

Food Safety Applications

Mitigating Matrix Effects: Examination of Dilution, QuEChERS, and Calibration Strategies for LC-MS/MS Analysis of Pesticide Residues in Diverse Food Types

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Abstract

Liquid chromatography tandem mass spectrometry (LC-MS/MS) is popular for monitoring pesticide residues in food. The increased selectivity and sensitivity of LC-MS/MS have impacted how multiresidue methods are performed, sometimes decreasing the need for rigorous sample preparation. However, this approach suffers from matrix effects causing poor data quality and difficult quantification. Matrix effects can be mitigated by sample preparation that reduces the concentration of coextracted matrix material or by experimental strategies like matrix-matched calibration that compensate for matrix effects. We considered these two aspects of multiresidue methods, sample preparation and calibration strategies, in order to determine recommendations that balanced data quality as well as time and financial investments.

We performed matrix effects studies investigating two approaches for reducing matrix interferences, QuEChERS sample preparation and dilution, in combination with the compensation strategy of matrix-matched calibration compared to solvent calibration. There are compromises with each method regarding time and financial resources. A variety of food types were tested including high water (celery), high pigment (kale), high fat (avocado), citrus (lime), and dry (brown rice flour) foods, with subsequent pesticide residue analysis by LC-MS/MS. Samples were fortified at high and low ppb levels with over 100 pesticides representing multiple classes.

We determined that with the easiest commodities, the dilution method and solvent-only calibration gave acceptable recovery values. However, for other commodities either a matrix-matched calibration curve and/or cleanup were needed to obtain good recovery values. The high carbohydrate and citrus commodities proved to be too difficult with the specific methods we tested here. In almost every case, use of matrix-matched calibration provided improvement.

Introduction

There are many challenges for chemists performing trace analysis like pesticide residues. Some challenges are associated with the diverse commodities that need to be tested and the large number of analytes. In recent years, the trend has been for analytical methods to become faster and simpler, but at the same time detect lower levels and test many analytes in one analysis (multiresidue methods).

Liquid chromatography tandem mass spectrometry (LC-MS/MS) is popular for this type of testing because it addresses some of these challenges and is amenable to many more pesticides than gas chromatographic techniques. Retention times, ion transitions, and transition ratios are used for pesticide identification and quantification. By monitoring ion transitions, tandem mass spectrometry increases selectivity by filtering specific ions. This selectivity removes noise, resulting in a large increase in the signal-to-noise ratio, thereby enhancing sensitivity.

Although tandem mass spectrometry is highly selective and sensitive, coeluting compounds can cause interferences at the LC-MS interface during electrospray ionization (ESI), which occurs before mass analysis and signal generation. Inconsistent ionization causes poor data quality and makes accurate quantification extremely difficult. We use the term "matrix effect" to discuss this ionization issue. The matrix effect of a compound is the change in ionization in solvent compared to ionization in matrix. Matrix coextractives can change the ionization efficiency of an analyte causing signal suppression or enhancement. Matrix effects must be considered to ensure acceptable quantitative results for any LC-MS/MS method.

Matrix effects can be compensated for by experimental design, for example, by employing labeled isotopic standards or matrix-matched calibration. Matrix-matched calibration is proven to be effective but adds time and cost to analysis. Also, it can be challenging to find commodities that do not contain pesticides that are target analytes. Efforts have been made to use one matrix-matched sample set for different commodities, but this has shown varying success [1,2,3].

Matrix compounds can be selectively removed by applying sample cleanup procedures. This lowers the concentrations of coextractives while maintaining the concentrations of target analytes, but it can be labor-intensive and costly. There is also the potential of losing analytes during sample processing. Common sample preparation techniques include liquid-liquid extraction, solid phase extraction, and the QuEChERS approach. QuEChERS has extraction and cleanup steps that remove matrix compounds, while maintaining analytes at initial concentrations. Another approach is to reduce matrix effects by directly diluting the sample. This decreases the matrix coextractives concentration, but it also decreases the concentration of target analytes. Higher dilutions reduce or eliminate matrix effects more successfully, but better and better sensitivity is required as dilution factors increase. Dilutions can range from 10 to 100-fold [4] and choosing the proper dilution factor depends on the concentrations of analytes and coextractives, as well as on chemical properties and instrument sensitivity. For example, 10 ppb is often used as a benchmark concentration because it is a common maximum residue limit for many pesticides in many foods. If we diluted the sample 50-fold, we need to detect and quantify pesticides at 0.2 ppb or 200 ppt (parts-per-trillion). The excellent sensitivity of tandem mass spectrometry can sometimes accommodate this inherent sensitivity challenge of dilution-based methods.

The dilution technique has been heavily promoted in recent years and sometimes endorsed as a universal method. It has been shown to be successful in many applications, including pesticides residue analysis. Dilution is attractive because it eliminates sample preparation and associated costs. However, pesticide residue testing involves a wide variety of food types as well as a large number of pesticides that vary in physiochemical properties and generally speaking a single method is not adequate to address this complexity. For that reason, it is important to evaluate the effectiveness of sample preparation techniques and define recommendations for applying different strategies.

We evaluated a variety of pesticides that are typical of multiresidue methods. We tested approximately 100 pesticides from different chemical classes including carbamate, organophosphorus, aniline, conazole, macrocyclic lactone, phenylurea, benzoylphenylurea, and strobilurin pesticides. We tested a variety of commodities representing different food types and ranging from easy to difficult. High water, low fat, low carbohydrate, and low pigment foods are considered the easiest commodities to analyze because their extracts do not contain the high amount of coextracted compounds that are more common with high fat, high carbohydrate, high pigment, and dry commodities.

Method performance was evaluated using matrix effect values, acceptable pesticide recovery values, and the number of pesticides detected. We fortified foods and processed samples according to QuEChERS and dilution methods. We then determined recovery values. Quantification was performed two ways, by using both a solvent calibration curve and a matrix-matched calibration curve. Calibration methods were evaluated by comparing recovery values. The experimental design yields four recovery value data sets for each commodity: dilution with solvent calibration, dilution with matrix-matched calibration, QuEChERS with solvent calibration, and QuEChERS with matrix-matched calibration. This allows assessment of sample preparation/calibration method combinations.

Experimental

Chemicals and Materials

All solvents were LC-MS grade and purchased from Fisher Scientific (Pittsburgh, PA). Formic acid was obtained from Sigma-Aldrich (Milwaukee, WI) and ammonium formate from Alfa Aesar (Ward Hill, MA). QuEChERS extraction and dispersive solid phase extraction (dSPE) tubes, as well as QuEChERS internal and quality control standards, were from Restek Corporation (Bellefonte, PA). The approximately 100 pesticide mix in acetonitrile was a custom standard that was combined with AOAC spike mix (cat.# 31999). Internal standards, atrazine-d5 and diazinon-d10, were purchased from Sigma-Aldrich (Milwaukee, WI). Food commodities were purchased at a local grocery store and included celery, kale, avocado, lime, and brown rice flour. Commodities were stored at -20 °C.

Sample Preparation

Commodity Selection

Celery is considered the easiest commodity with high water content, medium pigment, and low fat and carbohydrates. Kale represents a high water, high pigment commodity that produces an intensely colored extract. Lime is the representative citrus fruit. Although citrus fruits are known to be difficult for LC-MS/MS techniques, we included lime (with peel) to observe any differences among methods. Avocado represents the high fat category. Brown rice flour is a dry grain, which has very high carbohydrate content. Table I shows the nutritional composition of each food tested.

Table I: Nutritional content for each commodity as grams per 100 grams of edible material.[1]

	Water	Sugar	Lipids	Carbohydrates	Protein	Fiber
Celery	95	2	0	3	1	2
Kale	84	na	1	10	3	2
Lime	88	2	0	10	1	3
Avocado	73	1	15	8	2	7
Brown rice flour	12	1	3	77	7	5

[1] USDA National Nutrient Database for Standard Reference, http://ndb.nal.usda.gov/ (last modified: Dec 7, 2011).

Commodity Fortification

Frozen commodities were homogenized using a food processor, weighed, and fortified with pesticides at 10 and 500 µg/kg (ppb) levels. Internal standards atrazine-d5, diazinon-d10, and AOAC IS mix (cat.# 31963) were added at 250 ppb final extract concentration. Unfortified samples were prepared to determine incurred pesticides and produce matrix-matched standards.

AOAC QuEChERS Extraction

The AOAC QuEChERS extraction method was used for celery, kale, and lime. For these samples, 15 mL of acetonitrile with 1% acetic acid (v/v) was added to 15 g of fortified homogenized sample [5]. Q-sep® AOAC buffering extraction salts (cat.# 26237) containing 6 g $MgSO_4$, 1.5 g sodium acetate were added. At this point, lime samples were pH adjusted by adding 900 µL of a 5 N (or M, mol/L*) sodium hydroxide solution to the extraction tube. This pH adjustment was based on the EN method recommendation, but scaled for a 15 g sample [6]. Following 1 minute of manual shaking, samples were centrifuged for 5 minutes at 3,000 xg with a Q-sep® 3000 centrifuge (cat.# 26230). The top acetonitrile layer was removed to a clean vial.

For dilution samples, the extract was diluted 20-fold with initial mobile phase, 90:10 (v:v) ratio of water with 4 mM ammonium formate and 0.1% formic acid to methanol with 4 mM ammonium formate and 0.1% formic acid.

*A 5 N sodium hydroxide solution is equivalent to a 5 M (molar, mol/L) solution.

Unbuffered QuEChERS Extraction

The original unbuffered extraction method was used for avocado and brown rice flour with adjustments for sample wetting [7]. For brown rice flour, 5 g of brown rice flour, 15 mL of deionized water, and 10 mL of acetonitrile were combined in a 50 mL centrifuge tube. The sample was vortexed briefly and shaken for one hour using a shaker table. For avocado, 3 mL water, 10 g of partially thawed homogenized avocado, and 10 mL of acetonitrile were added to a 50 mL centrifuge tube and shaken for one minute. The samples were centrifuged for 5 minutes at 3,000 xg with a Q-sep® 3000 centrifuge (cat.# 26230). The top acetonitrile layer was removed to a clean vial.

For dilution LC-MS/MS analysis, the extract was diluted 20-fold with initial mobile phase, 90:10 (v:v) ratio of water with 4 mM ammonium formate and 0.1% formic acid to methanol with 4 mM ammonium formate and 0.1% formic acid.

QuEChERS Dispersive Solid Phase Extraction (dSPE) Cleanup

Restek Q-sep® dSPE tubes were used and sorbent formulations were chosen based on commodity nutrient composition and preliminary experiments examining pigment removal. Table II shows the dSPE formulation, amount of extract processed, manual shake time, and specific dSPE formulation method and catalog number. Cleanup tubes contained one or more of three sorbents: primary secondary amine (PSA), octadecyl (C18), and graphitized carbon black (GCB). In addition to the contents listed in Table II, each dSPE tube contained 150 mg magnesium sulfate per mL extract processed. After the acetonitrile extract was added to the dSPE tube, the tube was shaken for the period specified in Table II. The samples were then centrifuged for 5 minutes at 3,000 xg. For LC-MS/MS analysis, the extract was diluted 10-fold with initial mobile phase, 90:10 (v:v) ratio of water with 4 mM ammonium formate and 0.1% formic acid to methanol with 4 mM ammonium formate and 0.1% formic acid.

