Reduce Cost of Pesticide Residue Analysis

An Application for Mini-QuEChERS with GC/MS/MS and a High-Efficiency Source

Application Note

Food Testing and Agriculture

Authors

Melissa Churley and Joan Stevens
Agilent Technologies, Inc.

Abstract

Laboratories responsible for ensuring food safety seek to reduce the cost per analysis without compromising the accuracy and reliability of their results. A promising approach involves miniaturization of the QuEChERS extraction method and the use of smaller sample injection volumes. We found cost savings of >40% due to reductions in solvent, sorbent, and labeled ISTD. Use of a high-efficiency source and only 0.5 µL injection further reduces sample cost through decreased maintenance, while still enabling lower limits of quantitation (LOQs). In carrot, tomato, and celery, recovery-based LOQs for 86 to 90% of the 126 pesticides analyzed were 1 ng/g, and 95 to 98% of the pesticides had LOQs ≤5 ng/g. This is half the accepted default maximum residue limit (MRL), of 10 ng/g. Challenging captan and folpet residues were quantified at ≤5 and 1 ng/g, respectively, by using commercially available ISTDs captan-d_6 and folpet-d_4 for only $0.04 per sample.

Introduction

The QuEChERS extraction method for pesticide residues analysis in foods was first introduced by the USDA in 2003 [1]. The method was modified to address problematic pesticides by including buffered extraction systems [2]. The two resulting improved methods were formalized and adopted as AOAC 2007.01 [3] and EN 15662 [4]. In summary, this widely used method involves 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) uses a combination of sorbents and $MgSO_4$.

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Many foodstuffs are very complex due to their composition or processing, and so several interfering compounds can be observed during analysis. GC/MS/MS is often used for screening, confirming, and quantitating trace-level target compounds in complex food matrices because tandem MS allows for selective transition monitoring. This approach excludes or minimizes the presence of background interferences. Since QuEChERS does not exhaustively remove all interfering matrix, extra techniques are implemented to remove contaminants from the analytical system and improve robustness. For instance, backflushing the GC column ensures that the high-boiling compounds in the matrix are not passed through the column, reduces column bleed, eliminates ghost peaks, and minimizes contamination of the mass spectrometer [5,6].

Since QuEChERS is based on either a 10 g (EN method) or 15 g (AOAC method) homogenized representative food sample, it is advantageous to move to smaller sample sizes. Smaller samples are easier to handle, use less solvent and labeled standards, produce less solvent waste, and require a smaller storage area. In this application note, we proportionately reduced the amount of sample, solvent, and salts to maintain the ratio of sample to solvent to salt documented in the QuEChERS method. To demonstrate this approach using a real-world scenario, three commodities for the autumn testing period in the northern hemisphere specified by the United States Department of Agriculture (USDA), Agriculture Marketing Service under the Pesticide Data Program (PDP) [7] were used. Limits of quantitation (LOQ) for 126 pesticide residues were established based on recovery [8] from fortified carrot, tomato, and celery matrices, all of which were purchased as organic. 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. The default value of 0.01 mg/kg, or 10 ng/g, applies when the MRL for a given residue:commodity combination does not appear in the database. MRLs serve to prevent illegal or excessive use of pesticide, thus protecting the health of consumers and the environment. Therefore, the use of pesticides that can affect consumer safety and the environment are continually monitored to ensure product safety and legislation compliance [9].

A recent development in GC/MS is the Agilent high-efficiency source (HES), which maximizes the number of ions that are created and transferred out of the source body and into the quadrupole analyzer. Since sensitivity depends on the number of ions measured, this leads to better precision at low concentration levels and, thus, lower detection limits. In the case of pesticide residue analysis in foods, a practical benefit is that reduced injection volumes, such as 0.5 µL instead of 2 µL, may be used while still achieving the required detection levels for target analytes [10].

