Optimize Food Analysis with Miniaturized QuEChERS and Agilent 7010 Triple Quad GC/MS

Application Note

Food Testing and Agriculture

Authors

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Abstract

There are many advantages in miniaturizing sample preparation. The use of less solvent reduces solvent cost and waste. Smaller sample sizes are easier to handle, store, and process in the lab. Reduction in sample amount provides cost savings on sample preparation sorbent and substantial decreases in cost associated with the use of labeled compounds as internal standards. With a smaller sample size, it may be possible to use additional labeled compounds for troublesome analytes that are cost prohibitive with larger sample sizes.

We analyzed pesticides in food using miniaturized QuEChERS extraction and the ultra-efficient ionization source of the Agilent 7010 Triple Quadrupole GC/MS, which reduces injected sample volume by 75%. On average, limits of quantitation (LOQ) ≤ 10 ng/g were reached for 95% of the 126 pesticides studied in apple, carrot, and broccoli. Less matrix injected delivers prolonged uptime and sustained performance and, therefore, lower maintenance costs. By injecting only 25% of the standard 2 µL injection volume and implementing our recommended pesticide analysis method, we analyzed the pesticides at or below threshold MRLs of the EPA, EU, and Japan, 0.01 mg/kg (10 ng/g), which was adequate to monitor exposure.

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Introduction

The Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method for pesticide analysis was first introduced by the USDA in 2003 [1]. It was later modified to address problematic pesticides by including buffered extraction systems [2]. The two improved methods were formalized and adopted in AOAC 2007.01 [3] and EN 15662 [4]. In summary, the methods use a single-step buffered-acetonitrile extraction while simultaneously salting out water from the sample with magnesium sulfate ($MgSO_4$) to induce liquid-liquid partitioning. For cleanup, a dispersive solid phase extraction (dispersive SPE) is employed using a combination of sorbents and $MgSO_4$.

We analyzed apple, carrot, and broccoli, as they are specified in the harvest testing period by the Agriculture Marketing Service of the United States Department of Agriculture (USDA), under the Pesticide Data Program (PDP) [5]. The EPA, EU, and Japan have established maximum residue limits (MRLs), which are described as safe limits that define the maximum expected levels of pesticide for a food commodity. These MRLs prevent illegal or excessive use of pesticides, thus protecting the health of consumers and the environment. Therefore, pesticides that can effect consumer safety and the environment are continually monitored to ensure product safety and legislation compliance [6].

Many foodstuffs are very complex due to their composition or processing. This can cause a large number of interfering compounds that lead to elevated background interferences. GC/MS/MS is often employed for screening, confirming, and quantitating trace-level target compounds in these complex matrices. Tandem MS allows for selective transition monitoring and, therefore, eliminates or minimizes the presence of background interferences. The QuEChERS sample preparation method extracts compounds from these complex matrices, but does not exhaustively remove all interfering matrix. Therefore, additional techniques are required to remove contaminants from the analytical system. For instance, backflushing the GC column ensures that high-boiling compounds in the matrix are not passed through the column and reduces column bleed. Backflushing also eliminates ghost peaks and minimizes contamination of the mass spectrometer [7,8]. Additional techniques such as improving ionization within the source (ultra-efficient ionization) allow for smaller injection volumes while still achieving low sensitivity of target analytes.

The QuEChERS method is based on a 10 or 15 g homogenized, representative food sample. It is, therefore, advantageous to move to smaller sample sizes since they are easier to handle, use less solvent and labeled standards, and require less storage space. In this application note, we investigated scaling down the QuEChERS extraction method. Sample, solvent, and salts could be reduced proportionally while maintaining the same sample/solvent/salt ratio as defined in the validated QuEChERS methods [3,4]. Therefore, we did not anticipate negative effects on recovery or analytical results. The miniaturized QuEChERS approach, when combined with the ultra-efficient ionization source of the Agilent 7010 Triple Quadrupole GC/MS, delivers substantial cost savings associated with sample preparation and substantially smaller injection volume to achieve the recommended limit of detection (LOD) and reduction in matrix effects.

