A QuEChERS Strategy for Juice Concentrates with Unknown Properties Analyzed by GC/MS/MS

Application Note

Food Testing & Agriculture

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Abstract

QuEChERS is a widely used sample preparation method in the food and beverage industries for multiresidue contaminant analysis. Many references and guidelines are published by regulatory organizations, as well as food scientists. However, issues sometimes arise regarding the uncertainty of how to apply QuEChERS methods to uncommon and complex sample matrixes. Juice concentrates with unknown properties can be very challenging during modification of the QuEChERS protocol when there is a need to accommodate variables such as the concentration factor, and whether or not the samples contain peel. In this application note, method development for sample preparation using QuEChERS is discussed for orange and lemon juice concentrates, followed by analysis with GC/MS/MS.

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Introduction

Preparing juice concentrate samples for the analysis of multiclass pesticide residues can be challenging due to the extreme chemical and physical conditions of the sample. For example, juice concentrates from lemon can be extremely acidic, or have unusually thick textures depending on the concentration factor. Optimization of QuEChERS sample preparation is needed to account for these problematic variables and significantly improve data quality. Method development steps for the preparation of lemon and orange juice concentrates are described here in detail as potential solutions for complex matrixes from other sources.

Experimental

Materials and reagents

Acetonitrile:	LC/MS grade
Water:	Milli-Q filtered or LC/MS grade
Lemon and orange juice concentrates:	Provided by a worldwide beverage company with unknown sample properties (no information on concentration factors, origin, peel content, brix, and so forth)
Analytes:	A mixture of compounds in ACN + 1% acetic acid from a worldwide beverage company. Analytes are listed in Appendix 1.
Sample preparation:	Agilent QuEChERS EN extraction kit (p/n 5982-5650CH), Agilent QuEChERS dispersive SPE kit for general fruits and vegetables (p/n 5982-5021)

GC conditions

Column 1:	Agilent J&W HP-5ms Ultra Inert, 5 m (cut from a 15 m column), 0.25 mm, 0.25 µm (p/n 19091S-431UI)
Flow rate 1:	1.1 mL/min
Column 2:	Agilent J&W HP-5ms Ultra Inert, 15 m × 0.25 mm, 0.25 µm
Flow 2:	1.2 mL/min
Oven temperature:	60 °C for 1.5 minutes, then 50 °C/min to 160 °C, then 8 °C/min to 240 °C, then 50 °C/min to 280 °C (2.5 minutes hold), then 100 °C/min to 290 °C (3.1 minutes hold)
Run time:	20 minutes
Instrument:	Agilent 7000 and 7890A GC/MS/MS

Injection

Inlet type:	Multi-mode Inlet (MMI)
Liner:	2 mm id Agilent dimpled Ultra Inert liner (p/n 5190-2297)
Mode:	PTV solvent vent
Injection volume:	2 µL (syringe size 10 µL)
Solvent washes:	Pre-injection, once; post-injection, five times
Sample wash:	2 µL, twice
Sample pumps:	5
Inlet temperature program:	60 °C for 0.35 minutes, then 900 °C/min to 280 °C (15 minutes hold), then 900 °C/min to 300 °C (to end of the analysis)
Purge flow to split vent:	50 mL/min at 1.5 minutes
Vent flow:	25 mL/min
Vent pressure:	5 psi to 0.3 minutes
Gas saver:	20 mL/min at 5 minutes
Septum purge flow:	3 mL/min
Cryo:	On at 200 °C

Sample preparation for lemon and orange juice concentrates

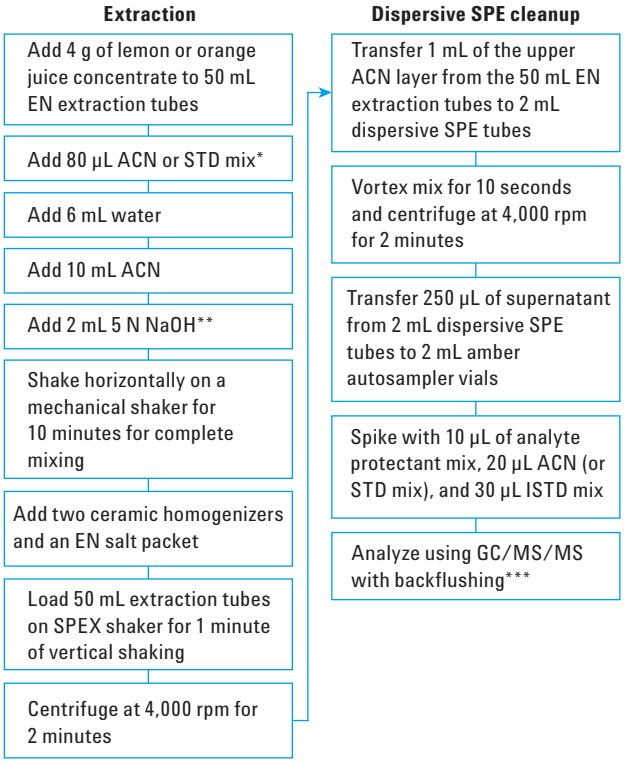
The QuEChERS optimized workflow for lemon and orange juice concentrates is shown in Figure 1.

Results and Discussion

Choosing the right extraction and dispersive SPE QuEChERS products

There are three main commercially available preweighed QuEChERS extraction products, as well as bulk materials for method customization. Optimization using bulk materials is not discussed here since it is dependent on the sample matrix, analyte type, required detection limits, and so forth, and can be more time-consuming, which may not be ideal in a routine analysis laboratory. In this study, AOAC and EN extraction methods were tested. AOAC extraction gave upper ACN layer volumes of 17 to 18 mL, whereas EN extraction consistently produced 10 mL. Due to different salt effects imposed on the sample matrix during the extraction in each product, differing results such as these can be expected. No sample information was provided by the beverage company, rather than investigating the different salt effects, the EN extraction method was selected due to the consistent volume of the upper ACN layer.

Having chosen EN extraction, different dispersive SPE tubes were tested. The general fruits and vegetables dispersive SPE kit gave higher peak area than other dispersive SPE kits and was therefore selected as the cleanup kit. The EN extraction with general fruits and vegetables dispersive SPE kit was used as the final QuEChERS sample preparation method and the additional optimization parameters were adjusted accordingly. Selecting the right combination of extraction and dispersive SPE is the first stage in QuEChERS method development and is further discussed elsewhere [1].



* Add 80 µL ACN for matrix-matched standard calibrators and 80 µL STD mix at appropriate concentration levels for recovery samples.

** After optimization of pH values, 2 mL and 0 mL 5 N NaOH solution were used for lemon juice and orange juice, respectively.

*** Backflushing is recommended to improve throughput and minimize system contamination by excluding high boiling matrix components in each run.

