Quantitative and Repeatability Analysis of Trace Level Pesticides in Plantation Food by GC/MS/MS

Application Note

Food Safety

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Abstract

A multi-residue pesticides analysis method by GC/MS/MS was evaluated for trace analysis of 33 representative pesticides in six different plantation food matrices extracted by QuEChERS method.

This study showed 1 ng/mL or lower LOQ for most pesticides, excellent linearity from LOQ to 100 ng/mL, and great repeatability from 10 injections at 10 ng/mL in matrix.

Introduction

Multi-residue analysis of pesticide in fruits, vegetables, and other foods is always a challenge in sample preparation as well as analytical detection. The required quantitation limit for many pesticides falls below 10 ng/mL (ppb) which demands more sophisticated analytical processes.

Compared to widely used GC/MS analyses, GC/MS/MS techniques provide much better selectivity thus significantly lower system detection limits. For target pesticide analysis in complex matrices, the Agilent 7890/7000 GC/MS Triple Quad (GC/QQQ) Analyzer has a Pesticides and Environmental Pollutants MRM database (p/n G9250AA) of over 1,000 compounds which makes the analytical task easier and productive.

The QuEChERS sample preparation technique was first introduced for pesticide analysis in foods by USDA scientists in 2003. [1] It has been rapidly accepted worldwide for multi-residue pesticide analysis due to its special features known as Quick, Easy, Cheap, Effective, Rugged, and Safe. The QuEChERS extracts can be analyzed by LC and GC combined with MS to determine a wide range of pesticide residues. Agilent QuEChERS extraction kits and dispersive SPE clean-up kits have demonstrated excellent recoveries for the frequently used pesticides in different food matrices. [2-3] However, food extracts processed by QuEChERS method are still very complex containing various matrix residues such as high-boiling indigenous compounds.

The QuEChERS extracts used in GC/MS analyses can cause contamination and deterioration of GC analytical column and MS ion source, resulting in poor data quality due to poor peak shape and loss of responses for active analytes. It also leads to shorter life-time of GC analytical columns and frequent MS maintenance. Therefore, it is necessary to use best techniques and supplies to achieve reliable results and to protect the analytical column and MS ion source.

Column backflushing can be beneficial for the analysis of food extracts because it significantly reduces analysis time and reduces both column head trimming and MS ion source cleaning frequency. [4] Agilent's capillary flow technology (CFT) makes column backflushing routine. [5,6]

Agilent's new Ultra Inert deactivation process significantly improves the inertness and robustness of wool liners. The wool surface area is deactivated thoroughly. The Ultra Inert splitless liners with wool have demonstrated excellent inertness in quantitative analysis of active and difficult pesticides in fruit and vegetable matrices. The Ultra Inert liners with wool also protect the sample flow path better, resulting in extended column lifetime and less frequent MS source maintenance. [7]

Experimental

A representative group of 33 challenging pesticides were selected for trace level analysis in six different plantation food matrices. Plantation food matrix blanks, extracted by QuEChERS AOAC method [1-3], were spiked with the pesticide standards. The spiked matrix samples were then analyzed by GC/MS/MS under Multiple Reaction Monitoring (MRM) mode. A calibration curve from 1 – 100 ng/mL was used for linearity evaluation. A 10 ng/mL QC sample was used to evaluate analysis repeatability. Liner to liner reproducibility study was conducted using four liners.

Chemicals and Reagents

All reagents and solvents were HPLC or analytical grade. Acetonitrile (AcN) was from Honeywell B&J (Muskegon, MI, USA). Ultra Resi-analyzed grade Acetone was from J.T.Baker (Phillipsburg, NJ, USA). Acetic acid was from Sigma-Aldrich (St Louis, MO, USA). The pesticide standards and internal standard (triphenyl phosphate, TPP) were purchased from Sigma-Aldrich (St Louis, MO, USA), Chem Service (West Chester, PA, USA), or Ultra Scientific (North Kingstown, RI, USA).

Solutions and Standards

A 1% acetic acid in AcN reagent blank solution was prepared by adding 1 mL of glacial acetic acid into 100 mL of AcN. This solution was also used as extraction solvent for the QuEChERS method.

Individual pesticide standard stock solutions, at 2 mg/mL each, were made in Acetone and stored at -20 °C. A 20 µg/mL 33-pesticide mixture was made in Acetone by dilution of individual pesticide stock solutions, and stored at 4 °C. In order to minimize matrix dilution in the calibration standards, a 500 ng/mL intermediate spiking solution was freshly made in the corresponding matrix blank from the 20 µg/mL standard mixture. The intermediate spiking solution in matrix was then used to spike five matrix-matched calibration standards of 1, 5, 10, 50, and 100 ng/mL and a 10 ng/mL QC standard.

Internal standard (IS) stock solution of triphenyl phosphate (TPP) at 2 mg/mL was made in Acetone. A 20 µg/mL IS spiking solution in Acetonitrile was made from the IS stock solution, and stored at 4 °C. Proper volume of IS spiking solution was then added into all samples to generate a concentration of 100 ng/mL.

Matrix Blank Preparation

White flour, strawberry, pear, orange, pepper, and spinach were selected as matrix samples in this study. The extraction procedure was described in detail in Agilent Application Notes [2,3]. The fruits and vegetables were frozen, chopped, and then homogenized thoroughly. In brief, 15 g of homogenous sample (except flour sample) was extracted using 15 mL of Acetonitrile with 1% acetic acid and separated into aqueous phase by the addition of BondElut QuEChERS AOAC extraction salt packet (p/n 5982-5755). For flour sample, 5 g of homogenous sample was mixed with 10 mL of water and soaked overnight. This mixture was then extracted following the QuEChERS procedure. After centrifugation, the supernatant was transferred and cleaned up using the general dispersive SPE kit (p/n 5982-5022). After vortex and centrifuge, the supernatant was transferred into vials as matrix blank for subsequent experiments. These individual matrix blanks were stored at 4 °C.

Instrumentation

All analyses were done on an Agilent 7890 GC equipped with an Agilent 7693B autosampler and an Agilent 7000 series GC/MS Triple Quadrupole system. [7] An Agilent Ultra Inert GC column, HP-5MS UI, was used to provide analyte separation and a highly inert flow path into the detector. Table 1 lists the instrument parameters used in this study. Table 2 lists consumable supplies used in this study, and Table 3 lists the MRM settings for 33 target analytes. Agilent MRM Database (p/n G9250AA) was used directly to build up the MS acquisition method for the target analytes.

Backflushing was used to shorten analysis time for samples that contain high-boiling matrix residues and reduce system maintenance. [2, 4] The instrument configuration was very similar to the configuration shown in Figure 1B in a previous setup [4], except no retention gap was used in this study. Retention time locking (RTL) was used to eliminate the need for adjusting time segment windows of MRM groups. [6] The runtime was 23 minutes with an additional 2 minutes for backflush. For each pesticide, two MRM transitions were selected for quantitation and qualification. However, different transitions might be used for quantitation in different matrices to minimize matrix effect. Therefore, it is critical to review the data in matrix before setting up a quantitation method for this matrix.

Table 1. Instrument Parameters for Agilent GC/MS/MS System

GC	Agilent 7890 Series GC
Autosampler	Agilent 7693 Autosampler and sample tray 5-µL syringe (p/n 5181-5246), 1 µL injection volume Postinj solvent A (Acetone) washes: 3 Postinj solvent B (Acetonitrile) washes: 3 Sample pumps: 3
Carrier gas	Helium, constant pressure
Inlet	Multi-Mode Inlet (MMI)
Inlet temperature	280 °C
Injection mode	Pulsed splitless mode
Injection pulse pressure	36 psi until 1 min
Purge flow to split vent	50 mL/min at 1 min
Inlet pressure	18.35 psi (RT locked) during run, and 1.0 psi during backflush
RT locking	Chlorpyrifos methyl at 8.298 min
Oven profile	100 °C for 2 min, to 150 °C at 50 °C/min, to 200 °C at 6 °C/min, to 280 °C at 16 °C/min and hold for 6 min
Post run	2 min at 280 °C
Capillary flow technology	Purged Ultimate Union (p/n G3182-61580) - used for backflushing the analytical column and inlet. Aux EPC gas: Helium plumbed to Purged Ultimate Union
Bleed line	0.0625-in od × 0.010-in id × 100 cm, 316 SS tubing, on top of the oven
Aux pressure	4 psi during run, 75 psi during backflushing
Analytical column	HP-5MS UI, 0.25 mm × 15 m, 0.25 µm (p/n 19091-431UI)
Column connections	Between Inlet and Purged Ultimate Union (p/n G3182-61580)
Restrictor	Inert Fused Silica tubing, 0.65 m × 0.15 mm (p/n 160-7625-5)
Restrictor connections	Between Purged Ultimate Union and the MS
MS	Agilent 7000 Triple Quadrupole GC/MS
Mode	MRM
Database	Agilent Pesticides and Environmental Pollutants Database (p/n G9250AA)
Transfer line temperature	280 °C
Source temperature	300 °C
Quad temperature	Q1 and Q2 = 150 °C
Solvent delay	2.3 min
Collision gas flows	Helium quench gas at 2.35 mL/min, N_2 collision gas at 1.5 mL/min
MS resolution	MS1 and MS2 = 1.2 amu (Low resolution or Wide setting)

