JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Evaluation and Validation of a Commercially Available Enzyme-Linked Immunosorbent Assay for the Neonicotinoid Insecticide Imidacloprid in Agricultural Samples

EIKI WATANABE,*,† HEESOO EUN,† KOJI BABA,† TOMOHITO ARAO,† YASUO ISHII,† SHOZO ENDO,† AND MASAKO UEJI§

Environmental Chemical Analysis Laboratory, Chemical Analysis Research Center, and Department of Research Planning and Coordination, National Institute for Agro-Environmental Sciences, Kannondai 3-1-3, Tsukuba, Ibaraki 305-8604, Japan

The performance of a commercially available microtiter plate ELISA kit for the determination of the neonicotinoid insecticide imidacloprid was evaluated for sensitivity, selectivity, influence of organic solvent used for extraction procedure, matrix interference originated from agricultural sample, accuracy, and method comparison with conventional HPLC analysis. The limit of detection for the kit (0.1 or 0.5 ng/mL) was determined. The working range (1−39 ng/mL) experimentally calculated on the basis of a criterion, which is determined as the range from I_{20} to I_{80}, was comparable to that established by the manufacturer (1−50 ng/mL). The linearity of the standard curve based on the kit-assembled standard solutions agreed with the one based on the self-made standard solutions. Specificity studies indicate that the imidacloprid monoclonal antibody can readily distinguish the target compound from other structurally related neonicotinoid analogues and some metabolites, with the exception of clothianidin, the cross-reactivity of which was ∼12%. To extract imidacloprid from an agricultural sample (apple) as simply and rapidly as possible, some extraction methods were examined. Consequently, the extraction method with hand-shaking for 5 min was the best among the examined methods. For the analysis of imidacloprid in apple samples, it was extracted directly with methanol and the extracts were diluted 10-fold (100-fold in the well) with water prior to ELISA analysis. No significant matrix interference was observed with the dilution factor. Recoveries of imidacloprid from fortified apple samples ranged from 87.7 to 112.0%. The results obtained with the ELISA kit correlated well with those by the reference method (conventional HPLC analysis) for apple samples ($r > 0.998$). These findings strongly indicate that the ELISA kit may be employed routinely for an on-site imidacloprid residue analysis of apple samples.

KEYWORDS: Neonicotinoid insecticide; imidacloprid; ELISA kit; validation; cross-reactivity; matrix interference

INTRODUCTION

Imidacloprid, 1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylideneamine, which was introduced under the commercial name Admire in 1991 by Bayer AG (*1*), is one of the neonicotinoid insecticides, which acts as an antagonist by binding to postsynaptic nicotinic receptors in the insect central nervous system (*1*). Its binding leads to the accumulation of acetylcholine, resulting in the paralysis and death of insects. On the basis of its action, the insecticide is used for the control of sucking insects, including aphids, whiteflies, thrips, scales, psyllids, plant bugs, leafhoppers, plant hoppers, and other various harmful pest species including resistant strains. Due to its high insecticidal activity at very low application rates, and its safety for humans and the environment, imidacloprid has been attraacting interest as a promising insecticide (*2*). For these reasons, development of a sensitive analytical method has been needed for monitoring the low levels of imidacloprid residues in soil, water, and agricultural products. Various analytical methods have been proposed for the determination of imidacloprid, such as gas chromatography−mass spectrometry (GC-MS) (*3*−*5*), high-performance liquid chromatography (HPLC) (*6*−*9*), HPLC-MS (*7*, *10*−*13*), HPLC-MS/MS (*14*), and capillary electrophoresis (CE) (*15*). However, because imidacloprid is thermolabile and has low volatility, some troublesome procedures in sample preparation, for example, hydrolysis in basic medium (*3*, *4*) or derivative (*5*), accompany direct analysis with GC-MS. Hence, it seems that it would be wise to choose HPLC analysis for imidacloprid in various matrices. Sample

* Author to whom correspondence should be addressed (telephone +81 29 838 7351; fax +81 29 838 7352; e-mail eikiw@niaes.affrc.go.jp).
† Environmental Chemical Analysis Laboratory.
§ Department of Research Planning and Coordination.