Figure 1. Schematic workflow of optimized QuEChERS application for lemon and orange juice concentrates of unknown properties.

Optimization of sample amount

Many QuEChERS applications use a fixed amount of sample during the extraction in 50 mL tubes. The AOAC and EN methods recommend 15 g and 10 g of sample in the extraction step, respectively. It is clear that the more sample in the extraction tube, the more analytes are in the tube, providing a higher signal for compounds of interest in the chromatogram. However, with more sample there are also more matrix components extracted with the analytes of interest. Having the optimal amount of sample in the extraction is an essential step in QuEChERS method development; thus there is a balance between using the right amount of sample and producing enough signals from the compounds of interest, while also minimizing matrix effects. Different sample amounts were tested in the extraction step using 3, 5, and 7 g of juice concentrate, followed by extraction and dispersive SPE. This experiment showed that no more than 4 g of sample was necessary to reach the desired detection limits (10 ppb for most of the analytes), therefore, 4 g samples were used throughout the study. Figure 2 shows the effect of different sample loading amounts in the QuEChERS extraction step.

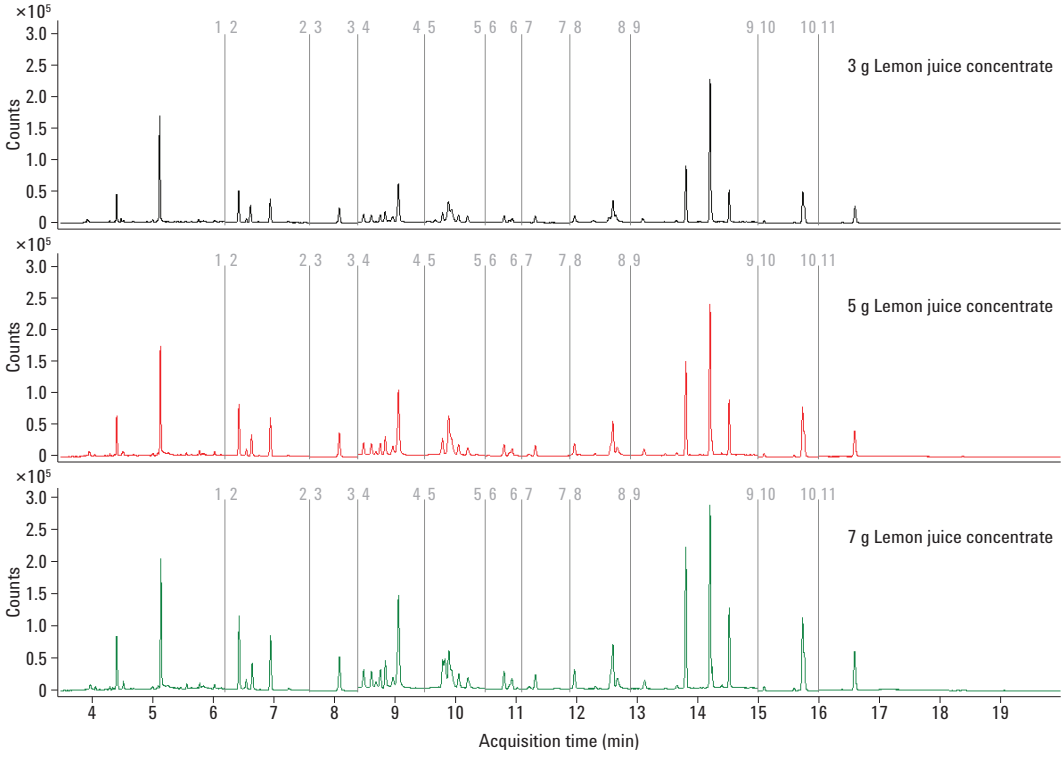


Figure 2. Different amounts of lemon juice concentrate used in QuEChERS extraction. More than 3 g of sample was needed for all analytes to be detected at MRL values (10 ppb for the majority of the compounds). The final sample amount for this application was 4 g, providing the best balance between sensitivity and matrix interference.

Adjustment of pH during extraction

Some pesticides are base-sensitive, which often results in loss of recovery during the analysis. Improvement to QuEChERS extraction using acid has been implemented to address this issue [2]. In this study, lemon and orange juice concentrates are extremely acidic matrixes and have not been extensively studied for QuEChERS-based protocols. The control of the pH value during the extraction process plays a very important role and different pH adjustments were performed to better understand their influence on performance. After adding 4 g of sample, 6 mL water, and 10 mL ACN were added as shown in the workflow in Figure 1. Four different amounts of 5 N NaOH were then added (0, 0.6, 1, and 2 mL) to vary the pH. Some compounds showed very good improvement when 2 mL of 5 N NaOH solution was added in the extraction step, as shown in Figure 3.

