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Development and Interlaboratory Validation of a QuEChERS-Based Liquid Chromatography−Tandem Mass Spectrometry Method for Multiresidue Pesticide Analysis[†]

JON WONG,[§] CHUNYAN HAO,[#] KAI ZHANG,[§] PAUL YANG,*[,#] KAUSHIK BANERJEE,[⊗] DOUGLAS HAYWARD,[§] IMRAN IFTAKHAR,[#] ANDRE SCHREIBER,[△] KATHERINE TECH,[⊥] CHRIS SACK,[⊥] MICHAEL SMOKER,[⊥] XIANGRU CHEN,[#] SAGAR C. UTTURE,[⊗] AND DASHARATH P. OULKAR[⊗]

[§]Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, College Park, Maryland 20740-3835, [#]Ontario Ministry of the Environment, Laboratory Services Branch, Toronto, Ontario M9P 3V6, Canada, [⊥]U.S. Food and Drug Administration, Kansas City, Kansas 66215, [⊗]National Research Centre for Grapes, P.O. Manjri Farm, Pune 412 307, India, and [△]AB Sciex, 71 Four Valley Drive, Concord, Ontario L4K 4V8, Canada

A high-throughput, QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) sample preparation and liquid chromatography−tandem mass spectrometry (LC-MS/MS) analytical method has been developed and validated for the determination of 191 pesticides in vegetation and fruit samples. Using identical LC analytical column and MS/MS instrumentation and operation parameters, this method was evaluated at the U.S. Food and Drug Administration (FDA), National Research Centre for Grapes (NRCG), India, and Ontario Ministry of the Environment (MOE) laboratories. Method validation results showed that all but 1 of these 191 pesticides can be analyzed by LC-MS/MS with instrument detection limits (IDL) in the parts per trillion (ppt) range. Matrix-dependent IDL studies showed that due to either the low ionization efficiency or matrix effect exerted, 14 of these 191 pesticides could not be analyzed by this method. Method recovery (%R) and method detection limits (MDLs) were determined by the three laboratories using four sample matrices in replicates ($N = 4$). With >79% of %R data from the fortification studies in the range from 80 to 120%, MDLs were determined in the low parts per billion range with >94% of MDLs in the range from 0.5 to 5 ppb. Applying this method to the analysis of incurred samples showed that two multiple reaction monitoring (MRM) transitions may not be enough to provide 100% true positive identification of target pesticides; however, quantitative results obtained from the three laboratories had an excellent match with only a few discrepancies in the low parts per billion levels. The %R data from the fortification studies were subjected to principal component analysis and showed the majority of %R fell into the cluster of 80% < %R < 120%. Due to the matrix effect exerted by ginseng and peach, outliers were observed at the lowest spiking levels of 10 and 25 ppb. The study also showed that QuEChERS samples should be analyzed as soon as prepared or stored in a freezer to avoid any adverse affect on the analytes evaluated.

KEYWORDS: Pesticides; liquid chromatograph−tandem mass spectrometer (LC-MS/MS); QuEChERS; principal component analysis

INTRODUCTION

The design of an atmospheric pressure ionization source (*1*) in 1984 resulted in the development of an electrospray ionization (ESI) based liquid chromatography−tandem mass spectrometry (LC-MS/MS) instrument (*2*) in 1989. The availability of LC-MS/MS revolutionized analytical methods used for the analysis of thermally labile and/or nonvolatile organic molecules including pharmaceutically active compounds, veterinary drugs, antibiotics, and pesticides (*3−5*). The state-of-the-art LC-MS/MS instrumentation has superb sensitivity and allowed the development of mega LC-MS/MS methods that allowed the simultaneous analysis of at least 50 pesticides with superior data quality and efficiency (*4*, *6*, *7*). This, concomitant with the implementation of artificial intelligence based data acquisition software such as Scheduled Multiple Reaction Monitoring (*Scheduled* MRM), Dynamic MRM, and Timed Selected Reaction Monitoring (*8*), allowed the development of LC-MS/MS methods for the effective determination of many pesticides.

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*Author to whom correspondence should be addressed [fax (416) 235-5900; e-mail paul.yang@ontario.ca].

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In this paper, a LC-MS/MS-based multiresidue method for the measurement of the residues of 191 pesticides including carbamates, polar organophosphates, phenylureas, anilides, benzoyl phenylureas, conazoles, macrocyclic lactone, neonicotinoids, strobilurines, and triazines is described. Using the QuEChERS extraction procedure (*9*), the method was validated at three laboratories utilizing similar LC mobile phases and gradient elution for the separation and the same ESI and MS/MS operating parameters in the analysis. Sample fortification studies were done using orange, peach, spinach, and ginseng for the determination of method detection limits (MDL) as well as proficiency tests for the three laboratories using blind, incurred samples of orange, peach, spinach, and ginseng.

