# Benefits of EMR—Lipid Cleanup with Enhanced Post Treatment on Pesticides Analysis by GC/MS/MS

## Application Note

Food Testing

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## Abstract

Agilent Bond Elut Enhanced Matrix Removal—Lipid (EMR—Lipid) is a next-generation sample preparation product designed for the selective cleanup of lipids in fatty samples. The product is implemented in a convenient dispersive solid phase extraction (dSPE) format for the treatment of extracts from widely accepted workflows such as QuEChERS and protein precipitation. The EMR protocol is modified after the EMR—Lipid cleanup, with the use of anhydrous $MgSO_4$ in a pouch format. Anhydrous $MgSO_4$ is used for the separation of the aqueous and acetonitrile solvent phases, and the subsequent drying step to completely remove residual water and any water-soluble residues. The enhanced post-sample treatment has significant impact on GC-type applications by improving instrumental analysis reproducibility, especially for labile analytes. This study investigates the modified EMR protocol for the analysis of GC amenable pesticides in avocado by GC/MS/MS. The modified EMR protocol improves instrumental analytical reproducibility, reliability, and long-term usability, especially for labile pesticides, while maintaining high matrix removal efficiency and acceptable analyte recovery.

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# Introduction

The analysis of pesticide residues in food commodities is routine for many laboratories. The adoption of the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method [1,2], allows the analysis of hundreds of pesticides at low concentrations. The methodology has worked well for various fruits and vegetables. However, foods high in fat such as avocado, nuts, and foods of animal origin present new challenges [3,4]. Overcoming these challenges is a high priority for laboratories tasked with reaching the stringent validation criteria required by government agencies to ensure that food is safe for consumption.

Agilent Bond Elut Enhanced Matrix Removal—Lipid (EMR—Lipid) is a novel sorbent material that selectively removes major lipid classes from the sample extract without unwanted analyte loss. A previous application note demonstrated the exceptional cleanliness that EMR—Lipid provides for complex, fatty samples such as avocado. EMR—Lipid also meets the recovery and precision requirements for multiclass pesticide residues [5]. Advancements in post-sample treatment have determined that removal of NaCl from the polish step is advantageous. The presence of NaCl could allow a small percentage of water and, therefore, nonmatrix water-dissolved residues to be present in the final extract. Complete removal of water residue is important for reliable GC and GC/MS analysis.

The enhanced post sample treatment incorporates anhydrous $MgSO_4$ for phase separation and sample drying. This significantly improves the removal of water and water-dissolved residue without sacrificing the matrix removal of EMR—Lipid cleanup. This study demonstrates the benefits of using enhanced post-sample treatment after EMR—Lipid cleanup for pesticide analysis in avocado by GC/MS/MS. The four difficult labile pesticides: captafol, phosmet, coumaphos, and pyraclostrobin, were added to evaluate the impact of water residue on labile pesticide analysis.

# Experimental

## Reagent and chemicals

All regents and solvents were HPLC or analytical grade. Acetonitrile (ACN) and methanol were from Honeywell (Muskegon, MI, USA). Reagent grade acetic acid (AA) was from Sigma-Aldrich. The pesticide standards and internal standard were purchased from Sigma-Aldrich (St Louis, MO, USA).

## Solution and standards

A solution of 1% AA in ACN was prepared by adding 10 mL of acetic acid to 990 mL of ACN. Standard and internal standard (IS) stock solutions were made in either ACN or methanol at 2.0 mg/mL. A combined working solution was prepared in ACN at 25 µg/mL. A 25 µg/mL solution of combined IS working solution was prepared in ACN, including TPP, Parathion ethyl d10, and ^{13}C-DDT.

