Residue analysis of picoxystrobin in oriental melon using gas chromatography coupled with electron capture detection and mass spectrometric confirmation: application to dissipation kinetics and risk assessment

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Abstract This study was carried out to determine the residual amounts of picoxystrobin in oriental melon (*Cucumis melo* L.) grown under plastic house conditions at two different sites. Samples collected over 10 days were extracted using acetonitrile and salting out (using solid sodium chloride) and purified using Florisil SPE cartridges. The analyte was determined using GC–ECD and field-incurred residues were verified using GC–MS. The calibration curve was linear over the range 0.02–2.0 mg/L with a $R^2 = 0.9998$. The LOD and LOQ were 0.003 and 0.01 mg/kg, respectively. Recoveries, tested at three spiking levels, were satisfactory with rates in the range 87.7–101.5% and relative standard deviations ≤ 9.6 . The dissipation half-lives were 3.4 and 3.7 days for sites 1 and 2, respectively. Hazard estimates obtained using hazard quotients revealed

no health risk from the suggested pesticide application dosage when considering an adult's body weight, oriental melon consumption, and the acceptable daily intake of picoxystrobin.

Keywords Picoxystrobin · GC–ECD · Dissipation kinetics · Risk assessment · Confirmation · Residue determination

Introduction

Oriental melon (*Cucumis melo* L.), the most widely cultivated horticulture crop, originated in Africa and Southwest Asia and then spread worldwide [1, 2]. Currently, the global production of oriental melon is over 31 million tons per year [3]. Oriental melons contain protein, fat, carbohydrates, vitamins (A, B1, B2, B6, C, and E), and minerals (sodium, potassium, calcium, magnesium, phosphorous, iron, and zinc). In the Republic of Korea, oriental melons are grown under greenhouse conditions and are also known as Korean melons [4].

Fungicides are widely used in agricultural practice to protect crops from fungal attack. There are two types of fungicides: protectant and specific fungicides. Specific fungicides act on a specific functional groups on fungi. For instance, strobilurin fungicides are products derived from naturally occurring β -methoxyacrylates [5, 6], which exert their fungicidal activity through inhibiting the mitochondrial respiration [7–9]. Picoxystrobin (methyl (2*E*)-3-methoxy-2-{2-[6-(trifluoromethyl)-2-pyridyloxy-methyl]phenyl}-acrylate) is a broad spectrum strobilurin fungicide with a unique redistribution property (showing both vapour activity and xylem systemicity) amongst the commercial strobilurin fungicides and those in development. These properties make

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the analyte possessing a good curative activity against a wide range of diseases [5]. In the Republic of Korea, picoxystrobin is registered for use on agricultural crops including oriental melon, ginseng, pepper, cucumber, apple, and onion to protect them from fungal attack, particularly downy mildew and plague [10]. Excess use of chemicals may lead to residues in agricultural commodities, which might have adverse effects on consumers. Considering public health, the regulatory authority of each country has set a maximum residue limit (MRL) for each compound in every crop.

So far, few reports have been published for the determination of picoxystrobin in different matrices. For instance, Kwon et al. [11] developed an analytical method for the determination of picoxystrobin in apple, green pepper, potato, hulled rice, and soybean using gas chromatography (GC)–electron capture detection (ECD) and GC–mass spectrometry (MS). In their study, they extracted the samples using acetone, then partitioned into *n*-hexane using brine, and undertook further cleanup with Florisil SPE cartridges. Wang et al. [12] reported the dissipation and residues of picoxystrobin in banana and soil under field conditions. They extracted the samples using methanol, partitioned with brine and ethyl acetate, and carried out further cleanup by open column chromatography using alumina. The abovementioned methodologies are not cost effective as they are time consuming and use large volumes of solvents. Zhu et al. [13] reported the decline pattern of picoxystrobin residues in peanut and soil. In their study, they used the quick, easy, cheap, effective, rugged, and safe “QuEChERS” method for sample preparation and liquid chromatography–tandem mass spectrometry for determination. It has to be noted that the QuEChERS method with its dispersive-solid phase extraction (d-SPE) cleanup procedure is not suitable for conventional detectors, such as GC–ECD and sample amounts should be representative for accurate residue determination. Notably, there is neither a report on residue determination, nor dissipation kinetics and risk assessment of picoxystrobin in oriental melon. Thence, the current study was conducted to determine the above mentioned objectives using GC–ECD and GC–MS confirmation for field-incurred samples.

