Multiresidue Pesticide Analysis of Agricultural Commodities Using Acetonitrile Salt-Out Extraction, Dispersive Solid-Phase Sample Clean-Up, and High-Performance Liquid Chromatography—Tandem Mass Spectrometry

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S *Supporting Information*

ABSTRACT: A multiresidue method analyzing 209 pesticides in 24 agricultural commodities has been developed and validated using the original Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) procedure and high performance liquid chromatography-positive electrospray ionization—tandem mass spectrometry (LC-MS/MS) analysis. Using solvent-only calibration standards (SOCSs) and matrix-matched calibration standards (MMCSs), it was demonstrated that a minimal concentration of $5-10$ μg/kg (part per billion, ppb) of analytes in matrix is required for the consistent identification of targeted pesticides with two MRM transitions. Method performance was validated by the precision and accuracy results obtained from fortification studies at 10, 25, 100, and 500 ppb and MMCSs. The method was demonstrated to achieve an average recovery of $100 \pm 20\%$ ($n = 4$) for >75% of evaluated pesticides at the low fortification level (10 ppb) and improved to >84% at the higher fortification concentrations in all 24 matrices. Matrix effects in LC-MS/MS analysis were studied by evaluating the slope ratios of calibration curves ($1.0-100$ ng/mL) obtained from the SOCSs and MMCSs. Principal component analysis (PCA) of LC-MS/MS and method validation data confirmed that each matrix exerts its specific effect during the sample preparation and LC-MS/MS analysis. The matrix effect is primarily dependent on the matrix type, pesticide type and concentration. Some caution is warranted when using matrix matched calibration curves for the quantitation of pesticides to alleviate concerns on matrix effects. The QuEChERS method with LC-MS/MS was used to identify and quantitate pesticides residues, with concentrations ranging from 2.5 to >1000 ppb in a variety of agricultural samples, demonstrating fitness for screening and surveillance applications.

KEYWORDS: QuEChERS, LC-MS/MS, matrix effects, identification criteria, multiresidue pesticide analysis

■ INTRODUCTION

There are approximately 1500 pesticides used frequently around the world for pest control applications.[1] Because of potential overuse and adverse health effects of pesticides, there is a need for multiresidue methods to screen, quantitate, and identify as many pesticides as possible to achieve consistent data quality and optimized operational effeciency. Gas chromatography (GC) coupled to element selective and mass spectrometric detectors were the primary instruments used because they were successful to analyze organochlorine, organophosphorus, pyrethroid, and other semivolatile and thermally stable pesticides.[2,3]

However, there are classes of polar, thermally labile, and newly registered pesticides that are not susceptible or not stable to the high temperature conditions typically used for GC analysis, therefore requiring alternative procedures such as high performance liquid chromatography methods. The availability of LC-MS/MS has improved the selectivity and sensitivity of the

analysis and the workflow for identification and quantitation of other pesticide classes in various agricultural products. These reasons led to the development and use of LC-MS/MS multi-residue methods in many pesticide laboratories. The result was consistent, targeted quantitative multiresidue pesticides analysis from a single injection beginning in 2002.[4] With increased instrument sensitivity the number of target pesticides screened in one injection increased.[5−16]

The sample preparation methods used involve an organic solvent extraction step to extract pesticides from the sample, followed by a cleanup procedure to remove matrix coextractives from the solvent extract. The coextractants potentially interfere with the analysis of the pesticides, i.e. matrix effects. Solvents that have been used for extraction include methanol,[6,10] ethyl acetate,[7,8] acetone,[9,12] and acetonitrile,[8,9] with acetonitrile being the most popular due to the development of the QuEChERS and generic extraction procedures.[11,13−16] QuEChERS, developed by Anastassiades et al.,[17] is a relatively simple and quick procedure that makes use of acetonitrile salt-out extraction involving excess amounts of salts (anhydrous magnesium sulfate mixed with sodium chloride, sodium acetate, or sodium citrate/disodium citrate sesquihydrate), followed by a solid-phase dispersive cleanup step involving the acetonitrile extract and a mixture of magnesium sulfate and primary−secondary (PSA) sorbent. Additional cleanup treatments of the extract can also include other dispersive sorbents such as C_{18} and graphitized carbon, as well as using solid-phase extraction cartridges in place of solid-phase dispersion tubes.[14] The sample extracts are diluted prior to LC-MS/MS analysis to minimize matrix effects at the cost of reduced LC-MS/MS sensitivity. Few studies have been performed on the fitness of these methods to perform quantitative analysis under the influence of sample matrices. Nor has there been any evaluation of the recently developed data acquisition software, i.e. *Scheduled* MRM, to enhance selectivity thus increasing the signal-to-noise ratio (SNR), hence improved data quality, of the LC-MS/MS experiment.

Documented in this paper is a QuEChERS sample preparation and LC/MS-MS analysis based multiresidue method for the measurement of 209 targeted pesticides including carbamates, polar organophosphates, phenylureas, anilides, benzoyl phenylureas, conazoles, macrocyclic lactone, neonicotinoids, strobilurines, triazines, and other thermally labile pesticides. The analytical results presented here were obtained using the *Scheduled* MRM data acquisition algorithm and include instrument detection limits (IDL) and method recovery. Method recovery data were obtained using replicate samples ($n = 4$) at 10, 25, 100, and 500 ppb fortification concentrations in 20 fresh produce and four agricultural commodities. Method recovery data were also used to estimate method detection limits according to the U.S. Environmental Protection Agency (EPA) protocols.[18,19] The validity of using two MRM transitions and their peak area ratios to identify target pesticide compounds to meet the European Commission criteria for the mass spectrometric identification of target compounds[20] was investigated. Matrix effects were also studied during the method validation stage. The same method recovery data were used to determine the method detection limits (MDL) as well as the effects of the sample matrix on LC-MS/MS ion suppression and quantitative results (Supporting Information Table S4). Principal components analysis (PCA) is used to investigate analytical data to gain an in depth understanding of the matrix effects derived from these 24 different food matrices. Finally, the method was applied to the analysis of incurred residues in a variety of agricultural commodities.

■ MATERIALS AND METHODS

Chemicals. Most of the 201 pesticide standards used in this work were obtained from the United States Environmental Protection Agency (US EPA) Pesticide Repository (Ft. Meade, MD), while others were obtained through Fluka/Sigma Aldrich (St. Louis, MO), EQ Laboratories (Dr. Ehrenstofer, Atlanta, GA) and Wako Chemicals USA (Richmond, VA) and are listed in Supporting Information Table S1. Methanol, acetonitrile, HPLC-grade water, formic acid, ammonium formate, anhydrous $MgSO_4$, and NaCl were purchased from Fisher Scientific (Pittsburgh, PA). Six deuterium (^2H) isotope labeled internal standards, diazinon-d_{10} (diethyl-d_{10}), dimethoate-d_6 (O,O-dimethyl-d_6), diuron-d_6 (dimethyl-d_6), linuron-d_6 (dimethyl-d_6), dichlorvos-d_6 (dimethyl-d_6), and malathion-d_6 (dimethyl-d_6) were purchased from CDN-Isotopes (Montreal, Quebec, Canada). Two QuEChERS products, i.e. 4 g of anhydrous magnesium sulfate and 1 g of sodium chloride packets with 50 mL centrifuge tubes and 15 mL centrifuge tubes containing 1.2 g of anhydrous magnesium sulfate and 400 mg of primary−secondary amine sorbents, were purchased from United Chemical Technologies (UCT, Bristol, PA). Fresh produce consisting of apple, avocado, beet, bell pepper, blueberry, broccoli, cabbage, carrot, corn, cucumber, eggplant, grape, green bean, onion, orange, peach, potato, spinach, strawberry, and tomato and agricultural commodities, ground hazelnuts, honey, milled wheat flour, and raisins, were purchased as organic products from commercially available sources. Incurred produce samples were obtained from FDA Office of Regulatory Affairs laboratories and from the EPA Analytical Chemistry Laboratory, Ft. Meade, MD.

