Multiresidue Pesticide Analysis of Dried Botanical Dietary Supplements Using an Automated Dispersive SPE Cleanup for QuEChERS and High-Performance Liquid Chromatography–Tandem Mass Spectrometry

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Ⓢ Supporting Information

ABSTRACT: An automated dispersive solid phase extraction (dSPE) cleanup procedure as part of the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method, coupled with liquid chromatography–tandem mass spectrometry using electrospray ionization in positive mode, was used for the simultaneous analysis of 236 pesticides in three dried powdered botanical dietary supplements (ginseng, saw palmetto, and gingko biloba). The procedure involved extraction of the dried powdered botanical samples with salt-out acetonitrile/water extraction using anhydrous magnesium sulfate and sodium chloride, followed by an automated dSPE cleanup using a mixture of octadodecyl- (C_{18}) and primary–secondary amine (PSA)-linked silica sorbents and anhydrous $MgSO_4$ and online LC-MS/MS analysis. Dynamic multiple-reaction monitoring (DMRM) based on the collection of two precursor-to-product ion transitions with their retention time windows was used for all of the targeted pesticides and the internal standard. Matrix-matched calibration standards were used for quantitation, and standard calibration curves showed linearity ($r^2 > 0.99$) across a concentration range of 0.2–400 ng/mL for the majority of the 236 pesticides evaluated in the three botanical matrices. Mean recoveries (average %RSD, $n = 4$) were 91 (6), 93 (4), 96 (3), and 99 (3)% for ginseng, 101 (9), 98 (6), 99 (4), and 102 (3)% for gingko biloba, and 100 (9), 98 (6), 96 (4), and 96 (3)% for saw palmetto at fortification concentrations of 25, 100, 250, and 500 μg/kg, respectively. The geometric mean matrix-dependent instrument detection limits were 0.17, 0.09, and 0.14 μg/kg on the basis of the studies of 236 pesticides tested in ginseng roots, gingko biloba leaves, and saw palmetto berries, respectively. The method was used to analyze incurred ginseng samples that contained thermally labile pesticides with a concentration range of 2–200 μg/kg, indicating different classes of pesticides are being applied to these botanicals other than the traditional pesticides that are commonly used and analyzed by gas chromatography techniques. The method demonstrates the use of an automated cleanup procedure and the LC-MS/MS detection of multiple pesticide residues in dried, powdered botanical dietary supplements.

KEYWORDS: QuEChERS, multiresidue pesticide residue analysis, botanical dietary supplements, LC-MS/MS, dynamic multiple-reaction monitoring, automated dispersive SPE

INTRODUCTION

Botanical products are used by consumers to prevent disease and to maintain or improve health, energy, and vitality.[1] The use of botanical medicines reached global retail sales of more than U.S. $18 billion in 2001,[2] and sales of herbal supplements reached more than U.S. $5 billion in 2010 in the United States alone.[3] Despite the impression that these botanicals or herbals are cultivated in the wild, many of these products are farmed using conventional agricultural practices, including pesticide application to control insects, molds, and other pests. The increasing risks to human health generated by the widespread use of pesticides in the environment and food supply are well established. To ensure that those risks are low, food is routinely monitored by the U.S. Food and Drug Administration (FDA) for purity and compliance to established regulations and tolerances (Federal Food, Drug, and Cosmetic Act (FFD&C Act), 21 USC 342(a)(2)(b)).[4] In 1994, Congress amended the FFD&C Act with the passage of the Dietary Supplement Health and Education Act of 1994 (DSHEA) (Pub. L. 103-417).[5] This law established a new paradigm for the regulation of dietary supplements.[6] Among other things, DSHEA defined dietary supplements to include certain products that contain herbs and botanicals (21 USC 321(ff)).[7] DSHEA also provided the FDA with the authority to establish good manufacturing practice requirements to govern the preparation, packaging, and

holding of dietary supplements and to ensure that these products are not adulterated. In 2003, due to public and industry concerns, the FDA proposed requiring dietary supplement manufacturers to adhere to current Good Manufacturing Practices (cGMP) standards. The final rule was issued in full effect in June 2010.[8] Because many dietary supplements are largely derived from botanical sources, they must be tested for pesticide contaminants to meet cGMP regulations.

Validated methods are needed for analyzing pesticide residues in botanicals, but the complexity of these matrices presents difficulties in their analysis. Most of these botanical products are dried and concentrated, which creates a greater challenge to the analysis because smaller sample sizes are used to avoid the interfering effects of the matrix, and sensitive instrumentation is needed for detecting trace levels of pesticides. Organic solvent extraction and sorbent cleanup procedures followed by gas chromatography coupled to element selective detectors (i.e., electron-capture and flame photometric detection) or mass spectrometry operated in selective ion monitoring mode (GC-MS/SIM) have been traditionally used for the analysis of pesticides.[9−11] These procedures have been improved with the more recent availability of commercial solid phase extraction products and use of gas chromatography—tandem mass spectrometry (GC-MS/MS) to detect the pesticide residues.[12,13] However, many of the newly registered pesticides that are replacing the traditional organochlorine and organophosphorus pesticides of the past are not amenable to the elevated temperatures used in GC methods. Liquid chromatography coupled to mass spectrometry is now commonly used to detect a variety of thermally labile compounds because of its stability, sensitivity, and selectivity. Many challenges remain in multiresidue pesticide analysis such as the presence of a large number of pesticides and their metabolites (>1000) that require monitoring and a wide variety of botanical and herbal products that present matrix complexities that can interfere in the detection of pesticides.[12,14]

The Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) sample preparation method has been used for the multiresidue pesticide analysis of a wide variety of plant- and animal-based matrices and is highly amenable to LC-MS/MS analysis.[15,16] QuEChERS involves a salt-out acetonitrile extraction/partitioning step with the sample, followed by a cleanup step using dispersive solid phase extraction (dSPE) prior to analysis by GC-MS or LC-MS/MS.[17,18] The standard cleanup for fresh produce samples typically uses a sorbent consisting of primary—secondary amine (PSA)-linked to silica, but additional sorbents such as octadodecyl-linked silica (C_{18})-sorbent and graphitized carbon black (GCB) in combination with PSA in the dSPE cleanup have been used to remove nonpolar components such as lipids, carotenoids, chlorophyll, and other matrix coextractives.[16] Lehotay et al. combined C_{18} and PSA for dSPE cleanup to extract pesticides in foods of fat content of 2−20% such as milk, egg, and avocado.[17] Several sorbents (i.e., PSA, C_{18}, GCB, and anhydrous $MgSO_4$) were evaluated in dSPE for cleanup of foods containing 2−20% fat, and the combination of PSA, C_{18} and anhydrous $MgSO_4$ was selected in those cases.[19]

This study uses an automated dSPE cleanup method for QuEChERS, followed by LC-MS/MS analysis of 236 pesticides in three commonly used botanicals: Asian and American ginseng roots (*Panax ginseng* and *Panax quinquefolius*,

respectively), ginkgo (*Ginkgo biloba*) leaves, and saw palmetto (*Serenoa repens*) berries. The ability to automate the dSPE cleanup of QuEChERS extracts using the LC-MS/MS system could result in improved laboratory productivity and consistent results. dSPE using a combination of 50 mg of PSA, 50 mg of C_{18}, and 150 mg of anhydrous $MgSO_4$ was used for the multiresidue pesticide cleanup for the three botanical matrices. Even with effective extraction and cleanup techniques, the analysis of dietary supplements can be very challenging. Coelution of matrix coextractives with the pesticides may affect the atmospheric pressure ionization process commonly used in liquid chromatography—mass spectrometry, resulting in signal suppression or enhancement that leads to inaccurate results. Therefore, solvent-only calibration standards and matrix-matched calibration standards for the botanicals were prepared to determine matrix effects, signal suppression, and/or signal enhancement. Method recovery data were obtained by fortifying samples at 25, 100, 250, and 500 μg/kg in the three botanicals. Once the method was validated, it was used to analyze commercial ginseng samples for incurred pesticide residues.

■ MATERIALS AND METHODS

Chemicals and Botanical Matrices. A majority of the pesticide standards were obtained from the U.S. Environmental Protection Agency (U.S. EPA) Pesticide Repository (Fort Meade, MD), whereas others were obtained through Fluka/Sigma-Aldrich (St. Louis, MO), EQ Laboratories (Dr. Ehrenstorfer, Atlanta, GA), and Wako Chemicals USA (Richmond, VA), and their relevant information (pesticide name, CAS Registry No., molecular formula, and molecular weight) is provided in the Supporting Information (Table S1). Methanol, acetonitrile, HPLC grade water, anhydrous $MgSO_4$, and NaCl were purchased from Fisher Scientific (Pittsburgh, PA). Formic acid (98% pure) and ammonium formate (99% pure) were obtained from Sigma-Aldrich (St. Louis, MO). Deuterium (^{2}H) isotope labeled internal standard (diazinon-d_{10}) was purchased from CDN-Isotopes (Montreal, QC, Canada).

Two QuEChERS products, that is, 4 g of anhydrous magnesium sulfate and 1 g of sodium chloride packets with 50 mL centrifuge tubes and 2 mL microcentrifuge tubes containing 50 mg of PSA, 50 mg of C_{18}, and 150 mg of magnesium sulfate, were obtained from Agilent Technologies (Wilmington, DE). The botanical samples, Asian and American ginseng roots (*P. ginseng* and *P. quinquefolius*), ginkgo (*G. biloba*) leaves, and saw palmetto (*S. repens*) berries, were purchased from commercially available sources. Incurred ginseng samples were obtained from various FDA field laboratories.

Preparation of Analytical Standards. Separate stock solutions of analytical standards, including those for the isotope-labeled internal standards, were prepared for the 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, QC, Canada). Intermediate solutions and spike solutions (20 μg/mL) were prepared in 200 mL volumetric flasks by mixing the individual stock solutions to be used in the preparation of solvent-only and matrix-matched calibration standards (SOCSs and MMCSs, respectively) and method recovery studies.

A pesticide standard mixture containing 236 pesticides used in this study was obtained by diluting the intermediate stock solution mixtures with acetonitrile to working concentrations of 0.5, 1, 2, 5, 10, 20, 50, 100, 200, 500, 1000, 2000, and 5000 ng/mL. The deuterium isotope labeled internal standard was prepared at 6000 ng/mL in acetonitrile. Standards were kept in the freezer at −20 °C.