10.1021/jf0498867 CCC: $27.50
Published on Web 04/14/2004

preparation procedures such as extraction from samples, liquid−liquid partition, column chromatography, and solid-phase extraction (SPE) are indispensable for these instrumental analytical methods. Recently, these procedures have been made very efficient by the introduction of SPE, gel permeation chromatography (GPC), and so on. However, realistically, these procedures require a large quantity of time and money, and use of harmful organic solvents may cause health hazards to analytical chemists and load to the environment. Furthermore, there are limitations on sample throughput. Hence, especially, these analytical methods may be unsuitable for on-site screening because quickness and simplicity are needed. Recently, Li and Li (*16*) and Lee et al. (*17*) developed polyclonal antibody-based enzyme-linked immunosorbent assays (ELISAs) for the detection of imidacloprid in some kinds of actual samples. As is known well, immunochemical methods, including ELISA, based on a specific antigen−antibody interaction, are very useful as on-site screening analytical methods for pesticide residues (*18*), because the methods are rapid, sensitive, and reliable and are generally cost-effective for large sample loads. Many antibodies (monoclonal or polyclonal antibodies), including the above introduced two papers, to various pesticides have been developed (*16*, *17*, *19*−*23*), and ELISA methods based on the developed antibodies have been applied to environmental (*16*, *17*, *19*−*23*) and agricultural matrices (*16*, *19*, *21*, *23*). Moreover, some ELISA systems have been produced as kits and have been available as handy screening analytical tools. No matter how easily one may perform ELISA kits, one should know their analytical characteristics such as influences of organic solvent for extraction procedure, pH, or ion strength on ELISA sensitivity, cross-reactivity of antibody used in a kit, and matrix interferences originated from sample matrices. This paper describes the evaluation of a commercially available microtiter plate type ELISA kit for imidacloprid in agricultural products, among which apple was selected as a model sample in this paper. The objectives of this study are (1) to evaluate the kit for sensitivity, selectivity (specificity), influence of variety and concentration of organic solvents, which are commonly used in extraction procedure, on assay sensitivity, matrix interference, precision, and accuracy; and (2) to compare the quality of ELISA results with those obtained by conventional HPLC methodology.

MATERIALS AND METHODS

Chemicals and Materials. Pesticide-grade imidacloprid with a purity of 99.9% (by HPLC) and other standards for cross-reactivity studies were from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), Kanto Chemical Co., Inc. (Tokyo, Japan), and Aldrich Chemical Co. (Milwaukee, WI). Clothianidin was a gift from Sumitomo Chemical Takeda Agro Co., Ltd. (Ibaraki, Japan). Dinotefuran was a gift from Mitsui Chemicals (Chiba, Japan). HPLC-grade acetonitrile and distilled water, the pesticide-grade organic solvents acetonitrile, ethyl acetate, *n*-hexane, and methanol, diatomaceous earth, anhydrous sodium sulfate, and environmental analysis-grade silica gel containing 5% water were purchased from Wako Pure Chemical Industries, Ltd. or Kanto Chemical Co., Inc. Chem Elut SPE disposable cartridges were from Varian (Harbor City, CA). The ELISA kits for imidacloprid (SmartAssay series) were purchased from Horiba Biotechnology (Kyoto, Japan).

Sample Preparation for ELISA Analysis. To 5 g of the finely chopped samples of apples grown pesticide-free was added 25 mL of methanol, and then the mixture was vigorously shaken by hand for 5 min. The methanolic extract was filtered through a disk type filter (GL Chromatodisk, 0.45 μm, GL Science Inc., Tokyo, Japan). The filtrate extract (1 mL) was diluted with water (9 mL) for ELISA analysis.

ELISA Analysis. The imidacloprid kit consists of a split-type microtiter plate (12 strips, 8 wells each) coated with an anti-imidacloprid monoclonal antibody, two standard solutions (2 and 100 μg/g), a horseradish peroxidase (HRP)-labeled conjugate (HRP-conjugate), a color solution consisting of a substrate (hydrogen peroxide) and a chromogen (3,3′,5,5′-tetramethylbenzidine), a stopping solution (0.5 M H_2SO_4 solution), and a washing solution. Absorbances were measured with a SmartReader MPR-01 (Horiba Biotechnology) in a single wavelength mode (450 nm). Each well was washed with a washing solution with detergent using a MiniLab washer (Lifetec Co., Ltd., Saitama, Japan). The attached standard solutions were used by dissolving in 1 mL of water/methanol (9:1, v/v). Another stock solution (1000 μg/g) was prepared by dissolving 20 mg of imidacloprid in 20 mL of methanol. Some working standard solutions for ELISA were also prepared with water/methanol (9:1, v/v) for the evaluation of the ELISA kit by using the stock solution. Standard solutions and extracts from fortified samples were analyzed according to the following procedure: 150 μL of either standard solution or sample extract, properly diluted with water, was added to tubes, followed by 150 μL of a HRP-conjugate solution. After the mixed solutions (100 μL) were added to the above microtiter plate in duplicate at least, the wells were covered with plate seal to minimize evaporation and incubated at ambient temperature for 1 h. After incubation, the seal was removed, and the wells were washed with a washing solution four times and tapped dry. The amount of the bound HRP-conjugate is revealed by the addition of a substrate solution (100 μL) for color development. The wells were incubated for 10 min at room temperature. After the incubation period was complete, 100 μL of a stopping solution was added to each well. Quantitation was based on the optical density of the wells at 450 nm using a SmartReader MPR-01.