Separate stock solutions of analytical standards, including those for the isotope-labeled internal standards, were prepared for individual compound by weighing 10−75 mg each and dissolving in 10 or 25 mL of acetonitrile, methanol, or methanol:water (50:50/v:v) in volumetric flasks or calibrated plastic tubes (Simport, Quebec, Canada). Intermediate solutions and spike solutions were prepared in 200 mL volumetric flasks by mixing the stock solutions and used in the preparation of solvent-only calibration standards (SOCSs) and matrix-matched calibration standards (MMCSs) and method recovery studies. SOCSs were transferred to amber vials containing PTFE screw caps and stored at −20 °C.

Sample Preparation. All sample matrices (apple, avocado, beet, blueberry, broccoli, cabbage, carrot, corn, cucumber, eggplant, grape, green bean, onion, orange, peach, potato, spinach, strawberry, tomato, and raisin) were cryo-grounded by blending broken pieces of a half-size block of dry ice in a Blixer 4 blender (Robot Coupe USA Inc., Jackson, MS) until a powdery consistency is obtained. The food samples including skins were cut into portions to allow thorough blending with the dry ice powder. The food portions were blended with the dry ice until powdery, sand-like consistencies were obtained. Approximately 10 lb of the sample were composited in batches and later pooled and mixed together. The homogenized samples were stored in double plastic (polyethylene) freezer bags, left opened to allow the dry ice to evaporate, and later sealed. The samples were stored in a −40 °C freezer until further use.

Method recovery study samples were prepared, in quadruplicates, from the homogenized sample matrices at four spiking concentrations. Method blank samples were also prepared in each sample batch as quality control (QC) samples, as well as used in the preparation of MMCSs in the matrix effects studies. These samples were prepared by weighing 10 ± 0.2 g of cryo-grounded sample in 50 mL disposable screw capped polypropylene centrifuge bottles (Fisher Scientific, Pittsburgh, PA). For fortification studies, spiking solutions at concentration of 0.2, 0.5, and 2.0 μg/mL (0.5 mL) and 5 μg/mL (1 mL) were added into sample tubes to achieve levels of 10, 25, 100, and 500 ppb. These sample tubes were vortexed for 1 min to achieve a homogeneous sample,

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followed by the addition of 10 mL of acetonitrile, 4 g of anhydrous MgSO$_4$, and 1 g of sodium chloride (UCT, Bristol, PA). After hand-shaking the sample to prevent clumping of the salts, 200 μL of internal standard solution was added into each sample and the sample tube placed on a GenoGrinder mechanical shaker (SPEX Sample Prep, LLC, Metuchen, NJ) for 1 min at 1000 strokes/min. Samples were centrifuged at 4500 rpm (4000g) for 5 min. The final extracts ($\sim$9 mL) were transferred to a centrifuge tube containing 400 mg of PSA sorbents and 1200 mg of MgSO$_4$ (UCT, Bristol, PA). The sample tubes were shaken on the GenoGrinder for 1 min (500 strokes/min) and centrifuged at 3500 rpm (2000g) for 5 min. Sample extracts were removed from the centrifuge tube (about 6.5−7.0 mL recovered) and transferred to clean, 15 mL glass centrifuge tubes and stored in freezer until LC-MS/MS analysis. Prior to LC-MS/MS analysis, QC blank and fortified sample extracts were prepared by diluting 200 μL of sample extracts with 300 μL of acetonitrile and 500 μL of sample buffer and filtered to concentrations of 2, 5, 20, and 100 μg/mL for the 10, 25, 100, and 500 ppb fortified samples, respectively. Samples were cloudy at this stage and were filtered using 0.2 μm nylon membrane filters (Sun SRi, Rockwood, TN) directly into the autosampler vials, upon which the solutions became clear and were analyzed as is.

Matrix effects study samples were prepared in pure solvent (i.e., SOCSs) and blank QC sample extracts (i.e., MMCSs) prepared in the method recovery study. In the preparation of a matrix effects study, nine sample batches involving 24 sample matrices were used. The matrix effect study was run over a 72 day period. Seven different concentrations of SOCSs and MMCSs were prepared in quadruplicate for each sample batch. A batch may contain 1−3 different sample matrices. Seven MMCSs were prepared by mixing 300 μL of 3.33. 6.67, 16.7, 33.3, 66.7, 167, 333, and 667 ng/mL standard solutions with 200 μL of matrix blank extracts and 500 μL of sample buffer (8 mM ammonium formate) and filtering with a 0.2 μm nylon membrane. This procedure forms concentrations of 1.0, 2.0, 5.0, 10, 20, 50, 100, and 200 ng/mL. Seven SOCSs were prepared in the same manner with the 200 μL matrix blank extracts replaced by 200 μL of acetonitrile. These SOCSs were used as calibration standards for the LC-MS/MS analysis and as quality control samples for the IDL evaluation and used to evaluate matrix effects on the LC-MS/MS quantitatively.

Samples of agricultural commodities, e.g. 5.0 ± 0.5 g of milled wheat flour, cryo-grounded raisin, and grounded hazelnuts, were used in the study and fortified with 0.25 mL of the 2, 0.5, and 0.2 μg/mL spike solutions to final concentrations of 0.10, 0.025, and 0.01 ppm. For the 0.5 ppm fortified sample, 0.5 mL of the 5 ppm spike solution was used. For honey samples, 10 g were used and the fortification amounts were the same as fresh produce. The wheat flour, raisin, hazelnut and honey samples were vortexed for 10 s and allowed to sit before adding 10 mL of HPLC-grade water. A steel ball bearing was added to the aqueous sample mixture and the samples were shaken on the GenoGrinder at 1000 stroke/min for 1 min prior to the addition of acetonitrile and salts. The raisin sample required additional blending and was shaken with the GenoGrinder until a homogenized mixture was obtained. Once the samples were well homogenized with water, the salts are added, the sample test tubes were hand shaken, and 200 μL of the internal standard was added to each tube. The rest of the procedure for these commodities is the same as for the fresh produce samples.

Instrumentation. Liquid chromatography separation was achieved using a Shimadzu Prominence/20 series (Columbia, MD) and was interfaced to an Applied Biosystems (Forest City, CA) 4000 Q Trap mass spectrometer through an electrospray ionization (ESI) interface. *Scheduled* MRM data were acquired and processed for all compounds in positive ion mode. Identification of target pesticides in incurred samples was done using two specific MRM transitions for each pesticide to achieve an identification point (IP) of 4 according to the European Commission (EC) criteria.[20] Quantification was carried out using
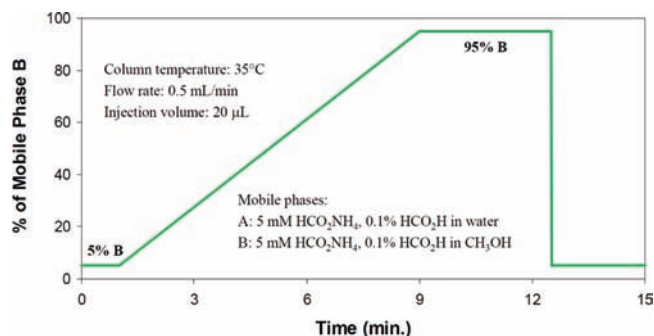
Figure 1. Mobile phases, column temperatures, injection volume, flow rate, and LC gradient parameters used in the separations.

internal standard calibration with ^{2}H$_{10}$-diazinon as internal standard. Nitrogen gas of 99% purity from a nitrogen generator (Parker Balston, Haverhill, MA) was used in the ESI source and the collision cell. A Restek LC column (Bellefonte, PA, USA, Ultra Aqueous, C-18, 100 mm × 2.1 mm, 3 μm) and a 10 mm × 2.1 mm guard cartridge (in guard cartridge holder) were used in the analysis. Mobile phases, column temperatures, injection volume, flow rate, and LC gradient parameters used in the separations are listed in Figure 1. Curtain, collision, nebulizer, and auxiliary gases of the 4000 Q Trap were set at 20, 6, 35, and 45 psi, respectively. Source temperature and entrance potential (EP) were kept at 400 °C and 10 V for the analysis. Ion spray voltage and collision cell entrance potential used were 5000 and 10 V, respectively. Using direct infusion for each analyte, the declustering potential (DP), collision energy (CE), collision cell exit potential (CXP), and the two most intense ion pairs of each analyte were optimized and chosen for the analysis. Values of DP, EP, CE and CXP and the two specific, most intense MRM pairs are listed in Supporting Information Table S1 and used for *Scheduled* MRM data acquisition.

