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ARTICLES

Simultaneous Determination of Neonicotinoid Insecticides in Agricultural Samples by Solid-Phase Extraction Cleanup and Liquid Chromatography Equipped with Diode-Array Detection

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Effective sample pretreatment procedures based on solid-phase extraction (SPE) for multiresidue determination of seven neonicotinoid insecticides in agricultural products were investigated. After extraction with acetone and concentration, the insecticides in aqueous sample extracts were transferred into organic solvent phases with a Chem Elut SPE cartridge. Finally, the eluate from the cartridge was cleaned up with a SPE cartridge packed with graphitized carbon black and aminopropyl silica gel, which showed a higher cleanup efficiency than the classical silica gel SPE cartridge. Seven insecticides were separated on a reversed-phase C18 column and a gradient system of methanol and phosphate solution based on high-performance liquid chromatography. The established multiresidue determination has been applied to several artificially spiked agricultural samples, with the result that the average recoveries were excellent, with the exception of nitenpyram. The limit of detection of the method ranged from 0.01 to 0.03 mg/kg for the insecticides.

KEYWORDS: Neonicotinoid insecticides; pesticide residue analysis; solid-phase extraction (SPE); HPLC; diode-array detection; agricultural products

INTRODUCTION

Current analytical methods for pesticide residues in agricultural and environmental matrices are entirely based on chromatographic techniques, which are typically gas chromatography (GC) and high-performance liquid chromatography (HPLC) as shown in many reports (*1*−*5*). As is well-known, each chromatographic method could be accurately amenable to various pesticides at trace levels in complex matrices by making the best use of the characteristics of the detectors. Moreover, the analytical methods using GC or HPLC coupled to mass spectrometry (MS) or tandem MS (MS/MS) have been applied to pesticide residue analyses in various matrices (*1*, *3*−*7*). The sample pretreatment procedures prior to determination are important keys to accurately determine residual pesticides with the above-mentioned techniques. The procedures are roughly classified into three steps: (i) extraction, (ii) transfer into organic solvent phase (re-extraction) and concentration, and (iii) final cleanup. If these are not adequately accomplished, satisfactory results may not be obtained while using chromatographic methods.

Here, a new multiresidue determination for seven neonicotinoid insecticides, acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid, and thiamethoxam (**Figure 1**), in several agricultural products based on HPLC equipped with a diode array detector (DAD) is proposed. These neonicotinoid insecticides are mainly determined with HPLC as described in several reports (*3*, *5*, *8*). Recently, a residue analysis for three neonicotinoid insecticides based on HPLC/DAD has been proposed by Obana et al. (*8*). Moreover, several residue analyses for the insecticides in agricultural samples (*3*, *5*), honey samples (*9*), and drinking water samples (*10*) with HPLC/MS have been developed. On the other hand, enzyme-linked immunosorbent assays (ELISAs) for imidacloprid and acetamiprid in agricultural and processed food samples also have been evaluated by validating with HPLC as a rapid and simple screening method. The reports concluded that the ELISAs could be suitable for a rapid and simple screening method for a single neonicotinoid insecticide residue in the samples (*11*−*14*).

Although HPLC/MS or HPLC/MS/MS techniques for multiresidue determination of various pesticides have been frequently used as demonstrated in several reports (*3*, *5*, *7*, *9*, *10*), the instrumentation used is fairly expensive and may not yet be available as a routine analytical method in a common analytical laboratory for pesticide residue. On the other hand, conventional HPLC equipped with DAD or ultraviolet detection

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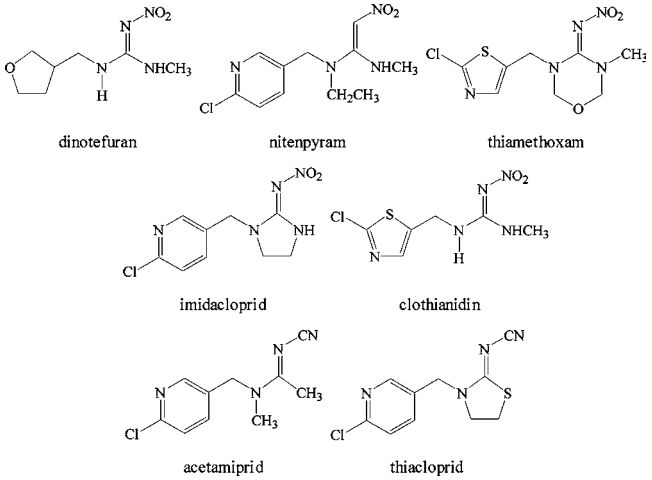

Figure 1. Chemical structures of neonicotinoid insecticides studied in the report.

is low cost (as compared to HPLC/MS or HPLC/MS/MS) and uses quite common instrumentation. However, the sensitivity and the specificity of these techniques are generally lower than those of HPLC/MS or HPLC/MS/MS. Thus, it is essential to discreetly investigate sample pretreatment procedures prior to conventional HPLC methods. Currently, solid-phase extraction (SPE) procedures have been well-established in multiresidue determinations for various pesticides (*1*−*5*, *8*−*10*) and have shown higher cleanup efficiencies and smaller organic solvent consumptions than classical methods (such as liquid−liquid partition or column chromatography). Historically, two kinds of SPE cartridges with a primary secondary amine (PSA) sorbent and a classical silica gel have been applied to sample pretreatment procedures for three neonicotinoid insecticides in agricultural samples prior to the determination with HPLC/DAD by Obana et al. (*8*).

