JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 3271–3278 3271
DOI:10.1021/jf904045j

Simultaneous Determination of Seven Neonicotinoid Pesticide Residues in Food by Ultraperformance Liquid Chromatography Tandem Mass Spectrometry

Shaoying Liu,[†] Zuntao Zheng,[‡] Fanglin Wei,[†] Yiping Ren,[§] Wenjun Gui,[†] Huiming Wu,[†] and Guonian Zhu*,[†]

[†]Institute of Pesticide and Environmental Toxicology, Zhejiang University, Hangzhou, 310029, P. R. China, [‡]Institute for the Control of Agrochemical, Ministry of Agriculture, Beijing, 100125, P. R. China, and [§]Laboratory of Chemistry and Physics, Zhejiang Province Center for Disease Prevention & control, Hangzhou 310009, P. R. China

The present study developed an improved analytical method for simultaneous quantification of seven neonicotinoids in food by ultraperformance liquid chromatography combined with electrospray ionization triple quadrupole tandem mass spectrometry (UPLC−MS/MS) under the multiple reaction monitoring (MRM) mode. The optimization of extraction, cleanup, UPLC separation and MS/MS parameters of analytes were especially focused on. The low limits of quantification (LOQs) of neonicotinoids ranged from 0.1 to 6 μg kg^{-1}. Meanwhile, reasonable recoveries (65−120%) of seven neonicotinoids for food including apple, cabbage, potato, rice, tea, milk, chicken, pork and egg were demonstrated in different spiked levels within their respective linear range (0.025−150 μg kg^{-1}). The developed analytical method would be appropriate for the routine, high throughput, high sensitivity quantification of seven neonicotinoids using simple sample pretreatment.

KEYWORDS: Neonicotinoid; UPLC−MS/MS; meat; vegetable; rice; tea; milk

INTRODUCTION

Neonicotinoid insecticides represent the fastest growing class of insecticides, including imidacloprid as the biggest selling insecticide worldwide (*1*). Nowadays, there are seven neonicotinoids commercialized, imidacloprid, acetamiprid, nitenpyram, thiacloprid, thiamethoxam, clothianidin and dinotefuran. Neonicotinoid insecticides act as agonists at the insect nicotinic acetylcholine receptor (nAChR), which are active against many sucking and biting pest insects, including aphides, whiteflies and some lepidoptera species (*2*). The use of neonicotinoid insecticides at various stages of cultivation and during postharvest storage plays an important role in food protection and quality preservation. Therefore, monitoring of pesticide residues is crucial for proper assessment of human exposure to pesticide through food products. Maximum residues limits (MRLs) in foodstuffs have been set by several agencies. European Union defined that the residue content of acetamiprid in tea should not be more than 100 μg kg^{-1} (*3*). Japan declared the MRL of imidacloprid was 20 μg kg^{-1} in milk, egg and chicken (*4*).

Current analytical methods for the determination of neonicotinoid residues mainly include GC, HPLC (*5*, *6*) and LC−MS (*7*−*9*). During recent years, a commercial system using columns with sub 2 μm particle size at elevated pressure up to 15000 psi was introduced by Waters (Manchester, U.K.).This technique, named ultraperformance liquid chromatography (UPLC), can be coupled to mass spectrometry for the detection of compounds such as pesticides (*10*−*12*). Of neonicotinoids, only imidacloprid (*13*) and acetamiprid (*14*) have been published using UPLC−MS/MS, while the other five neonicotinoids have not been applied onto UPLC.

Several papers have been published for the determination of neonicotinoids in water (*15*, *16*), milk (*17*), rice (*18*), tea (*19*), honey (*20*, *21*), juice (*22*), fruit and vegetables (*7*, *9*, *13*). However, no methods have been published for determination of neonicotinoids in egg, chicken and pork samples. In this study, we established a method to determine the residues in egg, chicken and pork to meet the requirements of domestic and international legislation. There were few papers about the multiresidue determination of neonicotinoids. Two neonicotinoid residues (imidacloprid and thiamethoxam) were simultaneously analyzed in grapes by LC−MS/MS (*23*). A method for simultaneous analysis of four neonicotinoid residues including acetamiprid, imidacloprid, thiamethoxam and thiacloprid in fruit and vegetables was developed by using LC−MS (*1*). Five neonicotinoids (except nitenpyram and dinotefuran) were analyzed by LC−MS/MS in mango (*24*). Previously, there was only one paper about determination of seven neocicotinoids in vegetables and fruit by HPLC-DAD (*25*). The sensitivity and the specificity of HPLC-DAD were generally lower than those of UPLC−MS/MS.

The goal of this study was to develop a method for quantification of seven neocicotinoid residues in food using solid phase extraction and ultraperformance liquid chromatography

*Corresponding author. Tel: 86-571-86971220. Fax: 86-571-86430193. E-mail: zhugn@zju.edu.cn.

Published on Web 02/26/2010

Table 1. MS/MS Parameters on the Parent and Quantitative Daughter Ion (*m/z*) and Collision Energy of Seven Neocotinoids and Internal Standard (Imidacloprid-d_4)

compound	precursor ion (*m/z*)	quantitative product ion (*m/z*)	collision energy (eV)	confirmation product ion (*m/z*)	collision energy (eV)
nitenpyram	271.3	225.2	12	189.2	12
thiamethoxam	292.0	211.0	8	132.2	16
thiacloprid	252.9	126.2	12	186.1	12
dinotefuran	203.1	129.3	8	157.2	8
acetamiprid	223.2	126.2	12	56.1	12
clothianidin	250.1	169.1	8	132.2	12
imidacloprid	256.0	209.2	12	175.2	12
imidacloprid-d_4	260.0	213.3	12	179.2	12

electrospray ionization tandem mass spectrometry (SPE−UPLC−ESI-MS/MS). The method was validated using several artificially spiked samples of different food which cross plant and animal tissue types and proved to be rapid, sensitive and precise.

MATERIALS AND METHODS

Chemicals and Reagents. The standards of seven neonicotinoids, i.e. imidacloprid (99.0%), nitenpyram (99.0%), acetamiprid (99.0%), thiamethoxam (99.0%), thiacloprid (98.0%), clothiandin (99.5%), dinotefuran (97.5%), and the internal standard of neonicotinoids, imidacloprid-d_4 (98.0%), were all purchased from Dr. Ehrenstorfer (Ausburg, Germany). Methanol and acetonitrile were purchased from Merck (Rahway, NJ) while formic acid was purchased from Tedia (Dayton, OH). Milli-Q quality water (Millipore, Bedford, MA) was used during the whole analysis.