Data Analysis. Pesticide concentrations from LC-MS/MS analysis were determined using Analyst software version 1.5 using ^{2}H$_{10}$-diazinon as the internal standard. These data were exported to Microsoft Excel 2003, and averages, standard deviations (SD) and relative standard deviations (RSD) from instrument analysis and fortification studies were determined. Principal components analysis was carried out using the Pirouette 4.0 software package (Infometrix Inc., Bothell, WA) to compare matrix effects exerted to pesticides by different matrices.

■ RESULTS AND DISCUSSION

Multiresidue pesticides analytical methods discussed in this paper involved two major components, i.e. QuEChERS sample preparation and LC-MS/MS analysis. Therefore, the instrument must first be evaluated as fit for purpose, using parameters such as instrument sensitivity, stability, and linearity. Once the instrument is fit for purpose, the method performance which includes the QuEChERS sample preparation and LC-MS/MS analysis, could be carried out to obtain method recovery data and related statistics for method validation documentation. Listed in Supporting Information Table S1 are names and characteristics of the 209 pesticides that were validated in the method by a single LC-MS/MS analysis in positive ionization mode using deuterium labeled internal standards. These 209 pesticides were selected because of their wide usage and applications on agricultural products and based on their wide range of chemical and physical properties such as volatility, polarity, and nominal molecular weight range between 141.1 (methamidophos) and 899.1 (doramectin).

Scheduled MRM, because of its ability to enhance signal-to-noise ratio (SNR), was used to efficiently screen a large number

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Table 1. Comparison of the MD-IDL Values with Slope Ratios (R_{slope}) and Nutrient Composition of the 24 Agricultural Matrices Studied[a]

	<1 ppb %	1 to 2 ppb %	> 2 ppb %	ND %	R_{slope}	RSD-R_{slope}	water %	protein %	total lipid (fat) %	ash %	carbohydrate %	fiber (total dietary) %	sugars %	total fatty acids %
solvent	94	1	2	3	1	NA	NA	NA	NA	NA	NA	NA	NA	NA
avocado	52	13	29	5	0.52	41.3	73.2	2	14.66	1.6	8.53	6.7	0.66	11.935
apple	77	4	12	7	0.93	8.9	85.6	0.26	0.17	0.2	13.81	2.4	10.4	0.086
bean	78	5	13	4	1.02	10.7	90.3	1.83	0.22	0.7	6.97	2.7	3.26	0.173
beet	59	22	12	7	0.79	12	87.6	1.61	0.17	1.1	9.56	2.8	6.76	0.119
blueberry	74	8	13	5	0.91	11.4	84.2	0.74	0.33	0.2	14.49	2.4	9.96	0.221
broccoli	65	6	27	3	0.93	5.3	89.3	2.82	0.37	0.9	6.64	2.6	1.7	0.088
cabbage	79	5	8	8	0.9	10.7	92.2	1.28	0.1	0.6	5.8	2.5	3.2	0.068
carrot	72	6	5	14	0.87	21.2	88.3	0.93	0.24	1	9.58	2.8	4.74	0.168
corn	79	8	7	6	1.0	11.6	76	3.22	1.18	0.6	19.02	2.7	3.22	1.088
cucumber	68	3	25	4	0.84	8.95	95.2	0.65	0.11	0.4	3.63	0.5	1.67	0.074
eggplant	72	6	17	5	0.88	9.6	92.4	1.01	0.19	0.7	5.7	3.4	2.35	0.126
grape	75	10	11	3	1.06	8.51	80.5	0.72	0.16	0.5	18.1	0.9	15.5	0.109
hazelnut	74	7	6	12	0.79	18	5.31	14.95	60.75	2.3	16.7	9.7	4.34	50.116
honey	58	18	18	6	0.5	13.4	17.1	0.3	0	0.2	82.4	0.2	82.1	0.195
onion	69	11	15	4	0.76	29.05	89.1	1.1	0.1	0.4	9.34	1.7	4.24	0.072
orange	79	5	3	13	0.72	26.2	82.3	1.3	0.3	0.6	15.5	4.5	NA	0.13
peach	79	3	5	13	0.98	15.5	88.9	0.91	0.25	0.4	9.54	1.5	8.39	0.172
pepper	70	7	17	5	0.96	25.7	92.2	0.99	0.3	0.5	6.03	2.1	4.2	0.1
potato	73	8	15	4	0.87	8.5	79.3	2.02	0.09	1.1	17.47	2.2	0.78	0.071
raisin	78	3	6	13	0.92	13.5	16.6	2.52	0.54	1.9	78.47	6.8	NA	2.739
spinach	78	3	5	13	0.82	16.8	91.4	2.86	0.39	1.7	3.63	2.2	0.42	0.852
strawberry	76	10	10	3	0.95	9.36	91	0.67	0.3	0.4	7.68	2.0	4.89	0.213
tomato	70	8	17	5	1.04	6.6	94.8	1.16	0.19	0.7	3.18	0.9	0.03	0.104
wheat flour	77	3	6	13	0.94	19	10.3	13.7	1.87	1.6	72.57	12.2	0.41	0.554

[a] Percentages of pesticides with matrix-dependent instrument detection limit (MD-IDL) values <1 ppb, from 1 to 2 ppb, greater than 2 ppb and those could not be detected/quantitated (ND). NA: not applicable. R_{slope} and relative standard deviations of R_{slope} (RSD-R_{slope}) determined using solvent-only calibration and matrix-matched calibration standards ranging from 1 to 100 ng/mL. Nutrient composition information provided by the USDA National Nutrient Database (www.nal.usda.gov/fnic/foodcomp/search/).

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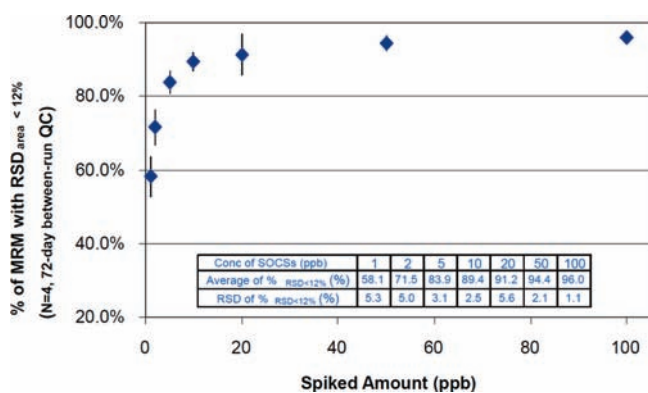
Figure 2. Long-term quality assurance data (nine sample batches in 72 days) obtained from the RSD of area counts for each pesticide in the seven-level solvent-only calibration standards (SOCSs, in quadruplicates). The average percentage of the 418 MRM transitions with a RSD $\leq$ 12%, along with their respective RSD (n = 4), are shown to demonstrate the minimal concentration requirement to fulfill positive identification of the target compounds.

of target pesticides in a single LC-MS/MS analysis. Different from previous multitargeted screening methods in which data acquisition is done by the legacy approach using wide range time segment windows for all analyte transitions that would elute within the time segment,[4−14] *Scheduled* MRM acquires transitions of the analytes in time windows by their predicted retention times. These MRM detection windows have a long enough dwell time, permitting sufficient data collection to accurately define the chromatographic peak. Optimizing the duty cycle and dwell times for each individual analyte ensures the best SNR is generated for each analyte as well as the optimal data points to characterize chromatographic peak. This improves the precision of the analyte peak area. From a previous study,[21] SNR of pesticides obtained from *Scheduled* MRM data acquisition can be significantly increased by 52−611% compared to segmented MRM data acquisition.