Materials and methods

Chemicals and reagents

Analytical grade standard picoxystrobin (purity 99.0%) was provided by Dr. Ehrenstorfer GmbH (Ausburg, Germany). HPLC grade acetonitrile (ACN), acetone, and *n*-hexane were purchased from Honeywell Burdick and Jackson (Ulsan, Republic of Korea). Extra pure (EP) grade ACN was obtained from Daejung Chemicals and Metals

Co. Ltd. (Gyeonggi-Do, Republic of Korea). Analytical grade granular anhydrous sodium sulfate (Na_2SO_4) and sodium chloride (NaCl) were supplied by Junsei Chemical Co. Ltd (Tokyo, Japan). Solid-phase extraction (SPE) Florisil cartridges (1 g, 6 mL) were supplied by Phenomenex (Torrance, CA, USA).

Preparation of standard and working solutions

A picoxystrobin standard stock solution (100 mg/L) was prepared by dissolving of picoxystrobin (5.08 mg) in acetone (50 mL). A working standard solution (20 mg/L) was prepared by diluting the picoxystrobin standard stock solution with acetone. The fortifications and calibration levels were prepared from the working solution by diluting with acetone.

Field experiment

Oriental melon was grown in two different plastic houses located in Seoungju, Republic of Korea. In both plastic houses, one plot of 33 m² was allocated for the application of commercial picoxystrobin (Gyungnong Co. Ltd. Seoul, Republic of Korea) and one control with no pesticide application. Both plots were divided into three parts, each for three replicate samplings. Commercial picoxystrobin formulation (25% a.i suspension concentrate (SC)) was sprayed according to the manufacturer's recommended dose (10 mL/20 L). The calculated sprayed amounts were 17.5 and 15.0 L, for site 1 and 2, respectively, and the harvesting times were April and September 2015, respectively. Samples were randomly collected at 0 (2 h after application) 1, 2, 3, 5, 7, and 10 days, post-application. For each replicate, 10 oriental melons (weighing approximately 2 kg) were collected, sent to Chonnam National University laboratory, cut into small pieces, and then mixed properly in a blender. A representative amount was transferred into a plastic zipper bag and stored at −24 °C until analysis.

Extraction and cleanup

A 20 g sample was placed in a plastic bottle, ACN (100 mL) was added and the bottle was manually shaken for 1 min. Then, sodium chloride (NaCl, 15 g) was added to the mixture, shaken with a mechanical shaker (DS-31F, Dasol Scientific Co. Ltd. Gyeonggi-do, Republic of Korea) for 30 min, and centrifuged at 2000 rpm for 10 min. Subsequently, 40 mL from the upper layer was aspirated and transferred into a 100 mL round-bottomed flask and evaporated to dryness using a rotary vacuum evaporator (R-114; Büchi, Flawil, Switzerland) at 40 °C. Afterwards, the residues were dissolved in *n*-hexane (6 mL) for further cleanup. A SPE Florisil (1 g, 6 mL) cartridge was pre-

conditioned and washed with *n*-hexane (6 mL each) and the eluent was discarded. The analyte was eluted with 10 mL acetone/*n*-hexane (10/90; *v/v*). The eluate was evaporated to dryness using a rotary vacuum evaporator and finally dissolved in acetone (4 mL) and transferred into a GC-vial for analysis.