The scaling down of the QuEChERS extraction method, when combined with analysis using the HES of the Agilent 7010 Triple Quadrupole GC/MS, saves substantial costs associated with sample preparation, improves robustness of the analytical method, and reduces costs due to instrument maintenance. More internal standards such as deuterated captan and folpet could be included without significant cost increase per sample, thus improving routine analysis for these challenging, base-sensitive compounds [11]. Savings resulting from less frequent maintenance are the result of smaller required injection volumes while enabling lower LOQs.

Experimental

Reagents and chemicals

All reagents and solvents were analytical grade or above. Acetonitrile (ACN) was from Honeywell (Muskegon, MI, USA). Acetic acid, L-gulonic acid γ-lactone (L-gulonolactone), and D-sorbitol at > 95% purity were from Sigma-Aldrich, Corp. (St. Louis, MO, USA). 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 (Round Rock, TX). Captan-d_6 and folpet-d_4 were the products of Toronto Research Chemicals (Toronto, Ontario, Canada).

Solutions and standards

A 1% acetic acid (HAc) in ACN solution was prepared by adding 5 mL of acetic acid to 500 mL of ACN. The L-gulonolactone and D-sorbitol stocks and analyte protectant (AP) solution preparation instructions are provided in the Agilent GC/MS/MS Pesticide Analysis Reference Guide. To request a copy [12], contact an Agilent sales or support representative.

Equipment, instrument, and material

Agilent supplies

- Agilent 7890 GC
- Agilent 7010 Triple Quadrupole GC/MS
- Agilent 7693A Automatic Liquid Sampler (ALS)
- Agilent Bond Elut QuEChERS AOAC Extraction Packets (p/n 5982-6755)
- Agilent Bond Elut QuEChERS AOAC Dispersive SPE kit for general fruits and vegetables (p/n 5982-5022)
- Agilent Bond Elut QuEChERS AOAC Dispersive SPE kit for all food types (p/n 5982-0028)
- Agilent Bond Elut QuEChERS Ceramic Homogenizers for 15 mL tubes (p/n 5982-9312)
- Manual syringes 10 µL (p/n 5190-1491), 25 µL (p/n 5190-1504), 100 µL (p/n 5190-1518), and 250 µL (p/n 5190-1525)
- Autosampler vials (p/n 5182-0733)
- Autosampler vial inserts, deactivated glass, flat bottom (p/n 5183-2086)

For maximum GC/MS sample path inertness, we used Agilent components.

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

Other supplies

- Robot Coupe blender
- 2010 Geno/Grinder
- VWR VX-2500 multitube vortexer
- Heraeus Labofuge 400 centrifuge
- Eppendorf microcentrifuge

The GC system was equipped with electronic pneumatic control (EPC), a multimode inlet (MMI) with air cooling, and a backflushing system based on a Purged Ultimate Union controlled by an AUX EPC module [13]. Agilent MassHunter software was used for instrument control, and for qualitative and quantitative data analysis.

Sample preparation

Preparation of the fruit and vegetable extracts was based on the AOAC version of the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method [3] using Agilent extraction salts and dispersive kits. Organically grown produce were finely chopped, frozen, and then homogenized with dry ice in a Robot Coupe blender. The homogenized sample was then stored in a –20 °C freezer until extraction.

Extraction/partitioning

Two grams of homogenized sample were weighed into a 15 mL centrifuge tube, and two ceramic homogenizers were added to the sample. QC samples were fortified with a 1 µg/mL pesticide stock solution (126 pesticides) yielding QC samples with concentrations of 1, 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′*-$^{13}C_{12}$, TPP, captan-d_6, and folpet-d_4) was added to all samples, except the control blank, to yield a 50 ng/g concentration in each sample. Sets of 16 tubes were capped and multivortexed as a batch for one minute. A 2 mL volume of 1% HAc in ACN was added to each tube. Tubes were capped and vortexed for one minute, then 1 g of Bond Elut AOAC QuEChERS salts from p/n 5982-6755 was added directly to the tubes. Sample tubes were sealed tightly and shaken using the Geno/Grinder for one minute. Sample tubes were centrifuged at 4,000 rpm for five minutes.