Instrument Performance. The instrument detection limit (IDL) of LC-MS/MS for each analyte was obtained by using procedures in the U.S. Environmental Protection Agency's (EPA) protocol for the determination of the method detection limit (MDL).[19] Rather than using the standard deviations of recovery results obtained from the method used to calculate the MDL, the standard deviations of the responses obtained from the two MRM transitions from eight consecutive analyses of SOCSs at concentrations 1.0, 2.0, 5.0, and 10 ng/mL were used instead to calculate the IDL for each analyte (i.e., 2.998 × SD (critical $t_{0.010}$ = 2.998 at degree of freedom (d_f) of 7)). Supporting Information Table S2 contains the calculated IDLs for each MRM transition. Because of the difference in the relative abundances for the different transitions, the true IDLs would be for the transitions with the lower relative abundances. Matrix dependent IDL (MD-IDL) was determined by analyzing eight replicates of MMCSs prepared at concentrations 1.0, 2.0, 5.0, and 10 ng/mL for each of the 24 agricultural matrices. Because of matrix effects and depending on the ionization efficiency of each pesticide, some pesticides were not detected at the lower levels, and analytical data from the next higher level were used to determine the IDL. The MD-IDLs were calculated using the same procedure used for the determination of the IDLs. Supporting Information Table S2 lists typical IDLs and MD-IDLs for

the 209 pesticides in 24 matrices. For each pesticide, there are two listings which correspond to the two MRM transitions used for each analyte. The limit of quantitation (LOQ) can be calculated at three times the LOD values and were not listed in this table. From the summary data in Supporting Information Table S2, it is clear that the LC-MS/MS experiment described above can achieve an IDL of 2 ppb or better for 95% of the two MRM transitions used for each pesticide.

Using the data in Supporting Information Table S2, percentages of pesticides with IDL and MD-IDL values less than 1 ppb, between 1 to 2 ppb, greater than 2 ppb, and those could not be detected/quantitated (ND) were summarized and listed in Table 1. Table 1 also lists percent composition of major nutrients in the 24 matrices studied for reference (USDA National Nutrient Database, www.nal.usda.gov/fnic/foodcomp/search/). The data in Table 1 indicate 2% of the pesticides in solvent only had IDL values greater than 2 ppb and 3% could not be detected or quantitated. The pesticides that could not be detected or quantitated were primarily the macrocyclic lactone insecticides, avermectin B_{1a} and B_{1b}, doramectin, eprinomectin, ivermectin, moxidectin, or compounds whose primary or secondary transition ions were poorly responsive such as amitraz, butocarboxim, cymoxanil, flubendiamide, fludioxinil, mesotrione, and zoxamide. Because all IDL values were derived by using the EPA protocol, they should be considered as conservative and potentially these IDLs could be 10 times higher than those derived through the traditional signal-to-noise ratio (SNR) approach. Such use of the U.S. EPA protocol also formed a consistent metric for any laboratories to carry out follow-up experiments for comparison purposes.

The European Commission (EC) criteria [20] for confirmation of identity specify relative ion ratios of primary and secondary MRM transitions for identification. To fulfill these criteria, it is important to determine the minimal concentrations at which the two MRM transitions of pesticides have stable and consistent responses. During the 72-day study period the peak areas of SOCSs at concentrations of 1.0, 2.0, 5.0, 10, 20, 50, and 100 ppb of nine sample batches were used to estimate the minimal concentrations. As indicated in Figure 2, at 10 ppb or higher concentrations, the majority (>89.4%) of pesticides have consistent responses to ensure true-positive identification of target compounds. This fact was also reported in previous single-laboratory and interlaboratory validation studies.[14,16,19]

From Table 1, MD-IDL values were affected by the matrices and have much larger values than the IDL. In fact, 93% of analytes evaluated have a < 1 ppb IDL value and only 3% of analytes are deemed to be not detected/not quantitated (ND) when using standards in solvent only (SOCSs) and the LC-MS/MS parameters. From Table 1, depending on the matrix, it can be determined that only 52−79% of the analytes can achieve at the low MD-IDL of <1 ppb. The total percentage of analytes that were deemed to be ND increased to at least 3% for broccoli and as high as 14% in the carrot matrix. There was no observable correlation between the percentage distribution of MD-IDL values or the number of ND for each sample matrix and individual sample nutrient compositions (water, protein, total lipid, ash, carbohydrate, fiber, sugar, and total fatty acid). The only observation is that matrix effects will affect LC-MS/MS sensitivity and are independent of the overall nutrient compositions in the sample matrices.

Another indicator that the sample matrix affects the analysis is by a change in the slope of the calibration curve. To determine what slope ratio between the standard and the sample is
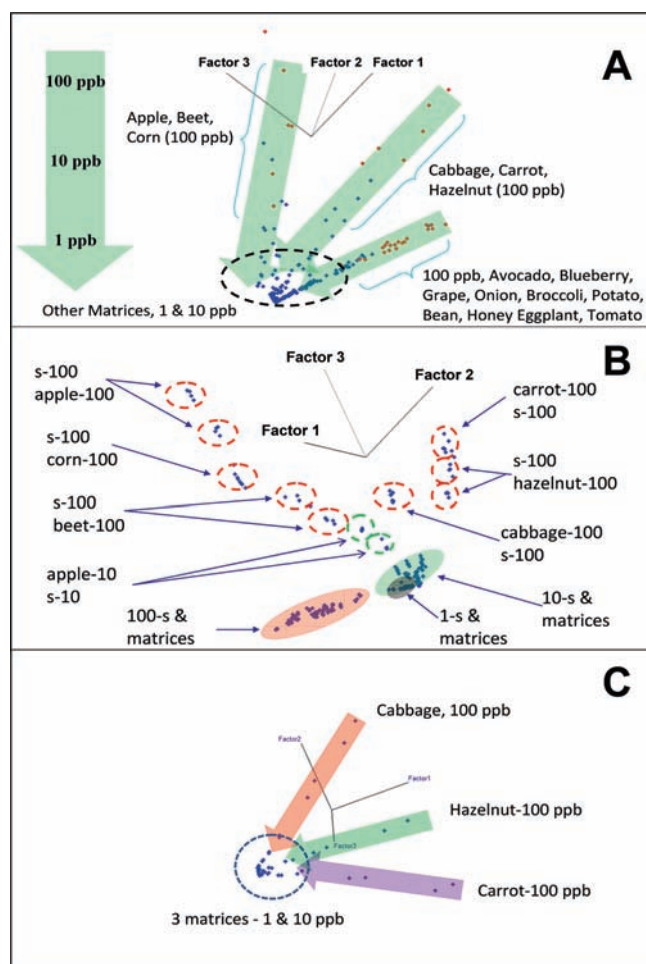
Figure 3. Scores of the three most significant factors (represent >96% weight of the ~70000 data points) obtained from the principal component analysis (PCA). PCA was used to investigate the relationship between the sample matrices, sample concentrations, and the area counts obtained from the IDL and MD-IDL studies. The two plots (A and B) show the same data set viewed from two different Cartesian axes. Plot (C) shows PCA results from a smaller data set (carrot, hazelnut, and cabbage group) to reveal their respective matrix effects. The results indicate there are no clear correlations between the different fresh produce matrices.

experimentally significant, the confidence interval for the slope using least-squares regression can be determined for the standard (SOCS). From the confidence interval for the slope, the computed ratio that suggests a matrix effect can be determined. The data from a number of analytes and matrices suggest a ratio of less than 0.9 and greater than 1.1 are true matrix effects on the calibration with MMCSs.

