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Rapid communication

Application of a commercial immunoassay to the direct determination of insecticide imidacloprid in fruit juices

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Abstract

An ELISA was used to directly determine residual imidacloprid in fruit juices. Imidacloprid could be determined by only diluting samples without any pre-treatments such as filtration, centrifugation, and clean-up procedures. The ELISA enabled imidacloprid to accurately determine down to about 5 μg/L in apple and grape juice samples and down to about 20 μg/L in orange juice sample. Recovery and precision of the ELISA were evaluated by spiking fruit juice samples with imidacloprid in the 10–400 μg/L ranges. Coefficients of variation were lower than 20% in all cases, and average recoveries were 94.2%, 113.2%, and 104.2% for apple, grape, and orange juice samples, respectively. No false positive results were found. The results obtained with the proposed ELISA well correlated with the reference HPLC for each fruit juice sample ($r > 0.99$).

Keywords: Immunoassay; ELISA; Imidacloprid; Pesticide; Food analysis; Fruit juice

1. Introduction

The advantage of immunoassays (enzyme-linked immunosorbent assays, ELISAs) relative to chromatographic techniques have been widely discussed by some researchers (Hennion & Barcelo, 1998; Meulenberg, Mulder, & Stoks, 1995), and included the following: (1) low limit of detection, (2) high analyte selectivity (specificity), (3) high throughput of samples, (4) minimal sample preparation, (5) cost effectiveness for large numbers of samples, and (6) adaptability to field use. Moreover, no complex or sophisticated instrumentation is required and the use of toxic organic solvents is minimal. Consequently, numerous ELISAs for residual pesticides in diverse food or environmental samples have been developed. Since a target pesticide in solid samples such as crop or soil samples is generally possible to directly detect only giving extraction procedure when using an ELISA method, the required time for acquirement of analytical results could considerably be shortened comparing with conventional chromatographic analysis. Furthermore, when handling liquid samples such as environmental water, juice, or biological samples, there could be no requirement for even extraction procedure (Abad, Manclús, Moreno, & Montoya, 2001; Abad & Montoya, 1995; Abad, Moreno, & Montoya, 1997; Bushway, 1996; Mastin et al., 1998; Miyake et al., 1998).

Neonicotinoids are a new type and an important class of pesticides now widely used in agriculture, instead of persistent organochlorine pesticides, as insecticides due to their broad spectrum of activity and their low bioaccumulation potential. Target imidacloprid in the present report is one of the main compounds belonging to this class of insecticides that act as an antagonist by binding to postsynaptic nicotinic receptors in the insect central nervous system (Tomlin, 2000). Because of the possibility of the extensive application of the neonicotinoid insecticides including imidacloprid to agricultural operations, their residues may occur in fruits and vegetables, and

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therefore, pose a potential hazard for consumers. The problems on residual pesticides incline to focus on environments or agricultural products. However, the residual pesticides in the processed foods such as juices, baby foods, or jams should not be definitely neglected. Especially, children who preferably eat them are an extremely sensitive against various chemicals including pesticides and are one of the vulnerable consumers groups. So, the appropriate control and monitoring for residual pesticides in general foods is indispensable to secure human health and security to food supply.

Till now, various sensitive ELISAs for residual pesticide detection including imidacloprid (Lee, Ahn, Park, Kang, & Hammock, 2001; Li & Li, 2000) have been developed because of the advantages mentioned above. However, since kinds and contents of matrix components widely vary with kinds of analytical samples, especially crop and processed food samples, it could be important to determine the influence on ELISA performance when quantitatively analyzing a pesticide in a sample made up of complicated components. Consequently, it should be essential to inspect the utility of ELISA by applying to samples as many as possible to extend applicability of ELISA for residual pesticide analysis. So, we have already demonstrated the utility of a commercial ELISA for the detection of imidacloprid in major agricultural samples, and suggested that the ELISA could serve to rapidly, simply, and quantitatively detect the pesticide, especially the check of the residual level before shipment (Watanabe et al., 2004a, 2004b). The objectives of this study are (1) to evaluate matrix interference coming from juice samples, (2) to inspect the accuracy using pesticide-spiked samples, and (3) to compare the quality of the ELISA results with those obtained by the reference HPLC method. Finally, we will exhibit someone a new application method of the ELISA as a consequence of the obtained results.

2. Materials and methods

2.1. Chemicals

Pesticide-grade imidacloprid (98% purity by HPLC) was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). All other chemicals were of analytical grade, and solvents were of pesticide-grade and HPLC-grade. The ELISA kits for imidacloprid were gift from Horiba Biotechnology Co., Ltd. (Kyoto, Japan) as approvals.