Dispersive SPE cleanup

A 1 mL aliquot of the upper ACN layer from the extracts was transferred to an Agilent Bond Elut QuEChERS Dispersive SPE 2 mL tube. For tomato and celery extracts, an Agilent Bond Elut QuEChERS AOAC dispersive SPE kit for general fruits and vegetables was used, containing 50 mg PSA and 150 mg $MgSO_4$. In the case of carrot, a Bond Elut QuEChERS AOAC Dispersive SPE kit for all food types, containing 25 mg PSA, C18, 2.5 mg GCB, and 150 mg $MgSO_4$, was used. The tubes were tightly capped and multivortexed as a batch for one minute. The tubes were centrifuged in a microcentrifuge at 13,000 rpm for three minutes. A 250 µL aliquot from the extract containing ISTD (or matrix blank) was transferred to a 400 µL deactivated glass flat bottom insert in a 2 mL autosampler vial. To the insert, 50 µL of 1% HAc in ACN was added except in the case of matrix blank samples, which were spiked using 50 µL of combined volume to prepare calibration standards (see Batch Analysis section). To all samples, 10 µL of AP (analyte protectants) was also added [12].

Figure 1 shows the work flow for the miniature QuEChERS sample extraction procedure.

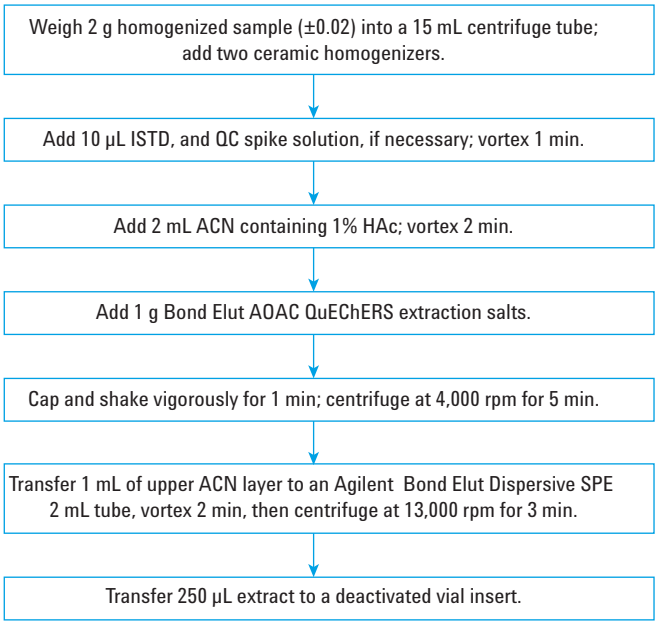

Figure 1. Work flow for the miniature Agilent QuEChERS sample extraction procedure.

Batch analysis

Calibration standards for a mixture of 126 pesticides and groups of pesticide isomers were prepared by spiking extracted blank matrix at levels of 0.5, 1, 5, 10, and 50 ng/g, and adding ISTD. A linear curve fit was used with 1/x weighting. The sample batch to be analyzed was set up such that a solvent blank was injected between sets of recovery samples at each concentration level (1, 5, 10, and 50 ng/g), and also before the set of calibration standards.

