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Determination of Neonicotinoid Pesticide Residues in Vegetables and Fruits with Solid Phase Extraction and Liquid Chromatography Mass Spectrometry

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A rapid and simple extraction method for the simultaneous analysis of five neonicotinoid insecticides has been developed. Twelve different fruit and vegetable matrixes were extracted with methanol and cleaned up using a graphitized carbon solid phase extraction cartridge loading with a 20% methanol solution. The concentrated eluate after methanol elution was then analyzed for pesticide residues by liquid chromatography/mass spectrometry in the APCI positive mode. The five pesticides including nitenpyram, thiamethoxam, imidacloprid, acetamiprid, and thiacloprid were recovered at 70−95% at spike levels of 0.1 and 1 mg/kg in bell pepper, cucumber, eggplant, grape, grapefruit, Japanese radish, peach, pear, potato, rice, and tomato. Relative standard deviations were less than 10% for all of the recovery tests. The proposed method is fast, easy to perform, and could be utilized for regular monitoring of pesticide residues.

KEYWORDS: Neonicotinoid; pesticide; insecticide; nitenpyram; thiamethoxam; imidacloprid; acetamiprid; thiacloprid; LC-MS; food

INTRODUCTION

The neonicotinoids are a relatively new class of insecticides with a distinct mode of action (*1*, *2*). The structures of five neonicotinoids (nitenpyram, thiamethoxam, imidacloprid, acetamiprid, and thiacloprid) are shown in **Figure 1**. Nitenpyram, imidacloprid, and acetamiprid were introduced in Japan during the 1990s, while thiamethoxam and thiacloprid were registered in 2001.

Single analyte methods for the neonicotinoid pesticides have been developed. Tsumura determined nitenpyram in foods with an high-performance liquid chromatography (HPLC)−UV system (*3*). Fernandez-Alba determined imidacloprid in foods by HPLC−UV and confirmed them by liquid chromatography with mass spectrometry (LC-MS) (*4*). Navalon analyzed imidacloprid residues in vegetables after hydrolysis for gas chromatography with mass spectrometry (GC-MS) determination (*5*). Tokieda analyzed acetamiprid with GC with electron capture detection after hydrolysis and oxidation (*6*). These methods use dichloromethane or chloroform as the extraction solvent. However, the use of chlorinated solvent is not favorable because of environmental concerns. Acetamiprid in foods was determined by GC with a nitrogen phosphorus detector in a Japanese official method (*7*). Sasaki pointed out that acetamiprid, a highly polar compound, might cause overestimated values with GC determination because of the matrix-induced enhancement effect (*8*). These reports indicate that neonicotinoids should be determined with LC because of their low volatility. We have previously reported the determination of three neonicotinoid pesticides (nitenpyram, imidacloprid, and acetamiprid) with HPLC with a diode array detector (DAD) (*9*), which required a two step cleanup process because of the less selective DAD.

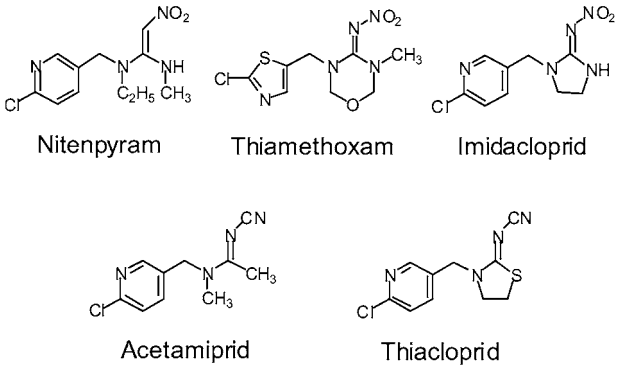

Figure 1. Chemical structures of five neonicotinoid pesticides.

