Improving Pesticides Analysis on GC/MS/MS for Complicated Sample Matrices by Increasing Matrix Removal

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Introduction

The adoption of the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method, 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. 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 next generation sample preparation product designed for the selective clean-up of lipids in fatty samples. EMR-Lipid was demonstrated to provide exceptional matrix cleanliness for complex, fatty samples like avocado, as well as meeting the recovery and precision requirements for multiclass pesticides residues. The EMR protocol is then modified, 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 following 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 maintains high matrix removal efficiency and acceptable analyte recovery.

Experimental

Instrumental Parameters for GC/MS/MS

GC Parameters	QQQ Parameters
• Agilent 7980A GC • Agilent J&W DB-5ms Ultra Inert, 0.25 mm x 15 m, 0.25 µm • Helium, constant pressure • Agilent Ultra Inert single taper splitless liner with wool • MMI inlet at pulsed cold splitless mode, 75ºC to 350ºC at 750ºC/min • Pulsed splitless injection • 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: UltiMetal Plus Purged Ultimate Union for backflushing the analytical column and inlet • 1 µL injection volume	• Agilent 7000C Triple Quadrupole GC/MS, inert, with performance electronics • Performance turbo • MRM mode • Transfer line temp: 280°C • Source temp: 300°C • Quad temp: 150°C for Q1 and Q2 • Solvent delay: 2.57 min • Collision gas flow: He quench gas at 2.35 mL/min, N_2 collision gas at 1.5 mL/min • MS resolution: MS1 and MS2 = 1.2u

Sample Preparation Workflow

QuEChERS extraction and EMR-Lipid cleanup procedure (unchanged)

Accurately weigh 15 g comminuted Avocado sample in 50 mL centrifuge tube

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Add 15 mL of 1% Acetic Acid in Acetonitrile, and AOAC QuEChERS extraction kit

↓

Cap and shake vigorously on mechanical shaker for 2 min, and then centrifuge @ 5000 rpm for 5 min.

↓

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

↓

Vortex and Centrifuge @ 5000 rpm for 5 min

↓

Enhanced post sample treatment (modified)

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

↓

Vortex or shake immediately, centrifuge@5000 rpm for 5 min

↓

Transfer 1 mL of upper ACN supernatant into a pre-weighed 2 mL drying tube containing 300 mg MgSO4 (from EMR-Polish pouch)

↓

Centrifuge @ 13000 rpm (on a microcentrifuge) for 5 min

↓

Transfer 200 µL the supernatant into 2mL sample vial with insert, and samples are ready for GC-QQQ analysis

Results and Discussion

Chromatographic Improvement

The enhanced post treatment after EMR-Lipid cleanup removes the residual water and dissolved solid residue, therefore improves analytes chromatographic responses, especially for labile pesticides.

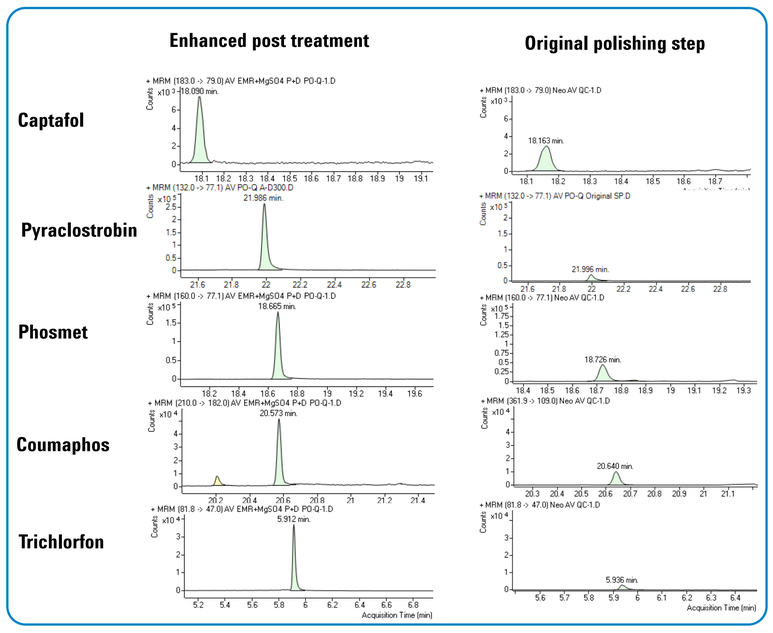

Figure 1, Chromatographic comparison for labile compounds responses and peak shape on GC/MS/MS using enhanced post treatment and original polishing step after EMR-Lipid clean-up.