Also documented in this paper are method performance and validation data such as the LC-MS/MS short-term stability, instrument detection limits (IDLs), matrix-dependent IDL (MD-IDL), and method detection limits (MDLs) determined using the U.S. Environmental Protection Agency's (U.S. EPA) protocol (*12*). In addition to the method validation and quality control and quality assurance (QC/QA) data, we also investigated the effect of solvents used to reconstitute the sample prior to the LC-MS/MS analysis. Using the principal component analysis (PCA) algorithm, we also evaluated method recovery data obtained from the three laboratories to demonstrate their similarity and the applicability of laboratories to use a single LC-MS/MS method for interlaboratory validation. Analytical results obtained from the *Scheduled* MRM data acquisition algorithm, validity of using two MRM transitions to identify target pesticide compounds to meet the European Union criteria for the mass spectrometric identification of target compounds (*10*), and the need to use other technologies to ensure true-positive identification of target pesticides will be discussed.

EXPERIMENTAL PROCEDURES

Chemicals. Most of the pesticide standards were obtained from the U.S. EPA Pesticide Repository (Ft. Meade, MD), whereas others were obtained through Fluka/Sigma Aldrich (St. Louis, MO) and Wako Chemicals USA (Richmond, VA) and are listed in Table S1 of the Supporting Information. Methanol, acetonitrile, HPLC-grade water, formic acid, ammonium formate, anhydrous $MgSO_4$, and NaCl were purchased from Fisher Scientific (Pittsburgh, PA). Six deuterium (^{2}H) isotope labeled internal standards (ILIS) listed in Table S1 of the Supporting Information were purchased from CDN-Isotopes (Montreal, QC, Canada). High-quality water (pure water) used to prepare method blank and method spike samples was produced by passing osmosis water through a Barnstead NANOpure water purification system. QuEChERS products, (1) 4 g of anhydrous magnesium sulfate, 1 g of anhydrous sodium acetate, and 50 mL centrifuge tubes and (2) 15 mL centrifuge tubes containing 1.2 g of anhydrous magnesium sulfate and 400 mg of primary−secondary amine (PSA) sorbents, were purchased from United Chemical Technologies (Bristol, PA). Dried and powdered ginseng samples, *Panax quinquefolius* (American ginseng) used to prepare blanks and matrix-matched standards were provided by the Wisconsin Ginseng Board (Wausau, WI). Peach, orange, spinach, and ginseng were purchased in bulk packages from commercially available sources.

Separate stock solutions of analytical standards, including those for ILIS, were prepared for individual compounds 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, QC, Canada). Intermediate solutions were prepared in 100 mL volumetric flasks by mixing the stock solutions. Five levels of matrix-matched calibration standards were prepared from the intermediate solutions by using sample matrix extract and matrix buffer (20 mM ammonium formate) in concentrations of 1, 5, 10, 50, and 100 ppb. The ILIS solution was added prior to sample preparation and used as an internal standard in the quantitative analysis.

Sample Preparation. Four different sample matrices (orange, peach, spinach, and ginseng) were used during the method validation stage. Samples were prepared at the FDA laboratory in College Park, MD, and were analyzed at the FDA, Ontario Ministry of the Environment (MOE), and National Research Centre for Grapes (NRCG) laboratories. For orange, peach, and spinach sample matrices, fortified samples were prepared in quadruplicate by weighing 10 ± 0.1 g of cryoground sample in 50 mL disposable screw-capped polypropylene centrifuge bottles (Thermo-Fisher Scientific, San Jose, CA). A 0.5 mL of 5, 2, 0.5, and 0.2 ppm spike solutions was added into each sample tube to achieve fortification levels of 0.25, 0.10, 0.025, and 0.010 ppm. Each 50 mL sample tube was vortexed for 3 min to achieve a homogeneous sample followed by the addition of 10 mL of 1% acetic acid/acetonitrile, 4 g of anhydrous $MgSO_4$, and 1 g of anhydrous sodium acetate. After the sample had been shaken by hand, 200 μL of surrogate solution and a steel ball bearing were added into each sample, and the sample tube was 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 for 5 min.

The final extracts (~9 mL) were transferred to a centrifuge tube containing 300 mg of PSA sorbents and 900 mg of $MgSO_4$. The sample tubes were shaken on GenoGrinder for 1 min (500 strokes/min) and centrifuged at 4500 rpm for 5 min. Sample extracts were removed from the centrifuge tube (about 6.5−7.0 mL recovered), transferred into 2 mL vials, and sent in a cooler on ice pack to laboratories in Canada and India. Samples arrived at the MOE in 36 h, whereas those sent to India were delayed at Customs for more than 168 h.