This study reports the development of multiresidue analysis of five neonicotinoid pesticides, nitenpyram, thiamethoxam, imidacloprid, acetamiprid, and thiacloprid, in foods with LC-MS after an easy solid phase extraction (SPE). SPE is widely used for pesticides residue analysis for liquid samples such as water (*10*, *11*) and juice (*12*, *13*). Iijima et al. reported that in order to apply food samples to SPE, samples were first extracted with water miscible solvents such as acetone. The solvent in the extract was then evaporated to near dryness before SPE loading (*14*, *15*).

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The removal of solvent from crude extract is a laborious and time-consuming in sample preparation process. It has been reported that low alcoholic beverages such as wine were directly loaded to a SPE cartridge and eluted pesticides were determined by HPLC or GC-MS without cleanup (*16*, *17*). This indicates that the solvent removal process could be eliminated by using a SPE cartridge with the strong affinity for pesticides and the appropriate organic solvent concentration. Kaufmann pointed out that retention affinities of graphitized carbon and polymer cartridge were stronger than those of C18 and C8 (*17*).

In this study, we present a simple sample preparation method using a graphitized carbon cartridge. Methanol extract diluted with water was used to reduce the solvent's concentration so that pesticides were retained in the SPE cartridge. This method eliminated the laborious evaporation process. By using selective LC-MS determination, five pesticides were analyzed without further cleanup.

MATERIALS AND METHODS

Reagents. HPLC grade of methanol, acetamiprid, imidacloprid, and nitenpyram standards were purchased from Wako Pure Chemical Ind. (Osaka, Japan). Other reagents were residue analysis grade from Wako. Thiamethoxam and thiacloprid were extracted from the respective formulations and recrystallized for use as analytical standards. Bariard, granular formulation of thiacloprid with 30% active ingredient, was supplied by Nihon Bayer Agrochem K. K. (Tokyo, Japan). Beetle Cop, granular formulation of thiamethoxam with 23.5% active ingredient, was manufactured by Novartis Agro K. K. (Tokyo, Japan).

Purification of Reference Standard Material. Thirty grams of Bariard was extracted with acetone (150 mL) twice, and the acetone extract was reextracted with ethyl acetate (100 mL) twice after addition of water. The ethyl acetate layer was concentrated to about 50 mL and recrystallized in a refrigerator after addition of a small amount of hexane. Small needle crystals were washed with hexane and dissolved in ethyl acetate and recrystallized. Colorless needles of pure thiacloprid were finally obtained at the end. Thiamethoxam was also recrystallized in a similar way as thiacloprid. Both pesticides were confirmed with LC-MS by comparing with pure standard solutions supplied by Dr. Shibamoto, University of California, Davis. The purity of crystals was measured with their melting points. The melting points of thiacloprid and thiamethoxam were 135.4 and 139.2 °C, respectively, which were in agreement with reported values (*18*, *19*).

Water was purified with a Milli-Q SP TOC system (Nippon Millipore, Tokyo, Japan). Each standard was dissolved in methanol to make a stock solution of 1 mg/mL. Stock solutions were equally mixed and diluted with methanol to make spiking mixture and working standard solutions. Standard solutions were stored at 4 °C in the dark.

Materials. The graphitized carbon cartridge used was Supelclean ENVI-Carb, 0.5 g/6 mL (Supelco, Bellefonte, PA), and the polymer cartridge used was Supelclean ENVI-Chrom P, 0.5 g/6 mL (Supelco). The sorbent was connected to a 75 mL reservoir (Parts no. 1213-1012 Varian, Harbor City, CA) and rinsed with 20 mL of methanol, followed by 20 mL of water for conditioning.

Food Commodities. Apple, bell pepper, cucumber, eggplant, grape, grapefruit, Japanese radish, peach, pear, potato, rice, and tomato were obtained in Osaka, Japan, for recovery tests. About 500 g of roughly chopped sample from 5 to 10 individual commodities were chopped in a conventional food processor for 2 min to obtain thoroughly mixed homogenates.

LC-MS. LC-MS analysis was carried out with a Platform-II mass spectrometer (Micromass, Manchester, U.K.) equipped with positive atmospheric pressure chemical ionization (APCI) probe and Jasco LC system (Jasco, Tokyo, Japan). The Microsoft Windows NT based software, Mass lynx, was used to control the instrument and for data acquisition and processing.