Sample Preparation for HPLC Analysis. Sample (20 g) was vigorously shaken with 100 mL of acetonitrile for 30 min. After the sample mixture was filtered through a funnel spread with diatomaceous earth by suction, the residue was similarly treated the second time. All filtrate was concentrated to ∼20 mL with a rotary evaporator and then loaded to a Chem Elut SPE cartridge. After standing for 15 min, the cartridge was washed with 50 mL of *n*-hexane, and then imidacloprid was eluted with 100 mL of ethyl acetate/*n*-hexane (1:1, v/v). The eluate was concentrated, and then the residue was dissolved in 5 mL of ethyl acetate/*n*-hexane (1:1, v/v). Next, the resulting solution was loaded to a silica gel column, which was packed with 10 g of silica gel containing 5% water and 2 g of anhydrous sodium sulfate suspended in adequate amounts of ethyl acetate/*n*-hexane (1:1, v/v), and then the column was washed with 50 mL of ethyl acetate/*n*-hexane (1:1, v/v) and 40 mL of ethyl acetate. After imidacloprid was eluted with 100 mL of ethyl acetate, the eluate was concentrated, and then the residue was dissolved in acetonitrile up to 5 mL.

HPLC Analysis. The HPLC system consisted of an Agilent 1100 series equipped with a quaternary pump, an autosampler, a column oven, and a diode array detector. The detection wavelength was 270 nm. The column used was a Zobrax Eclipse XDB-C8 (4.6 mm × 150 mm, 3.5 μm). The mobile phase was water/acetonitrile (8:2, v/v), and the flow rate was 1.0 mL/min. The injection volume was 20 μL.

RESULTS AND DISCUSSION

Kit Characteristics. The standard curves, which were produced with the self-made standard solutions and the kit-assembled standard solutions based on triplicates, are shown in **Figure 1**. According to the kit manufacturer, the working range of the kit is from 1 to 50 ng/g. We also determined the working range based on the criteria reported by Midgley et al. (*24*). As a consequence of the criteria, it was experimentally calculated as 1−39 ng/g. Moreover, as shown in **Figure 1**, the linearity of the standard curve based on the kit-assembled standard solutions (slope = −0.2531) agreed with that based on the self-made standard solutions (slope = −0.2485). The limit of detection (LOD) of the kit was determined. According to this criterion, it is calculated as the I_{10} value, where it is an analyte's concentration at which 10% of maximum binding of the antibody can be inhibited. The LOD of the kit was 0.5 ng/g. The experimental LOD was calculated as 3 times the mass

