Ultra Inert (UI) Wool Liner Performance Using an Agilent J&W DB-35ms UI Column with and without an Analyte Protectant for Organophosphorus (OP) Pesticides

Application Note

Food Safety

Authors

Ken Lynam and Doris Smith
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, Delaware 19808
USA

Abstract

Liners with wool have traditionally been avoided for pesticide analysis due to high levels of activity from the wool. Using an Agilent Ultra Inert (UI) Wool Liner coupled with an Agilent J&W DB-35ms UI column an effective organophosphorus (OP) pesticides analysis is shown in an olive oil matrix with and without analyte protectant.

Introduction

Chromatographically active compounds such as organophosphorus (OP) pesticides can adsorb onto active sites in the sample flow path, compromise an analyte's response, and in extreme cases cause trace level signal to effectively disappear. These pesticides tend to show peak tailing due to interactions with active sites in a chromatographic system. Minimizing surface activity throughout the flow path is essential to achieving consistent results for OP pesticides [1]. Methamidophos, acephate, omethoate and dimethoate are particularly difficult due to a P = O bond in their structure. These analytes are focal points in this study.

A potential source of activity in the sample path is within the GC inlet. In residue analyses, repeated injections of matrix samples can lead to a gradual accumulation of nonvolatile matrix components in the inlet liner and column head, producing active sites and the need for maintenance. This matrix-induced effect can impact peak shape, response, and retention. Agilent's Ultra Inert liner with wool minimizes liner activity and helps prevent matrix component buildup at the inlet base and column head by trapping the non-volatiles on the deactivated wool [2].

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The GC column can be another potential source of activity in the analyte flow path. The column has a large surface area relative to the inlet liner. It is essential to minimize surface activity that can lead to misshapen peaks or loss of active analytes. Agilent's J&W DB-35ms Ultra Inert (UI) columns are inertness verified to deliver the most consistent inertness performance available through rigorous evaluation with demanding test probes [3,4].

All reasonable means of eliminating flow path activity need to be pursued prior to analysis of these pesticides. One good practice is to conduct thorough inlet maintenance by installing a fresh gold seal and an exceptionally well deactivated Ultra Inert inlet liner. Additionally, an inertness performance verified Ultra Inert column can be installed. Injection parameters should also be optimized to get the best results possible from the chromatographic system as a whole.

Once all reasonable means to eliminate flow path activity have been accomplished, another factor to consider when analyzing pesticide residues in sample matrices is matrix-induced signal enhancement effect. This effect is seen in the improved peak shape and signal of affected analytes when injected in a matrix versus non-matrix. This enhancement is believed to result from sample matrix components acting as protectants, reducing thermal degradation and masking active sites within the injector. OP pesticides containing P = O bonds, such as methamidophos, acephate, omethoate, and so forth, often benefit from this matrix effect, yielding a higher response for the analyte in matrix than in matrix-free standards, which can lead to inaccurate recoveries for fortified samples.

Anastassiades et al. suggested the use of analyte protectants to help minimize the errors caused by matrix-induced signal enhancements [5]. These analyte protectants (APs) are compounds which can be added to matrix extracts to protect the susceptible analytes from degradative interactions. A wide variety of compounds were evaluated as viable APs in a subsequent study by Anastassiades et al.[6] Based on their efforts of L-glulonic acid γ-lactone (gulonolactone), was chosen as the analyte protectant for evaluation in this study. Here the focus is narrowed to look at the benefit of using an analyte protectant with an Ultra Inert (UI) liner with wool and an DB-35ms UI column on four specific P = O bond containing pesticides.

Experimental

An Agilent 7890 GC/5975C MSD equipped with a flame photometric detector and an Agilent 7683B autosampler was used for this series of experiments. A purged two-way capillary flow technology (CFT) device was used to split the effluent 1:1 to the MSD:FPD. The CFT device also enabled post column backflush [7]. Table 1 lists the chromatographic conditions used for these analyses. Table 2 lists flow path consumable supplies used in these experiments.

