Determination of Acetamiprid, Imidacloprid, and Nitenpyram Residues in Vegetables and Fruits by High-Performance Liquid Chromatography with Diode-Array Detection

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Determination of 3 neonicotinoid insecticides, nitenpyram, imidacloprid, and acetamiprid, was studied. Vegetables and fruits were extracted with acetonitrile. The crude extract was passed through a weak anion-exchange cartridge (PSA). The effluent was subjected to silica gel cartridge. Imidacloprid and acetamiprid were eluted with 10 mL of 4:6 (v/v) acetone/hexane, followed by nitenpyram with acetone (20 mL). Pesticides were determined by HPLC with a C-18 column and diode-array detection system. Imidacloprid and acetamiprid were recovered at about 90% at the spike levels with 0.2 and 2 mg/kg in cucumber, potato, tomato, eggplant, Japanese radish, and grape. Nitenpyram was recovered at 64−80%. Relative standard deviations were less than 10% throughout all the recovery tests. In the residue analysis, agriculturally incurred pesticides at 0.08−0.14 mg/kg were designated with UV spectra compared with respective reference standards.

KEYWORDS: Neonicotinoid; insecticides; nitenpyram; imidacloprid; acetamiprid; HPLC; diode-array; pesticide; food

INTRODUCTION

The neonicotinoids, which are also called neonicotinyls, chloronicotines, and chloronicotinyls, are a relatively new class of insecticides with a distinct mode of action (*1*). The structures of 3 neonicotinoids, nitenpyram, imidacloprid, and acetamiprid, which were introduced in Japan during the 1990s, are shown in **Figure 1**. Sales of these three neonicotinoids were $76.7 million in 1998 and accounted for 6.8% of the total insecticides distributed in Japan (*2*).

Single-analyte methods for the three pesticides have been developed. Tsumura determined nitenpyram in foods with an HPLC−UV system (*3*). The method consisted of acetone extraction, diatomaceous earth column, and silica gel column chromatography. Nitenpyram was eluted from a diatomaceous earth column with dichloromethane. The use of dichloromethane in the method is not favorable because of environmental concerns. Fernandez-Alba determined imidacloprid in foods by HPLC−UV and confirmed them by LC−MS (*4*). Imidacloprid was extracted with acetone and transferred to the mixture of dichloromethane and petroleum ether to reduce interferences. Navalon analyzed imidacloprid residues in vegetables after hydrolysis for GC−MS determination (*5*). Acetamiprid in foods was determined by GC−NPD in a Japanese official method (*6*). Sasaki pointed out that acetamiprid, a highly polar compound, might cause overestimated values with GC determination because of the matrix-induced enhancement effect (*7*).
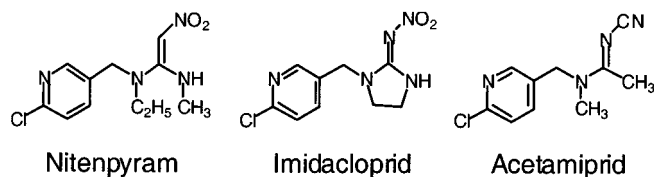

Figure 1. Chemical structures of nitenpyram, imidacloprid, and acetamiprid.

This study reports the development of multiresidue analysis of 3 neonicotinoid pesticides of nitenpyram, imidacloprid, and acetamiprid in foods with an HPLC−UV diode-array detection system. The aim of our work is to develop a routine method which preferably extends the current conventional multiresidue methods (*8, 9*) to incorporate neonicotinoid pesticides into the regular monitoring program. Chlorinated solvents (e.g., chloroform and dichloromethane) used in the individual method for residue analysis (*3−5*) were avoided as an extraction solvent. HPLC was chosen for their analysis because nitenpyram and imidacloprid are nonvolatile, and to avoid matrix effect on GC quantitation of acetamiprid.

MATERIALS AND METHODS

Reagents. Methanol was HPLC grade, and acetone and hexane were pesticide analysis grade (Wako Pure Chemical Ind., Osaka, Japan). Pesticide standards of acetamiprid, imidacloprid, and nitenpyram and other reagents were analytical grade (Wako). 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

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make spiking mixture and working standard solutions. Standard solutions were stored at 4 °C in the dark.

