Representative, Two-gram Incurred Food Samples Using Mini-QuEChERS, Cryomilling and GC/MS/MS Analysis with a High Efficiency Ion Source

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Introduction

A novel approach in food analysis involves miniaturization of the QuEChERS extraction method with cryomilling and the ability to inject less into the instrument. Various incurred fruits and vegetables underwent homogenization and cryomilling, which reduced particle size and provided a uniform, representative sampling of 2 g, or 87% less commodity than the 15 g sample required by AOAC.

Analytical results for a 2 g sample correlated with those using a 15 g sample. The mini-QuEChERS protocol for 2 g sample with cryomilling was evaluated for pesticide residue analysis relative to the 15 g sample required in the validated AOAC method. The extraction efficiency in several matrices showed that the results from the original 15 gram sample size for the AOAC QuEChERS compared to the 2 gram mini-QuEChERS are comparable and that the smaller sample size can be implemented without additional changes in equipment and or protocol. For example, results in peach were as follows: pyriproxyfen, 0.14 ng/g in a 15 g sample as compared with 0.13 ng/g in 2 g sample; iprodione, 2.0 ng/g (15 g) versus 2.0 ng/g (2 g); fenbuconazole, 11.8 ng/g (15 g) versus 11.0 ng/g (2 g). The mini-QuEChERS sample preparation procedure is easier to handle since it uses far less solvent, salts and labeled standards and was shown to produce the same results. In fact, the use of additional labeled standards for problematic compounds like captan and folpet is an acceptable option with the mini-QuEChERS since it is not cost prohibitive as it might be for a 15 gram sample size.

Cost savings ranged between 42-48% due to less solvent, sorbent and labeled ISTD. Use of a 0.5 µL injection and the High Efficiency Source (HES) further reduced cost-per-sample through less frequent maintenance and allowed for improved limits of quantitation for incurred residues in a variety of fruits and vegetables.

Method

Refer to GC/MS/MS "Pesticide Analysis Reference Guide", Agilent Technologies publication 5991-2389EN.

Sample Homogenization and Cryomilling

Various commodites were chopped frozen and homogenized in a Robot Coupe

Frozen homogenized matrices were cryomilled in a SPEX Freezer Mill with Liquid Nitrogen

Method

Sample Preparation: Extraction/Partitioning (AOAC)

2 g of sample, 2 ceramic homogenizers
2 mL ACN (1% HAc), vortex
1 g of Agilent Bond Elut AOAC salts; shake and centrifuge

Dispersive SPE:

Transfer 1 mL of extract to 2 mL Agilent Bond Elut dSPE: General fruit & veg [PSA] or Universal [PSA, C18, GCB]
vortex and centrifuge
transfer 250 µL to vial for analysis

QC samples were fortified with a 1 µg/mL pesticide stock solution (126 pesticides) yielding concentrations of 1, 5, 10, and 50 ng/g. A 10 µL volume of internal standard spiking solution (10 µg/mL of Parathion-d10, DDT, p,p'-13C12, TPP, captan-d6 and folpet-d4) was added to all samples except the control blank to yield a 50 ng/g concentration in each sample. Calibration standards were prepared by spiking the extracted blank at 0.5, 1, 5, 10, and 50 ng/g.

Analysis by GC/MS/MS

An Agilent 7890 GC coupled to a 7010 Triple Quadrupole GC/MS system with the High Efficiency Source was used. The GC system was equipped with a Multi-Mode Inlet (MMI) with air cooling and a back flushing system based on a Purged Ultimate Union controlled by an Aux EPC module. Agilent Mass Hunter Software was used for instrument control, and for qualitative and quantitative data analysis.

High Efficiency Source

High Efficiency Source, magnet removed

Method Transfer– No Re-optimization

What is new to this method:

- ***2 g QuEChERS sample instead of 15 g sample***
- ***0.5 µL injected instead of 2 µL***

The GC/MS/MS analysis method, with no change except injection volume, was seamlessly transferred to the 7010 GCMS with its High Efficiency Source, which maximizes ion production and leads to greater sensitivity.

Incurred Samples: Results of 15 g Homogenized and 2 g Cryomilled

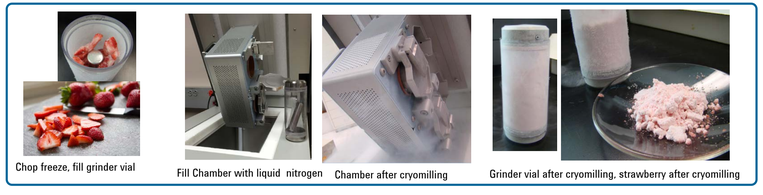
Preliminary Evaluation of the miniaturized 2 g QuEChERS extraction protocol with spiked matrix:

LOQs are based on %RSD ≤ 20 for n=6 Recovery Samples, where Average Recovery is in the range of 70 ≤≥ 120%

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.
In the case of carrot, 86% of the pesticides had LOQs of 1 ng/g. For tomato, 89% had LOQs of 1 ng/g and for celery 90% of the 126 pesticides had LOQs of 1 ng/g. Refer to 5991-5507EN and 5991-6069EN.

Comparison of 15 g homogenized to 2 g cryomilled matrix

In order to determine the efficacy of a 2 g matrix in the mini-QuEChERS protocol, homogenized matrices were obtained from a monitoring agency. The commodities had already been analyzed by the independent laboratory and pesticide residues had been documented. The homogenized matrices were subjected to cryomilling and then extracted by the mini-QuEChERS approach, Figure 1.



