Extraction of Permethrin Pesticides from Spinach Using QuEChERS Methodology with Automated Shaking

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Introduction

Sample preparation procedures for pesticide analysis in fruits and vegetables were simplified with the introduction of QuEChERS methodology in 2003.[1] Multi-residue pesticide extraction was accomplished through a 1 minute shaking procedure using acetonitrile as an extraction solvent followed by salt partitioning and dispersive solid phase extraction (SPE) cleanup. This extraction procedure has been successfully applied to a variety of fruits and vegetables. The QuEChERS procedure has grown in popularity and has gained worldwide acceptance during the past 10 years.

It is common to evaluate and validate pesticide residue methods by spiking representative blank matrix samples with a standard mix of pesticides. Pesticide-free produce samples, such as organically grown produce, are often used for this purpose. The pesticides are typically added to a matrix sample 1-2 hours prior to testing in order to allow pesticide incursion. They are then easily extracted using the 1 minute shaking protocol. However, recently, it was noted that existing residues may not be readily accessible for extraction as they can be "enclosed into cells or wax particles" of the produce.[2] The 1 minute shaking time specified in the QuEChERS protocol was not sufficient to fully extract existing pesticide residues in approximately 50% of tests conducted. It was later observed that a 10-15 minute shaking time with the use of an automated shaker was required to obtain acceptable extraction efficiencies.[3]

Recently the BenchMixer™ shaker was added to the Supelco/Sigma-Aldrich product listing to compliment the Supel QuE product line. Spinach, a vitamin and mineral-rich green leafy vegetable, was recently cited in the Environmental Working Group's "Dirty Dozen 2014" for having high levels of pesticide contamination.[4] For this study, the BenchMixer shaker was used for the extraction and cleanup of existing pesticide residues from non-organically grown spinach. The results were compared to a standard QuEChERS protocol involving the manual shaking of the spinach extracts for 1 minute

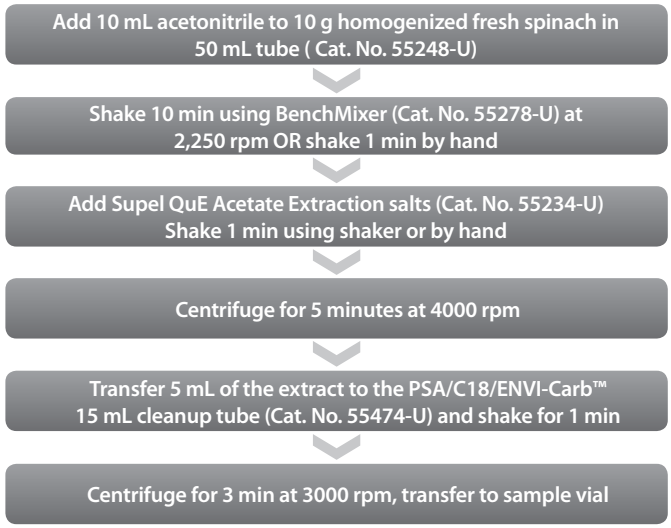
Experimental

Spinach was extracted with acetonitrile using 1 minute manual shaking (by hand) or 10 minute automated shaking using the BenchMixer vortex shaker. Previous work showed that using the BenchMixer for 1 minute and 5 minutes resulted in incomplete extractions. A minimum of 10 minutes shaking time at the 2250 rpm setting was required for full extraction of existing pesticides. The extraction was performed in Supel QuE 50 mL centrifuge tubes. For each pesticide extraction, 5 replicates were completed. The extraction procedure is presented in **Figure 1**.

The samples were then analyzed by GC/MS/SIM, using m/z 183 for quantitation, and m/z 163 as a qualifier.

Calibration was done using matrix-matched standards. Extracts of organically grown spinach were obtained and were used to construct the calibration curve. The calibration curve standards ranged from 25 ng/mL to 5,000 ng/mL.

Figure 1. QuEChERS Procedure Used for the Extraction of Pesticides from Spinach



Results and Discussion

Two permethrin isomers were resolved chromatographically (**Figure 2**) and quantitated separately. As illustrated in **Table 2**, the yield of permethrin isomer 1 was found to be 4.18 ppm using the manual method, but 4.76 ppm using the shaker method. Permethrin isomer 2 gave a level of 0.90 ppm from the hand-shaken samples, but a level of 1.03 ppm from BenchMixer shaken samples. For both isomers, the manual method extracted 14% less permethrin than the shaker method. This difference is significant. Similar results were observed in an experiment by Anastassiades et al. when 10-15 minutes of automated shaking was employed for the initial acetonitrile extraction step in the QuEChERS procedure.[3] The reproducibility of this work was similar for both shaking techniques (2-5%) when hand-shaking was performed by an experienced laboratory technician.