Materials. The PSA used was Bond Elut PSA, 0.5 g/3 mL (Varian, Harbor City, CA). The sorbent was rinsed with 10 mL of acetone, followed by 10 mL of 5:5 (v/v) acetone/hexane for conditioning. Hereafter, all the solvent ratios are expressed in (V/V). The silica gel used was Mega Bond Elut SI, 1 g/6 mL (Varian). The sorbent was rinsed with 10 mL of acetone, followed by 10 mL of 3:7 acetone/ hexane for conditioning. The water absorbent polymer was Aquapearl A3, a polymer of acrylic acid. It is an industrial product from Mitsubishi Chemical Industry Ltd. (Tokyo, Japan). The details of this polymer have been described elsewhere (*8*).

Food Commodities. Cucumber, potato, tomato, eggplant, Japanese radish, and grape for recovery tests were obtained in Osaka. About 500 g of roughly chopped sample from 5 to 10 individual commodities were chopped in a conventional food processor for 5 min to obtain thoroughly mixed homogenates. Incurred tomatoes, containing acetamiprid, were found during the analysis of samples presumably free of pesticides for recovery tests during the method development. Pesticide-containing cucumber and eggplant, which were agriculturally incurred, were obtained from a neighboring farmer.

HPLC. HPLC analysis was carried out with a Shimadzu CLASS-LC10 system (Shimadzu, Kyoto, Japan) equipped with a SPD-M10Avp photodiode-array detector in the range of 200−300 nm. HPLC separation was conducted using a Cadenza CD-C18 (3 μm particle size) column (75 × 4.6 mm i.d.) (Imtakt, Kyoto, Japan). Mobile phase was a gradient elution of methanol/0.05 M KH_2PO_4 solution 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%; 30−35 min, 5%, at the flow rate of 0.8 mL/min. The column temperature was maintained at 50 °C. An aliquot of 10 μL was injected with SIL-10A auto sampler.

Extraction. Sample (20 g) was extracted with acetonitrile (100 mL) for 2 min with a Polytron-type homogenizer. The extract, with a paper filtration, was transferred to a 200-mL separatory funnel. Sodium chloride (5 g) was added, and the solution was shaken with a mechanical shaker for 1 min to salt out the water layer. An aliquot of the extract (50 mL), equivalent to 10 g of sample, was collected.

Cleanup. The acetonitrile extract (50 mL), in a round-bottom flask, was evaporated to near dryness, and the residue was dissolved in acetone (2 mL). The flask was washed with another 2 mL of acetone, which was combined with hexane (4 mL) to make an acetone concentration of 50%. The mixture was added to a PSA cartridge which was connected with a glass syringe (10 mL) as reservoir. A 5-mL mixture of 1:1 acetone/hexane was further used to elute the pesticides from the PSA column. The PSA elution was evaporated and dissolved in about 2 mL of 3:7 acetone/hexane. The solution was loaded onto a silica gel cartridge. The cartridge was washed with 3:7 acetone/hexane (10 mL) and the elution was discarded. An aliquot of 10 mL of 4:6 acetone/ hexane was further eluted for acetamiprid and imidacloprid collection, followed by acetone (20 mL) for nitenpyram elution. Each elution was evaporated separately and dissolved in methanol to make up to 2 mL.

Recovery Test. Recovery tests were conducted with cucumber, eggplant, grape, Japanese radish, potato, and tomato. The targeted pesticides are registered for each of these commodities in Japan. The pesticide solution (0.5 mL) was spiked to homogeneous sample (20 g) at the concentration of 0.2 mg/kg or 2 mg/kg and allowed to stand for 1 h. Five experiments were carried out individually at respective spiking levels.

Extraction Studies. *Extraction with Acetonitrile.* A 1-mL aliquot of 3-pesticides-mixture solution (100 μg/mL each) was added to 20 mL of water in a separatory funnel. The water was thoroughly mixed with acetonitrile (100 mL) and then the solution, after which sodium chloride (5 g) was added, and the mixture was shaken to salt out the water layer. The acetonitrile layer (50 mL) was collected and concentrated to near dryness with a rotary evaporator. The residue was dissolved in methanol to make up to 5 mL.

