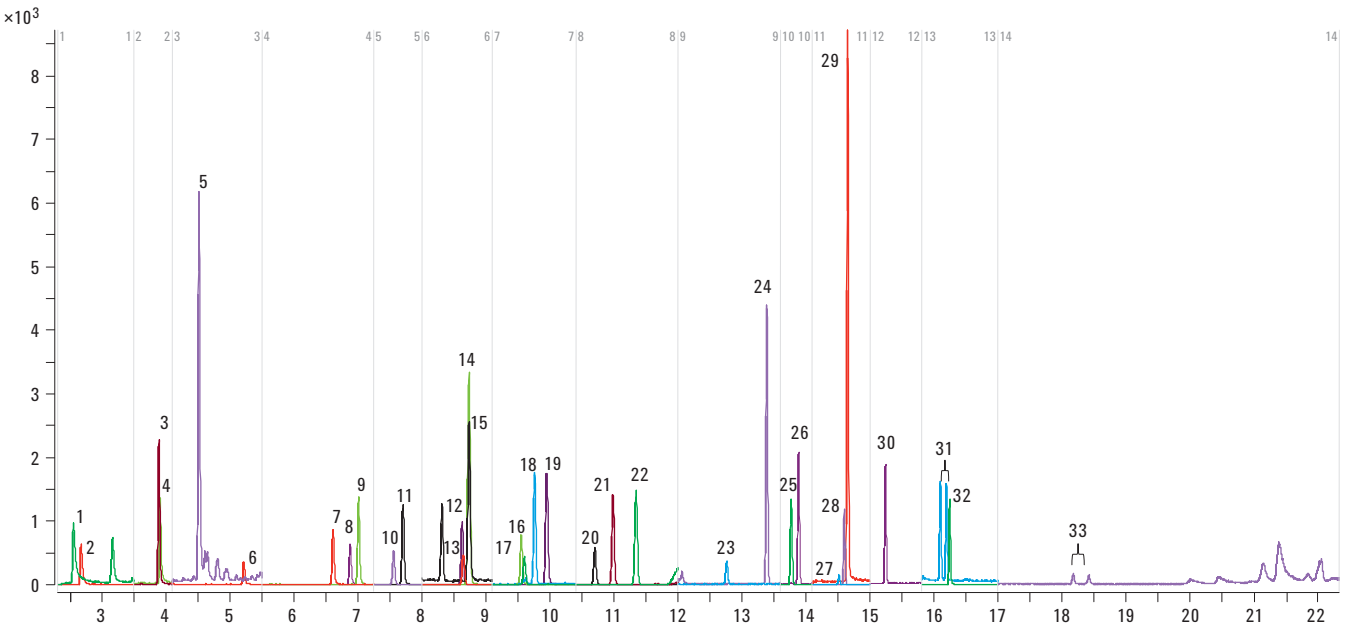


*Figure 1.  GC/MS/MS chromatogram (MRM) for 10 ppb spiked QuEChERS sample using Ultra Inert liner with wool (p/n 5190-2293).*
*Peak identification: 1. Methamidophos, 2. Dichlorvos, 3. Mevinphos, 4. Acephate, 5. σ-Phenylphenol, 6. Omenthoate, 7. Dimenthoate, 8. Altrazine, 9. Lindane, 10. Diazinon, 11. Chlorothalonil, 12. Chloropyrifos methyl, 13. Vinclozolin, 14, Carbaryl, 15, Tolclofos methyl, 16. Dichlorfluanid, 17. Aldrin, 18. Malathion, 19. Dichlorobenzophenone, 20. Pirimiphos ethyl, 21. Tolyfluanid, 22 Procymidone, 23. Endrin, 24. Ethion, 25. Endosulfan sulfate, 26. DDT, 27. Endrin ketone, 28. Iprodione, 29. Phosmet, 30. Phosalone, 31. Permethrin isomers, 32. Coumaphos, 33. Deltamethrin isomers.*