Table II: The dSPE sorbent formula and extract volume used during sample cleanup for each food are listed below. The shake time required, method associated with each formulation, and Restek catalog number are also presented.

	dSPE Contents						
	PSA (mg)	C18 (mg)	GCB (mg)	Extract (mL)	Shake (min)	Method	Cat.#
Celery	25	—	7.5	1	2	EN 15662	26218
Kale	300	—	150	6	2	NA	26126
Lime	25	—	2.5	1	2	EN 15662	26217
Avocado	50	50	—	1	0.5	AOAC 2007.01	26125
Brown rice flour	50	50	—	1	0.5	AOAC 2007.01	26125

Solvent and Matrix-Matched Calibration Standards

Two calibration strategies commonly used for LC-MS/MS pesticide residue analysis are a solvent-only calibration curve and a matrix-matched calibration curve. The solvent curve uses analytes in a common solvent. The solvent calibration curve generated contained pesticides at various levels in the initial mobile phase of 90% water with 4 mM ammonium formate and 0.1% formic acid to 10% methanol with 4 mM ammonium formate and 0.1% formic acid (v/v). Solvent curves for the dilution method were generated daily with calibration levels of 0.25, 0.5, 1, 2, 10, 25, and 50 ppb with internal standard compounds at 12.5 ppb. Because only 5 g of brown rice flour was initially processed, the expected injection concentration of the 10 ppb spike samples was 0.25 ppb. Therefore, a 0.1 ppb calibration level was added for the brown rice flour samples. Solvent calibration curves for the QuEChERS method were also prepared daily with concentrations of 0.5, 1, 2, 10, 25, 50, and 75 ppb and internal standard compounds at 25 ppb. All solvent standards were made in 90% water with 4 mM ammonium formate and 0.1% formic acid to 10% methanol with 4 mM ammonium formate and 0.1% formic acid (v/v).

Dilution method matrix-matched calibration curves were produced by combining 50 µL matrix solution (pre-cleanup) with the pesticide stock standard solution and water with 4 mM ammonium formate and 0.1% formic acid totaling 1 mL and resulting in a 20-fold dilution of the matrix. This was done for calibration standards at 0.25, 0.5, 1, 2, 10, 25, and 50 ppb with internal standard compounds at 12.5 ppb. Again, a 0.1 ppb calibration standard was added for brown rice flour samples. The QuEChERS method matrix-matched curves combined 100 µL matrix solution (post cleanup), with pesticide stock solution and water with 4 mM ammonium formate and 0.1% formic acid totaling 1 mL and resulting in a 10-fold dilution of the matrix. Calibration standards included pesticides at 0.5, 1, 2, 10, 25, 50, and 75 ppb and internal standard compounds at 25 ppb.

LC-MS/MS Analysis

A Shimadzu UFLCXR LC (Columbia, MD) and Applied Biosystems/MDS SCIEX AB SCIEX API 4000™ LC-MS/MS system with Turbo V™ source (Foster City, CA) were used for LC-MS/MS pesticide residue analysis. Testing was performed using a 100 mm x 2.1 mm, 3 µm Ultra Aqueous C18 column (Restek, cat.# 9178312) and 20 µL injections. The column was held at 50 °C. A mobile phase gradient of solvent A, water with 4 mM ammonium formate and 0.1% formic acid, and solvent B, methanol with 4 mM ammonium formate and 0.1% formic acid, and 0.5 mL/min flow rate were used. The mobile phase gradient is shown in Table III and includes a 3 minute re-equilibration step. Compounds were ionized by positive electrospray ionization. The interface parameters are as follows: interface temperature at 450 °C, ion spray voltage of 5.5 kV, curtain gas at 30 psi (206.8 kPa), ion source gas 1 at 40 psi (275.8 kPa), and ion source gas 2 at 45 psi (310.3 kPa). Two transitions were optimized for each compound and monitored in Scheduled MRM™ (sMRM) mode. The MRM window was 45 seconds and target scan time was 0.33 seconds. The optimized MRM transitions and retention times for each analyte are listed in Table IV and the optimized voltages can be found in Table V. Pesticide identification was based on retention time matching and MRM transition ratios.

Table III: LC-MS/MS mobile phase gradient program.

Time (min)	%A	%B
0	90	10
1.5	90	10
6	30	70
9	30	70
10	0	100
12	0	100
12.01	90	10
15	90	10

Table IV: LC-MS/MS MRM transitions and retention times for each analyte.

Component Name	Q1/Q3 Transition 1, m/z	Q1/Q3 Transition 2, m/z	t_R (min)
Methamidophos	142.1 / 94.1	142.1 / 112.2	1.27
Acephate	184.1 / 125.1	184.1 / 95.1	1.78
Propamocarb	189.2 / 102.0	189.2 / 144.2	2.65
Omethoate	214.1 / 125.2	214.1 / 155.1	2.94
Aldicarb sulfone	223.2 / 148.0	223.2 / 76.2	3.45
Aldicarb sulfoxide	207.2 / 132.1	207.2 / 89.1	3.71
Pymetrozine	218.1 / 105.0	218.1 / 78.2	3.82
Oxamyl	237.1 / 71.9	237.1 / 90.1	3.9
Methomyl	163.1 / 88.1	163.1 / 106.2	4.02
Monocrotophos	224.1 / 127.1	224.1 / 98.1	4.38
Dimethoate	230.1 / 125.2	230.1 / 171.2	4.81
Mevinphos E	225.2 / 193.3	225.2 / 127.2	4.99
Thiabendazole	202.2 / 175.0	202.2 / 131.2	4.99
Imidacloprid	256.3 / 209.1	256.3 / 175.2	5.02
Mevinphos Z	225.1 / 193.2	225.1 / 127.1	5.43
Aldicarb	208.2 / 116.2	208.2 / 89.1	5.55
Carbetamide	237.1 / 192.0	237.1 / 118.1	5.73
Imazethapyr	290.1 / 245.2	290.1 / 177.3	5.9
Thidiazuron	221.2 / 102.0	221.2 / 128.1	5.93
Thiophanate methyl	343.2 / 151.1	343.2 / 93.1	5.96
Propoxur	210.2 / 168.1	210.2 / 111.2	5.99
Bendiocarb	224.1 / 109.2	224.1 / 167.2	6.00
Dichlorvos	220.9 / 109.2	220.9 / 95.0	6.01
Carbofuran	222.3 / 165.2	222.3 / 123.1	6.09
Pirimicarb	239.2 / 72.2	239.2 / 182.2	6.15
Carbaryl	202.3 / 127.1	202.3 / 117.2	6.32
Imazalil	297.1 / 159.0	297.1 / 173.1	6.40
Isoprocarb	194.3 / 95.2	194.3 / 137.3	6.53
Metalaxyl	280.4 / 192.3	280.4 / 160.2	6.72
Metalaxyl-M	280.4 / 220.3	280.4 / 192.1	6.72
Atrazine	216.2 / 174.3	216.2 / 132.1	6.75
Isoproturon	207.2 / 72.3	207.2 / 134.3	6.86
Azinphos methyl	318.2 / 160.0	318.2 / 132.1	6.96
Diuron	233.1 / 72.1	233.1 / 160.0	6.97
Phosmet	318.1 / 160.2	318.1 / 133.0	6.97
Demeton-O	259.0 / 89.1	259.0 / 61.2	6.98
Demeton-S	259.2 / 88.9	259.2 / 61.1	6.98
Nuarimol	315.0 / 252.1	315.0 / 81.0	7.08
Propanil	218.2 / 162.0	218.2 / 127.0	7.11
Azoxystrobin	404.3 / 372.3	404.3 / 344.0	7.12
Malathion	331.1 / 127.1	331.1 / 99.1	7.13
Methiocarb	226.1 / 169.1	226.1 / 121.1	7.14
Chlorpropham	214.1 / 154.0	214.1 / 126.1	7.20
Linuron	249.2 / 160.1	249.2 / 182.1	7.20
Crotoxyphos	332.2 / 211.2	332.2 / 167.2	7.21
Promecarb	208.8 / 109.2	208.8 / 151.3	7.21
Propetamphos	282.1 / 138.0	282.1 / 110.2	7.22
Boscalid	343.2 / 307.2	343.2 / 140.0	7.25
Triadimefon	294.3 / 197.1	294.3 / 69.2	7.26
Triadimenol	296.3 / 70.2	296.3 / 227.1	7.35
Fenhexamid	302.1 / 97.1	302.1 / 55.0	7.40

Component Name	Q1/Q3 Transition 1, m/z	Q1/Q3 Transition 2, m/z	t_R (min)
Myclobutanil	289.3 / 70.2	289.3 / 124.9	7.43
Dichlofluanid	332.9 / 224.0	332.9 / 123.1	7.44
Triazophos	314.1 / 162.0	314.1 / 119.2	7.48
Alachlor	270.2 / 238.1	270.2 / 162.2	7.60
Fenarimol	331.0 / 268.0	331.0 / 81.0	7.61
Iprodione	330.3 / 245.2	332.3 / 247.0	7.66
Ethoprop	243.1 / 131.0	243.1 / 173.0	7.70
Parathion	292.1 / 236.0	292.1 / 140.1	7.77
Fenamiphos	304.4 / 217.2	304.4 / 202.0	7.83
Diflubenzuron	311.1 / 158.2	311.1 / 141.1	7.85
Fenoxycarb	302.1 / 88.0	302.1 / 116.1	7.87
Etaconazole-1	328.2 / 159.1	328.2 / 123.0	7.89
Fenbuconazole	337.3 / 125.3	337.3 / 70.3	7.89
Kresoxim methyl	314.2 / 115.9	314.2 / 131.0	7.9
Tolyfluanid	364.0 / 238.0	364.0 / 137.1	8.00
Etaconazole-2	328.3 / 159.2	328.3 / 123.1	8.01
Fenthion	279.1 / 169.1	279.1 / 105.1	8.06
Quinalphos	299.3 / 243.1	299.3 / 163.2	8.07
Cyprodinil	226.1 / 93.3	226.1 / 77.1	8.16
Tebuconazole	308.3 / 70.1	308.3 / 125.1	8.24
Chlorfenvinphos	359.2 / 155.1	359.2 / 99.2	8.32
Diazinon	305.2 / 169.3	305.2 / 153.1	8.34
Pirimiphos methyl	306.1 / 164.3	306.1 / 108.1	8.35
Phosalone	368.1 / 182.1	368.1 / 138.0	8.38
Coumaphos	363.1 / 227.2	363.1 / 211.1	8.46
Propiconazole-1	342.3 / 159.0	342.3 / 69.3	8.60
Pyraclostrobin	388.0 / 164.2	388.0 / 194.3	8.61
Chlorpyrifos methyl	323.9 / 125.0	323.9 / 291.8	8.72
Propiconazole-2	342.4 / 159.1	342.4 / 69.4	8.77
Dialifos	394.3 / 208.0	394.3 / 187.0	8.81
Prochloraz	376.1 / 308.1	376.1 / 266.0	9.01
Indoxacarb	528.6 / 218.0	528.6 / 150.2	9.04
Trifloxystrobin	409.4 / 186.0	409.4 / 145.1	9.18
Spinosyn A	733.1 / 142.4	733.1 / 98.4	9.58
Difenoconazole-1	406.3 / 251.1	408.2 / 253.1	9.60
Triflumizole	346.2 / 278.2	346.2 / 73.1	9.79
Difenoconazole-2	406.4 / 251.2	408.3 / 253.2	9.85
Ethion	385.3 / 199.0	385.3 / 171.0	10.20
Spinosyn D	746.8 / 142.4	746.8 / 98.3	10.38
Chlorpyrifos	350.0 / 198.0	350.0 / 97.0	10.40
Pendimethalin	282.3 / 212.2	282.3 / 194.3	10.47
Emamectin B1a Benzoate	887.2 / 158.3	887.2 / 126.3	10.48
Propargite	368.4 / 175.1	368.4 / 231.2	10.61
Fenpropathrin	350.3 / 125.0	350.3 / 97.4	10.67
Flufenoxuron	489.5 / 158.2	489.5 / 141.1	10.72
Lambda-cyhalothrin	467.4 / 225.1	467.4 / 181.0	10.72
Deltamethrin	523.3 / 280.9	523.3 / 181.0	10.78
trans-Permethrin	408.4 / 183.3	408.4 / 153.2	10.90
Leptophos	413.2 / 171.0	413.2 / 77.1	10.92
cis-Permethrin	408.5 / 183.1	408.5 / 153.2	10.95
Bifenthrin	440.3 / 181.2	440.3 / 166.2	10.98

Table V: LC-MS/MS optimized voltages for MRM mode. Voltages for first and second transitions are designated by 1 and 2, respectively.