This report summarizes effective sample pretreatment procedures based on SPE using diatomaceous earth material and with graphitized carbon black and aminopropyl silica gel to effectively eliminate matrix components coming from agricultural samples, and we validate this method using several artificially spiked samples of different agricultural commodities in establishing a multiresidue determination of the neonicotinoid insecticides based on conventional HPLC/DAD. This research represents a first trial on the development of the method covering major neonicotinoid insecticides.

MATERIALS AND METHODS

Chemicals, Materials, and Samples. Neonicotinoid standards and pesticide residue analysis-grade and HPLC-grade organic solvents were supplied from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and Kanto Chemical Co., Inc. (Tokyo, Japan). HPLC-grade water was produced with a Milli-Q water purification system (Millipore, Bedford, MA). A stock standard solution of 1 mg/mL of each insecticide was prepared in methanol. Working standard solutions were prepared by diluting the stock solution with methanol. Both solutions were stored at 4 °C. Chem Elut SPE cartridges packed with diatomaceous earth material were purchased from Varian (Harbor City, CA). Envi-Carb/NH_2 SPE cartridges (500 mg of graphitized carbon black and 500 mg of aminopropyl silica gel), Sep-Pak Florisil SPE cartridges (1 g of Florisil), and Sep-Pak silica gel SPE cartridges (1 g of silica gel) were from Supelco (Bellefonte, PA) and Waters Co. (Milford, MA), respectively.

Agricultural samples were purchased from a local market. After confirmation with HPLC analysis that they did not contain the target insecticides, each matrix was used for each experiment. For the recovery portion of the study, an aliquot of working standard solution at 10 μg/mL was spiked to 20 g of homogenized sample matrix at concentrations of 0.1 and 1.0 mg/kg. The spiked samples were exposed to the neonicotinoid insecticides overnight prior to extraction and were maintained at 4 °C in the dark.

Extraction and Cleanup Procedures. Whole agricultural samples were chopped and homogenized with a home food processor (National MK-K78, Matsushita Electric Industrial Co., Ltd., Osaka, Japan). A 20 g aliquot of the homogenized sample was weighed into an Erlenmeyer flask. Neonicotinoid insecticides were extracted with 100 mL of acetone by vigorously shaking for 30 min at about 300 rpm with a reciprocating shaker (Recipro Shaker SR-2s, Taitec, Saitama, Japan). The sample mixture was then filtered through a Büchner funnel (60 mm in diameter) containing an adequate amount of diatomaceous earth on filter paper, and the residue cake on the funnel was washed with an additional 50 mL of acetone. The extract was concentrated to about 70 mL with a rotary evaporator (temperature of water bath, 30 °C), and then, the extract was made up to 100 mL with acetone in a graduated cylinder. A 50 mL aliquot of the extract, equivalent to 10 g of sample, was concentrated to about 15 mL, and then, the aqueous extract was applied to a Chem Elut SPE cartridge. After it stood for 10 min, the cartridge was washed with 80 mL of *n*-hexane, and then, the neonicotinoid insecticides were eluted with 100 mL of dichloromethane. The eluate was concentrated to about 1 mL and then evaporated to dryness by a gentle nitrogen stream. The residue was reconstituted with 2 mL of acetonitrile/toluene (3:1), and the solution was applied to an Envi-Carb/NH_2 SPE cartridge preconditioned with 20 mL of acetonitrile/toluene (3:1). The neonicotinoid insecticides were eluted with 20 mL of acetonitrile/toluene (3:1). The eluate was concentrated to about 1 mL and then evaporated to dryness by a gentle nitrogen stream. The residue was reconstituted with 2 mL of methanol, and then, the solution was filtered with a PTFE membrane syringe-driven filter unit (0.45 μm, Millipore, Billerica, MA).

Studies of Extraction Efficiency, Transfer Efficiency, and Selection of SPE Cartridge. *Extraction Efficiency.* Each insecticide of 20 μg was spiked with 20 mL of Milli-Q water in a separatory funnel. The water sample was mixed with 100 mL of each tested extractant (acetone, acetonitrile, or ethyl acetate), and then, the solution was vigorously shaken for 30 min. After the addition of 5 g of sodium chloride for salting out, the mixture was vigorously shaken for 10 min. The obtained organic solvent phase was concentrated to near dryness, and then, the residue was reconstituted with 20 mL of methanol. The extraction efficiency of each extractant was shown as the recovery value obtained from each organic solvent.