Experimental

All reagents and solvents were analytical grade or above. Acetonitrile (ACN) was from Honeywell International, Inc. (Muskegon, MI, USA) and acetic acid from Sigma-Aldrich, Corp. (St. Louis, MO, USA). L-gulonic acid, γ-lactone, L-gulonolactone, and D-sorbitol at > 95% purity were also purchased from Sigma-Aldrich, Corp. Custom pesticide sets (15 unique mixes) at 100 µg/mL in acetone were purchased from AccuStandard, Inc. (New Haven, CT, USA). Triphenyl phosphate (TPP), parathion-d_{10}, and DDT, *p,p*-$^{13}C_{12}$ were purchased from Sigma-Aldrich, Corp. and Cerilliant Corp. (Round Rock, TX).

A 1% acetic acid (HAc) in ACN solution was prepared by adding 5 mL acetic acid to 500 mL ACN. Preparation of L-gulonolactone, D-sorbitol stock, and analyte protectant (AP) is shown on page 87 of the Agilent GC/MS/MS Pesticide Analysis Reference Guide. Contact an Agilent sales or support representative to request a copy of the guide [9].

Instrumentation

This study was performed using an Agilent 7890 GC coupled to an Agilent 7010 Triple Quadrupole GCMS with an ultra-efficient ionization source. The GC system was equipped with electronic pneumatic control (EPC), a Multi-Mode Inlet (MMI) with air cooling, an Agilent 7693A Automatic Liquid Sampler, and a backflushing system based on a Purged Ultimate Union controlled by an AUX EPC module [7,8]. Agilent MassHunter Software was used for instrument control, and for qualitative and quantitative data analysis.

Agilent inert flow path components

Columns:	Agilent J&W HP-5ms Ultra Inert, 5 m × 0.25 mm, 0.25 µm (p/n G3903-61005) and 15 m × 0.25 mm, 0.25 µm (p/n 19091S-431UI)
Liners:	Ultra Inert 2-mm dimpled liner (p/n 5190-2297)
Ferrules:	UltiMetal Plus Flexible Metal ferrules at the purged Ultimate Union used for column backflushing (p/n G3188-27501)

Other Agilent supplies

Sample extraction:	Agilent Bond Elut QuEChERS AOAC Extraction packets (p/n 5982-6755); Bond Elut QuEChERS AOAC dispersive SPE kit for General Fruits and Vegetables (p/n 5982-5022) and EN dispersive SPE kit for Pigmented Fruits and Vegetables (p/n 5982-5221)
Homogenizers:	Bond Elut QuEChERS Ceramic Homogenizers for 15 mL tubes (p/n 5982-9312)
Syringes:	Manual syringes, 10 µL (p/n 5190-1491), 25 µL (p/n 5190-1504), 100 µL (p/n 5190-1518), 250 µL (p/n 5190-1525)
Vials:	Autosampler vials (p/n 5182-0733)
Vial insets:	Autosampler vial inserts, deactivated glass, flat bottom (p/n 5183-2086)

Other equipment

- Robot Coupe blender
- VWR vortexer
- Heraeus Labofuge 400 Centrifuge
- Eppendorf microcentrifuge

Sample preparation

Preparation of the fruit and vegetable extracts was based on the AOAC version of the QuEChERS method [3] using Agilent extraction salts and dispersive kits. Organically grown produce was finely chopped, frozen, and then homogenized with dry ice in a Robot Coupe blender. The homogenized sample was stored at -20 °C until extraction.

Extraction/partitioning

Two grams of homogenized sample were weighed into a 15 mL centrifuge tube and two ceramic homogenizers were added. QC samples were fortified with a 1 µg/mL pesticide stock solution (126 pesticides) yielding QC samples with concentrations of 5, 10, and 50 ng/g. A 10 µL volume of internal standard spiking solution (10 µg/mL of parathion-d_{10}, DDT, *p,p*-$^{13}C_{12}$, and TPP) was added to all samples except the control blank to yield a 50 ng/g concentration in each sample. Tubes were capped and vortexed for 1 minute. A 2 mL volume of 1% HAc in ACN was added to each tube. Tubes were capped and vortexed for 1 minute, then 1 g Bond Elut AOAC QuEChERS salts from p/n 5982-6755 was added directly to the tubes. Sample tubes were sealed tightly and vigorously shaken by hand for 1 minute. Finally, sample tubes were centrifuged at 4,000 rpm for 5 minutes.