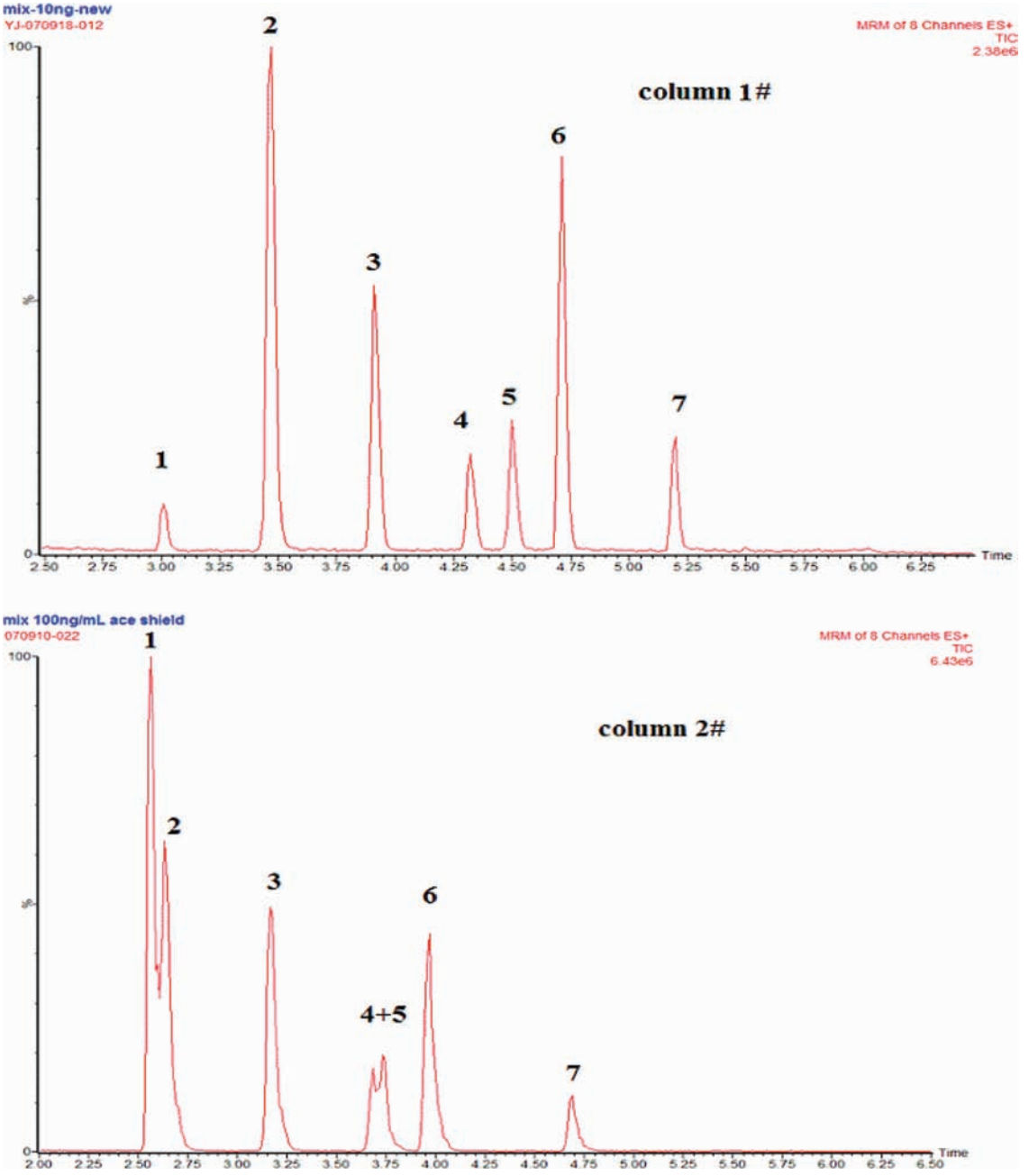

Figure 1. Comparison of separation effects among two different candidate columns. Column #1: Waters ACQUITY UPLC HSS T3 column (100 mm × 2.1 mm i.d., 1.8 μm). Column #2: Waters ACQUITY UPLC BEH SHIELD RP18 column (100 mm × 2.1 mm i.d., 1.7 μm). Mobile phase: solvent A (0.1%, v/v, of formic acid in water) and solvent B (acetonitrile). Flow rate: 0.3 mL min^{-1}. (1) Dinotefuran, (2) nitenpyram, (3) thiamethoxam, (4) clothianidin, (5) imidacloprid, (6) acetamiprid, (7) thiacloprid.

Preparation of Standard Solutions. Stocks solutions of individual analytes were prepared in methanol (1 mg mL^{-1}) and stored at 4 °C. The calibration was performed using a series of diluted solutions including 10, 25, 50, 100, 250, 500, 750, 1000, 1500 ng mL^{-1}. The internal standard (d_4-labeled imidacloprid) was added to the dilute solutions (final concentration of 500 ng mL^{-1}).

Sample Pretreatment. As for sampling, approximately 1 g of each prehomogenized sample (expect apple, potato and cabbage 10 g) was weighed into a 50 mL centrifuge tube and isotope internal standard (50 μg kg^{-1} imidacloprid-d_4) was added. After it stood for 10 min, 20 mL of acetonitrile was added and the tube was rotated for 30 s with the vortex mixed (tea and rice samples were extracted in an ultrasonic bath for 15 min), and then centrifuged at 12000 rpm (RCF value 13200) for 10 min (4 °C). The supernatant was transferred into a 100 mL flat-bottomed flask. The above extraction process was then repeated, and the supernatant was combined. The mixed supernatant was concentrated by a rotatory evaporator and dried by nitrogen gas at 40 °C. The residue was dissolved in 5 mL of water and submitted to solid phase extraction cleanup.

SPE HLB cartridge (3 cm^3/60 mg) (Waters; Milford, MA) was used. The HLB cartridge was first conditioned with 3 mL of methanol and 3 mL of water. Then, 3 mL of redissolved extract was loaded onto the cartridge and washed with 3 mL of water. Finally, the cartridge was eluted with 3 mL of methanol and the eluant was collected. This eluant was evaporated to dryness at 40 °C under a stream of nitrogen and dissolved in 1 mL of methanol/water solution (methanol:water = 1:1, v/v). The solution was filtrated via a 0.22 μm microporous film and injected into the UPLC−MS/MS system.