Component Name	Declustering Potential (DP), V	Entrance Potential (EP), V	Collision Energy (CE), V	Collision Cell Exit Potential (CXP), V
Methamidophos 1	57	11	20	6
Methamidophos 2	57	11	17	8
Acephate 1	53	5	25	7
Acephate 2	53	5	32	6
Propamocarb 1	61	10	25	8
Propamocarb 2	48	11	19	11
Omethoate 1	55	5	31	7
Omethoate 2	55	5	22	10
Aldicarb sulfone 1	71	5	14	11
Aldicarb sulfone 2	71	5	12	14
Aldicarb sulfoxide 1	58	3	10	10
Aldicarb sulfoxide 2	58	3	20	5
Pymetrozine 1	66	10	29	6
Pymetrozine 2	66	10	53	4
Oxamyl 1	34	5	20	6
Oxamyl 2	34	5	12	6
Methomyl 1	33	5	13	6
Methomyl 2	33	5	15	7
Monocrotophos 1	60	5	21	6
Monocrotophos 2	60	5	18	6
Dimethoate 1	56	11	29	6
Dimethoate 2	56	11	21	11
Mevinphos E 1	59	5	20	8
Mevinphos E 2	59	5	10	6
Imidacloprid 1	62	11	21	6
Imidacloprid 2	62	11	25	10
Thiabendazole 1	91	9	36	11
Thiabendazole 2	91	9	47	9
Mevinphos Z 1	59	5	10	6
Mevinphos Z 2	59	5	20	8
Aldicarb 1	30	5	11	9
Aldicarb 2	30	5	22	5
Carbetamide 1	56	10	13	12
Carbetamide 2	56	10	19	10
Atrazine d5 (IS) 1	64	10	26	11
Atrazine d5 (IS) 2	64	10	36	5
Imazethapyr 1	77	5	30	15
Imazethapyr 2	77	5	38	10
Thidiazuron 1	52	11	22	6
Thidiazuron 2	52	11	22	7
Thiophanate methyl 1	69	11	28	11
Thiophanate methyl 2	69	11	68	6
Propoxur 1	56	5	21	8
Propoxur 2	56	5	12	11
Bendiocarb 1	60	11	25	34
Bendiocarb 2	60	11	110	15
Dichlorvos 1	74	5	27	7
Dichlorvos 2	74	5	46	7
Carbofuran 1	64	10	17	11
Carbofuran 2	64	10	30	8
Pirimicarb 1	48	10	32	5
Pirimicarb 2	48	10	23	10
Carbaryl 1	57	15	37	11
Carbaryl 2	57	15	34	9
Imazalil 1	79	11	30	11
Imazalil 2	79	11	35	11
Isoprocarb 1	62	10	22	6
Isoprocarb 2	62	10	14	11
Metalaxyl-M 1	60	11	20	6
Metalaxyl-M 2	60	11	26	14
Metalaxyl 1	54	5	41	5
Metalaxyl 2	54	5	63	15

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Component Name	Declustering Potential (DP), V	Entrance Potential (EP), V	Collision Energy (CE), V	Collision Cell Exit Potential (CXP), V
Atrazine 1	72	9	25	10
Atrazine 2	72	9	32	8
Isoproturon 1	68	5	31	5
Isoproturon 2	68	5	31	7
Phosmet 1	64	4	17	12
Phosmet 2	64	4	50	10
Demeton-S 1	50	5	16	6
Demeton-S 2	50	5	48	7
Demeton-O 1	40	5	14	6
Demeton-O 2	40	5	47	6
Diuron 1	74	10	44	5
Diuron 2	74	10	36	12
Azinphos methyl 1	57	5	11	11
Azinphos methyl 2	57	5	21	10
Nuarimol 1	81	10	31	16
Nuarimol 2	81	10	45	14
Azoxystrobin 1	58	10	20	9
Azoxystrobin 2	58	10	34	9
Propanil 1	66	15	23	11
Propanil 2	66	15	36	9
Malathion 1	63	15	19	7
Malathion 2	63	15	33	7
Methiocarb 1	61	5	15	10
Methiocarb 2	61	5	27	8
Promecarb 1	63	5	22	9
Promecarb 2	63	5	14	11
Crotoxyphos 1	34	4	13	6
Crotoxyphos 2	34	4	21	11
Chlorpropham 1	54	5	25	11
Chlorpropham 2	54	5	35	8
Linuron 1	69	10	25	11
Linuron 2	69	10	23	10
Propetamphos 1	60	6	23	11
Propetamphos 2	60	6	41	8
Boscalid 1	98	10	28	11
Boscalid 2	98	10	27	11
Triadimefon 1	59	11	22	6
Triadimefon 2	59	11	32	6
Triadimenol 1	42	4	26	5
Triadimenol 2	42	4	14	6
Fenhexamid 1	81	10	35	6
Fenhexamid 2	81	10	71	10
Myclobutanil 1	65	11	36	5
Myclobutanil 2	65	11	39	9
Dichlofluanid 1	70	4	16	15
Dichlofluanid 2	70	4	44	15
Triazophos 1	76	9	26	11
Triazophos 2	76	9	50	9
Alachlor 1	46	5	12	15
Alachlor 2	46	5	28	11
Fenarimol 1	61	10	31	4
Fenarimol 2	61	10	49	15
Diazinon d10 (IS) 1	74	10	31	11
Diazinon d10 (IS) 2	74	10	32	11
Iprodione 1	82	4	21	6
Iprodione 2	82	4	31	7
Ethoprop 1	64	11	21	11
Ethoprop 2	64	11	29	9
Parathion 1	70	4	22	15
Parathion 2	70	4	33	10
Parathion d10 1	74	4	49	28
Parathion d10 2	74	4	49	28
Fenamiphos 1	82	5	31	6
Fenamiphos 2	82	5	47	6

(Continued on page 8)

(Continued from page 7)

Component Name	Declustering Potential (DP), V	Entrance Potential (EP), V	Collision Energy (CE), V	Collision Cell Exit Potential (CXP), V
Diflubenzuron 1	70	4	22	10
Diflubenzuron 2	70	4	46	8
Fenoxycarb 1	66	10	31	6
Fenoxycarb 2	66	10	17	8
Fenbuconazole 1	78	10	38	6
Fenbuconazole 2	78	10	43	5
Kresoxim methyl 1	66	10	33	8
Kresoxim methyl 2	66	10	19	6
Etaconazole (isomer 1) 1	93	10	35	10
Etaconazole (isomer 1) 2	93	10	75	9
Etaconazole (isomer 2) 1	93	10	35	10
Etaconazole (isomer 2) 2	93	10	75	9
Tolyfluanid 1	46	10	39	8
Tolyfluanid 2	46	10	19	14
Fenthion 1	71	10	25	11
Fenthion 2	71	10	34	7
Quinalphos 1	56	11	24	15
Quinalphos 2	56	11	34	11
Cyprodinil 1	92	11	49	5
Cyprodinil 2	92	11	61	14
Tebuconazole 1	75	11	46	6
Tebuconazole 2	75	11	48	7
Chlorfenvinphos 1	78	5	19	11
Chlorfenvinphos 2	78	5	44	7
Diazinon 1	58	10	31	11
Diazinon 2	58	10	28	12
Phosalone 1	78	9	20	9
Phosalone 2	78	9	41	11
Pirimiphos methyl 1	75	11	31	11
Pirimiphos methyl 2	75	11	43	9
Coumaphos 1	92	11	35	6
Coumaphos 2	130	10	52	12
Pyraclostrobin 1	46	10	18	6
Pyraclostrobin 2	46	10	26	11
Propiconazole (isomer 1) 1	81	5	37	11
Propiconazole (isomer 1) 2	81	5	37	5
Propiconazole (isomer 2) 1	81	5	37	11
Propiconazole (isomer 2) 2	81	5	37	5
Chlorpyrifos methyl 1	74	5	28	9
Chlorpyrifos methyl 2	74	5	22	18
Dialifos 1	71	5	12	7
Dialifos 2	71	5	20	6
Indoxacarb 1	90	11	33	14
Indoxacarb 2	90	11	34	11
Prochloraz 1	51	11	17	11
Prochloraz 2	51	11	24	18
Trifloxystrobin 1	56	6	22	13
Trifloxystrobin 2	56	6	61	11
Difenoconazole (isomer 1) 1	86	11	34	6
Difenoconazole (isomer 1) 2	76	10	33	4
Spinosyn A 1	111	11	44	11
Spinosyn A 2	111	11	93	7
Triflumizole 1	53	5	15	12
Triflumizole 2	53	5	26	5
Difenoconazole (isomer 2) 1	86	11	34	6
Difenoconazole (isomer 2) 2	76	10	33	4
Ethion 1	71	5	15	6
Ethion 2	71	5	22	11
Spinosyn D 1	112	11	41	11
Spinosyn D 2	112	11	93	7
Chlorpyrifos 1	71	10	45	6
Chlorpyrifos 2	71	10	23	12

(Continued on page 9)

(Continued from page 9)

Component Name	Declustering Potential (DP), V	Entrance Potential (EP), V	Collision Energy (CE), V	Collision Cell Exit Potential (CXP), V
Pendimethalin 1	45	4	16	6
Pendimethalin 2	45	4	26	7
Emamectin B1a Benzoate 1	127	5	50	11
Emamectin B1a Benzoate 2	127	5	62	6
Propargite 1	61	5	15	7
Propargite 2	61	5	23	11
Fenpropathrin 1	103	10	17	11
Fenpropathrin 2	103	10	44	5
Lambda-cyhalothrin 1	59	5	22	6
Lambda-cyhalothrin 2	59	5	49	15
Flufenoxuron 1	91	5	27	11
Flufenoxuron 2	91	5	65	12
Deltamethrin 1	56	4	22	19
Deltamethrin 2	130	10	52	12
trans-Permethrin 1	50	10	22	6
trans-Permethrin 2	50	10	61	11
Leptophos 1	85	11	32	13
Leptophos 2	85	11	77	6
cis-Permethrin 1	49	10	27	13
cis-Permethrin 2	49	10	61	11
Bifenthrin 1	53	5	18	9
Bifenthrin 2	53	5	59	11

Determination of Matrix Effects

Matrix effect values were determined for each pesticide in each commodity. Data were generated from the solvent and matrix-matched calibration curves discussed previously. Matrix effect values were calculated by comparing the ratio of the solvent curve slope to the matrix-matched curve slope. Equation 1 shows the calculation used to determine matrix effect. Values above 100% indicate enhanced ionization in matrix and values below 100% define ion signal suppression due to matrix.

$$\text{Equation 1: } \%ME = \left(\frac{\text{slope MM curve}}{\text{slope sol curve}}\right) * 100$$

Pesticide Percent Recovery Determination

Fortified samples at two levels, 10 and 500 µg/kg (ppb), were prepared for both the QuEChERS method and the dilution method. Atrazine-d5 was used as an internal standard. Quantification was performed two ways: one way used a solvent calibration curve and the other way used a matrix-matched calibration curve. Calculated concentrations were compared to the expected concentration, assuming 100% recovery. For each pesticide there is a set of four recovery values per fortification level: two recovery values for the QuEChERS method and two values for the dilution method. These values are based on pesticide quantification using a solvent curve and a matrix-matched curve. The four percent recovery categories will be abbreviated as shown in Table VI.

Table VI: Abbreviations for the sample preparation and calibration method combinations.

Abbreviation	Sample Preparation Method	Calibration Method
Q/Sol	QuEChERS	solvent calibration curve
Q/MM	QuEChERS	matrix-matched calibration curve
D/Sol	dilution	solvent calibration curve
D/MM	dilution	matrix-matched calibration curve

Figure 1: Chromatogram of QuEChERS extract of kale fortified with pesticides.