Extraction with Ethyl Acetate and Polymer (8). The water-absorbent polymer (3 g) was put into the pesticide-containing solution (20 mL) (described above) to absorb water. Ethyl acetate (100 mL) was added to the mixture and vigorously extracted for 2 min with a Polytron-type

Table 1. Comparison of Extraction Procedures

	recoveries (%)[a] of pesticides					
	nitenpyram		imidacloprid		acetamiprid	
procedure	mean	%RSD	mean	%RSD	mean	%RSD
acetonitrile	82	5	93	2	92	3
polymer	61	5	100	3	98	4
ethyl acetate	27	4	95	1	93	1

[a] Mean of 3 replicates.

homogenizer. Ethyl acetate extract (50 mL) was collected, evaporated, and made up to 5 mL with methanol.

Extraction with Ethyl Acetate. One milliliter of the 3-pesticides-mixture solution (100 μg/mL each) was added to 50 mL of 10% sodium chloride solution in a 200-mL separatory funnel. Ethyl acetate (100 mL) was added to the solution, which was vigorously shaken for 5 min and then allowed to separate into two phases. The water layer was collected into another funnel and extracted again after addition of ethyl acetate (50 mL). Both ethyl acetate layers were put together, evaporated, and made up to 5 mL with methanol.

RESULTS AND DISCUSSION

HPLC Condition. A gradient system was applied to separate 3 pesticides as independent peaks. Nitenpyram is a highly water soluble compound whose log Po/w is −0.64, whereas these values are 0.57 and 0.80 for imidacloprid and acetamiprid, respectively (*10*). HPLC elution required <5% methanol in the gradient to maintain a sharp peak of nitenpyram. Addition of KH_2PO_4 (0.05 M) in water phase and small injection volume (10 μL) were also effective in obtaining a sharp peak of nitenpyram.

Nitenpyram, imidacloprid, and acetamiprid were detected on their UV spectra at 270, 270, and 245 nm, respectively. Imidacloprid and acetamiprid showed linear calibrations from 0.05 to 10 μg/mL with correlation coefficients of 0.999 for both compounds. Nitenpyram showed linearity from 0.1 to 10 μg/ mL with a correlation coefficient of 0.999.

Extraction Studies. Three procedures for extracting pesticides from aqueous solution were compared for a method development. Recoveries are listed in **Table 1**. The acetonitrile extraction and the ethyl acetate partition method are commonly used in pesticide residue analysis. The polymer extraction was developed to extract multiresidues of pesticides in foods (*10*). The polymer extraction was particularly applicable to water soluble pesticides such as acephate and methamidofos (*11*), thus, the method was tested for highly water soluble nitenpyram.

Imidacloprid and acetamiprid were sufficiently partitioned into the extraction solvents under the tested procedures with 92 to 100% recoveries. Nitenpyram recovery was 82% with acetonitrile extraction. Polymer extraction showed 61% recovery for nitenpyram, which could not be accepted. Thus, we selected the acetonitrile method for the extraction of neonicotinoid residues.

Cleanup. The neonicotinoid pesticides were determined with a reversed-phase C18 column. We speculated that a normal phase cleanup, based on a separation mechanism different from that of C18 separation, would be effective to remove interferences from a food matrix before HPLC determination. Because Florisil and alumina absorbed nitenpyram, its elution was varied from Florisil and it was not eluted from alumina under the solvent condition for obtaining a good cleanup in the preliminary experiments (data not shown). Thus, silica gel cartridge was applied for cleanup. Elution profile of 3 pesticides with the mixture of acetone and hexane is listed in **Table 2**. Imidacloprid

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Table 2. Elution of Neonicotinoid Pesticides from Silica Gel Cartridge Column

elution mixture acetone/hexane (v/v, 10 mL)	elution rate from (%)		
	nitenpyram	imidacloprid	acetamiprid
1/9	0	0	0
2/8	0	0	0
3/7	0	1	4
4/6	0	93	91
5/5	0	93	92
6/4	0	97	97
7/3	0	93	92
8/2	48	97	96
9/1	69	86	85
10/0; first 10 mL	83	91	90
second 10 mL	5	1	1

and acetamiprid were retained when the acetone concentration was less than 3:7 in the elution mixture and completely eluted when the acetone concentration was increased to 4:6. Nitenpyram was eluted with 20 mL of acetone. It was eluted by 88% with 10 mL of acetone twice, and the additional 10 mL of acetone after 20 mL elution did not increase yield (data not shown). At first, we tried to elute the 3 pesticides together with acetone (20 mL) after washing with 3:7 acetone/hexane (10 mL). However, the obtained chromatogram was not clean enough to distinguish the 3 pesticides from matrix interferences.

