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Water-Based Extraction and Liquid Chromatography−Tandem Mass Spectrometry Analysis of Neonicotinoid Insecticides and Their Metabolites in Green Pepper/Tomato Samples

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Water-Based Extraction and Liquid Chromatography−Tandem Mass Spectrometry Analysis of Neonicotinoid Insecticides and Their Metabolites in Green Pepper/Tomato Samples

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Supporting Information

ABSTRACT: This paper proposes an environmentally friendly method involving water-based extraction of the samples, cleanup of the extracts by solid-phase extraction, and subsequent liquid chromatography coupled with tandem mass spectrometry, which was used for simultaneous determination of seven hydrophilic neonicotinoid insecticides as well as their metabolites in agricultural samples. The effects of sample matrix on detection of the target compounds were negligibly small. Mean recoveries obtained at spiked concentrations between 0.01 and 1.00 mg/kg were 71.2−122.3% with relative standard deviations of ≤7.5%. When the method was applied to crop samples sprayed with commercial formulations of the target compounds, the residual concentrations of the compounds determined by the proposed method (0.015−0.27 mg/kg in green peppers and 0.017−0.31 mg/kg in tomatoes) were equivalent to those determined by the official Japanese method (0.017−0.26 mg/kg in green peppers and 0.013−0.30 mg/kg in tomatoes).

KEYWORDS: *water extraction, neonicotinoid insecticides, LC-MS/MS, agricultural samples*

INTRODUCTION

Neonicotinoid insecticides, which have a high affinity for insect nicotinic acetylcholine receptors,[1,2] are widely used to protect crops against a broad range of pests, including aphids, whitefly, thrips, and mealybugs. The commerically available neonicotinoid insecticides are acetamiprid, clothianidin, dinotefuran, imidaclo-prid, nitenpyram, thiacloprid, and thiamethoxam (Table 1). Their high polarity[2] and other physicochemical properties make them useful for a wide range of application techniques, including seed treatment, soil drench, and foliar and stem application. Because some pesticides can be applied to crops up to the day before harvest, the residual pesticide concentrations in crops immediately after harvest may be relatively high.

Various methods for neonicotinoid insecticide residue analysis have been reported.[3−24] Imidacloprid and acetamiprid have been analyzed by gas chromatography,[3−5] and high-performance liquid chromatography (HPLC) is useful for the analysis of neonicotinoid insecticides in various matrices owing to the high polarity and low volatility of these insecticides.[2] The simultaneous determination of multiple neonicotinoid insecticides by HPLC coupled with diode array or UV detection[6−12] and by liquid chromatography coupled with mass spectrometry (LC-MS)[12−15] or with tandem mass spectrometry (LC-MS/MS)[10,16−24] has been reported.

Sample preparation, which is required for reliable results, represents a bottleneck in pesticide residue analysis. Sample preparation generally involves extraction with an organic solvent followed by liquid−liquid partitioning and cleanup with a solid-phase extraction (SPE) cartridge. Large volumes of hazardous organic solvents are used, which presents a health risk to the analyst and is not environmentally friendly. The quick, easy, cheap, effective, rugged, and safe (QuEChERS) method[18,21] and the dispersive liquid−liquid microextraction method,[8−10,23] which are available for successful extraction and cleanup with small volumes of organic solvents, could be reasonable in terms of reduction of organic solvents in sample preparation. The use of water as an extractant instead of an organic solvent can be an important option for environmentally friendly analysis and can be available for recovering hydrophilic pesticides and their polar metabolites simultaneously. However, few multiresidue methods based on water as an extractant have been developed.[20,25]

The aim of the current study was to develop a sample preparation method involving water-based extraction and versatile SPE cleanup for simultaneous determination of seven hydrophilic neonicotinoid insecticides, as well as some of the major metabolites of nitenpyram and thiacloprid, in agricultural samples by means of LC-MS/MS. To verify the applicability of the proposed sample preparation method, we compared analytical results obtained using the proposed method for samples prepared by spraying crops with several commercial neonicotinoid insecticide formulations with results obtained by means of a reference method (the official Japanese multiresidue method[18]).

MATERIALS AND METHODS

Chemicals and Reagents. Certified neonicotinoid insecticide standards, two major metabolites of nitenpyram [2-[*N*-(6-chloro-3-pyridylmethyl)-*N*-ethylamino]-2-(methylimino)acetic acid (CPMA)

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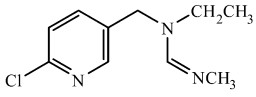
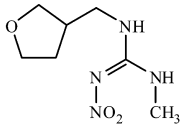
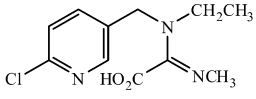
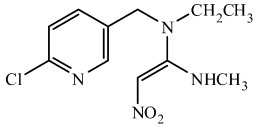
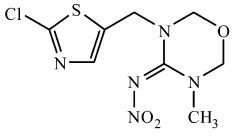
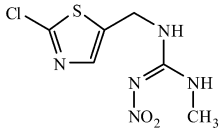
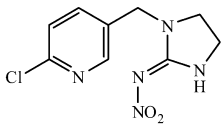
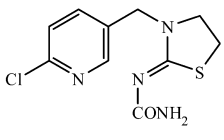
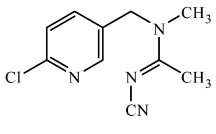
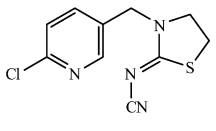
Table 1. Neonicotinoid Insecticides and Metabolites Analyzed in This Study