Instrumentation. Pesticide analysis was performed using an Agilent 1200 series HPLC and an Agilent 6460 triple-quadrupole LC-MS/MS with jet stream technology (Agilent Technologies, Santa Clara, CA). The automated dSPE cleanup was performed using a Gerstel MPS 2XL dual head, multipurpose sampler (Gerstel,
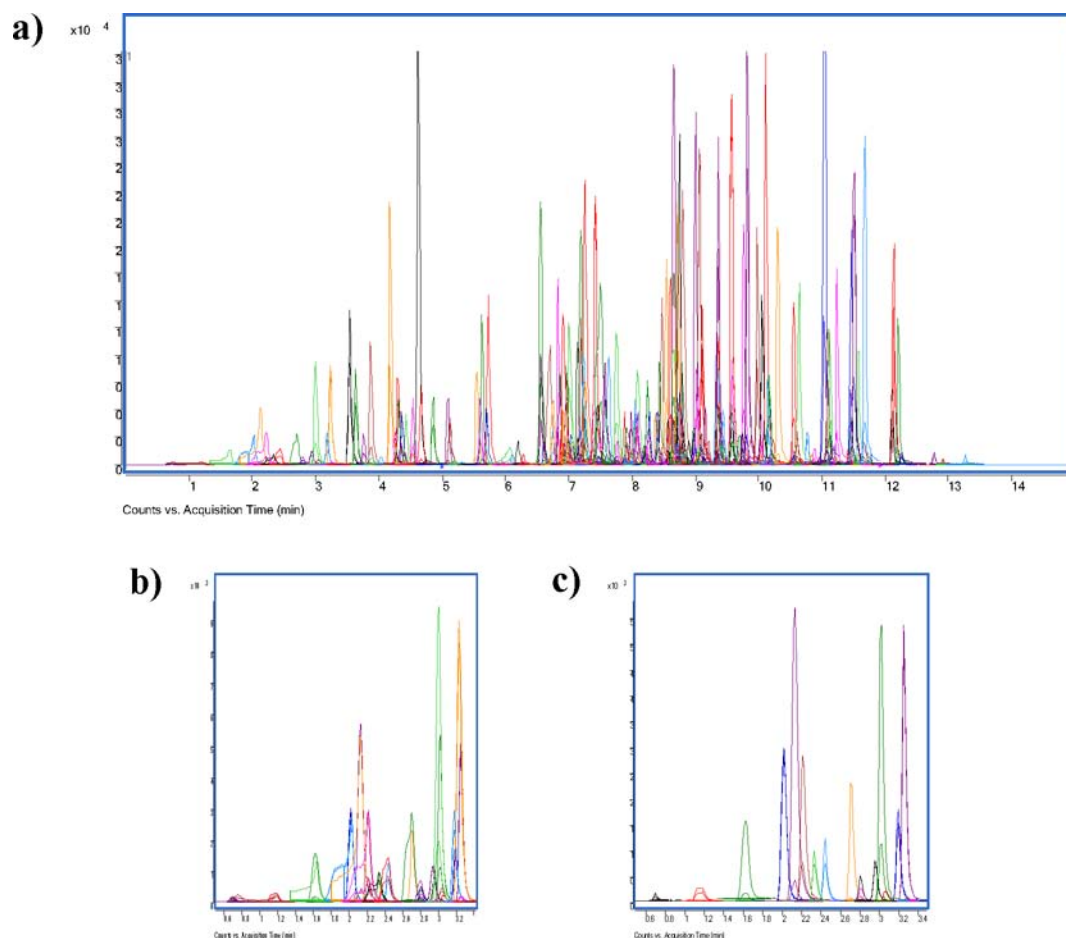
Figure 1. LC-MS/MS of pesticides extracted from botanical dietary supplements. Chromatogram of a 236 pesticide standard mixture at a concentration of 4 ng/mL in acetonitrile/mobile phase A (1:2.5) (a) and separation of early-eluting pesticides in acetonitrile/mobile phase A ratio of 1:2.5 (b) and 1:5 (c).

Linthicum, MD) configured with the LC-MS/MS, which was operated using Maestro software. A 5 μL stainless steel sample loop was used for sample injection to ensure the accuracy and precision of the sample delivery. Separation was performed on an Agilent Zorbax Eclipse Plus, C_{18} column (C_{18} RRHT, 2.1 mm × 100 mm × 1.8 μm). A gradient mode was used for separating the pesticides at 55 °C starting at 94% of A (5 mM ammonium formate in water with 0.01% formic acid) for 0.3 min to 95% of B (0.01% formic acid in acetonitrile) in 12.5 min with a total run time of 15 min. The mass spectrometer was operated in positive electrospray ionization mode using dynamic multiple-reaction monitoring (DMRM) with a retention time window of 0.6 min. Two transitions were chosen for each pesticide and optimized for the best sensitivity. The drying gas (N_2) temperature was 225 °C, and the flow rate was 8 L/min. The nebulizer was set at 40 psi, and the sheath gas (N_2) temperature and sheath flow rate were 325 °C and 10 L/min, respectively. The capillary and nozzle voltages were set at 4000 and 500 V, respectively.

Sample Preparation and Recovery Studies. The blank matrix samples were used for preparation of matrix-matched standards and recovery studies. These samples were screened to determine the presence of any of the targeted pesticide residues. Method blank samples were prepared for each sample batch as quality control samples as well as matrix-matched calibration standards for the matrix effect studies. The incurred ginseng samples were used for verification of the method.

Matrix-matched standards of three matrices were prepared by adding 6–240 μL of the pesticide standard mixture to 960–1194 μL of blank matrix extracts cleaned by dSPE to make a total final volume of 1200 μL and achieve pesticide concentrations of 0.5, 1, 2, 5, 10, 20, 50, 100, 200, 500, and 1000 ng/mL. The internal standard was added to

the matrix-matched standards at a concentration of 25 ng/mL prior to further dilution with mobile phase A using the automated dSPE system. The final concentrations of the matrix-matched standards before injection on the LC-MS/MS system were 0.2, 0.4, 0.8, 2, 4, 8, 20, 40, 80, 200, and 400 ng/mL.

The recovery studies were conducted by adding 5, 20, 50, and 100 μL of the 5000 ng/mL pesticide standard mixture to 1.0 ± 0.05 g of blank matrices to achieve the fortification levels of 25, 100, 250, and 500 μg/kg, respectively. These fortified samples were thoroughly vortexed before the addition of 10 mL of ultrapure water for hydration. For the incurred samples, the fortification step was eliminated. The samples were then vortexed for approximately 30 s to ensure homogeneity, followed by the addition of 10 mL of acetonitrile containing 25 ng/mL of the deuterated internal standard solution. The samples were again vortexed for 10 s followed by the addition of the extraction salts (4 g of anhydrous magnesium sulfate and 1 g of sodium chloride). Samples were then shaken vigorously by hand and vortexed at high speed for 1 min, followed by centrifugation at 1258 g for 5 min. A 1 mL aliquot supernatant was transferred to a 2 mL amber glass autosampler vial containing 50 mg of C_{18} adsorbent, 50 mg of PSA, and 150 mg of magnesium sulfate. The sample vials were placed on the Gerstel MPS 2XL dual-head multipurpose sampler configured for automated dSPE-LC-MS/MS analysis.