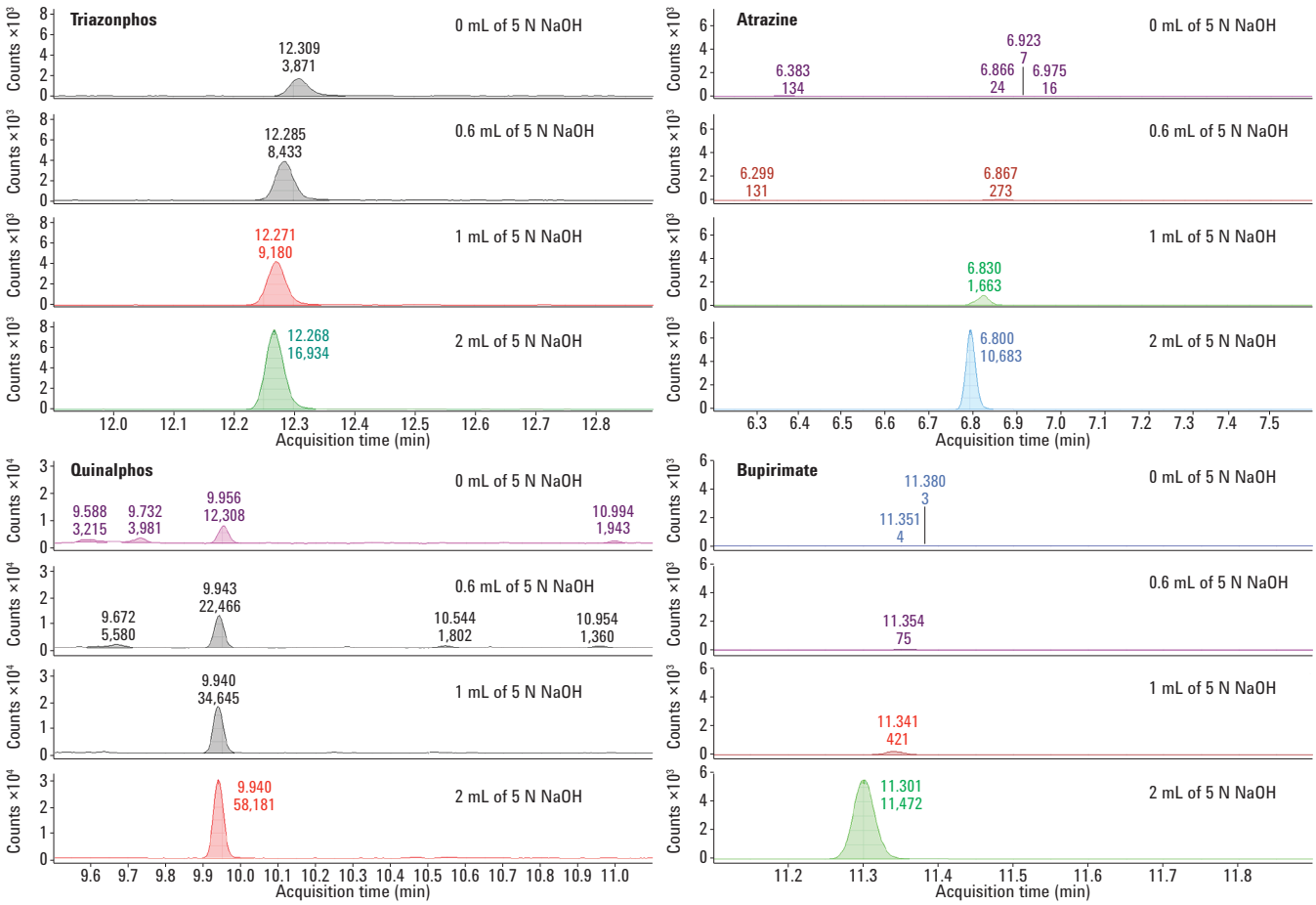


Figure 3 (cont. p.6). Chromatographic improvements as a result of increasing the pH during extraction of lemon juice concentrate. From top to bottom in every chromatogram, 5 N NaOH at 0, 0.6, 1, and 2 mL was added. Some chromatograms show area-count improvements at all levels, such as triazonphos, whereas some compounds only appear when 2 mL 5 N NaOH was added, such as bupirimate. Folpet showed better recovery with 2 mL 5 N NaOH, while captan showed better recovery with 0.6 mL 5 N NaOH. The addition of 2 mL 5 N NaOH during extraction of lemon juice concentrate was chosen for pH adjustment. More chromatograms showing pH variation during QuEChERS extraction are shown in Appendix 2.

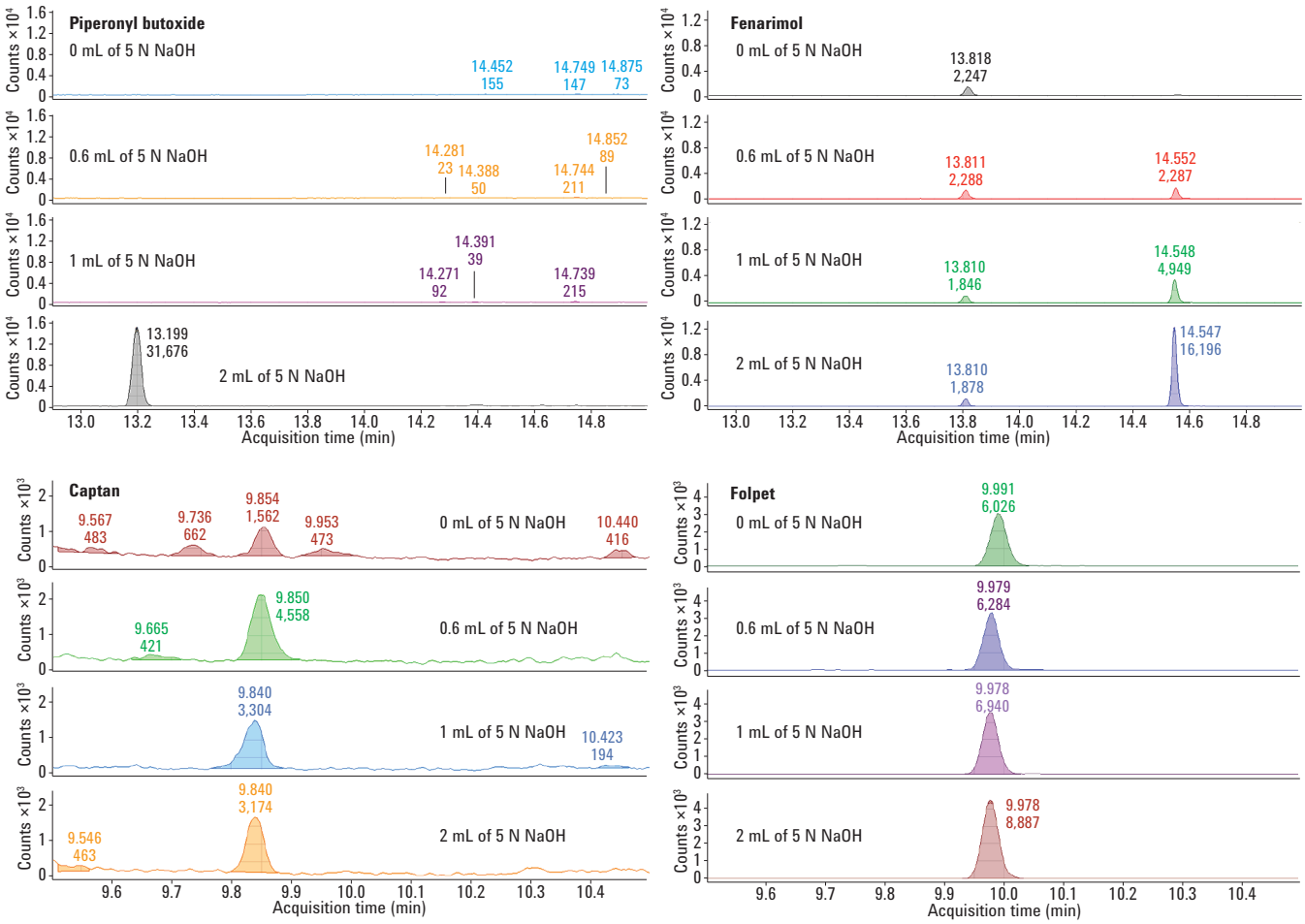


Figure 3 (cont.). Chromatographic improvements as a result of increasing the pH during extraction of lemon juice concentrate. From top to bottom in every chromatogram, 5 N NaOH at 0, 0.6, 1, and 2 mL was added. Some chromatograms show area-count improvements at all levels, such as triazonphos, whereas some compounds only appear when 2 mL 5 N NaOH was added, such as bupirimate. Folpet showed better recovery with 2 mL 5 N NaOH, while captan showed better recovery with 0.6 mL 5 N NaOH. The addition of 2 mL 5 N NaOH during extraction of lemon juice concentrate was chosen for pH adjustment. More chromatograms showing pH variation during QuEChERS extraction are shown in Appendix 2.