## Equipment and materials

Equipment and material used for sample preparation included:

- Geno Grinder (Metuchen, NJ, USA)

- CentraCL3R centrifuge (Thermo IEC, MA, USA)

- Eppendorf microcentrifuge (Brinkmann Instruments, Westbury, NY, USA)

- Vortexer and Multi-Tube Vortexer (VWR, Radnor, PA, USA)

- Bottle top dispenser (VWR, So. Plainfield, NJ, USA)

- Eppendorf pipettes and repeater

- Agilent Bond Elut AOAC extraction kit (p/n 5982–5755)

- Agilent Bond Elut EMR—Lipid dSPE (p/n 5982–1010) and EMR—$MgSO_4$ polish pouches (p/n 5982–0102)

## Instrument conditions

The GC and MS conditions were used in previous application notes [5]. Analysis was completed on an Agilent 7890A GC equipped with an Agilent 7693B Autosampler and an Agilent 7000C Triple Quadrupole GC/MS system. Column backflushing was used, which is highly recommended for complex sample matrices.

## GC conditions

| Parameter | Value |
|---|---|
| GC: | Agilent 7890A GC |
| Column: | Agilent J&W DB-5ms Ultra Inert, 0.25 mm × 15 m, 0.25 µm (p/n 122–5512UI) |
| Carrier: | Helium, constant pressure |
| Gas filter: | Gas Clean carrier gas filter kit, 1/8 inch (p/n CP17974) |
| Inlet liner: | Agilent Ultra Inert single taper splitless liner with wool (p/n 5190–2293) |
| Inlet: | MMI inlet at pulsed cold splitless mode, 75 °C initially, hold for 0.02 min, then ramp to 350 °C at 750 °C/min |
| Pulsed splitless injection: | 36 psi until 0.75 min |
| Purge flow to split vent: | 60 mL/min at 0.75 min |
| Inlet pressure: | 17 psi during run, and 1.0 psi during backflushing |
| Oven: | 60 °C for 2.57 min, then to 150 °C at 50 °C/min, to 200 °C at 6 °C/min, to 300 °C at 16 °C/min, hold for 3 min |
| Postrun: | 2 min at 300 °C |
| Capillary Flow Technology: | Agilent UltiMetal Plus Purged Ultimate Union (p/n G3182-61581) for backflushing the analytical column and inlet |
| Autosampler: | Agilent 7693 Autosampler and sample tray 10 µL syringe (p/n G4513-80220), 1 µL injection volume |

## MSD conditions

| Parameter | Value |
|---|---|
| MSD: | Agilent 7000C Triple Quadrupole GC/MS, inert, with performance electronics |
| Vacuum pump: | Performance turbo |
| Mode: | MRM |
| Transfer line temp: | 280 °C |
| Source temp: | 300 °C |
| Quad temp: | 150 °C for Q1 and Q2 |
| Solvent delay: | 2.57 min |
| MS resolution: | MS1 and MS2 = 1.2u |

Table 1 lists the MRM transitions for the four additional labile pesticides used in this study. The MRM transitions for other pesticides were listed in reference [5].

Table 1. GC/MS/MS MRM parameters and retention times for the additional labile pesticides used in this study.
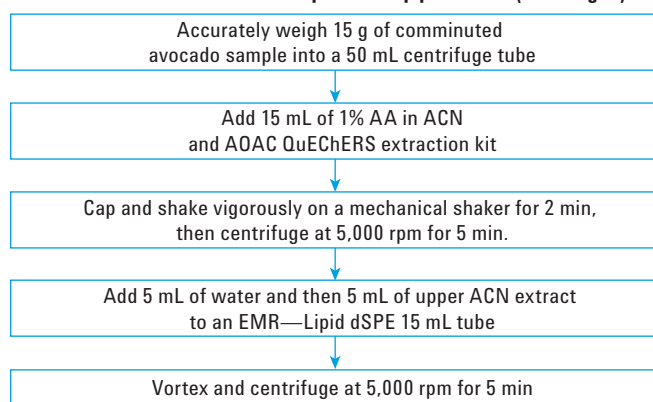
| Labile analyte | RT (min) | MRMs | | | |
|---|---|---|---|---|---|
| | | Quant channel | CE (v) | Qual channel | CE (v) |
| Captafol | 18.20 | 183 → 79 | 10 | 150 → 79 | 5 |
| Phosmet | 18.77 | 160 → 77.1 | 20 | 160 → 133.1 | 20 |
| Coumaphos | 20.67 | 361.9 → 109 | 10 | 210 → 182 | 10 |
| Pyraclostrobin | 22.03 | 132 → 77.1 | 20 | 164 → 132 | 15 |