We also compared the slope ratios (R_{slope}) of calibration curves obtained from SOCSs and MMCSs at levels 1.0−100 ng/mL to evaluate matrix effects for all analytes. In the absence of experimental error, a value of 1.00 for the R_{slope} shows no matrix effects, while values deviating from 1.00 indicates the existence of matrix effects and can be suppression (<1.00) or an enhancement (>1.00). Matrix effects, as expected, increase with increase deviation of R_{slope} from 1.00. Supporting Information Table S1 shows 24 plots of R_{slope} values obtained from the 24 matrices studied and plotted against the analyte number for the 209 pesticides. The R_{slope} averages and RSDs of all analytes in each
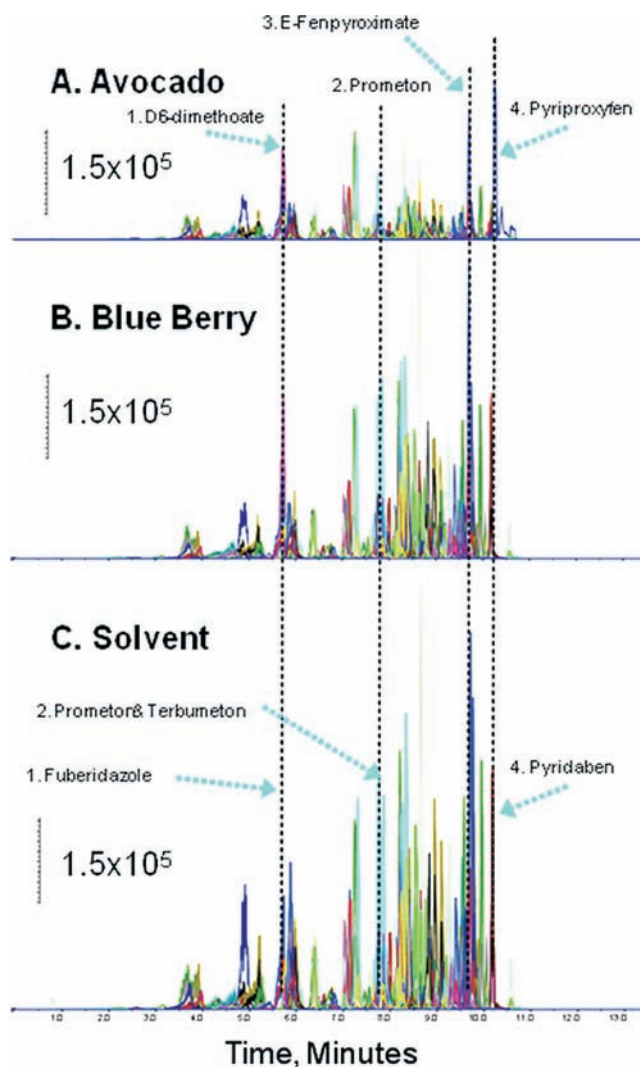
Figure 4. Typical reconstructed MRM chromatograms for the 209 pesticide mixture obtained from two matrix-matched calibration standards (MMCSs) of avocado (A), blueberry (B), and one solvent-only calibration standard SOCS (C) at concentration of 10 ng/mL.

matrix are listed in Table 1 and provide a quantitative statement of the "average matrix effect". From Supporting Information Table S1, three general trends can be seen depending on sample matrices: matrix effects can be minimal and consistent for all 209 analytes studied (e.g., apple, tomato, broccoli, potato), severe but consistent (e.g., honey, beet, eggplant, avocado and cucumber), or severe and randomly affecting each analyte (e.g., onion, orange, raisin, and peach). These observations matched well with trends observed in the average values of R_{slope} and RSD listed in Table 1.

To determine whether matrices with similar nutrient components would present similar matrix effects, principal component analysis (PCA) was used to further investigate the relationship between the sample matrices, sample concentrations, and the area counts obtained from the IDL and MD-IDL studies. The PCA scores (Figure 3) were based on the area counts that correspond to the primary MRM transitions of each analyte obtained from both SOCSs and MMCSs at 1.0, 10, and 100 ng/mL in the 24 matrices. The three most significant factors represent >96% weight of the data set that includes around 70000

Table 2. Results of Fortification Studies and % Nutrient Composition of Each Sample Matrix (ND, Not Detected/Quantitated; RSD, Relative Standard Deviation)

| | average recovery ± relative standard deviation (%) | | | | | % of analytes with 100 ± 20% recoveries | | | |
| | fortification levels (μg/kg, ppb) | | | | | fortification levels (μg/kg, ppb) | | | |
commodity	10	25	100	500	no. of "ND" analyte	10	25	100	500
apple	96 ± 14	99 ± 14	99 ± 14	105 ± 14	14	80	86	85	87
avocado	90 ± 27	99 ± 20	86 ± 12	83 ± 13	15	59	74	74	67
bean (green)	91 ± 17	92 ± 13	94 ± 15	98 ± 12	15	76	82	84	90
beet	91 ± 13	83 ± 12	86 ± 14	91 ± 9	11	82	66	73	90
blueberry	90 ± 17	93 ± 15	99 ± 16	89 ± 16	11	77	82	84	82
broccoli	102 ± 16	98 ± 16	99 ± 15	102 ± 15	9	81	86	88	91
cabbage	96 ± 14	99 ± 14	90 ± 15	89 ± 15	14	80	86	85	87
carrot	100 ± 33	100 ± 16	96 ± 10	88 ± 12	34	73	76	79	77
corn	98 ± 14	82 ± 12	91 ± 10	87 ± 12	9	83	64	89	83
cucumber	114 ± 12	108 ± 13	121 ± 14	108 ± 11	12	68	81	38	91
eggplant	101 ± 20	109 ± 14	94 ± 14	77 ± 16	11	79	74	86	61
grape	96 ± 13	97 ± 13	106 ± 14	103 ± 14	10	81	87	87	92
hazelnut	102 ± 24	101 ± 13	98 ± 12	97 ± 12	21	75	79	83	82
honey	108 ± 17	103 ± 16	111 ± 17	95 ± 15	10	75	86	71	90
onion	107 ± 15	98 ± 13	105 ± 13	102 ± 12	10	77	84	89	91
orange	109 ± 35	105 ± 29	101 ± 9	105 ± 9	16	78	84	90	92
peach	107 ± 33	110 ± 33	107 ± 10	102 ± 9	16	82	84	86	92
pepper (bell)	116 ± 16	110 ± 13	97 ± 13	97 ± 13	12	57	72	86	87
potato	98 ± 16	98 ± 15	103 ± 17	91 ± 15	10	81	85	87	89
raisin	109 ± 58	107 ± 51	102 ± 14	97 ± 9	24	72	77	81	85
spinach	100 ± 32	93 ± 26	92 ± 10	89 ± 10	19	75	77	84	81
strawberry	100 ± 11	90 ± 13	104 ± 13	106 ± 11	11	85	82	85	91
tomato	98 ± 13	94 ± 10	100 ± 12	78 ± 12	12	85	87	90	70
wheat flour	111 ± 16	109 ± 14	112 ± 12	110 ± 12	24	50	65	51	64
average	101	99	100	92	-	75	79	81	84
RSD	8	8	8	20	-	9	7	9	10
maximum	116	110	121	110	-	85	87	90	92
minimum	90	82	86	83	-	50	64	38	61

data points. In Figure 3A, the three main contributing groups of matrix effects were lined up by the three arrows going in the direction from 100 to 1.0 ng/mL, indicating that the scores are proportional to the concentrations and there is minimal correlation between matrix effects and the nutrient compositions listed in Table 1.

Figure 3B illustrates the same PCA data plotted by arranging the Cartesian axis from a different angle. Similar to Figure 3A, most of the main contributing factors (100 ppb) can be seen in Figure 3B along the three main groups. In addition, one could see that there are smaller groups within the three main groups, with the less significant components falling into three clusters at the bottom of Figure 3B. The appearance of these same three groups in Figure 3A is an aberration from scores of other matrices that force each other into the three observed groups. To confirm this statement, we carried out a simpler PCA using data obtained from cabbage, carrot, and hazelnut with results plotted for the three most significant factors shown in Figure 3C. Clearly, the three matrices reveal its own characteristic groups and confirm that each matrix exerts a different amount of effects to the analytes and needs to be addressed separately. For food matrices

with similar nutrient composition (e.g., fresh produce), there is no clear pattern regarding matrix effects in the 24 matrices studied, with the exception of the concentration level (1, 10, and 100 ppb) effect.