2.2. Instruments and chromatographic conditions

ELISA plates were washed with a MiniLab washer (Lifetec Co., Ltd., Saitama, Japan) and each well absorbance was read with a SmartReader MPR-01 with a 450 nm filter (Horiba Biotechnology Co., Ltd.).

The HPLC system consisted of an Agilent 1100 series equipped with a quaternary pump, an autosampler, a column oven, and a diode array detector. HPLC column SunFire C_{18} (250 mm × 4.6 mm, 5 μm, Waters, Milford, MA) with mobile phase water/acetonitrile (8:2, v/v) was used. The detection wavelength was 270 nm. The flow rate was 1.0 mL/min, column temperature was 40 °C, and the injection volume was 20 μL.

2.3. Sample preparation

Apple, grape, and orange juices (100% pure) were purchased from a local supermarket in Tsukuba, Ibaraki, Japan. Some known concentrations of imidacloprid were spiked to each fruit juice sample from stock solution in methanol to make the appropriate concentrations prior to sample preparations for both ELISA and HPLC analyses.

For ELISA determination, apple and grape juice samples were diluted at 10-fold and orange juice samples were done at 50-fold with 10 mM phosphate-bufferd saline (PBS; 7.75 mM disodium hydrogen phosphate, 2.25 mM potassium dihydrogen phosphate, 154 mM sodium chloride, pH 7.2) prior to the analysis. Standard solutions and diluted fruit juice samples were analyzed according to the following procedure: 150 μL of either standard solution or sample solution was added to borosilicate glass tubes, followed by 150 μL of a horseradish peroxidase-labeled conjugate (HRP-conjugate) solution properly diluted with water. After the mixed solutions (100 μL/well) were carefully added to the ELISA plate coated with anti-imidacloprid monoclonal antibody 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 was revealed by the addition of a color solution (100 μL/well) consisting of a substrate (hydrogen peroxide) and a chromogen (3,3′,5,5′-tetramethylbenzidine) for color development. The wells were incubated for 10 min at room temperature. After the incubation period was complete, a stopping solution (100 μL/well) (0.5 M sulfuric acid) was added to each well. Quantitation was based on the optical density of the wells at 450 nm. The analytical results obtained from the optical density multiplied by the dilution factor (20-fold for apple and grape juice samples and 100-fold for orange juice samples).

Fruit juice samples for HPLC analysis were prepared according to the proposed method (“Kongetsu No Nogyo” Henshushitsu, 1998) with slight modifications as follows: a 20 g of fruit juice sample, which was weighted into an Erlenmeyer flask, was diluted with 20 mL of water, it was transferred to 250 mL of separating funnel, and then 50 mL of dichloromethane was added it. The mixture was vigorously shaken for 5 min, and then it was stand for about 20 min. After the organic phase was transferred to 200 mL of Erlenmeyer flask, the other 50 mL of dichloromethane was added to aqueous phase, and then the same procedure was repeated. All organic phase was dehydrated

with anhydrous sodium sulfate, and then the dried dichloromethane was evaporated to dryness using a rotary evaporator in a water bath at 40 °C. The residue was dissolved in about 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 10% water and 2 g of anhydrous sodium sulfate suspended in adequate amount of ethyl acetate/*n*-hexane (1:1, v/v), and then the column was washed with 45 mL of ethyl acetate/*n*-hexane (1:1, v/v) and 40 mL of ethyl acetate. After imidacloprid was eluted with 80 mL of ethyl acetate, the eluate was concentrated, and then the residue was dissolved in acetonitrile up to 5 mL.

3. Results and discussion

3.1. Standard curve for imidacloprid ELISA

Fig. 1 shows a representative standard curve for imidacloprid, which was prepared in PBS based on triplicates. The sensitivity (I_{50} value), dynamic range, and limit of detection of the ELISA calculated with the criteria of Midgley, Niswender, and Rebar (1969) were 3.9, 0.9–27, and 0.22 μg/L, respectively. Although the dynamic range under the condition was somewhat narrower than that when using water containing 5% (v/v) methanol as final concentration in each well, which was experimentally estimated as 1–39 μg/L, the I_{50} value and limit of detection were lower than those which were calculated as 8 μg/L and 0.5 μg/L, respectively (Watanabe et al., 2004a), and the sensitivity of the ELISA was rather higher than those of already reported polyclonal antibody-based ELISAs (Lee et al., 2001; Li & Li, 2000).