Dispersive SPE cleanup

A 1 mL aliquot of the upper ACN layer from the extracts was transferred to a Bond Elut QuEChERS dispersive SPE 2 mL tube. For apple extracts, a Bond Elut QuEChERS AOAC dispersive SPE containing 50 mg PSA and 150 mg $MgSO_4$ was used. For broccoli and carrot, a Bond Elut QuEChERS EN dispersive SPE containing 25 mg PSA, 2.5 mg GCB, and 150 mg $MgSO_4$ was used. The tubes were tightly capped, vortexed for 1 minute, and then centrifuged in a microcentrifuge at 13,000 rpm for 3 minutes. A 250 µL aliquot from the extract was transferred into a 400 µL deactivated glass flat bottom insert within a 2 mL autosampler vial. Fifty microliters of 1% HAc in ACN (or this combined volume of solutions in the case of post-extraction spiking) and 10 µL of AP (analyte protectant) was also added to the insert [9]. Figure 1 shows the work flow for the miniature QuEChERS sample extraction procedure.

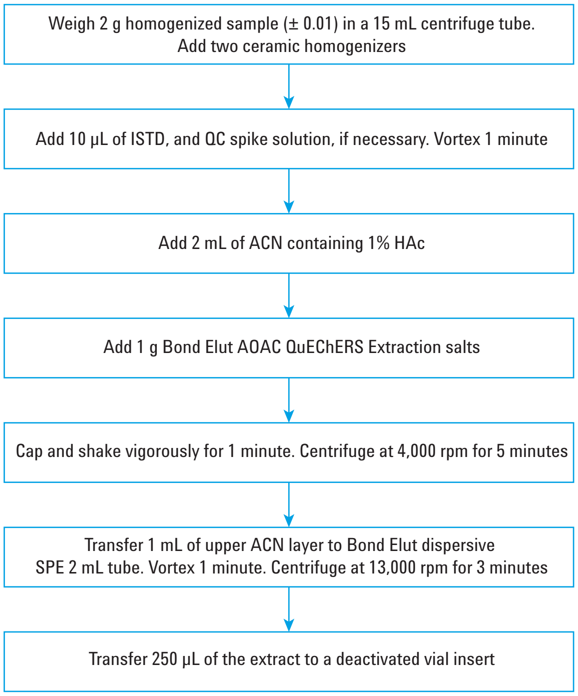

Figure 1. Work flow for the miniature Agilent Bond Elut QuEChERS sample extraction procedure.

Instrumental conditions

GC conditions

Column 1:	Agilent J&W HP-5ms UI, 5 m × 250 µm, 0.25 µm, configured from MMI to AUX EPC
Column 2:	Agilent J&W HP-5ms UI; 15 m × 250 µm, 0.25 µm, configured from AUX EPC to vacuum
Carrier:	Helium
Injection mode:	Solvent vent
Injection volume:	0.5 µL (syringe size 5 µL)
Solvent washes:	Pre-injection, 1x solvent A, methanol:water (4 µL) and 1x solvent B, acetonitrile (4 µL) Post-injection, 7x solvent A; methanol:water, and 7x solvent B, acetonitrile (4 µL each)
Sample pumps:	5
Injection speed:	Fast
MMI temp program:	60 °C for 0.35 min, 900 °C/min to 280 °C (18 min hold), 900 °C/min to 300 °C until end of the analysis
Purge flow to split vent:	50 mL/min at 1.5 min
Vent flow:	25 mL/min
Vent pressure:	5 psi until 0.3 min
Gas saver:	Off
Septum purge flow:	3 mL/min
Air cooling (cryo):	On at 125 °C (MMI liquid N_2 option selected on GC for air cooling)
Oven temp program:	60 °C for 1.5 min, 50 °C/min to 160 °C, 8 °C/min to 240 °C, 50 °C/min to 280 °C (2.5 min hold), 100 °C/min to 290 °C (1.6 min hold)
Column 1 flow program:	0.897 mL/min for 15.2 min, 100 mL/min to -1.706 mL/min (flow balanced with the Column 2 flow to achieve 2 psi inlet pressure) until end of the analysis for concurrent column backflush, post run -10.683 mL/min
Column 2 flow program:	0.997 mL/min until end of the analysis, post run 4 mL/min
Retention time locking:	Chlorpyrifos-methyl locked at 8.524 min
Total run time:	18.5 min
Post-run:	0.5 min at 290 °C

MS conditions

MS source:	-70 eV
Source temperature:	280 °C
Quadrupole temperature:	150 °C
Transfer line temperature:	280 °C
Solvent delay:	4.0 min
Helium quench gas:	2.25 mL/min
Nitrogen collision gas:	1.5 mL/min
Acquisition mode:	Multiple reaction monitoring (MRM)
MS1/MS2 resolution:	Wide
Time segments:	Page 94 of reference [9]
Acquisition parameters:	Pages 95 to 105 of reference [9]