## Sample preparation

The modifications only apply to the polishing step after EMR—Lipid cleanup. There are no changes to the QuEChERS extraction step and the EMR—Lipid cleanup step. After the EMR—Lipid cleanup, the ACN layer was phase separated from the aqueous phase, and further dried with anhydrous $MgSO_4$. Figure 1 shows the protocol diagram. There are two points to be emphasized for the modified procedure after EMR—Lipid cleanup:

- First, adding $MgSO_4$ to the sample minimizes the exothermic effect of $MgSO_4$ and water, and reduces salt clumping.

- Second, drying tubes were preweighed into 2 mL tubes using 300 mg of anhydrous $MgSO_4$ salt (from an EMR—Polish pouch) for 1 mL of ACN extract after EMR—Lipid cleanup.

**QuEChERS extraction and EMR-Lipid cleanup procedure (unchanged)**

| |
|---|
| Accurately weigh 15 g of comminuted avocado sample into a 50 mL centrifuge tube |

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| |
|---|
| Add 15 mL of 1% AA in ACN and AOAC QuEChERS extraction kit |

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| |
|---|
| Cap and shake vigorously on a mechanical shaker for 2 min, then centrifuge at 5,000 rpm for 5 min. |

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| |
|---|
| Add 5 mL of water and then 5 mL of upper ACN extract to an EMR—Lipid dSPE 15 mL tube |

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| |
|---|
| Vortex and centrifuge at 5,000 rpm for 5 min |

↓

**Enhanced post sample treatment (modified)**

| |
|---|
| Decant entire supernatant into a 15 mL tube, and carefully add polishing salt from EMR—Polish pouch |

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| |
|---|
| Vortex or shake immediately, centrifuge at 5,000 rpm for 5 min |

↓

| |
|---|
| Transfer 1 mL of the upper ACN supernatant into a preweighed 2 mL drying tube containing 300 mg $MgSO_4$ (from EMR—Polish pouch) |

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| |
|---|
| Centrifuge at 13,000 rpm (on a microcentrifuge) for 5 min |

↓

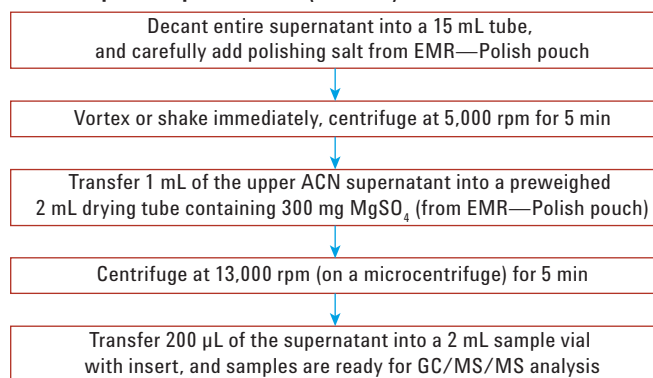| |
|---|
| Transfer 200 µL of the supernatant into a 2 mL sample vial with insert, and samples are ready for GC/MS/MS analysis |

Figure 1. Sample preparation workflow showing the recommended protocol for the analysis of pesticides in avocado by GC/MS/MS, using unchanged QuEChERS extraction and EMR—Lipid cleanup steps followed with the enhanced post sample treatment procedure.

## Sample matrix impact on GC/MS/MS system performance

To directly measure the impact of the sample matrix on GC/MS/MS instrument performance, avocado matrix blank sample was prepared following the original polish step and the enhanced post sample treatment after EMR—Lipid cleanup. The matrix blank was then post-spiked with pesticide standards at 50 ppb to determine matrix effects on GC/MS/MS system performance.

Labile compounds were investigated for analyte responses (peak area), peak shape, and reproducibility over multiple injections.