Instrumental analysis

The samples were analysed by GC (Agilent 7890 A, Agilent Technologies, Inc., Wilmington, USA) equipped with a micro electron capture detector (μ ECD, ^{63}Ni) and an auto sampler (Agilent 7683 B, USA). An Agilent Ultra 2 capillary column (50 m $\times$ 320 μm i.d. $\times$ 0.17 μm film thickness, Agilent Technologies, USA) was used for chromatographic separation, with a N_2 gas flow rate of 1 mL/min. A standard split/splitless injector was used and set up to the split injection mode at a ratio of 10:1 at 260 $^\circ\text{C}$ using an injection volume of 2 μL . The detector temperature was set to 300 $^\circ\text{C}$ with N_2 as make-up gas at a flow rate of 60 mL/min. The oven temperature was maintained at 150 $^\circ\text{C}$ for 1 min then increased to 280 $^\circ\text{C}$ at a rate of 15 $^\circ\text{C}/\text{min}$ and hold for 7 min. A peak corresponding to picoxystrobin appeared at 7.7 min. The Agilent ChemStation software was used for data collection and data processing.

Confirmation of mass was performed with an Agilent Technologies 6890 gas chromatograph equipped with an Agilent 5973 N mass-selective quadrupole detector and an Agilent 7683B autosampler. The chromatographic separation was performed using an HP-5MS capillary column (30 m $\times$ 0.25 mm. i.d. $\times$ 0.25 μm film thickness, J & W Scientific Products). The injector temperature was maintained at 250 $^\circ\text{C}$ and the oven temperature was held at 100 $^\circ\text{C}$ for 2 min and increased at a rate of 20 $^\circ\text{C}/\text{min}$ to 280 $^\circ\text{C}$ and then hold for 2 min. Helium was used as the carrier gas with a flow rate of 1.4 mL/min. The mass spectrometer was operated in electron ionization mode (70 eV) and analysis was carried out in selected ion monitoring (SIM) mode in which four characteristic ions (m/z , 145.0, 173.0, 303.0, and 335.0) were selected. Using the above mentioned conditions, the analyte's retention time was confirmed at 11.12 min.

Method validation

The method was validated in terms of linearity, specificity, limit of detection and quantification, accuracy, and precession. The linearity was expressed in terms of coefficient of determination (R^2), which was obtained from a seven-point calibration curve. There was no interference peak at or around the same retention time of target analyte in blank matrices, indicating method specificity. The limit of detection is the minimum amount of analyte required for a

response peak to be detected and the limit of quantification is the minimum amount that can be quantified by the instrument. Accuracy and precession were obtained from recovery studies; the accuracy was expressed as recovery (%) and the precession as relative standard deviation (%).

Dissipation kinetics

The dissipation kinetics is a parameter that describes how a pesticide declines under the environmental conditions. It is calculated using the first-order kinetics equation $C_t = C_0 e^{-kt}$, where C_t (mg/kg) is the concentration of pesticide at time t and C_0 (mg/kg) is the initial concentration. The decay half-life was calculated using $t_{1/2} = (\ln 2)/k$, where k (day^{-1}) is the dissipation rate constant.

Pre-harvest residue limits (PHRLs)

The PHRL was calculated using the computational software (SPSS 2010, NAQS, Gwangju, Republic of Korea) using the following equation:

$$\text{PHRL} = \text{MRL} \times e^{k_{\text{mint}}}$$

where MRL is the maximum residue limit and k_{mint} is the minimum dissipation constant at time t (calculated computationally by the software). The dissipation constant is the proportional constant representing the amount of residue remaining in an agricultural product.

Dietary risk assessment

The dietary risks of picoxystrobin were evaluated by comparing the acceptable daily intakes (ADIs) with the estimated daily intakes (EDIs). EDIs were estimated by multiplying the average daily-per-capita consumption of oriental melons by the residues quantified in the present study. The dietary risk was expressed as hazard quotients (HQs) [14, 15] and calculated as follows:

$$\text{HQ} = \text{EDI}/\text{ADI} \times 100\%$$

where ADI (mg/kg bw) is the acceptable daily intake and EDI (mg/kg bw) is the estimated daily intake. The risk is considered unacceptable when a HQ is greater than 100% [16].