UPLC Conditions. The UPLC system consisted of an Acquity ultraperformance liquid chromatograph (Waters, Milford, MA). Chromatographic separations of neonicotinoids were performed on a UPLC HSS T3 column (1.8 μm, 100 mm × 2.1 mm i.d., Waters). A gradient program was used with mobile phase, consisting of solvent A (0.1%, v/v, of formic acid in water) and solvent B (acetonitrile) as follows: 90:10 A:B (initial), 90−60% A with 10−40% B (0−3 min), 60−0% A with 40−100% B (3−4 min), 0:100 A:B (4−4.5 min), 0−90% A with 100−10% B (4.5−5 min). A subsequent re-equilibration time (3 min) was performed before next injection. The flow rate was 0.3 mL min^{-1}, the injection volume was 10 μL, and the column and sample temperatures were maintained at 30 and 25 °C, respectively.

MS/MS Conditions. MS/MS was performed on a Micromass Quattro Ultima triple-quadrupole mass spectrometer equipped with an ESI source (Micromass, Manchester, U.K.). The parameters used for the mass spectrometery under the ESI^+ mode were as follows: capillary voltage 3.50 kV, cone voltage 45 V, source block temperature 120 °C, cone gas 60 L h^{-1}, desolvation temperature 350 °C, desolvation gas (nitrogen gas) 500 L h^{-1}. The parameters of the m/z and collision energy of precursor ions and quantitative product ions from neonicotinoids are shown in **Table 1**.

RESULTS AND DISCUSSION

Optimization of UPLC Conditions. Previously, there was only one paper about determination of seven neonicotinoids in vegetables and fruit by HPLC-DAD (*25*). We improved separations available commercially using UPLC that offered speed and improved separation methods with low solvent usage.

Selection of UPLC Column. Two selective UPLC columns with different particle sizes, i.e., (1) column #1, HSS T3 column (100 mm × 2.1 mm i.d., 1.8 μm, Waters ACQUITY UPLC), and (2) column #2, BEH SHIELD RP18 column (100 mm × 2.1 mm i.d., 1.7 μm, Waters ACQUITY UPLC), were tested for the signal intensity and separation efficiency of seven neonicotinoids. The mixed standard solution (10 ng mL^{-1}) was used for this optimization test. **Figure 1** showed the UPLC−MS/MS chromatograms of neonitinoide standards with different columns. The separation efficiency and sensitivity of the HSS T3 column were obviously better than those of the BEH SHIELD RP18 column because the former had stronger retention ability for the neonicotinoids. Therefore, the HSS T3 column was selected as the separation column.

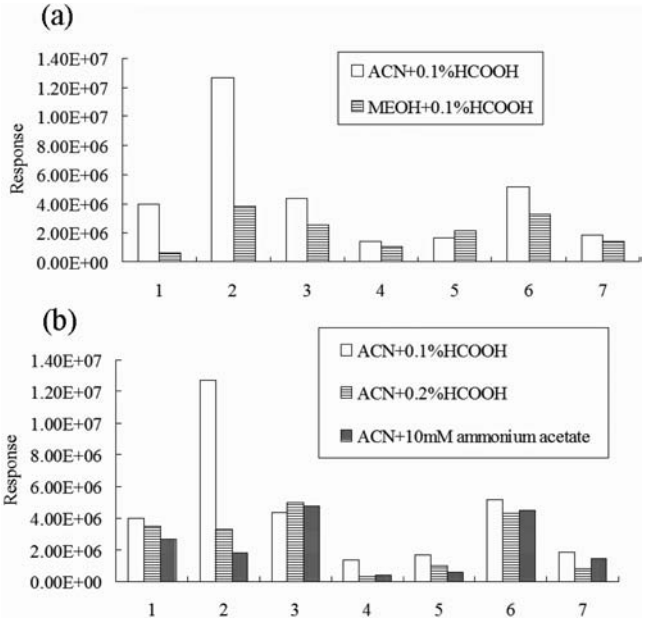

Figure 2. Response of neonicotinoids using different mobile phases. (**a**) Different organic phases: acetonitrile and methanol. (**b**) Different aqueous phases: 10 mM ammonium acetate, 0.1%, v/v, of formic acid in water and 0.2%, v/v, of formic acid in water. (1) Dinotefuran, (2) nitenpyram, (3) thiamethoxam, (4) clothianidin, (5) imidacloprid, (6) acetamiprid, (7) thiacloprid.

Selection of Mobile Phase. In order to achieve good separation of these neonicotinoids with high sensitivity, different mobile phases were compared in the test. Compared with methanol, acetonitrile provided better ionization conditions. 10 mM ammonium acetate and the ratio candidate of formic acid (designated as 0.1 and 0.2%, v/v) were added into mobile phase as ionization regents (**Figure 2**). Considering the combined factors between separation and ionization efficiency, acetonitrile/0.1% (v/v) of formic acid in water system was a compromise and reasonable choice.

Optimization of MS/MS Conditions. ESI^+ was chosen to select the parent ions according to chemical ionization characteristics of neonicotinoids. Initially, the precursor ion with the highest relative intensity in full scan was selected, and then its fragmentation was done with the help of collision energy in the form of argon gas. We selected the most abundant and stable fragment ion as quantitative product ion, whereas we selected the next abundant ion as confirmation product ion. For each ion, different voltages were applied to achieve the highest stable signal. Analysis was done in multiple reaction monitoring (MRM) mode. The MRM transitions are shown in **Table 1**. We also tried to elucidate the molecular structure of the parent ions selected for MRM transitions. **Figure 3** shows the mass spectra obtained after fragmentation of the precursor ions and proposed chemical structures of every selected product ion chosen for seven neonicotinoids using the Daughter Scan acquisition mode.

Optimization of Sample Pretreatment. We used only two sample preparation steps (extraction and SPE cleanup) whereas Watanabe et al. (*25*) used three (extraction, liquid−liquid partition and SPE cleanup) for determination of seven neonicotinoids. In our study, the procedure was quick, easy and efficient, which could adapt to routine analysis with high throughput and low cost.