Results and Discussion

Accurate calibration

This study used apple, carrot, and broccoli to assess the method for routine analysis. Specific dispersive SPE kits were employed depending on the matrix; PSA for apple, and PSA and 2.5 mg GCB for carrot and broccoli. Figure 2 shows a representative overlay of chromatograms for a fortified broccoli matrix extract with 126 pesticides at 10 ng/g. Calibration standards for a mixture of 126 pesticides and groups of pesticide isomers were prepared by spiking the extracted blank matrix (apple, carrot, and broccoli) at 1, 5, 10, 20, 50, and 100 ng/g. Sets of these six standards were injected consecutively six times, with calibration on a middle set, using a linear curve fit with 1/x weighting. The other five sets of standards were designated as QC and appear as blue triangles in Figure 3 as an indication of the precision of the method. Percent RSD (n = 6) was determined based on the calculated amount for each level from the calibration curve. Calibration sets yielded correlation coefficient values (R^2) that were > 0.99 for 95% of the 126 pesticides spiked in all matrixes. One solvent blank was injected between each set of six calibration standards. Challenging compounds monitored in these commodities are shown in Figure 3.

The amount of analyte in a 5 ng/g standard injected in a 0.5 µL injection volume is 2.5 pg, as opposed to 10 pg when using a 2 µL injection volume. This is a 75% reduction in sample amount. Even with this reduction in injected sample, optimal chromatography for some of the most challenging pesticides was maintained at half the default MRL, or 5 ng/g.

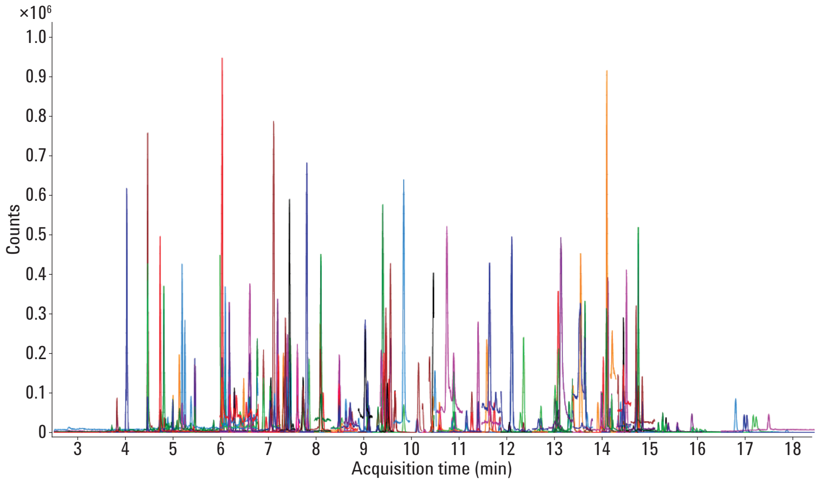

Figure 2. GC/MS/MS overlay chromatogram for 126 pesticides fortified in broccoli matrix at 10 ng/g, 0.5 µL injection.