The use of the shaker was convenient as the QuEChERS horizontal rack can hold seven 50 mL tubes during the extraction step, and the rack for 15-mL tubes can hold up to 50 tubes for the cleanup step. Both 50 mL extraction tubes and the new Supel QuE 15 mL cleanup tubes were compatible with the shaker. In addition to producing higher yields of existing pesticides, use of the BenchMixer shaker may also decrease the physical strain experienced by laboratory personnel when performing multiple daily QuEChERS extractions.

(continued on next page)

Figure 2. GC/MS of Permethrin Pesticide Isomers Extracted from Spinach Using the BenchMixer Shaker

column: SLB-5ms, 20 m x 0.18 mm I.D., 0.18 µm (28564-U)
oven: 120 °C (1 min), 10 °C/min to 330 °C (3 min)
inj. temp.: 250 °C
carrier gas: helium, 0.7 mL/min
detector: Agilent GC 7890 with 5975 MS Detector, selected ion mode (SIM) m/z = 163 (quant), 183 (qual)
MSD interface: 325 °C, source = 250 °C, quads = 200 °C
injection: 1 µL
liner: 2 mm I.D., split/splitless type, single taper wool packed FocusLiner™ design (2879525-U)

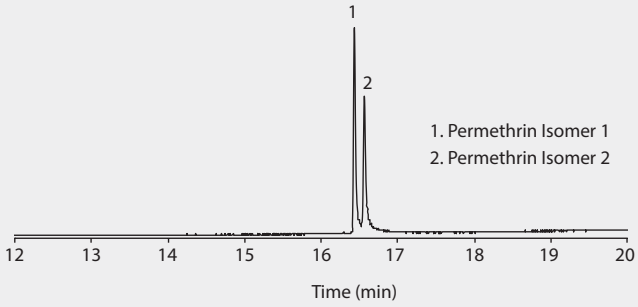


Table 2. Detected Permethrin Level and Reproducibility of Two Shaking Methods for Pesticide Extraction (n=5)

Shaking Method	Permethrin Isomer 1		Permethrin Isomer 2	
	Level Detected (ppm)	RSD	Level Detected (ppm)	RSD
Manual, 1 min	4.18	2%	0.90	4%
Shaker, 10 min	4.76	3%	1.03	5%

Conclusion

Introduction of the BenchMixer shaker into the QuEChERS protocol resulted in a 14% increase in the observed yield of permethrin residues existing in commercially bought spinach. The shaker can be successfully used for analysis of pesticide residues when an extended shaking time of 10-15 minutes is required to achieve better extraction efficiencies.

References

1. Anastassiades, M.; Lehotay, S.J.; Stajnbaher, D.; Schenck, F.J. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and dispersive solid-phase extraction for the determination of pesticide residues in produce. *J AOAC Int.* **2003**, *86*, 412-431.
2. Anastassiades, M.; Hepperle, J.; Roux, D.; Barth, A. Factors affecting the extractability of Incurred Pesticides residues, O-8. *Proceedings of North American Chemical Residue Workshop (NACRW)*, St. Petes Beach, FL, USA, July 22-24, 2014.
3. Anastassiades, M.; Hepperle, J.; Roux, D.; Sigalov, I.; Mack, D. Extractability of Incurred Residues using QuEChERS, PA-136. *Proceedings of European Pesticide Residue Workshop (EPRW)*, Strasbourg, France, June 20-24, 2010.
4. Environmental Working Group Site. **http://www.ewg.org/foodnews/summary.php** (accessed May 2014).

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Supel QuE QuEChERS Products	
Empty Centrifuge Tube, 50 mL, 50 ea	55248-U
Acetate Extraction Tube, 12 mL, 50 ea	55234-U
PSA/C18/ENVI-Carb Tube, 15 mL, 50 ea	55474-U
SLB-5ms Capillary GC Column	
20 m × 0.18 mm I.D., 0.18 µm	28564-U
Analytical Solvents and Reagents	
Permethrin (isomers), 100 mg	442748
Acetonitrile, for pesticide residue analysis	34481

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Description	Cat. No.
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2 mL QuEChERS Cleanup tube shaker rack, pack of 1	Z765554

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The BenchMixer is compatible with 50 mL extraction tubes, as well as 15 mL, 12 mL, and 2 mL cleanup tubes. It is available for both US and EU voltage requirements.