The samples containing the sorbents were agitated for 1 min and centrifuged (575 g for 3 min). The resulting filtrate (200 μL) was mixed with 300 μL of the aqueous mobile phase (5 mM ammonium formate/H_2O/0.01% formic acid), transferred to a 2 mL vial, agitated for 30 s, and filtered through a 0.45 μm PTFE membrane syringe filter (Millipore, MA, Billerica, MA) prior to LC-MS/MS injection (5 μL injection volume). All extracts, including solvent-only and matrix-

Table 1. Summary of Validation Results from Three Botanical Matrices (Ginseng, Gingko, and Saw Palmetto) Including Average Recoveries and Average Standard Deviations of Fortified Matrices at 25, 100, 250, and 500 $\mu g/kg$ for the 236 Pesticides Studied in the LC-MS/MS Targeted Screen[a]

| | av recovery/av SD (%) | | | | | | | no. of nondetects | | | | |
botanical	25 $\mu g/kg$	100 $\mu g/kg$	250 $\mu g/kg$	500 $\mu g/kg$	MD-IDL ($\mu g/kg$)	LOD ($\mu g/kg$)	LOQ ($\mu g/kg$)	25 $\mu g/kg$	100 $\mu g/kg$	250 $\mu g/kg$	500 $\mu g/kg$	matrix suppression
ginseng	91/6	93/4	96/3	99/3	0.17	4	13	16	7	7	7	0.97 ± 0.22
gingko	101/9	98/6	99/4	102/3	0.09	2	7	23	13	12	12	1.02 ± 0.33
saw palmetto	100/9	98/7	96/5	96/3	0.14	4	11	12	4	3	3	1.02 ± 0.31

[a]Geometric mean matrix-dependent instrument detection limits (MD-IDL), limits of detection (LOD), and limits of quantitation (LOQ); number of nondetected pesticides in the different matrices; and average (± standard deviation) ratio of slopes of matrix-matched calibration standards to solvent-only calibration standards.

matched calibration standards, sample blanks, and incurred samples, were prepared using the same cleanup procedure.

Data Analysis. Pesticide identification and quantitation analyses were performed using Mass Hunter software (version B.04.00). Pesticide concentrations in ginseng, saw palmetto, and ginkgo were quantitated using a deuterated internal standard (diazinon-d_{10}). Statistical analysis was performed using Microsoft Excel 2007.

■ RESULTS AND DISCUSSION

Instrument Performance and Method Optimization. This study investigated the use of an LC-MS/MS coupled with
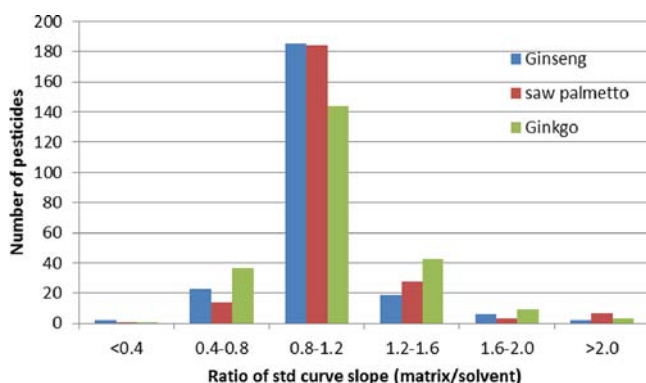

Figure 2. Distribution of matrix effects from ginseng, saw palmetto, and gingko. The matrix effects were assessed by the slope ratios obtained from the slopes from the matrix-matched and solvent-only calibration curves.

a QuEChERS automated dSPE cleanup system for multiresidue pesticide analysis. DMRM was used for data acquisition based on the retention time and peak width of the precursor-to-product ion transition rather than using MRM time segments typically used in older LC-MS instruments operating in MS/MS mode.[13,20,21] The analyte transitions in DMRM are monitored using their specified retention times and time windows to accommodate their peak widths to improve and optimize the MS duty cycle times and allow for increased sensitivity. A typical retention time window was 3 times the base width of a peak to allow for accurate determination of the baseline. DMRM allows for the analysis and determination of a large number of pesticides in a single LC-MS/MS run. The pesticides evaluated in this study along with their retention times, precursor and product ions, and fragmentation and collision voltages are provided in the Supporting Information (Table S1). Results from the studies of SOCSs and MMCSs revealed a concentration range of 0.2−400 ng/mL with a regression coefficient (r^2) > 0.99 and instrument sensitivity as

low as 2 pg on-column mass for the majority of the pesticides (228 of 236) shown in the Supporting Information (Table S2). Nine pesticides (benoxacor, bifenthrin, dichlorvos, ethofumesate, fenpropathrin, hexaflumuron, novaluron, lufenuron, and pyridalyl) were calculated to have a LOD >0.8 ng/mL in SOCSs and MMCSs. These particular pesticides have been shown either to be better detected with negative electrospray ionization or to not ionize well under any atmospheric pressure ionization process.

For optimization of the automated dSPE cleanup step, the pesticide standard mixtures were used to fortify the matrices (ginseng, saw palmetto, and ginkgo) at 25 $\mu g/kg$. The use of the automated dSPE has the advantage of increasing throughput in method development to evaluate and optimize different combinations of cleanup sorbents. Three types of dSPE combinations were evaluated for automated cleanup: (a) 50 mg of PSA, 50 mg of C_{18}, 7.5 mg of graphitized carbon black (GCB), and 150 mg of magnesium sulfate; (b) 50 mg of PSA, 50 mg of C_{18}, and 150 mg of magnesium sulfate; and (c) 50 mg of PSA and 150 mg of magnesium sulfate. The automated system was programmed to perform the cleanup and resulted in freshly prepared cleanup extracts for matrix-matched calibration standards, recovery, and incurred samples, just prior to LC-MS/MS analysis. Once prepared, these extracts were immediately injected into the LC-MS/MS as programmed by the automated dSPE system. This minimized variations that could occur from hydrolysis of pesticides in acetonitrile/water extracts that were prepared manually and allowed the samples to remain in sequence until injected into the LC-MS/MS as determined by the batch sample queue.