GC conditions

Column 1:	Agilent J&W HP-5ms UI, 5 m × 0.25 mm, 0.25 µm, configured from the MMI to AUX EPC
Column 2:	Agilent J&W HP-5ms UI, 15 m x 0.25 mm, 0.25 µm, configured from the AUX EPC to vacuum
Carrier gas:	Helium
Inj mode:	Solvent vent
Inj vol:	0.5 µL (syringe size 5 µL)
Solvent washes:	Pre-injection: 1x solvent A, 1:1 methanol:water (4 µL) and 1x solvent B, acetonitrile (4 µL) Post-injection: 7x solvent A, 1:1 methanol:water (4 µL) and 7x solvent B, acetonitrile (4 µL)
Sample pumps:	5
Inj speed:	Fast
MMI temp program:	60 °C for 0.35 min, 900 °C/min to 280 °C, 18 min hold, then 900 °C/min to 300 °C to end of analysis
Purge flow to split vent:	50 mL/min at 1.5 min
Vent flow:	25 mL/min
Vent pressure:	5 psi to 0.3 min
Gas saver:	Off
Septum purge flow:	3 mL/min
Air cooling (cryo):	On at 125 °C (MMI liquid N2 option selected on GC for air cooling)
Oven temp program:	60 °C for 1.5 min, then 50 °C/min to 160 °C, 8 °C/min to 240 °C, 50 °C/min to 280 °C, 2.5 min hold), then 100 °C/min to 290 °C, 1.6 min hold
Column 1 flow program:	1.01 mL/min for 15.2 min, then 100 mL/min to 1.703 mL/min (flow balanced with column 2 flow to achieve 2 psi inlet pressure) to end of analysis for concurrent column backflush
Post run:	10.683 mL/min
Column 2 flow program:	1.11 mL/min to end of 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 temp:	280 °C
Quadrupole temp:	150 °C
Transfer line temp:	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

For time segments, refer to p. 94 of the GC/MS Pesticide Analysis Reference Guide, available on request from Agilent [12]. A full list of the MRM transitions is on pp. 95 to 105 of the same publication.

Results and Discussion

Calibration standards prepared at concentration levels of 0.5, 1, 5, 10, and 50 ng/g yielded correlation coefficient values (R^2) that were ≥0.992 for 97% of the 126 pesticides used to fortify the carrot, tomato, and celery matrices.

LOQ determination

Reported LOQs are based on six replicate recovery samples at a given concentration level for which average recovery falls in the range of 70 to 120% and %RSD ≤20 [8]. Recovery was evaluated by spiking the pesticide standards into comminuted carrot, tomato, and celery at levels of 1, 5, 10, and 50 ng/g. These QC samples were quantitated against a calibration curve prepared by spiking extracted blank matrix at levels of 0.5, 1, 5, 10, and 50 ng/g. The analysis was performed in replicates of six at each level. The distribution of recovery percentages for the prespiked matrices is shown in Figure 2. LOQs of 5 ng/g or lower were reached for 95, 98, and 97% of the 126 pesticides analyzed in carrot, tomato, and celery, respectively. An LOQ of 1 ng/g was reached for 86% of the pesticides in carrot, 89% in tomato, and 90% in celery. It should be noted that the QuEChERS extraction/partitioning step was performed using mechanical shaking, and that this technique leads to increased pesticide recoveries, up to 35% over manual shaking [14-16].

