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Determination of Field-Incurred Chlorfluazuron Residues in the Peach

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Abstract Chlorfluazuron residues were determined in the peaches that were sprayed at dosage (a.i. 0.0167 kg $10a^{-1}$), using an analytical method that was validated as follows; $r^2 = 0.9999$, 0.02 mg kg^{-1} (LOQ) and 87.8–93.6% (recovery). The residues from all samples were lower than the MRL (0.5 mg kg^{-1}, Korea). A maximum 0.27 mg kg^{-1} of chlorfluazuron was detected in the samples applied at 6 days before harvest. The results signify that the 10% SC product would be used safely as an insecticide if it is applied two or three times onto peaches, with applications given until 6 days prior to harvest.

Keywords Chlorfluazuron · Peach · Field-incurred residues · HPLC-UVD

Chlorfluazuron is a member of the benzoylureas (BUs) which have been recognized as promising insecticides with great potential use in controlling insect attack on a broad range of fruits and vegetables (Tomšej and Hajšlová 1995). BUs are the fourth generation of insecticides with many attractive properties, including high selectivity, low acute toxicity in mammals, and profound biological activity (Tomlin 2000), which make them suitable for inclusion in integrated pest management programs for fruits and vegetables, and have been recommended for the control of Lepidoptera, Phyllide, Diptera, and Coleoptera on a wide variety of crops, including fruit trees (Tomlin 2000; Shim et al. 2007). Chlorfluazuron functions as a powerful growth regulator, inhibiting cuticle chitin synthesis in target pests, thereby inducing death or abortive development (Hajjar and Casida 1978). Sammour et al. (2008) described the pronounced efficacy of chlorfluazuron in reducing egg hatchability and fecundity in the most notorious and destructive phytophagus insect pest, *Spodoptera littoralis*.

As the peach is a major fruit-accounting for the sixth-largest cultivation area and production levels in 2006 (13,383 ha and 193,816 tons, respectively), in the Republic of Korea (KOSIS 2010) it has been treated with a broad variety of pesticides, including insecticides, fungicides, herbicides, and plant growth regulators, in order to control pests or weeds in peach cultivation. Although the pesticide usage carries some potential risks to consumers if pesticide residues remain, public concerns can be assuaged by the creation of maximum residue limits (MRLs) or pre-harvest intervals of pesticides, both of which may foster safer pesticide use. However, pesticide residue analysis is also critically important to food safety and public health.

Several analytical methods have been previously proposed for the determination of the BUs in fruits and vegetable matrices (Hiemstra et al. 1999). Owing to thermal instability and poor GC properties, reverse-phase high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection is the predominant method employed for the determination of BUs. Wimmer and Smith (1991) have reported the degradation of diflubenzuron by the heat of GC into three fragments. Tomšej and Hajšlová (1995)

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analyzed five BUs in the apple using a HPLC apparatus equipped with a diode-array detector, and Kaihara et al. (2002) conducted a multiresidue analysis of 18 pesticides, including chlorfluazuron, in fruits, vegetables, and rice using supercritical fluid extraction (SFE) followed by liquid chromatography-electrospray ionization mass spectrometry. Liquid chromatography with electrospray tandem mass spectrometry (LC/ESI-MS/MS) was employed for the determination of seven BUs by Sannino and Bandini (2005), and Barnes et al. (1995) employed high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry (HPLC-APCI-MS) to determine diflubenzuron levels in mushrooms. SFE or mass spectrometric detections are valuable regarding solvent consumption and reduction of analysis time; however, these methods require sophisticated and expensive apparatuses, as well as certain preconditioning techniques (Shim et al. 2007; Nochetto et al. 2009). In this study, we conducted chlorfluazuron residue analysis in the peach after treatment with different programs using the HPLC-UV detection system and estimated the safety of the residues considering the MRL of chlorfluazuron in the peach.

Materials and Methods

Pure standard chlorfluazuron (purity 99.6%) and its commercial product (Atabron®, 10% suspension concentrate) were generously provided by Kyung Nong Co. (Seoul, Republic of Korea) and its structure is shown in Fig. 1. Acetonitrile, *n*-hexane and ethyl acetate were of HPLC-grade and were obtained from Merck (Darmstadt, Germany). Sodium sulfate (anhydrous) 99.0% (Merck, Darmstadt, Germany) was purified by 3 h of heating at 600°C. Analytical-grade Celite 545 and sodium chloride were obtained from Duksan Pure Chemicals Co. Ltd (Ansan, Republic of Korea). Florisil at a purity suitable for pesticide residue analysis was purchased from Sigma–Aldrich (Missouri, USA). All other chemicals and reagents utilized herein were of analytical grade, unless otherwise noted. Gloves were worn when appropriate during the experimental procedure and solvent/extract transfers were conducted in well-ventilated exhaust hoods, owing to the toxicity of a number of the compounds and solvents employed in the following procedures.