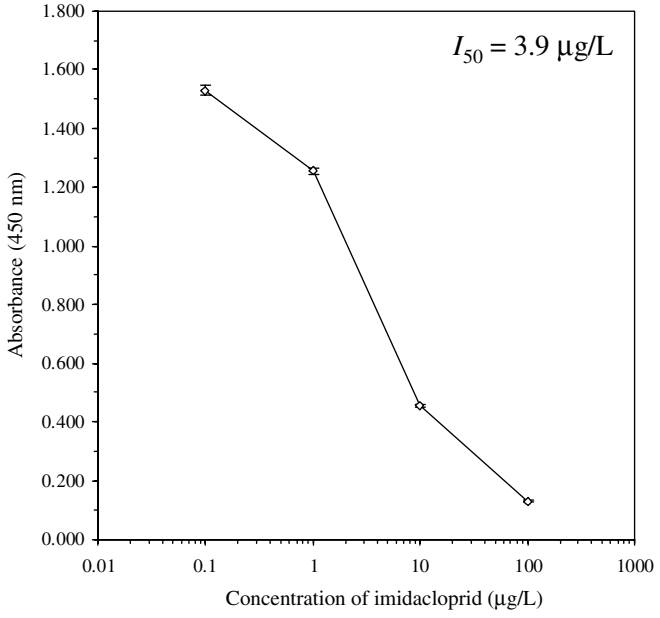

Fig. 1. ELISA inhibition curve for imidacloprid prepared in PBS. Each point is the mean of triplicate determinations. Vertical bars indicate ±SD about the mean.

3.2. Determination of matrix interference

ELISAs are not completely free from interferences caused by unidentified compounds of the food matrix notwithstanding antibody's high selectivity. Thus, it is advisable to determine the importance of these matrix interferences before the application of the assay to samples containing the interest. The easiest and most immediate way to minimize and to overcome matrix interferences is sample dilution (Abad et al., 1997, 2001; Abad & Montoya, 1995; Mastin et al., 1998; Miyake et al., 1998). Imidacloprid standards were prepared in PBS containing a variable proportion of each juice sample, and the matrix interference on the standard curves was determined. Fig. 2 shows the resulting standard curves for three kinds of fruit juices. In apple and grape juice samples, no significant matrix interference was observed only when diluting at 10-fold (20-fold in the assay). On the other hand, in case of orange juice samples, minor matrix interference was found, as shown by the fact that the curves were only slightly shifted at the side of the lower concentration. So, the orange juice samples should be diluted at least 50-fold (100-fold in the assay) to be accurately analyzed by the ELISA. Taking account of the required dilution factor to minimize matrix interference, and the limit of detection estimated above section, the practical limit of detections were about 5 μg/L in apple and grape juices and about 20 μg/L in orange juice, respectively.

3.3. Recovery study in spiked juice samples

Apple, grape, and orange juices were spiked with imidacloprid at four levels, and directly analyzed by the ELISA without any pre-treatment other than dilution with PBS. Each ELISA plate included its own imidacloprid standard curve, and absorbances from samples were interpolated on the curve performed in the same plate. Table 1 gives the results obtained for each spiked juice sample determined 3 times at best dilution factors as previously described. The average recovery data for these fruit juice samples were very excellent as follows: 94.2% (varying from 82.0% to 100.0%), 113.2% (varying 94.7% to 128.7%), and 104.2% (varying 95.8% to 113.3%) for apple, grape, and orange juice samples, respectively. On the other hand, the precision obtained for all fruit juices samples can also be considered very excellent for a residual method, since the averages of the coefficients of variation were 10.9%, 8.2%, and 10.4% for apple, grape, and orange juice samples, respectively.

The ELISA results are often inclined to rather overestimate comparing with theoretical (spiked) concentrations as observed in previously reported results (Abad et al., 1997; Watanabe et al., 2004b). However, the results on the apple juice samples were contradictory to the general tendency. Probably, it suggested that the findings might be due to matrix interference and the phenomena may vary with the types of the juice samples.