Neonicotinoids were chromatographed on a Cadenza CD-C18 (3 μm particle size) column (75 mm × 4.6 mm i.d.) (Imtakt, Kyoto, Japan). The mobile phase was a linear gradient elution of methanol/water with the following methanol content: 0−3 min, 5%; 3−10 min, 5−40%; 10−15 min, 40%; 15−20 min, 40−100%; 20−25 min 100%; 25−30 min, 100−5%; and 30−35 min, 5% at the flow rate of 0.8 mL/min. The column temperature was maintained at 50 °C. An aliquot of 5 μL was injected.

Table 1. Monitoring Ions of Neonicotinoid Pesticides with LC-MS[a]

pesticide	molecular weight	major	ref
nitenpyram	270.7	271 (100)	126 (81)
thiamethoxam	291.7	211 (100)	248 (83)
imidacloprid	255.7	256 (100)	212 (72)
acetamiprid	222.7	223 (100)	126 (19)
thiacloprid	252.7	253 (100)	126 (12)

[a] Values in parentheses are the relative intensities.

MS operating parameters were as follows: ionization, APCI positive; corona, 3.50 kV; HV lens, 0.10 kV; and skimmer lens offset, 5 V. The source temperature was 140 °C, and the APCI probe temperature was 550 °C. Cone voltages were set to 30 V. Monitoring ions are shown in **Table 1**.

Extraction. The sample (20 g) was extracted with methanol (95 mL) for 2 min with a Polytron type homogenizer. The extract, after a coarse paper filtration, was collected into a 100 mL graduated cylinder with a cap. Food sediments and the filter paper were washed with a small amount of methanol, and the filtrate was put together with the extract. The extract was made up to 100 mL with methanol.

SPE. A 10 mL aliquot of the methanol extract was taken to the column reservoir, in which 40 mL of water was filled in advance, and the solution was stirred around with a glass stick to make the methanol concentration 20% (v/v). The sorbent, vertically connected under the reservoir, was placed onto the vacuum manifold (VacMaster, International Sorbent Technology Ltd., Hengoed, U.K.). The solution load took about 30−40 min under a slight vacuum. The column was transferred to an another column holder after the solution load was finished. The manifold was not suitable for eluate collection in a 50 mL round bottom flask, which is suitable for evaporation. The pesticides were eluted with 20 mL of methanol. The eluate was evaporated to near dryness. The residue was dissolved in methanol and further concentrated with warm nitrogen stream to make up to 1 mL. The test solution corresponded to 2 g sample/mL.

Recovery Test. Recovery tests were conducted with bell pepper, cucumber, eggplant, grape, grapefruit, Japanese radish (leaf and root), peach, pear, potato, rice, and tomato. A 0.5 mL aliquot of pesticide solution at 4.0 or 40 μg/mL was spiked to homogeneous sample (20 g) at the concentration of 0.1 or 1 mg/kg and allowed to stand for 1 h. Five experiments were carried out individually at respective spiking levels.

RESULTS AND DISCUSSION

LC-MS Condition. A gradient system was applied to separate five pesticides as independent peaks. Nitenpyram is a highly water soluble compound (*20*); thus, HPLC elution started from 5% methanol in the gradient to hold it on a HPLC column and elute as a peak. Typical chromatograms of five pesticides spiked at 0.1 mg/kg on eggplant are shown in **Figure 2**. Nitenpyram was a relatively broad peak with tailing, which could be corrected by adding phosphate salts to a mobile phase (*9*), whereas others were sharp peaks. Because phosphate is nonvolatile compound, it could not be introduced into a LC-MS mobile phase. Two ions were monitored for each pesticide under APCI positive mode as listed in **Table 1**. Molecular ions of nitenpyram, imidacloprid, acetamiprid, and thiacloprid were dominant and primarily monitored for peak determination. Because thiamethoxam was well-fragmented, *m*/*z* 211 was chosen as a major monitoring ion. Reference ions were used for peak identification and determination of peak area when the primary ions were interfered.