The injection sequence consisted of injecting four matrix blank samples followed by a post-spiked sample. This injection pattern was repeated until 100 injections, therefore, 80 matrix blank sample injections and 20 post-spiked sample injections were run in the testing sequence. The liner was replaced, and the column head was trimmed between sequences using original polish or enhanced post sample treatment. Since both UI single taper splitless liner with wool and UI dimple liner have been usually used for the analysis of complicated matrix samples, they were evaluated for their appearance after 100 injections of avocado samples prepared using enhanced post sample treatment.

## Matrix removal efficiency and analyte recovery

Matrix removal efficiency was confirmed by running the avocado matrix blank by GC/MS under full scan mode, and comparing the entire chromatographic profile using the efficiency calculation, as previously described [5]. Analyte recovery was evaluated by comparing the pre-spike and post-spike peak area of each analyte at 50 ppb.

# Results and Discussion

## Higher analyte responses and better peak shape

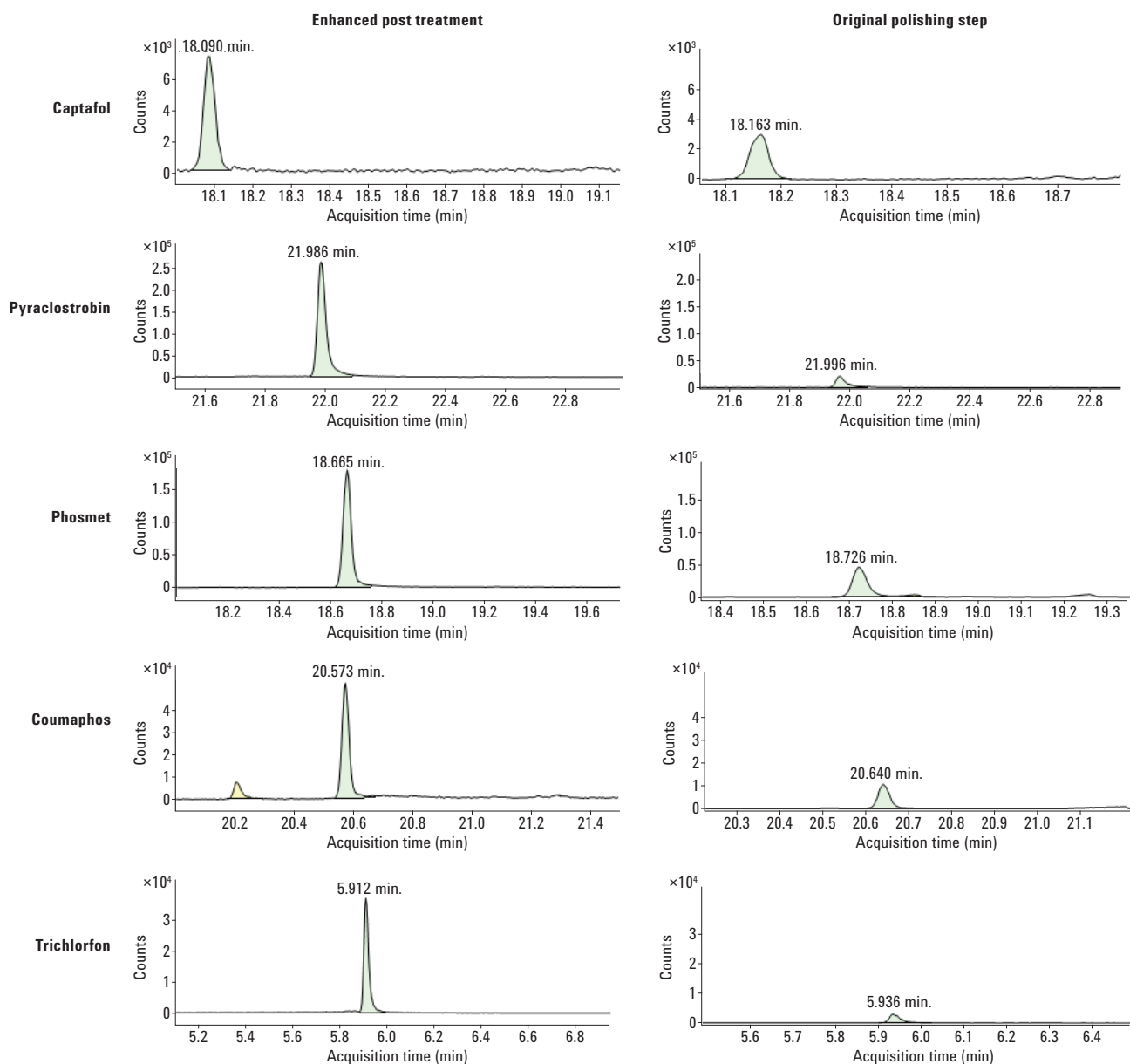The enhanced post treatment after EMR—Lipid cleanup removes the residual water and water-dissolved residues. Figure 2 shows the chromatographic comparison for labile compound response and peak shape on GC/MS/MS using enhanced post treatment versus the original polishing step. Analyte responses were increased more than threefold, especially for pyraclostrobin and trichlorfon, where a 10 fold increase was observed. Chromatography was also improved, with more symmetrical peak shape and less tailing, providing easier data processing. These improvements indicated that these labile compounds passed through the GC flow path without significant interactions on the flow path surface.