The effects of the matrix are illustrated in Figure 4, which shows typical reconstructed MRM chromatograms for the 209 pesticide mixture obtained from two MMCSs (avocado and blueberry, Figure 4A,B) and one SOCS at 10 ng/mL. From Figure 4, one can see that due to matrix effects, the relative peak intensities differ in each of the two matrix and solvent standards. The SOCS (Figure 4C) generally showed higher peak intensities, while the blueberry (Figure 4B) and avocado matrices (Figure 4A) exerted moderate and severe (lower) matrix effects, respectively, on the peak intensities in the MMCS samples. For example, the intensities of fuberidazole, E-Fenpyroximate, and pyridaben (Figure 4C, peaks 1, 3 and 4) were reduced as much as 25% from the SOCS compared to the blueberry MMCS and 67% compared to the avocado MMCS. The reduction in the intensities of the fuberidazole and pyridaben peaks (peaks 1 and 4, Figure 4C) due to matrix suppression is indicated by the replacement of dimethomate-d_6 and pyriproxyen (peaks 1 and

4, Figure 4A) as the more dominating peaks. The two blue traces of prometon and terbumeton (peak 2, Figure 4C) have their intensities reduced by at least 75% in the avocado matrix, resulting in a chromatogram that reveals prometon (peak 2, Figure 4A) as the only observable peak present in the avocado matrix.

Matrix effects can impact the data quality for the LC-MS/MS analysis of pesticides and other chemical contaminants. One type of matrix effect, isobaric interference, is a result of coeluting compounds of equal nominal masses and does not depend on the ionization method. This type of matrix effect can be resolved by changing the MRM transition pair, improving the LC separation to resolve the analyte from the interfering matrix component or using an orthogonal method such as HPLC-high resolution mass spectrometry to distinguish the exact masses of the analyte from the matrix component.[22,23] The second matrix effect, suppression/enhancement, is atmospheric pressure ionization-dependent. This is caused by the interaction between the analyte and matrix coextractives in the prepared sample that could either suppress or enhance the ionization of the analyte in the ion source, resulting in a lower/higher measured signal and affecting the accuracy of the quantitative result. The result can be in the form of signal suppression or enhancement when compared to the pure analytical standard prepared in solvent only. This is a challenging issue for LC-MS/MS analysis because this type of matrix effect can depend on the analyte, sample matrix, or mode of ionization.

Several approaches have been used to circumvent the problems resulting from the matrix components. These include extensive sample cleanup, improving LC separation to avoid coeluting with the matrix components, serially dilution of the final extract such that fewer matrix components will be injected into the analytical system, or postcolumn infusion.[24−26] Splitting the LC eluent flow before entering the mass spectrometer may also help eliminate matrix effects.[27] It was also suggested that MMCSs be used to establish a calibration curve.[26,28] In this study, we used a 1:5 dilution and MMCSs to study matrix effects in terms of instrument performance (IDL and MD-IDL) and method performance (recoveries and MDL).

Method Validation. Method validation is performed by fortifying quadruplicate samples of fresh produce and agricultural commodities at four concentration levels of 10, 25, 100, and 500 ppb. Diazinon-d_{10} was used as an internal standard and seven levels of MMCSs fitted to $1/C$ weighted (C being the concentration of each calibration curve) linear equations with correlation coefficients (r^2) greater than 0.99 were used for quantitative analysis in their respective sample matrices. Listed in Supporting Information Table S3 are the average method recoveries (%R, $n = 4$) and RSD of each target pesticide in the 24 matrices and at the four fortification levels obtained from the 96 fortification experiments.

From Supporting Information Table S3, we summarized the average recoveries of all analytes in each matrix at four fortification levels in Table 2. The range of results from the fortification studies list average recoveries and relative standard deviations (RSDs) of each commodity between 82 (corn, 25 ppb) and 121% (cucumber, 100 ppb) recovery. The average recoveries (±RSDs) at the 10, 25, 100, and 500 ppb levels were 101 ± 8, 99 ± 8, 100 ± 8, and 92 ± 20%, respectively. Higher RSDs (>20%) at the two lower fortification (10 and 25 ppb) concentrations were observed in matrices (carrot, hazelnut, orange, peach, raisin, and spinach). Although higher RSDs (>25%) were
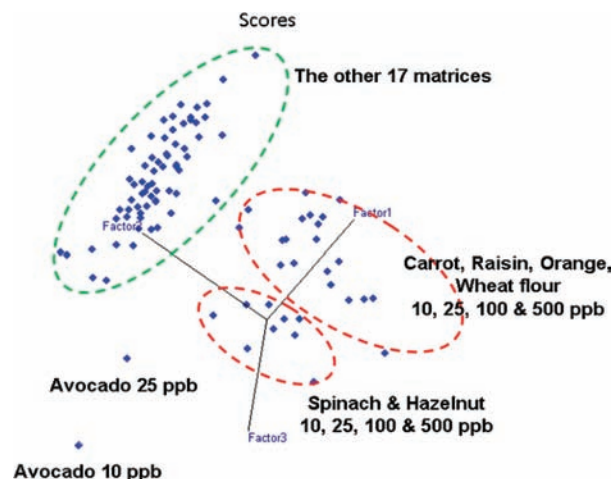
Figure 5. Results of PCA analysis of the % recovery data obtained from the fortification studies. The PCA analysis was determined by a total of 20096 recovery data points to reach the scores for the 24 matrices at the four fortification level.

observed at the 10 and 25 ppb concentrations for individual commodities such as avocado, orange, peach, raisin, and spinach, a larger variation in the average recovery was observed at the 500 ppb level for eggplant (77 ± 16%) and tomato (78 ± 12%) to cucumber (108 ± 11%) and wheat flour (110 ± 12%). These results suggest that specific coextractives in the different commodities may affect specific pesticides at the lower fortification concentrations. The variability at the 500 ppb concentration could be a result of variability in the sample preparation which becomes more evident at the higher fortification concentration.

The method at the lowest fortification level of 10 ppb was shown to achieve an average recovery at 100 ± 20% for 75% of the analytes in all 24 matrices. Method performance improves with increasing fortification concentrations as indicated by the increasing average recoveries in the 24 matrices, with values of 79%, 81% and 84% at the higher fortification concentrations of 25, 100, and 500 ppb. Using the lowest SDs obtained from the fortification experiment (Supporting Information Table S3), MDLs for each pesticide were calculated at the 95% confidence level according to the U.S. EPA protocol and listed in Supporting Information Table S3.Supporting Information Table S3. The number of analytes that could not be detected in a specific matrix (i.e., no MDL values and labeled as ND in Supporting Information Table S3) were also listed in Table 2. At the outset, and for reasons unknown, the method performed poorly for five of the 96 fortification experiments (i.e., corn at 25 ppb, cucumber at 10 and 100 ppb, and eggplant and tomato at 500 ppb), and these five data experiments were treated as outliers in the method validation data shown in Table 2.

Method performance data documented in Table 2 and Supporting Information Table S3 were determined using MMCSs and were expected to facilitate correcting for matrix effects.[26] Therefore, method performance measured by the average recoveries of all analytes in each sample matrix should be similar, improve with increasing fortification concentrations, and not be affected by matrix effects. From Table 2, the percentage of the number of analytes with recoveries at 100 ± 20% in each matrix at the four fortification levels was used as a metric for method performance. This table shows similar trends to the MD-IDL data in Table 1. Both studies showed that avocado, wheat, honey, peach,