Preliminary optimization studies demonstrating recoveries of <50% for planar pesticides were determined in low-pigment ginseng samples when GCB was used with the automated dSPE cleanup step. The planar pesticides, pesticides that contain nonpolar aromatic structures, may adsorb to the GCB sorbent, resulting in low recoveries.[22,23] However, the dSPE containing the PSA, C_{18}, GCB, and magnesium sulfate provided better recoveries for high-pigment matrices (saw palmetto and gingko) due to the removal of pigments by GCB (data not shown). This is in agreement with Zhou et al.,[11] who determined that among 81 pesticides on ginkgo leaves, 62 had recoveries ranging from 70 to 110% after GCB and NH$_2$ cleanup. Inconsistent recoveries were observed for some pesticides fortified at the 25 $\mu g/kg$ level in gingko samples when only PSA was used (data not shown). Acceptable recoveries in the 80−120% range were demonstrated for the majority of the pesticides in all three matrices using a dSPE cleanup mixture containing the PSA and C_{18}. Therefore, the
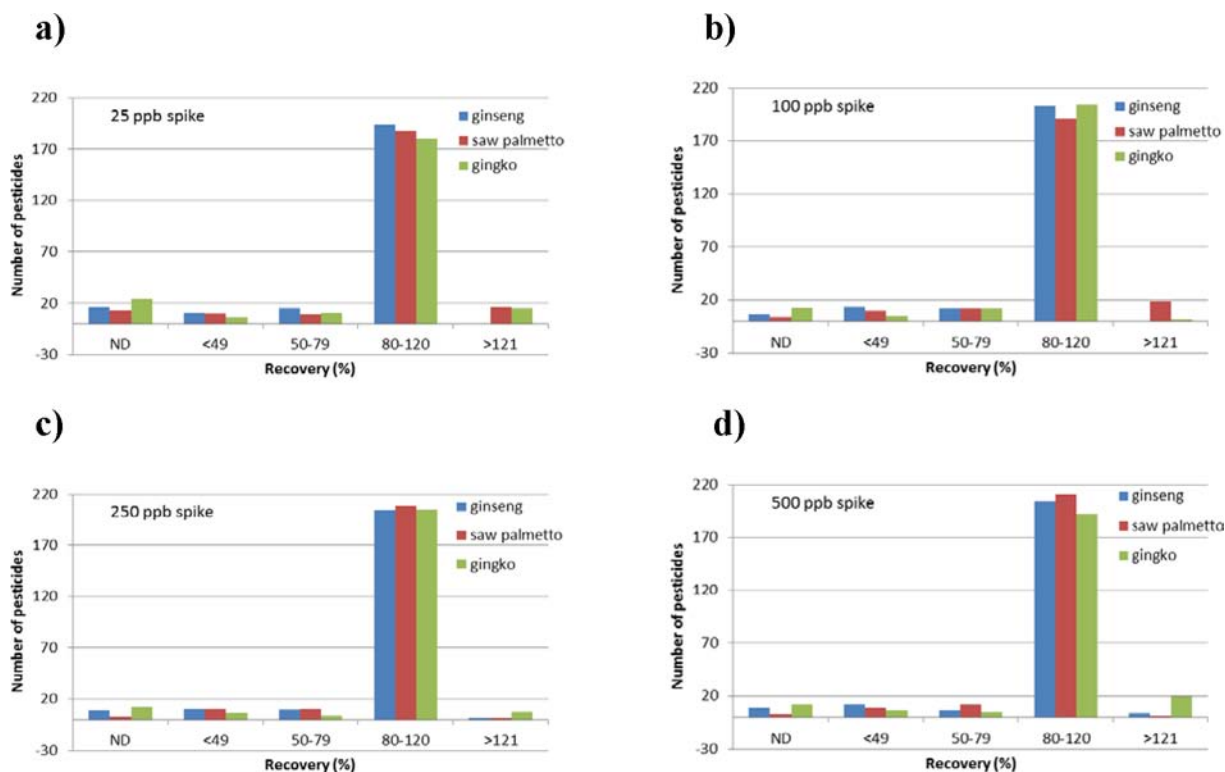
Figure 3. Distribution of recoveries of pesticides fortified in three matrices (ginseng, saw palmetto, and gingko) at four levels: (a) 25, (b) 100, (c) 250, and (d) 500 μg/kg. Average recoveries of each pesticide were determined using four replicates at each fortification level and matrix.
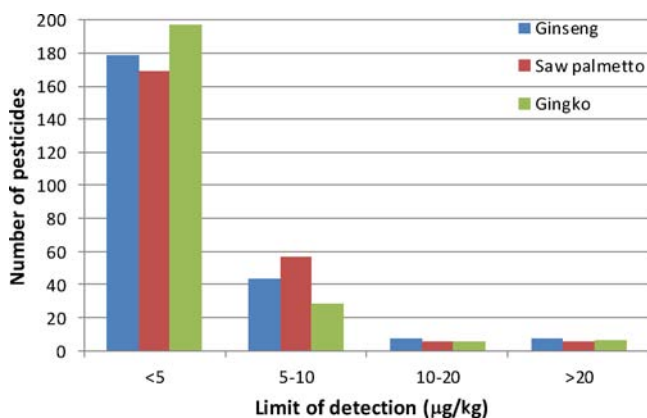

Figure 4. Distribution of the limit of detection of pesticides in different matrices (ginseng, saw palmetto, and gingko).

dSPE with a combination of PSA and C$_{18}$ was chosen for the validation study.