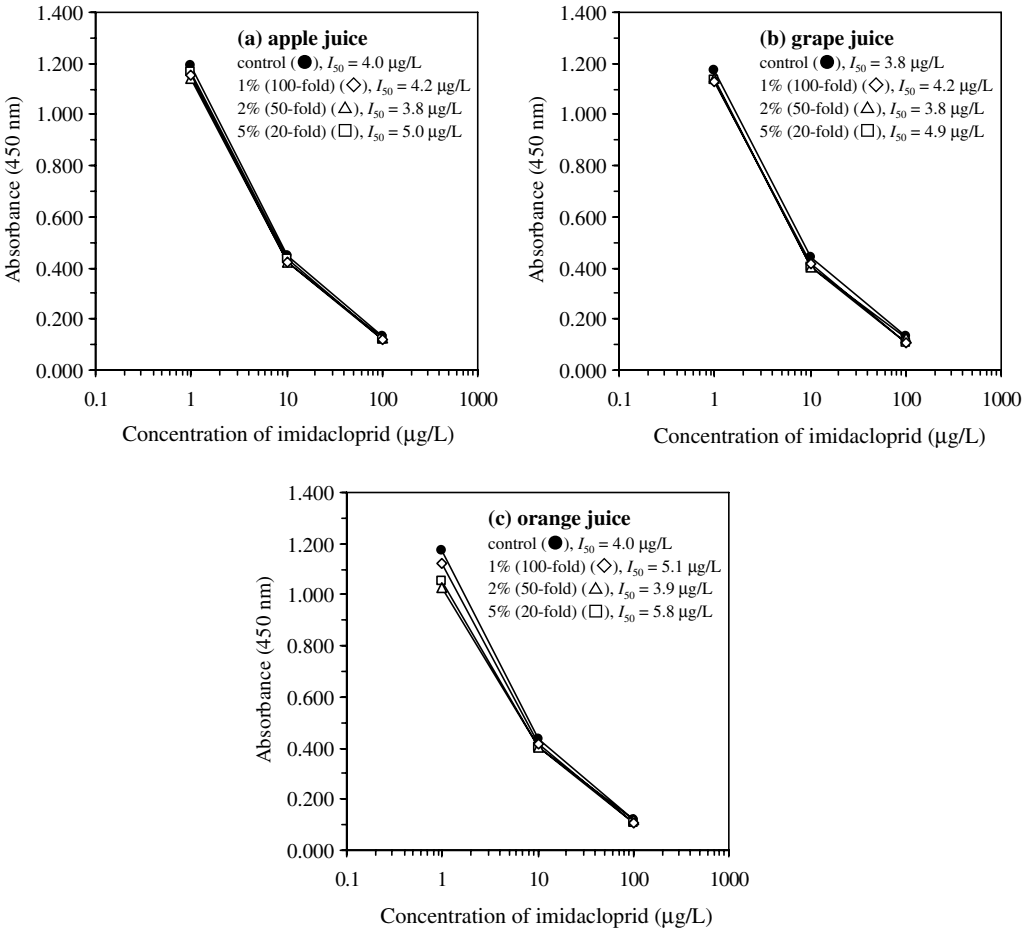

Fig. 2. Influence of matrix interference on the imidacloprid ELISA inhibition curve. The ELISA was performed in assay buffer (PBS) containing different proportions of each fruit juice.

Table 1
Reproducibility and accuracy of the imidacloprid ELISA for spiked fruit juice samples[a]

Matrix	Spiked level (μg/L)	Detected concentration (μg/L)	Average recovery (%, $n = 3$)	% CV
Apple juice	10	8.2 ± 1.6	82.0	19.0
	20	20.0 ± 2.0	100.0	10.0
	50	49.3 ± 4.2	98.7	8.4
	100	96.0 ± 6.0	96.0	6.3
Average			94.2	10.9
Grape juice	10	10.9 ± 1.3	109.3	11.8
	20	24.0 ± 2.0	120.0	8.3
	50	47.3 ± 3.1	94.7	6.5
	100	128.7 ± 8.1	128.7	6.3
Average			113.2	8.2
Orange juice	50	54.7 ± 5.7	109.3	10.4
	100	113.3 ± 15.3	113.3	13.5
	200	196.7 ± 15.3	98.3	7.8
	400	383.3 ± 37.9	95.8	9.9
Average			104.2	10.4

[a] For ELISA analysis, each sample was diluted with PBS in assay as follows: apple and grape juices, 10-fold; orange juice, 50-fold, and then the diluted samples were mixed with a HRP-conjugate solution at equal volume. So, total dilution factor were 20-fold for apple and grape juices and 100-fold for orange juice, respectively. Data are the average of three determinations performed on the same plate at the same days.