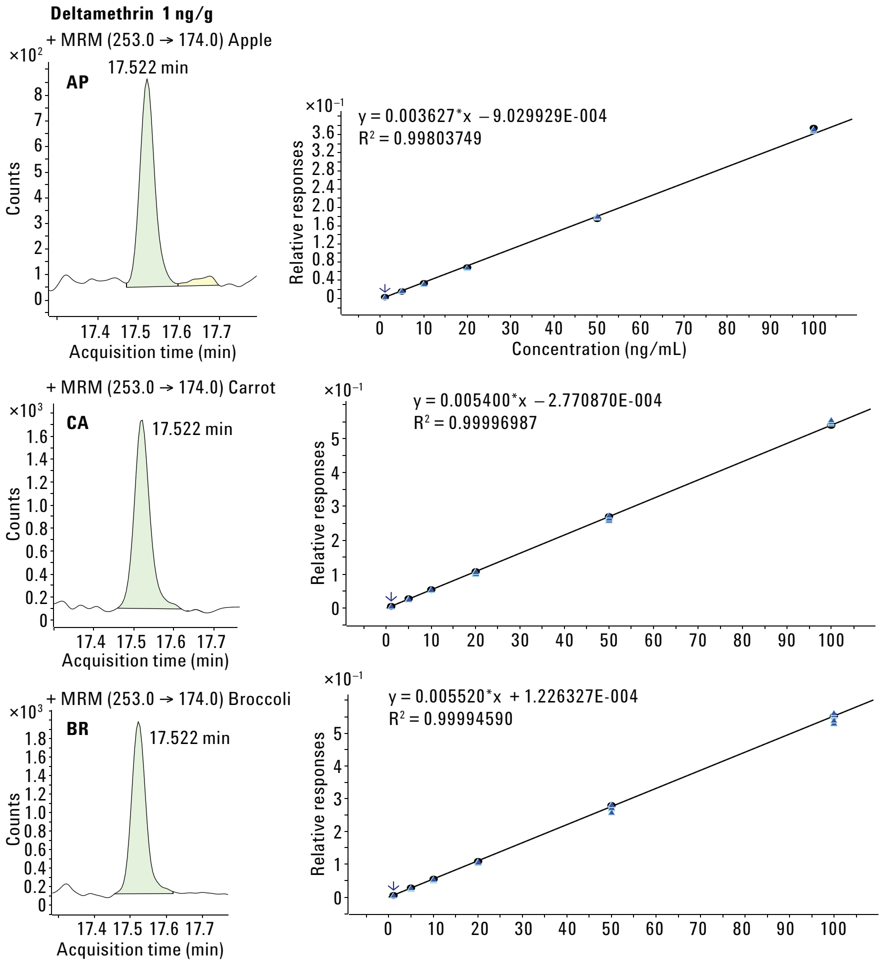

Figure 3. Quantitative transition (MRM) and calibration curves (n = 6) for deltamethrin at 1 ng/g (0.5 pg injected) and resmethrin I and II at 5 ng/g each (2.5 pg each isomer injected) for apple (AP), carrot (CA), and broccoli (BR).

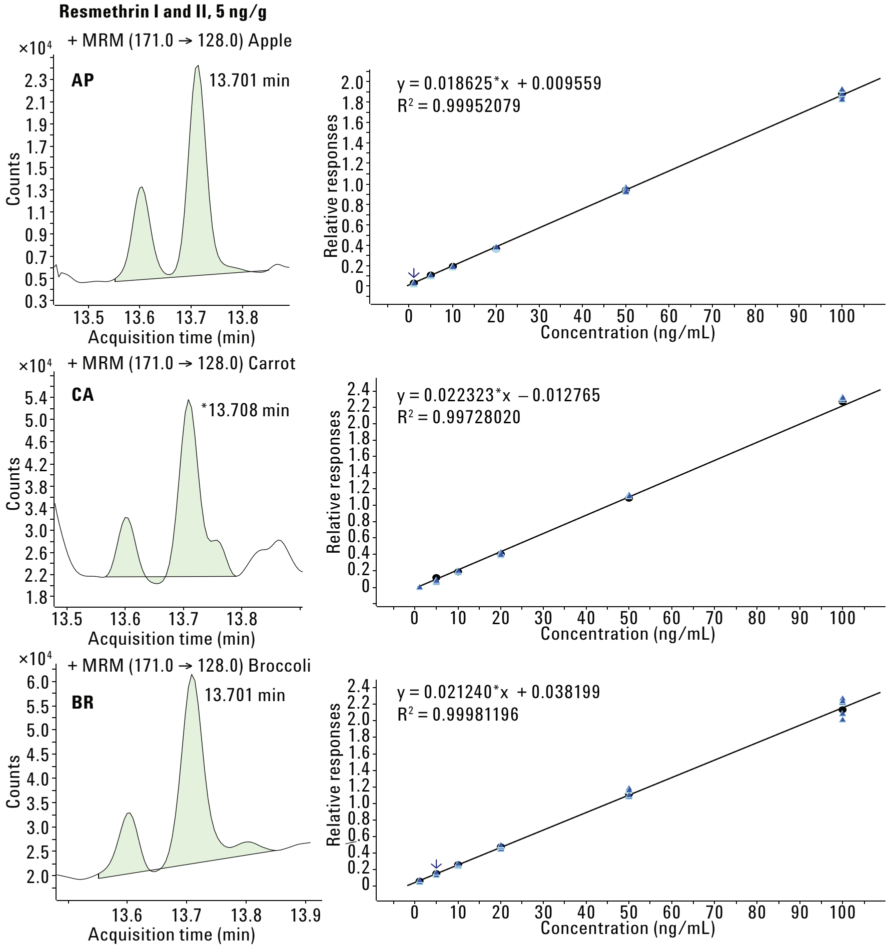

Figure 3 (cont). Quantitative transition (MRM) and calibration curves (n = 6) for deltamethrin at 1 ng/g (0.5 pg injected) and resmethrin I and II at 5 ng/g each (2.5 pg each isomer injected) for apple (AP), carrot (CA), and broccoli (BR).