Extraction Solvent. The selection of solvent was essential for efficient extraction. In the present experiments, acetonitrile, acetone and ethyl acetate were studied regarding their extraction

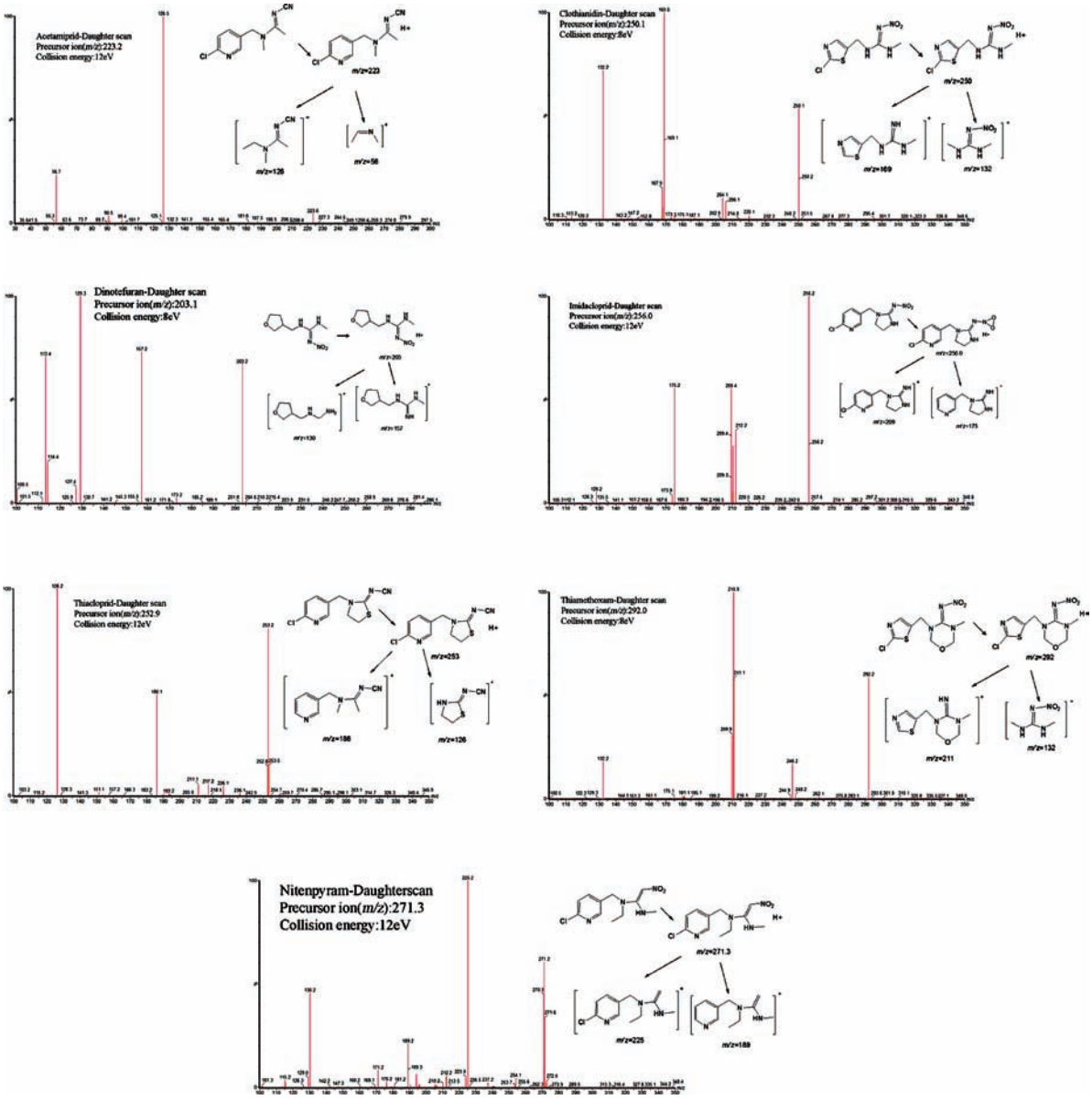

Figure 3. Proposed chemical structures and respective mass spectra of every selected product ion chose for seven neonicotinoids.

efficiencies. Neonicotinoid insecticides are polar compounds. Dinotefuran, nitenpyram and acetamiprid had higher water solubility than the other four neonicotinoids. Ethyl acetate extraction showed 5%, 3% and 29% recoveries for dinotefuran, nitenpyram and thiamethoxam respectively, which was not acceptable, as has been reported previously (*5*). Similarly acetone recoveries of dinotefuran and nitenpyram (17% and 18% for each) were too low. Thus, we selected acetonitrile extraction because it showed 80−103% recoveries for all seven neonicotinoids (**Table 2**).

Selection of SPE Cartridges. For samples with high fatty acid content, such as pork and chicken, a cleanup procedure after extraction with acetonitrile was necessary, because a small amount of fatty acid can be coextracted during acetonitrile extraction. Thus, we selected Oasis HLB cartridges (3 cm^3, 60 mg) (Waters; Milford, MA), which had been employed in pesticide residue detection (*26*−*28*) but had not been reported in neonicotinoid detection. We optimized the eluant with different levels of methanol concentrations. Dinotefuran was eluted at a methanol ratio of more than 10%, nitenpyram at more than 30%, and the other five neonicotinoids at 40−60%. When the methanol ratio was more than 80%, the seven neonicotinoids were completely eluted from HLB (**Figure 4**). In order to choose the most favored washing solution, we compared the cleanup efficiency between 10% methanol and water. The results suggested that 10% methanol reduced the recovery of dinotefuran. Dinotefuran recovery with 10% methanol as washing solution was only 51%, which was not acceptable. For the other six neonicotinoids, the two candidate washing solutions had no significant differences. As a result, we chose 3 mL of water as washing solution and 3 mL of methanol as eluant for SPE cleanup.

Table 2. Recovery Test of Three Candidate Extraction Solvents ($n = 3$)

compound	acetonitrile (%)	acetone (%)	ethyl acetate (%)
dinotefuran	85 ± 6.3	18 ± 0.6	4.8 ± 4.4
nitenpyram	80 ± 2.6	18 ± 1.6	2.8 ± 0.4
thiamethoxam	94 ± 7.9	90 ± 3.2	29 ± 3.4
clothianidin	90 ± 4.5	98 ± 7.6	80 ± 2.5
imidacloprid	103 ± 5.6	97 ± 6.7	84 ± 1.8
acetamiprid	87 ± 6.0	104 ± 4.7	56 ± 3.8
thiacloprid	91 ± 5.2	97 ± 7.9	95 ± 15

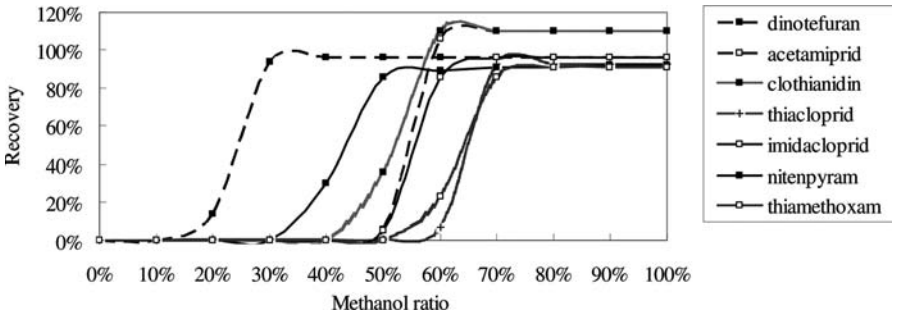

Figure 4. Recovery of neonicotinoids as determined by amount of methanol (percent methanol vs water in 10 mL) used to elute them from HLB cartridges.

