



A Green HPLC-PDA Technique for Detecting Acetamiprid and Its Metabolite IM-2-1

N. Furusawa*

Graduate School of Human Life Science, Osaka City University, Osaka 558-8585, Japan.

ARTICLE DETAILS

Article history:

Received 02 March 2015

Accepted 16 March 2015

Available online 23 March 2015

Keywords:

International Harmonized Analytical Method

Acetamiprid

IM-2-1

100 % Water Mobile Phase

High-Performance Liquid Chromatography

ABSTRACT

This paper describes a harmless HPLC technique for detecting acetamiprid (ATP) and its metabolite, IM-2-1 (IM2), using an isocratic 100 % water mobile phase. Chromatographic separations were performed on an Inertsil® WP300 C4 with water mobile phase and a photodiode-array detector (PDA). The total run time was < 7 min. The system suitability was well within the international acceptance criteria. The detection limits were 0.013 µg/mL for ATP and 0.006 µg/mL for IM2, respectively. A harmless HPLC method for simultaneous detecting ATP and IM2 was developed and may be further applied to the quantification in animal-derived foods.

1. Introduction

Overuse of pesticides is always associated with risk of food residues. In December 2013, acetamiprid (ATP), a widely/frequently-used neonicotinoid insecticide, 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 ATP be lowered while further research is carried out to provide more reliable data on so-called developmental neurotoxicity [1].

Previous ATP metabolism studies on animals (rats, lactating goats and laying hens) have found that the main metabolic pathway of ATP in rats is the transformation to N-desmethyl ATP, IM-2-1 (IM2), by demethylation; the predominant residue in most animal products (goat milk, goat liver/kidney, and hen's liver/muscle/skin) is the IM-2-1 [2]. To assure the safety of foods of animal origin for the consumer, the Codex sets maximum residue limits (MRLs) for the sum of ATP and IM2, expressed as ATP. Because determinations for ATP together with IM2 in the animal foods are therefore an important specific activity to guarantee food safety, a validated analytical method for the simultaneous determining ATP and IM2 is presently required.

In response to 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 ideal harmonized method must be easy-to-use, economical in time and cost, and must cause no harm to the environment and analyst.

Previous techniques based on high-performance liquid chromatographic (HPLC) for the monitoring ATP and IM2 [3-6] have crucial drawbacks: 1) consuming large quantities of toxic organic solvents, acetonitrile and/or methanol [7], 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 [8,9]; 2) 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 the first examination problem in the establishment of an international harmonized method for the residue monitoring of ATP and IM2, this paper describes an isocratic 100 % water mobile phase HPLC conditions to detect ATP and IM2 without the organic solvent/reagent consumption.

2. Experimental Methods

2.1 Chemicals and Reagents

Standards of acetamiprid (ATP) and IM-2-1 (IM2) (N^1 -[(6-chloro-3-pyridyl)methyl]- N^2 -cyanoacetamidine) and distilled water (HPLC grade) were purchased from Wako Pure Chem. Ltd. (Osaka, Japan).

2.2 Equipment

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 CO-810 column oven (Thosoh Corp., Tokyo, Japan), as well as a model SPD-M10A VP photodiode-array (PDA) detector (Shimadzu Scientific Instruments, Kyoto, Japan).

The following three types of non-polar sorbent columns (5 µm dP; 4.6 mm i.d.; 150 mm length) for HPLC analysis were used: Inertsil® HILIC (diol); Inertsil WP300 C4; Inertsil TMS (C1) (GL Sciences, Tokyo, Japan). Table 1 lists the particle physical specifications.

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 at 50 °C. PDA detector was operated at 190 – 350 nm: the monitoring wavelengths were adjusted to 234 and 245 nm which represent maximums for IM2 and ATP, respectively (Fig. 1). The injection volumes were 10 – 20 µL.

Preparation of Stock Standards and Working Mixed Solutions. Stock standard solutions of ATP and IM2 were prepared by dissolving each compound in water followed by water to a concentration of 100 µg/mL. Working mixed standard solutions of these two compounds were prepared by suitably diluting the stock solutions with water. These solutions were kept in a refrigerator (5 °C).

