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ARTICLES

Determination of Imidacloprid in Paddy Water and Soil by Liquid Chromatography Electrospray Ionization-Tandem Mass Spectrometry[1]

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Abstract—A method for the determination of imidacloprid in paddy water and soil was developed using liquid chromatography electrospray ionization-tandem mass spectrometry (**LC/ESI-MS/MS**). Separation of imidacloprid was carried out on a Shimadzu C18 column (150 mm × 4.6 mm, 4.6 μm) with an acetonitrile–water (50 : 50, v/v) mobile phase containing 0.1% of acetic acid. The flow rate was 0.3 mL/min in isocratic mode. The product ion at 209 *m/z* was selected for quantification in multiple-reaction monitoring scan mode. Imidacloprid residues in soil were extracted by a solid-liquid extraction method with acetonitrile. Water samples were filtered and directly injected for analysis without extraction. Detection limits of 0.5 μg/kg and 0.3 μg/L were achieved for soil and water samples, respectively. The method had recoveries of 90 ± 2% ($n = 4$) for soil samples and 100 ± 2% ($n = 4$) for water samples. A linear relationship was observed throughout the investigated range of concentrations (1–200 μg/L), with the correlation coefficients ranging from 0.999 to 1.000.

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Imidacloprid (1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine) is a systemic insecticide used to control sucking insects in rice and other agricultural crops. It can be applied to crops in many forms, including broadcasting or spraying treatments. In Japan, it is usually applied in rice nursery boxes before transplanting [1]. Because of its high insecticidal activity, it is used at a low application rate (0.2 kg a.i/ha in Japan). Therefore, levels of imidacloprid residues in the paddy environment are very low.

Various analytical methods such as ELISA, GC and HPLC [2] have been developed to quantify imidacloprid in environmental matrixes [2]. However, these methods require either high concentrations of imidacloprid in water and soil samples (50–2000 μg/L), or complicated sample extraction procedures for those samples with low imidacloprid concentrations [3]. Several methods have also been developed to determine multiple pesticide residues, including imidacloprid, using LC-MS and LC-MS/MS in agricultural products. However, the matrixes are more complex than paddy samples and require complicated clean-up and extraction procedures [4–6]. No studies have been reported to develop an analytical method for low imidacloprid concentrations in the paddy water and soil matrixes. Consequently, a less, time and solvent consuming method to determine imidacloprid in paddy water and soil is required.

The aim of this study was to develop a sensitive, selective, rapid and simple method to quantify imidacloprid residue in paddy water and soil samples using LC/ESI-MS/MS, combined with a simple sample treatment.

EXPERIMENTAL

Reagents. All solvents (acetonitrile, acetic acid) and imidacloprid standard (purity >99%) were of analytical grade and purchased from Wako Pure Chemical Industries (Osaka, Japan). Water was produced with a Milli-Q Water Purification System (Millipore, Billerica, MA, USA). Glass filters and syringe filters were from Whatman (Maidstone, UK).

Instruments. A Waters Alliance HPLC System (Waters, Milford, MA, USA) 2695 linked with a Micromass Quattro micro API Tandem Quadrupole system (Waters) was used. The chromatograph was equipped with a Shimadzu HPLC column C-18, (150 mm × 4.6 mm, 4.6 μm particle size), kept at 40°C. The pump was set in isocratic mode at a flow rate of 0.3 mL/min using a mobile phase of acetoni-

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Table 1. Physico-chemical properties of paddy soil [7]

Physico-chemical properties	Value
pH (H_2O)	6.5
Organic carbon content, %	3.96
Total carbon content, %	4.77
Total nitrogen content, %	0.44
Cation exchange capacity, $cmol_c/kg$	22.5
Particle density, g/cm	2.50
Sand, %	37.6
Silt, %	31.8
Clay, %	30.6
Soil texture, ISSS	Light clay (LiC)

ISSS: International Society of Soil Science.

trile-water (50 : 50, v/v), acidified with 0.1% of acetic acid. Mass analysis was performed with a Z-spray source for positive electrospray ionization (**ESI**) using multiple-reaction monitoring (**MRM**) scan mode. Both HPLC and MS/MS were controlled by MassLynx 4.0 software (Waters). The capillary voltage was adjusted to 3.0 kV. The ionization source was heated to 125°C and the desolvation temperature was set to 250°C. Dry nitrogen (≥9.5%) was used as the desolvation and nebulization gas and argon (>99.999%) was used as the collision gas. Ions were fragmented by collision with argon gas at 3.06×10^{-3} mbar. The nitrogen flow rate and cone gas flow were set to 680 L/h and 100 L/h, respectively. Dwell times and an interscan delay time of 0.2 s were used. To optimize the MS/MS conditions, cone voltages and collision energy were set at 15 to 35 V and 10 to 30 eV, respectively. The sample injection volume was 10 μL.