Table 2. Comparison of Solvents on SPE of Neonicotinoid Pesticides

SPE cartridge	solvent[a]	% recovery				
		nitenpyram	thiamethoxam	imidacloprid	acetamiprid	thiacloprid
graphitized carbon	methanol	93	93	89	90	88
	acetone	11	34	71	80	83
	acetonitrile	19	49	80	82	82
polymer	methanol	102	95	88	87	85
	acetone	0	10	24	25	47
	acetonitrile	0	11	21	22	53

[a] At the concentration of 20% (v/v).

Table 3. Effect of Methanol Concentrations on SPE of Neonicotinoid Pesticides

SPE cartridge	methanol concn (%)	% recovery				
		nitenpyram	thiamethoxam	imidacloprid	acetamiprid	thiacloprid
graphitized carbon	20	94	94	92	91	91
	30	99	100	97	96	96
	40	95	91	98	98	97
polymer	20	102	100	96	95	95
	30	66	101	100	99	99
	40	29	58	99	100	101
graphitized carbon (food extracts)	20	78	90	97	98	98
	30	57	45	76	91	94
	40	45	45	68	96	98

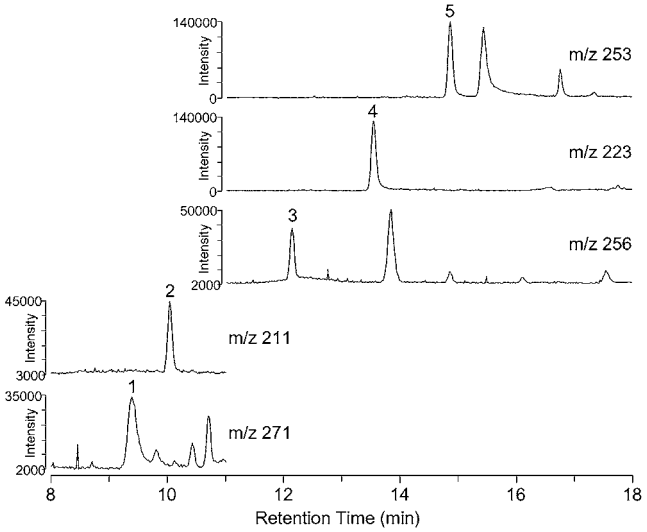

Figure 2. Chromatograms of extracts of eggplant spiked at 0.1 mg/kg. Peak labels: 1, nitenpyram; 2, thiamethoxam; 3, imidacloprid; 4, acetamiprid; and 5, thiacloprid.

Imidacloprid, acetamiprid, and thiacloprid showed linear calibration from 0.01 to 5 μg/mL with correlation coefficients of 0.999. Thiamethoxam showed linearity from 0.02 to 5 μg/mL with a correlation coefficient of 0.999. Nitenpyram showed linearity from 0.02 to 2.5 μg/mL with a correlation coefficient of 0.999.

SPE. The SPE of five pesticides was compared for three solvent systems and two SPE cartridges (**Table 2**). Fifty milliliters of simulated solution, which contained 20% of the respective solvent and 0.2 μg/mL of each pesticide, was loaded to a graphitized carbon cartridge (ENVI-Carb) and a polymer cartridge (ENVI-Chrom P). Twenty milliliters of methanol was necessary to elute out five pesticides from the carbon sorbent (data not shown).

Pesticides were retained when methanol solution was loaded to both cartridges. Nitenpyram and thiamethoxam were not well-recovered in the carbon sorbent, and none of pesticides were recovered well in the polymer sorbent when acetone or acetonitrile solution was loaded. The carbon sorbent showed good retention of pesticide from the extraction solution. The results indicated that load solvent should be methanol in SPE extraction; thus, samples also should be primarily extracted with methanol.