Compound	Chemical structure	Water solubility (g/L) [a]	log K_{ow} [a]
CPMF [b]		— [c]	—
Dinotefuran		54.3	−0.644
CPMA [b]		—	—
Nitenpyram		≥59	−0.66
Thiamethoxam		4.1	−0.13
Clothianidin		0.340	0.7
Imidacloprid		0.61	0.57
Thiacloprid amide [d]		—	—
Acetamiprid		4.25	0.80
Thiacloprid		0.185	0.73

[a] All values were obtained from ref 2. [b] Major metabolite of nitenpyram. [c] Not reported. [d] Major metabolite of thiacloprid.

and *N*-(6-chloro-3-pyridylmethyl)-*N*-ethyl-*N*′-methylformamidine (CPMF)], and a major metabolite of thiacloprid (thiacloprid amide), all of >95% purity, were purchased from Wako Pure Chemical Industries (Osaka, Japan), Dr. Ehrenstorfer (Augsburg, Germany), and Fluka-Sigma-Aldrich (Steinheim, Germany), respectively. Pesticide analysis grade and HPLC grade organic solvents, HPLC grade formic acid, and other analytical grade reagents were obtained from Kanto Chemical Co. (Tokyo, Japan) or Wako Pure Chemical Industries. Pure water for sample preparation and LC-MS/MS analysis was prepared in the laboratory by means of a water purification system (Milli-Q, Millipore Corp., Bedford, MA, USA). ENVI-Carb II/PSA SPE cartridges (500 mg of graphitized carbon black and 500 mg of ethylenediamine-*N*-propyl silica gel) and ENVI-Carb/LC-NH_2 SPE cartridges (500 mg of graphitized carbon black and 500 mg of aminopropyl silica gel) were purchased from Supelco (Bellefonte, PA, USA).

Stock and Working Solutions. Stock solutions of CPMA in water and the other target compounds in methanol were prepared, and the solutions were stored in the dark at 4 and −20 °C, respectively. Multicomponent working solutions (1, 10, and 100 mg/L) were prepared by diluting each stock solution with 20:80 (v/v) methanol/water at every recovery test and LC-MS/MS analyses, and the working solutions were used as calibration standards and for spiking agricultural samples.

Agricultural Samples. Green peppers and tomatoes were used as model agricultural crops. Green peppers and tomatoes were chopped and homogenized by a home food processor (KC-D627, TWINBIRD Corp., Niigata, Japan). For the preparation of spiked samples for recovery tests, green peppers and tomatoes were grown on arable land of the Japan Plant Protection Association (Miyazaki, Japan) without the use of neonicotinoid insecticides. The absence of the target compounds in the vegetables was confirmed by LC-MS/MS prior to each experiment. For the recovery tests, a homogenized sample (5 g) was spiked with 50 μL of the 1, 10, or 100 mg/L multicomponent working solution (0.01, 0.10, or 1.00 mg/kg, respectively). The spiked samples were exposed to the target compounds for 30 min prior to extraction.

In addition to the spike−recovery tests, we also conducted tests on green peppers and tomatoes sprayed with commercial formulations of the target compounds. The plants were grown in a plastic greenhouse on arable land of the National Institute for Agro-Environmental Sciences and, at the harvesting stage, were sprayed with a mixture of four neonicotinoid insecticide formulations: Mospilan (water-soluble powder), containing 20.0% acetamiprid (Nippon Soda Co., Tokyo, Japan); Admire (water-soluble granules), containing 20% imidacloprid (Bayer Crop Science, Tokyo, Japan); Bariard (water-soluble granules), containing 30% thiacloprid (Bayer Crop Science); and Actara (water-soluble powder), containing 10% thiamethoxam (Syngenta Japan K.K., Tokyo, Japan), all diluted with water according to the manufacturers' instructions. The samples were harvested at 1, 3, and 7 days after spraying. After harvesting, the samples were chopped and homogenized by a home food processor.

Sample Preparation. *Proposed Water-Based Extraction Method.* A 5 g aliquot of homogenized sample was weighed into a 100 mL centrifuge tube, and 25 mL of water was added to the tube. The sample was shaken vigorously for 30 min on a shaker (SA-400, Yamato Scientific Co., Tokyo, Japan) and then centrifuged at approximately 1400*g* for 20 min. The sample mixture was suction-filtered through a glass fiber filter (60 mm in diameter, GF-B, Kiriyama Glass Co., Tokyo, Japan), and the solid residue on the filter was washed with 15 mL of water. The volume of the extract was brought to exactly 50 mL with water, and then 2 mL aliquots (equivalent to 0.2 g of sample) were added to 10 mL of acetonitrile. The solution obtained by adding 2 mL aliquots to 10 mL of acetonitrile was cleaned up according to the procedure established by Kobayashi,[16] as follows. The solution was loaded onto an ENVI-Carb II/PSA SPE cartridge preconditioned with 5 mL of acetone and 5 mL of *n*-hexane. The target compounds were eluted with 5 mL of acetonitrile/toluene (3:1, v/v). The eluate was concentrated and evaporated to dryness with a gentle stream of nitrogen. The residue was reconstituted with 1 mL of 20:80 (v/v) methanol/water, and the resulting solution was filtered with a polytetrafluoroethylene membrane syringe-driven filter unit (0.45 μm, GL Sciences Inc., Tokyo, Japan).

