

Rapid and Space-Saving Sample Preparation Followed by a 100 % Water Mobile Phase HPLC-PDA Analysis for Quantifying Acetamiprid and Imidacloprid in Unpolished Rice

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Abstract – This paper presents a rapid, simple, and space-saving technique of sample preparation followed by a 100% water mobile phase high-performance liquid chromatography (HPLC) coupled photo-diode array detector (PDA) for quantifying acetamiprid (ATP) and imidacloprid (ICP) in unpolished rice. The analytes were extracted from the sample using a handheld ultrasonic homogenizer with water, and purified by MonoSpin[®] C18-CX, a centrifugal monolithic silica spin mini-column, and quantified within 20 min sample⁻¹. The accuracy, precision, and system suitability are well within the international method acceptance criteria.

Keywords – International Harmonized Analytical Method, Acetamiprid, Imidacloprid, Centrifugal Monolithic Silica Spin Mini-Column.

I. INTRODUCTION

Neonicotinoids are a class of neuro-active/systemic insecticides that act on certain kinds of receptors in the nerve synapse, like nicotine, and are used worldwide as agricultural crop protection and environmental pest management, and control fleas on domestic animals [1].

In the early 2000s some kinds of neonicotinoids began to come under increasing scrutiny over potential environmental impacts. The use of neonicotinoids was linked in a range of studies to a number of adverse ecological effects, including honey-bee colony collapse disorder and loss of birds due to reduction in insect populations. Increased scrutiny eventually led to restrictions and bans on the use of different neonicotinoids in several countries [2-4].

In December 2013, two neonicotinoid insecticides, acetamiprid (ATP) and imidacloprid (ICP), may affect the developing human nervous system, disclose the European Food Safety Authority (EFSA). Experts from the Authority propose that some guidance levels for acceptable exposure to the two neonicotinoids be lowered while further research is carried out to provide more reliable data on so-called developmental neurotoxicity [5].

Rice is an internationally important food because it is a staple for nearly half of the world's seven billion people [6]. The Japanese Ministry of Health Labor and Welfare has set maximum residue limit (MRL) for the ICP in unpolished rice at 1 ppm [7] to ensure the safety and appropriateness of rice for human consumption. Monitoring the presence of ATP and ICP in rice is, therefore, an important means of guaranteeing food safety.

Depending on the recent expansion and diversification in the international food trade, the development of international harmonized methods to determine chemical

residues in foods is essential to guarantee equitable international trade in these foods and ensure food safety for consumers. Whether in industrial nations or developing countries, an international harmonized method for residue monitoring in foods is urgently-needed. The optimal harmonized method must be easy-to-use, economical in time and cost, and must cause no harm to the environment and analyst. Although several techniques based on high-performance liquid chromatographic (HPLC) detection have been developed for the monitoring ATP and ICP [8-14], these methods have crucial drawbacks: 1) they involve several analytical steps in the sample preparation, which are time-and cost-consuming and do not permit the determination of large number of samples; 2) all of the methods consume large quantities of toxic organic solvents, acetonitrile and/or methanol [15], in the mobile phases. Risk associated with these solvents extend beyond direct implications for the health of humans and wildlife to affect our environment and the ecosystem in which we all reside. Eliminating the use of toxic solvents and reagents is an important goal in terms of environmental conservation, human health and the economy [16,17]; 3) most of the recent methods are based on LC-MS or -MS/MS. The facilities that LC-MS/MS system is available are limited to part of industrial nations because these are hugely expensive, and the methodologies use complex and specific. These are unavailable in a lot of laboratories for routine analysis, particularly in developing countries. No optimal method that satisfies the aforementioned requirements has yet been identified.

As a technique that can be encouraged as an international harmonized analytical method for the residue monitoring of ATP and ICP, this paper describes a simplified/space-saving sample preparation with minimized organic solvent consumption followed by an isocratic 100 % water mobile phase HPLC for determining ATP and ICP in unpolished rice.

II. MATERIALS AND METHODS

2.1 Chemicals, Reagents, and Blank Samples

All chemicals including acetamiprid (ATP) and imidacloprid (ICP) standards were purchased from Wako Pure Chem. Ltd. (Osaka, Japan). Ethanol (non-toxic class, the human or environmental toxicity is negligible [15]) and distilled water were of HPLC grade. Unpolished rice produced by pesticide-free was used as rice samples for the present study. The rice was brayed fully (in fine powder) and used as blank rice samples.