Transfer Efficiency. Sodium chloride solution (5%, 20 mL) spiked with 20 μg of each insecticide was extracted with each tested organic solvent [ethyl acetate, ethyl acetate/*n*-hexane (1:1), or dichloromethane, 50 mL × 2] by shaking for 5 min. The collected organic solvent phase was dehydrated with a suitable amount of anhydrous sodium sulfate and then concentrated to near dryness. The residue was reconstituted with 5 mL of methanol. In a method with a Chem Elut SPE cartridge, 15 mL of Milli-Q water spiked with the same amounts of insecticides was subjected to the cartridge. After the cartridges stood for 10 min and were washed with 80 mL of *n*-hexane, the bound insecticides were eluted with each tested organic solvent. The collected organic solvent phase was concentrated to near dryness, and then, the residue was reconstituted with 5 mL of methanol. The transfer efficiency was shown as the recovery value obtained from each organic solvent.

Selection of SPE Cartridge for Cleanup. For Florisil and silica gel SPE cartridges, 2 mL of acetone/*n*-hexane (2:8) spiked with 20 μg of each insecticide was subjected to each cartridge preconditioned with 10 mL of acetone/*n*-hexane (2:8). After the bound insecticides were eluted with 10 mL of each elution solvent, the eluates were concentrated to near dryness, and then, the residue was reconstituted with 5 mL of methanol. For an Envi-Carb/NH_2 SPE cartridge, 2 mL of acetonitrile/toluene (3:1) spiked with the same amounts of insecticides was subjected to the cartridge preconditioned with 20 mL of acetonitrile/toluene (3:1). After the bound insecticides were sequentially eluted with the same solvent (10 mL × 3), each eluate was concentrated to near dryness, and then, the residue was reconstituted with 5 mL of methanol.

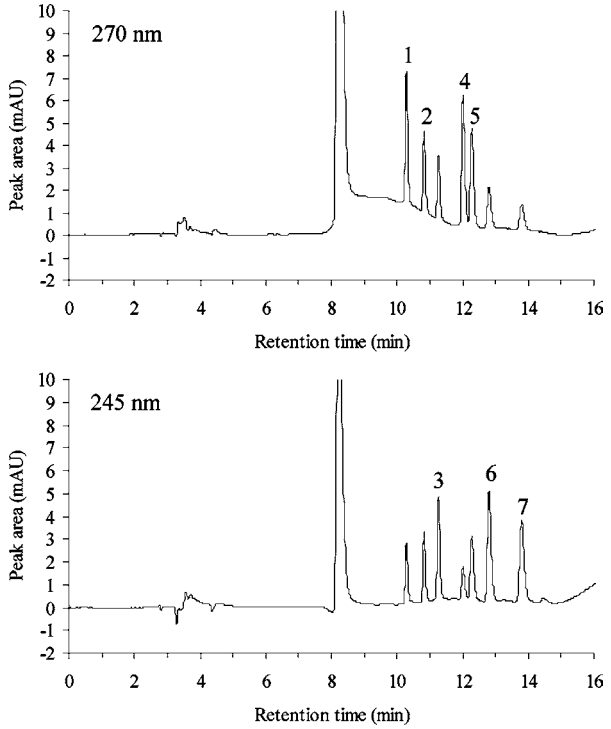

Figure 2. Typical chromatogram of standard solution (0.5 mg/kg). The numbered peaks are as follows: 1, dinotefuran; 2, nitenpyram; 3, thiamethoxam; 4, imidacloprid; 5, clothianidin; 6, acetamiprid; and 7, thiacloprid. For chromatographic conditions, see the text.

Instrumentation. The HPLC system consisted of an Agilent 1100 series equipped with a quaternary pump, an autosampler, a column oven, and a DAD. Analytical separations for the insecticides were achieved on Agilent Zorbax Extend-C18 column (5 μm, 250 mm $\times$ 4.6 mm i.d.) at 40 °C. The mobile phase used was a gradient system of methanol and 50 mM KH_2PO_4 solution (pH 4.5) in which the percentage of methanol was changed as follows: 0 min, 5%; 3 min, 5%; 7 min, 50%; 10 min, 50%; 15 min, 5%; and 21 min, 5%. The flow rate was 0.8 mL/min. The detections were performed at 270 nm for dinotefuran, nitenpyram, imidacloprid, and clothianidin and at 245 nm for thiamethoxam, acetamiprid, and thiacloprid, respectively. The injection volume was 10 μL.

RESULTS AND DISCUSSION

HPLC Performance. Analytes were separated as shown in **Figure 2** using a C18 reversed-phase column as described previously. A gradient elution profile starting at 5% methanol was required to maintain the peak shape of the highly water-soluble analytes, dinotefuran and nitenpyram [log $P_{o/w}$ −0.644 and −0.66, respectively (*15*)], as suggested by Obana et al. (*8*).