Japanese Official Method (Reference Method). To verify the applicability of the proposed water extraction method, we used the

Table 2. Detection Parameters, Calibration Data, and Limits of Detection of Target Compounds

compound	retention time (min)	transition mass (m/z)[a]	transition mass (m/z)[b]	cone voltage (V)	collision energy (eV)	calibration curve	linearity (μg/L)	correlation coefficient (r)	limit of detection (μg/L)
CPMF	2.01	212 → 126	212 → 157	20	33	$y = 1015.23x - 36.43$	0.10−5	0.9997	0.05
dinotefuran	2.66	203 → 129	203 → 157	12	26	$y = 319.99x + 6.97$	0.10−5	0.9997	0.05
CPMA	2.90	256 → 126	256 → 176	20	25	$y = 271.39x - 0.90$	0.10−5	0.9997	0.05
nitenpyram	3.31	271 → 225	271 → 130	10	31	$y = 104.36x + 6.11$	0.5−5	0.9996	0.10
thiamethoxam	5.69	292 → 211	292 → 181	17	26	$y = 224.32x + 6.14$	0.05−5	1.0000	0.02
clothianidin	7.86	250 → 169	250 → 132	13	24	$y = 226.19x + 37.17$	0.5−5	0.9995	0.10
imidacloprid	8.07	256 → 175	256 → 209	22	25	$y = 188.91x + 1.54$	0.05−5	0.9998	0.03
thiacloprid amide	8.13	271 → 126	271 → 228	25	25	$y = 530.47x - 5.39$	0.05−5	0.9998	0.01
acetamiprid	8.62	223 → 126	223 → 90	20	32	$y = 1837.52x + 21.67$	0.05−5	1.0000	0.01
thiacloprid	9.74	253 → 126	253 → 56	25	36	$y = 2146.54x + 0.60$	0.05−5	1.0000	0.01

[a]MS-MS transition used for quantification. [b]MS-MS transition used for conformation.

Japanese official method[26] as a reference method as follows. Acetonitrile (50 mL) was added to 20 g of homogenized sample, and the slurry was extracted for 3 min with a high-speed homogenizer (Polytron PT2100, Kinematica AG, Lucerne, Switzerland). The sample mixture was suction-filtered, and the solid residue on the filter was extracted again with 20 mL of acetonitrile. The volume of the combined extracts was brought to exactly 100 mL with acetonitrile, and 20 mL aliquots of the extract (equivalent to 4 g of sample) were mixed with 10 g of sodium chloride and 20 mL of 0.5 M phosphate buffer (pH 7). The resulting mixture was shaken vigorously for 10 min and then allowed to stand for about 10 min. After the aqueous phase was discarded, the acetonitrile phase was dried with anhydrous sodium sulfate and filtered, and the filtrate was concentrated and evaporated to dryness with a gentle stream of nitrogen. The residue was reconstituted in 2 mL of acetonitrile/toluene (3:1, v/v), and the resulting solution was loaded onto an ENVI-Carb/LC-NH_2 SPE cartridge preconditioned with 10 mL of acetonitrile/toluene (3:1, v/v). After the target compounds were eluted with 20 mL of acetonitrile/toluene (3:1, v/v), the eluate was concentrated. The residue was reconstituted in 10 mL of acetone. The resulting solution was concentrated, and 5 mL of acetone was added to the residue. The acetone was evaporated under a gentle stream of nitrogen. After the residue was reconstituted with 1 mL of methanol, the solution was diluted five times with methanol/water (20:80, v/v) and filtered through a polytetrafluoroethylene membrane syringe-driven filter unit.

Evaluation of Matrix Effects. To evaluate matrix effects, we used the proposed water extraction method and the reference method described above to prepare cleaned up extracts of nonspiked samples. The cleaned up extracts were dissolved in 1 mL of methanol/water (20:80, v/v), and then 0.04 μg of each target compound was added to the extracts. Matrix effects were evaluated according to a method described in an earlier paper,[27] and the magnitude of the effects was calculated by means of the following equation:

$$\text{matrix effect (\%)} = \left[\frac{\text{peak area of standards in cleaned up extract}}{\text{peak area of standards in pure solvent}} - 1\right] \times 100$$

LC-MS/MS Analysis. LC-MS/MS analysis was carried out with an HPLC system (Alliance 2695 series, Waters, Milford, MA, USA) equipped with a pump, a degasser, an autosampler, and a column oven. The target compounds were separated on an Atlantis T3 analytical column (50 mm × 2.1 mm i.d., 3.5 μm particle size) fitted with an Atlantis T3 guard column Atlantis T3 (10 mm × 2.1 mm i.d., 3.5 μm particle size), both from Waters. The column oven temperature was kept at 30 °C. The injection volume was 10 μL, and the flow rate was 0.2 mL min^{-1}. Mobile phases A and B were methanol and 5 mM ammonium acetate containing 0.1% formic acid, respectively. A gradient mobile was used, with the A:B ratio varied as follows: 0 min, 20:80; 5 min, 20:80; 10 min, 95:5; 13 min, 95:5; 13.5 min, 20:80; 25 min, 20:80.