Table 3. LOQ of Neonicotinoids in the Nine Sample Matrixes

	LOQ (μg kg^{-1})								
compound	apple	cabbage	rice	potato	tea	chicken	pork	milk	egg
dinotefuran	0.42	0.45	4.0	0.08	0.26	2.3	2.4	1.7	1.2
nitenpyram	0.39	0.27	2.3	0.06	0.36	1.3	2.2	0.71	1.2
thiamethoxam	0.29	0.66	1.3	0.14	0.68	0.68	0.97	0.76	0.58
clothianidin	0.69	1.4	2.6	0.23	1.2	1.4	1.4	1.3	2.0
imidacloprid	1.2	1.6	1.1	0.10	1.7	1.5	0.42	2.0	1.6
acetamiprid	0.36	1.2	1.3	0.51	0.16	0.53	0.31	0.65	0.33
thiacloprid	0.52	5.2	0.43	3.0	1.1	0.29	0.23	0.37	0.14

Table 4. Recoveries of the Neonicotinoids at Spiking Levels 10, 50, and 100 μg kg^{-1} (n = 3)

		recovery (%)								
compound	spiked level (μg kg^{-1})	rice	tea	apple	cabbage	potato	chicken	pork	milk	egg
dinotefuran	10	113 ± 12	117 ± 5.1	96 ± 9.3	74 ± 4.9	97 ± 3.7	103 ± 3.5	101 ± 2.1	116 ± 6.9	115 ± 1.9
	50	117 ± 14	83 ± 9.2	95 ± 5.6	82 ± 11	83 ± 8.1	95 ± 5.9	104 ± 3.8	99 ± 3.3	116 ± 8.1
	100	99 ± 13	82 ± 3.3	93 ± 2.7	98 ± 9.7	83 ± 2.6	91 ± 6.3	94 ± 2.7	93 ± 7.9	118 ± 12
nitenpyram	10	71 ± 5.4	71 ± 4.8	72 ± 1.2	65 ± 4.4	65 ± 3.6	67 ± 6.5	76 ± 11	102 ± 2.1	90 ± 6.0
	50	91 ± 11	67 ± 3.9	89 ± 14	69 ± 11	68 ± 4.1	70 ± 2.2	85 ± 1.4	81 ± 5.6	91 ± 11
	100	89 ± 7.1	72 ± 3.6	73 ± 2.7	71 ± 1.1	66 ± 2.5	67 ± 7.8	79 ± 0.89	91 ± 9.3	90 ± 17
thiamethoxam	10	97 ± 5.3	110 ± 11	78 ± 6.6	83 ± 3.6	102 ± 17	101 ± 8.1	101 ± 8.6	90 ± 13	119 ± 4.7
	50	103 ± 6.5	95 ± 12	84 ± 5.4	90 ± 10	95 ± 12	101 ± 4.3	93 ± 4.2	96 ± 7.5	120 ± 10
	100	90 ± 8.1	94 ± 6.7	82 ± 12	81 ± 8.0	94 ± 8.0	116 ± 7.1	108 ± 3.8	102 ± 7.7	110 ± 1.7
clothianidin	10	82 ± 7.9	114 ± 6.9	112 ± 8.6	74 ± 9.0	76 ± 11	88 ± 11	96 ± 1.9	98 ± 3.4	98 ± 2.2
	50	87 ± 7.1	118 ± 11	110 ± 11	79 ± 6.0	104 ± 5.7	88 ± 5.5	92 ± 3.3	92 ± 6.0	103 ± 7.5
	100	91 ± 13	116 ± 5.3	103 ± 4.5	92 ± 1.2	102 ± 8.2	91 ± 3.7	85 ± 6.1	102 ± 2.8	106 ± 8.1
imidacloprid	10	81 ± 4.9	109 ± 5.6	95 ± 11	77 ± 5.1	87 ± 6.4	118 ± 16	110 ± 7.8	107 ± 8.5	114 ± 1.8
	50	98 ± 8.5	97 ± 5.9	94 ± 6.2	81 ± 13	98 ± 8.8	101 ± 4.5	102 ± 6.7	96 ± 3.2	114 ± 7.2
	100	100 ± 4.3	106 ± 3.1	95 ± 2.8	82 ± 16	94 ± 1.5	108 ± 3.1	99 ± 5.6	100 ± 1.6	108 ± 4.3
acetamiprid	10	108 ± 12	120 ± 8.8	103 ± 7.7	72 ± 7.9	100 ± 9.4	104 ± 2.4	99 ± 5.4	109 ± 14	105 ± 3.9
	50	116 ± 10	115 ± 15	105 ± 14	75 ± 5.8	109 ± 10	99 ± 9.8	94 ± 1.4	93 ± 14	109 ± 10
	100	111 ± 2.1	109 ± 5.3	105 ± 3.7	75 ± 2.5	107 ± 3.4	113 ± 6.4	92 ± 1.7	99 ± 6.3	110 ± 6.5
thiacloprid	10	87 ± 2.9	98 ± 1.6	108 ± 15	94 ± 5.8	95 ± 5.6	116 ± 2.7	105 ± 2.4	106 ± 1.6	111 ± 11
	50	91 ± 9.9	94 ± 5.3	105 ± 5.4	93 ± 6.5	100 ± 8.9	110 ± 7.8	103 ± 7.2	89 ± 12	93 ± 12
	100	88 ± 7.3	90 ± 2.7	95 ± 7.3	95 ± 4.1	96 ± 5.0	116 ± 10	98 ± 2.8	96 ± 6.7	95 ± 11

METHOD VALIDATION

Standard Curve. Previously, the external standard quantitation method has been used accompanied by matrix-matched calibration methods (*15*, *21*, *23*) to avoid possible matrix effects with certain matrixes. Xie et al. (*19*) used the isotope internal standard to validate the concentration of the six neonicotinoids in tea sample with adding imideacloprid-d_4 into the sample solutions. In our study, we chose to use isotope labeled internal standard (imidacloprid-d_4) accompanied by matrix-matched solution for quantification of neonicotinoids which has been successfully applied to many other important problems concerning food safety (*29*−*31*). Instrumental methods using standard solution and complete methods using spiked sample surrogates were validated for UPLC−MS/MS of neonicotinoids. The mixed standard solution with the concentration gradient of 50, 100, 250, 500, 750, 1000, 1500 ng mL^{-1} was diluted with the noncontaminated sample matrix (each sample type used a separate matrix), and that internal standard was added to achieve 500 ng mL^{-1} in each standard mix. The calibration curves of neonicotinoids using the isotope dilution method were created after the injection (10 μL) of mixed standard solution. Good linear relationships and coefficients of determination (R^2 > 0.99) were achieved over the concentration range of 0.025−150 ng mL^{-1}.