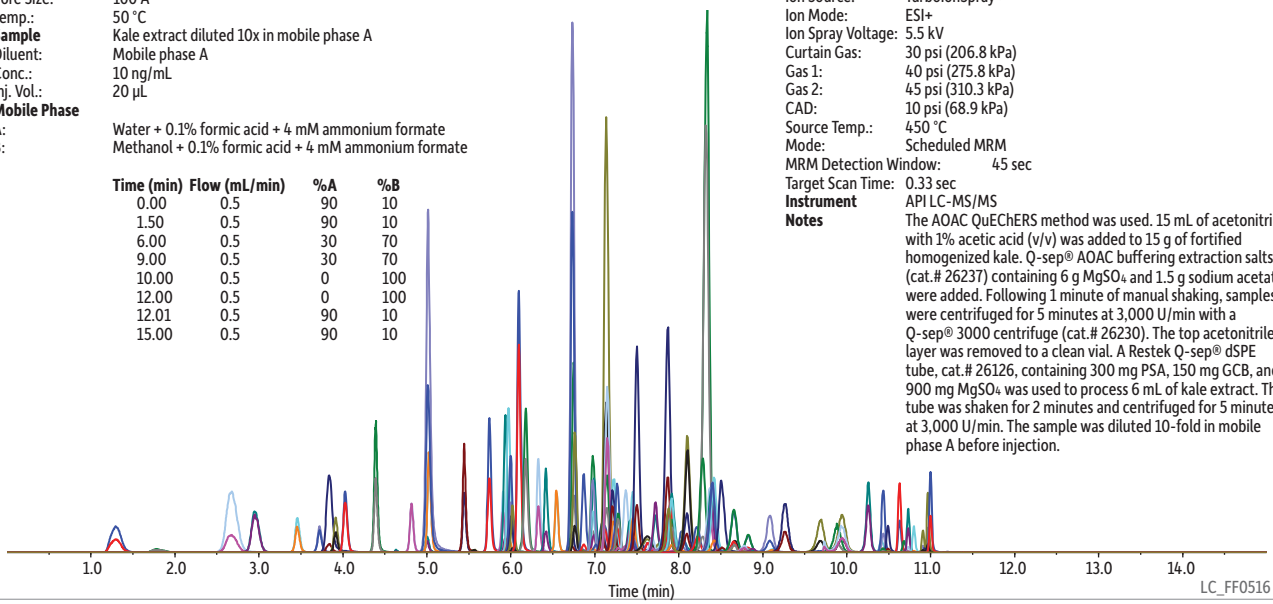
	Peaks	t_R (min)	MRM 1	MRM 2
1.	Methamidophos	1.27	142.1 / 94.1	142.1 / 112.2
2.	Acephate	1.78	184.1 / 125.1	184.1 / 95.1
3.	Propamocarb	2.65	189.2 / 102.0	189.2 / 144.2
4.	Omethoate	2.94	214.1 / 125.2	214.1 / 155.1
5.	Aldicarb sulfone	3.45	223.2 / 148.0	223.2 / 76.2
6.	Aldicarb sulfoxide	3.71	207.2 / 132.1	207.2 / 89.1
7.	Pymetrozine	3.82	218.1 / 105.0	218.1 / 78.2
8.	Oxamyl	3.90	237.1 / 71.9	237.1 / 90.1
9.	Methomyl	4.02	163.1 / 88.1	163.1 / 106.2
10.	Monocrotophos	4.38	224.1 / 127.1	224.1 / 98.1
11.	Dimethoate	4.81	230.1 / 125.2	230.1 / 171.2
12.	Mevinphos E	4.99	225.2 / 193.3	225.2 / 127.2
13.	Thiabendazole	4.99	202.2 / 175.0	202.2 / 131.2
14.	Imidacloprid	5.02	256.3 / 209.1	256.3 / 175.2
15.	Mevinphos Z	5.43	225.1 / 193.2	225.1 / 127.1
16.	Aldicarb	5.55	208.2 / 116.2	208.2 / 89.1
17.	Carbetamide	5.73	237.1 / 192.0	237.1 / 118.1
18.	Imazethapyr	5.90	290.1 / 245.2	290.1 / 177.3
19.	Thidiazuron	5.93	221.2 / 102.0	221.2 / 128.1
20.	Thiophanate methyl	5.96	343.2 / 151.1	343.2 / 93.1
21.	Propoxur	5.99	210.2 / 168.1	210.2 / 111.2
22.	Bendiocarb	6.00	224.1 / 109.2	224.1 / 167.2
23.	Dichlorvos	6.01	220.9 / 109.2	220.9 / 95.0
24.	Carbofuran	6.09	222.3 / 165.2	222.3 / 123.1
25.	Pirimicarb	6.15	239.2 / 72.2	239.2 / 182.2
26.	Carbaryl	6.32	202.3 / 127.1	202.3 / 117.2
27.	Imazalil	6.40	297.1 / 159.0	297.1 / 173.1
28.	Isoprocarb	6.53	194.3 / 95.2	194.3 / 137.3
29.	Metalaxyl	6.72	280.4 / 192.3	280.4 / 160.2
30.	Metalaxyl-m	6.72	280.4 / 220.3	280.4 / 192.1
31.	Atrazine	6.75	216.2 / 174.3	216.2 / 132.1
32.	Atrazine-d5 (IS)	6.77	221.1 / 179.0	221.1 / 101.2
33.	Isoproturon	6.86	207.2 / 72.3	207.2 / 134.3
34.	Azinphos-methyl	6.96	318.2 / 160.0	318.2 / 132.1
35.	Diuron	6.97	233.1 / 72.1	233.1 / 160.0
36.	Phosmet	6.97	318.1 / 160.2	318.1 / 133.0
37.	Demeton-O	6.98	259.0 / 89.1	259.0 / 61.2
38.	Demeton-S	6.98	259.2 / 88.9	259.2 / 61.1
39.	Nuarimol	7.08	315.0 / 252.1	315.0 / 81.0
40.	Propanil	7.11	218.2 / 162.0	218.2 / 127.0
41.	Azoxystrobin	7.12	404.3 / 372.3	404.3 / 344.0
42.	Malathion	7.13	331.1 / 127.1	331.1 / 99.1
43.	Methiocarb	7.14	226.1 / 169.1	226.1 / 121.1
44.	Chlorpropham	7.20	214.1 / 154.0	214.1 / 126.1
45.	Linuron	7.20	249.2 / 160.1	249.2 / 182.1
46.	Crotoxyphos	7.21	332.2 / 211.2	332.2 / 167.2
47.	Promecarb	7.21	208.8 / 109.2	208.8 / 151.3
48.	Propetamphos	7.22	282.1 / 138.0	282.1 / 110.2
49.	Boscalid	7.25	343.2 / 307.2	343.2 / 140.0
50.	Triadimefon	7.26	294.3 / 197.1	294.3 / 69.2
51.	Triadimenol	7.35	296.3 / 70.2	296.3 / 227.1
52.	Fenhexamid	7.40	302.1 / 97.1	302.1 / 55.0
53.	Myclobutanil	7.43	289.3 / 70.2	289.3 / 124.9
54.	Dichlofluanid	7.44	332.9 / 224.0	332.9 / 123.1
55.	Triazophos	7.48	314.1 / 162.0	314.1 / 119.2
56.	Alachlor	7.60	270.2 / 238.1	270.2 / 162.2
57.	Fenarimol	7.61	331.0 / 268.0	331.0 / 81.0
58.	Iprodione	7.66	330.3 / 245.2	332.3 / 247.0
59.	Ethoprop	7.70	243.1 / 131.0	243.1 / 173.0
60.	Parathion	7.77	292.1 / 236.0	292.1 / 140.1
61.	Fenamiphos	7.83	304.4 / 217.2	304.4 / 202.0
62.	Diflubenzuron	7.85	311.1 / 158.2	311.1 / 141.1
63.	Fenoxycarb	7.87	302.1 / 88.0	302.1 / 116.1
64.	Etaconazole isomer 1	7.89	328.2 / 159.1	328.2 / 123.0
65.	Fenbuconazole	7.89	337.3 / 125.3	337.3 / 70.3
66.	Kresoxim-methyl	7.90	314.2 / 115.9	314.2 / 131.0
67.	Tolyfluanid	8.00	364.0 / 238.0	364.0 / 137.1
68.	Etaconazole isomer 2	8.01	328.3 / 159.2	328.3 / 123.1
69.	Fenthion	8.06	279.1 / 169.1	279.1 / 105.1
70.	Quinalphos	8.07	299.3 / 243.1	299.3 / 163.2
71.	Cyprodinil	8.16	226.1 / 93.3	226.1 / 77.1
72.	Tebuconazole	8.24	308.3 / 70.1	308.3 / 125.1
73.	Chlorfenvinphos	8.32	359.2 / 155.1	359.2 / 99.2
74.	Diazinon	8.34	305.2 / 169.3	305.2 / 153.1
75.	Pirimiphos methyl	8.35	306.1 / 164.3	306.1 / 108.1
76.	Phosalone	8.38	368.1 / 182.1	368.1 / 138.0
77.	Diazinon-d10 (IS)	8.39	315.3 / 170.1	315.3 / 154.1
78.	Coumaphos	8.46	363.1 / 227.2	363.1 / 211.1
79.	Propiconazole isomer 1	8.60	342.3 / 159.0	342.3 / 69.3
80.	Pyraclostrobin	8.61	388.0 / 164.2	388.0 / 194.3
81.	Chlorpyrifos methyl	8.72	323.9 / 125.0	323.9 / 291.8
82.	Propiconazole isomer 2	8.77	342.4 / 159.1	342.4 / 69.4
83.	Dialifos	8.81	394.3 / 208.0	394.3 / 187.0
84.	Prochloraz	9.01	376.1 / 308.1	376.1 / 266.0
85.	Indoxacarb	9.04	528.6 / 218.0	528.6 / 150.2
86.	Trifloxystrobin	9.18	409.4 / 186.0	409.4 / 145.1
87.	Spinosyn A	9.58	733.1 / 142.4	733.1 / 98.4
88.	Difenoconazole isomer 1	9.60	406.3 / 251.1	408.2 / 253.1
89.	Triflumizole	9.79	346.2 / 278.2	346.2 / 73.1
90.	Difenoconazole isomer 2	9.85	406.4 / 251.2	408.3 / 253.2
91.	Ethion	10.20	385.3 / 199.0	385.3 / 171.0
92.	Spinosyn D	10.38	746.8 / 142.4	746.8 / 98.3
93.	Chlorpyrifos	10.40	350.0 / 198.0	350.0 / 97.0
94.	Pendimethalin	10.47	282.3 / 212.2	282.3 / 194.3
95.	Emamectin B1a benzoate	10.48	887.2 / 158.3	887.2 / 126.3
96.	Propargite	10.61	368.4 / 175.1	368.4 / 231.2
97.	Fenpropathrin	10.67	350.3 / 125.0	350.3 / 97.4
98.	Flufenoxuron	10.72	489.5 / 158.2	489.5 / 141.1
99.	Lambda cyhalothrin	10.72	467.4 / 225.1	467.4 / 181.0
100.	Deltamethrin	10.78	523.3 / 280.9	523.3 / 181.0
101.	*trans*-Permethrin	10.90	408.4 / 183.3	408.4 / 153.2
102.	Leptophos	10.92	413.2 / 171.0	413.2 / 77.1
103.	*cis*-Permethrin	10.95	408.5 / 183.1	408.5 / 153.2
104.	Bifenthrin	10.98	440.3 / 181.2	440.3 / 166.2

Isomers were designated 1 or 2 by elution order

Column Ultra Aqueous C18 (cat.# 9178312)
Dimensions: 100 mm x 2.1 mm ID
Particle Size: 3 µm
Pore Size: 100 Å
Temp.: 50 °C
Sample Kale extract diluted 10x in mobile phase A
Diluent: Mobile phase A
Conc.: 10 ng/mL
Inj. Vol.: 20 µL
Mobile Phase
A: Water + 0.1% formic acid + 4 mM ammonium formate
B: Methanol + 0.1% formic acid + 4 mM ammonium formate

Time (min)	Flow (mL/min)	%A	%B
0.00	0.5	90	10
1.50	0.5	90	10
6.00	0.5	30	70
9.00	0.5	30	70
10.00	0.5	0	100
12.00	0.5	0	100
12.01	0.5	90	10
15.00	0.5	90	10

Detector ABSCIEX MS/MS
Model #: API 4000
Ion Source: TurboIonSpray®
Ion Mode: ESI+
Ion Spray Voltage: 5.5 kV
Curtain Gas: 30 psi (206.8 kPa)
Gas 1: 40 psi (275.8 kPa)
Gas 2: 45 psi (310.3 kPa)
CAD: 10 psi (68.9 kPa)
Source Temp.: 450 °C
Mode: Scheduled MRM
MRM Detection Window: 45 sec
Target Scan Time: 0.33 sec
Instrument API LC-MS/MS
Notes The AOAC QuEChERS method was used. 15 mL of acetonitrile with 1% acetic acid (v/v) was added to 15 g of fortified homogenized kale. Q-sep® AOAC buffering extraction salts (cat.# 26237) containing 6 g $MgSO_4$ and 1.5 g sodium acetate were added. Following 1 minute of manual shaking, samples were centrifuged for 5 minutes at 3,000 U/min with a Q-sep® 3000 centrifuge (cat.# 26230). The top acetonitrile layer was removed to a clean vial. A Restek Q-sep® dSPE tube, cat.# 26126, containing 300 mg PSA, 150 mg GCB, and 900 mg $MgSO_4$ was used to process 6 mL of kale extract. The tube was shaken for 2 minutes and centrifuged for 5 minutes at 3,000 U/min. The sample was diluted 10-fold in mobile phase A before injection.