Table 2. Consumable Supplies

Vials	Amber, write-on spot, 100/pk (p/n 5182-0716)
Vial caps	Blue screw cap, 100/pk (p/n 5182-0717)
Vial inserts	150 µL glass w/polymer feet, 100/pk (p/n 5183-2088)
Septum	Advanced Green Non-Stick 11 mm, 50/pk (p/n 5183-4759)
Column ferrules	0.4 mm id, 85/15 Vespel/Graphite, 10/pk (p/n 5181-3323)
Liner O-rings	Non-stick liner O-ring, 10/pk (p/n 5188-5365)
Capillary flow technology	Purged Ultimate Union (p/n G3182-61580) Internal nut, 1/pk (p/n G2855-20530) SilTite metal ferrules, for 0.10–0.25 mm id columns, 10/pk (p/n 5188-5361)
Inlet liners	Agilent Ultra Inert deactivated single taper splitless liner with wool, 1/pk (p/n 5190-2293), 5/pk (p/n 5190-3163)

Table 3. Quantifier and Qualifier MRM Transitions for 33 Pesticides

Analytes (Peak number on chromatogram)	MRM 1 (CE)	MRM 2 (CE)
Methamidophos (1)	141.0 → 95.0 (6)	95.0 → 79.0 (13)
Dichlorvos (2)	185.0 → 93.0 (15)	108.9 → 79.0 (5)
Acephate (3)	136.0 → 42.0 (6)	136.0 → 94.0 (14)
Mevinphos (4)	127.0 → 109.0 (10)	191.9 → 127.0 (10)
2-phenylphenol (5)	169.9 → 115.0 (30)	169.9 → 141.0 (15)
Omethoate (6)	156.1 → 79.0 (15)	156.1 → 110.0 (20)
Dimenthoate (7)	125.0 → 47.0 (15)	143.0 → 111.0 (10)
Altrazine (8)	214.9 → 58.0 (11)	200.0 → 94.1 (20)
Lindane (9)	180.8 → 145.0 (12)	218.8 → 183.0 (20)
Diazinon (10)	304.0 → 178.9 (15)	178.9 → 121.0 (28)
Chlorothalonil (11)	265.8 → 133.0 (53)	265.8 → 169.9 (28)
Chloropyrifos methyl (12)*	285.8 → 271.0 (16)	287.8 → 93.0 (26)
Vinclozolin (13)	211.8 → 172.0 (15)	211.8 → 145.0 (15)
Carbaryl (14)	143.9 → 116.0 (15)	143.9 → 89.0 (50)
Tolclofos methyl (15)	264.8 → 250.0 (15)	264.8 → 93.0 (50)
Dichlorfluanid (16)	223.9 → 123.0 (8)	223.9 → 77.0 (45)
Aldrin (17)	262.8 → 193.0 (30)	262.8 → 191.0 (30)
Malathion (18)	173.0 → 99.0 (15)	157.9 → 125.0 (5)
Dichlorobenzophenone (19)	249.9 → 139.0 (5)	249.9 → 214.9 (15)
Pirimiphos ethyl (20)	318.0 → 166.0 (12)	333.1 → 318.0 (5)
Toloyfluanid (21)	237.9 → 137.0 (15)	237.9 → 91.1 (50)
Procymidone (22)	282.9 → 96.0 (10)	282.9 → 67.1 (40)
Endrin (23)	262.8 → 193.0 (35)	262.8 → 191.0 (35)
Ethion (24)	230.8 → 129.0 (25)	230.8 → 175.0 (35)
Endosulfan sulfate (25)	271.7 → 236.8 (20)	386.7 → 253.0 (5)
DDT (26)	234.9 → 165.0 (20)	236.8 → 165.0 (5)
TPP (IS)	325.9 → 169.0 (30)	325.9 → 233.0 (27)
Endrin ketone (27)	316.7 → 101.0 (20)	316.7 → 245.0 (20)
Iprodione (28)	313.8 → 56.0 (20)	186.9 → 123.0 (25)
Phosmet (29)	159.9 → 77.0 (30)	159.9 → 133.1 (20)
Phosalone (30)	181.9 → 138.0 (5)	366.9 → 182.0 (5)
Permethrin (31)	183.0 → 168.1 (15)	183.0 → 153.1 (15)
Coumaphos (32)	361.9 → 109.0 (15)	361.9 → 81.0 (35)
Deltamethrin (33)	180.9 → 152.0 (26)	252.8 → 93.0 (20)

* Chloropyrifos methyl was used for RT locking.

Results and Discussion

The purpose of this study was to evaluate the GC/MS/MS performance using representative pesticides in six different matrices at trace level, including pear, orange, strawberry, flour, pepper, and spinach. With effective deactivation on wool, the Agilent Ultra Inert liners with wool provided excellent inertness as well as superior protection to the column and MS ion source. Thus, Ultra Inert liners provide better peak shape and response consistency, especially for the very active pesticides. [7] In this study, Ultra Inert liner with wool was also compared with Siltek Cyclosplitter liner on peak shape and response repeatability.

The system performance evaluated in this study includes: linearity in the range of 1 to 100 ng/mL, the limit of quantitation (LOQ), the injection repeatability at 10 ng/mL, and liner-to-liner reproducibility. All these evaluations were done in all six matrices. Matrix effect, regarding matrix interferences and impact on the system robustness, was also part of the study. Some pesticides showed consistent responses in different matrices, but many pesticides had different responses in different matrices due to either matrix enhancement or matrix suppression. Therefore, it is important to use matrix-matched calibrations to achieve accurate quantitation results.

The testing sequence included 10 injections of 10 ng/mL QC samples in each matrix in the order of orange, pear, strawberry, flour, pepper, and spinach. Calibration standards and matrix blanks were also included in the sequence. There were more than 80 samples in a sequence for each liner evaluated. Because Omethoate is one of the most challenging pesticides, which can be negatively impacted by the matrix, it is used as the probe in Figure 1 to show the benefit of using Ultra Inert liner with wool.