The MS/MS system (Quattro Micro API, Micromass, Manchester, UK) was a triple-quadrupole tandem mass spectrometer equipped with an electrospray ionization (ESI) interface. For all compounds, the MS instrument was operated in the ESI positive-ion mode at a desolvation temperature of 350 °C and a source temperature of 120 °C and at an ESI voltage of 3.9 kV. Nitrogen was used as the desolvation gas at a flow rate of 600 L/h. For collision-induced dissociation, argon gas was used as the collision gas at a pressure of 4.5×10^{-3} mbar in the collision cell. Mass spectrometric detection was performed in multiple reaction monitoring mode. The multiple reaction monitoring transitions were selected and tuned by direct syringe pump infusion of a 5.0 mg/L standard solution of each compound prepared in methanol/water 20:80 (v/v) into the spectrometer at a flow rate of 10 μL/min. The dwell time for each transition was 300−600 ms. Optimized MS/MS transitions as well as specific cone voltages and collision energies are summarized in Table 2.

Under the chromatographic conditions described, calibration graphs were constructed by plotting peak areas versus concentrations. Excellent linearity and regression coefficient (r) were achieved for all of the target compounds in this study (Table 2). The limit of detection for each compound was determined as the lowest concentration of each compound that gave a signal-to-noise ratio of at least 3.

RESULTS AND DISCUSSION

Optimization of LC-MS/MS Conditions. To achieve good separation of the target compounds with high sensitivity, we analyzed all samples using a slightly modified version of the LC gradient program reported by Kobayashi.[16] This gradient program resulted in good sensitivity and peak shape for all of the target compounds. We optimized the MS parameters and multiple reaction monitoring transitions for the maximum abundance of fragmented ions under ESI positive-ion mode conditions by infusing standard solutions of the target compounds into the mass spectrometer (Table 2). Full-scan spectra were measured for selection of the most abundant m/z values. For each target compound, the addicted ion $[M + H]^+$ was determined as a precursor ion. The optimal collision energy for each target compound was selected, which yielded the most abundant product ion by dissociation of each precursor ion in the collision cell.

Matrix Effects. Matrix effects have been widely studied and are recognized as a source of error in quantitative LC-ESI-MS/MS analysis of food samples.[28] Matrix effects result from competition between matrix ions and analyte ions in the sprayed solution for access to the droplet.[29] Depending on the environment in which ionization and ion evaporation take place, this competition can either suppress or enhance the efficiency of analyte ion formation.[30]

We evaluated matrix effects on the results of analysis of the target compounds in green peppers and tomatoes by means of the proposed water-based extraction method and the reference method (Figure 1). For all of the target compounds, the

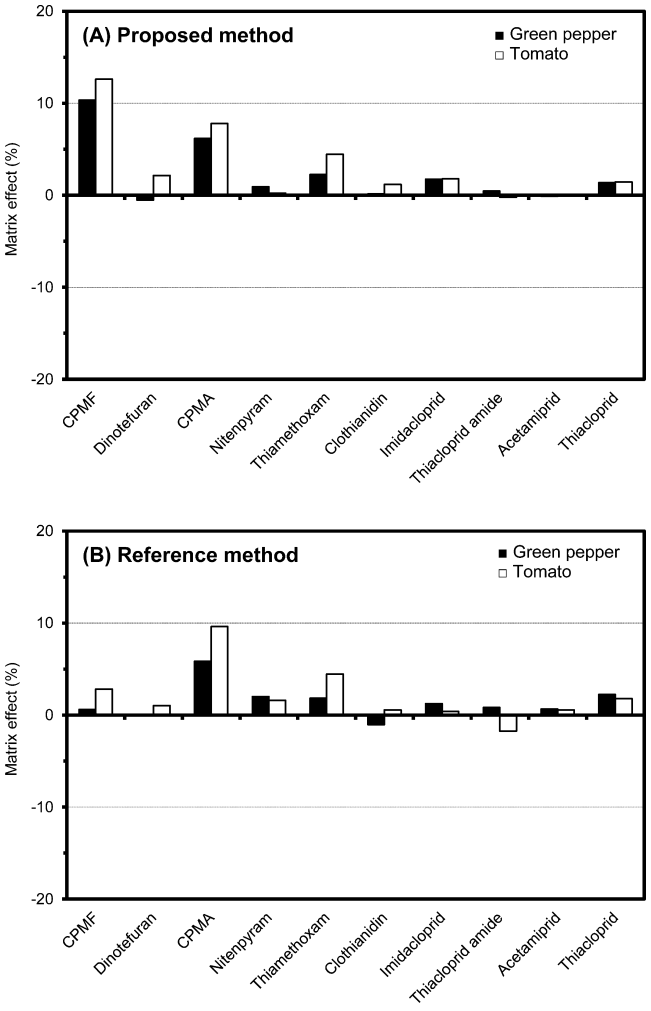


Figure 1. Matrix effects in the proposed water-based extraction method and the reference method.

magnitude of the matrix effect was between −1.7 and +12.6%; this result indicates that for all the combinations of target compounds and matrices in this study, there was no substantial signal suppression or enhancement (matrix effect within ±20%) that interfered with accurate determination by LC-MS/MS analysis according to the criteria explained by Mol et al.[31] On the basis of our results, we performed calibration of the target compounds with external standards diluted with 20:80 (v/v) methanol/water. Some studies[21,32,33] on the QuEChERS method reported that matrix-matched calibration was performed because there was substantial signal suppression and enhancement for the combinations of some pesticides and matrices. On the other hand, matrix effects in the proposed method were not significant; therefore, the target compounds in this study could be analyzed with good precision by calibration with external standards. From the viewpoint of calibration of the target compounds without matrix-matched standards, the proposed method might have an advantage over the QuEChERS method.