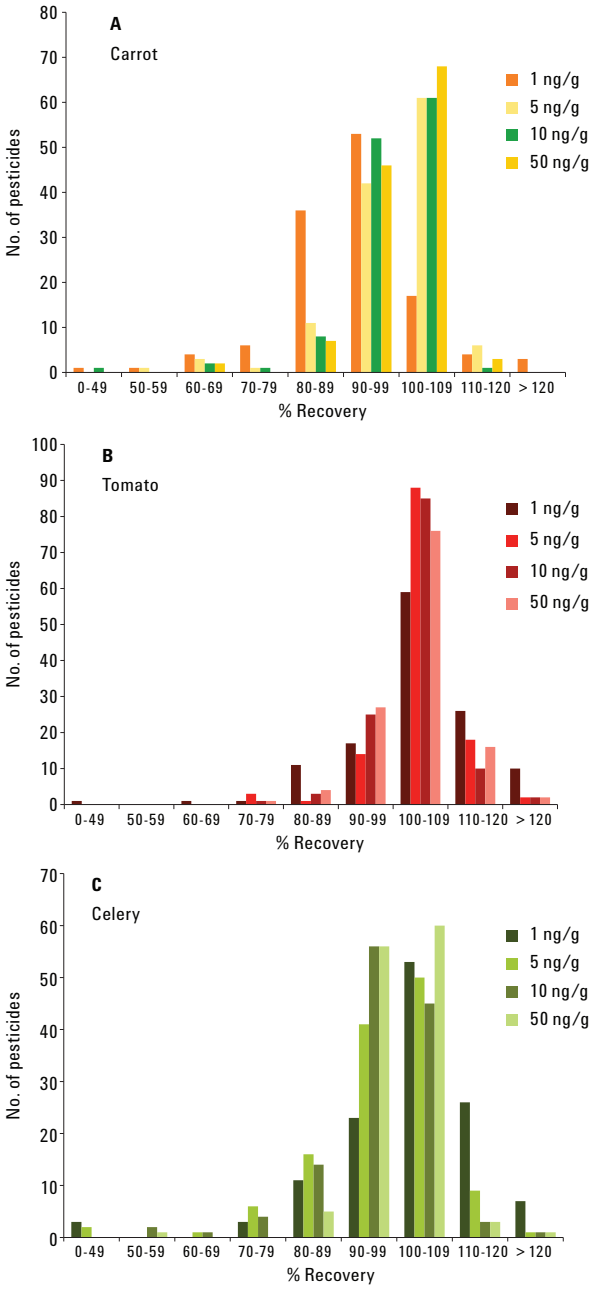

Figure 2. Distribution of average recoveries for 126 pesticides spiked at 1, 5, 10, and 50 ng/g in carrot (A), tomato (B), and celery (C) (n = 6).

Table 1. A partial list of determined LOQs (ng/g) for some challenging pesticides monitored in carrot, tomato, and celery.

	Carrot	Tomato	Celery
Bifenthrin	1	1	1
Bupirimate	1	1	1
Captan	5	5	1
Chlorothalonil	1	1	1
Chlorpropham	1	1	1
Clomazone	1	1	1
Cypermethrin	1	1	1
Cyprodinil	1	5	1
DDE-*p,p*'	1	1	1
Diazinon results	1	1	1
Dicloran	1	1	1
Dieldrin	1	1	1
Difenoconazole I	1	1	1
Diphenylamine	1	1	1
Endosulfan I	5	1	1
Endosulfan II	1	1	1
Endosulfan sulfate	1	1	1
Endrin	1	1	1
Etridiazole	1	1	5
Fenpropathrin	1	1	1
Fenvalerate	1	1	1
Fludioxonil	1	1	1
Folpet	1	1	1
Fuberidazole	5	5	1
Iprodione	1	1	1
Lenacil	1	1	1
Lindane (*gamma*-BHC)	1	1	5
Linuron	1	1	5
Metalaxyl	1	1	1
Methoxychlor-*p,p*	1	1	1
Nuarimol	1	1	1
Parathion-ethyl	1	1	1
Penconazole	1	1	1
Pendimethalin	1	1	1
Permethrin I	1	5	5
Permethrin II	1	1	1
Phenothrin I and II	10	1	1
Phosmet	1	1	1
Pirimicarb	1	1	1
Pirimiphos-methyl	1	1	1
Prochloraz	1	1	1
Pyridaben	1	1	5
Pyriproxyfen	1	1	1
Quinalphos	1	1	1
Resmethrin I and II	50	5	>50
Secbumeton	1	1	1
Tebuconazole	1	1	1
Tebufenpyrad	1	1	1
Tecnazene (TCNB)	1	1	1
Tefluthrin	1	1	1
Terbuthylazine	1	1	1
Tetrachlorvinphos, E-isomer	1	1	5
Tetraconazole	1	1	1
THPI	1	5	1
Triadimefon	1	1	1
Triadimenol	1	1	1
Trifluralin	1	1	1