Under the chromatographic conditions described, the calibration graphs were constructed by plotting peak areas vs concentrations. Excellent linearity and coefficients of regression (r) were achieved for the seven insecticides as given in **Table 1**. The limit of detection (LOD) for each insecticide was determined as the lowest concentration of each insecticide that gave a signal-to-noise ratio of 3 (*16*). This was as low as 0.01 mg/kg for imidacloprid, clothianidin, and acetamiprid, approximately 0.02 mg/kg for thiamethoxam and thiacloprid, and as high as 0.03 mg/kg for dinotefuran and nitenpyram, respectively (**Table 1**).

Extraction Efficiency. Water-miscible organic solvents such as acetone (*5*), acetonitrile (*1*, *2*, *4*, *8*), or methanol (*3*) have often been used to quantitatively extract pesticides, including neonicotinoid insecticides, from agricultural samples. On the other hand, the methods in which extraction and dehydration are carried out together by using ethyl acetate or dichloromethane and a dehydrator such as anhydrous sodium sulfate have also been offered (*6*, *7*). At the first stage, the most suitable extractant in three common organic solvents (acetone, acetonitrile, and ethyl acetate) was selected based on the extraction efficiency for the neonicotinoid insecticides. As shown in **Table 2**, although a high water-soluble nitenpyram was just not extracted with ethyl acetate, the other two extractants showed a quantitative extraction efficiency for all insecticides including nitenpyram. Finally, acetone was selected as the best extractant because it was easier to evaporate due to its low boiling point (56 °C) that was more amenable to subsequent cleanup procedures than acetonitrile (81−82 °C).

Elution Profiles of SPE for Cleanup. As described above, pesticides in the aqueous sample extract must be transferred

Table 1. Analytical Data: Equation of the Calibration Curve, Linearity, Coefficient of Regression (r), Retention Time, and LOD for the Neonicotinoid Insecticides

pesticide	equation of calibration curve	linearity (mg/kg)	r	retention time (min)	LOD (mg/kg)
dinotefuran	$y = 31.571x + 0.0413$	0.04-2	1.0000	10.2	0.03
nitenpyram	$y = 19.723x + 0.0741$	0.04-2	1.0000	10.8	0.03
thiamethoxam	$y = 24.888x + 0.4675$	0.03-2	0.9999	11.2	0.02
imidacloprid	$y = 38.633x + 0.036$	0.02-2	1.0000	12.0	0.01
clothianidin	$y = 31.437x + 0.0808$	0.02-2	0.9999	12.2	0.01
acetamiprid	$y = 39.032x + 0.2573$	0.02-2	0.9999	12.8	0.01
thiacloprid	$y = 34.147x + 0.0304$	0.03-2	0.9999	13.8	0.02

Table 2. Extraction Efficiency of Extractants for Multiresidue Determination of Neonicotinoid Insecticides

	acetone		acetonitrile		ethyl acetate	
	extraction efficiency (%)	CV (%)	extraction efficiency (%)	CV (%)	extraction efficiency (%)	CV (%)
dinotefuran	94.5 ± 0.7	0.7	95.0 ± 0.6	0.6	79.7 ± 0.5	0.6
nitenpyram	85.3 ± 0.3	0.3	91.0 ± 0.4	0.4	not extracted	
thiamethoxam	99.2 ± 1.0	1.0	98.6 ± 1.4	1.4	97.5 ± 1.3	0.8
imidacloprid	96.7 ± 1.7	1.8	97.2 ± 0.4	0.4	97.9 ± 2.0	2.0
clothianidin	95.1 ± 0.9	0.9	79.0 ± 0.5	0.7	94.0 ± 1.4	1.5
acetamiprid	97.0 ± 1.1	1.2	97.8 ± 0.2	0.3	95.4 ± 0.8	0.9
thiacloprid	94.9 ± 1.9	2.0	97.0 ± 1.3	1.4	94.2 ± 0.9	0.9

Table 3. Transfer Efficiency to Organic Solvent Phase with Liquid–Liquid Partition and Chem Elut SPE Cartridge