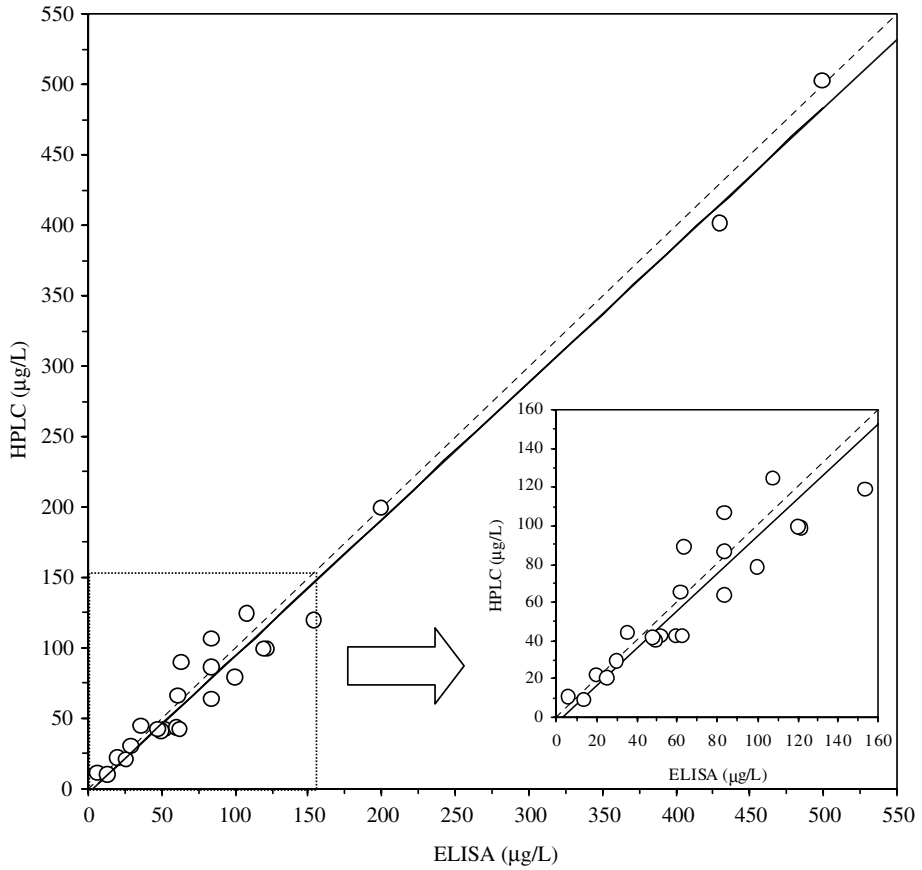

Fig. 3. Relationship between imidacloprid levels in the spiked fruit juice samples as the proposed ELISA method and the reference HPLC method. The formula and correlation coefficient (r) in tested samples is $y = 0.9733x - 3.1484$ and $r = 0.9916$. The dotted line corresponds to a perfect correlation ($y = x$).

3.4. Method comparison

To confirm the applicability of the proposed ELISA for the determination of imidacloprid in three kinds of fruit juice samples, 24 samples (8 samples per each fruit juice) were analyzed both by the ELISA and the reference HPLC. The results are given in Fig. 3. Regression analyses gave good linear relationships of both methods for all fruit juice samples ($y = 0.9733x - 3.1484$, $r = 0.9916$). The average recovery values of 109.3% for all fruit juice samples as determined by ELISA were somewhat higher than the corresponding concentrations from HPLC (100.4%).

The simplicity of the method clearly makes a large contribution to the advantages of ELISA methods as described in a review (Hennion & Barcelo, 1998). Probably, about 25 juice samples (in triplicates at least) with a standard curve composed of three or four standard series can be simultaneously handled on an ELISA kit. It takes little time to prepare the samples for the ELISA analysis, and only takes about 90 min to manage the ELISA procedures including addition of the standards and the samples into the wells, incubation step, washing, color developing, and stop reaction. Hence, assuming that three or four plates can be handled at a time, you can deal with seventy samples and upward in a working day. These findings demonstrate that the proposed ELISA method can be employed as the initial test and backed up by the HPLC method. Such a combination can be beneficial in the quantitation of imidacloprid in fruit juice samples because of the number of samples that can be analyzed in a cost-effective way. Finally, it was suggested that the proposed ELISA was possible to quantitatively determine residual imidacloprid not only in agricultural samples but also in processed food samples without any sample pre-treatment procedures. Consequently, ELISA method would be ranked with useful and prospective residual pesticide analyses, which can accurately determine them in various types of samples at trace levels.

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