*Corresponding Author

Email Address: furusawa@life.osaka-cu.ac.jp (N. Furusawa)

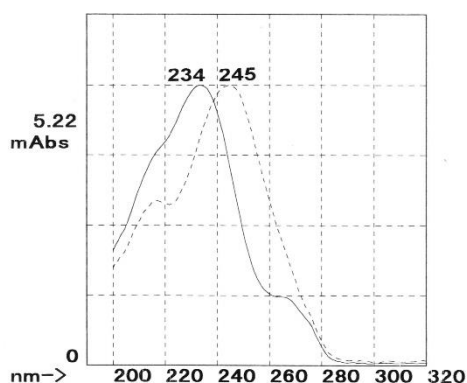


Fig. 1 Typical absorption spectra of peaks for ATP (dashed line) and IM2 (solid line) standards in the HPLC chromatogram.

2.4 HPLC validation

2.4.1 Linearity

The calibration curve was generated by plotting peak areas ranging from 0.025 to 10 $\mu\text{g/mL}$ versus their concentrations. The linearity was assessed from the linear regression with its correlation coefficient.

2.4.2 Detection limit

The detection limit should correspond to the concentration for which the signal-to-noise ratio. The value was defined as the lowest concentration level resulting in a peak area of three times the baseline noise.

2.4.3 System suitability test

The HPLC system suitability 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 areas and retention times calculated for 10 replicate injections of a mixed standard solution (0.5 $\mu\text{g/mL}$).

3. Results and Discussion

3.1 Optimum HPLC conditions

Using three types of non-polar sorbent columns [a) diol; b) C4; c) C1] (Table 1), the test was carried out to achieve the separation with a 100 % water mobile phase. This study used water as an isocratic mobile phase and examined column temperatures $\geq 25^\circ\text{C}$, the flow rates $\geq 0.75\text{ mL/min}$, and HPLC retention times $\leq 10\text{ min}$ (Table 1). Because the HPLC separations were performed serially, the time/run was critical for routine residue monitoring. The short run time not only increased sample throughput for analysis but also affected the method-development time.

The three columns were compared with regard to the separation between ATP and IM2 and the sharpness of peaks obtained upon injection of equal amounts. The chromatographic separations within the conditions ranges examined are also presented in Table 1.

The complete separation of the two compounds and their symmetrical peaks were obtained by a Column- b) and water mobile phase with column temperature of 50°C and flow rate of 1.0 mL/min . Fig. 2 displays that the resulting chromatogram obtained from the HPLC. The two target peaks are clearly distinguished at 5.75 and 6.49 min, respectively. The present HPLC-PDA analysis accomplished optimum separation in a short time without the need for a gradient system to improve the separation and pre-column washing after an analysis.

3.2 HPLC validation

Table 2 summarizes the validation data for the performance parameters. The linearity and system suitability values were well within the international acceptance criteria [10].

Table 1 Physical/chemical specifications of the reversed-phase columns^a used and chromatographic ATP and IM2 separations obtained under the HPLC conditions examined^b

Column	Silica type	Trade name	Pore diameter (nm)	Pore volume (mL/g)	Surface area (m ² /g)	Carbon load (%)	HPLC target compounds	
							Separation	Peak forms
a) diol		Inertsil HILIC	10	1.05	450	20	NE ^c	-
b) C4		Inertsil WP300 C4	30	1.05	150	3	Separated	Symmetrical/Sharp
c) C1		Inertsil TMP	10	1.05	450	3.5	NE	-

^a i.d. = 4.6 mm; length = 150 mm; $d_p = 5\text{ }\mu\text{m}$.

^b Isocratic mobile phase of water; flow-rates $\geq 0.75\text{ mL/min}$; column temperatures $\geq 25^\circ\text{C}$; HPLC retention times $\leq 10\text{ min}$.

^c No ATP and IM2 were eluted.