Results and discussion

Method optimization

The solubility of picoxystrobin in water is 3.1 mg/L, and in methanol, acetone, and ethyl acetate is ≥ 250 g/L; there is

Fig. 1 Chromatograms of picoxystrobin (A) blank sample of oriental melon, (B) standard (2.0 mg/L), (C) blank sample spiked with picoxystrobin (0.1 mg/kg), and (D) field-incurred sample collected on day zero

no available data of its solubility in ACN [17]. ACN was used for extraction rather than acetone because it can be separated from the aqueous layer easier and more

effectively than acetone. The QuEChERS method was not used because very small amounts of sample rarely represent the real data in dissipation studies. Moreover, this method is unsuitable for conventional detector, and the purity of the extracts is generally low. The partitioning step generally requires large volumes of solvent, such as dichloromethane or ethyl acetate, and consequently large

Table 1 Linear equation, average recovery % ($\pm$ RSD), LOD and LOQ of picoxystrobin in oriental melon ($n = 3$)

Pesticide	Linear equation (R^2)	Spiking level (mg/kg)	Average recovery % $\pm$ RSD	LOD (mg/kg)	LOQ (mg/kg)
Picoxystrobin	$y = 1484.4x + 17.949$ (0.9998)	0.01	87.7 ± 9.6	0.003	0.01
		0.1	101.1 ± 3.5		
		2.0	101.5 ± 7.9		

Table 2 Residue and hazard quotients (HQ) of picoxystrobin at sites 1 and 2

Days after treatment	Site 1				Site 2			
	Residue $\pm$ RSD (mg/kg)	Decline (%)	EDI (mg)	HQ	Residue $\pm$ RSD (mg/kg)	Decline (%)	EDI (mg)	HQ
0	0.346	–	0.0039	9.07	0.314	–	0.0035	8.14
1	0.228	34.10	0.0026	6.04	0.201	35.99	0.0023	5.35
2	0.214	38.15	0.0024	5.58	0.143	54.46	0.0016	3.72
3	0.149	42.83	0.0017	3.95	0.125	60.19	0.0014	3.25
5	0.104	69.94	0.0012	2.79	0.082	73.88	0.0009	2.09
7	0.097	71.96	0.0011	2.56	0.068	78.34	0.0008	1.86
10	0.036	89.059	0.0004	0.93	0.045	85.67	0.0005	1.16
Half life	3.4				3.7			
ADI	0.043 (mg)							
FAI	0.0113 (kg)

Fig. 2 (A) Average weight of oriental melons and (B) temperature and humidity at sites 1 (B1) and 2 (B2)

glassware, which makes it not cost effective and time consuming. In the current procedure, the uses of such solvents were avoided.

Specificity

The method specificity was confirmed by comparing the chromatograms of blank samples of oriental melon with spiked samples (in triplicate). As shown in Fig. 1, no interfering peaks are detected at or around the picoxystrobin peak in the blank sample.

Linearity

The linearity of the method was validated using a seven point standard calibration curve, constructed by plotting concentration of standard against peak area. Excellent linearity was obtained, with the linear equation $y = 1484.4x + 17.949$, and the coefficient of determination (R^2) = 0.9998 (Table 1).

Fig. 4 GC–MS chromatograms of 1.0 mg/L standard picoxystrobin (A1) chromatogram, (A2) relative mass spectrum, 0 day field-incurred sample (B1) chromatogram, and (B2) relative mass spectrum

Limit of detection and quantification (LOD and LOQ)

The LOD and LOQ were defined as S/N 3 and 10, respectively. The LOD and LOQ were 0.003 and 0.01 mg/kg, respectively (Table 1). The LOQ is much lower than the MRLs (2.0 mg/kg) established by the MFDS [18], which validates our GC– μ ECD method as effective in measuring the analyte in oriental melon.