Recovery

The recovery was evaluated by spiking the pesticide standards in comminuted apple, carrot, and broccoli at levels of 5, 10, and 50 ng/g. Specific dispersive SPE kits were employed depending on the matrix; PSA for apple, and PSA and 2.5 mg GCB for carrot and broccoli. These QC samples were quantitated against the matrix-spiked calibration curve. The analysis was performed in replicates of three (n = 3) at each level. The distribution of recovery for these prespiked matrices is shown in Figure 4. The percentages of pesticides that fell within the acceptable mean recovery of 70 to 120% in apple were 95% at 5 ng/g and 96% at both 10 and 50 ng/g. Acceptable recoveries in carrot were 96% at 5 ng/g, 95% at 10 ng/g, and 94% at 50 ng/g. For broccoli, mean recoveries were 97% at 5 ng/g and 96% at both 10 and 50 ng/g. In the case of carrot, 11 pesticides were outside the 70 to 120% range. However, nine of these pesticides had %RSDs ≤ 20, and so these mean recovery values were considered acceptable. For broccoli, 11 pesticides also fell outside the 70 to 120% range but seven of these were accepted based on %RSD ≤ 20. As stated within SANCO/12571/2013 guidelines, "in certain cases and typically with multiresidue methods, recoveries outside the range 70-120% may be accepted. Exceptionally, where recovery is low but consistent (that is, demonstrating good precision) and the basis for this is well established (for example, due to analyte distribution in a partitioning step), a mean recovery below 70% may be acceptable" [10].

Limits of quantitation (LOQ) reaching 1 ng/g

Calibration standards for the mixture of 126 individual pesticides and groups of pesticide isomers were prepared by spiking the extraction blank matrix at 1.0 to 100 ng/g. Figure 5 shows the distribution of %RSDs for 126 pesticides in apples, carrot, and broccoli at 1 and 5 ng/g using a 0.5 µL injection volume. The LOQs were estimated using %RSD ≤ 20 and S/N > 10 (n = 6) for calculated amounts. Thirty-five pesticides that are commonly monitored in the three matrices are listed in Table 1. Most LOQs across the matrices are 1 ng/g with a 0.5 µL injection; ongoing recovery testing at or below 1 ng/g to establish recovery-based LOQs is expected to bear out these estimates.

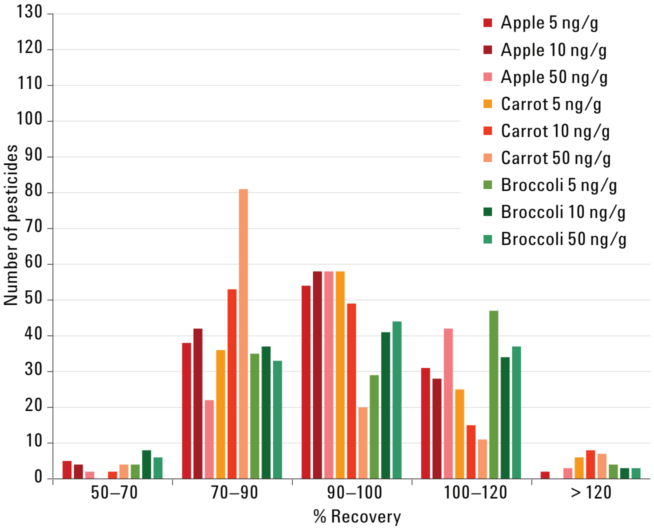

Figure 4. Distribution of recovery for 126 pesticides in prespiked apple, carrot, and broccoli, using a 0.5 µL injection of 5, 10, and 50 ppb extracts.