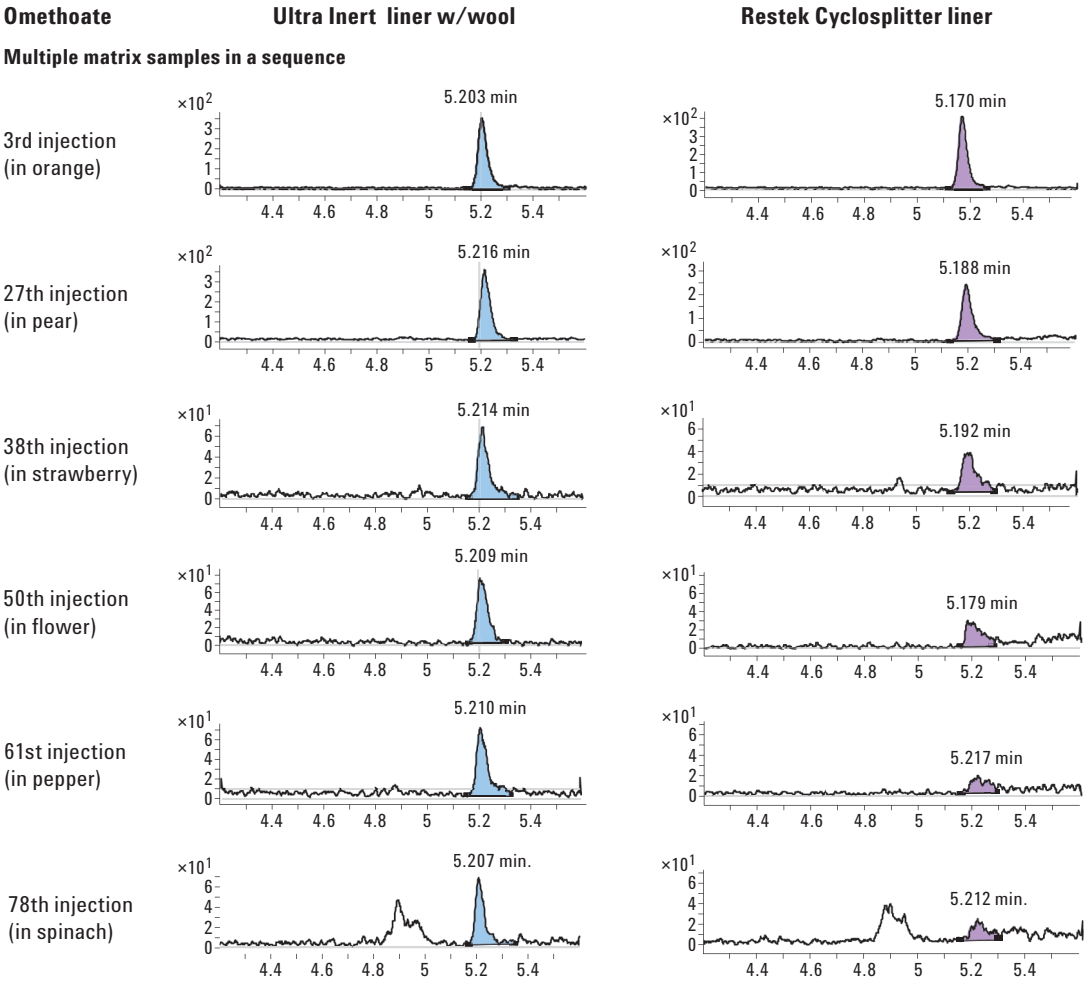

Figure 1. Peak shape comparison of Omethoate between Ultra Inert single-taper liner with wool and Restek Siltek Cyclosplitter double-taper liner. Sample was a 10 ng/mL pesticide mixture spiked in each matrix.

Trace analysis in pear

Figure 2 shows the GC/QQQ MRM chromatograms of pear matrix blank and pear matrix spiked at 1 ng/mL. After careful selection of MRM transitions based on matrix, the pear matrix blank still showed a few interference peaks in several MRM transitions. Most of the interfering peaks were chromatographically separated and did not interfere with the quantitation results. However, there was an interfering peak at the same retention time as Methamidophos. This interfering peak increased the target LOQ to 5 ng/mL. The same situation happened also to 2-phenylphenol, and the LOQ had to be increased to 5 ng/mL. Omethoate and Endrin ketone showed low responses in Figure 2, but they both gave acceptable S/N ratio at 1 ng/mL. Deltamethrin's response was always low. Although it was detected at 1 ppb with S/N ratio of 3, it was more reasonable to set the target LOQ at 5 ng/mL. Many pesticides could achieve lower LOQ (< 1 ng/mL) in pear matrix with acceptable S/N ratios. These pesticides are labeled with asterisks in Table 4. Table 4 shows the quantitation results, except Methamidophos, 2-phenylphenol, and Deltamethrin, all of the other 30 pesticides achieves 1 ng/mL or lower LOQ in pear matrix. The repeatability of 10 injections of the 10 ng/mL QC sample was excellent, showing less than 15% RSD for all 33 pesticides, even for the most challenging pesticides like Omethoate, Acephate, and DDT.

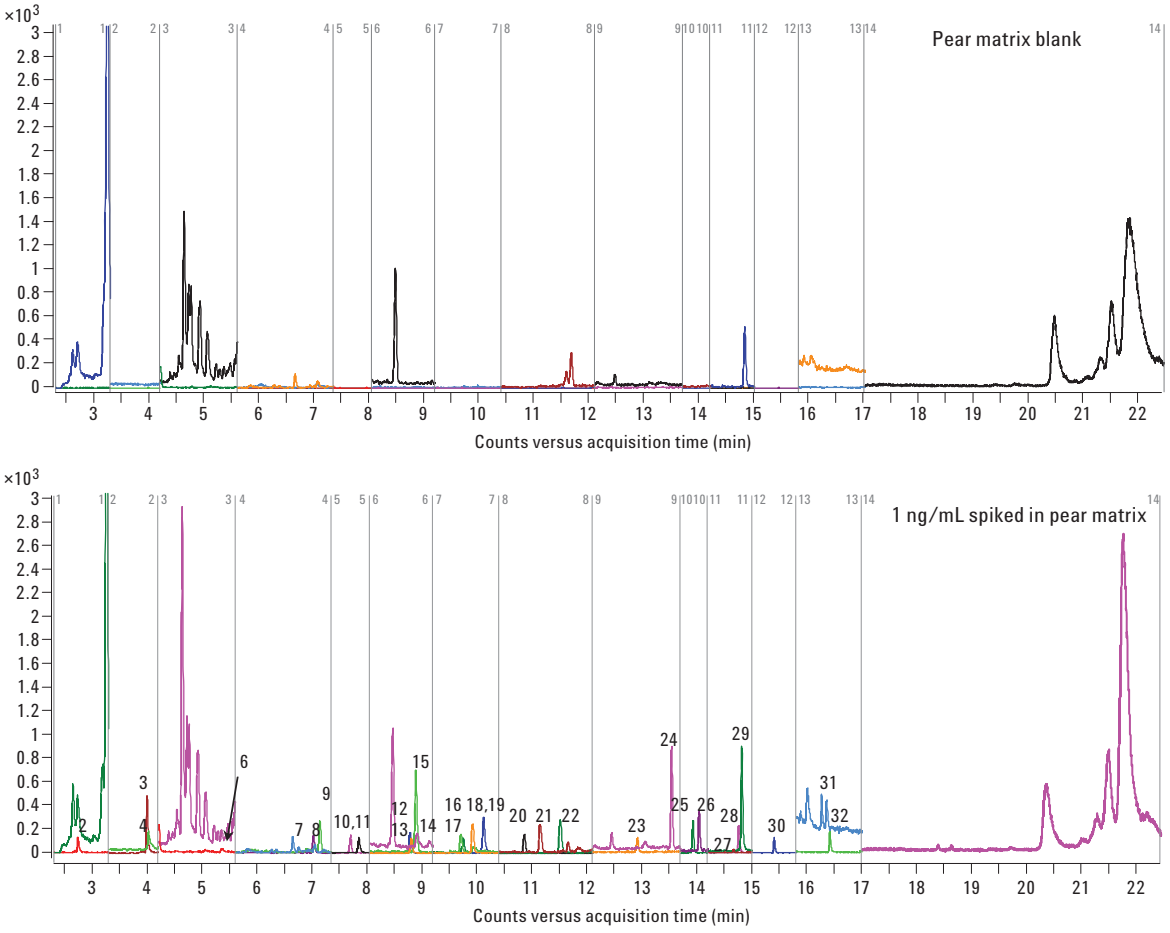

Figure 2. GC/QQQ MRM chromatograms for pear matrix blank and pear matrix spiked with 1 ng/mL pesticides. Refer to Table 3 for peak identification. Methamidophos (1), 2-phenylphenol (5), and Deltamethrin (33) were not identified at 1 ng/mL in pear matrix due to low responses or matrix interferences.

Table 4. Quantitation Results (Pear Matrix)

	RSD(%) for n = 10 injections of 10 ng/mL pear sample					
Pesticides	**UI liner number 1**	**UI liner number 2**	**UI liner number 3**	**UI liner number 4**	**LOQ (ng/mL)**	**R^2 (LOQ – 100 ng/mL)**
Methamidophos	10.7	8.9	8.5	9.7	5	0.9859
Dichlorvos *	3.5	3.5	1.5	1.4	1	0.9885
Mevinphos	2.5	1.4	2.0	1.2	1	0.9938
Acephate	4.5	5.6	3.6	2.5	1	0.9924
2-phenylphenol	2.2	1.4	1.6	2.9	5	0.9949
Omethoate	6.9	9.0	4.7	4.3	1	0.9936
Dimenthoate	3.1	2.9	2.6	1.6	1	0.9899
Altrazine *	1.4	2.1	2.0	1.8	1	0.9842
Lindane *	1.9	1.7	1.7	1.5	1	0.9836
Diazinon *	1.6	2.5	2.0	0.9	1	0.9962
Chlorothalonil *	3.8	6.2	3.3	1.3	1	0.9953
Chloropyrifos methyl *	1.7	1.6	2.4	1.3	1	0.9970
Vinclozolin *	3.2	4.4	3.0	1.0	1	0.9955
Tolclofos methyl *	2.1	2.3	3.1	1.0	1	0.9965
Carbaryl	1.8	6.5	1.3	2.1	1	0.9935
Dichlorfluanid *	2.3	2.7	2.3	1.9	1	0.9958
Aldrin *	2.0	1.7	2.4	2.4	1	0.9953
Malathion *	2.9	2.3	1.8	1.6	1	0.9975
Dichlorobenzophenone *	2.8	1.9	2.4	0.8	1	0.9959
Pirimiphos ethyl *	3.5	4.0	1.7	2.5	1	0.9944
Tolylfluanid *	1.7	2.2	3.1	2.1	1	0.9945
Procymidone *	1.9	1.2	1.9	1.3	1	0.9942
Endrin *	4.0	2.2	3.0	1.2	1	0.9971
Ethion *	3.5	1.0	2.1	2.1	1	0.9958
Endosulfan sulfate *	2.0	3.5	2.0	0.9	1	0.9987
DDT *	8.2	11.9	13.6	9.3	1	0.9965
Endrin ketone	4.9	6.0	6.2	2.5	1	0.9989
Iprodione *	2.6	3.6	3.0	1.4	1	0.9998
Phosmet *	2.5	5.6	6.1	3.7	1	0.9983
Phosalone	1.4	2.8	4.4	3.0	1	0.9937
Permethrin	2.0	2.7	3.0	1.9	1	0.9998
Coumaphos *	2.2	5.2	4.5	2.5	1	0.9972
Deltamethrin	5.0	7.5	3.5	7.3	5	0.9973

* Pesticides that can achieve lower LOQ with current method.