2.2 Equipment

The following apparatuses were used in the sample preparation: handheld ultrasonic-homogenizer (model HOM-100, 2 mm ID probe, Iwaki Glass Co., Ltd., Funabashi, Japan); micro-centrifuge (Biofuge® fresco, Kendo Lab. Products, Hanau, Germany); two type of MonoSpin® as centrifugal monolithic silica spin mini-column (sample throughput volume ≤ 300 µL), MonoSpin C18 (bonded with octadecyl group) and MonoSpin C18-CX (bonded with octadecyl group and benzene sulfonic acid group) (GL Sciences, Inc., Tokyo, Japan); three types of non-polar sorbent columns (5 µm_p; 4.6 mm i.d.; 150 mm length) for HPLC analysis were used, Inertsil® HILIC (diol), Inertsil WP300 C4, and Inertsil TMS (C1) (GL Sciences, Tokyo, Japan). The HPLC system, used for method development, included a model PU-980 pump and DG-980-50-degasser (Jasco Corp., Tokyo, Japan) equipped with a model CTO-10AS VP column oven (Shimadzu Scientific Instruments, Kyoto, Japan), as well as a model SPD-M10A VP photo-diode-array (PDA) detector (Shimadzu).

2.3 Operating Conditions

The analytical column was an Inertsil WP300 C4 (150 × 4.6 mm, 5 µm) column using an isocratic mobile phase of water at a flow rate of 1.0 mL min⁻¹ at 50°C. PDA detector was operated at 190 – 350 nm: the monitoring wavelengths were adjusted to 245 and 269 nm which represent maximums for ATP (at 245 nm) and ICP (at 269 nm), respectively. The injection volumes were 10 – 20 µL.

2.4 Preparation of Stock Standards and Working Mixed Solutions

Stock standard solutions of ATP and ICP were prepared by dissolving each compound in water followed by water to a concentration of 50 µg mL⁻¹. Each solution was stored at -20 °C. Working mixed standard solutions of these two compounds were freshly prepared by suitably diluting the stock solutions with water on the day of the analysis.

2.5 Preparation of Calibration Standards and Quality Control Samples

For method validation studies, calibration standards and quality control samples (QCs), terms defined in the FDA guideline [18], were prepared by spiking appropriate aliquots of the mixed standard solution in blank rice samples. Calibration standards were used to construct calibration curves from which the concentrations of analytes in unknown monitoring samples are determined practically. QCs used to evaluate the performance of the proposed method. In this study, the standards were

prepared in the range of 0.5 – 10 µg g⁻¹ for both analytes. Three QC levels (For both analytes, QC1 = 0.5 µg g⁻¹; QC2= 1 µg g⁻¹; QC3 = 5 µg g⁻¹) were prepared.

2.6 Sample Preparation

An accurate 0.1 g sample was taken into a 1.5 mL micro-centrifuge tube and homogenized with 0.6 mL of water with a handheld ultrasonic-homogenizer for 30 s. After being homogenized, the capped tube was centrifuged at 13,000 rpm for 5 min. A 0.1 mL of supernatant liquid was poured to a MonoSpin C18-CX and, immediately after, the capped mini-column was centrifuged at 3,500 rpm for 1 min. Under the similar centrifuging operation, the mini-column had been washed with 0.1 mL of 5 % (v v⁻¹) ethanol (in water) and then ATP and ICP was eluted with 0.1 mL of 30 % ethanol. The eluate was injected into the HPLC system.

2.7 Method Validation

The performance of the developed method was validated in terms of some parameters from the international guidelines for bio-analytical procedure [18-23].

III. RESULTS AND DISCUSSION

3.1. Sample Preparation

The present procedure is very easy and small-scale technique that minimizes organic solvent consumption in the preparation of analytes. The ultrasonic-homogenization enabled the satisfactory extraction of ATP and ICP from a rice sample with a 100% water. The extract obtained by the present operation was purified by subsequent centrifugal monolithic silica spin mini-column, MonoSpin®. The spin mini-column is a monolithic SPE column which is said to be excellent for the small volume sample with easy and quick operation by centrifuge [24]. The author used two types of the spin mini-columns, MonoSpinC18 and - C18-CX, and the retention profiles of analytes in these columns were compared. The possibility of eluting analytes from the columns by several eluents was evaluated. Since the reversed-phase sorbents were de-retained by adding polar solvents (normally use toxic class solvents like acetonitrile and methanol) to the eluent, the effect of the concentration of ethanol (= non-toxic class [15]) in the eluent (ethanol – water, v v⁻¹) on the recovery of ATP and ICP standards from the MonoSpin mini-columns was determined when the centrifugal acceleration and time were standardized at 3,500 rpm and 1 min, respectively.