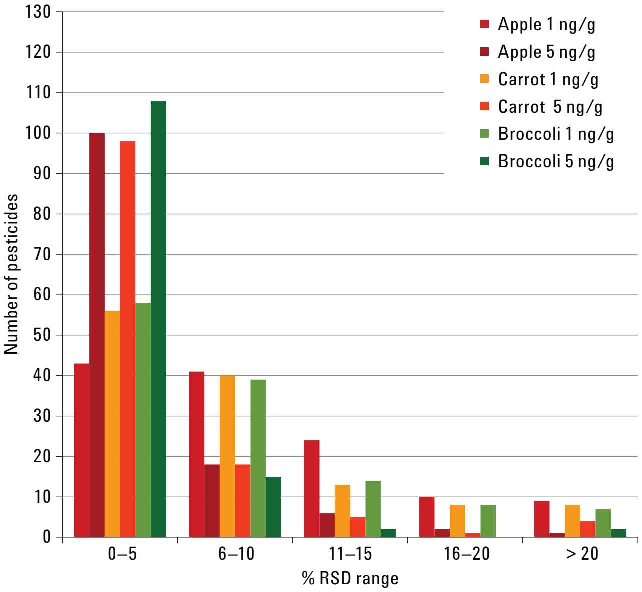

Figure 5. Distribution of %RSD for 126 pesticides in apple, carrot, and broccoli extract at 1 and 5 ng/g using 0.5 µL injection (n = 6).

Table 1. Published MRLs and LOQs for 35 commonly monitored pesticides in apple, carrot, and broccoli.

Pesticide	**EPA/EU MRL* in apples (ng/g)**	**Estimated LOQ(s) (ng/g)**	**EPA/EU MRL* in carrots (ng/g)**	**Estimated LOQ(s) (ng/g)**	**EPA/EU MRL* in broccoli (ng/g)**	**Estimated LOQ(s) (ng/g)**
Bifenthrin	500/300	1	100/50	1	600/200	1
Buprofezin (Z-isomer)	10[a]	1	10[a]	1	12,000/50	1
Captan	25,000/3,000	10	50/100	> 50	50/20	> 50
Chlorothalonil	10[a]	1	1,000/1,000	1	5,000/5,000	1
Chlorpyrifos	10/500	1	100/100	1	1,000/50	1
Clomazone	10[a]	1	10[a]	1	100/10	1
Cyhalothrin, *lambda*-I	100/300	10	10/20	10	400/100	10
Cypermethrin	10[a]	5	10[a]	1	2,000/1,000	1
Cyprodinil	1,700/1,000	1	750/2,000	1	1,000/50	1
DCPA (dacthal, chlorthal-dimethyl)	10[a]	1	10[a]	1	5,000/10	1
Deltamethrin	10[a]	1	200/50	1	50/100	1
Diazinon	500/10	1	750/10	1	700/10	1
Difenoconazole I	1,000/500	5	500/400	5	1,900/1,000	1
Diphenylamine	10,000/100	1	10[a]	1	10[a]	1
Endosulfan I & II	1,000/500	1,1	10[a]	1,1	10[a]	1,1
Fenarimol	300/100	1	10[a]	1	10[a]	1
Fenpropathrin	5,000/100	1	10[a]	1	10[a]	1
Fludioxonil	5,000/5,000	1	750/1,000	1	2,000/700	1
Folpet	10[a]	> 50	10[a]	> 50	10[a]	> 50
Iprodione	10[a]	1	5,000/500	1	25,000/100	1
Linuron	10[a]	1[b]	1,000/200	5[b]	10[a]	5[b]
Metalaxyl	200/1,000	1	500/100	1	2,000/200	1
Metolachlor	10[a]	1	400/50	1	600/50	1
Pendimethalin	10[a]	1	500/200	1	100/50	1
Permethrin I & II	50/50	1,1	10[a]	5,5	2,000/50	5,5
Phosmet	10,000/500	1	10[a]	1	10[a]	5
Piperonyl butoxide	8,000/10[a]	1	10,000/ 10[a]	1	10,000/10[a]	1
Pyridaben	500/500	5	10[a]	1	10[a]	5
Pyriproxyfen	10[a]	1	10[a]	1	700/50	1
Resmethrin I & II (summed)	3,000/100	5	3,000/100	5	3,000/100	5
Simazine	200/10	1	10[a]	1	10[a]	1
Triflumizole	500/500	1	10[a]	1	8,000/100	1
Trifluralin	10[a]	1	1,000/10	5	50/10	1

* MRLs from http://mrldatabase.com

[a] Not listed; default MRL applies (MRL = 0.01 mg/kg)