Acceptable methanol concentration in the load solution for SPE was studied in the absence of or the presence of sample matrix consisting of methanol extracts of cucumber, eggplant, and Japanese radish root (**Table 3**). The carbon sorbent extracted five pesticides up to 40% methanol solution in the absence of sample matrix. The polymer sorbent could retain five pesticides with 20% methanol solution while methanol solutions at 30 and 40% were not suitable for nitenpyram and solutions at 40% were not suitable for thiamethoxam. The addition of sample matrix reduced the SPE retention of the carbon sorbent, possibly due to matrix absorption to the carbon sorbent. The combination of carbon sorbent and 20% methanol solution was chosen for SPE.

Recovery Test. Five pesticides were spiked at two levels, 0.1 or 1 mg/kg, in 12 food samples as shown in **Table 4**. Five pesticides were recovered in the range of 70−95% with 2−10% of relative standard deviation (RSD) at both spiking levels. There was no significant difference in recoveries among sample types or spiking levels. Average recoveries of five pesticides in 12 samples were around 80%. Most chromatograms were clear enough to identify and determine each pesticide with primary ions detection (**Figure 2**). Interferences were occasionally found in certain samples, such as peach, grapefruit, and Japanese radish root. Acetamiprid and thiacloprid showed interfering peaks at both spiked concentrations in peach and grapefruit. Those pesticides were determined with secondary ion (*m/z* 126) without serious interferences as shown in **Figure 3**. No interference was detected in all of the control samples under the mentioned conditions.

Apples, which were presumed to be pesticide free, were analyzed for blank confirmation with the proposed method during the method development. Acetamiprid was detected in apples at 0.02 mg/kg (**Figure 4**). The acetamiprid peak was identified by relative peak intensities of primary and secondary

Table 4. Recoveries of Five Neonicotinoid Pesticides

		nitenpyram		thiamethoxam		imidacloprid		acetamiprid		thiacloprid	
sample	spike level (mg/kg)	mean[a] (%)	RSD (%)	mean[a] (%)	RSD (%)	mean[a] (%)	RSD (%)	mean[a] (%)	RSD (%)	mean[a] (%)	RSD (%)
bell pepper	0.1	78	8	91	5	87	2	95	5	84	3
	1	82	7	88	9	91	8	90	9	86	9
cucumber	0.1	80	6	85	5	84	3	77	5	76	5
	1	76	8	85	9	85	7	83	8	80	7
eggplant	0.1	76	2	88	10	77	5	75	7	78	6
	1	85	4	90	5	83	5	88	5	88	5
grape	0.1	83	3	86	5	84	3	80	4	79	4
	1	80	4	88	5	83	4	85	5	83	4
grapefruit	0.1	85	9	80	3	76	8	71	4	72	6
	1	70	5	76	7	76	8	78	5	79	4
Japanese radish leaf	0.1	70	5	82	4	82	3	82	3	84	3
	1	74	4	84	4	77	2	85	4	81	3
Japanese radish root	0.1	72	4	93	8	74	3	72	5	79	2
	1	83	6	86	5	82	4	82	4	81	4
peach	0.1	74	2	90	4	82	3	88	8	88	7
	1	78	6	86	7	83	6	88	9	89	10
pear	0.1	80	5	88	5	84	4	82	7	77	3
	1	77	6	84	6	86	5	83	7	83	5
potato	0.1	82	8	82	8	80	7	77	8	72	4
	1	80	9	79	9	79	4	78	6	74	6
rice	0.1	73	5	77	5	77	5	85	6	73	5
	1	71	4	81	3	83	4	85	4	77	4
tomato	0.1	75	2	82	5	82	4	80	5	78	2
	1	85	7	85	6	84	5	85	4	85	3
average of 12 samples		78	5	85	6	82	5	82	6	80	5

[a] Average of five replicates.