Table 1. Chromatographic Conditions

GC/MSD	Agilent 7890/5975C
Sampler	Agilent 7683B, 5.0 µL syringe (p/n 5181-1273)
CFT device	Purged 2-way splitter (p/n G3180B)
Split ratio	1:1 MSD:FPD
MSD restrictor	1.43 m × 0.18 mm ID deactivated fused silica tubing (p/n 160-2615-10)
FPD restrictor	0.53 m × 0.18 mm id deactivated fused silica tubing (p/n 160-2615-10)
Aux EPC	3.8 psi constant pressure
Inlet	2 µL splitless; 250 °C, Purge flow 60 mL/min at 0.25 min, Gas saver on at 2 min 20 mL/min
Column	DB-35ms UI 30 m × 0.25 mm × 0.25 µm (p/n122-3832UI)
Carrier	Helium, constant pressure 28.85 psi at 95 °C
Oven	95 °C (0.5 min), 25 °C/min to 210 °C, 10 °C/min to 250 °C (0.5 min), 20 °C/min to 290 °C (4.5 min)
Postrun backflush	8.75 min at 290 °C, Aux EPC pressure 45 psi during backflush, 2 psi inlet pressure during backflush
MSD	300 °C transfer line, 300 °C source, 150 °C quad
FPD	230 °C, Hydrogen 75 mL/min, Air 100 mL/min, Carrier + makeup (N2) 60mL/min

Table 2. Flow Path Supplies

Vials and caps	Crimp Top Amber MS Analyzed Vial kits (p/n 5190-2283)
Vial inserts	250 µL glass/polymer feet (p/n 5181-8872)
Syringe	5 µL (p/n 5181-1273)
Septum	Advanced Green (p/n 5183-4759)
Inlet liner	Ultra Inert single taper liner with wool (p/n 5190-2293)
Ferrules	0.4 mm id short ; 85/15 vespel/graphite (p/n 5181-3323)
PCT fittings	Internal nut (p/n G2855-20530)
PCT ferrules	SilTite ferrules, 0.25 mm id (p/n 5188-5361)
20 × magnifier	20 × Magnifier loop (p/n 430-1020)

Reagents and Chemicals

All reagents and solvents were HPLC or Ultra Resi grade. Acetonitrile (ACN) from Honeywell (Muskegon, MI, USA), toluene from Burdick & Jackson, and acetone from JT Baker were purchased through VWR International (West Chester, PA, USA). The neat pesticide standards were purchased from Chem Service, Inc. (West Chester, PA, USA), gulonolactone from Aldrich (St. Louis, MO), and triphenyl phosphate from Alfa Aesar (Ward Hill, MA).

Solutions and Standards

The individual OP Pesticides standards were prepared in acetone to yield neat solutions at a 1–2 mg/mL concentration. These neat solutions were then used to prepare a 50 µg/mL stock standard in acetone. Spiking solutions at concentrations of 1 and 5 µg/mL were prepared from the stock standard. A surrogate standard, triphenyl phosphate (TPP), was prepared at concentrations of 1, 15 and, 100 µg/mL in toluene. An analyte protectant solution was prepared by dissolving the neat gulonolactone in a minimum amount of water and appropriate amount of ACN to yield a 50 mg/mL concentration. The pesticide and surrogate standard spiking solutions were used to prepare the matrix standard in the matrix blank extract by appropriate dilution. The appropriate amount of gulonolactone solution was added to the calibration standards to yield a 0.5 mg/mL concentration in each standard.

Sample Preparation

A sample of extra virgin olive oil was purchased from a local grocery store. The sample extraction method utilized a modified QuEChERS approach. Figure 1 illustrates the sample preparation procedure graphically in a flow chart.