Limit of Detection and Limit of Quantification. LOD and LOQ were defined as the concentrations of a compound at which its signal-to-noise ratios were detected as 3:1 and 10:1, respectively (*32*). As for the confirmation of LOD and LOQ, seven neonicotinoids (10 ng mL^{-1}) were prepared. Noncontaminated

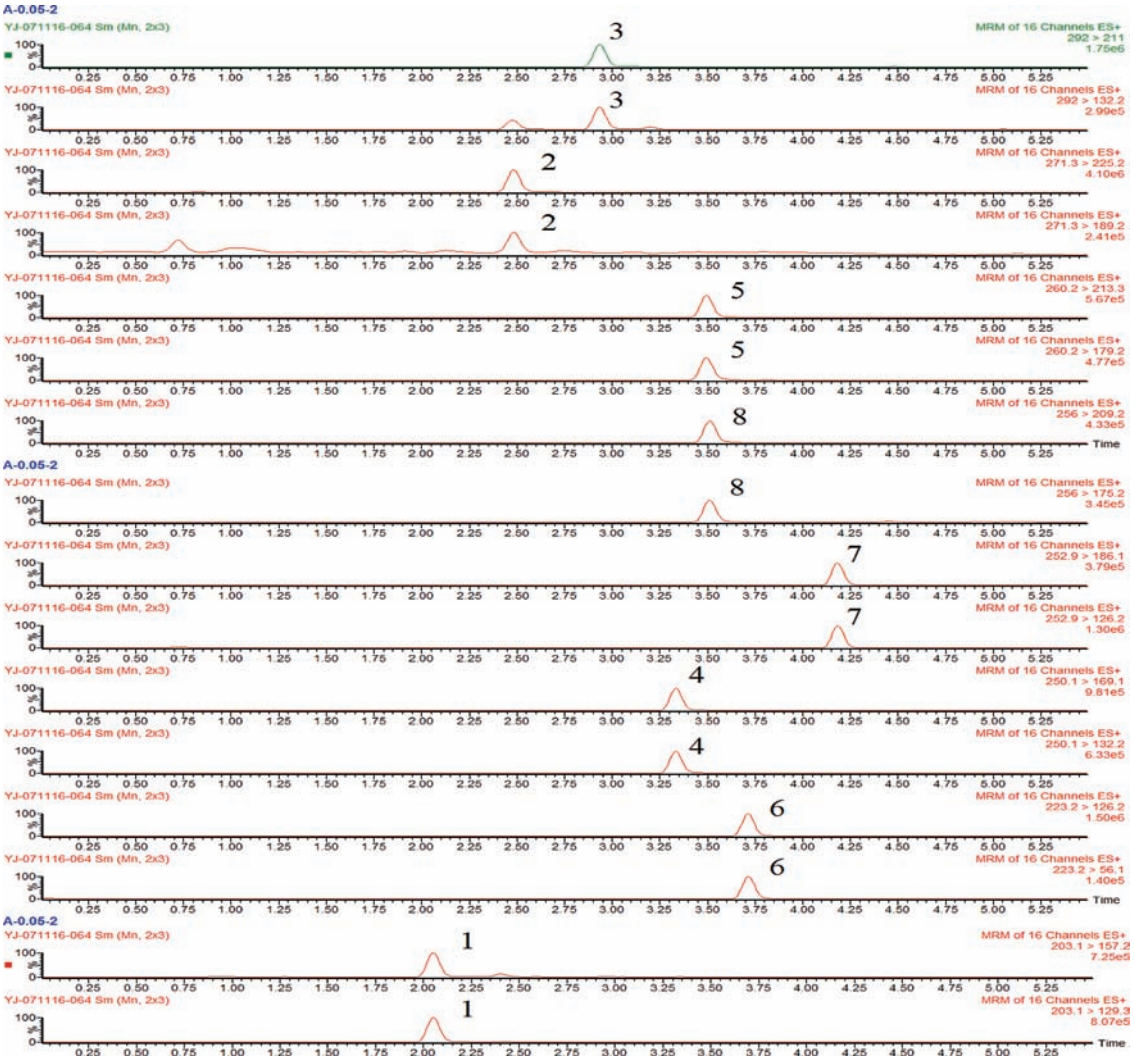

Figure 5. MRM extracted ion chromatograms of neonicotinoids (50 μg kg^{-1} in apple matrix). (1) Dinotefuran, (2) nitenpyram, (3) thiamethoxam, (4) clothianidin, (5) imidacloprid, (6) acetamiprid, (7) thiacloprid, (8) imidacloprid-d_4.

matrix was sampled and spiked with all of the above seven neonicotinoid standards. After sample pretreatment and injection, the LOD and LOQ levels of each neonicotinoid were analyzed by the instrumental software of MassLynx v4.0. The LOQ of seven neonicotinoids ranged from 0.1 to 6 μg kg^{-1} in the nine matrix samples (**Table 3**).

Extraction Recoveries from Various Food Matrixes. The evaluation of recoveries was performed in the noncontaminated apple, cabbage, rice, tea, potato, milk, chicken, pork and egg matrix employing the method of addition of standard to samples. Nine portions of matrix were spiked with the low (10 μg kg^{-1}), intermediate (50 μg kg^{-1}) and high levels (100 μg kg^{-1}) of neonicotinoid mixed standards and IS (50 μg kg^{-1}) while three additional portions were selected as the controls. The recovery was 65−120%, and the RSD was 1.3−16% (**Table 4**, **Figure 5**).

Intraday and Interday Precision. In the intra- and interday precision test, the neonicotinoid-free matrix sample was selected and spiked with all the seven neonicotinoid standards (50 μg kg^{-1}) and internal standard. Spiked samples were pretreated and injected. This experiment was repeated 5 times within a day for the intraday precision test and additionally performed once each day, continuously 5 days, for the interday test. The RSDs of seven neonicotinoids analyzed by the present method were 3.3−10% for the intraday test and 5.8−15% for the interday test, respectively (**Table 5**).