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Results and Discussion

LC-MS/MS Analysis

Figure 1 shows a chromatogram of kale extract processed with the QuEChERS method. This is representative of the chromatograms produced in this work. Analytes eluted in 11 minutes followed by a short high-organic rinse and an equilibration step. The high-organic rinse was important in helping to maintain the column and also in avoiding matrix carryover into the next sample. Elution of remaining matrix material during a subsequent analysis can cause unexpected matrix effects. There is evidence that late eluting matrix compounds in a reverse phase HPLC analysis can cause significant ion suppression [2].

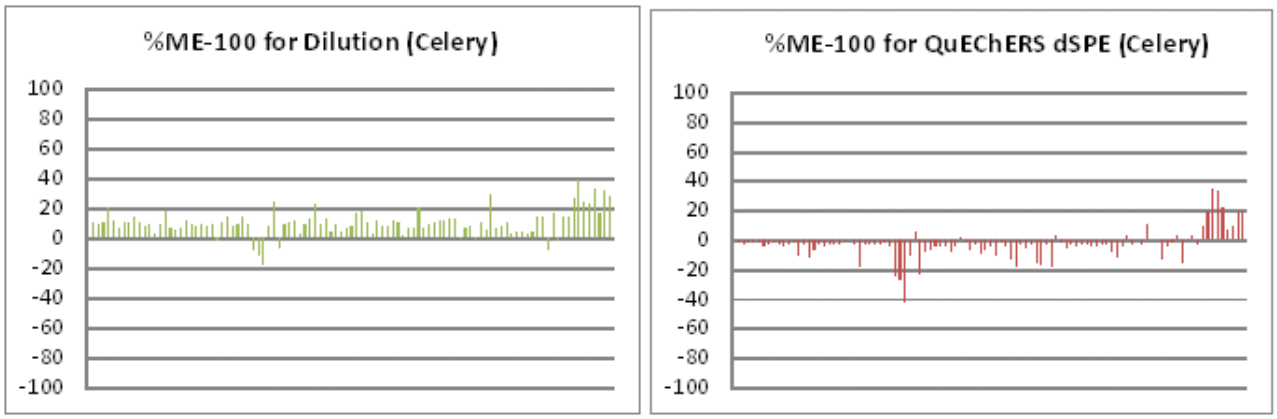
Retention times were an integral part of pesticide identifications and were used to produce the scheduled MRM program. For these reasons, retention time reproducibility is extremely important. We tested approximately 380 samples; each sample tracked 204 MRM transitions resulting in 77,520 MRM transitions. Only four transitions, or 0.005%, shifted and all of these were isomers that could be manually integrated and reported as one peak. Early eluting polar pesticides typically are difficult to analyze on C18 columns and usually are characterized by little or no retention and poor peak shape. However, we obtained excellent results using an Ultra Aqueous C18 column. We tested polar pesticides methamidophos, acephate, propamocarb, and omethoate using an Ultra Aqueous C18 column (100 mm x 2.1 mm, 3 µm). This column is a polar modified bonded C18 stationary phase, meaning it can interact with polar and nonpolar compounds. This resulted in significant retention and good peak shapes for these early-eluting polar pesticides. Also, the remaining analytes were distributed across the elution window. This can help ensure a proper scan rate for scheduled MRM methods with many analytes.

Matrix Effects Interpretation

The matrix effect for each compound was calculated as described by Equation 1 above. If we assume that the slopes of the solvent calibration curve and the matrix-matched curve are equal, then no matrix effect is present and the slope ratio is 1, meaning the signal of a compound in matrix and in solvent is exactly the same. A matrix effect value above 100% indicates signal enhancement when the analyte is tested in matrix. When the value is below 100% this means that the signal of an analyte in matrix is lower or suppressed compared to analyte in solvent.

We illustrate total matrix effects by plotting the calculated matrix effect minus 100 for each compound ordered by increasing retention time. These plots help identify matrix effect trends, like overall suppression or enhancement of a specific commodity or an association between retention time and matrix effects. These matrix effect plots were generated for samples processed by QuEChERS and dilution methods and can be used to compare trends. For example, the plots for celery in Figure 2 show that the dilution method tended to produce signal enhancement, while celery processed with QuEChERS tended to show signal suppression. However, both methods produce samples that show signal enhancement at the end of the chromatogram.

Figure 2: Matrix effect for celery plotted in retention time order. The dilution method tended to produce signal enhancement, whereas the QuEChERS method usually resulted in signal suppression.



More drastic matrix effects at specific points in the chromatogram can indicate what type of coextracted compounds might be in the sample. For example, strong matrix effects at the end of a reverse phase chromatographic analysis indicate that hydrophobic compounds, like lipids, might be causing ionization problems. If we look at the plots for high fat avocado in Figure 3, we see ion suppression at the end of the elution window for samples produced by both sample treatments. It is reasonable to conclude that lipid content remains in both samples.

Figure 3: Matrix effect for avocado plotted in retention time order. Signal suppression is evident for late eluting compounds for both dilution and QuEChERS samples.

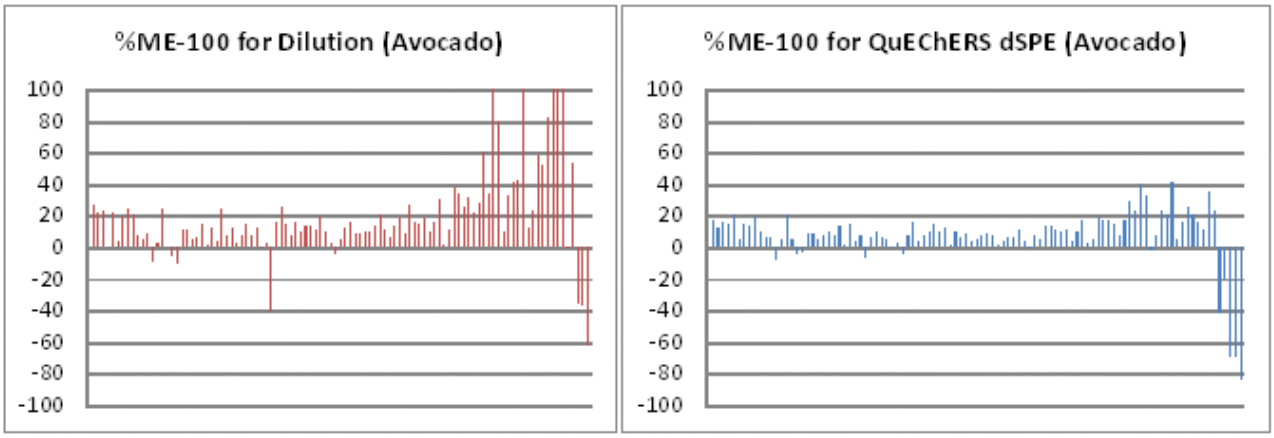
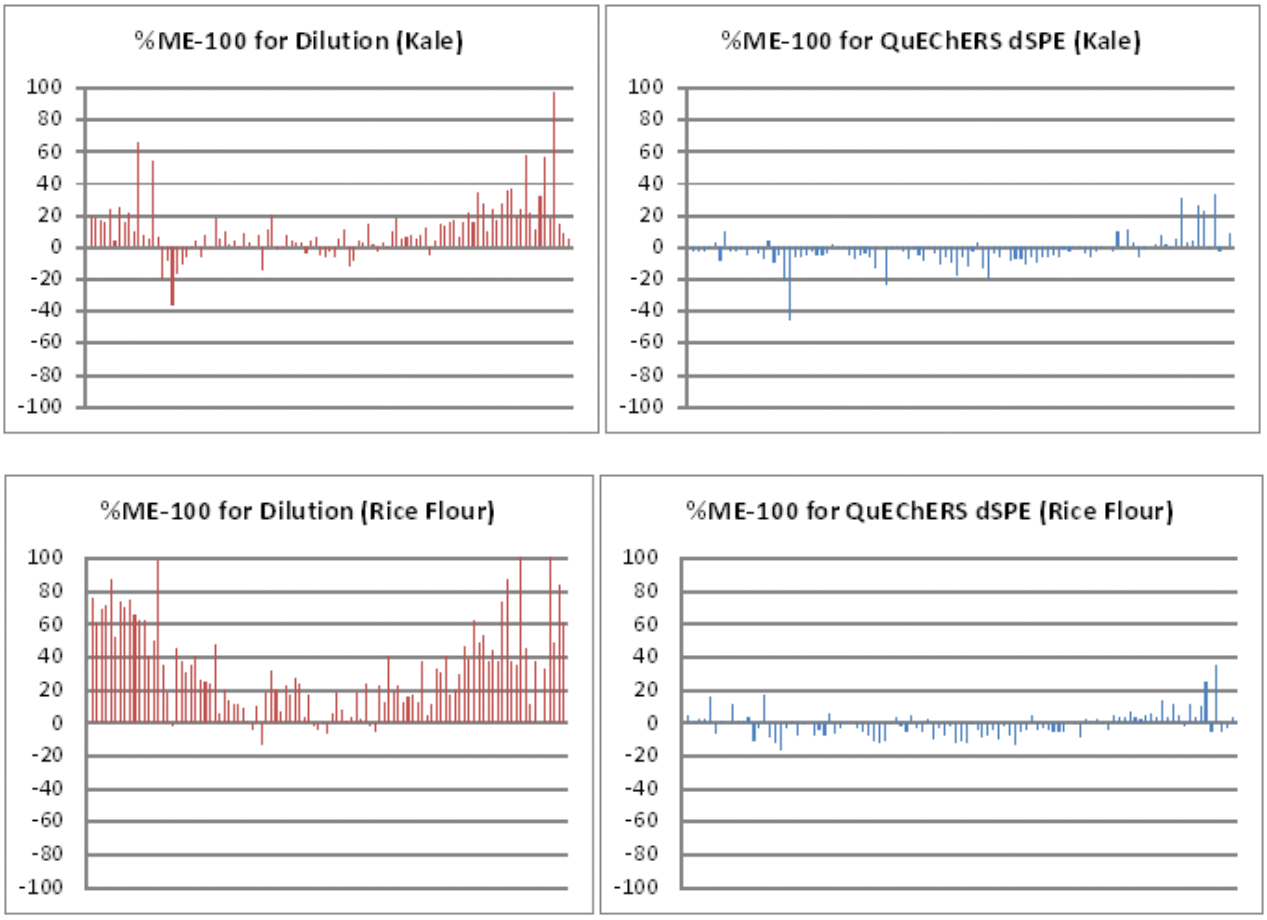


Figure 4: Matrix effects plots for kale (top) and brown rice flour (bottom). For many pesticides, the QuEChERS method reduced matrix effects.