## Sensitivity and linearity

5 ng/mL limits of quantitation (LOQ) for all of pesticides in QuEChERS extracts were obtained easily using GC/MS/MS. This limit was well below the maximum residue limits (MRL) for these pesticides. A 5 – 500 ng/mL calibration curve was used to cover an extensive direct quantitation range; and a series of standards spiked in the blank matrix were 5, 10, 50, 100, 250, and 500 ng/mL. The linear regression with $1/x^2$ fit was used for the calibration curves regression. All of analytes' calibration curves show excellent linearity with correlation coefficient ($r^2$) over 0.99. To demonstrate the linearity of difficult compounds, the response factor (RF) values at each calibration level and RSD across the calibration range were calculated. The lower the RSD across the calibration range, the better the linearity for the calibration curve, because lower RSD indicate consistent RF values for calibration standards. The results in Table 4 show that the calibration standards RSD values over 5 – 500 ng/mL are less than 13% for the 18 selected pesticides, indicating the excellent linearity for these difficult pesticides.

## Liner to liner reproducibility

In order to quantitatively evaluate liner-to-liner reproducibility, seven Ultra Inert liners from three different lots were tested. A group of 18 difficult active pesticides were selected for evaluation. The Response Factors (RFs) were calculated for each calibration level, and the average RFs values were used as evaluation criteria for the test. Results are shown in Table 5. This reproducibility test includes matrix variation since the standards were spiked into QuEChERS extracts. The results indicate good liner to liner performance reproducibility, with less than 16% RSD for difficult active pesticides.

*Table 4. Calibration Curve (5 – 500 ng/mL in QuEChERS Extract) Average RFs and RSD Values for Selected Active Pesticides Obtained by GC/MS/MS using Ultra Inert liners with Wool*

| Pesticides | Average RFs | RSD | Pesticides | Average RFs | RSD |
|---|---|---|---|---|---|
| Methamidophos | 1.117 | 11.4 | Tolyfluanid | 1.580 | 7.4 |
| Acephate | 0.905 | 6.2 | Procymidone | 1.547 | 3.4 |
| σ-Phenylphenol | 4.193 | 4.9 | Endrin | 0.298 | 3.7 |
| Omenthoate | 0.277 | 10.3 | Endosulfan sulfate | 0.982 | 4.0 |
| Dimenthoate | 0.758 | 5.6 | DDT | 1.233 | 9.0 |
| Lindane | 1.262 | 4.5 | Endrin ketone | 0.102 | 5.9 |
| Chlorothalonil | 0.983 | 4.6 | Iprodione | 0.780 | 4.4 |
| Carbaryl | 2.315 | 4.7 | Phosmet | 5.405 | 9.0 |
| Dichlorfluanid | 0.744 | 5.5 | Coumaphos | 0.871 | 12.9 |

*Table 5. Liner to Liner Reproducibility: 18 Difficult Active Pesticides Average RF Values Across the Calibration Range (5 – 500 ng/mL) and RSD for Seven Replicates of Ultra Inert Deactivated Liners With Wool (p/n 5190-2293).*