Trace analysis in orange

Figure 3 shows the GC/QQQ MRM chromatograms of orange matrix blank and orange matrix spiked at 1 ng/mL. Orange matrix showed less interfering peaks as seen in the matrix blank chromatogram. Peaks shown around Deltamethrin did not affect the analysis of Deltamethrin. Although 2-phenylphenol was present in the orange blank, the 1 ng/mL LOQ was achieved due to the high response and relatively clean matrix background. Overall, 32 pesticides reached the 1 ng/mL LOQ, except Deltamethrin at 5 ng/mL. Pesticides that can achieve lower LOQ (< 1 ng/mL) are labeled with asterisks in Table 5. Table 5 shows the quantitation and good linearity results in orange matrix. The repeatability of 10 injections of the 10 ng/mL QC sample was excellent for most analytes with less than 15% RSD. DDT's RSD was a little higher than 15% from 10 injections, due to decreasing response in orange matrix.

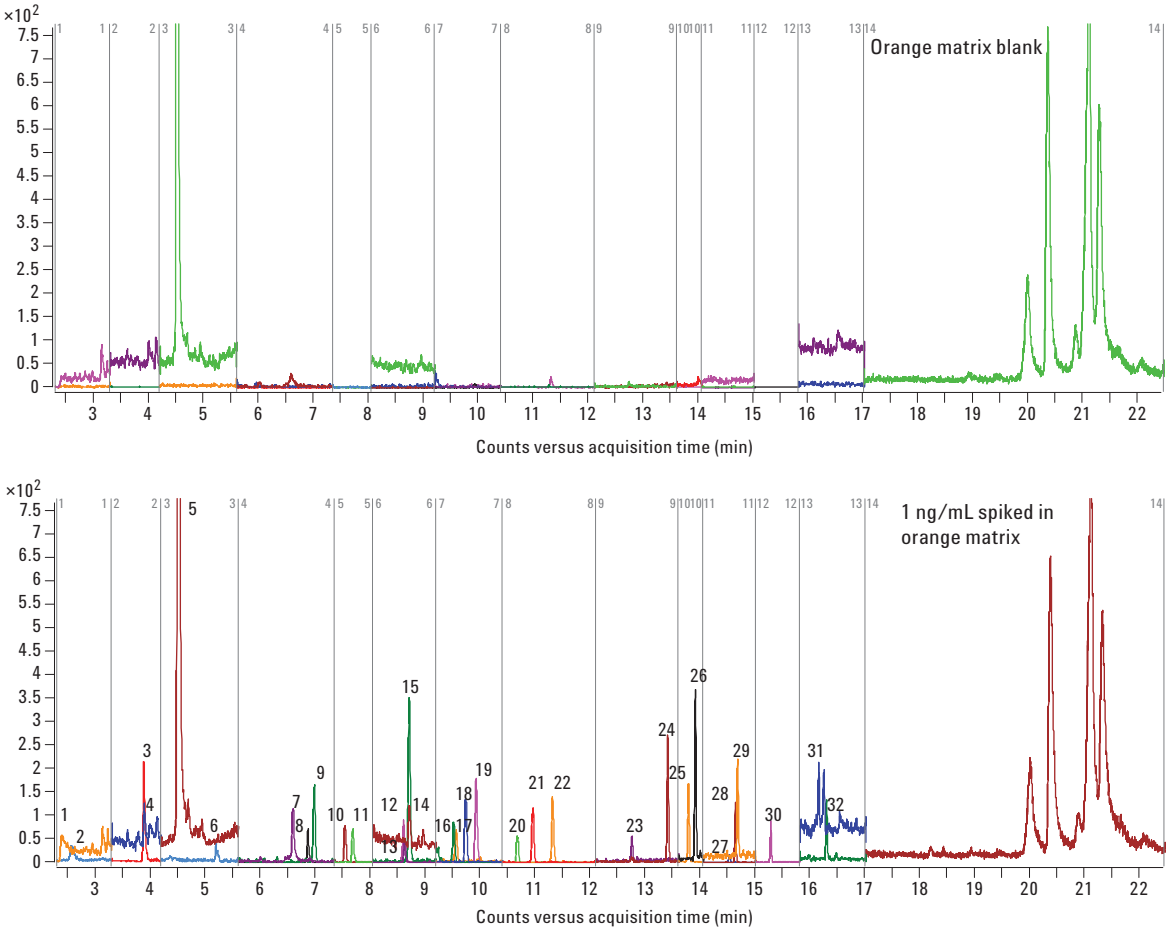

Figure 3. GC/QQQ MRM chromatograms for orange matrix blank and orange matrix spiked with 1 ng/mL pesticides. Refer to Table 3 for peak identification. Deltamethrin (33) was not identified at 1 ng/mL in orange matrix due to low responses.

Table 5. Quantitation Results (Orange Matrix)

	RSD(%) for n = 10 injections of 10 ng/mL orange sample					
Pesticides	**UI liner number 1**	**UI liner number 2**	**UI liner number 3**	**UI liner number 4**	**LOQ (ng/mL)**	**R^2 (LOQ – 100 ng/mL)**
Methamidophos	4.9	3.2	5.8	4.5	1	0.9966
Dichlorvos *	1.8	2.3	6.2	1.5	1	0.9996
Mevinphos*	2.6	2.2	8.4	0.7	1	0.9995
Acephate	11.2	4.4	10.3	8.6	1	0.9996
2-phenylphenol*	1.2	1.4	3.9	1.5	1	0.9977
Omethoate	12.0	8.9	9.5	14.6	1	0.9993
Dimenthoate	5.6	2.8	8.2	5.5	1	0.9995
Altrazine *	1.2	2.0	5.7	1.0	1	0.9998
Lindane *	2.5	2.8	5.2	1.4	1	0.9997
Diazinon *	1.5	2.8	3.0	2.1	1	0.9971
Chlorothalonil *	3.3	2.0	4.7	4.0	1	0.9987
Chloropyrifos methyl *	3.2	3.3	2.9	1.6	1	0.9996
Vinclozolin *	2.1	2.6	7.1	1.8	1	0.9998
Tolclofos methyl *	1.6	2.1	2.8	1.1	1	0.9999
Carbaryl	3.9	4.0	3.4	4.1	1	0.9991
Dichlorfluanid *	4.1	4.3	8.3	2.4	1	0.9965
Aldrin *	2.1	3.5	2.7	2.0	1	0.9997
Malathion *	4.2	4.0	4.5	2.2	1	0.9983
Dichlorobenzophenone *	2.2	1.1	3.2	2.7	1	0.9999
Pirimiphos ethyl *	3.0	2.2	5.0	1.3	1	0.9981
Tolylfluanid *	3.4	3.0	5.6	1.9	1	0.9976
Procymidone *	1.2	1.5	3.1	0.9	1	0.9993
Endrin *	2.9	1.7	3.2	2.9	1	0.9983
Ethion *	6.6	6.9	7.9	3.5	1	0.9937
Endosulfan sulfate *	6.4	3.1	8.4	3.7	1	0.9995
DDT *	14.1	15.1	15.8	11.3	1	0.9924
Endrin ketone	6.2	8.0	8.1	3.4	1	0.9987
Iprodione *	4.0	3.3	7.9	1.1	1	0.9992
Phosmet *	11.6	9.5	9.3	9.5	1	0.9993
Phosalone*	5.7	6.3	5.8	4.8	1	0.9955
Permethrin	2.4	2.2	4.0	1.7	1	0.9999
Coumaphos *	7.0	7.4	7.6	7.7	1	0.9979
Deltamethrin	5.6	6.1	4.3	7.1	5	0.9993

* Pesticides that can achieve lower LOQ with current method.