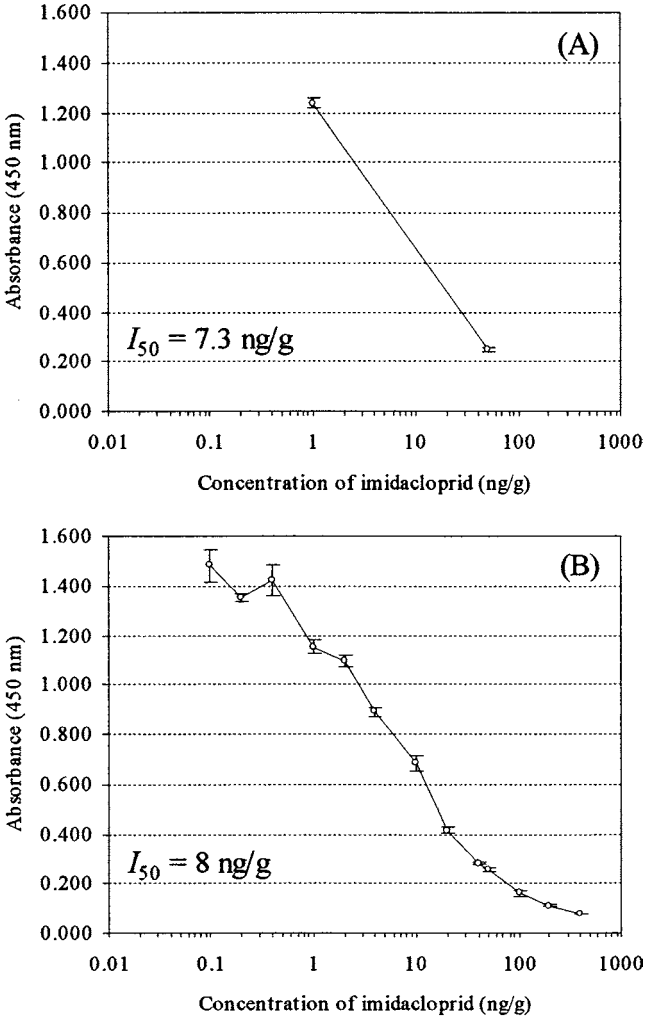


Figure 1. ELISA inhibition curves for imidacloprid, produced with the kit-assembled standard solutions (**A**) and the self-made standard solutions (**B**). Each point is the mean of triplicate determinations. Vertical bars indicate ±SD about the mean. The slope of each curve is as follows: (**A**) –0.2531; (**B**) –0.2485.

equivalence of the standard deviation of the negative control from its mean absorbance (*25*). The LOD calculated by this criterion (0.1 ng/g) suggested that the sensitivity is somewhat higher than that determined by the I_{10} criteria. Accordingly, the sensitivity of the kit (I_{50}, ~8 ng/g) was higher than those of already developed polyclonal antibody-based ELISAs in which I_{50} showed 35 ppb (*16*) and 17.3 ng/mL (*17*), respectively.

Cross-Reactivity. Cross-reactivity between antibodies and compounds that are structurally similar to the target compound is an inherent problem with ELISA. Cross-reactions can affect analytical results either by indicating that the target compound is present when it is not (false positive) or by elevating the predicted concentration of the target compound when both the target and one or more structurally similar compounds are present. Hence, the specificity of the kit toward the target compound and its most probable cross-reactants should be understood. Compounds tested for cross-reactivity included related neonicotinoid insecticides (acetamiprid, nitenpyram, thiacloprid, thiamethoxam, clothianidin, and dinotefuran) and other related compounds (metabolites, thiacloprid-amide, and 6-chloronicotinic acid). As shown in **Table 1**, cross-reactivities were calculated as a percentage obtained by calculating the ratio of the I_{50} value of imidacloprid to that of the given compound. On the basis of the results, the antibody used in the ELISA kit

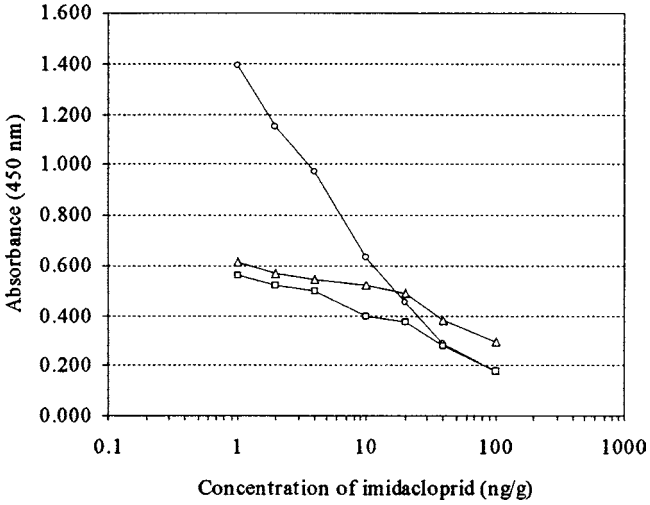
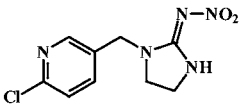
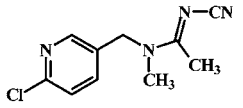
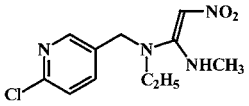
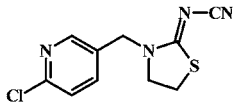
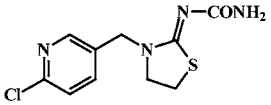
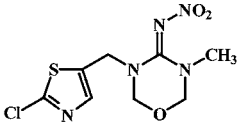
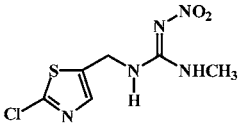
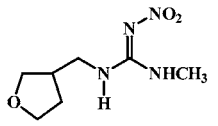
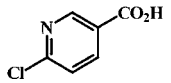


Figure 2. Selection of organic solvents for extraction procedure from agricultural samples, and their influence on the development of color and sensitivity of the ELISA kit. The data are the average of two replicates. The final concentration of each solvent (in the well) is 5%. A_{max} values are as follows: methanol (○), 1.581; acetone (□), 0.576; acetonitrile (△), 0.635. I_{50} values are as follows: methanol, 6.3 ng/g; acetone, 40 ng/g; acetonitrile, 80 ng/g.