| Compounds (peak number) | UI Liner 1 (Lot 1) | UI Liner 2 (Lot 1) | UI Liner 3 (Lot 1) | UI Liner 4 (Lot 2) | UI Liner 5 (Lot 2) | UI Liner 6 (Lot 3) | UI Liner 7 (Lot 3) | RSD |
|---|---|---|---|---|---|---|---|---|
| Methamidophos (1) | 1.211 | 1.314 | 1.292 | 1.235 | 1.123 | 0.862 | 1.204 | **12.9** |
| Acephate (4) | 0.622 | 0.877 | 0.934 | 0.929 | 0.946 | 1.017 | 1.01 | **14.8** |
| σ-Phenylphenol (5) | 3.876 | 4.416 | 4.426 | 4.434 | 4.219 | 4.383 | 3.897 | **5.9** |
| Omenthoate (6) | 0.356 | 0.238 | 0.267 | 0.256 | 0.271 | 0.291 | 0.259 | **13.9** |
| Dimenthoate (7) | 0.727 | 0.744 | 0.755 | 0.748 | 0.759 | 0.784 | 0.786 | **2.8** |
| Lindane (9) | 1.191 | 1.27 | 1.252 | 1.301 | 1.211 | 1.269 | 1.342 | **4.1** |
| Chlorothalonil (11) | 0.963 | 0.961 | 1.114 | 1.016 | 0.963 | 1.002 | 0.861 | **7.7** |
| Carbaryl (14) | 1.844 | 2.282 | 2.348 | 2.44 | 2.447 | 2.585 | 2.26 | **10.2** |
| Dichlorfluanid (16) | 0.715 | 0.751 | 0.769 | 0.772 | 0.696 | 0.717 | 0.786 | **4.6** |
| Tolylfluanid (21) | 1.99 | 1.642 | 1.47 | 1.467 | 1.437 | 1.519 | 1.538 | **12.2** |
| Procymidone (22) | 1.581 | 1.629 | 1.494 | 1.575 | 1.581 | 1.596 | 1.376 | **5.6** |
| Endrin (23) | 0.288 | 0.295 | 0.3 | 0.297 | 0.28 | 0.293 | 0.331 | **5.4** |
| Endosulfan sulfate (25) | 0.885 | 0.912 | 0.949 | 1.015 | 0.977 | 1.016 | 1.123 | **8.1** |
| DDT (26) | 1.326 | 1.52 | 1.449 | 1.115 | 1.009 | 1.1 | 1.112 | **16.0** |
| Endrin ketone (27) | 0.098 | 0.103 | 0.107 | 0.111 | 0.096 | 0.105 | 0.093 | **6.3** |
| Iprodione (28) | 0.628 | 0.747 | 0.774 | 0.848 | 0.872 | 0.897 | 0.695 | **12.6** |
| Phosmet (29) | 4.432 | 5.727 | 5.904 | 5.137 | 5.819 | 5.803 | 5.01 | **10.3** |
| Coumaphos (32) | 0.864 | 0.955 | 0.907 | 0.856 | 0.825 | 0.885 | 0.805 | **5.8** |

## Injection repeatability and performance stability

Injection repeatability and liner performance stability were tested by running 100 neat standards or matrix samples. A 50 ng/mL QC in solvent or matrix was run for every 10 injections, and solvent blanks or matrix blanks were run in the middle. A calibration curve was usually run within the first 10 injections for quantitation. The QC samples were back calculated with the calibration curve in the same sequence, and the calculated concentrations were used for RSD calculation. The results in Table 6 show the RSD values of 18 difficult pesticides for 100 neat standard injections and matrix samples. For the matrix samples repeatability, RSD values for 50 injections were also included.

As seen in Table 6, the repeatability for samples without matrix showed excellent consistency with < 12% RSD over 100 injections when using Ultra Inert liners with wool. The responses of active pesticides decrease as more matrix sample are run on the system due to matrix effect and its negative impact on liners and column. These decreases were more significant for certain very active pesticides such as Acephate, Omenthoate, DDT and Phosmet, resulting in much higher RSD over the sample runs. This system performance deterioration showed not only as the loss of active analyte responses, but also the loss of peak shape integrity for certain active pesticides. Figure 2 (column for Ultra Inert liner with wool) shows the peak shape difference for several critical active compounds at the beginning and end of 100 injections of 50 ng/mL pesticides spiked in matrix samples using Ultra Inert liners with wool. As seen in Figure 6, after 100 injections of matrix samples, the peak of Methamidophos deteriorates with slightly more tailing, while the peaks of other three critical compounds are basically consistent with good peak shape.

*Table 6. Injection Repeatability and Performance Stability: 100 Injections Repeatability (% RSD) by Ultra Inert Liners with Wool with 50 ng/mL Standards in Neat Solvent and QuEChERS Blank Extracts*