A representative chromatogram of a 236 pesticide standard mixture at a concentration of 4 ng/mL is shown in Figure 1a, revealing adequate separation and signal responses of both polar and nonpolar pesticides. In addition to the evaluation of dSPE cleanup, we also studied the effect of dilution (2.5 or 5 times) of the 10 ng/mL matrix-matched standards with the aqueous phase (mobile phase A) to minimize the matrix effect and to improve the peak shapes of the polar pesticides. The sample extracts prepared in a higher organic solvent content (40% acetonitrile) showed broader peaks for polar pesticides (acephate, cyromazine, methamidophos, and omethoate) with retention times of <2 min (Figure 1b). A reduction in the organic content from 40 to 20% resulted in sharper peak shapes (Figure 1c). However, reduction in the organic content had no

impact on the peak shapes of pesticides eluted after 2 min. Wong et al.[13] also reported improved peak shapes and hence better signal-to-noise ratios for polar pesticides with decreased organic solvent content in the final sample. The results of this study also revealed losses of pesticides in sample extracts containing 20% organic solvent after the extracts had been filtered using 0.45 μm PTFE syringe filters into the autosampler vials. Twenty-two of the 236 pesticides had a loss of >15%; in particular, 3 pesticides had losses of >80%. However, no obvious losses in pesticides were observed in extracts containing 40% organic solvent after filtration. To improve and maximize the sensitivities for both polar and nonpolar pesticides, 40% organic solution in the final sample extract was chosen as the final solvent content for further recovery studies.

Matrix Effects. Standard curves at a concentration range of 0.2−400 ng/mL for all pesticides in both solvent and matrix-matched standards were generated to evaluate matrix effects. Matrix effects in the form of either isobaric interference or suppression/enhancement can affect the analysis of pesticides. Isobaric interference can be resolved either by changing the precursor-to-product ion transition pair or by improving the LC separation to resolve the pesticide analyte from interfering matrix coextractives. Suppression/enhancement is attributed to ionization competition between the pesticide analyte and matrix coextractives in the atmospheric pressure ionization source, resulting in a lower/higher signal response. The suppression/enhancement effect was determined by the ratio of the matrix-matched and solvent-only slopes by dividing the slope of the matrix-matching calibration standard curve by the corresponding slope of the solvent-only calibration curve in solvent. The geometric mean values for each botanical are provided in Table 1, and slope ratios for each pesticide in each botanical are provided in the Supporting Information (Table

Table 2. Pesticides Detected and Their Concentrations (Average ± Standard Deviation, $n = 4$) in the Four Incurred Ginseng Samples Analyzed Using the Proposed Method[a]

pesticide found	American ginseng concn (μg/kg)	American ginseng ion ratio	matrix-matched standard concn (μg/L)	matrix-matched standard ion ratio	Chinese red ginseng concn (μg/kg)	Chinese red ginseng ion ratio	matrix-matched standard concn (μg/L)	matrix-matched standard ion ratio	white Korean ginseng concn (μg/kg)	white Korean ginseng ion ratio	matrix-matched standard concn (μg/L)	matrix-matched standard ion ratio	Wisconsin ginseng concn (μg/kg)	Wisconsin ginseng ion ratio	matrix-matched standard concn (μg/L)	matrix-matched standard ion ratio
methomyl	–	–	–	–	2.9 ± 0.1	1.45	5	1.51	7.1 ± 0.1	1.54	5	1.51	–	–	–	–
carbendazim	–	–	–	–	8.2 ± 0.4	7.69	10	7.78	12 ± 0.8	7.85	10	7.78	2.7 ± 0.2	7.35	5	7.41
boscalid	–	–	–	–	–	–	–	–	–	–	–	–	13 ± 1.2	2.89	20	2.81
zoxamide	–	–	–	–	–	–	–	–	–	–	–	–	23 ± 1.5	2.78	20	2.84
pyraclostrobin	4.7 ± 0.5	2.38	5	2.36	–	–	–	–	–	–	–	–	–	–	–	–
propoxur	–	–	–	–	7.0 ± 0.4	2.63	5	2.60	–	–	–	–	–	–	–	–
carbofuran	–	–	–	–	2.9 ± 0.1	2.04	5	1.92	4.6 ± 0.3	1.96	5	1.92	–	–	–	–
metalaxyl	–	–	–	–	–	–	–	–	–	–	–	–	60 ± 5.4	1.04	50	1.05
dimethomorph, E-	5.0 ± 0.2	4.76	5	4.69	–	–	–	–	–	–	–	–	–	–	–	–
dimethomorph, Z-	6.4 ± 0.1	3.45	5	3.39	–	–	–	–	–	–	–	–	–	–	–	–
azoxystrobin	43 ± 0.03	9.09	50	9.09	–	–	–	–	–	–	–	–	198 ± 6.7	8.93	200	9.05
diazinon	26 ± 1	1.41	20	1.45	–	–	–	–	–	–	–	–	30 ± 0.6	1.43	20	1.45
piperonyl butoxide	–	–	–	–	58 ± 1.9	5.26	50	5.26	3.7 ± 0.2	5.26	5	5.12	–	–	–	–

[a]Pesticides were quantitated using matrix-matched calibration standards and identified by comparing the ratios between the secondary and primary transition product ions and the precursor ion of the incurred sample to that of the matrix-matched calibration standard at the approximated concentration. (–) indicates no detection of pesticides from LC-MS/MS targeted screening procedure.

S2). A value of 1.0 for the ratio of the two standard curve slopes indicates no matrix effects, whereas value deviations from 1.0 indicate either suppression (<1.0) or enhancement (>1.0). Matrix effects increase with an increased deviation of the ratio from 1.0, and the distributions of the matrix effect are dependent on the pesticide analyte and the coextractive compounds in the matrix (Figure 2). The geometric mean slope ratios (and standard deviations, SD) of ginseng and saw palmetto were determined to be 0.97 ± 0.22 and 1.02 ± 0.31 μg/kg, respectively. Of the 236 pesticides analyzed in the fortified ginseng and saw palmetto matrices, 180 pesticides in the matrix-matched standards (ginseng and saw palmetto) did not show a substantive matrix effect, with slope ratios ranging from 0.8 to 1.2. The geometric mean value of 1.02 ± 0.33 μg/kg for ginkgo was similar to those for ginseng and saw palmetto, but only 140 pesticides were found to have slope ratios in the same range. This may be attributed to the high polyphenol or chlorophyll contents present in this pigmented botanical, which may have contributed to matrix suppression of the additional pesticides.