Upon receiving the sample extracts, the staff at each laboratory prepared five different levels of matrix-matched calibration standards for analysis. This was done by mixing 300 μL of 0.333, 0.167, 0.067, 0.033, and 0.0167 ppm standard solutions with 200 μL of matrix blank extracts and 500 μL of sample buffer (20 mM ammonium formate), and the solutions were used immediately for LC-MS/MS analysis. The 500 μL of sample buffer was added prior to LC-MS/MS analysis to ensure the integrity of analytes. Fortified samples were prepared by diluting 200 μL of sample extracts with 300 μL of acetonitrile and 500 μL of sample buffer prior to analysis at levels of 50, 20, 5, and 2 ppb.

Fortified ginseng samples were prepared by using 1.0 ± 0.05 g of ginseng and fortifying with 50 μL of 5, 2, and 0.5 ppm spike solutions to final concentrations of 0.25, 0.10, and 0.025 ppm. The samples were vortexed for 10 s and allowed to set before 10 mL of HPLC-grade water was added. A steel ball bearing was added to the ginseng−water mixture, and the samples were shaken on the GenoGrinder at 1000 strokes/min for 1 min prior to the addition of acetonitrile and salts. Matrix-matched standards were prepared by adding 100 μL of 1.6, 0.8, 0.333, 0.167, 0.067, and 0.033 ppm standard solutions to 400 μL of ginseng blank extracts, and 500 μL of sample matrix buffer was added prior to analysis to achieve matrix-matched calibration standards of 160, 80, 33.3, 16.7, 6.67, 3.33, and 1.67 ppb, respectively.

Samples were cloudy at this stage and were filtered using 0.2 μm nylon membrane filters (Sun SRi, Rockwood, TN) directly into the LC autosampler vials. Filtered samples were clear and can be stored in a freezer until ready for analysis.

Instrumentation and Data Analysis. Liquid chromatography separation was achieved using Shimadzu Prominence/20 series (Columbia, MD) systems in the FDA, MOE, and NRCG laboratories. The LC systems were interfaced to an AB Sciex (Forest City, CA) 4000 Q Trap mass spectrometer through an ESI interface (IonSpray). *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 (*10*, *11*). Quantification was carried out using either external standard calibration (NRCG) or internal standard calibration (FDA and MOE) with $^2H_{10}$-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; Ultra Aqueous, C-18, 100 × 2.1 mm, 3 μm) and guard column (Ultra Aqueous, C-18 cartridges, 10 × 2.1 mm 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 **Table 1**. Curtain, collision, nebulizer, auxiliary gases, and source temperature of the ESI source were set at 15, 6, 35, and 45 psi and 450 °C, respectively. Ion spray voltage used was 5200. Declustering potential (DP), collision energy

Table 1. Gradient Elution Parameters Used in the LC Separation

	NRCG	FDA	MOE
mobile phase	A: 5 mM ammonium formate, 0.1% formic acid in water	A: 5 mM ammonium formate, 0.1% formic acid in water	A: 10 mM ammonium acetate in water
	B: 5 mm ammonium formate, 0.1% formic acid in methanol	B: 5 mm ammonium formate, 0.1% formic acid in methanol	B: 10 mm ammonium acetate in methanol
column temperature	35 °C	35 °C	35 °C
flow rate	0.3 mL/min	0.5 mL/min	0.35 mL/min
total run time	14.0 min	12.0 min	16.0 min
gradient program	10% B at 0 min, hold for 1 min to 98% B at 8 min, hold for 6 min	5% B at 0 min to 70% B at 5 min, hold for 1 min to 90% B at 8 min, hold for 4 min	20% B at 0 min to 90% B at 10 min to 100% B at 12 min, hold for 4 min
injection volume	20 μL	20 μL	15 μL