Figure 2. Chromatographic comparison for labile compounds responses and peak shape on GC/MS/MS using enhanced post treatment and original polishing step after EMR—Lipid cleanup.

## Improved system reproducibility

Method reproducibility is arguably the most important aspect of analysis as it directly impacts the reliability of quantitation results. As matrix accumulates in the flow path over multiple injections, analyte responses can vary over multiple injections, especially for labile compounds. These inconsistent responses make the quantitation difficult and unreliable. Our previous results demonstrated significant improvements in GC/MS/MS system reproducibility over multiple injections of complex samples prepared using EMR—Lipid cleanup [5]. Despite these improvements, some labile compounds still showed variability over multiple injections. This variability is mostly caused by trace amounts of water residues remaining in the final sample extract. The enhanced post treatment after EMR—Lipid cleanup, $MgSO_4$ salt partition, and drying steps were implemented to eliminate water residue and water dissolved solid residue from the final sample extract, thus improving the GC/MS/MS system reproducibility.

In Figure 3, pyraclostrobin was used as an example to show the improved reproducibility when injecting avocado samples prepared by the enhanced post treatment after EMR—Lipid cleanup. The comparison includes results from samples prepared using the enhanced post treatment and original polishing step after EMR—Lipid cleanup, as well as using traditional PSA/C18 cleanup. The data clearly demonstrate the dramatically improved reproducibility of pyraclostrobin response in samples prepared using the enhanced post treatment after EMR—Lipid cleanup. When using traditional PSA/C18 cleanup or EMR original protocol to prepare samples, the pyraclostrobin signal drops to 30–40% of the initial response after 100 injections. This inconsistency will cause quantitative analysis to fail for this compound. However, when using enhanced post treatment after EMR—Lipid cleanup, excellent signal reproducibility for pyraclostrobin (±10% deviations) was obtained. The improved reproducibility gained using the enhanced post treatment after EMR—Lipid cleanup makes quantitative analysis of labile analytes reliable and robust.



Pyraclostrobin reproducibility on GC/MS/MS over 100 injections of avocado samples