Precision and Accuracy of the Proposed Water-Based Extraction Method. We conducted recovery tests on samples spiked with the target compounds at three concentrations (0.01, 0.10, and 1.00 mg/kg; Table 3). The recoveries and relative standard deviations were in the range of 71.2−112.8 and ≤7.5%, respectively, except for the recoveries of imidacloprid (122.3 ± 2.5%) and nitenpyram (121.7 ± 4.9%) in tomato samples spiked at 0.01 mg/kg. For most of the target compounds, the recoveries and relative standard deviations were within the criteria specified in Japanese and European guidelines for validation of pesticide residue analysis methods (70−120 and <20%, respectively).[34,35] These results are indicative of the good precision and accuracy of the proposed water-based extraction method. Although the water solubility and octanol−water partition coefficients of CPMA, CPMF, and thiacloprid amide have not been reported, these metabolites are thought to be at least as hydrophilic as the respective parent compounds. Xiao et al.[20] reported that neonicotinoid insecticides in bovine tissues can be recovered almost completely by means of extraction with pressurized pure water. Our results confirmed that the hydrophilic neonicotinoid insecticides and their metabolites targeted in this study could be extracted into water and recovered, suggesting that the proposed environmentally friendly water-based extraction method is potentially useful for the analysis of hydrophilic pesticides such as neonicotinoid insecticides and related metabolites.

Table 3. Recoveries of Target Compounds from Artificially Spiked Agricultural Samples Using the Proposed Water-Based Extraction Method (n = 4)

		green pepper		tomato	
compound	spiked concentration (mg/kg)	recovery (%)	RSD[a] (%)	recovery (%)	RSD[a] (%)
CPMF	0.01	88.6	6.4	99.8	6.7
	0.10	71.2	5.0	75.9	6.4
	1.00	106.1	0.7	85.0	3.5
dinotefuran	0.01	112.8	4.3	96.9	4.7
	0.10	81.8	4.0	77.1	6.2
	1.00	100.7	0.4	93.1	1.9
CPMA	0.01	88.8	2.3	93.0	4.5
	0.10	82.3	7.5	80.2	6.9
	1.00	100.1	0.8	97.6	1.9
nitenpyram	0.01	94.3	3.1	121.7	4.1
	0.10	83.5	4.7	96.6	4.3
	1.00	95.0	0.8	104.5	1.6
thiamethoxam	0.01	90.0	4.0	104.6	4.4
	0.10	84.0	5.4	85.7	6.8
	1.00	102.8	0.4	100.2	2.1
clothianidin	0.01	110.7	1.9	104.0	4.5
	0.10	85.0	2.9	87.0	7.4
	1.00	105.2	0.5	108.0	2.2
imidacloprid	0.01	106.2	2.4	122.3	2.0
	0.10	97.2	6.0	96.2	6.5
	1.00	103.9	0.3	100.5	1.8
thiacloprid amide	0.01	87.2	3.2	105.1	2.5
	0.10	81.9	5.6	86.5	6.3
	1.00	96.4	2.1	98.7	1.1
acetamiprid	0.01	86.8	2.9	100.6	1.5
	0.10	81.7	5.1	82.8	6.6
	1.00	101.4	0.2	102.2	1.4
thiacloprid	0.01	82.7	2.6	100.1	1.5
	0.10	79.0	6.1	81.9	5.5
	1.00	98.0	1.1	101.3	1.0

[a]Relative standard deviation.

Applicability of the Proposed Method to Samples Sprayed with Commercial Insecticide Formulations. We evaluated the relationship between the analytical results obtained by means of the proposed water-based extraction method and the reference method with green pepper and tomato samples that had been sprayed with four commercial neonicotinoid insecticide