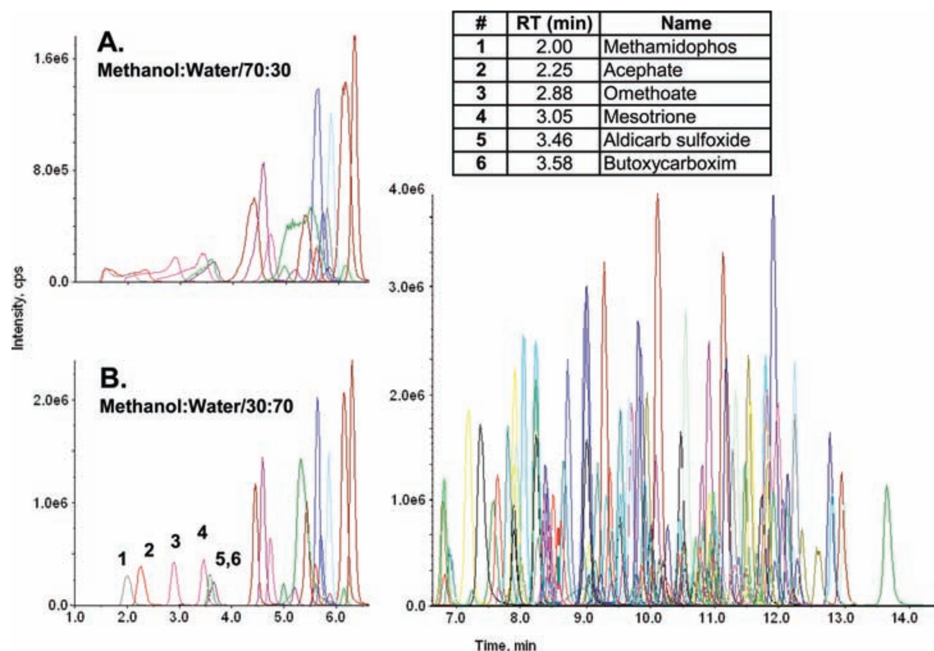

Figure 1. Typical, reconstructed MRM chromatograms for the 191 pesticides and 6 ILISs analyzed using this method: (**A**) methanol/water 70:30; (**B**) methanol/water 30:70.

(CE), and collision cell exit potential (CXP) were optimized by direct infusion, and the two most intense ion pairs of each analyte were chosen for the analysis. Values of DP, CE, and CXP and the two specific, most intense MRM pairs are listed in Table S1 of the Supporting Information and used for *Scheduled* MRM data acquisition. Principal component analysis (PCA) was carried out using Infometrix Pirouette 4 (Bothell, WA).

RESULTS AND DISCUSSION

Figure 1 shows reconstructed chromatograms for the pesticides and ILISs evaluated in this method. These pesticides have a wide range of polarity with a wide range of molecular weights between 179 and 920. One would therefore expect the solubility of these pesticides to vary with the solvent used in the final extract and affect the quality of chromatography. This is best demonstrated in **Figure 1** where the use of different compositions of methanol/water from 70:30 (**A**) to 30:70 (**B**) in the sample changes the chromatographic peak shape. The most dramatic changes were observed for those very polar elutes at the first 4 min of the analysis. As a result, solvent used in the final extract for LC-MS/MS analysis had at least 70% water to ensure good chromatographic peak shape.

Instrumental Performance. Instrument within-run precision (4.5 h, $N = 12$) was obtained from the relative standard deviation (RSD) of 12 consecutive analyses of an 8 ppb standard solution in 4 h and is listed in Table S1 of the Supporting Information. Instrument detection limits (IDL) were calculated according to the U.S. EPA's protocol (*12*) using the standard deviation (SD) of each analyte obtained from 12 consecutive analyses of 1 and 8 ppb calibration standards. The SD was multiplied by a critical $t_{0.010} = 2.718$ (degree of freedom (df) of 11) to obtain the IDL. Matrix-dependent IDLs (MD-IDL) were obtained by analyzing eight replicates of the four matrices at concentrations of 1, 2, 5, and 10 ppb (peach, orange, and spinach) and 1.67, 3.34, 8.35, and 16.7 ppb (ginseng). Due to the matrix effect and depending on the sample matrix, some pesticides were not detected at the lowest level (1 or 1.67 ppb), and analytical data from the next higher level were used. The MD-IDLs were then calculated by multiplying the SD of each analyte by 2.99 ($t_{0.010}$, df = 7) and are listed in Table S1 of the Supporting Information for the four matrices.

Table S1 of the Supporting Information demonstrates the inherent ruggedness of the method, which allowed the three LC-MS/MS systems to deliver excellent short-term (4.5 h, $N = 12$) precision of 5% RSD for the majority (>80%) of the compounds studied. Less than 10% of the 191 compounds studied had a short-term RSD of 10% or higher. Values of IDL for each target pesticide were determined at the low to high parts per trillion level using this short-term stability data. Using a similar approach, values of the MD-IDL ($N = 8$) were also determined from medium parts per trillion to low parts per billion levels. Worth noting is that IDL values were derived by using the

U.S. EPA protocol and they should be considered as conservative and could be 10 times higher than those derived through signal-to-noise ratio (SNR) approach. Such use of U.S. EPA protocol also formed a consistent baseline for the three laboratories to carry out follow-up experiments for comparison purposes.