| | Repeatability in neat standards (number liners = 5) | Repeatability in matrix samples (number liners = 7) | |
|---|---|---|---|
| **Pesticides (peak number)** | **by 100 injections** | **by 50 injections** | **by 100 injections** |
| Methamidophos (1) | 7.7 | 5.1 | 11.7 |
| Acephate (4) | 5.4 | 16.6 | 30.1 |
| σ-Phenylphenol (5) | 6.4 | 1.6 | 2.2 |
| Omenthoate (6) | 10.5 | 27.1 | 44.8 |
| Dimenthoate (7) | 4.8 | 7.3 | 13.4 |
| Lindane (9) | 11.5 | 3.6 | 6.5 |
| Chlorothalonil (11) | 8.6 | 9.3 | 15.2 |
| Carbaryl (14) | 8.7 | 11.7 | 19.9 |
| Dichlorfluanid (16) | 5.3 | 6.3 | 11.8 |
| Tolylfluanid (21) | 7.3 | 7.3 | 13.4 |
| Procymidone (22) | 8.0 | 0.9 | 1.9 |
| Endrin (23) | 6.6 | 3.2 | 6.0 |
| Endosulfan sulfate (25) | 7.7 | 9.5 | 14.1 |
| DDT (26) | 6.3 | 23.1 | 36.4 |
| Endrin ketone (27) | 7.3 | 9.3 | 14.2 |
| Iprodione (28) | 5.1 | 5.0 | 8.1 |
| Phosmet (29) | 6.9 | 15.4 | 27.2 |
| Coumaphos (32) | 5.9 | 7.8 | 15.0 |