Effect of analyte protectants

Analyte protectants (APs) can be important in pesticide analysis by GC/MS/MS, especially for some challenging samples such as juice concentrates with unknown properties. Without APs, some compounds can show poor peak shapes. The most recent GC/MS/MS development in inert flow paths has brought a whole new level of detection and robustness to laboratories that test for pesticide residues.

QuEChERS is a sample preparation method used to accommodate a wide range of analytes in some very complicated matrixes (usually food) and does not provide the comprehensive cleanup associated with cartridge-based SPE. Over time, QuEChERS samples will eventually introduce some matrix to the flow path components, resulting in peak tailing and gradually increasing matrix effects. To overcome this, APs are added to all samples to interact with the active sites that have accumulated in the flow path, thereby reducing analyte interactions. Many studies have been published showing the effect of APs in routine pesticide analysis by GC/MS/MS [3,4]. A mixture of D-sorbitol and L-gulonolactone is economical and readily available, and serves as a good analyte protectants. APs can be helpful components in any routine pesticide analysis. The extreme effects of APs for this application are shown in Figure 4.

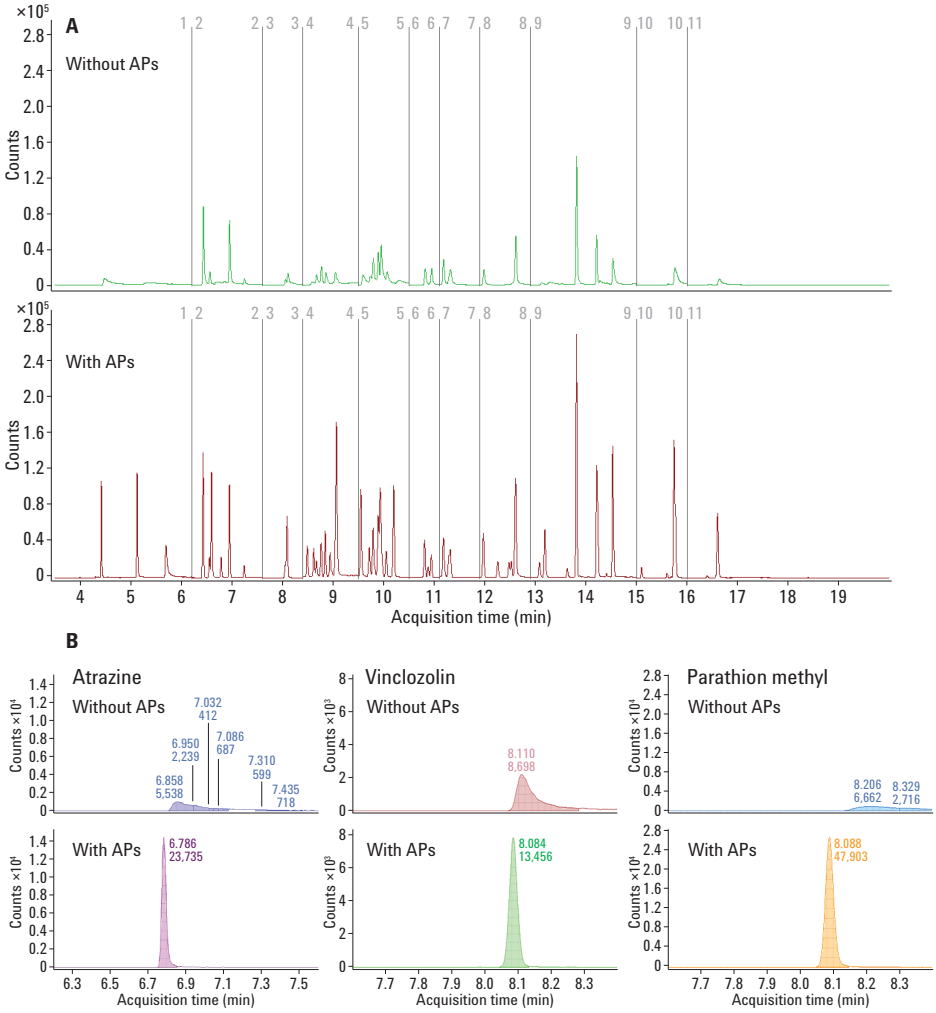


Figure 4. The effect of analyte protectants in pesticide analysis by GC/MS/MS. A) TIC comparison between samples with and without APs, B) upper, STD mix at 100 ppb in ACN without APs, lower, STD mix at 100 ppb in ACN with APs.

Calibration curves, precision, and accuracy with an optimized QuEChERS method

Precision and accuracy were measured at four different concentration levels (5, 10, 50, and 100 ppb) in the form of % recovery and % RSD. All compounds in the analyte panel showed excellent regression in their matrix-matched calibration curves. Most compounds of interest also demonstrated excellent performance with low-single-digit % RSD. Details of calibration curve linearity, precision, and accuracy are shown in Appendix 1.

Conclusions

QuEChERS method optimization for juice concentrates provided a comprehensive workflow for samples with unknown properties. This included selecting the right combination of extraction and dispersive SPE products, pH optimization, and identifying the correct amount of sample. Each optimization step resulted in a better peak shape and sensitivity for the majority of analytes, especially for traditionally problematic pesticides such as captan and folpet, which gave excellent recoveries and R^2 values in their matrix-matched calibration curves. The use of analyte protectants for this particular application was essential for achieving good chromatography and can provide similar benefits for other types of complex matrixes prepared using this QuEChERS workflow. The identification and flexibility of the variables identified in this study have implications for performance improvements in other matrixes and demonstrate the evolving practicality of the QuEChERS sample preparation method.

References

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2. S. J. Lehotay, K. Maštovská, A. R. Lightfield *J. AOAC Int.* **88**, 2 (2005).
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Appendix 1

List of compounds with recovery, precision, and accuracy of an optimized QuEChERS method for juice concentrates

Table 1 (cont. p.10). Recovery, precision, accuracy, and linearity data for lemon juice concentrate. Linear regression was used for all compounds without any weighting factor. Lambda, cyhalothrin I–II were combined. Cypermethrin I–IV were combined.