Table 5. Intra- and Interday Precision Test of Neonicotinoids

	concentration (μg kg^{-1})	
compound	intraday precision (n = 5)	interday precision (n = 5)
dinotefuran	47 ± 3.9	46 ± 3.6
nitenpyram	40 ± 3.2	42 ± 2.8
thiamethoxam	45 ± 4.8	46 ± 5.2
clothianidin	40 ± 3.9	40 ± 2.3
imidacloprid	45 ± 4.8	45 ± 4.7
acetamiprid	51 ± 4.2	48 ± 7.2
thiacloprid	46 ± 1.5	45 ± 6.0

Application of the Method for Real Samples. The proposed method has been applied for the routine analysis of approximately 25 real tea samples collected in an agricultural area, Zhejiang province, China. The results (**Table 6**) showed that only 2 neonicotinoids (imidacloprid and acetamiprid) were detected in tea samples. Of 25 samples, 11 samples contained acetamiprid (0.2−10 μg kg^{-1}) and 3 samples contained imidacloprid (2.8−6.3 μg kg^{-1}). These measured concentrations were below the maximum residue limits for tea set by either Japan (10000−50000 μg kg^{-1} for seven neonicotinoids) or EU (50 μg kg^{-1} for imidacloprid and 100 μg kg^{-1} for acetamiprid) (*3*, *4*).

From these results, a broad spectrum method for seven neonicotinoids was developed and validated, which could

Table 6. Concentration of Pesticide Residues in Tea Samples (μg kg^{-1}) Collected from an Agricultural Area, Zhejiang Province, China (n = 3)

sample	dinotefuran	acetamiprid	clothianidin	thiacloprid	imidacloprid	nitenpyram	thiamethoxam
#1	nd[a]	4.3 ± 0.19	nd	nd	2.8 ± 0.27	nd	nd
#2	nd	10 ± 0.92	nd	nd	4.9 ± 0.29	nd	nd
#3	nd	0.92 ± 0.029	nd	nd	nd	nd	nd
#4	nd	nd	nd	nd	nd	nd	nd
#5	nd	0.21 ± 0.013	nd	nd	nd	nd	nd
#6	nd	nd	nd	nd	nd	nd	nd
#7	nd	nd	nd	nd	nd	nd	nd
#8	nd	nd	nd	nd	nd	nd	nd
#9	nd	0.28 ± 0.024	nd	nd	nd	nd	nd
#10	nd	nd	nd	nd	nd	nd	nd
#11	nd	nd	nd	nd	nd	nd	nd
#12	nd	0.45 ± 0.023	nd	nd	nd	nd	nd
#13	nd	10	nd	nd	nd	nd	nd
#14	nd	nd	nd	nd	nd	nd	nd
#15	nd	nd	nd	nd	nd	nd	nd
#16	nd	0.44 ± 0.021	nd	nd	nd	nd	nd
#17	nd	10 ± 0.49	nd	nd	nd	nd	nd
#18	nd	nd	nd	nd	nd	nd	nd
#19	nd	nd	nd	nd	nd	nd	nd
#20	nd	1.4 ± 0.14	nd	nd	6.3 ± 0.45	nd	nd
#21	nd	0.22 ± 0.024	nd	nd	nd	nd	nd
#22	nd	nd	nd	nd	nd	nd	nd
#23	nd	nd	nd	nd	nd	nd	nd
#24	nd	nd	nd	nd	nd	nd	nd
#25	nd	nd	nd	nd	nd	nd	nd

[a] Not detected.

cross plant and animal tissue types. In addition, separation methods were improved by using ultraperformance liquid chromatography which offered rapid speed with low solvent usage. The pretreatment method included acetonitrile extraction and HLB cleanup procedures. After optimization of MS/MS, the neonicotinoids were detected in samples under the MRM mode of triple quadrupole mass spectrometer using imidacloprid-d_4 as internal standard. The neoniocotinoid contents were quantified by isotope dilution and matrix-spiked methods, which significantly improved the analytical method of neoniocotinoid residues. For a method to be practical, it is necessary to consider cost of analyses, including reagents, equipment, labor, and environmental restrictions. This method could be applied to the quantification of seven neonicotinoids in food only within eight minutes. It provided high sensitivity, selectivity, and efficiency and thus was suitable as a routine technique for regulatory monitoring purposes in neonicotinoid residue analysis.

LITERATURE CITED

(1) Di Muccio, A.; Fidente, P.; Barbini, D. A.; Dommarco, R.; Seccia, S.; Morrica, P. Application of solid-phase extraction and liquid chromatography-mass spectrometry to the determination of neonicotinoid pesticide residues in fruit and vegetables. *J. Chromatogr., A* **2006**, *1108*, 1–6.

(2) Casida, J. E.; Tomizawa, M.; Latli, B. Nicotinoid insecticides as probes for the insect nicotinic acetylcholine receptor. *Abstr. Pap. Am. Chem. Soc.* **1997**, *214*, 24-Agro.

(3) Commission Regulation (EC) No 149/2008 of 29 January 2008 amending Regulation (EC) No.396/2005 of the European Parliament and of the Council by establishing Annexes II, III and IV setting maximum residue levels for products covered by Annexes I thereto.

(4) Japan's Positive List System for regulation agricultural chemical residues in food 26 May **2006**.

(5) Obana, H.; Okihashi, M.; Akutsu, K.; Kitagawa, Y.; Hori, S. Determination of acetamiprid, imidacloprid, and nitenpyram residues in vegetables and fruits by high-performance liquid chromatography with diode-array detection. *J. Agric. Food. Chem.* **2002**, *50*, 4464–4467.

(6) Garcia, M. D. G.; Galera, M. M.; Valverde, R. S.; Galanti, A.; Girotti, S. Column switching liquid chromatography and post-column photochemically fluorescence detection to determine imidacloprid and 6-chloronicotinic acid in honeybees. *J. Chromatogr., A* **2007**, *1147*, 17–23.

(7) Blasco, C.; Font, G.; Pico, Y. Analysis of pesticides in fruits by pressurized liquid extraction and liquid chromatography-ion trap-triple stage mass spectrometry. *J. Chromatogr., A* **2005**, *1098*, 37–43.

(8) Botitsi, H.; Econornou, A.; Tsipi, D. Development and validation of a multi-residue method for the determination of pesticides in processed fruits and vegetables using liquid chromatography-electrospray ionization tandem mass spectrometry. *Anal. Bioanal. Chem.* **2007**, *389*, 1685–1695.

(9) Pan, J.; Xia, X. X.; Liang, J. Analysis of pesticide multi-residues in leafy vegetables by ultrasonic solvent extraction and liquid chromatography-tandem mass spectrometry. *Ultrason. Sonochem.* **2008**, *15*, 25–32.