Table 1: Effect of the Ethanol Concentration in the Eluent (Ethanol– Water) on the Recoveries of ATP and ICP from Mono Spin® Spin mini-Columns^a

	Ethanol concentration (% v v ⁻¹) in the eluent						
	0	5	10	15	20	25	30
MonoSpin C18:							
ATP	37.5	95.0 (2.4)	-	-	-	-	-
ICP	64.2	89.9 (2.8)	-	-	-	-	-
MonoSpinC18-CX:							
ATP	0	0	0	13.2	32.9	89.3(2.3)	101.1(1.1)
ICP	0	0	18.7	48.7	63.0	96.1(2.0)	100.3(0.9)

Data are averages (% , n=5).

^aA 0.1 mL portion of a mixed standard solution containing 0.1 µg of each compound was poured to MonoSpin[®] spin mini-column and centrifuged at 3,500 rpm for 1 min. The eluate was injected into the HPLC system.

Relative standard deviations in parentheses (%).

In this study, a 0.1 mL portion of a mixed standard solution containing 0.1 µg of each compound was applied to the spin mini-column and the elute was determined by the present HPLC system. The resulting profiles are given in Table 1. As shown, MonoSpin C18-CX showed best recoveries and repeatabilities for ATP and ICP when the eluent was 30 % ethanol. Because both analytes were not eluted with 5% ethanol from the spin mini-column, 5 % ethanol was used as the washing eluent to eliminate interfering compounds of sample origin. This isolated protein/fat constituents from the column. The present procedure can realize a small scale extraction and easy purification of ATP and ICP in a short time while significantly limiting the consumption of organic solvents (mere 35 µL sample⁻¹). The time required for the sample preparation of a single sample was <10 min. The procedure resulted in high recovery and reproducibility.

Fig. 1 illustrates that the resulting chromatograms were free of interfering compounds for the quantification and identification of ATP and ICP by the HPLC, with the PDA detector set at 245 (for ATP) and 269 nm (for ICP) (giving the maximum absorbance for ATP or ICP). The present HPLC system accomplished good separation with the need for a gradient system to improve the separation and pre-column washing after analysis. Fig. 1 demonstrates that the present method can provide the quantification and identification of the analytes.

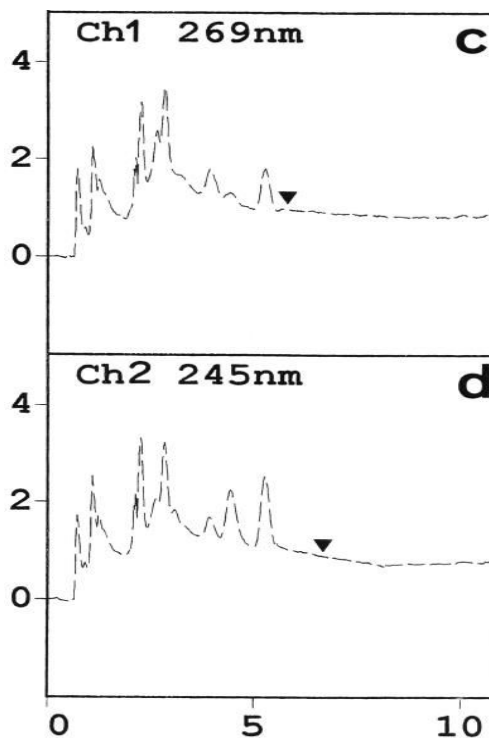
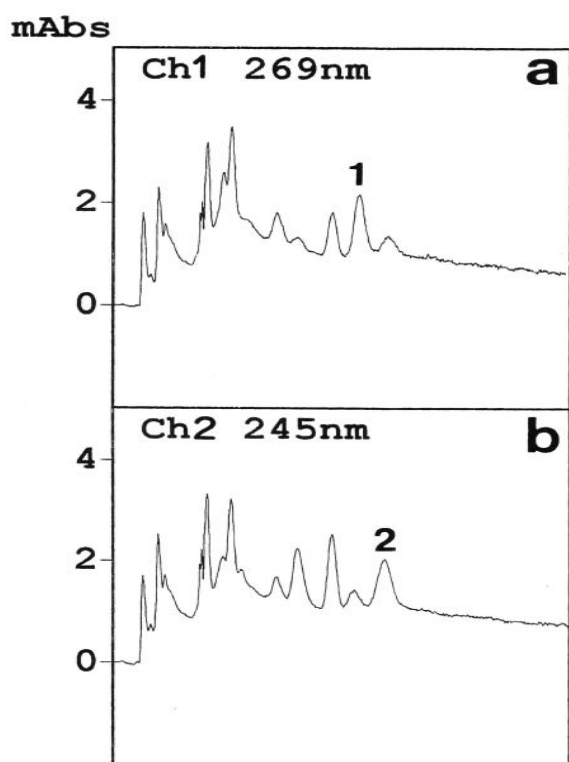


Fig.1. Chromatograms obtained from the HPLC system for a spiked (each compound 1 ppm) rice sample (a,b), a blank rice sample (c,d). PDA detector set at 269 nm (a,c) and 245 nm (b,d). Peaks, 1 = ICP (Retention time, Rt = 5.85 min); 2 = ATP (Rt = 6.54 min). Closed triangles (▼) indicate the retention times of ICP (c) and ATP (d), respectively.