was highly selective toward imidacloprid. Only clothianidin has been found to exhibit a significant cross-reactivity (11.9%, I_{50} = 42 ng/g). As shown in **Table 1**, the $=N-NO_2$ moiety is a common structure between imidacloprid and clothianidin, which significantly cross-reacts with the antibody. However, even though thiamethoxam and dinotefuran also have the moiety, the antibody showed negligible cross-reactivities toward them (cross-reactivity < 0.05%). The big difference in the chemical structure between clothianidin and dinotefuran is a property in the thiazole moiety or saturated furan moiety. It would account for the fact that clothianidin showed >200-fold higher cross-reactivity than dinotefuran. The antibody would probably recognize an aromatic moiety containing a nitrogen atom such as a pyridine moiety or a thiazole moiety. However, the result that the antibody showed negligible cross-reactivity toward thiamethoxam, which also has a thiazole moiety, could be due to a steric unfitness to combine with the antibody. At any rate, it is impossible to ignore its cross-reactivity toward clothianidin, and if the results obtained from the ELISA kit are unusual or doubtful or show false positive, it will be necessary to suspect the clothianidin residue in samples.

Influence of Organic Solvents on Assay Sensitivity. The use of water-miscible organic solvents such as acetone, acetonitrile, and methanol is essential to effectively extract from agricultural or environmental samples. Practically, these organic solvents are commonly used in sample extraction in the instrumental residue methods (*5*−*9*, *13*). Because the analyte in the sample extract competes with the HRP-conjugate to bind to the coated antibody in the well in the kit based on a direct competitive ELISA format and thus the organic solvent component directly touches the enzyme label, the organic solvent would directly affect the HRP activity and thus indirectly affect the development of color. So, it was desirable to assess the influence of these organic solvents on ELISA performance. First, the influence of solvents (acetone, acetonitrile, and methanol) on the kit's sensitivity was evaluated by preparing standard curves using water containing 5% of each solvent as their final concentration in the wells. The results are presented in **Figure 2**. These solvents, especially acetone and acetonitrile, signifi-

Table 1. Specificity of the Imidacloprid ELISA Kit toward Other Structurally Related Neonicotinoid Analogues and Metabolites

analogues	chemical structure	I_{50} (ng/g)	cross-reactivity (%) [a]
imidacloprid		5	100
acetamiprid		2,400	0.21
nitenpyram		>10,000	<0.05
thiacloprid		600	0.83
thiacloprid-amide		8,000	0.06
thiamethoxam		>10,000	<0.05
clothianidin		42	11.9
dinotefuran		>10,000	<0.05
6-chloronicotinic acid		>10,000	<0.05

[a] Cross-reactivity (%) = (I_{50} of imidacloprid/I_{50} of other compounds) × 100.

cantly influenced assay performance. The development of color (estimated from A_{max}) in the presence of acetone ($A_{max} = 0.576$) or acetonitrile ($A_{max} = 0.635$) was much slower than that in the presence of methanol ($A_{max} = 1.581$). I_{50} values in the presence of acetone ($I_{50} = 40$ ng/g) and acetonitrile ($I_{50} = 80$ ng/g) were much higher than that in the presence of methanol ($I_{50} = 6.3$ ng/g). Accordingly, we selected methanol as the most suitable cosolvent. Although the sensitivity of the indirect competitive ELISA for bromophos-ethyl developed by Kim et al. (*21*) tends to increase with increasing concentration of methanol, several other workers reached the conclusion that methanol caused the least negative effect on the performance of their assays (*16*, *20*, *21*, *23*). Therefore, the influence of methanol on the kit's sensitivity was also investigated by preparing standard curves in water containing various amounts of methanol (0, 1, 5, 10, 20, and 30% in water as final concentrations). As shown in **Figure 3**, A_{max} values at concentrations of ≥20% were much lower than those at concentrations of <10%. On the other hand, the I_{50} values in water containing ≥10% were gradually increased, and when the concentration of methanol exceeded 20%, the values significantly decreased (20%, $I_{50} = 30$ ng/g; 30%, $I_{50} > 100$ ng/g).