(10) Aguilera-Luiz, M. M.; Vidal, J. L. M.; Romero-Gonzalez, R.; Frenich, A. G. Multi-residue determination of veterinary drugs in milk by ultra-high-pressure liquid chromatography-tandem mass spectrometry. *J. Chromatogr., A* **2008**, *1205*, 10–16.

(11) Koivunen, M. E.; Dettmer, K.; Vermeulen, R.; Bakke, B.; Gee, S. J.; Hammock, B. D. Improved methods for urinary atrazine mercapturate analysis - Assessment of an enzyme-linked immunosorbent assay (ELISA) and a novel liquid chromatography-mass spectrometry (LC-MS) method utilizing online solid phase extraction (SPE). *Anal. Chim. Acta* **2006**, *572*, 180–189.

(12) Zhang, K.; Wong, J. W.; Hayward, D. G.; Sheladia, P.; Krynitsky, A. J.; Schenck, F. J.; Webster, M. G.; Ammann, J. A.; Ebeler, S. E. Multiresidue Pesticide Analysis of Wines by Dispersive Solid-Phase Extraction and Ultrahigh-Performance Liquid Chromatography-Tandem Mass Spectrometry. *J. Agric. Food Chem.* **2009**, *57*, 4019–4029.

(13) Frenich, A. G.; Vidal, J. L. M.; Pastor-Montoro, E.; Romero-Gonzalez, R. High-throughput determination of pesticide residues in food commodities by use of ultra-performance liquid chromatography-tandem mass spectrometry. *Anal. Bioanal. Chem.* **2008**, *390*, 947–959.

(14) Drozdzynski, D. Studies on residues of pesticides used in rape plants protection in surface waters of intensively exploited arable lands in Wielkopolska province of Poland. *Ann. Agric. Environ. Med.* **2008**, *15*, 231–235.

(15) Seccia, S.; Fidente, P.; Barbini, D. A.; Morrica, P. Multiresidue determination of nicotinoid insecticide residues in drinking water by liquid chromatography with electrospray ionization mass spectrometry. *Anal. Chim. Acta* **2005**, *553*, 21–26.

(16) Rodrigues, A. M.; Ferreira, V.; Cardoso, V. V.; Ferreira, E.; Benoliel, M. J. Determination of several pesticides in water by solid-phase extraction, liquid chromatography and electrospray tandem mass spectrometry. *J. Chromatogr., A* **2007**, *1150*, 267–278.

(17) Seccia, S.; Fidente, P.; Montesano, D.; Morrica, P. Determination of neonicotinoid insecticides residues in bovine milk samples by solid-phase extraction clean-up and liquid chromatography with diode-array detection. *J. Chromatogr., A* **2008**, *1214*, 115–120.

(18) Obana, H.; Okihashi, M.; Akutsu, K.; Kitagawa, Y.; Hori, S. Determination of neonicotinoid pesticide residues in vegetables and fruits with solid phase extraction and liquid chromatography mass spectrometry. *J. Agric. Food Chem.* **2003**, *51*, 2501–2505.

(19) Xie, W.; Qian, Y.; Ding, H. Y.; Chen, X. M.; Xi, J. Y.; Jiang, X. Y. Determination of Six Neonicotinoid Pesticides Residues in Tea Samples Using High Performance Liquid Chromatography Tandem Mass Spectrometry. *Chin. J. Anal. Chem.* **2009**, *37*, 495–499.

(20) Fidente, P.; Seccia, S.; Vanni, F.; Morrica, P. Analysis of nicotinoid insecticides residues in honey by solid matrix partition clean-up and liquid chromatography-electrospray mass spectrometry. *J. Chromatogr., A* **2005**, *1094*, 175–178.

(21) Pirard, C.; Widart, J.; Nguyen, B. K.; Deleuze, C.; Heudt, L.; Haubruge, E.; De Pauw, E.; Focant, J. F. Development and validation of a multi-residue method for pesticide determination in honey using on-column liquid-liquid extraction and liquid chromatography-tandem mass spectrometry. *J. Chromatogr., A* **2007**, *1152*, 116–123.

(22) 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.

(23) Venkateswarlu, P.; Mohan, K. R.; Kumar, C. R.; Seshaiah, K. Monitoring of multi-class pesticide residues in fresh grape samples using liquid chromatography with electrospray tandem mass spectrometry. *Food Chem.* **2007**, *105*, 1760–1766.

(24) Banerjee, K.; Oulkar, D. P.; Patil, S. B.; Jadhav, M. R.; Dasgupta, S.; Patil, S. H.; Bal, S.; Adsule, P. G. Multiresidue Determination and Uncertainty Analysis of 87 Pesticides in Mango by Liquid Chromatography-Tandem Mass Spectrometry. *J. Agric. Food Chem.* **2009**, *57*, 4068–4078.

(25) Watanabe, E.; Baba, K.; Eun, H. Simultaneous determination of neonicotinoid insecticides in agricultural samples by solid-phase extraction cleanup and liquid chromatography equipped with diode-array detection. *J. Agric. Food Chem.* **2007**, *55*, 3798–3804.

(26) Koesukwiwat, U.; Sanguankaew, K.; Leepipatpiboon, N. Rapid determination of phenoxy acid residues in rice by modified QuEChERS extraction and liquid chromatography-tandem mass spectrometry. *Anal. Chim. Acta* **2008**, *626*, 10–20.

(27) Hatakeyama, E.; Kajita, H.; Sugawara, T.; Takahashi, S. Determination of chlorothalonil residues in raw tea and green tea leaves by an enzyme-linked immunosorbent assay. *J. Pestic. Sci.* **2008**, *33*, 387–392.

(28) Gilbert-Lopez, B.; Garcia-Reyes, J. F.; Omega-Barrales, P.; Molina-Diaz, A.; Fernandez-Alba, A. R. Analyses of pesticide residues in fruit-based baby food by liquid chromatography/electrospray ionization time-of-flight mass spectrometry. *Rapid Commun. Mass. Spectrom.* **2007**, *21*, 2059–2071.

(29) Spliid, N. H.; Helweg, A.; Heinrichson, K. Leaching and degradation of 21 pesticides in a full-scale model biobed. *Chemosphere* **2006**, *65*, 2223–2232.

(30) Hegele, J.; Buetler, T.; Delatour, T. Comparative LC-MS/MS profiling of free and protein-bound early and advanced glycation-induced lysine modifications in dairy products. *Anal. Chim. Acta* **2008**, *617*, 85–96.

(31) Pedrero, Z.; Madrid, Y. Novel approaches for selenium speciation in foodstuffs and biological specimens: A review. *Anal. Chim. Acta* **2009**, *634*, 135–152.

(32) European Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results.

Received for review November 18, 2009. Revised manuscript received February 5, 2010. Accepted February 08, 2010.