Standard preparation. Stock solution (50 mg/L) of imidacloprid standard was prepared in acetonitrile. This stock solution was diluted to concentrations of 1, 5, 20, 50, 200 μg/L in acetonitrile–water (20 : 80, v/v). All standard samples were stored at 4°C. During analysis, the standard samples were run periodically after every ten samples. The amount of imidacloprid in the sample was calculated using an external standard curve.

Sample preparation. Paddy water and soil samples were collected from the experimental paddy field. The average pH value of the surface water fluctuated slightly from neutral (pH 7 in the morning) to alkaline (pH 8.3 in the afternoon) due to photosynthetic processes. The detail physico–chemical properties of paddy soil was reported by Watanabe et al., 2007 in Table 1 [7].

Water samples containing imidacloprid were filtered through 0.2 μm syringe filters (Whatman, Maidstone, UK). The filtrate was transferred to a 2 mL vial and kept at 4°C until LC analysis.

Soil samples (20 g) containing imidacloprid were extracted by sonication with 40 mL of acetonitrile for 20 min in a 200 mL Erlenmeyer flask. The sample was shaken and equilibrated for 2 h then allowed to settle for 1 h. The samples were filtered through a 1.2 μm glass fiber filter (Whatman, Maidstone, UK), and then washed with 10 mL acetonitrile three times. The combined filtrate was transferred to a 200 mL round bottomed flask and concentrated using a rotary vacuum evaporator at 40°C. The residue was dissolved in 2 mL of acetonitrile–water (20 : 80, v/v) and then filtered through 0.2 μm syringe filters (Whatman, Maidstone, UK) to a 2 mL vial and kept at 4°C until LC analysis.

Quantification and method validation. The repeatability of the calibration curves were determined by injecting the standard solutions five times during the same day and on three different days. The correlation factors (r^2) of the calibration curves were then calculated.

Precision and accuracy of the method were evaluated using spiked samples in recovery experiments. The four soil samples and the four water samples were each spiked with four known amounts of imidacloprid standard solution at different levels: 5, 20, 40, 60 μg/kg; and 5, 25, 50, 150 μg/L, respectively. Blank samples were also run using the same procedures in order to eliminate potential false positives caused by contamination in the operation process, instrument or chemicals used.

Limits of detection (**LODs**) and limits of quantification (**LOQs**) of the method for each of the matrixes were determined. Instrumental detection limit (**IDL**) and instrumental quantification limit (**IQL**) were also calculated following the reported method [8, 9].

RESULTS AND DISCUSSION

Optimization of LC-ESI-MS/MS conditions. The double mass selection enables highly specific quantification in MRM scan mode. However, optimization is required to increase the specificity and sensitivity, and to reduce background noise due to the complexity of matrix. Optimization of MS/MS conditions includes the selection of the ionization mode, identification of the parent and product ions, and selection of the cone and collision voltages.

Full-scan mass spectra were recorded in both positive (**ESI+**) and negative (**ESI–**) ionization modes, to select the most abundant mass fragments. The relative intensity for the most abundant m/z was used to evaluate the performance of each ionization mode. Higher signal intensities were obtained in the positive mode. Full-scan daughter mass spectra were obtained with continuous infusion of imidacloprid in product-ion scan mode. Two specific fragments of imidacloprid

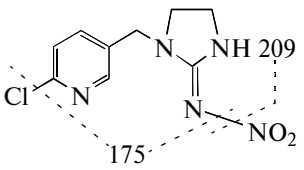

Fig. 1. The structures of two product ions of imidacloprid at $m/z = 209$ and $m/z = 175$.

were then monitored. The first fragment, at $m/z = 209$, is due to loss of NO_2 (Fig. 1). The second fragment, at $m/z = 175$, is due to loss of both NO_2 and ^{35}Cl (Fig. 1). The MRM transitions (Table 2) were used for quantification (209 m/z) and confirmation (175 m/z).