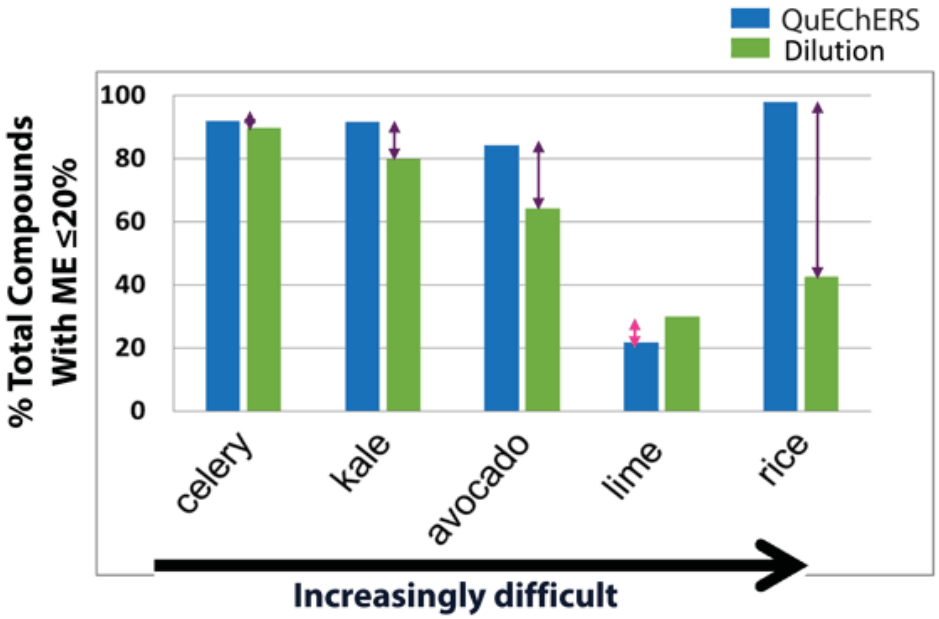


Corresponding plots for kale and rice show the QuEChERS method was able to mitigate matrix effects for some portions of the chromatogram. Signal enhancement at the beginning and end of kale chromatograms is decreased when the sample is treated with the QuEChERS method (Figure 4). The brown rice plots, also in Figure 4, demonstrate a clear example of matrix effect reduction with the QuEChERS sample for many pesticides, especially for early and late eluting pesticides as shown by the U-shaped plot of the dilution method. Lime plots did not show any clear trends.

Matrix Effects Evaluation

Matrix effect values from 80-120% are considered suitable values indicating minor matrix effects. The range is considered acceptable by many people testing for pesticide residues. Often, this 100±20% range is used as a cutoff value to justify using solvent calibration as opposed to matrix-matched standards. We evaluated the different testing strategies by comparing the percent of compounds tested that fell within ± 20% of the solvent curve values (Figure 5). The total number of compounds is 102, but it is adjusted here for incurred pesticides. Incurred pesticides determined by either QuEChERS or dilution methods were removed from both data sets. This ranged from three incurred pesticides for brown rice flour, to fifteen incurred pesticides in celery. Commodities are listed in order of increasing difficulty from left to right in Figure 5.

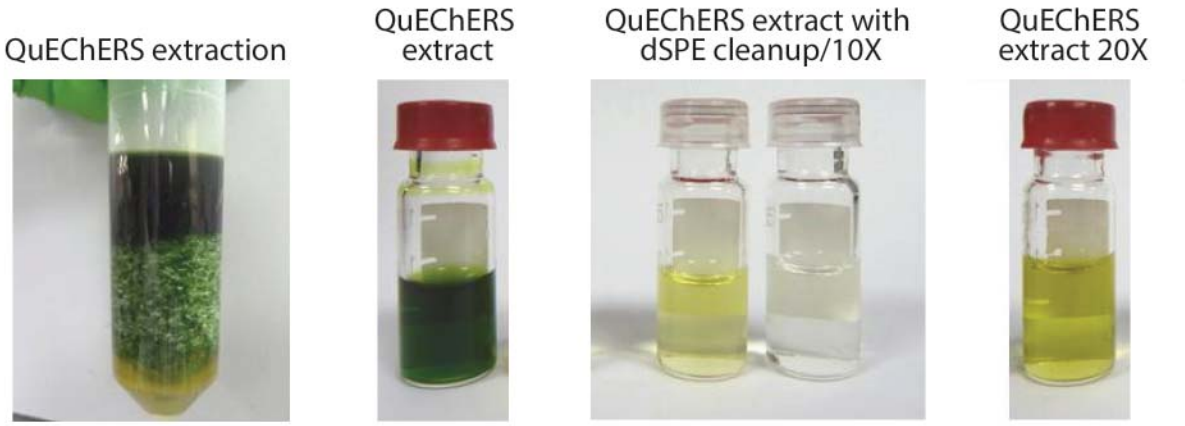
Figure 5: Comparison of the percent of analytes that have matrix effects within 80-120% by commodity and sample preparation method.



Celery has high water, intermediate color, and is low in fat. The performances of QuEChERS and dilution methods are almost identical, 92% and 90% matrix effects, respectively. Both strategies for decreasing the concentration of coextracted material are successful. For celery, the dilution method saved time and eliminated the potential loss of analytes by sample cleanup.

As shown in Figure 6, the initial QuEChERS extraction step was used for both the QuEChERS and the dilution methods. The dark top layer is the acetonitrile extract that was prepared two ways for analysis. For the dilution sample, this extract was diluted 20-fold and analyzed; whereas for the QuEChERS method, this extract was further processed by dispersive solid phase extraction and then diluted 10-fold and analyzed. Much of the pigment was removed by GCB during dSPE cleanup; however, even at this level of dilution significant pigment remains. This impacts the cleanliness of the LC-MS/MS interface requiring more frequent cleaning to maintain the same level of data quality. Based on Figure 5, the QuEChERS and dilution methods performed similarly well for kale with respect to analyte matrix effects. The QuEChERS method resulted in 91% of analytes having low matrix effects compared to the dilution method that achieved low matrix effects for 80% of analytes. That is a relatively small difference of about ten compounds. The QuEChERS method provides a slight advantage in minimizing matrix effects, which in general can help increase the time between interface cleanings. The dilution method performs well and requires less sample preparation time and expense. We estimate that the QuEChERS method added about one and a half hours to total processing time.

Figure 6: Preparation of kale samples for analysis. From left to right, sample layers separated in extraction tube, extract (solvent layer) removed to a clean vial, extract treated with dSPE cleanup, post-cleanup extract diluted 10x (QuEChERS sample for analysis), and pre-cleanup extract diluted 20x (dilution sample for analysis).



Avocado is more challenging chiefly because of its high fat content and lower water content. The QuEChERS cleanup used 50 mg of C18 sorbent per one milliliter of extract to help remove coextracted fat compounds. Figure 5 shows that both the QuEChERS and dilution methods were less effective at minimizing matrix effects in avocado than they were in easier commodities like celery and kale. The QuEChERS method produced low matrix effects for 84% of analytes, which can still be considered tolerable performance. In contrast, the dilution method produced low matrix effects for just 64% of analytes, indicating that it was not able to mitigate matrix effects as well at the QuEChERS method for this matrix. This difference in performance is equivalent to about 20 analytes or one fifth of all target analytes. Figure 3 shows that the removal of matrix lipid material by dSPE cleanup improved matrix effects of analytes in the latter part of the analysis where coelution with lipid type compounds is expected.

The poor performance of both methods for lime is not surprising as citrus fruits are known to be difficult to analyze by LC-MS/MS methods [2,3,4,8]. Despite the challenges, pesticide residue testing for citrus commodities is still performed by LC-MS/MS so we included lime in this work. The QuEChERS method produced low matrix effects for only 22% of analytes while the dilution method did so for 30% of analytes, which is a difference of about eight compounds. It is thought that compounds specific to citrus fruit peel interfere with ionization [7,8].

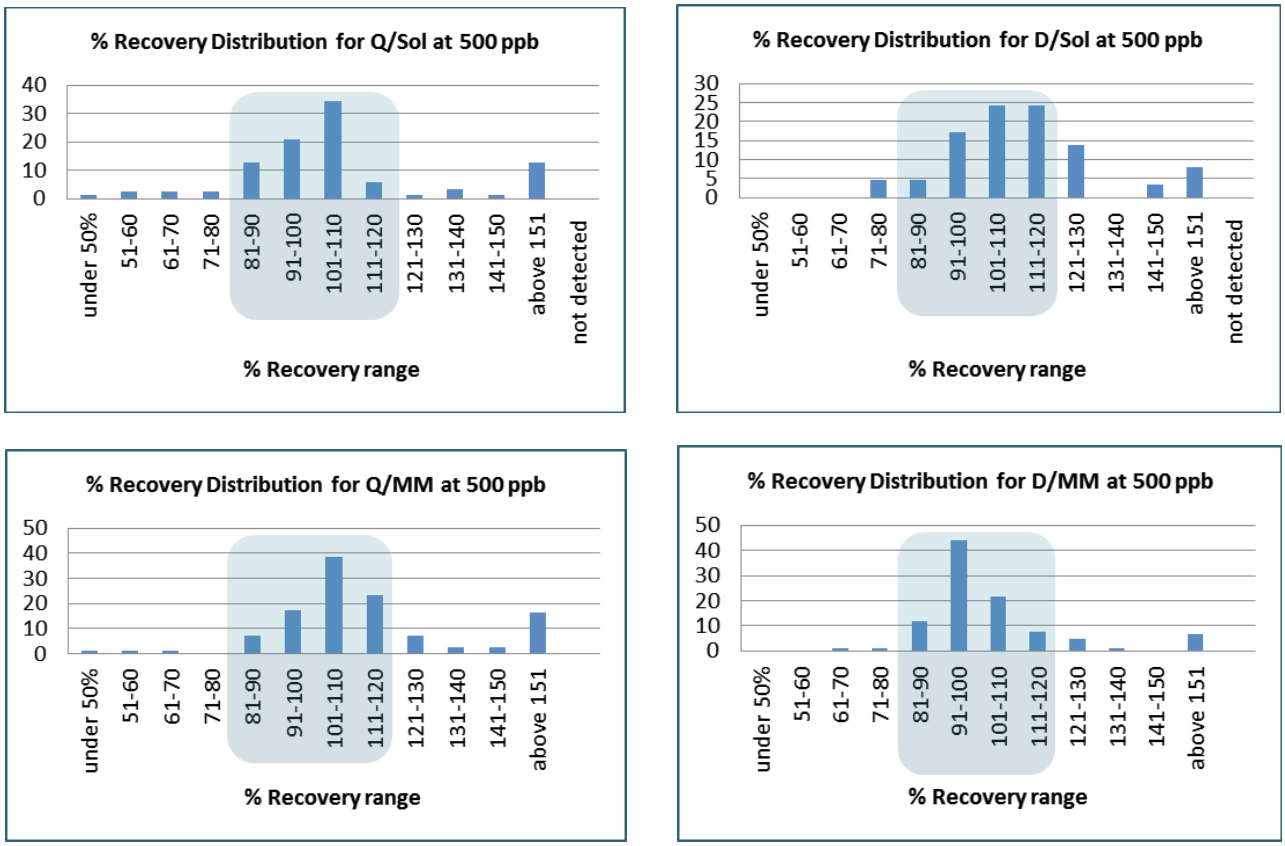
Grains also present challenges for sample preparation because they are dry and contain high levels of coextracted material. For these reasons, a modified QuEChERS method was used as described earlier. The high amount of coextractives can intensify matrix effects, making LC-MS/MS pesticide residue analysis difficult. For brown rice flour, the performance of the methods is significantly different (Figure 5). The modified QuEChERS method shows low matrix effects for 98% of analytes, while the dilution method did so for only 42%. This equates to about 55 analytes for which the QuEChERS method produces acceptably low matrix effects, but that fall outside the range of the dilution method. This indicates that the dilution factor of 20 was not able to reduce the coextractives concentration to the degree needed to produce workable matrix effects. The QuEChERS cleanup step was able to remove carbohydrates and fatty acids that are commonly found in high levels in grains. This was accomplished by the use of PSA sorbent and makes a significant difference with respect to matrix effects.

Both QuEChERS and dilution methods performed well for high water commodities. With these types of samples, the dilution approach offers time savings in both sample processing and also in standard preparation because solvent standards can be used for calibration. As commodities become more challenging with higher concentrations of coextractives, especially fat and carbohydrates, QuEChERS shows better performance by removing more coextracted material compared to the dilution method used in this work. This is demonstrated by the significant differences observed for avocado and brown rice flour. To mitigate matrix effects, QuEChERS provides a good option for pesticide residue testing because it works well for many foods and pesticides and the time and cost expense is small compared to other sample cleanup techniques.

