Proposal of an international harmonized analytical technique for quantifying of residual acetamiprid and imidacloprid in wheat

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Abstract

This paper proposes an international harmonized analytical method for residual monitoring of selected neonicotinoids in crops and presents a fast, easy, 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 wheat. 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. The accuracy, precision, and system suitability are well within the international method acceptance criteria.

Keywords: Internal harmonized analytical method, Acetamiprid, Imidacloprid, Centrifugal monolithic silica spin mini-column

1. Introduction

Some kinds of neonicotinoids, neuro-active/systemic insecticides, began to come under increasing scrutiny over potential environmental impacts, in the early 2000s. 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 [1-3].

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 [4].

Wheat is a very important food because it is grown on more than 216,000,000 hectares (530,000,000 acres)[5] larger than for any other crop; its world trade is greater than for all other crops combined; is the world's most favored staple food. The Codex, FAO/WHO Food Standards, has set maximum residue limit (MRL) for the ICP in wheat flour at 0.03 ppm [6] to ensure the safety and appropriateness of wheat for human consumption. Monitoring the presence of ATP and ICP in wheat 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 ï 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 [7-13], 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 [14], 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 [15,16]; 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 wheat.

2. 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 [14]) and distilled water were of HPLC grade.

Wheat produced by pesticide-free was used as wheat samples for the present study. The wheat was brayed fully (in fine powder) and used as blank wheat 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); a MonoSpin® as centrifugal monolithic silica spin mini-column (sample throughput volume Ò 300 εL), MonoSpin C18-CX (bonded with octadecyl group and benzene

sulfonic acid group) (GL Sciences, Inc., Tokyo, Japan). A non-polar sorbent columns, Inertsil WP300 C4 (5 εm d_P, 4.6 × 150 mm) (GL Sciences) for HPLC analysis was used.

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 $_{VP}$ photodiode-array (PDA) detector (Shimadzu).

2.3. Operating conditions

The analytical column was an Inertsil WP300 C4 (150 × 4.6 mm, 5 εm) column using an isocratic mobile phase of water at a flow rate of 1.0 mL/min at 50 ℃. PDA detector was operated at 190 ï 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 (Fig. 1). The injection volumes were 10 ï 20 εL.

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 εg/mL. Each solution was stored at -20 ℃. 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 [17], were prepared by spiking appropriate aliquots of the mixed standard solution in blank wheat 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 0.1 ï 5 εg/g for both analytes. Three QC levels (For both analytes, QC1 = 0.1 εg/g; QC2= 0.5 εg/g; QC3 = 1 εg/g) 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) 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 [17-22].

3. 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 wheat 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 [23]. 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 (an ethanol: non-toxic class) (mere 35 ε L/sample). The procedure resulted in high recovery and reproducibility.

Fig. 2 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.

3.2. Method Validation

3.2.1. Main method validation data

Table 1 summarizes the method validation parameters. The accuracy, precision, and system suitability are within the international method acceptance criteria [17-22].

3.2.2. Specificity and selectivity

The application of the proposed procedure to 6 blank wheat 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 a wheat sample by their retention times and absorption spectra. The ATP and ICP spectra obtained from the wheat 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 11, 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.

4. Conclusion

An idiotproof operating sample preparation followed by an isocratic 100 % water mobile phase HPLC-PDA method for simultaneous quantification of ATP and ICP in wheat 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, space-saving, and harmless and resulted in high recovery and repeatability with considerable saving of analysis time/cost. In particular, the present technique may be proposed as an international harmonized method for deterring ATP and ICP in wheat.

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Legends to figures

Fig. 1: Typical absorption spectra of peaks for ATP (dashed line, max. 245 nm) and ICP (solid line, max. 269 nm) standards in the HPLC chromatogram.

Fig. 2: Chromatograms obtained from the HPLC system for a spiked (each compound 0.5 ε g/g) wheat sample (A,B) and a blank wheat 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.

Table 1: Method Validation Data

Parameter	ATP	ICP
Linearity (r)[a]	0.9948	0.9971
Range (ε g/g)	0.1 ï 5	
Accuracy[b](%)	93.8	98.0
Precision[c](%)	1.8	1.6
Sensitivity[d] (ε g/g)	0.029	0.024
System suitability[e] (%) :		
Retention time	0.11	0.07
Peak area	0.73	0.54

[a] r is the correlation coefficient ($p < 0.01$) for calibration curve.
[b] Average recoveries from 18 replicates (=six replicates at three QC levels (0.1, 0.5, and 1 ε g/g for ATP and ICP, respectively).
[c] Values are relative standard deviations (RSD, n= 18).
[d] Quatitative limit as the concentration of analyte giving a signal-to-noise ratio = 10.
[e] Data as the relative standard deviations calculated for 20 replicate injections of the prepared eluate for a rice sample spiked with ATP and ICP (each 0.5 ε g/g).

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