Consequently, pesticide elution was separated into 3 fractions after addition of the extract. The first fraction was washing with 3:7 acetone/hexane (10 mL), which was discarded, and the second fraction was eluted with 4:6 acetone/hexane (10 mL) for imidacloprid and acetamiprid collection, followed by acetone (20 mL) for nitenpyram elution. Chromatograms of blank samples for the recovery test showed a flat baseline around respective retention time for pesticides except Japanese radish.

Additional cleanup with a different principle, which was ion-exchange cartridge of PSA, was introduced before silica gel cleanup. Three pesticides were not retained by PSA when eluted with 5:5 acetone/hexane while many interfering peaks were retained in the analysis of Japanese radish and other samples.

Before PSA cleanup, the crude extract was dissolved in acetone, followed by addition of an equal amount of hexane to make the acetone concentration 50%. This operation was effective to improve recoveries of nitenpyram which is practically insoluble in hexane. Once the targeted pesticides were dissolved in acetone, addition of hexane did not affect the solubility of nitenpyram, though insoluble matrix components were precipitated when hexane was added. PSA cleanup with 100% acetone elution was not effective to remove interferences, whereas a lower acetone concentration (<50%) made nitenpyram recoveries unstable or poor. Typical cleanup chromatograms are shown in **Figure 2**. Three pesticides, spiked at 0.2 mg/kg in Japanese radish, were detected without interruption by food matrix after PSA and silica gel cleanup.

Because food matrixes are complex, and they differ from sample to sample, a two-step cleanup was introduced to all the samples; although this extra fractionation step and second injection was an undesirable consequence of analyzing the 3 pesticides in the same method. As a result, HPLC determination was run twice in each sample: once for imidacloprid and acetamiprid determination and once for nitenpyram determination.

Recovery Test. Three pesticides were spiked at 2 levels, 0.2 or 2 mg/kg, in 6 food samples as shown in **Table 3**. Imidacloprid was sufficiently recovered in the range of 87−97% with 1−4%
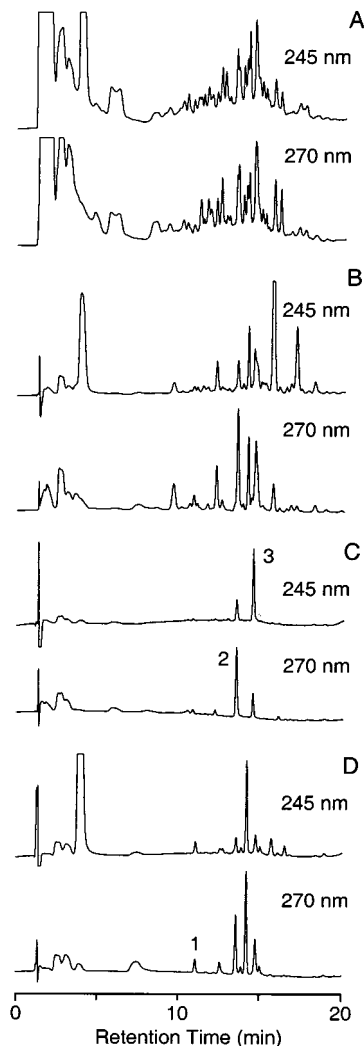
Figure 2. Chromatograms of extracts of Japanese radish spiked at 0.2 mg/kg. (A) crude extract; (B) after PSA; (C) silica 4:6 acetone/hexane elution; and (D) silica acetone elution. Peak labels: 1, nitenpyram; 2, imidacloprid; 3, acetamiprid.