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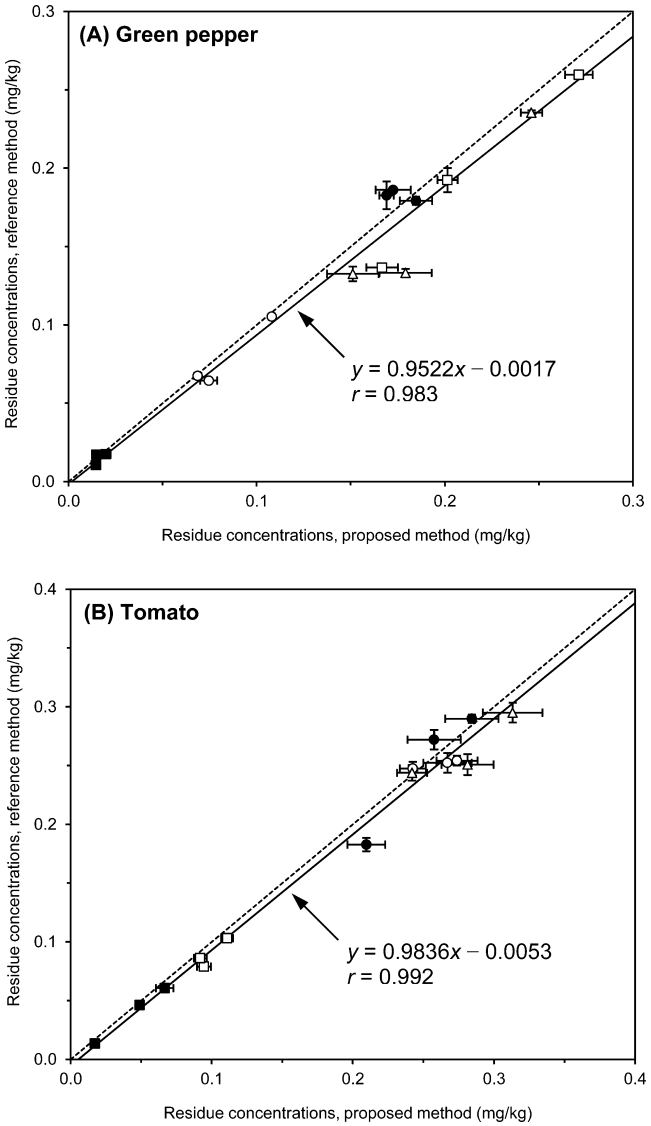

Figure 2. Relationship between residue concentrations in green pepper and tomato samples determined by the proposed water-based extraction method and by the reference method: (○) acetamiprid; (□) clothianidin (a metabolite of thiamethoxam); (△) imidacloprid; (●) thiacloprid; (■) thiamethoxam. Each point is the average of four replicate determinations. The dotted line corresponds to a perfect correlation ($y = x$).

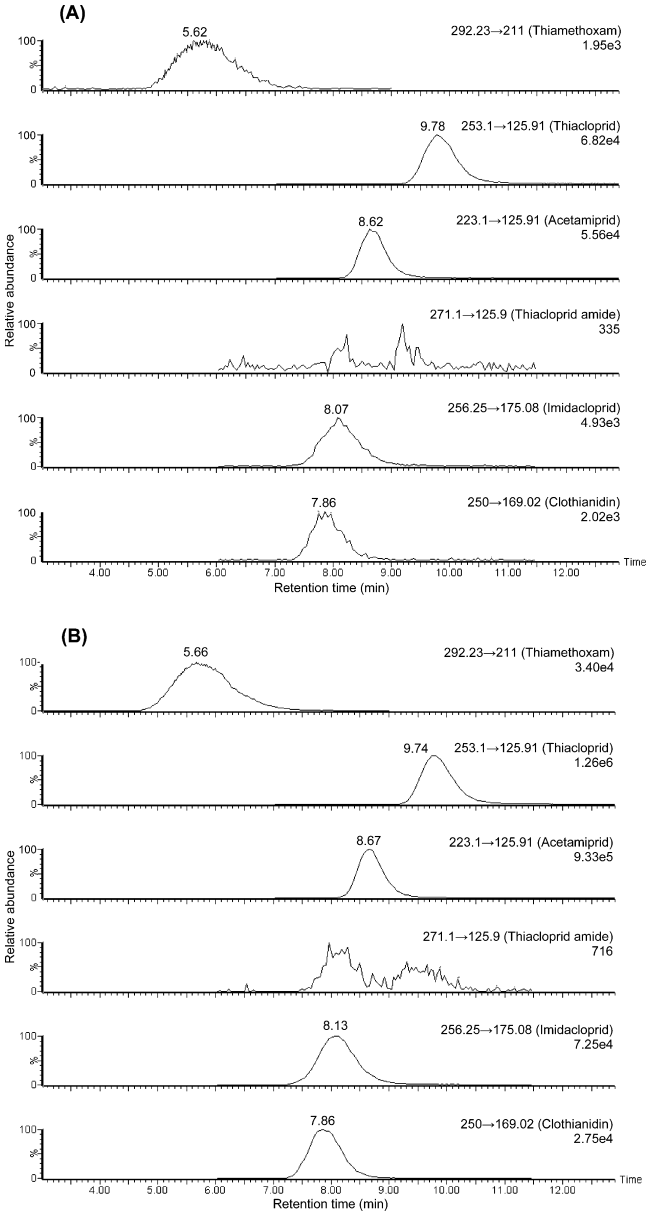

Figure 3. Chromatograms of target compounds obtained from tomato samples harvested 7 days after being sprayed with mixtures of the insecticide formulations by the proposed water-based extraction method (A) and by the reference method (B).

formulations (Figure 2). Acetamiprid, imidacloprid, thiacloprid, thiamethoxam, and clothianidin (a metabolite of thiamethoxam) were detected at >0.01 mg/kg in the samples. The residual concentrations of the target compounds in the green pepper samples determined by means of the proposed method and the reference method were 0.015−0.27 and 0.017−0.26 mg/kg, and those in the tomato samples were 0.017−0.31 and 0.013−0.30 mg/kg, respectively. The residual concentration of thiacloprid amide in the samples was <0.01 mg/kg. Figure 3 shows chromatograms of target compounds obtained from green pepper and tomato samples harvested 7 days after being sprayed with mixtures of the insecticide formulations. The slopes of the simple linear regression equations describing the relationship between the pesticide concentrations detected by means of the proposed method and the reference method were approximately 1; specifically, the slope for the green pepper samples was 0.95 ($r = 0.98$) and that for the tomato samples was 0.98 ($r = 0.99$), indicating that the concentrations detected by means of the two methods were equivalent. Our results suggest that the proposed method can be also applicable for spraying crops and market samples with hydrophilic pesticides.