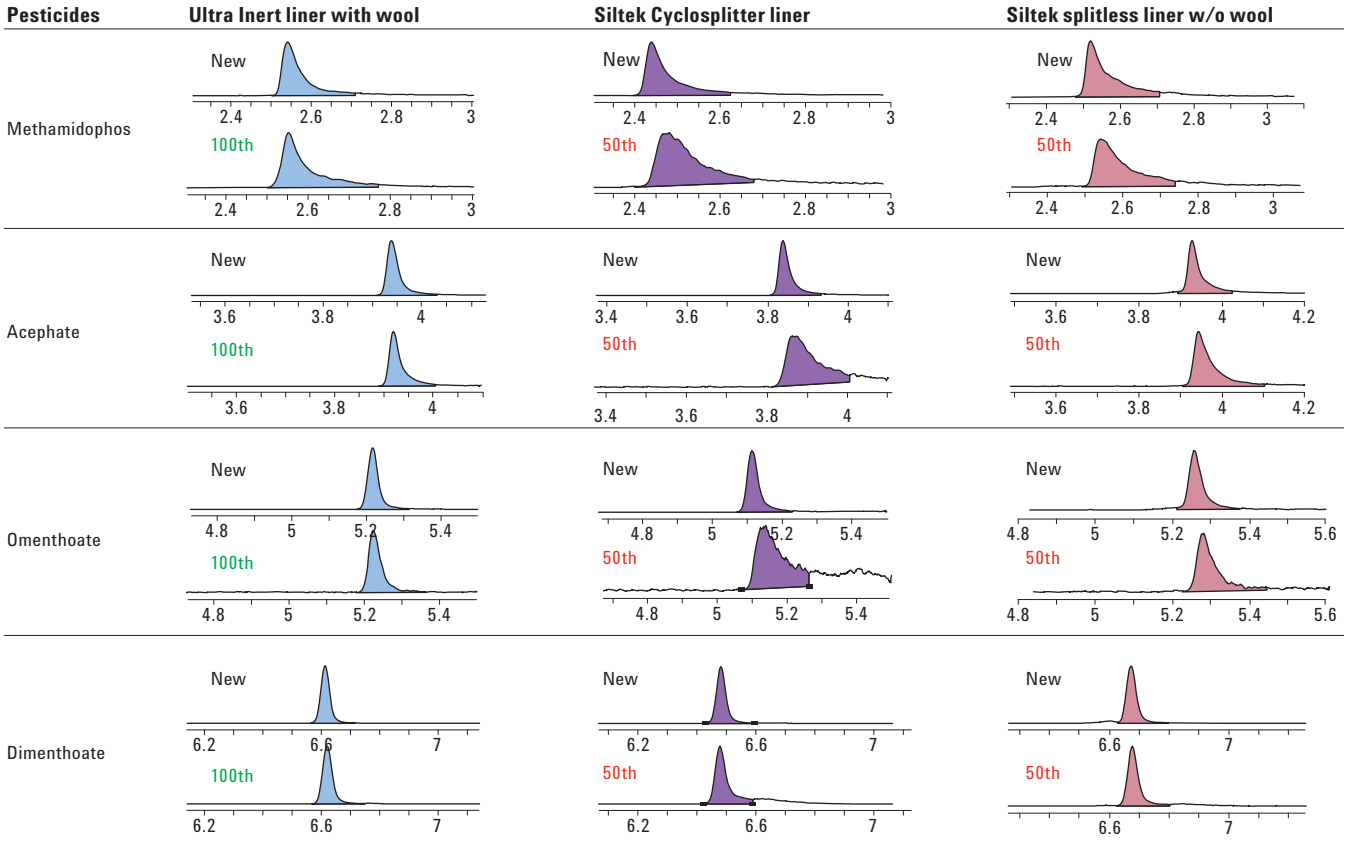


*Figure 2. Chromatographic comparisons for critical active compounds peak shape in QuEChERS extracts sample analysis using Ultra Inert single taper splitless liner with wool (p/n 5190-2293), Restek Siltek Cyclosplitter double taper splitless liner, and Restek Siltek gooseneck splitless liner without wool. Samples run were 50 ng/mL pesticides spiked in QuEChERS fruits and vegetables matrix blank.*

## Protection to the analytical column and MS source

Ultra Inert liners with wool provide better protection to the analytical column as well as the MSD source. The liner traps and filters more matrix interferences, especially the high-boiling indigenous compounds. This protection significantly extends the analytical column life time and decreases the frequency of MS source cleaning and maintenance. A new Ultra Inert column can run more than 300 matrix samples with acceptable performance. Changing a new Ultra Inert liner after 50 – 100 matrix samples run is usually necesary, when certain critical pesticides are the target analytes. System performance can be restored by installing a new Ultra Inert liner and short trimming the column head (< 10 cm) when necessary.

The long term protection on the MS source has not been fully evaluated, but we performed more than 3000 injections, including more than 2000 matrix samples and about 1000 neat samples, in our GC/MS triple quardrupole instrument for more than three months without conducting a source cleaning. Afterward, the MS tune profiles were still acceptable.

Ultra Inert liner with wool is more compatible with pulsed splitless injection. Pulsed splitless injection works well to reduce residence time and minimize solvent expansion volume, but this approach can force nonvolatile matrix components farther into the column than desirable. The use of liners with wool can definitely help this by trapping and filtering nonvolatile impurities, allowing better use of pulsed-splitless injection.

## Comparison with other popular liners used for pesticides analysis

Siltek deactivated liners have been widely used to analyze pesticides. Siltek Cyclosplitter double taper splitless liners and Siltek single taper splitless liners without wool were selected for the performance comparison to Ultra Inert liners with wool. Similar tests were done on the Siltek Cyclosplitter and single taper w/o wool liners at n = 3 replicates. Instead of 100 injections of matrix samples, these liners were tested with 50 matrix samples due to the rapid performance deterioration and poor column protection. The results were compared as the average RSD values over 50 matrix sample injections to the results obtained by Ultra Inert liners w/wool over 50 matrix samples as shown in Table 7.

As seen in Table 7, similar RSD values were obtained for most of the active pesticides by three kinds of liners. However, lower RSD numbers were achieved when using Ultra Inert liners with wool for those critical analytes such as Methamidophos, Acephate, Omenthoate, and Iprodione. This indicates that the Ultra Inert liners even with glass wool provide better inertness. The two types of Siltek liners generated slightly better RSD data for DDT and pass the 20% criteria. The flow path active sites may induce decomposition of DDT to DDE and DDD. However, this degradation is normally well controlled with low breakdown without matrix effect [7]. When matrix is introduced, the repeatability of DDT also seems to be influenced by matrix effect. The DDT peak intensity was sporadic across the matrix sample runs, rather than a continuous decrease as observed for other pesticides like Acephate and Omenthoate. More investigations are necessary to better understand DDT's stability in matrix during the GC/MS analysis.