	Recovery (%)				%RSD				
Compound	**5 ppb**	**10 ppb**	**50 ppb**	**100 ppb**	**5 ppb**	**10 ppb**	**50 ppb**	**100 ppb**	**R^2**
Biphenyl	78	92	100	101	16.3	7.7	5.3	5.2	1.000
Omethoate	90	95	93	95	6.6	8.6	9.7	6.3	0.995
BHC, alpha-	93	101	103	104	4.4	1.6	3.7	3.7	1.000
Hexachlorobenzene	93	98	96	97	6.4	2.7	3.0	4.1	1.000
Demeton S	78	100	102	105	12.2	10.5	2.9	1.8	0.999
Atrazine	104	106	102	103	4.6	4.5	2.8	2.7	1.000
Lindane (gamma-BHC)	100	102	103	104	3.0	2.3	2.6	3.3	1.000
Diazinon	116	112	99	101	5.0	3.9	2.6	2.5	1.000
Acetochlor	97	102	103	104	2.8	2.2	1.9	2.5	1.000
Vinclozolin	77	92	89	92	8.1	2.7	2.6	2.9	1.000
Chlorpyrifos methyl	105	105	101	102	2.9	0.5	2.6	1.6	1.000
Parathion methyl	107	106	101	104	3.3	3.1	2.5	1.3	1.000
Dicofol, o,p'-	95	101	103	103	4.5	3.9	3.7	3.3	1.000
Fenthion	126	117	106	104	38.0	19.7	8.9	6.8	0.995
Fenitrothion	121	112	99	102	2.7	0.9	2.7	1.9	1.000
Pirimiphos-methyl	115	111	103	104	4.1	5.2	4.3	3.6	1.000
Dichlofluanid	74	81	74	79	5.9	1.4	2.1	2.1	1.000
Malathion	100	102	96	100	3.3	2.2	2.2	1.0	1.000
Diethofencarb	98	101	104	106	3.6	1.5	3.0	2.1	1.000
Chlorpyrifos	109	101	100	101	3.0	2.8	2.0	2.3	1.000
Parathion	123	111	101	105	4.4	3.8	0.8	2.2	1.000
Dicofol, p,p'-	100	102	101	103	4.9	2.3	1.9	3.1	1.000
Cyprodinil	97	104	103	104	2.9	8.6	3.0	2.7	1.000
Penconazole	91	103	104	106	6.0	7.8	2.0	2.0	1.000
Tolyfluanid	78	84	75	82	2.1	4.5	1.3	2.2	1.000
Captan	51	66	68	79	39.3	11.9	7.0	7.3	0.999
Isofenphos	107	104	102	104	3.4	1.9	1.1	2.0	1.000
Mecarbam	114	97	91	97	21.1	7.3	3.8	3.2	1.000
Quinalphos	101	101	100	104	6.5	2.3	1.3	1.5	1.000
Phenthoate	46	86	107	106	13.6	4.2	3.1	2.8	0.997
Folpet	68	74	66	72	20.3	5.8	2.2	6.8	1.000

Table 1 (cont.). Recovery, precision, accuracy, and linearity data for lemon juice concentrate. Linear regression was used for all compounds without any weighting factor. Lambda, cyhalothrin I–II were combined. Cypermethrin I–IV were combined.

	Recovery (%)				%RSD				
Compound	**5 ppb**	**10 ppb**	**50 ppb**	**100 ppb**	**5 ppb**	**10 ppb**	**50 ppb**	**100 ppb**	**R^2**
Procymidone	97	99	97	99	3.6	2.5	2.8	1.8	1.000
Methidathion	102	101	100	107	4.0	5.0	3.7	2.5	1.000
Prothiofos	100	100	100	102	2.2	0.9	1.0	1.6	1.000
Profenofos	110	107	101	102	4.4	4.3	1.9	0.9	1.000
DDE-p,p'	97	100	98	100	5.1	2.3	2.4	2.4	1.000
Buprofezin	106	110	101	103	7.5	6.0	2.3	2.6	1.000
Bupirimate	103	115	102	102	4.7	10.3	4.0	3.8	1.000
Kresoxim methyl	96	100	98	101	3.9	2.8	1.4	2.1	1.000
Ethion	127	111	98	101	4.1	3.3	2.0	4.3	1.000
Triazophos	114	104	99	107	5.1	2.0	4.0	3.9	1.000
Quinoxyfen	94	100	102	104	2.7	6.4	2.6	1.9	1.000
Carfentrazone-ethyl	92	95	91	96	5.4	4.9	2.2	2.8	1.000
DDT-p,p'	101	100	98	99	1.5	3.8	1.7	1.2	1.000
Imazalil	131	108	103	106	17.0	13.9	8.3	2.7	0.999
Norflurazon	100	104	103	107	7.2	8.5	3.1	2.9	1.000
TPP	101	117	102	105	7.2	24.5	2.3	1.9	1.000
Piperonyl butoxide	120	113	101	98	2.3	3.2	1.0	1.6	1.000
Pyridaphenthion	98	100	99	105	4.7	4.5	4.5	2.4	1.000
Bifenthrin	94	98	100	102	2.7	8.6	1.2	2.9	1.000
Phosalone	118	105	96	102	2.4	3.0	3.4	3.5	1.000
Lambda, cyhalothrin I-II	128	111	95	98	2.0	4.4	2.5	2.9	0.998
Fenarimol	104	109	101	105	4.2	8.6	1.2	3.2	1.000
Coumaphos	107	106	99	106	5.0	3.6	4.0	5.3	1.000
Cypermethrin I-IV	127	111	95	99	2.6	4.1	2.6	3.1	0.999
Esfenvalerate I	126	108	91	97	3.1	5.2	3.6	4.5	0.999

Table 2 (cont. p.12). Recovery, precision, accuracy, and linearity data for orange juice concentrate. *Quadratic fit was used for phenthoate. Linear regression without weighting was used for all other compounds. Lambda, cyhalothrin I–II were combined. Cypermethrin I–IV were combined.