Samples containing 3.00 (± 0.05) g of olive oil were weighed into centrifuge tubes. Two ceramic bars (p/n 5982-9313) were added to each sample to aid in sample extraction. Each sample received a 7 mL aliquot of cold reagent grade water and vortexed 1 min. A 10 mL aliquot of ACN was added to the tube. The samples were vortexed for 1 min. An Agilent original QuEChERS extraction salt packet (p/n 5982-5550) containing 4 grams of $MgSO_4$ and 1 g sodium chloride was added to each centrifuge tube. The capped tubes were shaken on a mechanical shaker @1500 rpm for 1 min. The samples were centrifuged at 4000 rpm for 5 min.

An 8 mL aliquot of the upper layer was transferred to an Agilent QuEChERS AOAC dispersive SPE 15 mL tube for fatty samples (p/n 5982-5158). The dSPE tube was vortexed for 1 min and then centrifuged at 4000 rpm for 5 min. Approximately 5.5 mL of the extract was then transferred to a second fatty sample dispersive SPE 15 mL tube and the above vortex and centrifuge procedure repeated to complete the sample extraction. The extract from the second dSPE tube was collected and the appropriate amounts of pesticide and surrogate standard spiking solutions were added to prepare the matrix standard in the matrix blank extract. The gulonolactone solution was added to yield 0.5 mg/mL gulonolactone. The extract was then analyzed by GC/MS/FPD using the chromatographic conditions listed in Table 1.

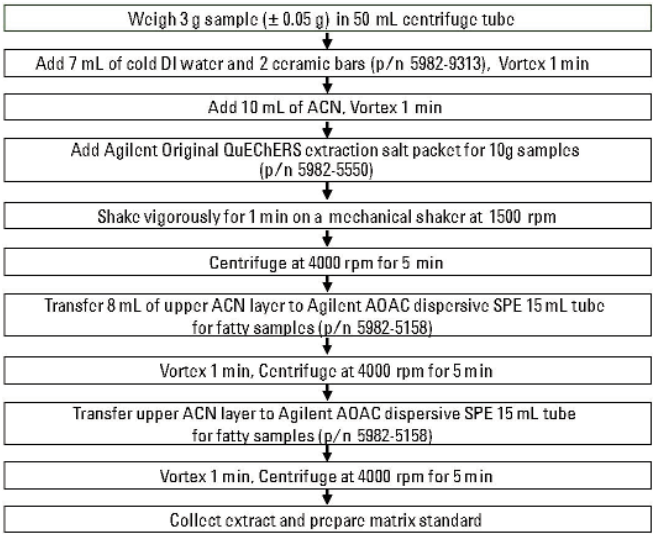

Figure 1. QuEChERS sample preparation workflow.

Results and Discussion

A large volume of olive oil sample matrix was prepared for repetitive injection of the matrix over the course of 100 injections on the GC/MS/FPD system and the same DB-35ms UI column. The performance of the Agilent Ultra Inert liner with wool was evaluated by running 100 injections of the 250 ng/mL matrix standard. A solvent blank was run after every 10 matrix injections. The first set of the injection study was done using an UI wool liner without analyte protectant. The head of the column was trimmed, a new gold seal, septa, UI liner with wool and O-ring were installed for the second set of injections, this time with gulonolactone added to the matrix standard as an analyte protectant. Methamidophos, acephate, omethoate and dimethoate results were carefully examined to evaluate the performance of the UI wool liner and the impact of the analyte protectant in the GC/MS/FPD system.

Figure 2 highlights the peak shapes observed after the first, 50th and 100th injections of a QuEChERS matrix on an Agilent Ultra Inert liner with wool. Signal strength and peak shape were consistent over the course of the study for methamidophos, acephate, omethoate and dimethoate. Good reproducibility was achieved over the course of 100 matrix injections with < 9% RSD for all four targeted OP pesticides. Table 3 shows the %RSD values for each pesticide after 10, 50, and 100 injections. The signal strength, peak shape and area consistency demonstrate that Ultra Inert liners with wool can be successfully used for 100 injections or more of QuEChERS matrix samples and effectively deliver problematic organophosphorus pesticides in a sample to the detector.