System Reproducibility Improvement

The enhanced post treatment after EMR-Lipid cleanup removes the residual water and dissolved solid residue, therefore improves analytes' system reproducibility over multiple injections, thus allowing more injections done before regular maintenance like liner change and column head cut.

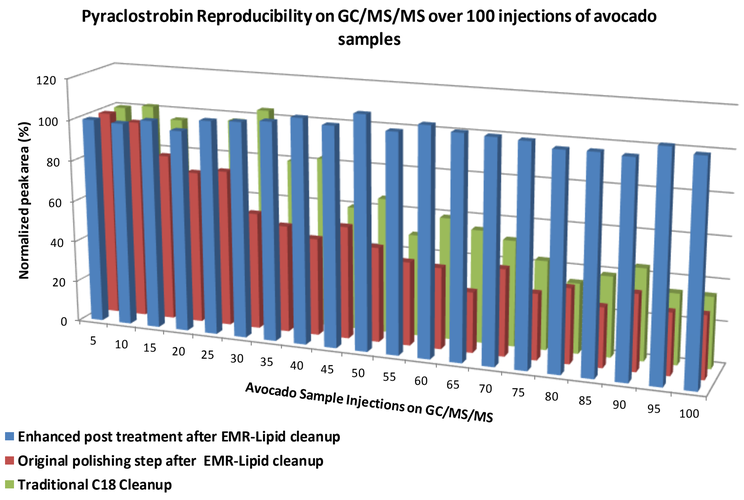

Figure 2. Labile compound pyraclostrobin responses reproducibility on GC/MS/MS over 100 avocado sample injections prepared using enhanced post treatment and original polishing step after EMR-Lipid clean-up, and traditional C18 cleanup.

Pesticide	Analyte RSD over 100 injections (n=20) **		
	EMR-Lipid clean-up with enhanced post treatment	EMR-Lipid clean-up with original polishing step	C18/PSA clean-up
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

Table 1. Analytes GC/MS/MS reproducibility (peak area RSD %) over 100 injections of avocado samples. *By using EMR-Lipid cleanup followed with enhanced post treatment, the 50 injections reproducibility for three extreme difficult pesticides: Captan, Folpet and Captafol, was less than 10% RSD. **An avocado QC sample spied with 50 ppb pesticide standard was injected every 5 injections, with avocado blank samples being injected in between. RSD > 20% in red.

Results and Discussion

Longer GC Inlet Liner and Column Lifetime

The use of enhanced post treatment after EMR-Lipid cleanup significantly reduced accumulation of nonvolatile salt residue and high-boiling interferences in the system, resulting in longer liner and column lifetime and less system maintenance.

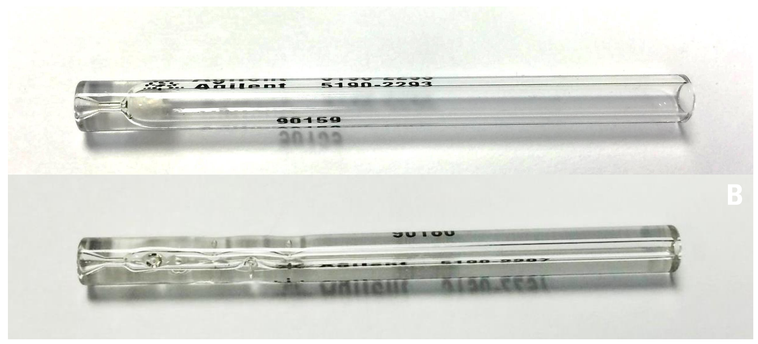

Figure 3. Typical GC inlet liners appearance after 100 injections of avocado samples prepared by EMR-Lipid cleanup followed with enhanced post treatment. A) UI single taper splitless liner with wool, B) UI dimple liner.