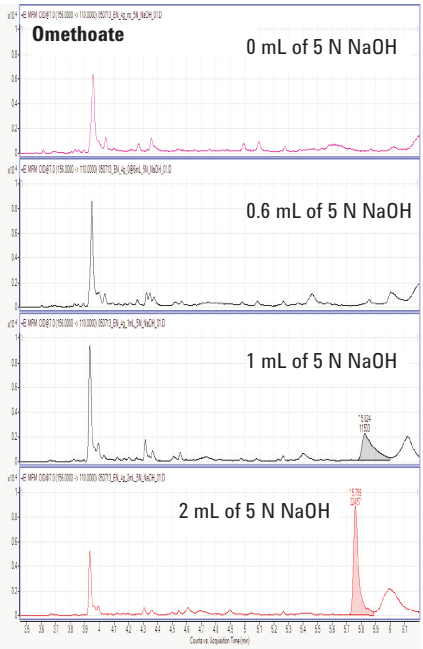
	Recovery (%)				%RSD				
Compound	**5 ppb**	**10 ppb**	**50 ppb**	**100 ppb**	**5 ppb**	**10 ppb**	**50 ppb**	**100 ppb**	**R^2**
Biphenyl	81	86	92	94	13.7	8.0	3.1	2.9	0.999
Omethoate	109	110	101	104	6.2	5.3	3.9	5.5	0.999
BHC, alpha-	86	96	104	106	6.6	2.0	2.0	3.2	1.000
Hexachlorobenzene	75	86	95	96	3.2	3.7	3.3	3.1	1.000
Demeton S	111	103	102	105	9.7	9.6	1.2	2.5	1.000
Atrazine	98	102	103	106	7.5	2.3	2.8	1.0	1.000
Lindane (gamma-BHC)	96	99	102	105	4.7	4.3	2.1	3.0	1.000
Diazinon	96	101	103	107	7.9	2.8	2.2	2.0	1.000
Acetochlor	102	106	103	107	2.5	1.7	1.6	2.5	1.000
Vinclozolin	90	101	103	106	4.0	4.3	1.7	2.9	1.000
Chlorpyrifos methyl	96	101	102	105	2.3	1.9	2.4	1.7	1.000
Parathion methyl	115	110	101	103	1.1	2.0	2.2	1.5	1.000
Dicofol, o,p'-	98	105	103	105	17.7	19.5	1.8	3.5	1.000
Fenthion	129	107	114	111	45.1	25.7	9.1	5.0	0.999
Fenitrothion	112	106	101	103	7.1	2.8	3.3	1.6	1.000
Pirimiphos-methyl	88	99	104	107	4.1	2.4	1.9	3.0	1.000
Dichlofluanid	31	37	50	53	9.6	8.5	3.9	2.3	1.000
Malathion	101	103	104	106	2.7	3.3	2.5	2.2	1.000
Diethofencarb	90	99	104	107	6.9	2.5	1.3	2.7	1.000
Chlorpyrifos	86	95	100	103	7.1	4.5	1.9	2.9	1.000
Parathion	124	112	100	101	2.8	2.4	2.4	3.0	0.999
Dicofol, p,p'-	95	99	100	102	3.4	2.9	2.2	2.5	1.000
Cyprodinil	100	103	103	105	3.8	2.3	1.7	2.0	1.000
Penconazole	88	99	104	107	3.6	1.8	1.8	2.7	1.000
Tolyfluanid	60	60	63	66	5.4	5.6	1.8	1.9	1.000
Captan	63	71	66	68	13.2	10.7	4.6	4.1	0.999
Isofenphos	102	105	103	107	2.0	2.6	2.4	2.4	1.000
Mecarbam	92	109	104	107	54.6	9.1	7.1	2.7	0.999
Quinalphos	103	106	102	105	3.1	6.7	1.5	2.0	1.000
Phenthoate*	95	103	106	96	4.0	2.6	2.3	2.9	1.000
Folpet	90	84	72	72	6.8	6.0	3.5	3.0	1.000

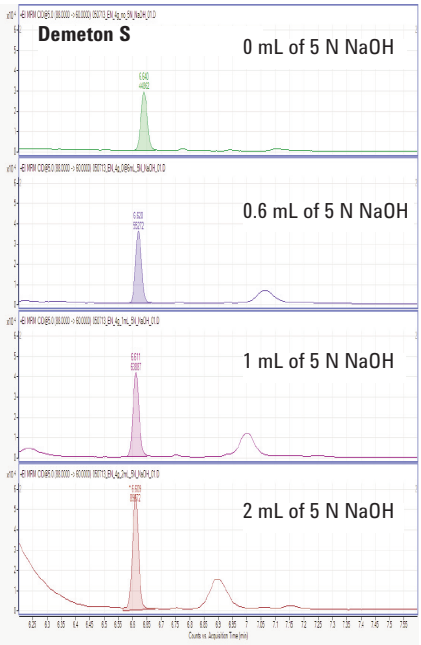
Table 2 (cont.). Recovery, precision, accuracy, and linearity data for orange juice concentrate. *Quadratic fit was used for phenthoate. Linear regression without weighting was used for all other compounds. Lambda, cyhalothrin I–II were combined. Cypermethrin I–IV were combined.

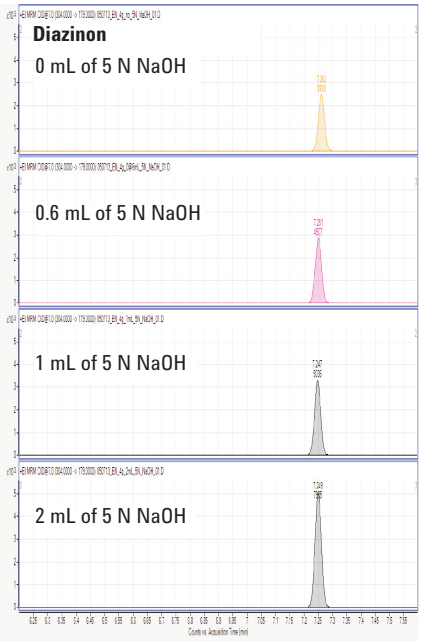
	Recovery (%)				% RSD				
Compound	**5 ppm**	**10 ppm**	**50 ppm**	**100 ppm**	**5 ppm**	**10 ppm**	**50 ppm**	**100 ppm**	**R^2**
Procymidone	104	107	104	106	3.5	2.1	2.2	2.7	1.000
Methidathion	105	108	102	106	4.6	5.1	2.7	1.7	1.000
Prothiofos	94	98	100	102	2.6	2.4	1.2	1.6	1.000
Profenofos	102	105	102	104	4.3	3.7	2.6	2.0	1.000
DDE-p,p'	91	99	98	99	3.6	2.3	2.7	2.1	1.000
Buprofezin	108	104	103	104	6.4	2.7	1.8	3.3	1.000
Oxyfluorfen	130	108	96	97	6.9	5.4	2.9	2.6	0.999
Bupirimate	98	102	104	108	4.9	2.6	2.7	1.6	1.000
Kresoxim methyl	102	104	103	107	3.0	2.6	2.2	2.1	1.000
Ethion	107	104	100	103	2.9	3.7	2.0	0.7	1.000
Triazophos	110	104	101	105	2.4	1.5	3.5	0.8	1.000
Quinoxyfen	96	101	101	103	2.4	5.5	2.3	2.0	1.000
Carfentrazone-ethyl	107	104	101	104	4.7	4.4	2.9	1.4	1.000
DDT-p,p'	104	104	98	99	3.4	1.1	1.3	1.2	1.000
Imazalil	234	115	104	106	32.5	18.8	3.5	1.2	0.999
Norflurazon	112	109	102	104	5.7	1.3	2.9	0.8	1.000
TPP	106	107	104	105	5.5	3.0	3.7	3.1	1.000
Piperonyl Butoxide	100	102	100	104	2.2	3.3	3.5	1.5	1.000
Pyridaphenthion	101	100	102	105	6.4	4.9	3.4	0.8	1.000
Bifenthrin	93	98	98	100	2.9	2.2	1.6	1.8	1.000
Phosalone	121	109	99	101	1.4	2.1	2.1	0.7	1.000
Lambda, cyhalothrin I -II	119	105	97	98	2.0	6.4	2.4	1.6	1.000
Fenarimol	97	101	101	102	6.8	3.9	2.6	2.0	1.000
Coumaphos	107	104	100	101	5.2	2.2	2.8	1.8	1.000
Cypermethrin I-IV	110	106	97	98	1.9	1.4	2.1	2.1	1.000
Esfenvalerate I	122	107	96	96	2.3	2.5	2.4	2.1	1.000