Sample Preparation/Calibration Method Combinations Evaluation

We evaluated the performance of the two sample processing strategies combined with two different calibration methods to determine the best combinations for different commodity types. We know from the discussion above that a dilution method can save time during sample processing. However, if a dilution method requires matrix-matched calibration, this adds significant time to the overall experiment. In some cases, it may be beneficial to process the samples with a more time consuming sample preparation method that would allow the use of solvent calibration. When feasible, analyzing dilution-only samples with solvent calibration is faster and less expensive; however, sometimes acceptable results can only be achieved by using sample cleanup and matrix-matched calibration, which requires significantly more resources. Our evaluation of sample preparations paired with different calibration methods will help establish recommendations for choosing which strategies to apply.

Figure 7: Percent recovery distribution comparing sample preparation and calibration strategy combinations for celery samples. (See Table VI for abbreviation key.)

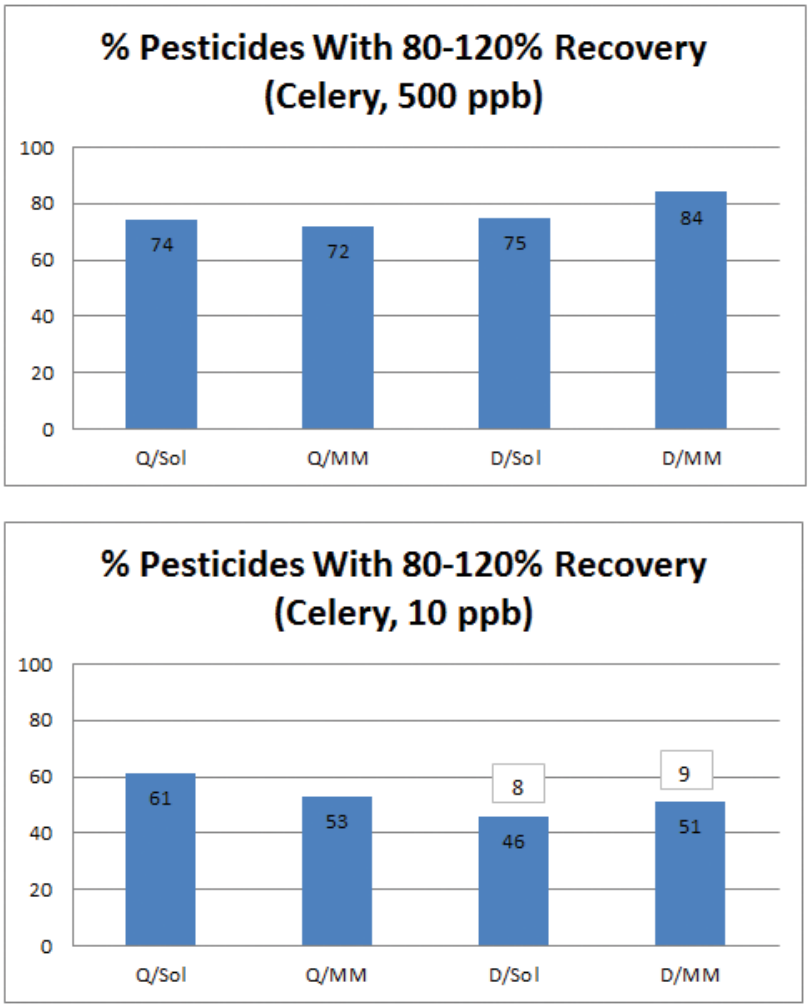


Percent recovery values for all analytes, excluding incurred pesticides, were calculated for each commodity and sample preparation approach using both solvent and matrix-matched calibrations. This yields percent recovery values for each pesticide for the four sample preparation/calibration combinations. This was done at two fortification levels, 500 ppb and 10 ppb. The 500 ppb level was selected to begin with because we thought based on experience that all or most of the pesticides at this level could be quantified even after 10x and 20x dilutions. Fortified samples were also prepared at 10 ppb because this is the default maximum residue limit (MRL) for pesticides in foods if a specific value is not set.

We sorted pesticides into recovery value ranges and plotted these to produce the graphs in Figure 7. The plot shows the percentage of total compounds that fell within the recovery range labeled on the x-axis for celery. The 80-120% recovery range is considered satisfactory for quantitative work. These plots can be used to compare biasing of recovery values between sample preparation/calibration strategies. When these plots were compared for each commodity, generally we saw the same biasing trend towards either high or low recovery values for all four strategies. There were a couple of exceptions where biasing was removed because of the use of matrix-matched calibration, not because of different sample preparation (e.g., lime using QuEChERS method at 500 ppb and avocado using the dilution method at 10 ppb [data not shown]). Biasing was only removed by the use of matrix-matched calibration.

We calculated the percent of compounds that fell within the acceptable recovery range (80 to 120%) and used this to compare sample preparation/calibration method combinations. This is done for each commodity at both the 500 and 10 ppb fortification levels (Figures 8-12). Graphs are used for easy visual comparison and the percent values are listed inside the top of each bar. During analysis, the signal response for some analytes fell below the quantification level or was not detected. In this case, the number of compounds that were not able to be detected or quantified is given above the bar. This is an important parameter for evaluating different testing strategies. For example, in Figure 8, all analytes could be quantified at the 500 ppb level as anticipated. At the 10 ppb level, the QuEChERS method with either solvent or matrix-matched calibration allowed quantification of all analytes. However, for the dilution method, eight analytes could not be quantified by solvent calibration and nine compounds could not be quantified using matrix-matched standards.

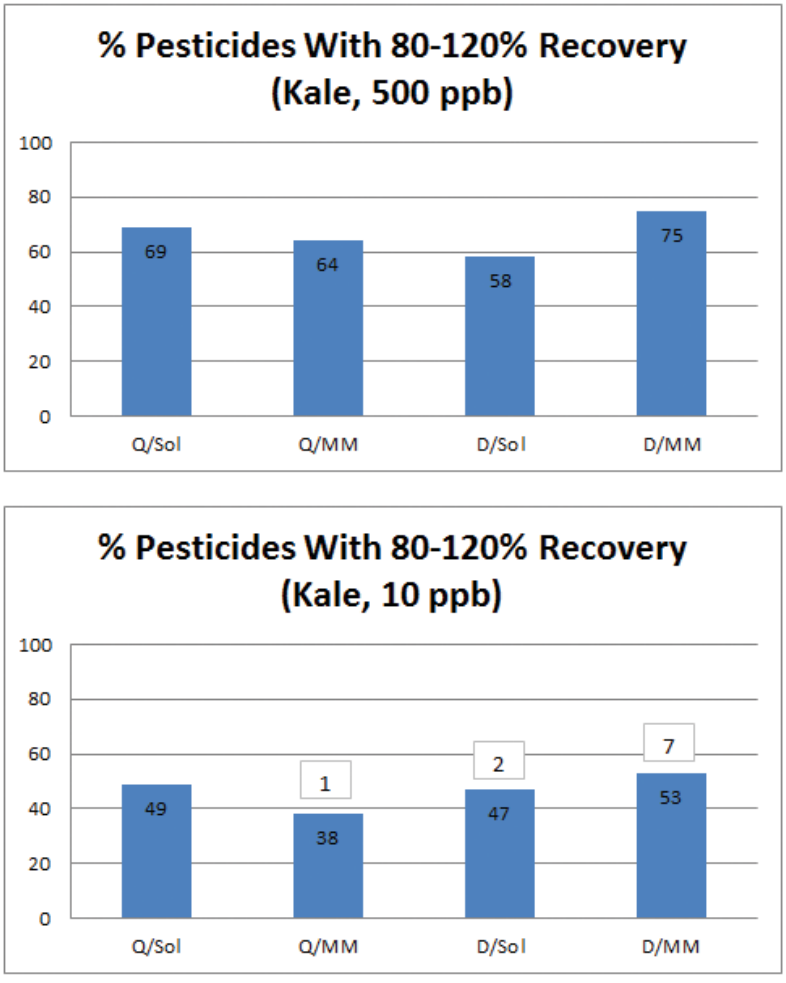
Figure 8: Comparison of percent of pesticides with acceptable recoveries (80-120%) by sample preparation/calibration strategy at 500 and 10 ppb fortification levels in celery. (See Table VI for abbreviation key.)



Of the commodities tested, celery is the easiest to analyze because it is mainly water. While the extract contained some pigmentation, color intensity was reduced significantly by both the dilution and QuEChERS methods. The 500 ppb data indicate that both the QuEChERS and dilution techniques work well, but there is some advantage using a matrix-matched calibration with the dilution method. This equates to about 10 compounds.

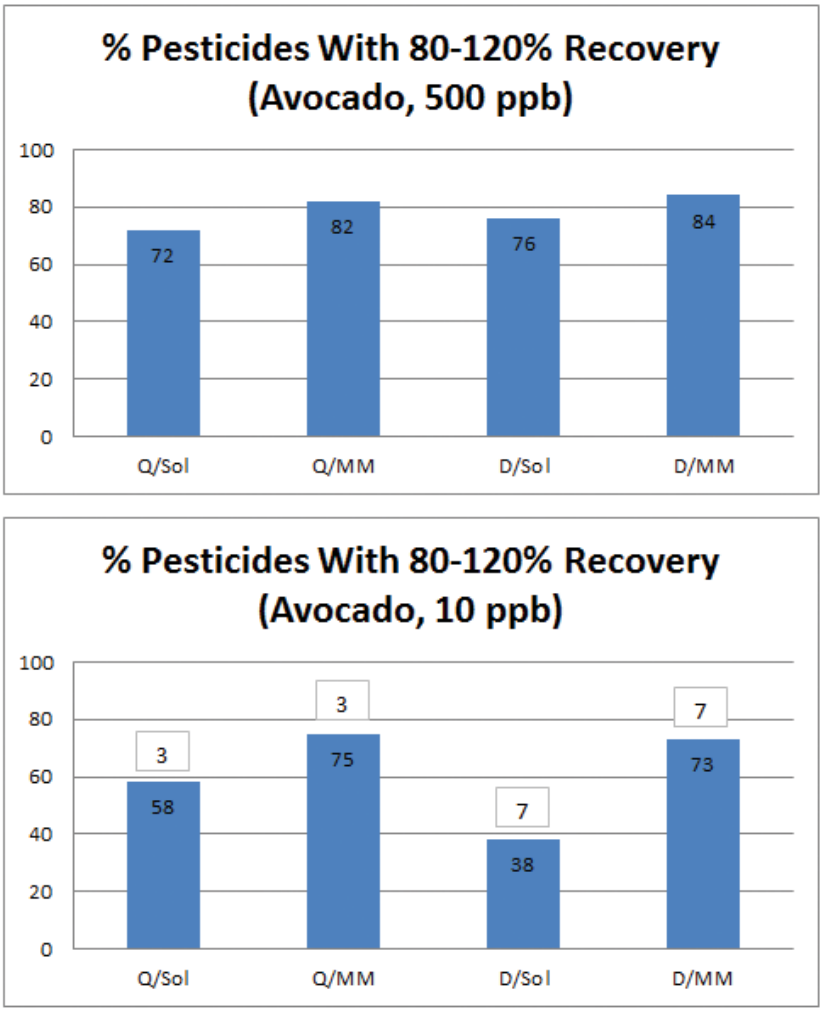
At the 10 ppb level results for the four strategies show similar results. However, the dilution method was not able to detect all of the pesticides regardless of calibration strategy. The injection concentration was 0.5 ppb, which is close the detection limit for some of the 102 pesticides tested. Because there is no advantage with respect to recovery values using the dilution method, choosing the QuEChERS method or at least decreasing the dilution factor are the best options for detectability reasons.

Figure 9: Comparison of percent of pesticides with acceptable recoveries (80-120%) by sample preparation/calibration strategy at 500 and 10 ppb fortification levels in kale. (See Table VI for abbreviation key.)