■ Enhanced post treatment after EMR—Lipid cleanup
■ Original polishing step after EMR—Lipid cleanup
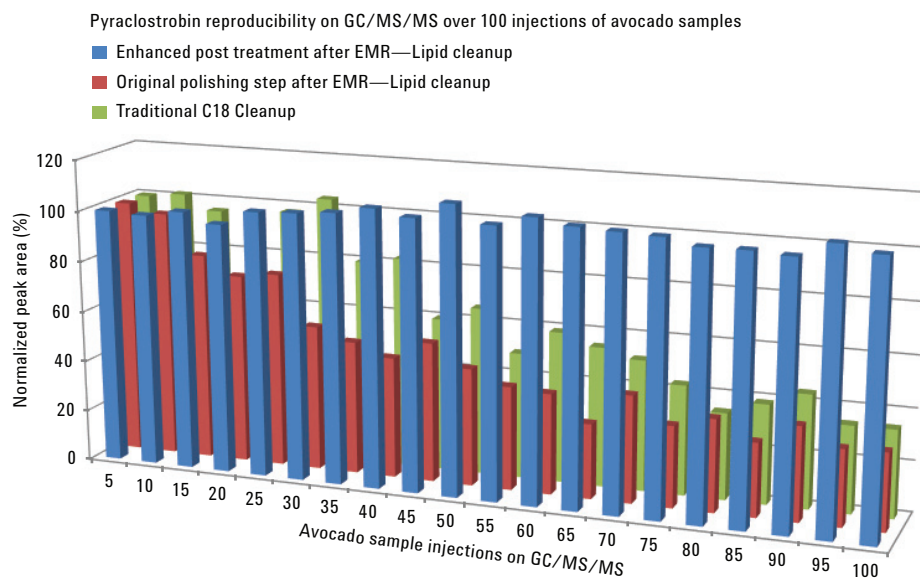■ Traditional C18 Cleanup

Figure 3. Labile compound pyraclostrobin response reproducibility on GC/MS/MS over 100 avocado sample injections prepared using enhanced post treatment and original polishing step after EMR—Lipid cleanup, and traditional C18 cleanup.

Table 2 lists all the pesticides tested in this study and their respective RSD over 100 injections of avocado using the method described. The EMR—Lipid cleanup followed with enhanced post treatment gives less than 10% RSDs for 24 of the 29 compounds. Captan, Folpet, Captafol, and DDT are problematic compounds on GC/MS/MS, and the high RSDs, especially from PSA/C18 cleanup, reflect a significant signal variation within 100 injections. However, the signal reduction for samples prepared by EMR—Lipid cleanup and enhanced post treatment was reduced, and the reproducibility of these four labile pesticides within 50 injections met the acceptance criteria, with less than 10% RSD.

Table 2. Analytes GC/MS/MS reproducibility (peak area RSD %) over 100 injections of avocado samples.

| Pesticide | Analyte RSD over 100 injections (n = 20) | | |
| | EMR-Lipid cleanup with enhanced post treatment | EMR-Lipid cleanup with original polishing step | C18/PSA cleanup |
|---|---|---|---|
| Dichlorvos | 8.5 | 6.2 | 10.5 |
| Trichlorfon | 9.2 | 35.0 | 73.0 |
| 2-Phenylphenol | 2.5 | 7.0 | 13.6 |
| Ethalfluralin | 4.6 | 12.4 | 18.8 |
| Sulfotep | 3.1 | 7.1 | 11.8 |
| Atrazin | 2.1 | 6.8 | 12.2 |
| Lindane | 3.1 | 8.5 | 10.8 |
| Chlorothanil | 2.2 | 12.5 | 11.7 |
| Diazinon | 2.6 | 6.6 | 11.7 |
| Chlorpyrifos-Me | 2.6 | 8.4 | 8.9 |
| Dichlorfluanid | 5.4 | 11.7 | 9.0 |
| Aldrin | 2.1 | 9.8 | 19.3 |
| Tolyfluanid | 6.6 | 10.5 | 6.6 |
| Captan | 29.8 | 29.9 | 51.9 |
| Folpet | 22.0 | 53.8 | 52.2 |
| Procymidone | 2.1 | 6.8 | 14.3 |
| Bupirimate | 3.1 | 6.8 | 10.4 |
| Endrin | 4.0 | 8.3 | 12.6 |
| Endosulfan sulfate | 3.6 | 8.5 | 12.1 |
| DDT | 16.1 | 21.6 | 22.4 |
| Captafol | 38.5 | 53.8 | 63.7 |
| Iprodione | 3.7 | 11.0 | 10.7 |
| Phosmet | 6.2 | 24.0 | 12.5 |
| Coumaphos | 4.3 | 19.8 | 9.7 |
| Permethrin | 3.0 | 6.8 | 11.8 |
| Pyraclostrobin | 3.7 | 43.7 | 38.8 |
| Deltamethrin | 8.7 | 22.5 | 9.8 |
| Parathion ethyl –d10 (IS) | 4.9 | 11.8 | 7.2 |
| TPP (IS) | 2.1 | 9.1 | 19.1 |