Accuracy and precession

Recovery studies were carried out in triplicate at three different fortification levels by spiking samples of 20 g with standard solution. The average recovery at

Fig. 3 Picoxystrobin (A) dissipation patterns and (B) PHRLs at sites 1 and 2

fortification levels of 0.01, 0.1, and 2.0 mg/kg ranged from 87.7 to 101.5% with RSDs of 3.5–9.6% (Table 1). These results are satisfactory according to SANTE guidelines [19].

Method application to dissipation pattern and PHRL

The developed method was successfully applied to determine the residue amounts of picoxystrobin in oriental melon cultivated in two different sites in plastic house condition. As shown in Table 2, the initial (0 day) residue deposits, 0.346 and 0.314 mg/kg for site 1 and site 2, respectively, are considerably lower than the MRL set by the MFDS [18]. The initial concentration at site 1 is slightly higher than site 2, which might be due to the difference in sprayed amounts of picoxystrobin (17.5 L for site 1 and 15 L for site 2). After 10-day post-application, the residual levels decline to 0.036 and 0.045 mg/kg, for site 1 and site 2, with a decline percentage of 89.059 and 85.67%, respectively. There are many factors that could affect the decline rate of pesticides, including environmental conditions, seasonal variation, pesticide formulation, and sample size [20–22]. In our experimental work, the temperature was slightly higher at site 1 than 2 (Fig. 2), and the fruit sizes were approximately the same for both sites (Fig. 2). The half-life of picoxystrobin was approximately the same, 3.4 and 3.7 days, for sites 1 and 2, respectively, suggesting that the season does not affect the decline behaviour. Zhu et al. [13] found the dissipation half-lives of picoxystrobin of 2.1–2.8 days in peanut seeds and 1.5–8.6 days in field soil. On the other hand, Wang et al. [12] reported dissipation half-lives of picoxystrobin of 10.7–12.1 days in banana and 12.5–13.4 days in soil. As the PHRL graph shows (Fig. 3), if the residue is $\leq 4.39/3.87$ mg/kg (site 1/site2) 5 days before harvest, then after harvest the residue will be below the MRL levels. As well, if the residue is $\leq 9.65/7.45$ mg/kg (site 1/site 2) 10 days before harvest then the residue will also be below the MRL after harvest.

GC–MS confirmation

The finding obtained by a conventional detector can sometimes be false-positive results. Thus, authentication by mass confirmation is an important tool, as it results in identification at the molecular structure level [23]. Therefore, the presence of picoxystrobin in real samples was confirmed by GC–MS (Fig. 4). Picoxystrobin standard (1.0 mg/L) and 0 day real samples were confirmed by mass spectrometry working within the scan range m/z 50–450 and four ions (m/z = 143.0, 173.0, 303.0, and 335.0) were

selected for mass confirmation from the relative mass spectrum.

Risk assessment

In the Republic of Korea, the MFDS has set the MRL for picoxystrobin in oriental melon to 2.0 mg/kg [18]. On average, 0.0113 kg of oriental melon are consumed per day by the average Korean adult (average weight = 55 kg) [24]. The acceptable daily intake (ADI) of picoxystrobin is 0.043 mg [25]. The risk quotients (RQ) were determined from the risk assessment. The RQ level at 0 day was 8.14–9.07%, which was far below the 80% consumer's health risk level. At 10 days, the RQ decreased to 0.93–1.16%, which is also well below the RQ at 0 day. Overall, these results suggest that picoxystrobin applied to oriental melon at the recommended doses is unlikely to induce adverse effects in Korean consumers.

In conclusion, a method with satisfactory validation parameters was developed for residue analysis of picoxystrobin in oriental melon cultivated in plastic house conditions at two different locations using GC– μ ECD and GC–MS for confirmation. The samples were extracted with ACN and NaCl, and purified using SPE Florisil cartridges. Dissipation of picoxystrobin in oriental melon followed a first-order exponential equation with dissipation half-lives of 3.4 and 3.7 days for site 1 and site 2, respectively. This data could be used as food safety guidance. Residues after harvest can be predicated from the data calculated from the PHRL curve. Hazard estimates obtained using hazard quotients (HQs) revealed no health risk.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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