	ethyl acetate		ethyl acetate/ *n*-hexane (1:1)		dichloromethane	
	transfer efficiency (%)	CV (%)	transfer efficiency (%)	CV (%)	transfer efficiency (%)	CV (%)
(a) liquid–liquid partition						
dinotefuran	76.3 ± 0.7	0.9	18.0 ± 1.3	7.2	89.5 ± 2.2	2.4
nitenpyram	4.7 ± 0.3	6.6	1.5 ± 0.1	3.4	7.4 ± 0.2	2.0
thiamethoxam	97.6 ± 4.9	5.0	57.1 ± 4.8	8.3	98.4 ± 2.0	2.1
imidacloprid	101.1 ± 0.6	0.6	86.9 ± 2.9	3.3	98.1 ± 2.3	2.3
clothianidin	94.8 ± 1.6	1.7	84.5 ± 5.7	6.8	97.1 ± 1.7	1.8
acetamiprid	33.0 ± 0.8	2.3	65.3 ± 4.1	6.3	97.2 ± 3.2	3.3
thiacloprid	24.9 ± 1.2	4.7	77.2 ± 5.5	7.1	97.1 ± 2.1	2.2
(b) Chem Elut SPE cartridge						
dinotefuran	89.9 ± 3.8	4.3	14.8 ± 0.7	5.0	95.4 ± 1.6	1.7
nitenpyram	49.5 ± 4.4	8.9	8.0 ± 0.8	10.4	94.6 ± 2.2	2.3
thiamethoxam	99.5 ± 5.2	5.2	70.3 ± 1.3	1.9	95.7 ± 2.7	2.8
imidacloprid	96.3 ± 2.1	2.2	97.1 ± 0.5	0.5	96.6 ± 2.4	2.5
clothianidin	96.9 ± 1.9	2.0	97.8 ± 0.7	0.7	96.7 ± 1.6	1.6
acetamiprid	95.3 ± 2.6	2.7	95.3 ± 0.6	0.7	96.5 ± 2.9	3.0
thiacloprid	95.4 ± 1.5	1.6	95.9 ± 0.9	0.9	95.4 ± 1.6	1.6

Table 4. Elution Profiles of Neonicotinoid Insecticides from Three SPE Cartridges

elution solvent (10 mL each)	dinotefuran	nitenpyram	thiamethoxam	imidacloprid	clothianidin	acetamiprid	thiacloprid
			Florisil SPE cartridge				
acetone/*n*-hexane (2:8)	NE[a]	NE	NE	NE	NE	NE	NE
acetone/*n*-hexane (3:7)	8.1	NE	NE	33.0	69.0	27.3	40.7
acetone/*n*-hexane (4:6)	94.5	NE	NE	103.5	104.0	103.7	101.8
acetone/*n*-hexane (5:5)	100.5	NE	13.3	104.3	103.1	104.6	102.0
acetone/*n*-hexane (6:4)	103.0	NE	64.0	105.3	105.0	104.4	102.9
acetone/*n*-hexane (7:3)	101.4	NE	97.5	103.7	103.6	103.6	102.0
acetone/*n*-hexane (8:2)	101.2	NE	103.8	105.0	103.3	105.1	103.9
acetone/*n*-hexane (9:1)	101.5	NE	103.5	104.6	104.4	103.6	104.5
acetone (first)	100.4	NE	102.9	103.6	103.5	103.7	103.1
acetone (second)	0.6	4.6	NE	NE	NE	NE	NE
acetone (third)	NE	23.9	NE	NE	NE	NE	NE
			silica gel SPE cartridge				
acetone/*n*-hexane (2:8)	NE	NE	NE	NE	NE	NE	NE
acetone/*n*-hexane (3:7)	NE	NE	NE	NE	8.1	NE	1.2
acetone/*n*-hexane (4:6)	101.3	NE	54.5	103.4	93.5	103.5	102.0
acetone/*n*-hexane (5:5)	100.3	NE	95.0	101.1	100.9	100.8	101.8
acetone/*n*-hexane (6:4)	102.1	36.3	102.4	101.0	101.7	101.1	102.3
acetone/*n*-hexane (7:3)	101.1	88.5	102.7	101.1	100.3	95.9	101.4
acetone/*n*-hexane (8:2)	100.5	95.6	100.2	99.1	99.1	98.6	100.3
acetone/*n*-hexane (9:1)	102.1	100.0	101.6	101.8	100.7	101.6	104.3
acetone (first)	106.0	102.8	108.6	105.1	105.0	102.6	106.2
acetone (second)	NE	NE	NE	NE	NE	NE	NE
acetone (third)	NE	NE	NE	NE	NE	NE	NE
			Envi-Carb/NH_2 SPE cartridge				
acetonitrile/toluene (3:1) (first)	96.3	90.7	92.8	89.5	91.9	94.3	95.2
acetonitrile/toluene (3:1) (second)	0.6	1.0	0.6	0.8	0.2	0.7	1.1
acetonitrile/toluene (3:1) (third)	NE	NE	NE	NE	NE	NE	NE

[a] Not eluted.