The proposed environmentally friendly water-based extraction method for agricultural samples here demonstrates that (1) the consumed volume of organic solvent (25 mL per sample) is much less than the volume consumed by the official Japanese multiresidue method (150 mL per sample) and (2) it has simple procedures without liquid−liquid partitioning and can simultaneously and suitably recover hydrophilic pesticides as well as polar metabolites such as CPMA and CPMF that cannot be simultaneously recovered by the official Japanese multiresidue method. To secure food safety, this proposed method could be utilized for regular monitoring of neonicotinoid insecticides and their metabolites in high water content crops, such as green

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peppers and tomatoes, and for monitoring before shipment of these crops.

■ ASSOCIATED CONTENT

Supporting Information

Residue concentrations in green pepper and tomato samples sprayed with formulations 1, 3, and 7 days after insecticide application determined by the proposed method and by the reference method ($n = 4$). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) In *Nicotinoid Insecticides and Nicotinic Acetylcholine Receptor*; Yamamoto, I., Casida, J. E., Eds.; Springer: Tokyo, Japan, 1999.

(2) In *The Pesticide Manual*, 15th ed.; Tomlin, C. D. S., Ed.; British Crop Protection Council: Hampshire, UK, 2009.

(3) Navalón, A.; González-Casado, A.; El-Khattabi, R.; Vilchez, J. L.; Fernández-Alba, A. R. Determination of imidacloprid in vegetable samples by gas chromatography-mass spectrometry. *Analyst* **1997**, *122*, 579−581.

(4) MacDonald, L. M.; Meyer, T. R. Determination of imidacloprid and triadimefon in white pine by gas chromatography/mass spectrometry. *J. Agric. Food Chem.* **1998**, *46*, 3133−3138.

(5) Mateu-Sánchez, M.; Moreno, M.; Arrebola, F. J.; Martínez Vidal, J. L. Analysis of acetamiprid in vegetables using gas chromatography-tandem mass spectrometry. *Anal. Sci.* **2003**, *19*, 701−704.

(6) 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.

(7) 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.

(8) Wu, Q.; Li, Z.; Wang, C.; Wu, C.; Wang, W.; Wang, Z. Dispersive solid-phase extraction clean-up combined with dispersive liquid-liquid microextraction for the determination of neonicotinoid insecticides in vegetables samples by high-performance liquid chromatography. *Food Anal. Methods* **2011**, *4*, 559−566.

(9) Wang, P.; Yang, X.; Wang, J.; Cui, J.; Dong, A. J.; Zhao, H. T.; Zhang, L. W.; Wang, Z. Y.; Xu, R. B.; Li, W. J.; Zhang, Y. C.; Zhang, H.; Jing, J. Multi-residue method for determination of seven neonicotinoid insecticides in grains using dispersive solid-phase extraction and dispersive liquid-liquid micro-extraction by high performance liquid chromatography. *Food Chem.* **2012**, *134*, 1691−1698.

(10) Campillo, N.; Viñas, P.; Férez-Melgarejo, G.; Hernández-Córdoba, M. Liquid chromatography with diode array detection and tandem mass spectrometry for the determination of neonicotinoid insecticides in honey samples using dispersive liquid-liquid micro-extraction. *J. Agric. Food Chem.* **2013**, *61*, 4799−4805.

(11) Hou, R.-Y.; Jiao, W.-T.; Qian, X.-S.; Wang, X.-H.; Xiao, Y.; Wan, X.-C. Effective extraction method for determination of neonicotinoid residues in tea. *J. Agric. Food Chem.* **2013**, *61*, 12565−12571.

(12) Tapparo, A.; Giorio, C.; Soldà, L.; Bogialli, S.; Marton, D.; Marzaro, M.; Girolami, V. UHPLC-DAD method for the determination of neonicotinoid insecticides in single bees and its relevance in honeybee colony loss investigations. *Anal. Bioanal. Chem.* **2013**, *405*, 1007−1014.

(13) 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.

(14) 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.

(15) Yáñez, K. P.; Bernal, J. L.; Nozal, M. J.; Martín, M. T.; Bernal, J. Determination of seven neonicotinoid insecticides in beeswax by liquid chromatography coupled to electrospray-mass spectrometry using a fused-core column. *J. Chromatogr., A* **2013**, *1285*, 110−117.

(16) Kobayashi, H. Development of residue analysis for pesticides by LC/MS and LC/MS/MS methods. *Bunseki Kagaku* **2009**, *58*, 985−997 (in Japanese).

(17) Liu, S.; Zheng, Z.; Wei, F.; Ren, Y.; Gui, W.; Wu, H.; Zhu, G. Simultaneous determination of seven neonicotinoid pesticide residues in food by ultraperformance liquid chromatography tandem mass spectrometry. *J. Agric. Food Chem.* **2010**, *58*, 3271−3278.

(18) Kamel, A. Refined methodology for the determination of neonicotinoid pesticides and their metabolites in honey bees and bee products by liquid chromatography−tandem mass spectrometry (LC-MS/MS). *J. Agric. Food Chem.* **2010**, *58*, 5926−5931.