From the instrument short-term RSD values listed in Table S1 of the Supporting Information, one would notice that only one compound (i.e., avermectin B_{1b}) could not be analyzed by LC-MS/MS and was listed as nondetectable (ND). Seventeen of the remaining 190 pesticides had inferior RSDs that ranged from 12 to 63%. The RSD was obtained from a calibration standard free from the effect of sample matrix, and these inferior RSDs could only be attributed to the low ionization efficiency of these 17 pesticides. One would therefore expect MD-IDLs of these 17 compounds to be worse than the other target pesticides. As can be seen in Table S1 of the Supporting Information, 14 of the 191 pesticides (highlighted in yellow) could not be measured in the matrix-matched standards. These are listed as nondetectable (ND) and not evaluated further. It is worth noting that six (pesticides 47, 48, 92, 139, 152, and 182) of these ND pesticides had superior RSD (1.8−4.5%), and because of the matrix effect exerted, we could not determine their MD-IDL. The cause of ND for the other seven pesticides (pesticides 11, 60, 104, 105, 112, 128, and 181) could be attributed to a combination of analyte instability, low ionization efficiency, and the matrix effect. Pesticides 12, 20, 35, 44, 64, 72, 99, 106, 137, and 150 had RSD values > 10% but were less affected by the matrices studied. These pesticides had an acceptable MD-IDL and were evaluated further.

Method Validation. Each of the three laboratories analyzed samples of orange, peach, and spinach fortified at four levels and ginseng fortified at three levels, at concentrations from 10 to 250 ppb. Four replicates of each fortification level were analyzed to produce a total of 60 samples for method validation. Both the FDA and MOE laboratories used D10-diazinon as an internal standard for quantitative analysis, whereas the NRCG used external calibration for quantitative analysis. Using five levels of matrix-matched calibration standards, calibration curves were least-squarely fitted to a $1/C$ weighted (C being the concentration of each calibration standard) quadratic equation with a correlation coefficient of > 0.99. These curves were used for the quantitative determination of target analytes in their respective sample matrices. Average method recoveries (%R, $N = 4$) and SD of target compounds at various fortification levels are listed in Tables S2−S5 of the Supporting Information. The nine pesticides having RSD values > 10% are highlighted in yellow in these four tables.

From the %R obtained from the three laboratories, we noted that FDA, MOE, and NRCG laboratories had, respectively, 90.4, 79.6, and 69.8% of their 177 target analytes recovered between 80 and 120%, resulting in a total of 79.8% of target analytes having %R values between 80 and 120%. This is summarized in **Table 2**. As the same samples, LC columns, and MS operational parameters were used by a proficient analyst at each laboratory, one may attribute these different recoveries to the reasons discussed below.

From **Table 1**, we noted that the NRCG, MOE, and FDA laboratories used flow rates of 0.3, 0.35, and 0.5 mL/min to achieve the LC separation. This would give the FDA laboratory narrower LC peaks, higher peak intensity, and a superior signal-to-noise ratio (SNR) for the analysis. Analytical data from the FDA would have a higher precision and accuracy than the other two laboratories, resulting in better recovery data. Additional validation works would be required to support this rationale. The second possibility could be the external standard calibration quantification approach used by the NRCG laboratory. This might partially explain why only 69.8% of NRCG's %R fell into the 80−120% range. The third possibility would have been the sample extract storage condition and holding time. The FDA laboratory prepared and analyzed these sample extracts immediately after the extraction. The NRCG and MOE laboratories started preparing and analyzing their samples at 168 and 36 h after sample extraction, respectively. Sample extracts were shipped in a cooler at a temperature < 12 °C to the MOE and NRCG laboratories and were prepared and analyzed > 48 and > 180 h after extraction. Uncertainty during the sample shipping and storage conditions would have contributed more to the observed %R discrepancy.

Table 2. Summaries of Method Validation Results Obtained from the Three Laboratories[a]

method attribute	FDA	MOE	NRCG
% of analytes with 80% < %R < 120%	90.4	79.6	69.8
% of analytes with 70% < %R < 130%	94.2	89.6	86.4
% of analytes with MDL < 1 ppb	29.7	25.8	19.8
% of analytes with 1 ppb < MDL < 2 ppb	41.2	29.2	31.8
% of analytes with 2 ppb < MDL < 5 ppb	22.5	39.7	41.7
% of analytes with MDL > 5 ppb	6.6	5.2	6.8

[a] %R, percent of method recovery of fortified samples; MDL, method detection limits.

As can be seen from Tables S2−S5, all three laboratories had difficulties analyzing replicates of "very low spike" and "low spike" of spinach and ginseng matrices as evidenced by the average %R and their respective SD. The four sample matrices showed minimal effect on the nine pesticides with inferior RSD (highlighted in yellow).