Matrix Interference. Immunoassay procedures are very rapid primarily because they usually do not require sample concentration and troublesome cleanup steps. However, this approach has some disadvantages as it may interfere with the reliability of immunoassays, which are, due to the protein nature of the antibodies, prone to matrix interferences (*26*). Chemical compounds present in samples or sample extracts, such as pigments, solvents, and others, can adversely and nonspecifically influence the antibody affinity toward the analyte. This so-called "matrix effect" or "matrix interference" can lead to decreased antibody binding capacity and therefore can reduce the sensitivity and reliability of the immunoassay. Hence, it is essential to understand the influence of the matrix component, which may lead to unreliable results in immunoassay data on the kit characteristics. In this study, apple was selected as a model matrix, and the influence of the matrix components originated from apple extracts on the kit was investigated. Typically, interferences are quantified by comparing a standard curve produced in a control matrix such as distilled water or buffered water with a calibration curve generated in the matrix of interest. The matrix interference of apple samples was fairly diminished by the 100-fold dilution, as shown in **Figure 4**; their inhibition

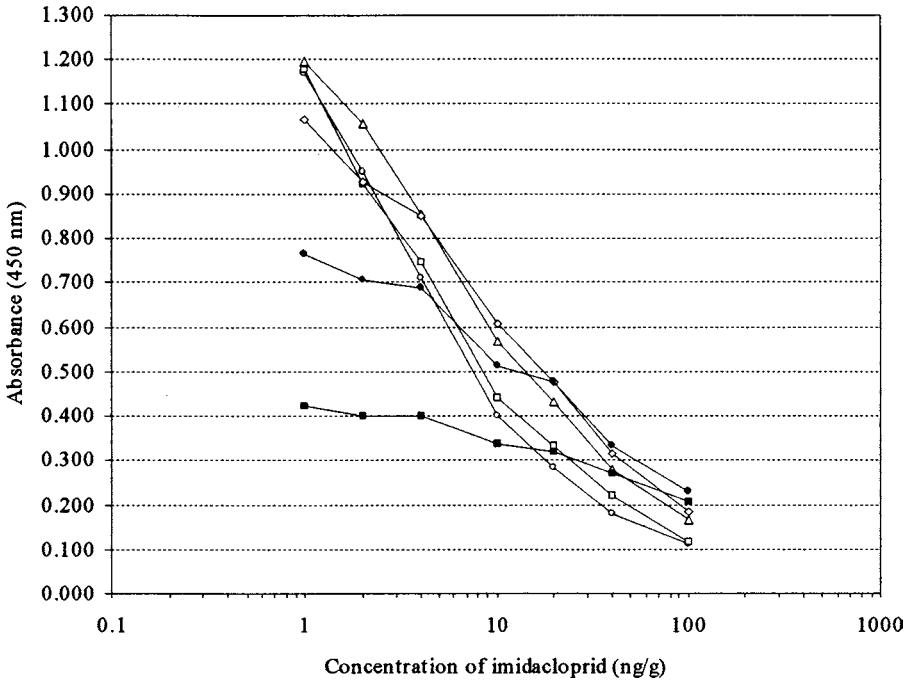


Figure 3. Influence of methanol concentration on the development of color and sensitivity of the ELISA kit. The data are the average of two replicates. A_{max} values are as follows: 0% (○), 1.452; 1% (□), 1.473; 5% (△), 1.414; 10% (◇), 1.267; 20% (●), 0.794; 30% (■), 0.398. I_{50} values are as follows: 0%, 3.8 ng/g; 1%, 4 ng/g; 5%, 6.3 ng/g; 10%, 9.2 ng/g; 20%, 30 ng/g; 30%, >100 ng/g.