into low-boiling medium-polarity organic solvents such as ethyl acetate or a mixture of ethyl acetate and *n*-hexane to remove water and water-soluble coextractives. Mainly, SPE using diatomaceous earth materials (*5*, *9*, *17*, *18*) or classical liquid−liquid partition (*19*) has been used in this procedure, that is, re-extraction. In this stage, the transfer efficiency based on these methods was investigated by using three kinds of extraction solvents as eluates. As given in **Table 3**, liquid−liquid partition based on ethyl acetate or ethyl acetate/*n*-hexane (1:1) showed an unsatisfactory extraction efficiency for most neonicotinoid insecticides, and dichloromethane showed the best efficiency to all neonicotinoid insecticides except for nitenpyram. On the other hand, when using a SPE cartridge packed with diatomaceous earth materials (Chem Elut SPE cartridge), dichloromethane showed the best efficiency in three kinds of extraction solvents and was the only extractant for nitenpyram, which can quantitatively extract it from aqueous sample extract. Although classical liquid−liquid partition has some disadvantages such as the large amount of organic solvent consumed, the formation of emulsions, and the extensive time-consuming cleanup procedures (*20*), SPE eliminates the above disadvantages of liquid−liquid partition. So, effective and convenient Chem Elut SPE cartridges and dichloromethane as a best quantitative eluate for the insecticides have been applied to the re-extraction procedure.

The eluate from the Chem Elut SPE cartridge contains target insecticides and various hydrophobic co-extractives such as colored matters or lipids coming together from agricultural samples. So, the eluate should be cleaned up with SPE cartridges packed with various materials (*1*−*4*, *8*, *18*) or classical column chromatography based on normal-phase mode (*19*). Nowadays, because SPE has been offered as an effective cleanup procedure, the method has been applied to the multiresidue determination for the insecticides in this report. When selecting the most suitable SPE cartridge for our purpose, classical SPE cartridges based on normal-phase mode, Florisil and silica gel, and a two-phase type SPE cartridge based on graphitized carbon black and aminopropyl silica gel (Envi-Carb/NH_2 SPE cartridge) have been investigated. The behavior of the insecticides on these SPE cartridges has been investigated by using a mixture of acetone and *n*-hexane with various mixture ratios and pure acetone for Florisil and silica gel SPE cartridges and acetonitrile/toluene (3:1) recommended by the manufacturer for an Envi-Carb/NH_2 SPE cartridge, respectively.

As given in **Table 4**, when the concentration of acetone in *n*-hexane was more than 60%, all insecticides were quantitatively eluted from a Florisil SPE cartridge except for nitenpyram. Nitenpyram was hardly eluted from it, although a more polar solvent such as pure acetone was used. On the other hand, a silica gel SPE cartridge gave ideal quantitative elution profiles for all insecticides by applying an eluate containing more than 60% acetone. However, the elution profiles from silica gel SPE cartridge differed from the result suggested by Obana et al. (*8*) in which nitenpyram was eluted with pure acetone because of unsatisfied elution with a mixture of acetone and *n*-hexane.

Possibly, the disagreement of the elution profiles between both results might be attributed to the difference of the cartridge manufacturer. Elution profiles of pesticides from SPE cartridges could fluctuate due to the difference of the manufacturers or the lots of SPE cartridges. Therefore, they should be regularly identified before use.

In our results, when applying a silica gel SPE cartridge to agricultural samples based on the optimum condition for the elution, enough cleanup efficiency for accurate HPLC analysis was not obtained because some coextractives (colored matters) were eluted with the target insecticides into each eluate (**Figure 3**). As the next experimental step, an Envi-Carb/NH_2 SPE cartridge that has an especially effective removal of colored matters was investigated. All insecticides were quantitatively recovered in first 10 mL of acetonitrile/toluene (3:1). Small