Using the lowest SD obtained from the fortification experiment (Tables S2−S5 of the Supporting Information), the MDL for each pesticide was calculated at the 95% confidence level according to the U.S. EPA protocol and these are listed in Table S6 of the Supporting Information. Depending on the performance of the instrumentation at the three laboratories, matrix effect, and sample condition, some pesticides could not be detected at the lowest fortification level (10 ppb), and SDs obtained from the next higher level were used. The MDLs were then calculated by multiplying the designated SD of each analyte by a critical $t_{0.050} = 2.353$ ($N = 4$). A detailed analysis of these MDLs showed that all three laboratories could achieve a sub-parts per billion MDL for 25.1% of the analytes, with the majority (68.7%) of the MDLs ranging from 1 to 5 ppb, and only a fraction (6.2%) had MDLs that were > 5 ppb. Details of the performance of each laboratory are listed in **Table 2** along with the performance of %R data.

Principal Component Analysis of Method Validation Data. Due to the large number of %R data, the complexity of the matrix effect, and the uncertainty associated with the separation parameters, we used PCA to explore the property of these data (*13*). This is done by combining %R data obtained from the three laboratories (60 samples/laboratory for a total of 180 samples) for the 177 pesticides at various fortification levels in four matrices. Also included in the data set was one class (categorical) variable representative source samples. Pirouette 4 was used to carry out the PCA and automatically select the optimal factors that would represent the whole data set. Those %R values corresponding to the optimal factors were used to carry out hierarchical cluster analysis (HCA) and the generation of clustering pattern to observe possible outliers in the analysis. In the current study, we used factors that would account for about 80% of the total analyte to achieve a better understanding of the data set.

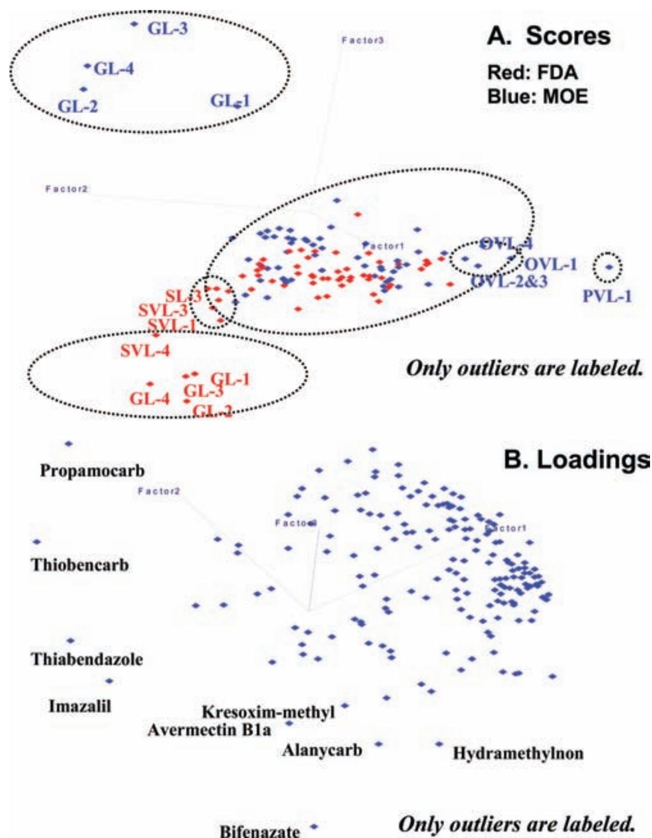

Figure 2. PCA results of FDA and MOE %R data in which variance representing 9 factors accounts for 81.4% of total sample (98 samples, **A**) and loadings of the 177 analytes in these 98 samples (**B**).

As discussed, %R data from the FDA and MOE laboratories obtained using internal standard quantification would have inherent consistency (similarity), unless affected by the low ionization efficiency and matrix effect, and were used first to characterize %R. **Figure 2A** shows the variance of 98 samples (81.4%) represented by the first 9 factors from the PCA. As expected, the majority of these samples clustered together, indicating similar recoveries for the 177 analytes in these 98 samples. Samples that were considered as outliers were labeled and include MOE (blue) and FDA (green) ginseng samples spiked at 25 ng/g and MOE spinach and FDA peach spiked at 10 ppb. The two individual clusters of the 25 ng/g ginseng fortification samples demonstrated the difficulty related to the analysis of this specific sample matrix, with the FDA and MOE showing some difficulty, respectively, analyzing spinach and orange sample matrices. **Figure 2B** shows contributions (loading) of the %R data of these 81.4% of the data set. Labeled outliers such as alanycarb (5), avermectin B1a1 (12), bifenazate (19), hydramethylnon (96), propamocarb (147), spirodiclofen (164), and thiobencarb (180), due to their higher or lower recoveries (>130 or <70%), had much higher contributions (loading) than those contributing in the normal range of %R. The results shown matched observations made from Tables S2−S5 of the Supporting Information as well.