[b] Preferred analysis method for linuron is LC/MS/MS

Captan and folpet are base-sensitive, and often present issues in terms of recovery from matrix and precision during analysis. Although not used in this study, the evaluation of captan-d_6 and folpet-d_4 ISTDs is recommended to control recovery and assure reliable results, especially for longer batches in which the number of injections exceeds 40 [11]. Here, without the use of specific labeled ISTD, and well over 40 injections, captan yielded estimated LOQs of 10 ng/g for apple (below MRL), and > 50 ng/g (above MRL) for carrot and broccoli. Conversely, folpet yielded estimated LOQs > 50 ng/g for all three matrices, which is above the established MRLs. The ability to use a smaller sample size (2 g) in the QuEChERS extraction equates to less volume of labeled ISTDs spiked into the samples and thus substantial reductions in cost per sample. Therefore, additional labeled compounds such as captan-d_6 and folpet-d_4 could be included without significant cost increase per sample, improving recovery and reliability.

Figure 6 shows GC inlet liners that had different sample volumes injected over the course of a sequence. In (A), the upper liner had over 65 injections of 0.5 µL sample. The lower liner had a greater amount of the same sample injected, 2.0 µL, over 65 times and clearly contains increased deposits that reduce usable liner lifetime and added additional maintenance costs. The liner (B) had reduced sample volume injected but over 200 injections (0.5 µL). It is almost free of visible deposits, shown clearly in the magnified view, which prolongs performance and decreases maintenance cost.

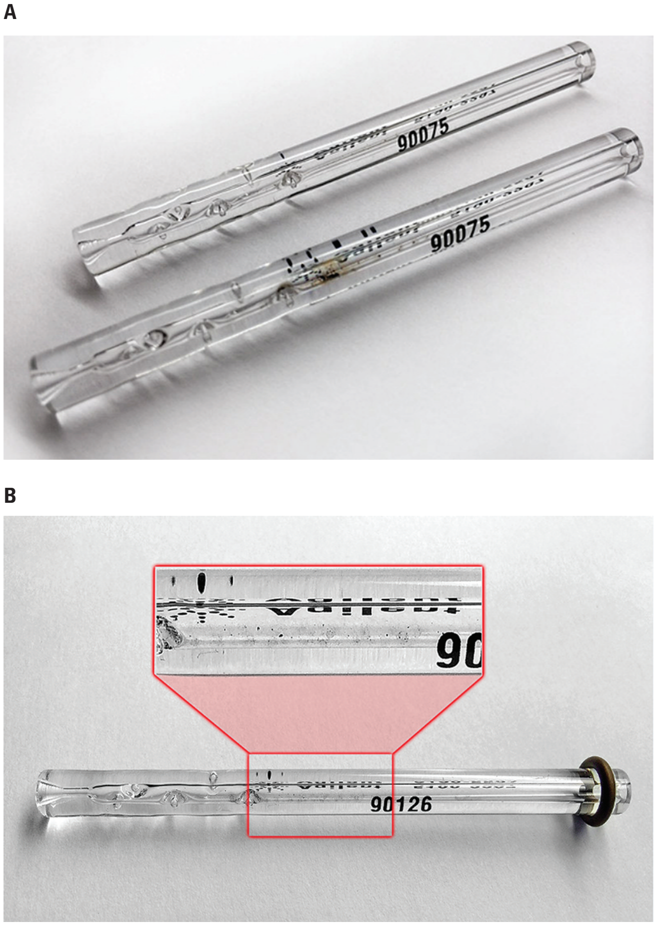

Figure 6. GC liners (A) residue deposit after 65 injections of 0.5 µL (upper) and 2 µL matrix extract (lower); (B) almost free of visible deposits after 200+ injections of matrix extract of 0.5 µL.

Conclusions

Miniaturization of the QuEChERS extraction method can substantially decrease sample preparation cost by 80%, with less solvent and solvent waste, and reduced amount of ISTD. Maintaining the same ratio of sample to solvent and salts, and, therefore, a 1 g/mL sample extract, delivered excellent results, as expected. The ultra-efficient ionization source reduces sample cost by allowing for an increased number of samples to be injected before GC liner replacement is required. By combining an existing analytical method, the ultra-efficient ionization source, which uses only 25% of the previously required injected sample volume, and QuEChERS miniaturization, 95% of pesticide residues in apples, carrots, and broccoli were quantitated at or below the default threshold of 10 ng/g.

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