Trace analysis in strawberry

Figure 4 shows the GC/QQQ MRM chromatograms of strawberry matrix blank and strawberry matrix spiked at 1 ng/mL. Strawberry matrix showed clean background for all MRM transitions, except 2-phenylphenol. The matrix peaks around 2-phenylphenol raised its LOQ to 5 ng/mL. Methamidophos, Omethoate, and Deltamethrin also had a 5 ng/mL LOQ due to low responses from the 1 ng/mL sample. Pesticides that can achieve lower LOQ (< 1 ng/mL) in strawberry matrix are labeled with asterisks in Table 6. Table 6 shows the quantitation and good linearity results. The repeatability of 10 injections of the 10 ng/mL QC sample was less than 15% RSD for most analytes. The repeatability of Omethoate was > 20% RSD. Interestingly, DDT showed good repeatability with < 10% RSD. The results showed that matrix affected pesticide responses (MRM transitions) differently.

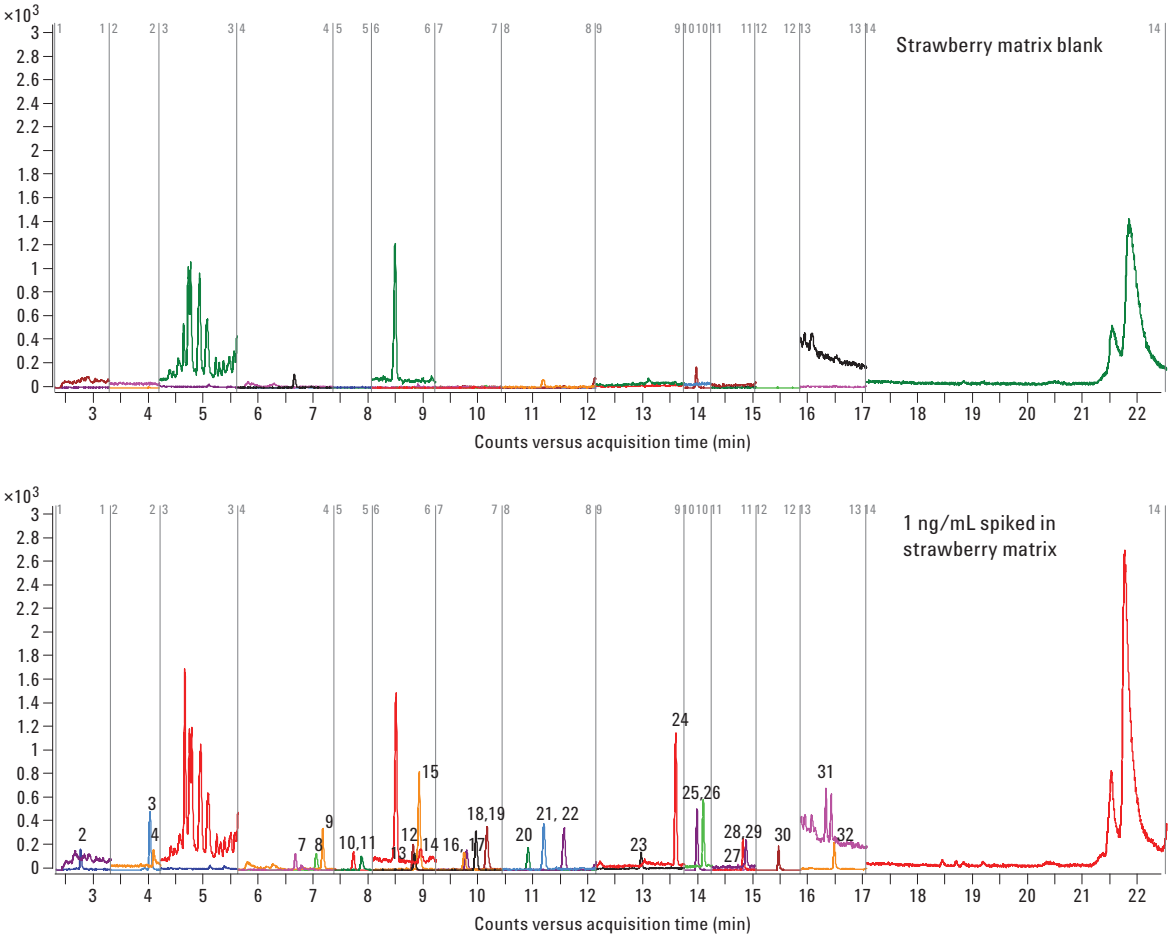

Figure 4. GC/QQQ MRM chromatograms for strawberry matrix blank and strawberry matrix spiked with 1 ng/mL pesticides. Refer to Table 3 for peak identification. Methamidophos (1), 2-phenylphenol (5), Omethoate (6) and Deltamethrin (33) were not identified at 1 ng/mL in strawberry matrix due to low responses or matrix interferences.

Table 6. Quantitation Results (Strawberry Matrix)

	RSD(%) for n = 10 injections of 10 ng/mL strawberry sample					
Pesticides	**UI liner number 1**	**UI liner number 2**	**UI liner number 3**	**UI liner number 4**	**LOQ (ng/mL)**	**R^2 (LOQ – 100 ng/mL)**
Methamidophos	7.8	5.1	5.5	4.2	5	0.9845
Dichlorvos *	2.0	1.8	3.5	2.0	1	0.9915
Mevinphos*	1.4	3.5	1.3	2.1	1	0.9914
Acephate	14.4	16.5	12.8	15.5	1	0.9946
2-phenylphenol	2.5	1.9	2.7	4.3	5	0.9946
Omethoate	26.1	27.2	20.9	24.1	5	0.9998
Dimenthoate*	5.1	7.0	5.3	10.2	1	0.9940
Altrazine *	2.4	2.7	1.3	2.4	1	0.9936
Lindane *	1.7	2.0	1.7	1.8	1	0.9914
Diazinon *	1.0	1.7	2.4	1.8	1	0.9910
Chlorothalonil *	4.6	5.0	3.8	4.5	1	0.9885
Chloropyrifos methyl *	2.2	3.9	2.4	2.3	1	0.9920
Vinclozolin *	2.4	2.0	1.8	1.8	1	0.9930
Tolclofos methyl *	1.0	1.5	2.0	0.9	1	0.9915
Carbaryl	5.2	6.2	4.6	7.0	1	0.9968
Dichlorfluanid *	4.3	6.2	4.1	4.8	1	0.9900
Aldrin *	2.7	2.5	3.1	2.6	1	0.9935
Malathion *	2.3	3.1	3.2	3.6	1	0.9901
Dichlorobenzophenone *	1.4	1.3	1.2	1.5	1	0.9937
Pirimiphos ethyl *	1.6	2.8	1.8	3.5	1	0.9907
Tolylfluanid *	7.8	9.0	6.3	6.9	1	0.9922
Procymidone *	1.7	2.0	1.2	1.8	1	0.9931
Endrin *	2.5	2.3	2.8	1.6	1	0.9953
Ethion *	2.2	2.3	1.9	1.9	1	0.9939
Endosulfan sulfate *	3.5	3.5	2.6	3.3	1	0.9962
DDT *	9.7	12.0	7.2	8.3	1	0.9931
Endrin ketone	5.1	5.3	4.0	5.2	1	0.9941
Iprodione *	3.0	2.2	3.0	1.8	1	0.9944
Phosmet *	10.4	9.8	9.6	9.7	1	0.9897
Phosalone	5.9	3.3	4.5	3.7	1	0.9914
Permethrin	1.3	1.9	2.3	2.7	1	0.9954
Coumaphos *	7.1	7.8	7.0	6.1	1	0.9939
Deltamethrin	5.0	7.0	9.5	11.6	5	0.9897

* Pesticides that can achieve lower LOQ with current method.

Trace analysis in flour

Figure 5 shows the GC/QQQ MRM chromatograms of flour matrix blank and flour matrix spiked at 1 ng/mL. Flour matrix showed clean background for all MRM transitions, except 2-phenylphenol. The LOQ of Carbaryl was set to 5 ng/mL due to its low response in flour. All of the other 32 pesticides achieved 1 ng/mL LOQ. The co-eluting interference peak contributed to less than 20% of 2-phenylphenol's response at 1 ng/mL level. The responses of Methamidophos, Omethoate and Deltamethrin were good and achieved 1 ng/mL LOQ in flour. Pesticides that can achieve lower LOQ (< 1 ng/mL) in flour matrix are labeled with asterisks in Table 7. Table 7 shows the quantitation and good linearity results. The repeatability of 10 injections of the 10 ng/mL QC sample was less than 15% RSD for all analytes.