Robust analysis of captan and folpet

Captan and folpet are base-sensitive compounds, and are often the most problematic in terms of recovery when using QuEChERS and precision during analysis. Miniaturization allows for 100 ng each of captan-d_6 and folpet-d_4 ISTD to be added to a 2 g sample at a cost of $0.012 and $0.028 per tube ($0.04 total). Resulting LOQs for captan and folpet are 1 ng/g in celery, which is well below the MRLs of 50 ng/g for each commodity. Individual recovery results for six replicate samples with %RSD at each level are shown in Figure 3. The LOQ for captan in both carrot and tomato was established to be 5 ng/g (%RSD 8 and 15, respectively) and 1 ng/g for folpet (%RSD 10 and 13, respectively). MRLs are 100 µg/g for captan and 20 ng/g for folpet in carrot, and 3 µg/g for both in tomato. Additional labeled internal standards such as captan-d_6 and folpet-d_4 could be included without significant cost increase per sample, as shown in Table 2.

Cost savings of 43 to 48% using Mini-QuEChERS

Cost savings were from 43 to 48% per sample depending on the type of dispersive cleanup chosen.

Conclusions

In an analysis of pesticide residues, significant cost savings were realized through scaled down sample preparation and decreased instrument maintenance. This was made possible by using a miniaturized QuEChERS method and a high-efficiency source for GC/MS/MS analysis. LOQs for 86 to 90% of pesticides spiked into carrot, tomato, and celery were 1 ng/g, despite injecting 75% less sample to improve ruggedness of the overall method.

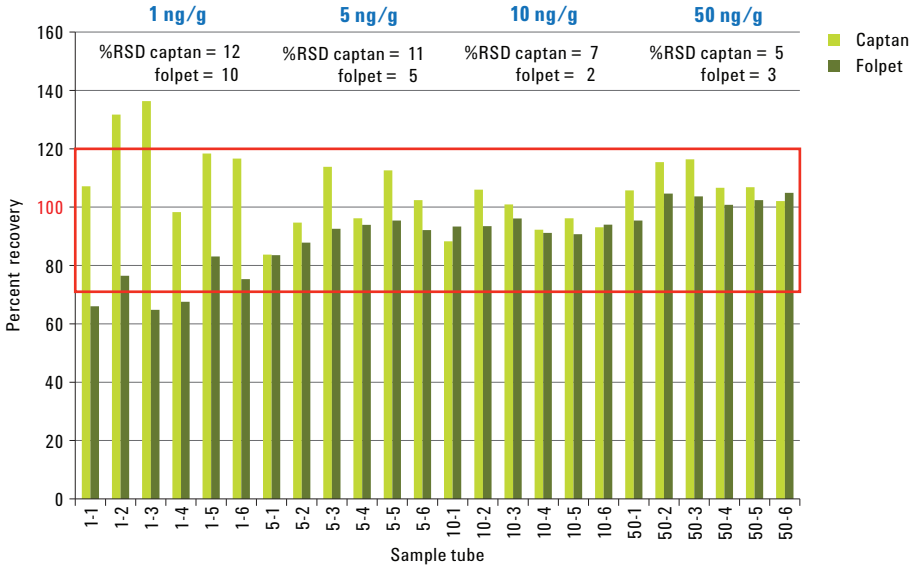

Figure 3. Individual recoveries of captan and folpet spiked at 1, 5, 10, and 50 ng/g in 2 g celery samples. The LOQ for folpet is reported as 1 ng/g since the average recovery at this level is 72% with %RSD = 10 (n = 6), which meets the specified criteria.

Table 2. Cost breakdown and savings for sample preparation with QuEChERS and mini-QuEChERS techniques.

Sample preparation cost/sample	Centrifuge tube	ACN	Salts	Internal standards: captan-d_6, folpet-d_4	dSPE general F&V or universal	Total cost/sample	Cost savings
QuEChERS	$0.43	$1.50	$2.96	$0.30	$1.32/$1.96	$6.51/$7.15	–
Mini-QuEChERS	$0.42	$0.20	$0.80	$0.04	$1.32/$1.96	$2.78/$3.42	**43%/48%**

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