Table 3. Results of Pesticides Found and Concentrations (μg/kg or ppb) in Incurred Samples Using QuEChERS Sample Preparation and LC-MS/MS Analysis for 11 Agricultural Commodities (Apple, Bean (Green), Black Rice, Blueberry, Cabbage, Goji Berry, Pepper (Bell), and Tomato) ("-" Not Detected in Sample)

pesticide	reporting value (μg/kg or ppb) ± RSD[c]										
	apple 1[a]	apple 2[a]	bean[a]	black rice[b]	blueberry[a]	cabbage 1[a]	cabbage 2[a]	goji berry[b]	pepper[a]	tomato[a] no. 123669	tomato[a] no. 123667
3-hydroxycarbofuran	-	-	-	-	-	-	-	4.5 ± 11	-	-	-
acetamiprid	-	-	-	-	-	-	-	305 ± 3	-	-	-
azoxystrobin	-	-	-	-	-	-	-	-	6.5 ± 8	-	-
buprofezin	-	-	-	9.0 ± 11	-	-	-	-	-	-	-
carbaryl	-	-	-	-	-	225 ± 9	-	-	-	-	-
carbendazim	-	-	-	-	-	-	-	115 ± 4	-	-	-
clofentezine	-	-	-	-	-	-	-	10.5 ± 14	-	-	-
cyazofamid	-	-	-	-	-	-	>1000	-	-	-	-
dimethoate	-	-	-	-	-	-	-	<0.5	-	-	-
flutolanil	-	-	-	45.5 ± 5	-	-	-	-	-	-	-
hexaconazole	-	-	-	17.0 ± 6	-	-	-	-	-	-	-
imidacloprid	-	-	-	9.5 ± 32	-	-	-	22 ± 5	-	50 ± 20	60 ± 25
isoprocarb	-	-	-	3.5 ± 14	-	-	-	-	-	-	-
novaluron	-	-	115 ± 13	-	-	-	-	-	80 ± 13	-	-
omethoate	-	-	-	-	-	-	-	11.5 ± 4	-	-	-
prometryne	-	-	-	-	-	-	-	-	3 ± 33	-	-
propamocarb	-	-	-	-	-	-	6.5 ± 8	-	-	-	-
spinosad	2.5 ± 4	4.0 ± 13	-	-	<0.5	-	-	-	-	-	-
spirodiclofen	-	-	-	-	515 ± 11	-	-	-	-	-	-
thiophanate-methyl	-	-	-	-	-	-	-	155 ± 3	-	-	-
tricyclazole	-	-	-	65 ± 8	-	-	-	-	-	-	-
triflumizole	-	-	-	-	-	340 ± 6	-	-	-	-	-

[a] n = 4. [b] n = 3. [c] relative standard deviation, %.

and orange exerted severe matrix effects, while apple, corn, cabbage, and tomato showed the least matrix effects at the 10 ppb fortification concentration. Similar observations can be made at the higher fortification concentrations. This observation gave reasonable indication that each matrix exerted different effects on the LC-MS/MS instrument and method performance. It is likely these matrix effects propagate through the sample preparation, cleanup, and dilution process and may be inherent in the LC-MS/MS analysis and may not always be resolved sufficiently using MMCSs.

Using PCA, we also examined and characterized recovery data obtained from the 96 fortification studies. At the outset, data from fortification studies are expected to behave better than those from the MD-IDL data because of the use of MMCSs. Method performance, i.e. variability during the QuEChERS preparation and LC-MS/MS analysis, may play a more important role and is reflected in the recovery data. As can be seen in Figure 5, PCA analysis showed three separate groups and two outliers (avocado matrix). The main contributors to the two red-circled groups were spinach and hazelnut in one and spinach, carrot, raisin, orange and wheat flour in the other. The rest of matrices fell into the third group (green circled) due to their smaller and similar contribution to the data set. Obviously, the application of MMCSs cannot eliminate the all the factors in the course of the entire analytical procedure such as instrument performance, sample preparation (using QuEChERS), and day-to-day variations and other random factors. If MMCSs could eliminate all of these factors, the clustering patterns in Figure 5 would only depend on how well the MMCSs can compensate for matrix effects (suppression or enhancement) in quantitating the pesticide analytes by using the appropriate matrices.

We note that in the MD-IDL study, sample matrices were extracted by QuEChERS, cleaned up by dispersive SPE, and then fortified into the pesticide standards. This is different from the fortification study in which pesticides were fortified with the matrix sample first and subjected to acetonitrile salt-out extraction and dispersive SPE cleanup. Matrix-matched calibration standards would alleviate matrix effects in quantification only if sample matrices remained the same before and after the sample preparation, which can be difficult to achieve. This is best illustrated in the PCA grouping of scores showed in Figure 5, where a majority of the 96 fortified samples cluster into three groups with few outliers (e.g., avocado 10 and 25 ppb).

Results of Incurred Sample Analysis. Grab samples collected from various sources were prepared in replicates of three or four and analyzed by the method validated in this work. In total, 11 samples encompassed nine matrices including seven different fresh produce samples and two dried commodities were prepared and analyzed. We found various pesticides in these incurred samples at various concentrations as shown in Table 3. Consistent with the results from the method validation study presented here, as expected, samples analyzed at the low ppb levels tend to have higher uncertainty as indicated by the RSD calculated from the replicate analysis. The higher the concentration of the pesticides found, the better the precision of the analytical results. Results obtained for imidacloprid in tomato behaved differently than those observed during the method validation. Imidacloprid had higher RSD values than other pesticides. As the tomato used in the method validation was a different species from that incurred samples, this may have contributed differently to the uncertainty in the analysis. Pesticides found in each sample represented those commonly used for that specific produce/commodity and are considered to be accurate. None of the pesticide found would be considered a violation of permitted uses.

■ CONCLUSIONS

Results obtained from this current work suggest that analytes must have concentrations of at least 5–10 ppb to obtain consistent

results using the EC identification criteria for targeted compounds using two MRM transitions. Matrix effects produced by coextractives from the sample that interfere with the LC-MS/MS response of the analyte were found to be significant for pesticide analyses in QuEChERS prepared extracts. To better evaluate the analytical procedure in terms of sensitivity and quantitation, MMCSs must be used to compensate signal suppression or enhancement resulting from coeluted matrix components that would interfere with the analyte response. Thus, it is important to assess the matrix effects by comparing MMCSs to SOCSs, which is a practical approach that can be incorporated into routine sample analysis. Extract dilution might be a solution to reduce matrix effects but the sensitivity of the LC-MS/MS system used in this study prevents further investigation to assess the applicability of this approach. The use of isotopically labeled internal standards to carry out isotope dilution mass spectrometric quantification would be more effective, but it is an impractical and expensive solution. Using high resolution mass spectrometry to resolve analytes from matrix effects, and/or the use of a higher sensitivity LC-MS/MS system for the effectiveness of sample dilution in removing matrix effects may offer useful and cost-effective solutions and will be investigated in the near future.

■ ASSOCIATED CONTENT

ⓢ Supporting Information. Pesticides information and LC-MS/MS parameters; estimated LOD (N = 8) and MDL (N = 4) (ppb); results of fortification studies and MDL calculated for each analyte in 24 matrices; ion suppression results of 200 pesticides tested in 24 agricultural commodities (A—X) by comparing slope ratios (based on concentration ranges (1.0—100 ng/mL) of matrix-matched calibration curves to solvent-only calibration curves (ordinate) to pesticide type (all PDF files). This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

C, concentration; EC, European Commission; EPA, United States Environmental Protection Agency; ESI, electrospray ionization; FDA, United States Food and Drug Administration; IDL, instrument detection limit; LC, liquid chromatography; LC-MS/MS, high performance liquid chromatography-tandem mass spectrometry; MD-IDL, matrix dependent-instrument detection limit; MDL, method detection limit; MMCSs, matrix-matched calibration standards; MRM, multiple reaction monitoring; ND, not detected/quantitated; PCA, principal component analysis; PFTE, polytetrafluoroethylene; QC, quality control; QuEChERS, Quick, Easy, Cheap, Effective, Rugged and Safe; R_{slope}, Slope ratio; RSD, relative standard deviation; SD, standard deviation; SNR, signal-to-noise ratio; SOCSs, solvent-only calibration standards; SPE, solid-phase extraction

■ REFERENCES

(1) Tomlin, C. D. S. *The Pesticide Manual- A World Compendium*, 13th ed.; British Crop Protection Council (BCPC) Publications: Hampshire, UK, 2003.

(2) Nakamura, Y.; Tonogai, Y.; Sekiguchi, Y.; Tsumura, Y.; Nishida, N.; Takakura, K.; Isechi, M.; Yuasa, K.; Nakamura, M.; Kifune, N.; Yamamoto, K.; Terasawa, S.; Oshima, T.; Miyata, M.; Kamakura, K.; Ito, Y. Multiresidue analysis of 48 pesticides in agricultural products by capillary gas chromatography. *J. Agric. Food Chem.* **1994**, *42*, 2508–2518.

(3) Fillion, J.; Sauvé, F.; Selwyn, J. Multiresidue method for the determination of residues of 251 pesticides in fruits and vegetables by gas chromatography/mass spectrometry and liquid chromatography with fluorescence detection. *J. AOAC Int.* **2000**, *83*, 698–713.