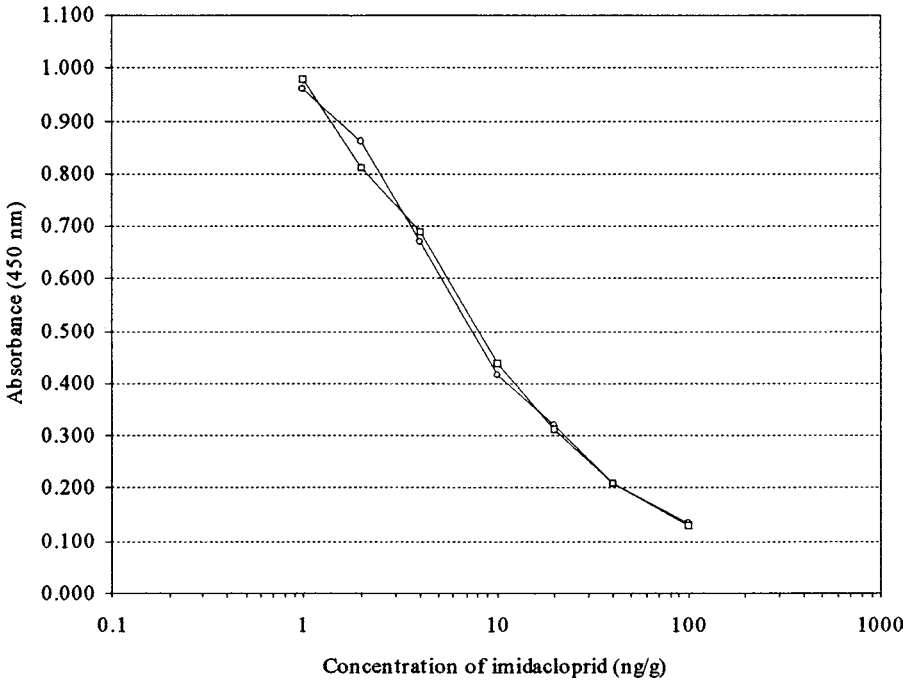


Figure 4. Influence of apple matrix on the ELISA inhibition curve. The data are the average of two replicates. I_{50} values are as follows: standard (○), 4.8 ng/g; apple extract (□), 5 ng/g.

curves were almost close to the standard control curve. These results indicate that the kit can determine imidacloprid residues in apple samples near the regulation value (1 μg/g) for apple in Japan only by simple dilution. Moreover, simple dilution of sample extract may contribute to an on-site screening for imidacloprid residue with the kit.

Comparison of Extraction Methods. Finally, to apply the ELISA kit to imidacloprid residue analysis, it should be simple and rapid not only to perform sample dilutions and ELISA procedures but also to extract the sample. Wang et al. (*27*) investigated some simple extraction techniques for development of a rapid on-site ELISA for diflubenzuron in grains. They proposed three different extraction methods and investigated the efficiency of each. Therefore, we also examined the efficiency of some extraction methods and selected the most suitable method for a screening purpose on the basis of the data (recovery and reproducibility) obtained from each method. The apple samples fortified with imidacloprid at 1 μg/g were extracted by four different methods as shown in **Table 2**. Although the recovery values obtained from the method by hand-shaking for 5 min were somewhat lower than those obtained from other methods, the method was reasonably

Table 2. Extraction of Imidacloprid Using Various Techniques[a]

method	detected concn[b] (ng/g)	mean recovery (%, $n = 3$)	coeff of variation[c] (%)
shaking by hand for 5 min	9.27 ± 0.15	92.7	1.6
mechanical shaking for 30 min (recommended by the manufacturer)	9.87 ± 0.23	98.7	2.3
homogenized for 2 min	9.87 ± 0.32	98.7	3.3
ultrasonicated for 15 min	9.63 ± 0.35	96.3	3.6

[a] The apple samples fortified with imidacloprid at 1 μg/g were extracted by each method, respectively. [b] Each extract of apple was diluted 10 times with water and 2 times with an HRP-conjugate solution (total dilution factor, 100-fold). Theoretical concentration in the ELISA is 10 ng/g. Data are the average of three determinations performed on the same days. [c] Coefficient of variation is defined as the standard deviation divided by the mean, expressed as a percentage.

Table 3. Recovery of Imidacloprid from Fortified Apple Samples with the ELISA Kit

fortified level (μg/g)	theor concn in ELISA[a] (ng/g)	detected concn[b] (ng/g)	mean recovery (%, $n = 3$)	coeff of variation[c] (%)
0.1	1	0.88 ± 0.21	87.7	24.4
0.2	2	1.97 ± 0.06	98.3	2.9
0.5	5	5.60 ± 0.52	112.0	9.3
1	10	9.87 ± 0.06	98.7	0.6
1.2	12	12.33 ± 0.58	103.0	4.7
1.5	15	14.33 ± 0.29	95.6	2.0
2	20	19.67 ± 0.58	98.3	2.9

[a] The extract of apple was diluted 10 times with water and 2 times with an HRP-conjugate solution (total dilution factor, 100-fold). [b] Data are the average of three determinations performed on the same days. [c] Coefficient of variation is defined as the standard deviation divided by the mean, expressed as a percentage.

efficient, extracting >90% of the imidacloprid residue in apple samples. Moreover, because the extraction method by hand-shaking for 5 min dose not require the instruments for extraction, such as a shaker, Polytron-type homogenizer, or ultrasonic bath, it is the simplest of the examined methods and reproducible enough to be used for extraction for an on-site screening purpose.