Recovery Studies. Recovery studies were conducted by fortifying the 236 pesticides into the blank matrices (ginseng, saw palmetto, and ginkgo) at fortification levels of 25, 100, 250, and 500 μg/kg ($n = 4$). The recovery data for each of the 236 pesticides, at each fortification level and each matrix, are provided in the Supporting Information (Table S3) and summarized in Figure 3, and the average values for each botanical are provided in Table 1. The mean recoveries of each pesticide at each of the four fortification levels and the three different botanical matrices were subjected to one-way ANOVA. The results indicate that there were no statistically significant differences ($p > 0.05$) observed in the recoveries at 250 and 500 μg/kg fortification levels. However, at fortification levels of 25 and 100 μg/kg, statistically significant differences ($p < 0.05$) were observed between the three botanical matrices. At the 25 μg/kg fortification level, 194, 188, and 180 of the 236 fortified pesticides had recoveries between 80 and 120%, with the average recovery/average SD being 91/6, 101/9, and 100/9% for ginseng, saw palmetto, and ginkgo, respectively. However, 16, 12, and 23 pesticides in the above respective sample types were not detected at this lowest fortification level evaluated due to the low instrument responses of the analytes. At the 500 μg/kg fortification level, larger numbers of pesticides, 204, 211, and 192 of the 236 fortified pesticides, showed recoveries between 80 and 120%, with the average recovery/average SD being 99/3, 96/3, and 102/3%, whereas 7, 3, and 12 pesticides were not detected for ginseng, saw palmetto, and ginkgo, respectively.

The nondetected or low recoveries of the pesticides are due to one or a combination of the following factors: (i) low instrument response and matrix suppression for benoxacor, bifenthrin, dichlorvos, ethofumesate, fenpropathrin, hexaflumuron, novaluron, and pyridalyl; (ii) chemical instability or adsorption to the dSPE sorbents for benzoximate, flucarbazone, lufenuron, penoxsulam, pyrosulam, sprodiclofen, spiromesifen, spirotetramat, and thiencarbazone-methyl; and (iii) nonoptimal pH effects in the extraction solvent for the extraction for pymetrozine. Lehotay et al.[24] reported improved recoveries of pymetrozine from apple−blueberry sauce, peas, and limes when the acetonitrile extraction solvent was acidified to pH 4.8.

Instrument Limit of Detection. Matrix-matched standards at concentrations of 0.2, 0.8, and 2 ng/mL, each with eight replicates, were analyzed and used for the determination of the
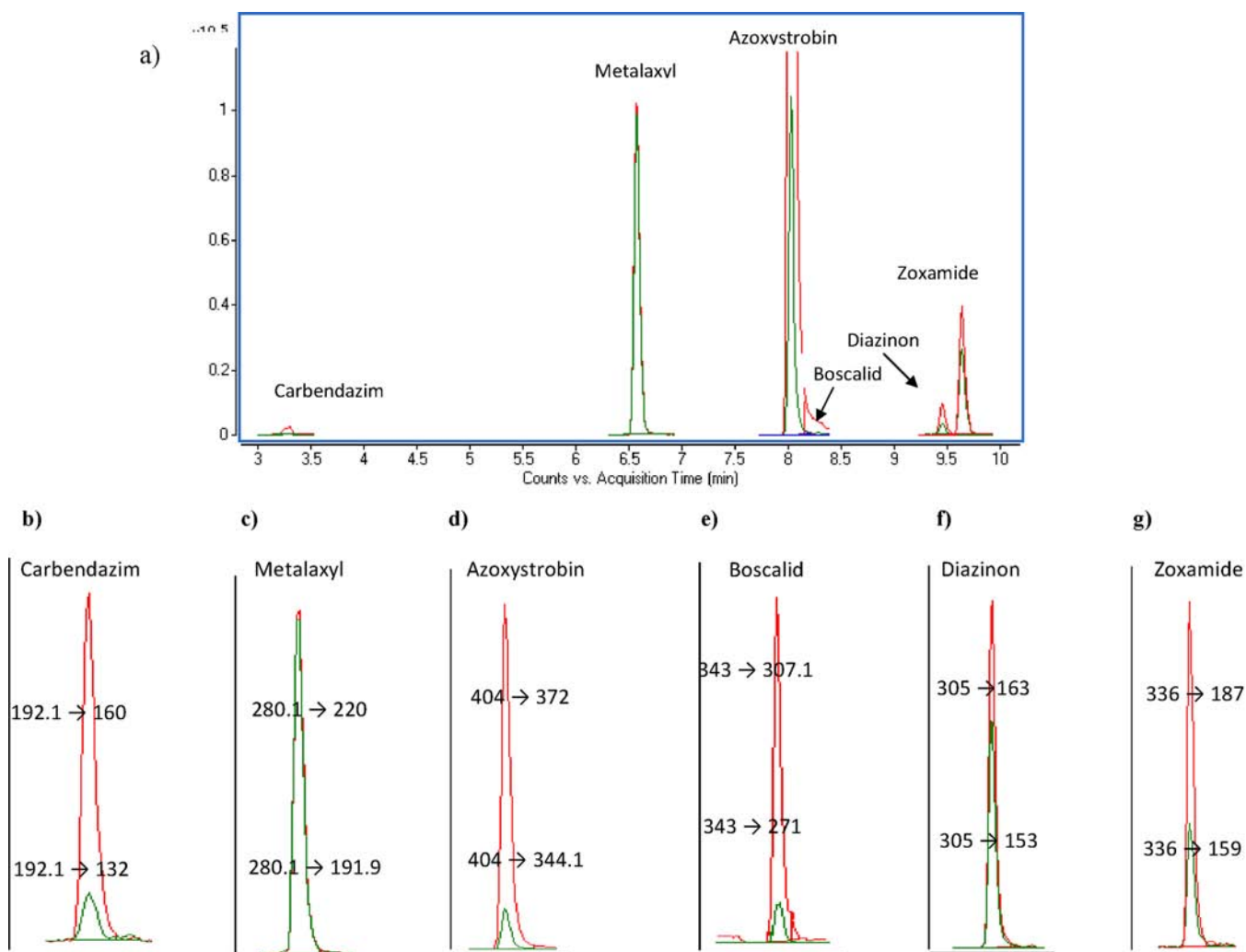
Figure 5. LC-MS/MS chromatogram of an incurred ginseng sample consisting of six pesticides. In (a) LC-MS/MS intensities of each pesticide are shown on the same scale, and the identification of each pesticide using two precursor → product ion transitions: (b) carbendazim, 192.1 → 160 and 192.1 → 132; (c) metalaxyl, 280.1 → 220, 280.1 → 191.9; (d) azoxystrobin, 404 → 372, 404 → 344.1; (e) boscalid, 343 → 307.1, 343 → 271; (f) diazinon, 305 → 163, 305 → 153; and (g) zoxamide, 336 → 187, 336 → 159.