Kale is considered slightly more difficult due to its higher pigment content, but it is still a relatively easy commodity because it has high water content. For the 500 ppb level shown in Figure 9, the performance is similar between the four sample preparation/calibration strategies. We see again a small increase for the dilution method when using a matrix-matched calibration curve. For the 10 ppb level, the methods performed similarly with the exception of the QuEChERS/matrix-matched calibration scheme. This is surprising as the trend is to see improvement with matrix-matched calibration. The dilution method performs as well as the QuEChERS method but the tradeoff at the lower concentration is failure to detect some pesticides.

Figure 10: Comparison of percent of pesticides with acceptable recoveries (80-120%) by sample preparation/calibration strategy at 500 and 10 ppb fortification levels in avocado. (See Table VI for abbreviation key.)



Avocado is a high fat commodity with about 15% lipid content. It can be a difficult matrix to analyze because some lipids are coextracted in acetonitrile along with the pesticides. At the 500 ppb level, the four different strategies produced similar results, but improvement was seen when using matrix-matched calibration (Figure 10). For the lower fortification level, the benefit seen from using matrix-matched calibration is even stronger. When employing solvent calibration, the QuEChERS approach resulted in more compounds with acceptable recovery values than the dilution method, likely because the QuEChERS procedure used C18 sorbent to remove some of the coextracted lipid material. The dilution method was noticeably inferior, probably because dilution was not able to decrease ionization problems caused by coextracted lipids. This is supported by the matrix effects data showing that matrix effects were more pronounced at the end of the chromatographic analysis where lipids would be expected to elute (Figure 3).

Matrix effects are significant for the dilution/solvent calibration scheme, but using matrix-matched calibration compensated well and produced better recovery values. The QuEChERS/matrix-matched calibration data is almost identical to the dilution/matrix-matched calibration method, but with fewer compounds lost due to sensitivity at the 10 ppb level. In the cases of the compounds that are not detected, the three pesticides not detected using QuEChERS methods are the same and are included in the seven that were not detected for the dilution methods. This is common for undetected pesticides and indicates that the losses are due to sensitivity limitation and are not associated with loss due to QuEChERS sample cleanup. However, there are occasions when the commodity (rice) or the cleanup (GCB) causes low recovery and at 10 ppb these pesticides are difficult to detect.

It is well known that LC-MS based techniques struggle with citrus fruits but we still wanted to compare the sample preparation and calibration methods. At the 500 ppb level, recovery values are poor for all methods (Figure 11). There is a 20% increase, or about 20 compounds, for the QuEChERS method when matrix-matched calibration is used. This demonstrates the influence matrix-matched calibration can have on data quality. The dilution method shows similar results between solvent and matrix-matched calibration. At 10 ppb, it is apparent that this is a difficult matrix for trace analysis by the 21 and 39% values produced with solvent calibration. It is also apparent that the use of a matrix-matched curve can compensate for matrix effects for many pesticides. Using a matrix-matched curve for both the dilution and QuEChERS sample preparation methods significantly increases the number of pesticides that show good recovery, by about 20 and 40 compounds respectively. It is important to note that the dilution methods lost detectability for 8 compounds.

Brown rice flour has low water content and high carbohydrate composition. Dry commodities, like grains, present analytical challenges as they produce extracts with a high concentration of coextractives. The dilution/solvent calibration strategy suffers greatly and is not a viable method for this type of matrix (Figure 12). Matrix-matched calibration is extremely advantageous when used with the dilution method at both concentration levels. For the QuEChERS method, with both solvent and matrix-matched calibration, data show the benefit of removing some of the coextractives with dSPE cleanup. This cleanup step used here included PSA, which can remove fatty acids. This should be helpful for grains that are known to contain high amounts of fatty acids in the extract. Because of the low values at the 10 ppb level for all four schemes, it is likely that some combination of rigorous cleanup, high dilution, and matrix-matched calibration will be needed to yield acceptable results.

Several ideas result from the evaluation of different sample preparation/calibration approaches. All strategies will struggle to produce good recovery values as commodity types become more difficult and are susceptible to stronger matrix effects. This might require adjustment to experimental design to achieve acceptable results. For all foods tested in this work, there is a trad-eoff between spending time on sample cleanup and losing detectability of some analytes due to sensitivity limitations. This can be alleviated by higher dilution factors when instrumentation allows, but for some commodities actual removal of coextractives may be needed. Matrix-matched calibration is a powerful strategy to ensure data quality.

Figure 11: Comparison of percent of pesticides with acceptable recoveries (80-120%) by sample preparation/calibration strategy at 500 and 10 ppb fortification levels in lime. (See Table VI for abbreviation key.)

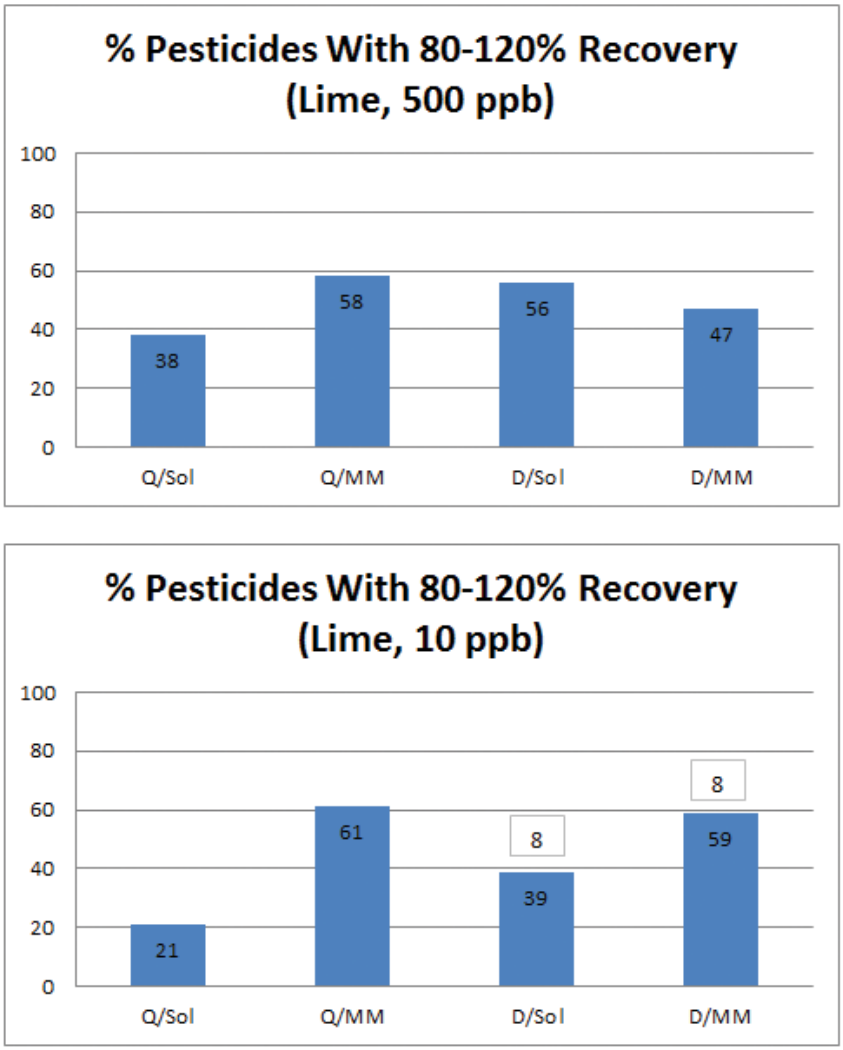
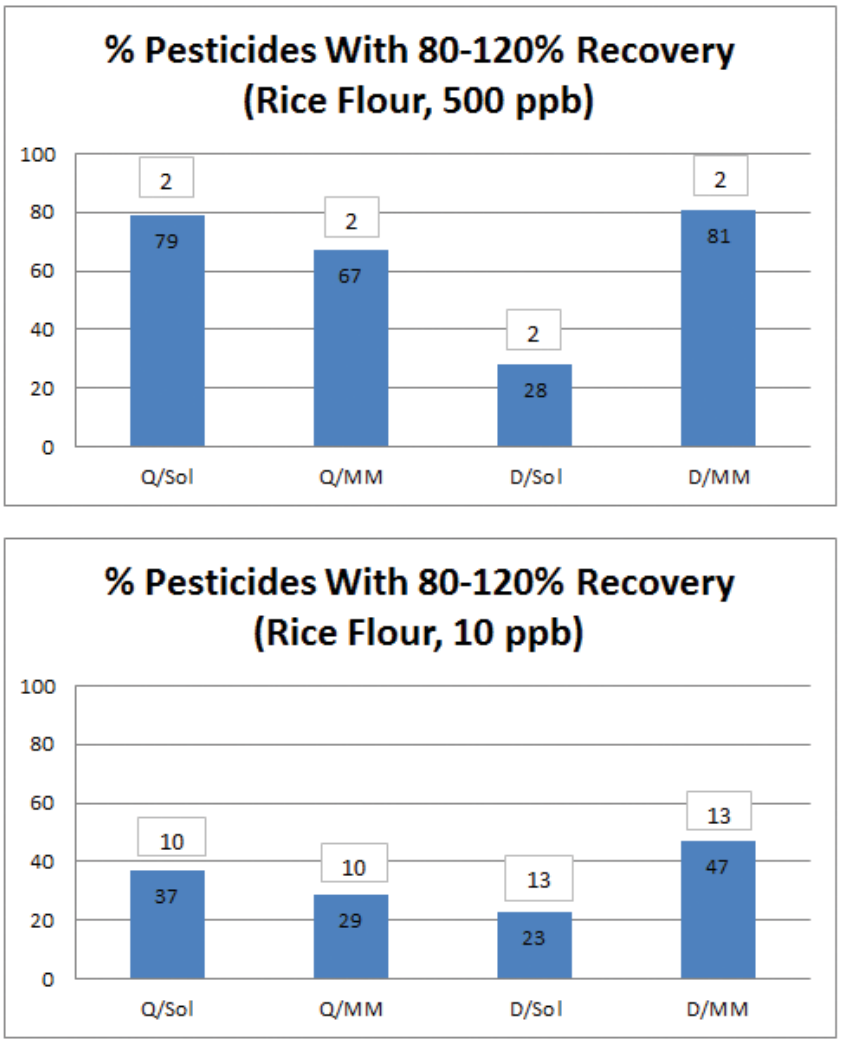


Figure 12: Comparison of percent of pesticides with acceptable recoveries (80-120%) by sample preparation/calibration strategy at 500 and 10 ppb fortification levels in brown rice flour. (See Table VI for abbreviation key.)



Conclusions

Matrix effects must be considered during method development of any pesticide residue method because of their drastic impact on data quality. Sample preparation strategies should be designed and tested to minimize matrix effects and ensure good data quality. Matrix effect studies can be useful for determining the regions of a chromatogram that are severely affected by matrix effects and, therefore, yield information about the nature of the interfering compounds. This information can steer changes to chromatographic conditions to minimize analytes eluting with interfering compounds or identify the best sample cleanup approach. In general, severe matrix effects require more sample preparation or higher dilution factors.

The choice of sample preparation and calibration methods is highly dependent on the commodity. Easy commodities with high water content are likely to be successful with either QuEChERS or dilution approaches, and the use of matrix-matched calibration may not be needed. Commodities that produce severe matrix effects, like grains, require some sample preparation or higher dilution factors, as well as matrix-matched calibration. Foods that have high fat content can be challenging and will require some sample cleanup, high dilutions and likely matrix-matched calibration. For the most difficult commodities, like dry grains, successful methods will likely involve substantial sample cleanup in combination with dilution and matrix-matched calibration. Matrix-matched calibration was the most effective way to improve results, regardless of sample preparation technique. Sample preparation is costly and time consuming and can sometimes result in the removal of analytes during processing, but it is sometimes needed for these types of commodities. Dilution methods do not require this time and financial investment during sample processing. However, high dilutions will be needed in many cases and this will cause some analytes to fall below limits of detection and may require the use of newer, more expensive instrumentation. These are some of the variables and tradeoffs that need to be considered when developing pesticide residue testing schemes.

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