## Longer GC inlet liner and column lifetime

Another advantage of using enhanced post treatment after EMR—Lipid cleanup is the reduction of nonvolatile salt residue, which can remain dissolved in trace water residues. We tested two types of UI liners for 100 injections of avocado samples, Agilent Ultra Inert single taper splitless liner with wool (p/n 5190-2293) and Agilent UI dimple liner (p/n 5190–2297). After the test, the appearance of the liner was visually inspected for residue deposition. Figure 4 shows that both liners are virtually clean after 100 injections. These results attest to the superior cleanliness achieved using EMR—Lipid cleanup following enhanced post treatment. It results in longer liner and column lifetime and less system maintenance.

## Equivalent matrix removal efficiency and analyte recovery

Matrix removal efficiency was evaluated using the GC/MS full scan profile comparison before and after cleanup [5]. Results showed that equivalent matrix removal efficiency can be achieved using the enhanced post treatment and the original polishing step after EMR—Lipid cleanup (Figure 5).
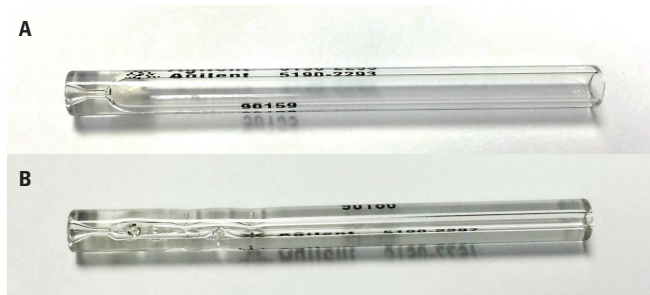


Figure 4. Typical GC inlet liners appearance after 100 injections of avocado samples prepared by EMR-Lipid cleanup followed with enhanced post treatment. A) Agilent Ultra Inert single taper splitless liner with wool, B) Agilent UI dimple liner.
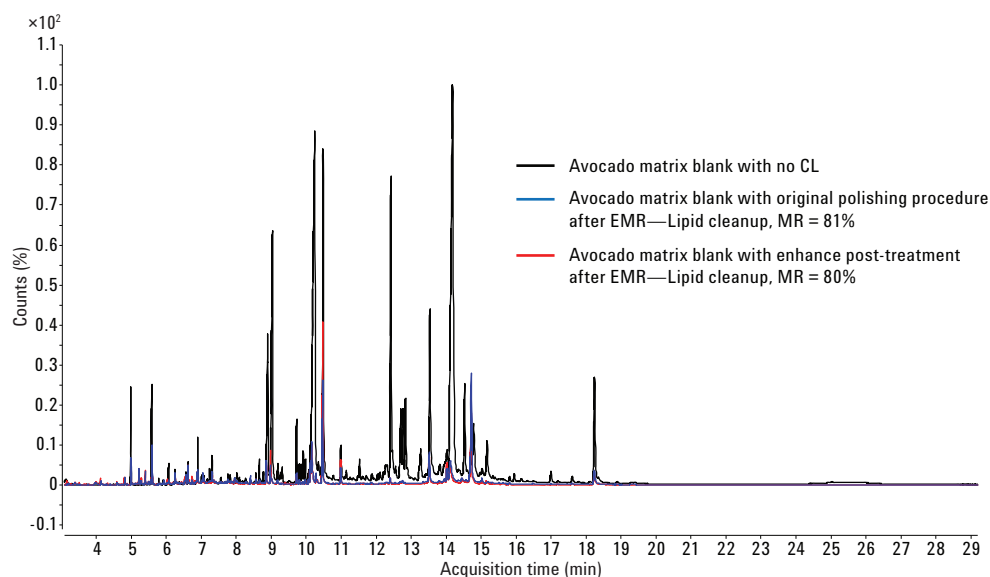


Figure 5. GC/MS full scan chromatograph comparison demonstrate the equivalent matrix removal efficiency provided by enhanced post treatment and original polishing step after EMR—Lipid cleanup.