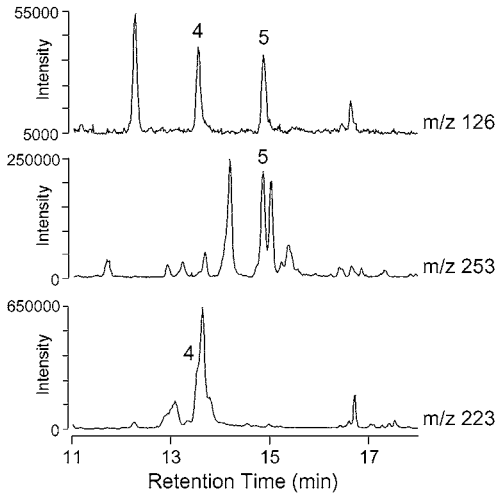

Figure 3. Chromatograms of extracts of peach spiked at 0.1 mg/kg. Peak labels: 4, acetamiprid; and 5, thiacloprid.

ion chromatograms. The suspected peak was not detected on the other ion chromatogram such as *m*/*z* 253 set for thiacloprid around the corresponding retention time for acetamiprid; thus, it showed that the selectivity was secured at low concentrations with sample matrix. The MS spectrum was not confirmed under a scan mode because of low concentration.

Limit of detection (LOD) was calculated from the peak intensity at 0.1 mg/kg and blank levels in recovery tests. LOD was defined as $S/N > 4$ so that it is in the linear range of the standard calibration. The LOD of thiamethoxam, imidacloprid, acetamiprid, and thiacloprid was 0.01 mg/kg and that of nitenpyram was 0.02 mg/kg in most samples. LODs were doubled in samples with too much interference such as grapefruit.

Advantage of SPE. We have previously tried to analyze nitenpyram, imidacloprid, and acetamiprid with acetonitrile

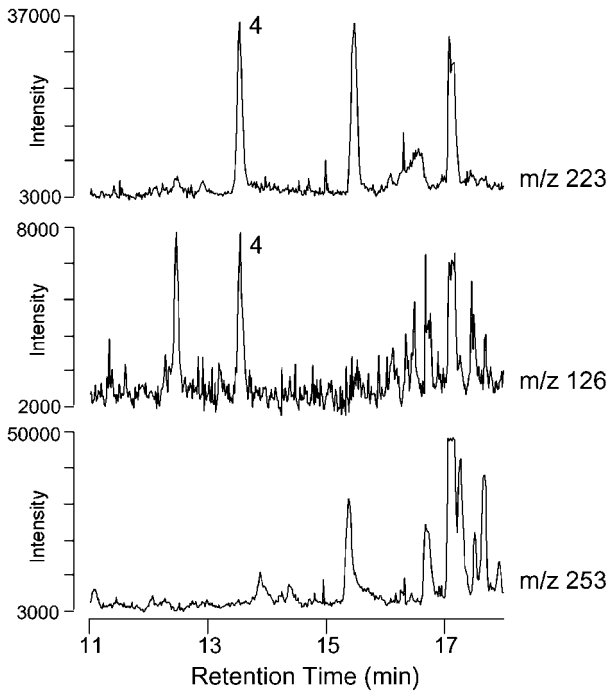

Figure 4. Chromatograms of extracts of agriculturally produced apples. Peak 4, acetamiprid at 0.02 mg/kg.

extraction. However, recoveries of nitenpyram were not always acceptable because of its high water solubility of 840 g/L (*20*). This study employed SPE to extract five neonicotinoids from the crude extract of food samples. By using SPE, water soluble nitenpyram and four other less water soluble pesticides (0.2−4.1 g/L) (*19*, *21*, *22*) were extracted in one step. Graphitized carbon is known to extract various kinds of pesticides including highly water soluble pesticides such as acephate. This study showed that its retention affinity was kept effective with the load of 20% methanol solution. Besides the stronger retaining

affinity, one advantage of choosing the graphitized carbon as the sorbent was carbon's cleanup effect. The methanol eluate after SPE was colorless or pale yellow. Food color does not necessarily mean interferences in a chromatogram; however, it might deteriorate the LC column by its adsorption.

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