(19) Xie, W.; Han, C.; Qian, Y.; Ding, H.; Chen, X.; Xi, J. Determination of neonicotinoid pesticides residues in agricultural samples by solid-phase extraction combined liquid chromatography−tandem mass spectrometry. *J. Chromatogr., A* **2011**, *1218*, 4426−4433.

(20) Xiao, Z.; Li, X.; Wang, X.; Shen, J.; Ding, S. Determination of neonicotinoid insecticides residues in bovine tissues by pressurized solvent extraction and liquid chromatography−tandem mass spectrometry. *J. Chromatogr., B* **2011**, *879*, 117−122.

(21) Zhang, F.; Li, Y.; Yu, C.; Pan, C. Determination of six neonicotinoid insecticides residues in spinach, cucumber, apple and pomelo by QuEChERS method and LC−MS/MS. *Bull. Environ. Contam. Toxicol.* **2012**, *88*, 885−890.

(22) Xiao, Z.; Yang, Y.; Li, Y.; Fan, X.; Ding, S. Determination of neonicotinoid insecticides residues in eels using subcritical water extraction and ultra-performance liquid chromatography-tandem mass spectrometry. *Anal. Chim. Acta* **2013**, *777*, 32−40.

(23) Jovanov, P.; Guzsvány, V.; Franko, M.; Lazić, S.; Sakač, M.; Šarić, B.; Banjac, V. Multi-residue method for determination of selected neonicotinoid insecticides in honey using optimized dispersive liquid-liquid microextraction combined with liquid chromatography-tandem mass spectrometry. *Talanta* **2013**, *111*, 125−133.

(24) Yoshida, T.; Murakawa, H.; Toda, K. Determination of nitenpyram and its metabolites in agricultural products by using hydrophilic interaction liquid chromatography-tandem mass spectrometry. *J. Pestic. Sci.* **2013**, *38*, 27−32.

(25) Watanabe, E.; Kobara, Y.; Baba, K.; Eun, H. Reduction of hazardous organic solvent in sample preparation for hydrophilic pesticide residues in agricultural products with conventional liquid chromatography. *J. Agric. Food Chem.* **2013**, *61*, 4792−4798.

(26) Japanese Ministry of Health, Labour and Welfare. Analytical methods for residual compositional substances of agricultural chemicals, feed additives, and veterinary drugs in food; http://www.mhlw.go.jp/english/topics/foodsafety/positivelist060228/dl/060526-1a.pdf (accessed Nov 19, 2013).

(27) Stahnke, H.; Kittlaus, S.; Kempe, G.; Alder, L. Reduction of matrix effects in liquidchromatography−electrospray ionization−mass spectrometry by dilution of the sample extracts: how much dilution is needed? *Anal. Chem.* **2012**, *84*, 1474−1482.

(28) Niessen, W. M. A.; Manini, P.; Andreoli, R. Matrix effects in quantitative pesticide analysis using liquid chromatography−mass spectrometry. *Mass Spectrom. Rev.* **2006**, *25*, 881−899.
(29) Choi, B. K.; Hercules, D. M.; Gusev, A. I. Effect of liquid chromatography separation of complex matrices on liquid chromatography−tandem mass spectrometry signal suppression. *J. Chromatogr., A* **2001**, *907*, 337−342.
(30) Soler, C.; Mañes, J.; Picó, Y. Routine application using single quadrupole liquid chromatography−mass spectrometry to pesticides analysis in citrus fruits. *J. Chromatogr., A* **2005**, *1088*, 224−233.
(31) Mol, H. G. J.; Plaza-Bolaños, P.; Zomer, P.; deRijk, T. C.; Stolker, A. A. M.; Mulder, P. P. J. Toward a generic extraction method for simultaneous determination of pesticides, mycotoxins, plant toxins, and veterinary drugs in feed and food matrixes. *Anal. Chem.* **2008**, *80*, 9450−9459.
(32) Kmellár, B.; Fodor, P.; Pereja, L.; Ferrer, C.; Martínez-Uroz, M. A.; Valverde, A.; Fernandez-Alba, A. R. Validation and uncertainty study of a comprehensive list of 160 pesticide residues in multi-class vegetables by liquid chromatography−tandem mass spectrometry. *J. Chromatogr., A* **2008**, *1215*, 37−50.
(33) Lee, S. W.; Choi, J.-H.; Cho, S.-K.; Yu, H.-A.; El-Aty, A. M. A.; Shim, J.-H. Development of a new QuEChERS method based on dry ice for the determination of 168 pesticides in paprika using tandem mass spectrometry. *J. Chromatogr., A* **2011**, *1218*, 4366−4377.
(34) Japanese Ministry of Health, Labour and Welfare. Revision of guideline on validation of analytical methods for residual compositional substances of agricultural chemicals, feed additives, and veterinary drugs in food; http://www.mhlw.go.jp/topics/bukyoku/iyaku/syoku-anzen/zanryu3/dl/101224-1.pdf (in Japanese) (accessed Nov 19, 2013).
(35) European Commission. Method validation and quality control procedures for pesticide residues analysis in food and feed, Document no. SANCO/10684/2009; http://ec.europa.eu/food/plant/protection/resources/qualcontrol_en.pdf#search=%27No.%20SANCO/10684/2009%27 (accessed Nov 19, 2013).