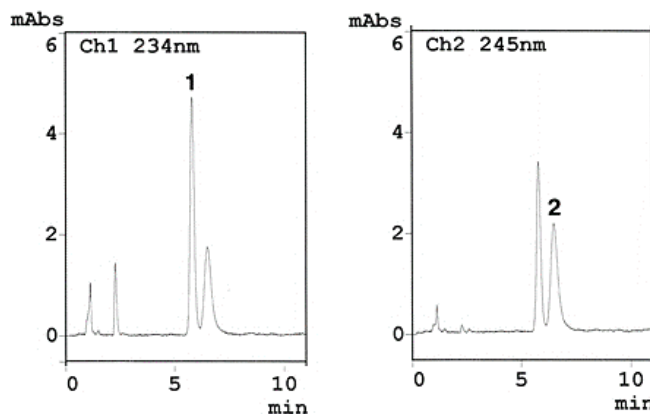


Fig. 2 Typical chromatograms of a standard mixture (0.5 $\mu\text{g/mL}$) obtained from the HPLC system. PDA set at 234 nm (Ch 1) or 245 nm (Ch 2). The injection volume was 15 μL . Peaks, 1= IM2 (retention time, $R_t = 5.75\text{ min}$); 2= ATP ($R_t = 6.49\text{ min}$).

Table 2 Chromatographic method validation data

	IM2 ^a	ATP ^b	Acceptance criterion ^c
Linearity (r) ^d	0.9997	0.9994	≥ 0.999
Range ($\mu\text{g/mL}$)	0.025 – 10		
Detection limit ^e ($\mu\text{g/mL}$)	0.006	0.013	
System suitability :			
a) Capacity factor (k')	4.08	4.67	>2
b) Injection repeatability ^f (RSD, %)			
Retention time	0.31	0.20	≤ 1
Peak area	0.42	0.44	≤ 1
c) Tailing factor	0.75	1.13	≤ 2

^a PDA set at 234 nm.

^b PDA set at 245 nm.

^c Recommendations in the FDA guidelines [10]

^d r is the correlation coefficient ($p < 0.01$) for calibration curve.

^e Detection limit as the concentration of analyte giving a signal-to-noise ratio = 3.

^f Data as the relative standard deviations calculated for 10 replicate injections (10 μL) of a mixed standard solution (0.5 $\mu\text{g/mL}$ of ATP and IM2, respectively).

4. Conclusion

In the present study, a green HPLC-PDA method for detecting ATP and IM2 using an isocratic 100 % water mobile phase has been successfully established. The water mobile phase method is harmless to the environment and to humans and has a short run time and high system suitability. The HPLC system may be proposed as an international harmonized method for detecting ATP and IM2. For the quantification in animal-derived foods, the proposed HPLC method will be applicable enough by performing a suitable sample preparation technique.

References

- [1] European Food Safety Authority (EFSA), EFSA assesses potential link between two neonicotinoids and developmental neurotoxicity. Press release 17 December 2013. <http://www.efsa.europa.eu/en/press/news/131217.htm>
- [2] FAO, JMPR 2005. www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Report11/Acetamidiprid.pdf (Accessed on 12.02.2015)
- [3] K.A. Ford, J.E. Casida, Chloropyridinyl neonicotinoid insecticides: diverse molecular substituents contribute to facile metabolism in mice, *Chem. Res. Toxicol.* 19 (2006) 944-951.
- [4] G. Tanner, C. Czerwenka, LC-MS/MS analysis of neonicotinoid insecticides in honey: methodology and residue findings in Austrian honeys, *J. Agr. Food Chem.* 59 (2011) 12271-12277.

- [5] European Food Safety Authority (EFSA), Review of the existing maximum residue levels (MRLs) for acetamiprid according to Article 12 of Regulation (EC) No 396/20051, EFSA Journal 9 (2011) 2328.
- [6] K. Taira, K. Fujioka, Y. Aoyama, Qualitative profiling and quantification of neonicotinoid metabolites in human urine by liquid chromatography coupled with mass spectrometry, Plos One 8 (2013) 1-12.
- [7] EU classification (The Dangerous Substances Directive 67/548/EEC), Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances, 1967.
- [8] P.T. Anastas, J.C. Warner, Green Chemistry: Theory and Practice, Oxford University Press, Oxford, United Kingdom, 1998.
- [9] T. Yoshimura, T. Nishinomiya, Y. Homda, M. Murabayashi, Green Chemistry: Aim for the Zero Emission-Chemicals, Sankyo Publishing Co. Ltd. Press, Tokyo, Japan, 2000.
- [10] FDA, Reviewer Guidance, Validation of Chromatographic Methods, Center for Drug Evaluation and Research (CDER), 1994.