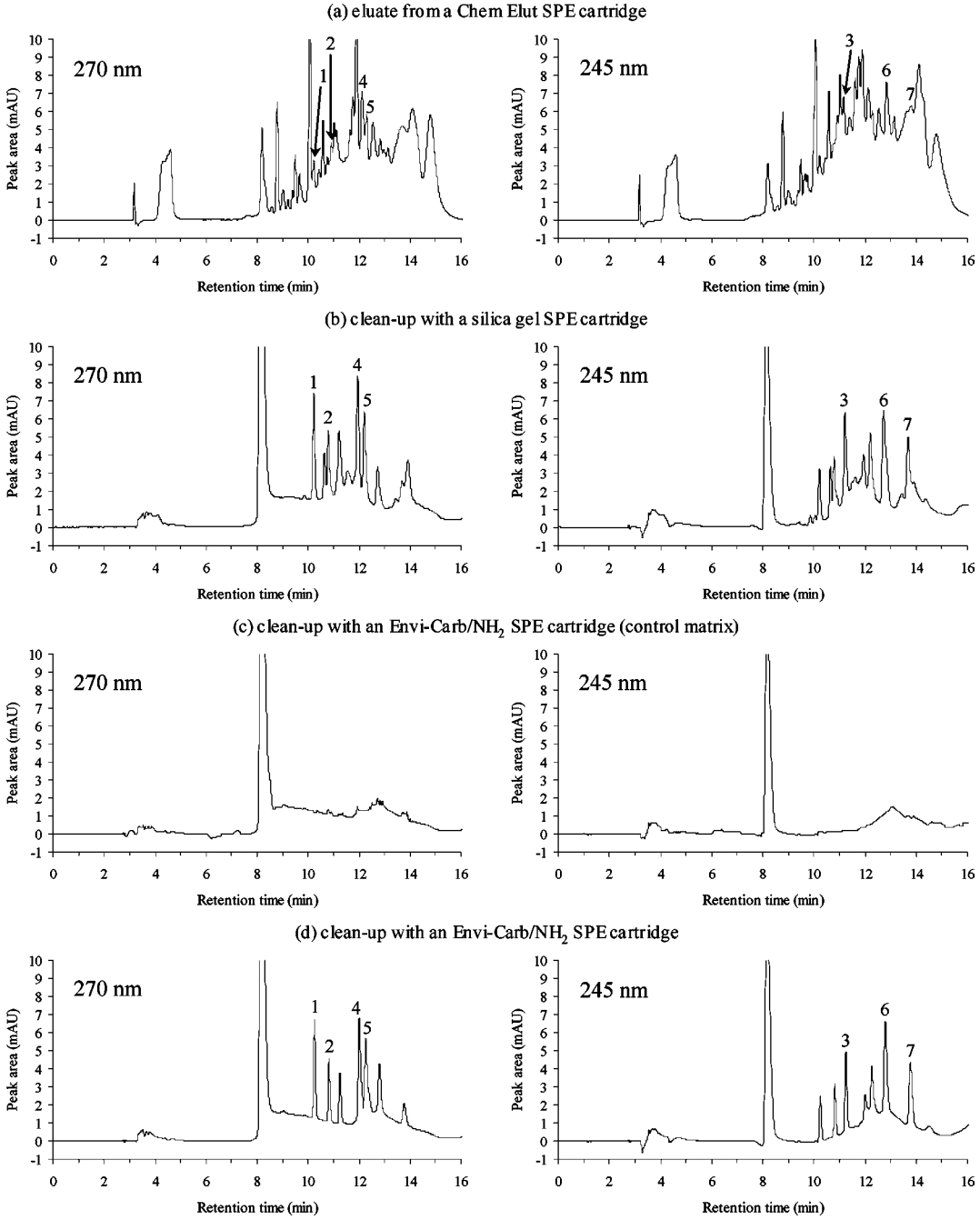

Figure 3. Typical chromatograms of artificially spiked spinach samples (spiked level, 0.1 mg/kg). For chromatographic conditions, see the text. For insecticide identification, see **Figure 2**.

amounts were also recovered in the second 10 mL of elution fraction (**Table 4**). Its cleanup efficiency was fairly higher than the one of the silica gel SPE cartridge, and no interference peak around the peak of each neonicotinoid insecticide was observed (**Figure 3**). Furthermore, although two-step procedures with PSA and silica gel SPE cartridges required achieving enough cleanup efficiency in the previous report (*8*), the one-step only procedure gave excellent removal of any coextractives by using the proposed cartridge. Therefore, the sample pretreatment procedures consisted of extraction with acetone, re-extraction with a Chem Elut SPE cartridge, and finally cleanup with an Envi-Carb/NH_2 SPE cartridge; they were used as optimum methods for the insecticides and were applied to residue analysis using several artificially spiked agricultural samples.

Application to Artificially Spiked Samples. The recovery results and the CV values obtained from analysis of fruit and vegetable samples at two spiked levels are shown in **Table 5**. The recoveries of the insecticides were very good in most cases and were independent of sample matrix and the spiked level except for nitenpyram. A good reproducibility from four repetitive determinations of recovery was also achieved for the insecticides except for nitenpyram. Although nitenpyram was quantitatively recovered in individual procedures as shown in previous sections, the recoveries from the tested agricultural samples were very low. To investigate the cause of the low recovery of nitenpyram, the recovery study based on the established sample pretreatment procedures by using water samples spiked with 0.1 and 1.0 mg/kg of seven neonicotinoid insecticides was examined. Interestingly, when analyzing the spiked water samples, nitenpyram was quantitatively recovered

Table 5. Recoveries from Artificially Spiked Agricultural Samples ($n = 4$ Replicates)