Chop freeze, fill grinder vial

Fill Chamber with liquid nitrogen

Chamber after cryomilling

Grinder vial after cryomilling, strawberry after cryomilling

Figure 1: Cryomilling Process

To compare the results 15 g of homogenized matrix was extracted by AOAC QuEChERS extraction and dSPE. Two grams of the same matrix after cryomilling was extracted by the mini-QuEChERS and dSPE in triplicate.

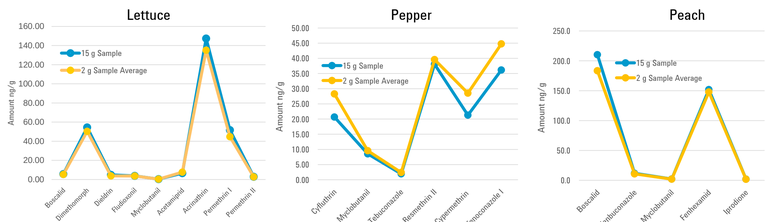


Figure 2: Comparison of extraction results after a 15 g homogenized matrix with QuEChERS extraction and 2 g cryomilled matrix with mini-QuEChERS

The graphs in Figure 2 suggest that reducing the amount of sample to 2 grams versus 15 grams with cryomilling produced similar results and the application identified the same pesticide residues as determined by the independent laboratory and additional pesticide residues within Multiresidue acquisition method.

Incurred Samples: Results 15 and 2 g Homogenized and 2 g Cryomilled

Tables 1-3: Comparison of Pesticide Residue Analysis from 15 g and 2 g Homogenized and 2 g Cryomilled

Incurred Residues in Tomato, ng/g

	2g Cryo	% RSD (n=6)	2g homogenized	% RSD (n=3)	15g homogenized	% RSD (n=2)
Chlorothalonil	7.32	20.3	8.49	7.0	6.56	2.2
Chlorpyrifos	0.32	17.9	0.39	13.1	0.35	16.6
Myclobutanil	0.13	11.6	0.15	7.3	0.10	12.4
Buprofezin (2-isomer)	45.22	3.6	54.82	10.6	41.51	1.2
Tebuconazole	0.36	12.9	0.32	7.2	0.25	0.1
Pyriproxyfen	12.63	6.1	14.05	2.7	10.50	2.5

Incurred Residues in cucumber, ng/g

	2g Cryo	% RSD (n=6)	2g homogenized	% RSD (n=3)	15g homogenized	% RSD (n=2)
Chlorpropham	0.544	12	0.528	2.6	0.674	3.8
Chlorothalonil	44.4	7.6	51.4	7.5	51.5	7.7
Metalaxyl	35.0	7.7	37.6	9.5	37.0	5.3
Dieldrin	1.86	7.4	1.92	12	1.83	0.4
Boscalid	14.4	6.1	15.3	7.7	15.1	7.6
Pyraclostrobin	1.79	7.6	1.89	15	1.86	0.3
Dimethomorph	7.63	7.5	7.42	3.1	7.18	10.2

Incurred Residues in Strawberry, ng/g

	2g Cryo	% RSD (n=6)	2g homogenized	% RSD (n=3)	15g homogenized	% RSD (n=2)
Chlorpropham	0.28	12.1	0.14	22.5	0.18	0.6
Cyprodinil	1.15	5.1	1.16	3.0	1.10	8.2
Fludioxonil	1.90	9.9	2.13	14.8	1.79	4.2
Myclobutanil	3.80	6.6	3.92	5.4	3.42	0.5
Bifenthrin	3.87	6.3	3.67	4.4	3.54	1.5
Boscalid Results	2.28	4.6	2.38	7.53	2.26	2.0
Pyraclostrobin	1.04	11.8	0.92	11.7	0.97	5.1

Store bought non-organic commodities were extracted by AOAC QuEChERS extraction and by mini-QuEChERS. The data presented in Tables 1-3 suggest that at a 2 gram sample size whether homogenized or homogenized and cryomilled will produce results similar to the 15 gram homogenized matrix. Therefore this initial miniaturization study suggests that a 2 gram homogenized matrix (no cryomilling) is acceptable for multi-residue analysis.

Reduce Sample Prep Costs by Over 40%

Cost Breakdown and Cost Savings for Sample Preparation with QuEChERS and Mini-QuEChERS Technique

Sample Preparation Cost/Sample	Centrifuge Tube	ACN	Salts	Internal Std: Captan-d6, Folpet-d4	dSPE Gen 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%**

Conclusions

- The QuEChERS method is a common sample preparation technique for multi-residue analysis in various matrices
- Miniaturizing QuEChERS has several advantages: easier sample handling, uses existing laboratory equipment, more samples per batch, less storage space, reduced salt and sorbent cost, reduced cost of labeled compounds, addition of labeled compounds for troublesome analytes, reduced solvent cost and waste, uses existing dSPE
- The 7010 Triple Quadrupole GC/MS system with its High Efficiency Source has several advantages: new hardware design, new achievable level of response, dilute sample prior to injection, inject less sample, only 25% of sample volume, less matrix injected offers prolonged uptime, increased performance
- Homogenized matrix is required in order to have a representative sample for QuEChERS extraction and multi-residue pesticide analysis
- Cryomilling is believed to be required for small sample size in order to have a very uniform and small particle that is representative of the entire matrix for mini-QuEChERS
- Initial data presented suggest that a 2 gram sample with mini-QuEChERS and dSPE was capable of producing similar results as found with a 15 gram sample amount used in AOAC QuEChERS
- Initial comparison of 2 gram sample homogenized versus homogenized and cryomilled produced similar results suggesting that cryomilling is not required for a 2 gram sample size employed in mini-QuEChERS, which offers laboratories an alternative approach to multi-residue analysis