Equivalent Matrix Removal and Analyte Recovery

The use of enhanced post treatment after EMR-Lipid cleanup does not compromise the EMR-Lipid matrix removal efficiency and analyte recovery.

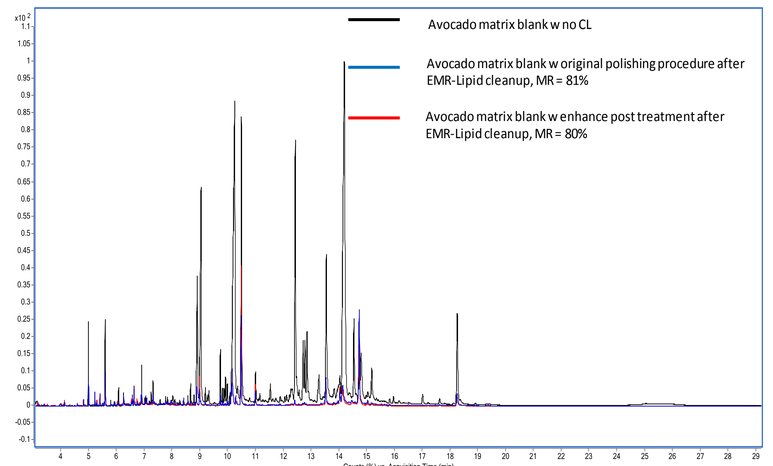

Figure 4. GC/MS full scan chromatograph comparison demonstrated the equivalent matrix removal efficiency provided by original polishing step and enhanced post treatment after EMR-Lipid clean-up.

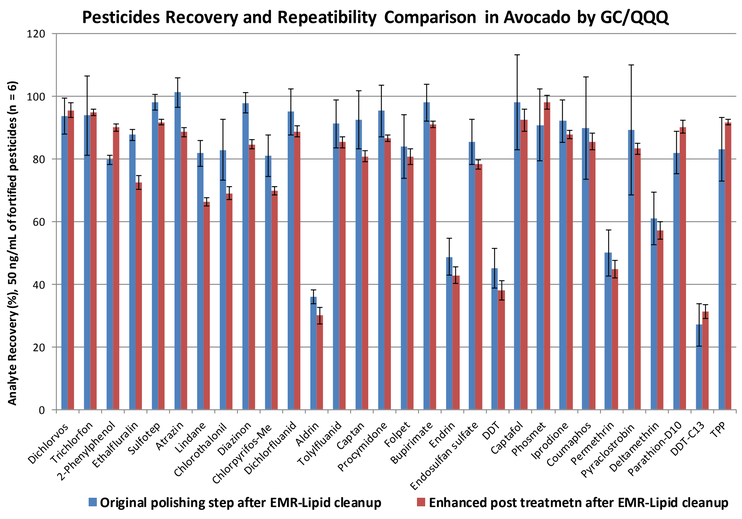

Figure 5. Pesticides recovery of avocado sample fortified at 50 ng/mL prepared by enhanced post treatment and original polishing step after EMR-Lipid clean-up.

Conclusions

The enhanced post sample treatment after EMR-Lipid cleanup implements a new polish step with anhydrous $MgSO_4$ and a final drying step to remove residual water and water dissolved residue before sample injection. It improves the GC/MS/MS analysis by:

- ✓ Better chromatographic response for labile analytes
- ✓ Excellent system reproducibility
- ✓ Extended GC inlet liner and column lifetime and reduced regular instrument maintenance frequency
- ✓ High matrix removal efficiency for complicated samples
- ✓ Acceptable analytes recoveries for multi-class multi-residue pesticides analysis

Additionally, the new polish salt is in a pourable pouch for easy dispensing and better storage.

Check our website for more information:

www.agilent.com/chem/EMR-Lipid