The voltage applied to the ion source (**ESI**), collision cell and quadrupoles was optimized in the MRM mode in order to achieve the highest sensitivity as possible. The optimized cone voltage and collision energy for imidacloprid were 25 V and 15 eV, respectively (Figs. 2 and 3). The observed peaks of the two MRM transitions, run using the above-optimized conditions, are indicated on Fig. 4. Similar results were obtained in other studies. Pirard et al., 2007 [6], used a cone voltage of 35 V and collision energy of 15 eV for determining multiple pesticide residues, including imidacloprid in honey. Bonmatin et al., 2003 [4] used a collision energy of 15 eV for analyzing imidacloprid in soil, plant matter, and honey. The optimized cone voltage for each compound could alter depending on the analysis equipment and conditions. Therefore, the optimized value must be determined before each analysis.

Extractions. Different solvents(dichlomethane [10], methanol/NH_4OH 0.05% [4], acetone [6], and acetonitrile–water (80 : 20, v/v) [11]) have been used to extract imidacloprid from various matrixes. Since acetonitrile is a common solvent for extraction of pesticides, we attempted to extract imidacloprid with 100% acetonitrile.

Analytical quality control parameters. Internal quality criteria were applied to ensure the quality of results when the proposed methods were applied to routine analysis. Satisfactory resolution was achieved on a LC reverse-phase column packed with C18 using isocratic elution with acetonitrile–water (50 : 50, v/v), acidified with acetic acid (0.1%). The retention time was 7.82 ± 0.08 min ($n = 60$).

External calibration curves of the peak area vs. concentration were constructed. The linear equation was $y = 0.0047x + 0.8746$ where y is the concentration of imidacloprid (ppb) and x is the peak area. The range of linearity was between 1 and 200 μg/L. It covered the concentration range studied (1, 5, 20, 50, 200 μg/L). The correlation coefficients (r^2) were higher than 0.999 ($r^2 = 0.9992 \pm 0.0004$, $n = 15$). Thus, a linearity with a confidence level of 99% was achieved in all the calibration ranges. Imidacloprid was not detected in

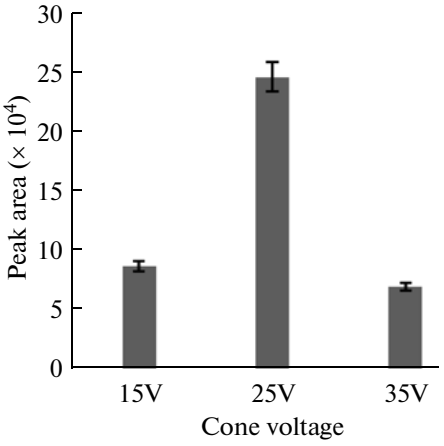

Fig. 2. Optimization of cone voltage.

either blank water or blank soil samples. Hence, no contamination occurred in the extraction process, instrument or chemicals used in this method. These results ensure high selectivity for the analytical method.

The main goal of the recovery experiment was to determine method accuracy. The recovery and repeatability data were in line with EU guidelines for pesticide residue analysis. Recovery studies of both soil and water sample preparation were performed at four fortification levels, the average percent recovery and standard deviations (**SDs**) were also determined (Table 3). The average percent recovery and SDs were excellent at all fortification levels. Average recoveries for soil extraction and water filtration were $90 \pm 2\%$ and $100 \pm 2\%$, respectively, in agreement with the EU criteria (European Communities, Commission decision 2002/657/EC and SANCO/10232/2006) [12, 13].

LODs and LOQs of the method for each matrix were determined by spiking water and soil samples with standard imidacloprid at signal-to-noise ratios of 3 and 10, respectively [8, 9]. The LODs were 0.3 μg/L in water, and 0.5 μg/kg in the soil. The LOQs were 1 μg/L and 1.7 μg/kg for water sample and soil sample, respectively. The IDL and IQL were estimated from the injection of a standard solution successively

Table 2. Acquisition parameters for imidacloprid analysis

Retention time, min	Precursor ions, m/z	Product ions, m/z	Dwell time, s	Collision voltage, eV
7.8	256	209*	0.2	15
	256	175	0.2	15

* Product ion 209 m/z was used for quantification.