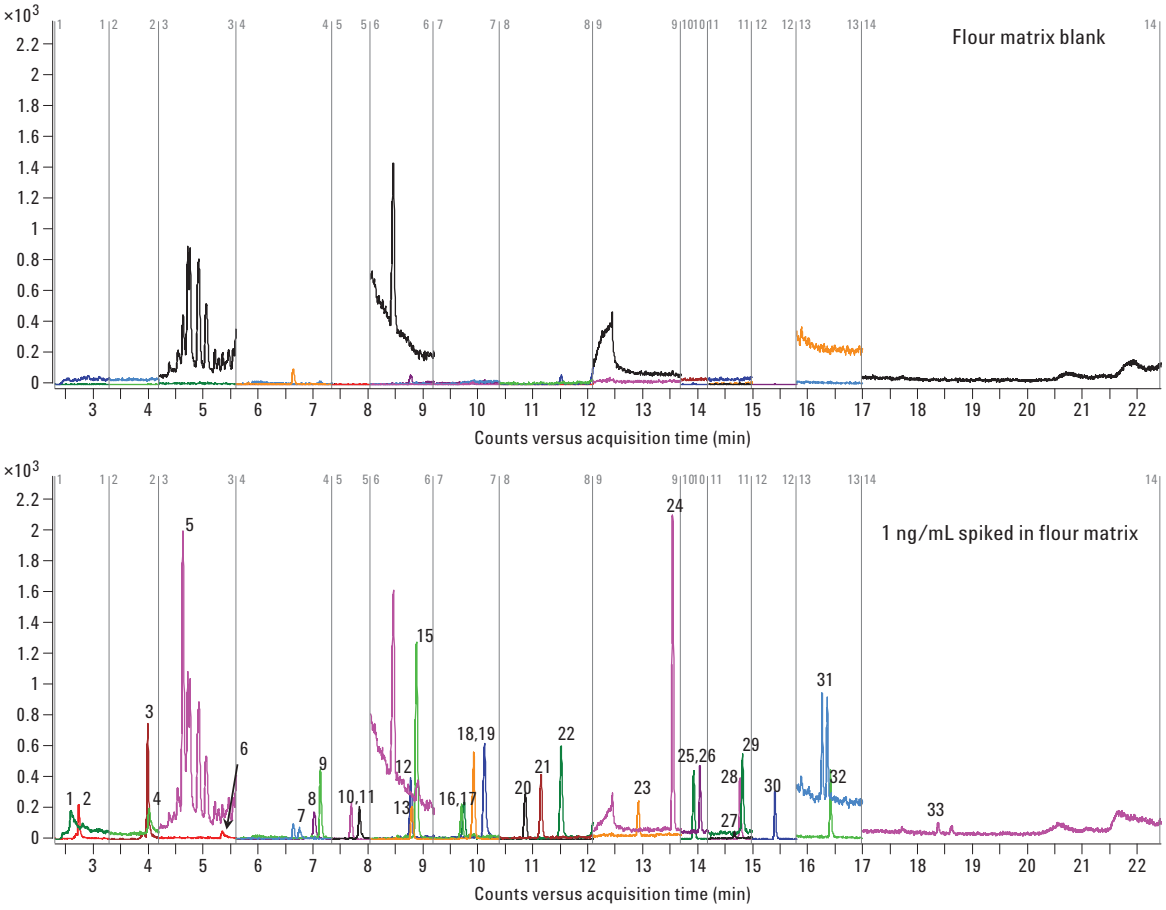

Figure 5. GC/QQQ MRM chromatograms for flour matrix blank and flour matrix spiked with 1 ng/mL pesticides. Refer to Table 3 for peak identification. Carbaryl (14) was not identified at 1 ng/mL in flour matrix due to low responses.

Table 7. Quantitation Results (Flour Matrix)

	RSD(%) for n = 10 injections of 10 ng/mL flour sample					
Pesticides	**UI liner number 1**	**UI liner number 2**	**UI liner number 3**	**UI liner number 4**	**LOQ (ng/mL)**	**R^2 (LOQ – 100 ng/mL)**
Methamidophos	3.4	2.8	4.4	3.4	1	0.9983
Dichlorvos *	1.1	2.4	2.1	2.8	1	0.9960
Mevinphos*	1.2	1.1	1.5	1.3	1	0.9960
Acephate	7.9	4.4	4.9	3.3	1	0.9994
2-phenylphenol	1.3	2.3	1.8	4.0	1	0.9929
Omethoate	13.4	10.7	4.5	3.9	1	0.9991
Dimenthoate*	4.9	4.3	4.6	3.6	1	0.9953
Altrazine *	2.7	2.0	1.8	2.3	1	0.9974
Lindane *	2.5	2.1	2.1	1.8	1	0.9947
Diazinon *	3.4	4.0	4.7	2.9	1	0.9943
Chlorothalonil *	4.2	3.7	3.4	4.6	1	0.9945
Chloropyrifos methyl *	3.3	2.9	3.6	2.4	1	0.9954
Vinclozolin *	2.4	2.2	2.4	2.4	1	0.9966
Tolclofos methyl *	2.5	2.3	2.3	1.9	1	0.9958
Carbaryl	6.5	7.9	7.8	12.5	5	0.9956
Dichlorfluanid *	4.5	5.1	4.0	4.9	1	0.9975
Aldrin *	2.7	1.9	2.8	1.4	1	0.9968
Malathion *	3.6	3.4	2.9	2.5	1	0.9959
Dichlorobenzophenone *	1.2	1.8	1.4	1.4	1	0.9965
Pirimiphos ethyl *	4.1	5.1	3.9	2.8	1	0.9956
Tolylfluanid *	5.7	5.9	3.0	5.8	1	0.9968
Procymidone *	1.6	1.2	2.1	1.1	1	0.9976
Endrin *	2.3	3.1	2.5	2.1	1	0.9966
Ethion *	4.8	4.7	4.0	2.8	1	0.9932
Endosulfan sulfate *	9.2	8.1	8.5	6.8	1	0.9963
DDT *	15.1	12.6	9.2	15.0	1	0.9933
Endrin ketone	7.4	10.9	5.1	8.3	1	0.9971
Iprodione *	7.5	5.8	6.1	8.2	1	0.9952
Phosmet *	5.6	4.9	3.2	5.1	1	0.9970
Phosalone*	4.1	4.2	2.9	3.2	1	0.9979
Permethrin	3.4	3.0	2.1	1.7	1	0.9976
Coumaphos *	7.9	6.4	4.5	4.7	1	0.9964
Deltamethrin	7.7	9.9	9.3	7.5	1	0.9963

* Pesticides that can achieve lower LOQ with current method.

Trace analysis in pepper

Figure 6 shows the GC/QQQ MRM chromatograms of pepper matrix blank and pepper matrix spiked at 1 ng/mL. Similar to flour and strawberry matrices, pepper matrix showed clean background for all MRM transitions. Although 2-phenylphenol was present in the pepper blank, the 1 ng/mL LOQ was achieved due to high response and relatively clean matrix background. Omethoate had a 5 ng/mL LOQ due to the low response at 1 ng/mL. The other 32 pesticides all achieved 1 ng/mL LOQ. Pesticides that can achieve lower LOQ (< 1 ng/mL) are labeled with asterisks in Table 8. Table 8 shows the quantitation results in pepper matrix. Similar to orange matrix results, DDT's RSD was close to 15%, which calls for attention in analyzing many samples. All other analytes achieved excellent system repeatability.