Organic Pesticides over 100 injections with Agilent's Ultra Inert Liner with wool

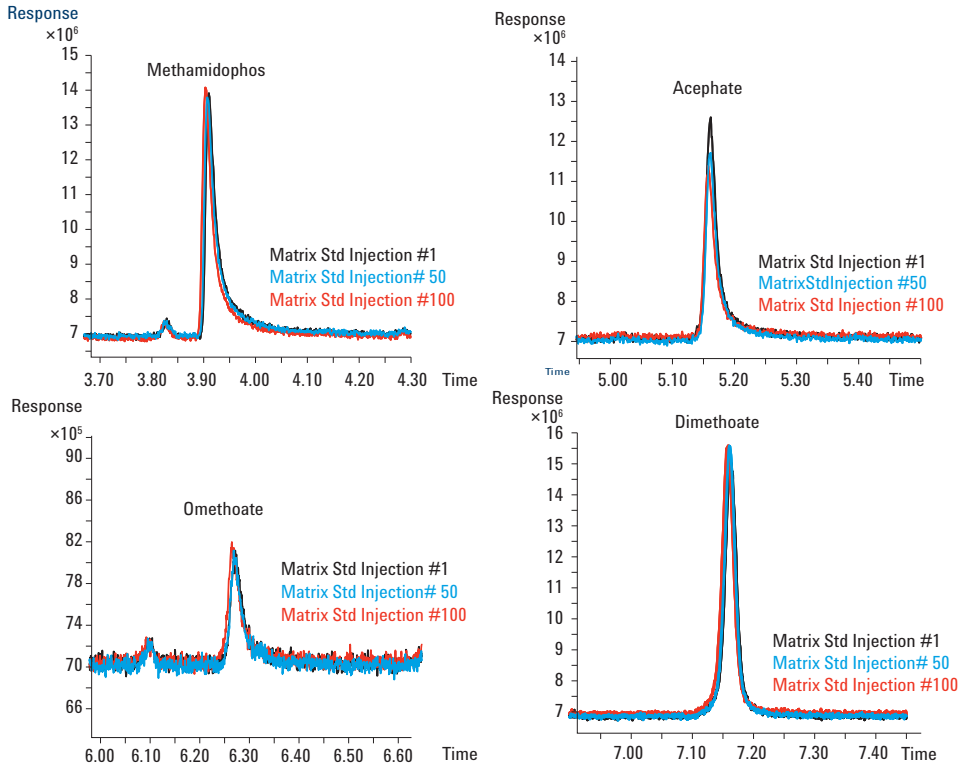

Figure 2. Overlay of first, 50th, and 100th injections of problematic OP pesticides on a UI wool liner without analyte protectant.

Agilent's Ultra Liner with Wool Repeatability (%RSD)

Table 3. Repeatability Over 100 Injections of 250 ng/mL Matrix Standard Without Analyte Protectant Using Agilent's Ultra Inert Liner with Wool

		%RSD	
Pesticide	**10 Injections**	**50 Injections**	**100 Injections**
Methamidophos	2.6	2.6	3.9
Acephate	2.2	4.6	8.7
Omethoate	3.8	4.1	4.6
Dimethoate	2.9	2.7	2.7

Figure 3 illustrates standard response after 100 matrix injections on UI wool liners with and without the use of analyte protection. This view indicates that improvements in signal responses are seen for most of the problematic OP pesticides when analyte protectant is used. Signal response for dimethoate was only marginally improved though use of the analyte protectant. Improvements in signal responses were on the order of 40% for acephate and omethoate, 20% for methamidophos, and 8% for dimethoate. The use of the analyte protectant improved peak shape for acephate, omethoate and methamidophos primarily. The peak tailing factors after 100 injections depicted in Figure 3 demonstrates the effect of analyte protectants on masking potential active sites in the flow path which can accumulate after a multitude of matrix samples.