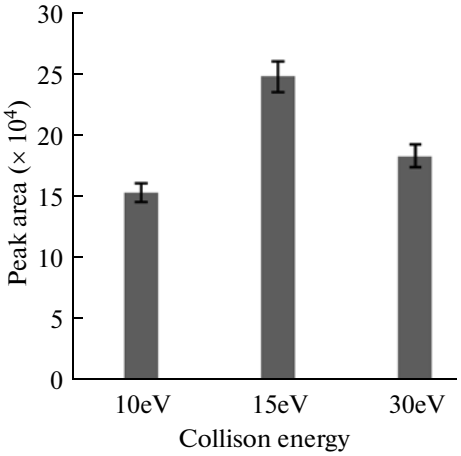

Fig. 3. Optimization of collision energy.

diluted until its concentration corresponded to signal-to-noise ratios of 3 and 10, respectively [8, 9]. Low IDL and IQL (0.15 and 0.5 μg/L) indicated that the equipment used had high sensitivity for the determination of pesticide in paddy environmental samples.

The method developed is also highly selective, and with the monitoring of two mass transitions, the risk of false positive results is reduced [14, 15]. By monitoring two transitions and their relative ion intensity within maximum permitted tolerances, the method achieves these results which meet the current requirements for unambiguous identification of organic residues proposed by the European Community (2002/657/EC) [12]. In addition, for the positive confirmation of analyte in the samples, strict criteria are to be met. These include no more than 2% variation in the chromatographic retention time of imidacloprid, and the relative abundance of the two transitions monitored has to lie within a 20% margin compared with the calibration standards. In this study, the retention time of imidacloprid was identical for every sample at 7.82 min with a relative standard deviation (RSD, $n = 60$) of 1%. Retention time fluctuations showed the maximum RSD obtained was 3%. The ratio between the two transitions (209 m/z : 175 m/z) was stable at 0.911 throughout the defined linearity range, with RSD = 12% ($n = 60$) for both water and soil samples. These results confirm that the method can be applied in a routine manner with a high level of confidence.

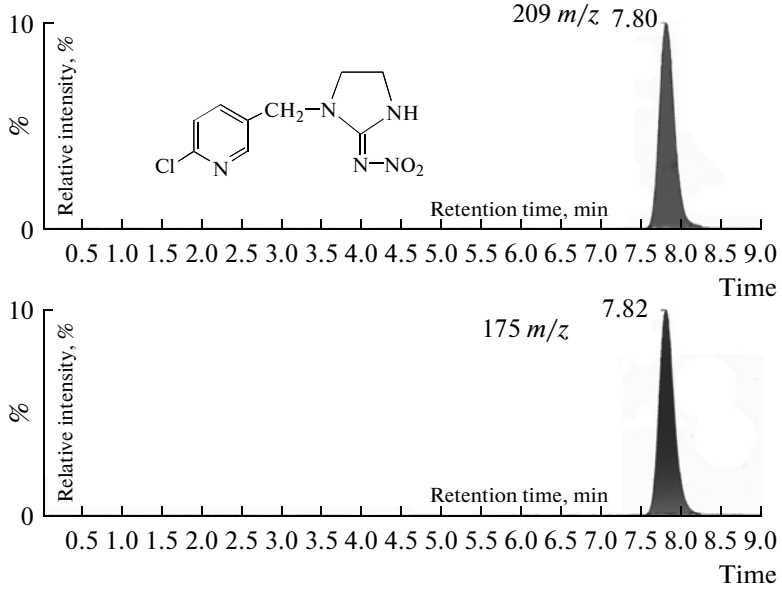

Fig. 4. LC/ESI-MS/MS chromatogram of two product tons of imidacloprid in positive mode.

Table 3. Recovery of imidacloprid from spiked water and soil samples

Sample No.	Water samples		Soil samples	
	Added (μg/L)	Recovery, %	Added (μg/kg)	Recovery, %
1	5	99	5	88
2	25	98	20	91
3	50	102	40	91
4	150	100	60	92
Average of recovery, %		100 ± 2		90 ± 2

The analytical method developed had satisfactory performance for linearity, repeatability, accuracy, selectivity and sensitivity. Its other advantages include fast analysis, minimum sample manipulation and cost efficiency.

CONCLUSIONS

An analytical method based on LC–MS/MS has been developed for fast and simple determination of the insecticide imidacloprid in paddy water and soil. The soil extraction method described in this study is rapid, does not require extensive clean-up and therefore allows for more efficient operation. The main advantages of the method are: effectiveness, simplicity, rapidity, safety, and high sensitivity. The limits of detection are 0.3 μg/L and 0.5 μg/kg for water and soil samples, respectively. The method has good reproducibility and high selectivity. The results obtained are reliable and attain the identification points required by the EU for confirmatory analysis.

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