Using the same approach, %R data from the three laboratories were analyzed, and the results are shown in **Figure 3**. The variance of 80.4% (145 samples) represented by the first 12 factors is shown in **Figure 3A**. The introduction of the NRCG data, which were derived from external calibration quantification, changed certain characteristics of the data set. However, the majority of the samples still clustered together, indicating similar recoveries for the 177 analytes in these 145 samples analyzed by the three laboratories. The effect of spinach matrix on the %R of the MOE and NRCG data (denoted “O−S*” and “N−S*” in **Figure 3A**) was much more pronounced than that exerted on the FDA data (denoted “F−S*”). This is a good indication that spinach exerted a higher level of matrix effect than the other three matrices. The peach matrix also exerted a certain matrix effect on the NRCG data that was not observed in the FDA and MOE data and could be attributed to the extended sample storage time of the NRCG samples. The matrix effect arising from the orange matrix in the MOE data remained about the same. **Figure 3B** shows loadings of the %R obtained from the 177 analytes in the 145 samples shown in **Figure 3A**. In addition to outliers observed in the FDA/MOE data set, we also observed the pesticides aldicarb sulfoxide (6), benfuracarb (17), ethiprole (67), formaetanate HCl (89), imazalil (97), spirodiclofen (164), and thiabendazole (180), as outliers.

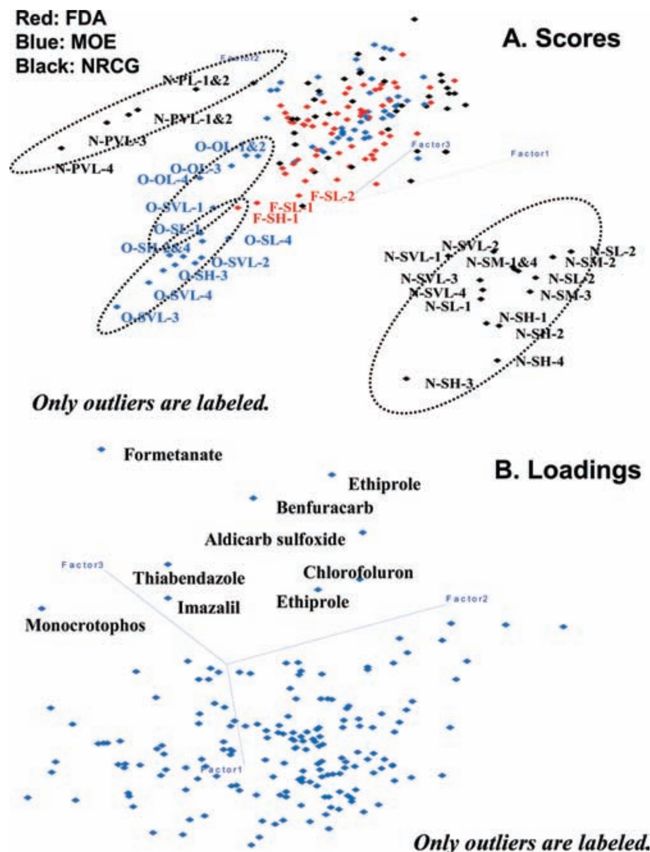

Figure 3. PCA results obtained from the %R data of three laboratories. Variance representing 11 factors accounting for 79.4% of total sample (145 samples, **A**) and loadings of the 177 analytes in these 145 samples (**B**) are displayed.

From **Table 2**, the FDA/MOE reported 85.1% of their %R in the range from 80 to 120%, whereas the FDA/MOE/NRCG had 79.6% of their %R reported in the same range. That result of 79.6% would have been better had the NRCG laboratory had the opportunity to receive their samples earlier, as well as use the internal standard calibration for quantification. It is difficult to interpret the number of factors that could be used to represent the FDA/MOE or FDA/MOE/NRCG data sets; however, PCA and HCA results showed that the majority of the samples analyzed were clustered together with minimal outliers. Furthermore, it is also logical to assume that the FDA/MOE data set was affected by up to 32 factors (%R data from two laboratories analyzing four sample matrices at four fortification levels) and the FDA/MOE/NRCG data might be affected by up to 48 factors.