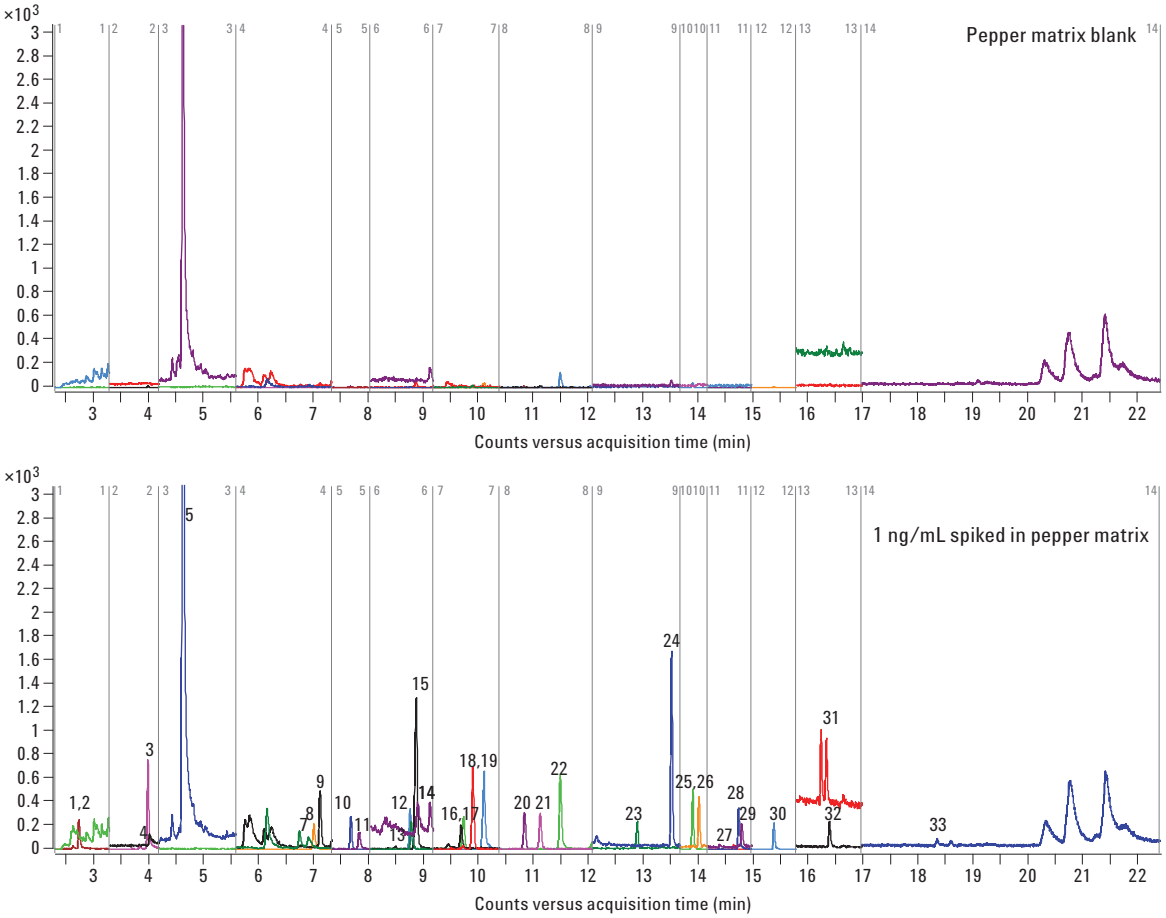

Figure 6. GC/QQQ MRM chromatograms for pepper matrix blank and pepper matrix spiked with 1 ng/mL pesticides. Refer to Table 3 for peak identification. Omethoate (6) was not identified at 1 ng/mL in pepper matrix due to low responses.

Table 8. Quantitation Results (Pepper Matrix)

	RSD(%) for n = 10 injections of 10 ng/mL pepper sample					
Pesticides	**UI liner number 1**	**UI liner number 2**	**UI liner number 3**	**UI liner number 4**	**LOQ (ng/mL)**	**R^2 (LOQ – 100 ng/mL)**
Methamidophos	4.9	3.1	4.4	2.9	1	0.9967
Dichlorvos *	2.3	1.8	1.7	1.9	1	0.9975
Mevinphos*	1.7	0.7	1.2	2.2	1	0.9948
Acephate	9.8	3.6	4.2	6.4	1	0.9891
2-phenylphenol*	1.2	0.9	1.3	2.6	1	0.9914
Omethoate	13.0	9.3	6.7	8.2	5	0.9983
Dimenthoate*	4.0	2.7	1.6	5.2	1	0.9866
Altrazine *	1.8	1.3	1.0	1.4	1	0.9949
Lindane *	1.3	1.5	3.0	2.0	1	0.9853
Diazinon *	1.7	2.3	1.7	2.5	1	0.9924
Chlorothalonil *	4.6	2.3	2.1	4.3	1	0.9930
Chloropyrifos methyl *	1.7	1.5	2.0	3.8	1	0.9935
Vinclozolin *	1.5	3.1	2.3	3.5	1	0.9946
Tolclofos methyl *	1.5	0.9	3.3	2.4	1	0.9949
Carbaryl	3.5	3.7	1.1	4.9	1	0.9957
Dichlorfluanid *	4.3	4.7	3.0	2.3	1	0.9910
Aldrin *	2.5	2.1	1.8	1.9	1	0.9939
Malathion *	2.4	1.7	1.7	4.5	1	0.9904
Dichlorobenzophenone *	0.8	1.7	1.2	2.6	1	0.9922
Pirimiphos ethyl *	2.1	1.5	1.6	3.7	1	0.9932
Tolylfluanid *	5.0	5.5	2.1	3.9	1	0.9815
Procymidone *	2.5	3.1	2.4	1.7	1	0.9950
Endrin *	2.5	3.8	3.2	2.7	1	0.9929
Ethion *	2.0	1.9	1.6	4.5	1	0.9859
Endosulfan sulfate *	7.3	3.3	4.8	4.8	1	0.9943
DDT *	14.9	14.3	16.9	15.9	1	0.9863
Endrin ketone	7.6	7.2	8.3	9.3	1	0.9913
Iprodione *	3.3	4.3	4.8	4.9	1	0.9954
Phosmet *	9.0	6.3	4.7	6.7	1	0.9902
Phosalone*	3.5	2.9	2.8	5.2	1	0.9885
Permethrin	2.6	3.3	3.3	3.3	1	0.9868
Coumaphos *	7.1	5.8	5.9	7.9	1	0.9831
Deltamethrin	5.5	3.9	5.8	5.2	1	0.9981

* Pesticides that can achieve lower LOQ with current method.

Trace analysis in spinach

Spinach matrix is a well-known challenging matrix, due to its complexity and recurrent matrix suppressions. Figure 7 shows the GC/QQQ MRM chromatograms of spinach matrix blank and spinach matrix spiked at 1 ng/mL. Matrix caused low responses or distorted peak shapes for Methamidophos, Dichlorvos, Acephate, Omethoate, Carbaryl, and Deltamethrin at 1 ng/mL. Therefore, the LOQ of these pesticides in Spinach was 5 ng/mL. The most abundant MRM transition for Lindane (180.8 > 145) couldn't be used for quantitation due to the background interference. As a result, a less abundant MRM transition (218.8 > 183) was used for quantitation. More than half of the pesticides (labeled with asterisks in Table 9) achieved lower LOQ (< 1 ng/mL) in spinach. Table 9 shows the quantitation and good linearity results. The repeatability of 10 injections of the 10 ng/mL QC sample was less than 15% RSD for most analytes. DDT, Omethoate, Endosulfan sulfate, and Endrin ketone showed RSDs higher than 15%. In addition, Acephate, Carbaryl, Phosmet, and Iprodione showed a slight trend of decreasing responses. Therefore, more frequent liner changes may be necessary to analyze multiple samples. Dichlorfluanid and Tolylfluanid are base label compounds. Their lack of stability in Spinach extract caused higher RSDs than in other matrices.

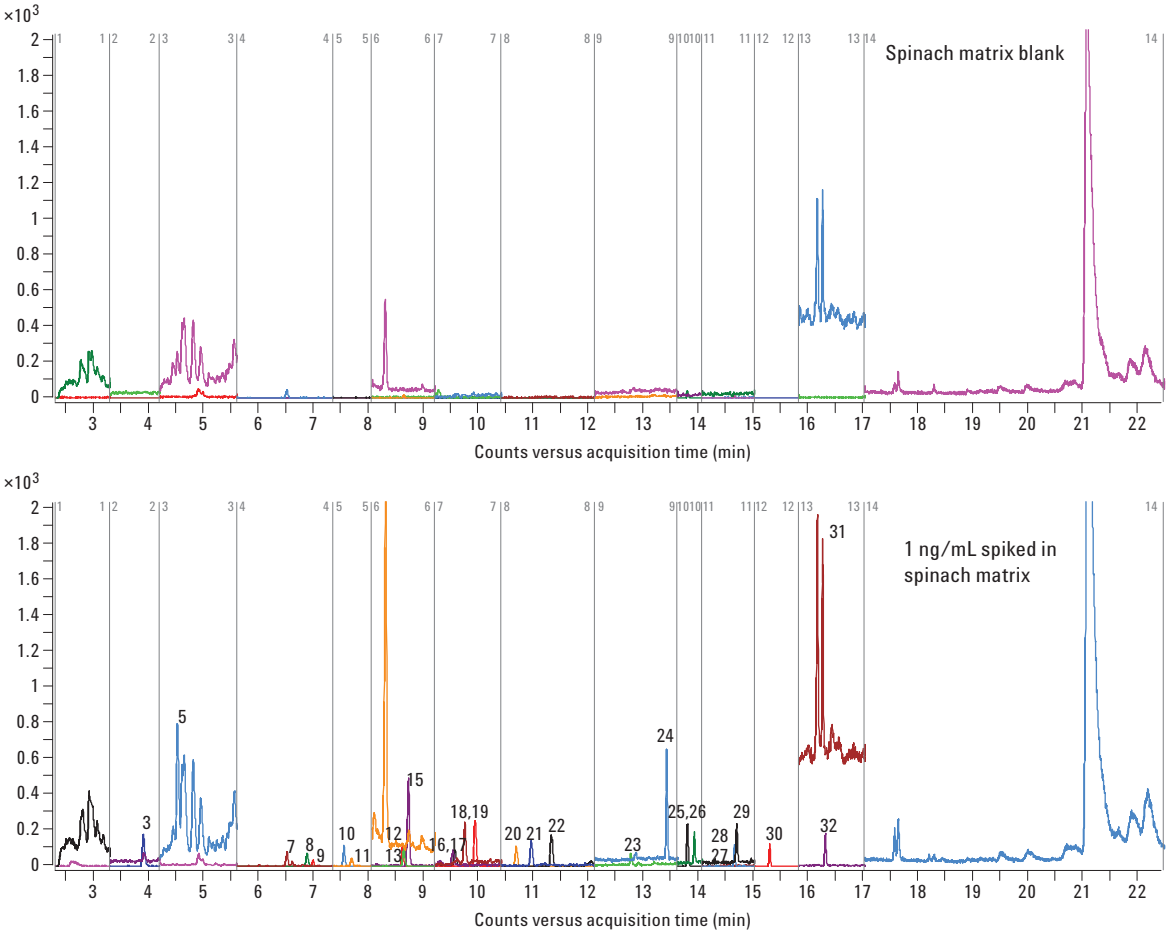

Figure 7. GC/QQQ MRM chromatograms for spinach matrix blank and spinach matrix spiked with 1 ng/mL pesticides. Refer to Table 3 for peak identification. Methamidophos (1), Dichlorvos (2), Acephate (4), Omethoate (6), Carbaryl (14) and Deltamethrin (33) were not identified at 1 ng/mL in spinach matrix due to low responses or matrix interferences.