matrix-dependent instrument limit of detection (MD-IDL) as given in the Supporting Information (Table S2). The MD-IDL for all pesticides was calculated according to the U.S. Environmental Protection Agency (EPA) procedure used to determine the method limit of detection.[25] The SDs of the responses were calculated from the eight injections for each pesticide [i.e., $2.998 \times$ SD (critical $t_{0.010} = 2.998$ at degree of freedom $(d_f) = 7$)]. The geometric mean MD-IDLs were 0.17, 0.14, and 0.09 μg/kg for ginseng, saw palmetto, and gingko, respectively, as presented in Table 1. Table 1 also lists the number of nondetected pesticides for each botanical. Sixteen, 12, and 23 pesticides in ginseng, saw palmetto, and gingko, respectively, were not detected at 25 μg/kg possibly because of poor ionization efficiency or matrix suppression, and data from the higher levels were then used to determine the MD-IDLs. The number of nondetected pesticides decreased at the higher fortification levels (100, 250, and 500 μg/kg), indicating the instrument showed no difference in its capability to detect these pesticides at each of these levels. The limit of detection (LOD) was calculated at 10×2.5 times (dilution factor) of the MD-IDL, and the limit of quantitation (LOQ) was calculated at $3 \times$ LOD. Of the 236 pesticides analyzed, 179, 170, and 198

pesticides had a LOD of <0.2 μg/kg in ginseng, saw palmetto, and gingko, respectively. The pesticides had geometric mean LODs of 4, 2, and 4 μg/kg within a 5–10 μg/kg range and geometric mean LOQs of 13, 7, and 11 μg/kg in ginseng, gingko, and saw palmetto, respectively, as given in Table 1. The distribution of the individual pesticide LODs and LOQs is shown in Figure 4. Just as the influence of the botanical matrices was evaluated on the method recoveries, one-way ANOVA ($p < 0.05$) was also used to determine whether the three different matrices also influenced the LODs of each pesticide. The results indicate that there were statistical differences observed in the LODs for the pesticides of the three botanical matrices. However, the LODs of a majority (>70%) of these pesticides in the three matrices are below the desirable detection level of 5 μg/kg.

Pesticide Detection in Incurred Samples. Ginseng samples were analyzed using the QuEChERS–automated dSPE cleanup procedure, followed by LC-MS/MS analysis. A total of 13 pesticides were detected in 4 matrices with an average of 5 pesticides in each matrix, except for one American ginseng sample, which contained none of the 236 pesticides in the targeted screen. The detected pesticide concentration levels

were in the range of 2−200 μg/kg, with % RSD <10% as shown in Table 2. An example LC-MS/MS chromatogram is shown in Figure 5a of a ginseng sample containing six pesticides, carbendazim, metalaxyl, azoystrobin, boscalid, diazinon, and zoxamide, revealing and comparing the different signal intensities of each pesticide. Identification is achieved in Figure 5b−g for each pesticide using the two characteristic precursor → product ion transitions, and the ion ratios of the two transitions of the suspected pesticide in the incurred sample are compared to the standard in the matrix-matched standard at a comparable concentration as given in Table 2. The pesticides detected in these ginseng samples come from different pesticide classes such as carbamate insecticides (carbofuran, methomyl, and propoxur), the pesticide synergist, piperonyl butoxide, and fungicides different from those of a previous study of ginseng samples analyzed by GC-MS analysis.[26] In the GC-MS studies, the focus of the screen was primarily on organochlorine and organophosphorus pesticides, which identified chlorinated fungicides such as quintozene and chlorothalonil and the environmentally persistent insecticides DDT and BHCs. The presence of pesticides in dried botanical products, including tea and other herbal extracts, has been documented by other studies as well.[9−12,27] These results reveal that growers and producers are utilizing a wide variety of pesticides to increase crop yield. Thus, there is a need for analytical tools to screen for an expanding list of pesticides in botanical products. LC-MS/MS analysis provides the ability to analyze for thermally labile pesticides and for other pesticides at lower detection levels in very difficult matrices, such as those commonly found in botanical dietary supplements.

In conclusion, results of the current study demonstrate that the automated dSPE cleanup system coupled with LC-MS/MS injection and analysis can be used for quantitation of multiple pesticide residues in botanical matrices with sufficient sensitivity, accuracy (70−120% average recoveries), and precision (<20% RSDs). There were a small number of pesticides that were not detected or had low recoveries (<70%) in the three matrices (ginseng, saw palmetto, and ginkgo) studied due to matrix suppression, adsorption of analytes onto the dSPE materials, and possibly the pH of the extraction solvent. Further studies are also required to evaluate other different types of botanicals. In addition, a manual method versus the automated QuEChERS method should be compared for these pesticides in the three matrices to determine whether sample processing strategy significantly affects results.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Pesticide information and LC-MS/MS parameters; linearity coefficients (r^2), matrix effect ratios (slope of matrix-matched calibration standards/solvent-only calibration standards), matrix-dependent instrument detection limits (ppb), limits of detection (LOD, ppb), and limits of quantitation (LOQ, ppb); average recoveries and average standard deviations from botanicals (ginseng, gingko, saw palmetto) fortified at 25, 100, 250, and 500 μg/kg, $n = 4$. This material is available free of charge via the Internet at http://pubs.acs.org.

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