Table 3. Analytical Results (Nanograms per Gram) of Incurred Samples

pesticide	orange		
	FDA	MOE	NRCG
carbendazim	1.0 ± 0.2	0.39 ± 0.06	0.88 ± 0.05
forchlorfenuron	ND	ND	3.22 ± 0.2
imazalil	365 ± 57	356 ± 34	357 ± 35
thiabendazole	64 ± 6	58.3 ± 2.9	72.4 ± 6.8

pesticide	ginseng, 355110			ginseng, 321657		
	FDA	MOE	NRCG	FDA	MOE	NRCG
azoxystrobin	ND	ND	ND	0.5 ± 0.1	0.7 ± 0.02	0.2 ± 0.01
thiabendazole	64 ± 6	58.3 ± 2.9	72.4 ± 6.8	ND	ND	ND
etaconazole	5.0 ± 0.5	0.7 ± 0.07	1.0 ± 0.10	ND	ND	ND
flusilazole	1.0 ± 0.02	0.7 ± 0.07	18.3 ± 0.9	ND	ND	ND
iprovalicarb	ND	1.3 ± 0.04	0.3 ± 0.06	ND	ND	ND
metalaxyl	3.5 ± 0.2	2.3 ± 0.06	0.4 ± 0.04	ND	ND	ND
myclobutanil	ND	ND	0.5 ± 0.2	ND	ND	ND
oxadixyl	40 ± 2	24 ± 1.5	54.4 ± 2.1	ND	ND	ND
paclobutrazole	ND	ND	0.5 ± 0.1	ND	ND	ND
propamocarb	112 ± 9	36 ± 3	1.4 ± 0.1	ND	ND	ND
tricyclazole	0.8 ± 0.04	0.6 ± 0.01	ND	ND	ND	ND

pesticide	peach, 249007			peach, 249018			peach, 167685			peach, 145850		
	FDA	MOE	NRCG	FDA	MOE	NRCG	FDA	MOE	NRCG	FDA	MOE	NRCG
bifenazate	ND	ND	ND	<0.5	0.8 ± 0.02	0.4 ± 0.2	ND	ND	ND	ND	ND	ND
boscalid	9.5 ± 0.1	9.5 ± 0.6	11.4 ± 0.8	ND	ND	ND	ND	ND	ND	ND	ND	ND
carbaryl	40 ± 0.5	43.2 ± 3.0	49.5 ± 0.6	ND	ND	ND	36 ± 2	37.4 ± 1.0	53.0 ± 6.6	ND	ND	ND
carbendazim	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	28.2 ± 1.4	32.2 ± 0.7
fenbuconazole	ND	ND	ND	ND	ND	ND	10 ± 1	9.1 ± 0.1	12.7 ± 0.6	15 ± 2	14.2 ± 1.7	19.2 ± 2.8
fludioxinil	56 ± 12	44.9 ± 11.6	75.7 ± 1.0	77 ± 9	57.1 ± 11.2	134 ± 9.2	ND	ND	ND	ND	ND	ND
propiconazole	5.6 ± 0.6	5.1 ± 0.7	5.3 ± 0.3	ND	ND	ND	ND	ND	ND	ND	ND	ND
pyraclostrobin	2.6 ± 0.3	2.1 ± 0.4	2.5 ± 0.4	ND	ND	ND	ND	ND	ND	ND	ND	ND
tebuconazole	ND	ND	ND	10 ± 0.2	10.1 ± 0.7	14.6 ± 1.5	ND	ND	ND	ND	ND	ND
thiophanate-methyl	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.4 ± 0.1	2.6 ± 0.03	1.25 ± 0.03

The fact that 9 and 11 factors could be used to represent, respectively, 81.4% of the FDA/MOE and 79.4% of the FDA/MOE/NRCG data sets showed the great similarity in %R. One would therefore conclude that the QuEChERS-based LC-MS/MS method described here is rugged enough for the three laboratories to obtain comparable analytical data.

Results of Incurred Samples. Grab samples collected from various sources were prepared in replicates and analyzed by the three laboratories using the same procedure described above. In total, one orange, two ginseng, and two spinach samples ($N = 4$ for each sample) and five peach samples ($N = 3$ for each sample) were prepared and analyzed. With the exception of the two spinach and one peach samples, we found various pesticides in the other eight samples with concentrations ranging from 1 to 365 ppb. The majority of quantitative results between the FDA and MOE matched very well. As expected, quantitative results obtained by the NRCG showed a higher level of discrepancy when compared to those obtained from the FDA and MOE because of the use of external standard calibration for quantification. These results are listed in **Table 3**.

It is worth noting that despite the use of two MRM transitions for the identification of target pesticides, there were observed discrepancies among reported results, indicating there could be a high percentage of false-negative or false-positive results obtained by the three laboratories. These data were observed in all matrices analyzed, at the lower parts per billion range, and suggested that the use of other technologies for the confirmation of these analytes may be beneficial. These include the measurement of product ion spectrum for library search (*14*) or high-resolution, accurate mass spectrometric analysis (*15*) to achieve required true-positive identification.

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Supporting Information Available: Additional details of the target pesticides, MRM transitions, instrumental performance data such as IDL and MD-IDL for each pesticides, validation data resulting from fortified samples using four different matrices, and MDLs of the 177 pesticides for each matrix. This material is available free of charge via the Internet at http://pubs.acs.org.

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