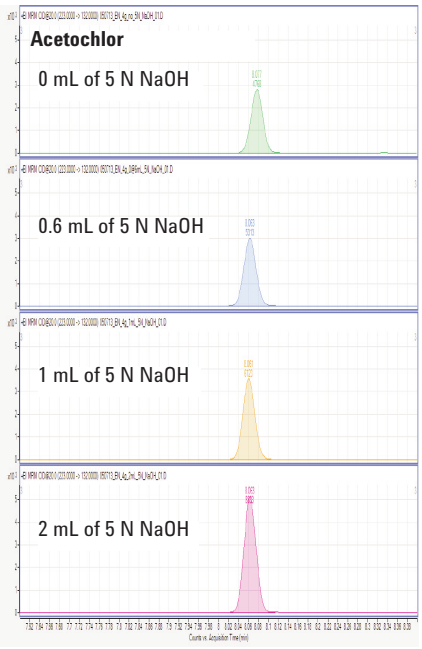
Appendix 2

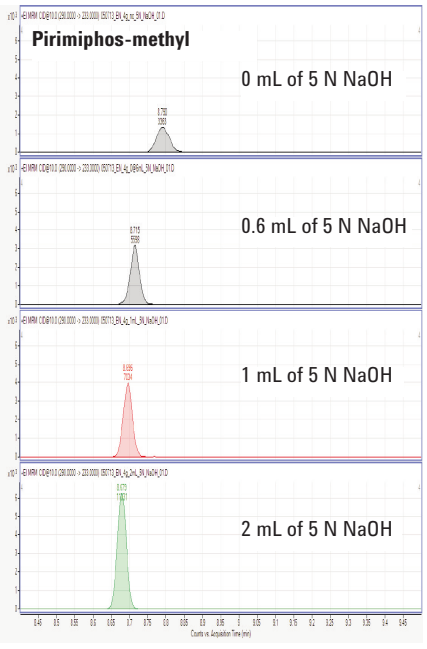
Chromatograms showing pH variation in QuEChERS extraction (from top to bottom, addition of 0, 0.6, 1, and 2 mL 5 N NaOH)