Figure 6 shows the pesticides recovery comparison for 50 ppb fortified avocado samples (n = 6) prepared by EMR—Lipid cleanup followed with the enhanced post treatment and original polishing step, respectively. Some analytes show slightly lower recoveries using enhanced post treatment. However, the drastic improvements in reproducibility with less than 5% RSD for all compounds is significant.
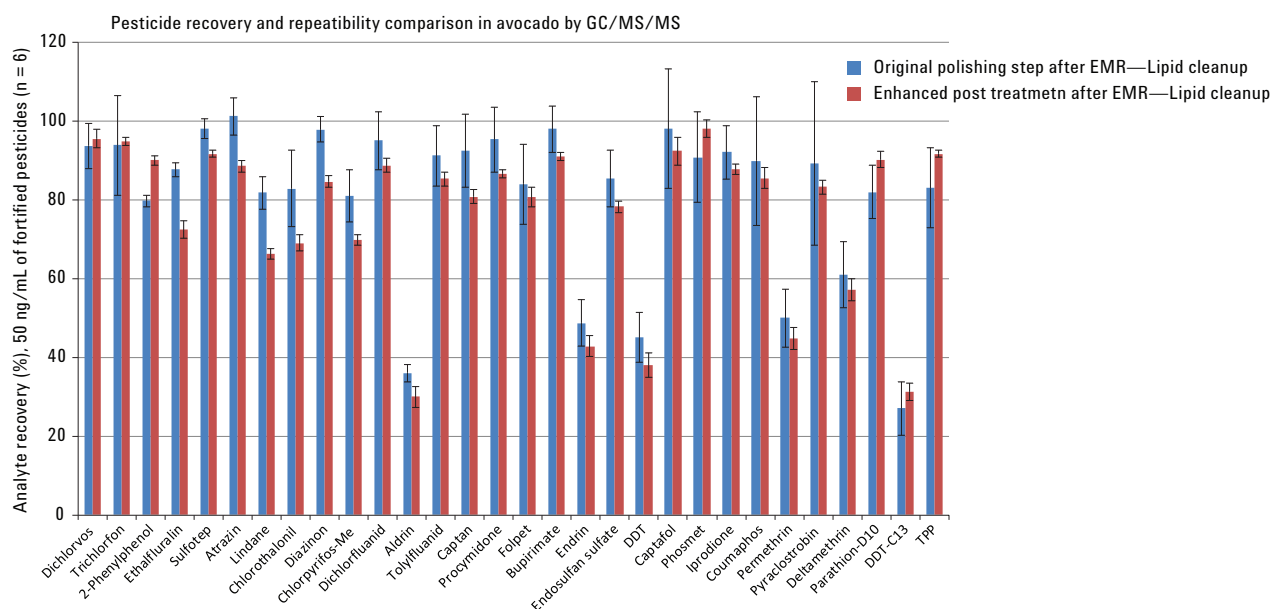


Figure 6. Pesticides recovery of avocado sample fortified at 50 ng/mL prepared by enhanced post treatment and original polishing step after EMR—Lipid cleanup.

## Conclusions

The enhanced post sample treatment after EMR—Lipid cleanup implements a polish step and a drying step with anhydrous $MgSO_4$ to remove residual water and water-dissolved residue before sample injection on GC/MS/MS. It improves the GC/MS/MS analysis by providing higher analyte response, better peak shape, excellent instrument reproducibility, and longer inlet liner and column life. This approach is ideal for analysts seeking to improve their sample preparation for complex, fatty samples, especially when labile analytes are of interest. The enhanced post sample treatment after EMR—Lipid cleanup also maintains high matrix removal efficiency for complicated samples, and delivers acceptable analyte recovery for multiresidue pesticides analysis. The polish salt (anhydrous $MgSO_4$) is available in a pourable pouch for easy dispensing into samples, and better storage.

## References

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