3.2 Method Validation

Main method validation data

Tables 2 and 3 summarize the method validation parameters. The quantitative limit for ICP, 0.13 ppm, is much lower than the MRL (1 ppm in unpolished rice) [7]. The system-suitability evaluation is an essential parameter of HPLC determination, and it ascertains the strictness of the system used. The suitability was evaluated as the relative standard deviations of peak area and retention time calculated for 20 replicate injections of a spiked rice sample with ATP and ICP (each 1 µg g⁻¹). The values for ATP and ICP RP were estimated to be ≤ 0.08 % for retention time and ≤ 0.61 % for peak area, respectively.

Table 2: Method Validation Data

Parameter	ATP	ICP
Linearity (<i>r</i>) ^a	0.9942	0.9979
Range (ppm)	0.5 – 10	
Accuracy ^b (%)	100.4	98.4
Precision ^c (%)	1.9	1.5
Sensitivity ^d (ppm)	0.17	0.13
System suitability ^e (%):		
Retention time	0.08	0.06
Peak area	0.61	0.45

^a *r* is the correlation coefficient (p<0.01) for calibration curve.

^b Average recoveries from 18 replicates (=six replicates at three QC levels, 0.5, 1, and 5 µg g⁻¹ for ATP and ICP, respectively).



^cValues are relative standard deviations (n= 18).

^dQuantitative limit as the concentration of analyte giving a signal-to-noise ratio = 10.

^eData as the relative standard deviations calculated for 20 replicate injections of the prepared eluate for a rice sample spiked with ATP and ICP (each 1 µg g⁻¹).

Table 3: Robustness^a Data

Parameter	Change (%)	ATP		ICP	
		Rt ^b	Peak area	Rt	Peak area
Flow rate ^c :					
	+5	-5.4	+0.2	-4.6	+0.4
	-5	+4.2	+0.6	+5.0	+0.3
Column temperature ^d :					
	+5	-3.4	+5.6	-2.3	+2.0
	-5	+2.0	-4.8	+2.7	-1.1

^aChanges of ±5 % units of the flow rate and the column temperature were determined.

The effect on the peak areas and the validations in the retention times were evaluated. Data are expressed as average rates (% , n=5).

^bRetention time (min); normal Rts for ATP and ICP were 6.54 and 5.85 min, respectively..

^cAt 1 mL min⁻¹ under the present HPLC system.

^dAt 50°C under the present HPLC system.

The accuracy, precision, and system suitability are within the international method acceptance criteria (Table 2) [18-23]. The other validation findings are as follows:

Specificity and Selectivity

The application of the proposed procedure to 10 blank rice samples demonstrated that no interference peak was presented around the retention times for ATP and ICP in any of the sample examined. The present HPLC-PDA system easily confirmed the peak identity of target compound. Both analytes were identified in an unpolished rice sample by their retention times and absorption spectra. The ATP and ICP spectra obtained from the rice sample were practically identical to those of the standards. Because of the complete separations, PDA detection at trace levels is fully available. It is, therefore, instructive to demonstrate purification effectiveness of the sample preparation. The system did not require the use of MS or MS/MS, which is very expensive and is not available in a lot of laboratories for routine analysis.

3.3. Cost and Time Performances

The total time and budget required for the analysis of a single sample was < 20 min and approximately US \$3.8 (as of December 5, 2014), respectively. For sequential analysis, a batch of 24 samples could be analyzed in < 4 h. These findings became term required for the routine assay. The short analytical time not only increased the sample throughput for analysis but also positively affected the cost.

IV. CONCLUSION

An idiot-proof operating sample preparation followed by an isocratic water mobile phase HPLC-PDA method for simultaneous quantification of ATP and ICP in unpolished

rice has been successfully established. The method validation data were well within the international method acceptance criteria. The present procedure provided an easy-to-use, rapid, and space-saving and resulted in high recovery and repeatability with considerable saving of analysis time and cost. In particular, the present technique may be proposed as an international harmonized method for deterring ATP and ICP in the rice.

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