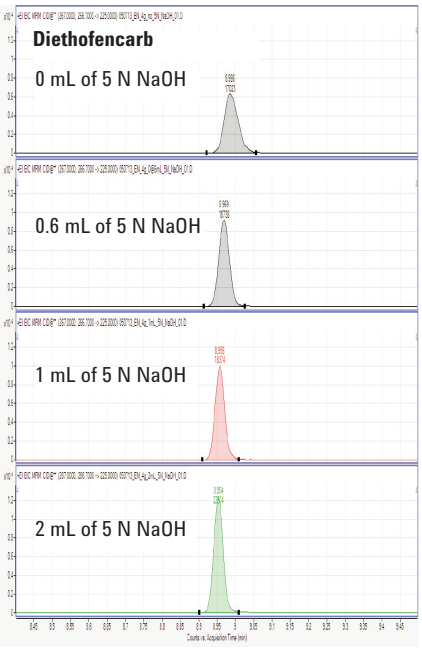


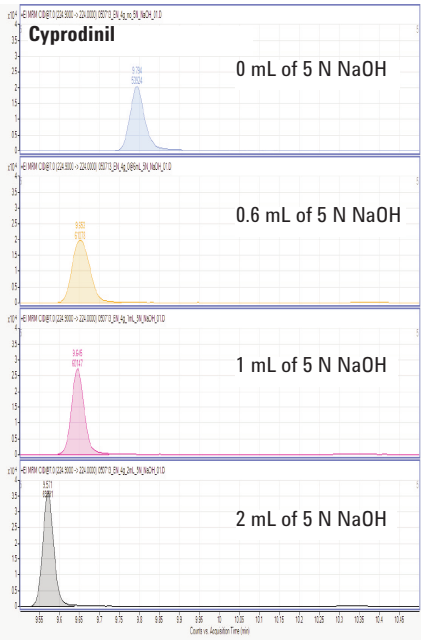


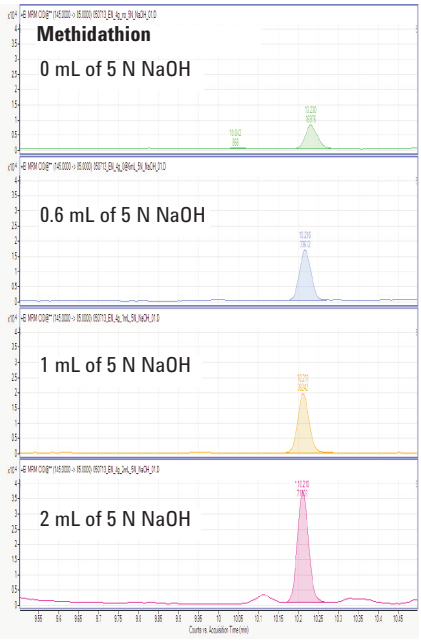


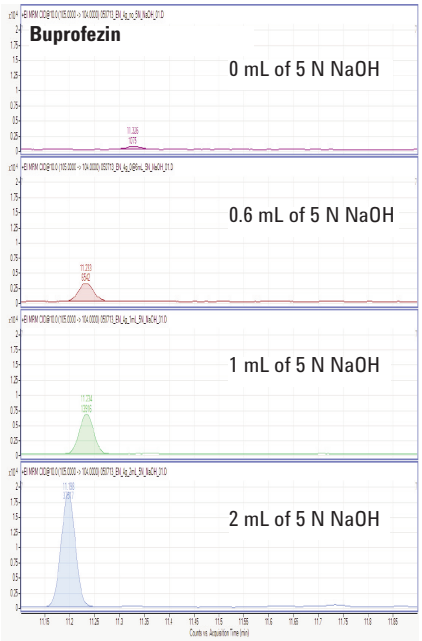


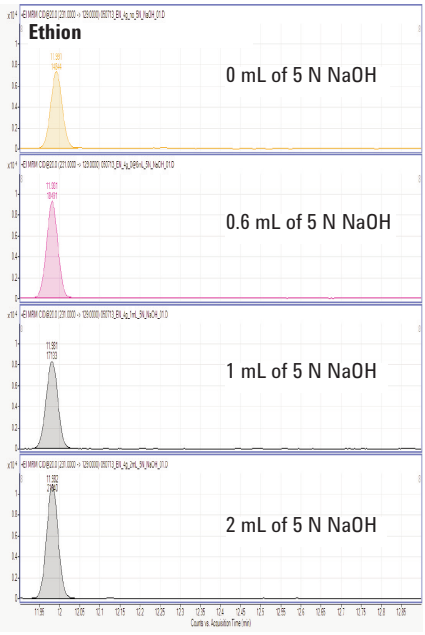


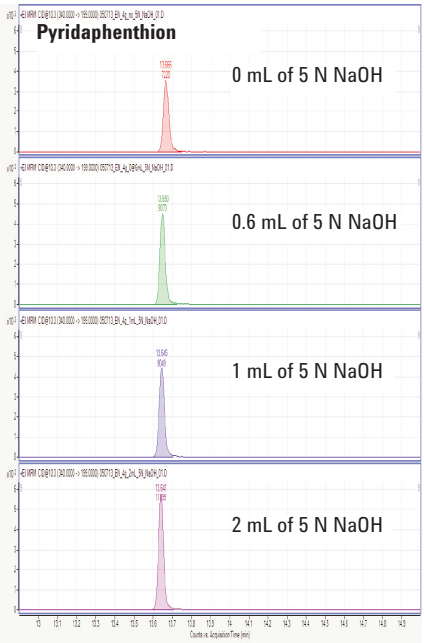


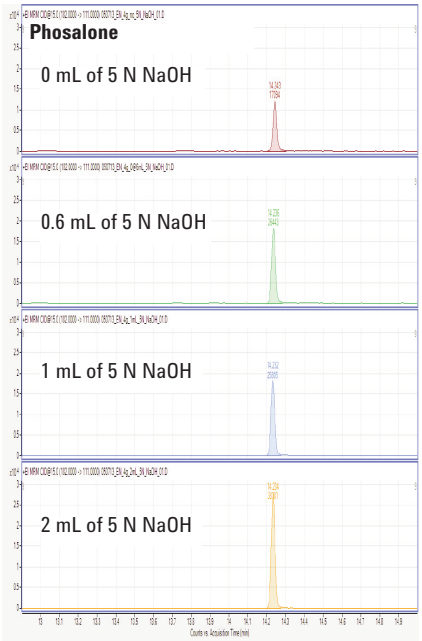

Cyprodinil
0 mL of 5 N NaOH
0.6 mL of 5 N NaOH
1 mL of 5 N NaOH
2 mL of 5 N NaOH

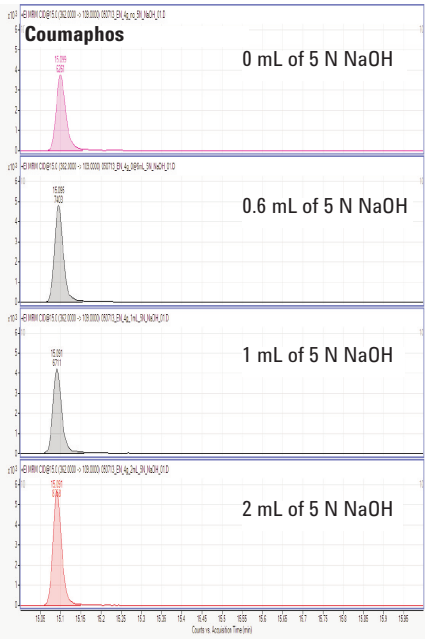

Methidathion
0 mL of 5 N NaOH
0.6 mL of 5 N NaOH
1 mL of 5 N NaOH
2 mL of 5 N NaOH


Buprofezin
0 mL of 5 N NaOH
0.6 mL of 5 N NaOH
1 mL of 5 N NaOH
2 mL of 5 N NaOH


Ethion
0 mL of 5 N NaOH
0.6 mL of 5 N NaOH
1 mL of 5 N NaOH
2 mL of 5 N NaOH


Pyridaphenthion
0 mL of 5 N NaOH
0.6 mL of 5 N NaOH
1 mL of 5 N NaOH
2 mL of 5 N NaOH


Phosalone
0 mL of 5 N NaOH
0.6 mL of 5 N NaOH
1 mL of 5 N NaOH
2 mL of 5 N NaOH



Appendix 3

Preparation of samples from QuEChERS final extract

For recovery samples, add 250 µL of the final extract from dispersive SPE tube, 10 µL AP mix, 20 µL STD mix, and 30 µL ISTD mix to 2 mL autosampler vials as shown in the Table 3.

Table 3. Preparation of final samples in 2 mL vials.

Component	Matrix-matched calibrators	Recovery samples
Final extract from dispersive SPE (µL)	250	250
STD mix (µL)*	20	0
ACN (µL)	0	20
ISTD mix(µL)*	30	30
AP mix (µL)	10	10
Total volume (µL)	310	310

* STD mix and ISTD mix concentration information is given in Appendix 4.

Appendix 4

STD mix, ISTD mix, and AP mix preparation

Table 4. Ten-concentration level STD mix. From the stock solution A, serial dilution was performed with ACN + 1% acetic acid to create 10 concentration levels. For matrix-matched calibrators, STD mix solutions from D to J were used, with the amount shown in Appendix 3 for seven concentration levels.

Solution	ng/mL in ACN + 1% acetic acid
A*	10,000
B	5,000
C	2,500
D	1,000
E	500
F	250
G	100
H	50
I	25
J	10

*A was provided by a worldwide beverage company as a stock solution.

Table 5. Individual stock ISTD preparation.

	Amount	
Component	D10-Parathion	^{13}C-DDT
ISTD (g)	0.01	0.005
ACN (mL)	10	0
Toluene (mL)	0	5
Stock ISTD conc. (ng/mL)	1,000,000	1,000,000

Table 6. Composite ISTD mix solution preparation from individual stock ISTD solutions.

Component	Amount
ACN (mL)	9.8
ISTD stock, d10-parathion (mL)	0.1
ISTD stock, ^{13}C-DDT (mL)	0.1
Total volume (mL)	10
Composite ISTD mix conc. (ng/mL)	10,000

Table 7. Spiking ISTD mix solution preparation.

Component	Amount
Composite ISTD mix (mL)	0.5
ACN + 1% acetic acid (mL)	9.5
Total volume (mL)	10
Spiking ISTD conc. (ng/mL)*	500

* Spiking ISTD mix solution at 500 ng/mL was used for all samples.

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