Peak Shape and Response Comparison with analyte protectant versus without analyte protectant

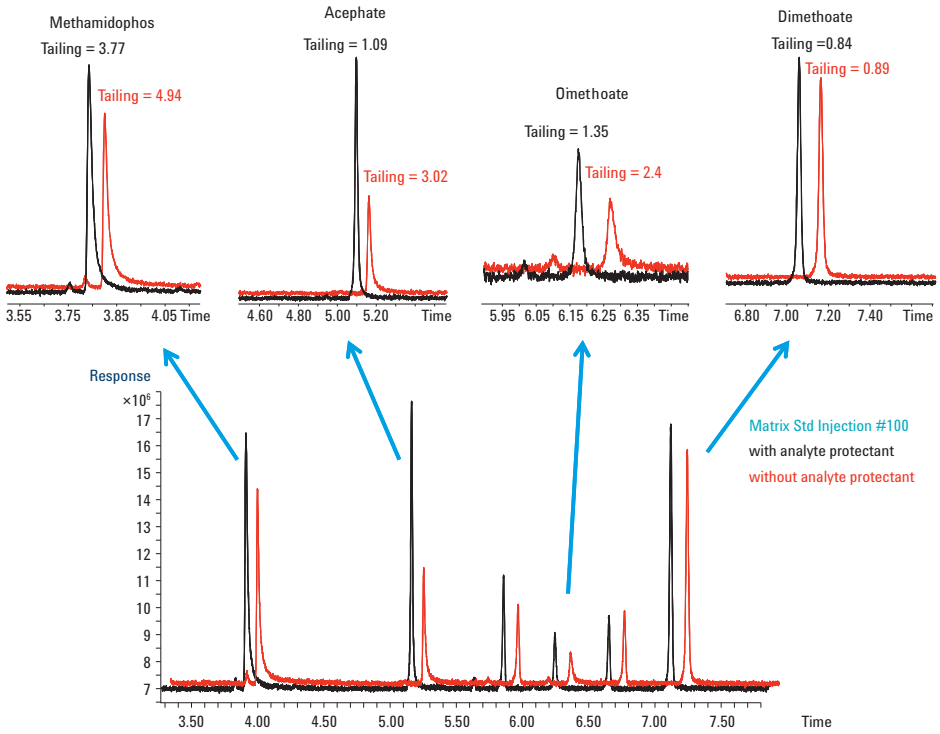

Figure 3. Overlay of 100th injection chromatograms with offset retention times for problematic OP pesticides with and without analyte protectant using UI wool liners.

Conclusions

These results clearly show that the use of Agilent Ultra Inert liners with wool is effective for organophosphorus pesticides over a course of at least 100 olive oil QuECHeRS matrix injections. Consistent results were seen in terms of signal response and peak shape between injections 1, 50 and 100 at the 250 ppb level for organophosphorus pesticides considered to be problematic. This consistency was observed both with and without the addition of the analyte protectant gulonolactone to final matrix extracts. The consistency observed demonstrates that the pesticides in the study were not lost due to
interaction with the wool in these Ultra Inert liners.

Addition of gulonlactone analyte protectant did improve area response and peak shapes for acephate and omethoate. Peak shapes and signal improvements were observed for methamidophos and dimethoate but were less dramatic. All reasonable means to render a system and its flow path inert need to be explored to obtain consistent and accurate results for these challenging P=O bond containing organophosphorus pesticides. A highly deactivated system with a freshly maintained inlet, an Ultra Inert Liner with wool, and an Agilent Ultra Inert DB-35ms column still showed benefit from the addition of the analyte protectant gulonlactone.

Ultra Inert Liners with wool and DB-35 ms UI columns proved to be excellent tools in combination to obtain consistent recoveries and peak shapes for the four P=O organophosphorus pesticides. Methamidophos, acephate, omethoate and dimethoate are considered problem pesticides and yet gave consistent results over the course of 100 matrix injections using Ultra Inert liners with wool and
DB-35ms UI columns both without and with anayte protectant.

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