		dinotefuran		nitenpyram		thiamethoxam		imidacloprid		clothianidin		acetamiprid		thiacloprid	
sample	spiked level (mg/kg)	average recovery (%)	CV (%)	average recovery (%)	CV (%)	average recovery (%)	CV (%)	average recovery (%)	CV (%)	average recovery (%)	CV (%)	average recovery (%)	CV (%)	average recovery (%)	CV (%)
apple	0.1	97.6	2.6	14.4	13.3	94.0	2.0	92.4	1.8	103.3	2.6	94.6	4.7	75.6	1.6
	1.0	95.2	1.3	40.6	13.0	98.2	2.3	99.3	1.4	99.6	1.2	99.7	4.7	99.3	6.5
carrot	0.1	83.9	10.6	ND[a]		98.9	4.5	88.0	3.4	83.8	5.7	94.3	2.4	90.6	12.4
	1.0	91.9	4.2	18.8	7.4	93.3	1.9	88.0	2.3	85.5	2.1	97.5	2.9	73.5	8.8
cucumber	0.1	85.0	12.2	ND		98.5	13.2	83.7	2.5	84.6	1.1	97.1	2.8	115.4	16.2
	1.0	85.0	1.7	14.0	13.0	92.4	1.9	97.0	2.7	96.0	2.5	95.9	0.5	97.7	1.7
grape	0.1	87.0	5.1	11.5	11.2	90.3	4.5	84.0	4.3	83.0	9.8	81.3	11.2	78.8	8.1
	1.0	89.9	10.4	11.8	10.7	91.1	6.7	86.4	6.1	87.1	7.6	88.1	5.7	81.6	6.4
peach	0.1	83.3	10.5	ND		100.4	7.2	91.0	4.4	88.3	11.0	95.2	8.4	84.3	3.2
	1.0	106.7	9.2	34.3	18.1	102.6	2.6	91.0	2.5	102.4	3.9	99.3	2.4	88.0	3.0
sweet pepper	0.1	92.1	6.5	11.2	8.6	84.9	3.0	88.5	7.9	96.9	6.4	108.3	4.8	91.6	11.7
	1.0	81.9	9.0	12.3	12.2	87.2	5.6	89.9	3.5	89.3	4.3	98.5	4.7	87.7	4.4
spinach	0.1	94.0	11.0	22.0	15.7	80.3	9.6	92.9	6.0	85.1	5.8	87.0	5.7	102.9	6.8
	1.0	89.4	1.7	19.0	5.0	96.5	1.9	86.0	2.5	85.9	2.9	91.5	1.8	86.3	2.3
tomato	0.1	85.3	8.1	10.5	12.3	85.5	5.6	87.8	7.7	90.3	9.7	88.3	6.6	85.5	10.0
	1.0	91.6	4.3	13.0	6.3	94.3	1.1	97.8	0.3	100.1	0.3	96.3	0.7	94.3	1.8

[a] Not detected.

Table 6. Recoveries from Spiked Water Samples ($n = 4$ Replicates)

	0.1 mg/kg spiked water		1.0 mg/kg spiked water	
	average recovery (%)	CV (%)	average recovery (%)	CV (%)
dinotefuran	90.9 ± 5.0	5.5	91.9 ± 4.2	4.6
nitenpyram	81.1 ± 4.9	6.0	88.5 ± 4.7	5.3
thiamethoxam	94.9 ± 4.1	4.4	93.4 ± 3.8	4.0
imidacloprid	96.3 ± 4.7	4.9	94.5 ± 3.6	3.8
clothianidin	91.9 ± 5.5	6.0	94.4 ± 3.6	3.9
acetamiprid	95.9 ± 2.4	2.4	92.8 ± 3.8	4.0
thiacloprid	91.2 ± 3.5	3.8	91.2 ± 2.9	3.2

from both samples (>81%) although the same sample pretreatment procedures as those for the spiked agricultural samples were used (**Table 6**). Next, the loss of nitenpyram in re-extraction procedure with a Chem Elut SPE cartridge and cleanup procedure with an Envi-Carb/NH_2 SPE cartridge was investigated using a spinach sample spiked with 0.1 mg/kg nitenpyram on the eve of subject to each cartridge. Consequently, although there was no loss of nitenpyram in the cleanup procedure (average recovery = 94.5%, CV = 1.9%, and n = 3), a significant loss of it was observed in the re-extraction procedure (average recovery = 59.6%, CV = 1.0%, and n = 3). On the other hand, no significant degradation of nitenpyram due to incubation time (2 and 24 h) at 4 °C prior to extraction was observed (data not shown). Hence, nitenpyram may be susceptible to decomposition by matrix components, especially in a re-extraction procedure with a Chem Elut SPE cartridge.

In conclusion, this work shows that it is essential to select the most suitable sample pretreatment procedures prior to conventional HPLC analysis for complex agricultural samples. With the multiresidue determination developed in the present report, an effective sample pretreatment procedure using a Chem Elut SPE cartridge and an Envi-Carb/NH_2 SPE cartridge was accomplished, and all neonicotinoid insecticides were successfully separated on a reversed-phase C18 analytical column based on gradient systems of methanol and phosphate solutions. However, it was impossible to quantitatively determine nitenpyram in the complex agricultural samples.

The major factor causing very low recoveries may be due to loss in the re-extraction procedure with a Chem Elut SPE cartridge because nitenpyram coexists with various coextractives; the insecticide could be decomposed by them over the elution with dichloromethane, which is suitable for the quantitative elution.

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