Table 9. Quantitation Results (Spinach Matrix)

	RSD(%) for n = 10 injections of 10 ng/mL spinach sample					
Pesticides	**UI liner number 1**	**UI liner number 2**	**UI liner number 3**	**UI liner number 4**	**LOQ (ng/mL)**	**R^2 (LOQ – 100 ng/mL)**
Methamidophos	4.9	3.0	4.4	4.2	5	0.9992
Dichlorvos	1.4	4.1	2.9	1.2	5	0.9977
Mevinphos	2.6	3.6	2.1	2.2	1	0.9989
Acephate	11.3	7.1	9.5	7.2	5	0.9999
2-phenylphenol	3.1	1.9	6.2	4.6	1	0.9985
Omethoate	19.1	11.1	10.2	14.0	5	0.9970
Dimenthoate*	4.7	4.9	4.1	7.0	1	0.9997
Altrazine *	1.7	1.7	1.6	2.1	1	0.9970
Lindane *	6.0	7.1	8.0	3.7	1	0.9980
Diazinon *	2.2	3.1	1.5	1.4	1	0.9996
Chlorothalonil *	5.5	12.7	8.6	4.3	1	0.9989
Chloropyrifos methyl *	2.4	2.4	2.3	1.9	1	0.9996
Vinclozolin *	2.1	1.9	2.4	2.2	1	0.9991
Tolclofos methyl *	1.5	1.1	1.6	1.3	1	0.9998
Carbaryl	9.7	10.2	13.0	8.6	5	0.9990
Dichlorfluanid	11.5	18.2	10.9	8.4	1	0.9992
Aldrin *	3.1	1.9	1.6	1.8	1	0.9985
Malathion *	2.7	3.6	2.1	4.5	1	0.9995
Dichlorobenzophenone *	1.4	0.9	0.9	1.8	1	0.9998
Pirimiphos ethyl *	2.7	4.1	2.9	2.8	1	0.9997
Tolylfluanid	13.0	18.7	11.8	9.6	1	0.9981
Procymidone *	1.4	1.4	1.4	1.3	1	0.9993
Endrin	5.1	5.3	3.0	2.5	1	0.9992
Ethion *	2.2	5.1	5.7	2.3	1	0.9988
Endosulfan sulfate *	6.5	17.4	10.0	4.9	1	0.9991
DDT *	45.5	37.9	32.1	15.7	1	0.9897
Endrin ketone	13.3	22.0	10.4	15.8	1	0.9991
Iprodione *	9.5	12.5	9.5	4.7	1	0.9987
Phosmet *	10.5	11.1	8.8	12.6	1	0.9992
Phosalone*	3.6	5.7	3.6	5.4	1	0.9996
Permethrin	1.2	3.9	2.8	2.2	1	0.9985
Coumaphos *	6.9	9.1	4.7	7.8	1	0.9992
Deltamethrin	6.8	6.0	5.0	5.2	5	0.9983

* Pesticides that can achieve lower LOQ with current method.

Conclusion

Multi-residue pesticide analysis in food matrix by GC/MS or GC/MS/MS has always been challenging. Different matrix shows different matrix effect on the analytes, especially active compounds like Omethoate, DDT, and Acephate. Matrix can cause quantitation interference, lower response (higher LOQ), and/or poor peak shape. Therefore, it is critical to use matrix-matched calibration curves to achieve accurate and reliable quantitation results.

A repeatability (%RSD in Response Factors) comparison of 10 injections in different matrices is shown in Figure 8. Backflush and an Agilent Ultra Inert liner with wool can effectively protect the whole system and improve system durability. However, for challenging matrix like spinach, more attention is needed to monitor the peak shape and repeatability of active analytes, such as Omethoate and DDT, in analyzing multiple samples.

This GC/QQQ study of 33 representative pesticides in six different plantation matrices showed 1 ng/mL or lower LOQ for most pesticides, excellent linearity from LOQ to 100 ppb, and good repeatability from 10 injections at 10 ng/mL in matrix. Methamidophos, Omethoate, Carbaryl, and Deltamethrin were unable to reach 1 ng/mL LOQ in certain matrices. Excellent linearity ($R^2 > 0.99$) and analysis repeatability (%RSD < 15%) were achieved for most pesticides in all tested matrices.

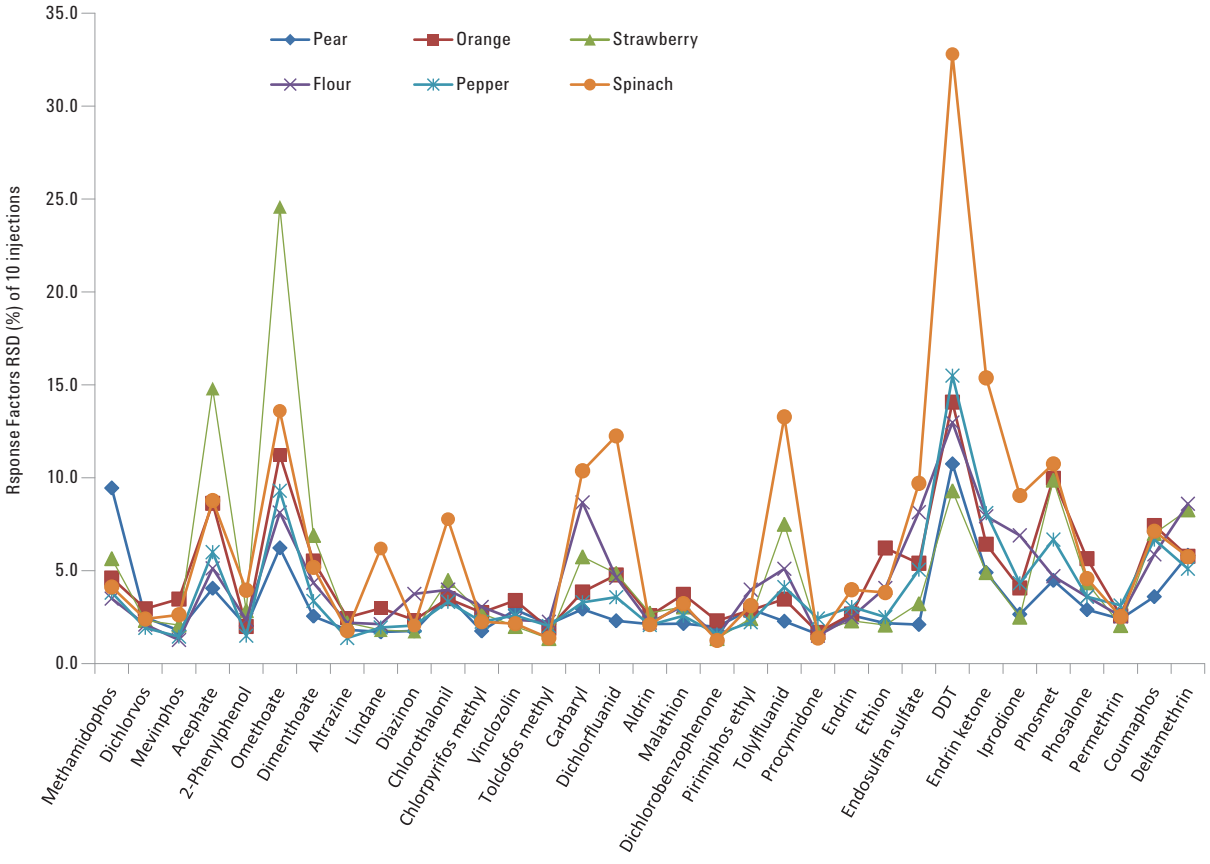

Figure 8. Repeatability (% RSD in Response Factors) of 10 injections in different matrices.

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