Recovery of Imidacloprid Residues from Some Fortified Apple Samples. The accuracy of the ELISA kit was investigated by performing recovery studies in which the measured concentrations in apple samples were determined and compared with theoretical values. The accuracy of the ELISA kit was determined by fortifying apple samples with imidacloprid (0.1, 0.2, 0.5, 1, 1.2, 1.5, and 2 μg/g) and analyzing all samples in triplicate. The results of these analyses are summarized in **Table 3**. According to the data shown in **Table 3**, both the precision and accuracy of the method with the ELISA kit may be considered very good for most fortification levels, with coefficients of variation below 10% and with recovery values ranging from 87.7 to 112.0%. The only exception was the coefficient of variation for the sample fortified at 0.1 μg/g (24.4%). When the samples containing imidacloprid at low level, such as ≤0.1 μg/g, were diluted 100-fold (in the well), the final concentrations result near the LOD of the ELISA kit. Because the ELISA data tend to vary for concentrations near the LOD, the data obtained from these samples may be lacking in reliability. However, because the recoveries and the coefficients of variation in other samples fortified at >0.1 μg/g were excellent, it was still thought that the ELISA kit is very useful to determine if residue in a sample is against the regulation value for apple or not.

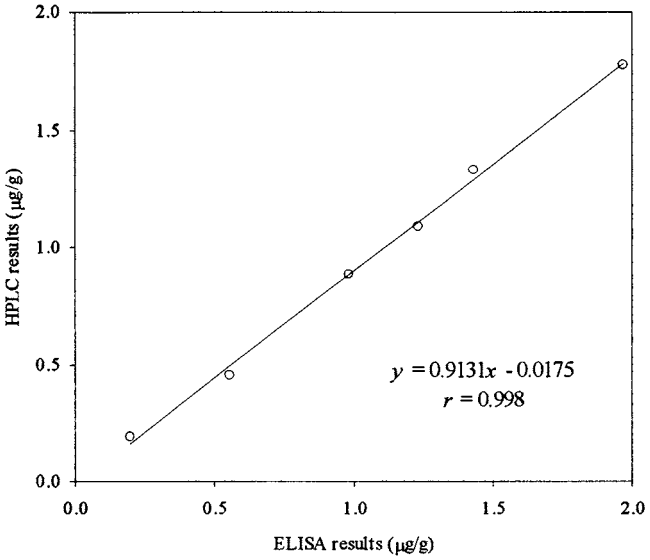


Figure 5. Correlation of the ELISA versus the conventional HPLC results for apple samples fortified with imidacloprid.

Validation of ELISA Results by HPLC. To ascertain the effectiveness of the ELISA kit for imidacloprid, a correlation study between methods was performed on six fortified apple samples. **Figure 5** shows the results of the ELISA and the HPLC analyses for apple samples containing detectable levels of imidacloprid. Despite the fact that the comparison was established on the samples subjected to different treatments, ranging in concentration from 0.2 to 2 μg/g, an excellent linear correlation was achieved ($r = 0.998$) with slopes of 0.9131. Because of the loss of imidacloprid during concentration and column chromatography steps of the HPLC method (average recovery = 90.5%) and because of the false positive results of the ELISA (average recovery = 99.0%), which indicates a concentration increment by cross-reaction or excessive response to the background, the slope could incline to the ELISA results (x-axis) slightly. Hence, the introduced ELISA kit for imidacloprid could be suitable for a screening analytical method with rapidity, simplicity, and high sample throughput.

Conclusions. The results of the present investigations of the characteristics (working range, LOD, cross-reactivity, and influence of organic solvent on the kit's sensitivity) of the ELISA kit for imidacloprid residue analysis and its applicability to actual agricultural samples, such as apple samples selected in the present study (matrix interference, and effective and simple extraction method) indicate that the ELISA kit is a suitable tool for an on-site screening purpose. The simplicity of the method is clearly evidenced by its sample throughput. With standard solutions (either kit-assembled or self-made) and samples run at least in duplicate, 30 samples or more can be determined on each plate. Because a few plates (three or four plates at least) can be handled simultaneously and the complete procedures take ∼2−3 h to perform, as many as ∼100 apple samples can be analyzed in a workday. Thus, the ELISA kit will be useful for an on-site screening for imidacloprid residues in apple samples. In addition, this kit could be a "comparatively" cost-effective and high selectivity assay for monitoring imidacloprid residues near the regulation values of agricultural samples. Hence, this ELISA kit could be used as an alternative and supplemental analytical tool to the conventional instrumental methods for monitoring imidacloprid residues in agricultural samples.

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Received for review January 22, 2004. Revised manuscript received February 27, 2004. Accepted March 4, 2004. This work was supported by the Research Project for Utilizing Advanced Technologies in Agriculture, Forestry and Fisheries.

JF0498867