Table 3. Recovery Studies of Neonicotinoid Pesticides

sample	spike level (mg/kg)	nitenpyram		imidacloprid		acetamiprid	
		mean[a] (%)	RSD (%)	mean[a] (%)	RSD (%)	mean[a] (%)	RSD (%)
cucumber	0.2	64	4	91	2	99	3
	2	75	5	94	1	95	1
potato	0.2	69	6	89	1	91	4
	2	80	1	87	2	88	2
tomato	0.2	74	4	90	4	86	5
	2	80	1	87	2	88	2
eggplant	0.2	69	7	90	1	84	9
	2	72	2	95	3	89	8
Japanese radish	0.2	76	2	99	2	87	2
grape	0.2	75	8	89	3	87	2
average		74	4	91	2	88	4

[a] Average of 5 replicates.

of RSD at 2 spiked levels. Acetamiprid was also sufficiently recovered in the range of 84−99% with 1−9% of RSD. There seems no bias in sample verifications and spiked levels for recoveries of imidacloprid and acetamiprid. Nitenpyram was recovered more than 70% when spiked at 2 mg/kg with less
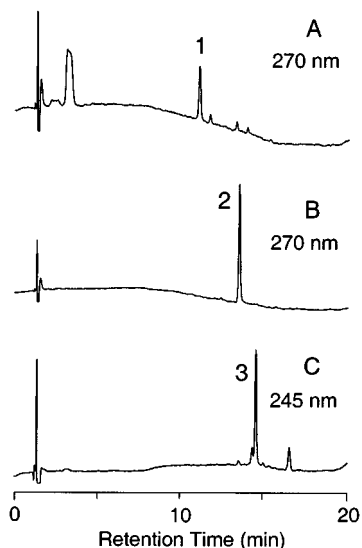
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Figure 3. Chromatograms of extracts of agriculturally incurred foods. (A) 1, nitenpyram in eggplant at 0.08 mg/kg; (B) 2, imidacloprid in cucumber at 0.14 mg/kg; (C) 3, acetamiprid in tomato at 0.12 mg/kg.
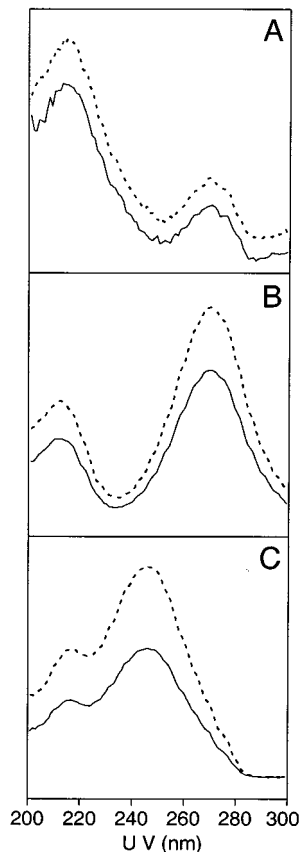

Figure 4. UV absorption spectra of pesticide residues found. Solid line, pesticide residues shown in **Figure 3**; dotted line, reference standards; (A) nitenpyram (0.5 μg/mL); (B) imidacloprid (1 μg/mL); (C) acetamiprid (1 μg/mL).

than 5% RSD, however its recovery was lower, ranging 64–76%, at 0.2 mg/kg with <7% RSD. Average recovery of nitenpyram was 74% throughout the test, which was more than 10% lower than those of imidacloprid and acetamiprid. The reason for low recovery of nitenpyram was not clear; however, it could be speculated that nitenpyram was lost during aceto-nitrile exaction and/or silica gel column cleanup, because its

recovery was somewhat lower than that of the other 2 pesticides in the two steps, as shown in **Tables 1** and **2**.

Residue Analysis. Agriculturally incurred samples were analyzed with the proposed method. Eggplant contained niten-pyram at 0.08 mg/kg, cucumber contained imidacloprid at 0.14 mg/kg, and tomato contained acetamiprid at 0.12 mg/kg (chromatograms are shown in **Figure 3**). The incurred pesticides were clearly detected without serious interferences. Detected pesticides were designated by respective retention times and UV spectra comparison with reference standards (**Figure 4**). From the recovery test and residue samples, LODs were defined the lowest level as those with standard calibration as serious interferences were not observed in every chromatogram. LODs are 0.02 mg/kg for nitenpyram and 0.01 mg/kg for imidacloprid and acetamiprid.

ABBREVIATIONS USED

PSA, primary/secondary amine; HPLC, high-performance liquid chromatography; C-18, octadecyl; UV, ultraviolet detection; HPLC–UV, high-performance liquid chromatography with ultraviolet detection; LC–MS, liquid chromatography with mass spectrometer; GC–MS, gas chromatography with mass spectrometer; GC–NPD, nitrogen phosphorus detector; GC, gas chromatography; RSD, relative standard deviation; LOD, limit of detection.

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