Ultra Inert liners with wool provide better protection to the analytical column than the Siltek Cyclosplitter liner and single taper splitless liner without wool. This protection allows shorter column trimming, easy system performance recovery, and longer column life-time for complicate samples analysis. The Siltek liners allow the matrix interferences to get into the analytical column easily, especially for the single taper splitless liner without wool. These interferences, especially the high-boiling impurities, accumulate in the column causing the column performance to deteriorate much faster, requiring longer column trimming from the front of the column to restore acceptable performance. This deterioration not only showed as a loss of active pesticides responses, but also as poor peak shapes after just 50 matrix samples run (Figure 2). Usually, a new column cannot run more than 150 matrix samples when using those Siltek liners. A new Siltek liner without wool cannot support more than 50 samples (see data in Table 7 and Figure 2) for critical active pesticides analysis. After 50 injections, a new Siltek liner and substantial column head trimming (30–50 cm) were needed to achieve acceptable (but not as good as original performance with new column) peak shape and intensity. Even with liner changes, the column performance continues to deteriorate. The column might "die" after 100 – 150 matrix injections.

Instead, when using Ultra Inert liner with wool, a new column can support more than 300 samples with same matrices. A new Ultra Inert liner with wool can support analysis of 50–100 matrix samples, and system performance can be recovered easily by changing a new liner and/or a short column trim.

The comparison results demonstrate that the Ultra Inert liners with wool provided equivalent or better inertness than Siltek Cyclosplitter liners and single taper splitless liners without wool for the active pesticides analysis in real fruits and vegetables matrices. Ultra Inert liners with wool provided better protection to the whole system than non-wool Siltek liners, thus extending the column life and decreasing the frequency of MS source maintenance.

*Table 7. Injection Repeatability and Performance Stability Comparison Among Ultra Inert Liner with Wool, Restek Siltek Dual Taper Cyclosplitter Splitless Liner and Single Taper Splitless Liner Without Wool*

| | Injection repeatability and performance stability with 50 matrix samples run (% RSD) | | |
|---|---|---|---|
| **Pesticides (peak number)** | **Ultra Inert single taper splitless liners with wool (n = 7)** | **Siltek dual taper Cyclosplitter liner (n = 3)** | **Siltek single taper splitless liner without wool (n = 3)** |
| Methamidophos (1) | 5.1 | 25.4 | 9.6 |
| Acephate (4) | 16.6 | 55.6 | 22.3 |
| σ-Phenylphenol (5) | 1.6 | 2.5 | 4.7 |
| Omenthoate (6) | 27.1 | 49.9 | 41.0 |
| Dimenthoate (7) | 7.3 | 14.9 | 6.8 |
| Lindane (9) | 3.6 | 4.2 | 3.2 |
| Chlorothalonil (11) | 9.3 | 11.7 | 5.4 |
| Carbaryl (14) | 11.7 | 13.4 | 3.2 |
| Dichlorfluanid (16) | 6.3 | 3.9 | 5.6 |
| Tolylfluanid (21) | 7.3 | 5.7 | 6.9 |
| Procymidone (22) | 0.9 | 1.6 | 3.9 |
| Endrin (23) | 3.2 | 2.7 | 3.7 |
| Endosulfan sulfate (25) | 9.5 | 10.5 | 5.6 |
| DDT (26) | 23.1 | 16.8 | 18.4 |
| Endrin ketone (27) | 9.3 | 9.4 | 8.5 |
| Iprodione (28) | 5.0 | 20.3 | 7.9 |
| Phosmet (29) | 15.4 | 16.3 | 13.6 |
| Coumaphos (32) | 7.8 | 7.5 | 8.6 |

## Conclusion

Agilent Ultra Inert splitless liners with wool demonstrated excellent inertness for the accurate quantitative analysis of active and difficult pesticides in fruit and vegetable matrices. The evaluation results demonstrated that Ultra Inert splitless liners with wool can provide excellent chromatography at lower concentrations, superior sensitivity (5 ng/mL of LOQ) and linearity ($R^2 > 0.99$), consistent liner to liner reproducibility, acceptable repeatability and performance stability with injections of real samples. The use of Ultra Inert liners with wool also protect the entire system better, resulting in extended column life-time and less frequent MS source maintenance. When compared to other liners used widely in pesticides analysis, the performance of Ultra Inert liners with wool was superior to Restek Siltek Cyclosplitter liners and splitless liners without wool. Ultra Inert liners with wool are shown to be an excellent choice for accurate analysis of active and difficult pesticides in fruits and vegetables, and thus will be used for more pesticides analyses.

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