(4) Taylor, M. J.; Hunter, K.; Hunter, K. B.; Lindsay, D.; Le Bouhellec, S. Multi-residue method for rapid screening and confirmation of pesticides in crude extracts of fruits and vegetables using isocratic liquid chromatography with electrospray tandem mass spectrometry. *J. Chromatogr., A* **2002**, *982* (2), 225–236.

(5) Klein, J.; Alder, L. Applicability of gradient liquid chromatography with tandem mass spectrometry to the simultaneous screening for about 100 pesticides in crops. *J. AOAC Int.* **2003**, *86* (5), 1015–1037.

(6) Granby, K.; Andersen, J. H.; Christensen, H. B. Analysis of pesticides in fruit, vegetables, and cereals using methanolic extraction and detection by liquid chromatography—tandem mass spectrometry. *Anal. Chim. Acta.* **2004**, *520* (1—2), 165–176.

(7) Jansson, C.; Pihlström, T.; Österdahl, B.-G.; Markides, K. E. A new multi-residue method for analysis of pesticides in fruits and vegetables using liquid chromatography with tandem mass spectrometric detection. *J. Chromatogr., A* **2004**, *1023*, 93–104.

(8) Ortelli, D.; Edder, P.; Corvi, C. Multiresidue analysis of 74 pesticides in fruits and vegetables by liquid chromatography—electrospray-tandem mass spectrometry. *Anal. Chim. Acta* **2004**, *520* (1—2), 33–45.

(9) Sannino, A.; Bolzoni, L.; Bandini, M. Application of liquid chromatography with electrospray tandem mass spectrometry to the determination of a new generation of pesticides in processed fruits and vegetables. *J. Chromatogr., A* **2004**, *1036*, 161–169.

(10) Tanizawa, H.; Shima, M.; Ikehara, C.; Kobata, M.; Sato, M. Multi-residue method for screening of pesticides in crops by liquid chromatography with tandem mass spectrometry. *J. Food Hyg. Soc. Jpn.* **2005**, *46* (5), 185–197.

(11) Pang, G.-F.; Fan, C.-L.; Liu, Y.-M.; Cao, Y.-Z.; Zhang, J.-J.; Li, X.-M.; Li, Z.-Y.; Wu, Y.-P.; Guo, T.-T. Determination of residues of 446 pesticides in fruits and vegetables by three-cartridge solid-phase extraction-gas chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry. *J. AOAC Int.* **2006**, *89* (3), 740–771.

(12) Hiemstra, M.; de Kok, A. Comprehensive multi-residue method for the target analysis of pesticides in crops using liquid chromatography—tandem mass spectrometry. *J. Chromatogr., A* **2007**, *1154* (1—2), 3–25.

(13) Payá, P.; Anastassiades, M.; Mack, D.; Sigalova, I; Tasdelen, B.; Oliva, J.; Barba, A. Analysis of pesticide residues using the Quick Easy Cheap Effective Rugged and Safe (QuEChERS) pesticide multiresidue method in combination with gas and liquid chromatography and tandem

7645

dx.doi.org/10.1021/jf2010723 |*J. Agric. Food Chem.* 2011, 59, 7636–7646

mass spectrometric detection. *Anal. Bioanal. Chem.* **2007**, *389* (6), 1697–1714.

(14) Takatori, S.; Okihashi, M.; Okamoto, Y.; Kitagawa, Y.; Kakimoto, S.; Murata, H.; Sumimoto, T.; Tanaka, Y. A rapid and easy multiresidue method for the determination of pesticide residues in vegetables, fruits, and cereals using liquid chromatography/tandem mass spectrometry *J. AOAC Int.* **2008**, *91* (4), 871–883.

(15) Wang, J.; Chow, W.; Leung, D. Applications of LC/ESI-MS/MS and UHPLC QqTOF MS for the determination of 148 pesticides in fruits and vegetables. *Anal. Bioanal. Chem.* **2010**, *396* (4), 1513–1538.

(16) Wong, J.; Hao, C.; Zhang, K.; Yang, P.; Banerjee, K.; Hayward, D.; Iftakhar, I.; Schreiber, A.; Tech, K.; Sack, C.; Smoker, M.; Utture, S. C.; Oulkar, D. P. Development and interlaboratory validation of a QuEChERS-based liquid chromatography/tandem mass spectrometry method for multi-residue pesticides analysis. *J. Agric. Food Chem.* **2010**, *58* (10), 5897–5903.

(17) Anastassiades, M.; Lehotay, S. J.; Stajnbaher, D.; Schenck, F. J. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *J. AOAC Int.* **2003**, *86* (2), 412–431.

(18) Mol, H. G.; Plaza-Bolaños, P.; Zomer, P.; de Rijk, T. C.; Stolker, A. A.; Mulder, P. P. Toward a generic extraction method for simultaneous determination of pesticides, mycotoxins, plant toxins, and veterinary drugs in feed and food matrices. *Anal. Chem.* **2008**, *80*, 9450–9459.

(19) Title 40—Protection of the Environment. Part 136—Guidelines establishing test procedures for the analysis of pollutants. Appendix B—Definition and Procedure for the Determination of the Method Detection Limit—Revision 1.11. *Code of Federal Regulations*; U.S. Government Printing Office: Washington, DC, 2010; http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&tpl=/ecfrbrowse/Title40/40cfr136_main_02.tpl.

(20) Commission Decision 2007/657/EC of 12 August 2002. Implementing Council Directive (96/23/EC) concerning the performance of analytical methods and the interpretation of results. *Off. J. Eur. Communities* **2002**, *L221*, 8–36.

(21) Hao, C.; Nguyen, B.; Zhao, X.; Chen, E.; Yang, P. Determination of residual carbamate, organophosphate, and phenyl urea pesticides in drinking and surface water by high-performance liquid chromatography/tandem mass spectrometry. *J. AOAC Int.* **2010**, *93* (2), 400–410.

(22) Title 40—Protection of the Environment. Part 136—Guidelines establishing test procedures for the analysis of pollutants. Appendix B—Definition and Procedure for the Determination of the Method Detection Limit—Revision 1.11. *Code of Federal Regulations*; U.S. Government Printing Office: Washington, DC, 2010; http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&tpl=/ecfrbrowse/Title40/40cfr136_main_02.tpl.

(23) Schürmann, A.; Dvorak, V.; Crüzer, C.; Butcher, P.; Kaufmann, A. False-positive liquid chromatography/tandem mass spectrometric confirmation of sebuthylazine residues using the identification points system according to EU directive 2002/657/EC due to a biogenic insecticide in tarragon. *Rapid Commun. Mass Spectrom.* **2009**, *23* (8), 1196–1200.

(24) Gómez, M. J.; Petrović, M.; Fernández-Alba, A. R.; Barceló, D. Determination of pharmaceuticals of various therapeutic classes by solid-phase extraction and liquid chromatography-tandem mass spectrometry in hospital effluent wastewaters. *J. Chromatogr., A* **2006**, *1114*, 224–233.

(25) Hernando, M. D.; Heath, E.; Petrovic, M.; Barceló, D. Trace-level determination of pharmaceutical residues by LC-MS/MS in natural and treated waters. A pilot-survey study. *Anal. Bioanal. Chem.* **2006**, *385*, 985–991.

(26) Stahnke, H.; Reemtsma, T.; Alder, L. Compensation of matrix effects by postcolumn infusion of a monitor substance in multiresidue analysis with LC-MS/MS. *Anal. Chem.* **2009**, *81*, 2185–2192.

(27) Kloepfer, A.; Quintana, J. B.; Reemtsma, J. Operational options to reduce matrix effects in liquid chromatography—electrospray ionisation-mass spectrometry analysis of aqueous environmental samples. *J. Chromatogr., A* **2005**, *1067*, 153–160.

(28) Schlüsener, M. P.; Spiteller, M.; Bester, J. Determination of antibiotics from soil by pressurized liquid extraction and liquid chromatography—tandem mass spectrometry. *J. Chromatogr., A* **2003**, *1003*, 21–28.

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