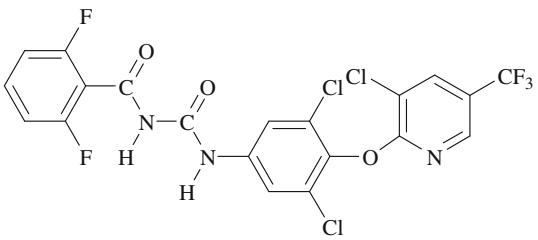

Fig. 1 The chemical structure of chlorfluazuron

Stock solution was prepared via the dissolution of 100.4 mg of chlorfluazuron (99.6%) in 100 mL of acetonitrile in order to achieve a concentration of 1,000 μg mL^{-1}. The theoretical amounts added to the stock solution were corrected and the actual concentrations were obtained. The stock chlorfluazuron solution was serially diluted with blank extracts of the peach samples in order to prepare working standard solutions of five different concentrations of 0.02, 0.04, 0.08, 0.2, and 0.4 mg kg^{-1}. A portion of 20 μL of each concentration was injected into HPLC-UVD to obtain the calibration curve. All of the standard solutions were stored at −4°C while not in use.

The experimental trials were conducted at Sol-Nongwon located in Hwasun, in the southern part of Gwangju, Republic of Korea. The experimental area consisted of five plots, each containing three trees, and chlorfluazuron SC (Atabron®; formulation, a suspension concentrate containing 10% chlorfluazuron) was applied in June 2006 to 14-year old trees at the manufacturer's recommended dose of a.i. 0.0167 kg $10a^{-1}$, using a pressurized handgun-style applicator. The pesticide was applied to the trees either two or three times except in the control group, and the schedules are described in Table 1. The treated peaches were grown without paper wrap, and the average daily temperature and the rainfall were 23.5°C and 3.6 mm, respectively, in June. Sampling was conducted in accordance with the recommendations of the Codex Alimentarius (CAC/GL 33 1999) via the random collection of a total of 16 peach samples from several positions in all trees from each plot. The samples were forwarded to the laboratory on the same day, and the collected peaches were chopped and mixed homogeneously. The chopped samples were then packed in plastic bags, labeled, and stored in individual bags at −24°C until extraction.

A 50-g representative sample of peach homogenized with 100 mL of acetonitrile for 2 min at 1,100 rpm in a

Table 1 Spraying schedule of chlorfluazuron 10% SC onto peach trees

Treatment frequency	Spraying date prior to harvest	Final spraying date	Harvest date
0	–	–	July 2, 2006
2	20-14	June 18, 2006	
2	14-6	June 26, 2006	
3	40-30-21	June 11, 2006	
3	30-21-14	June 18, 2006	
3	21-14-6	June 26, 2006	

blender (WiseMix^{TM} HG15A, Daihan Scientific, Seoul, Republic of Korea). The homogenate was filtered through Whatman filter paper (No. 6, i.d. 90 mm, England) containing 20 g of Celite 545, resting on a porcelain Büchner funnel connected to a suction flask. The filtrate was then transferred to a separatory funnel (1 L) to which 30 mL of saturated sodium chloride solution and 500 mL of distilled water were added. The content was thoroughly mixed via gentle shaking and was partitioned with 100 mL of ethyl acetate with vigorous shaking. The organic layer was separated and partitioning was again performed with an additional 50 mL of ethyl acetate. The combined organic layers were dehydrated by passing through anhydrous sodium sulfate over a cotton filter, after which the dehydrated filtrate was evaporated under vacuum until completely dried, and then taken for clean-up.

The dried extract was re-dissolved in 10 mL of *n*-hexane and cleaned-up through an open preparative chromatographic column. A glass column (1.1 cm, i.d.) was packed with 5.0 g of florisil (60–100 mesh) in *n*-hexane. The sample (10 mL) was loaded onto it and eluted with a 150 mL mixture of hexane and ethyl acetate (7:3, v/v). The eluate was then evaporated to dryness in vacuo, followed by reconstituted with 2 mL of acetonitrile, and then subjected to HPLC analysis.

The HPLC system (Kontron Instruments, Milano, Italy) utilized in this study consisted of a HPLC 335 Detector (UV–Vis) and a HPLC 322 System (pump) connected to a computing program. A mixture of acetonitrile and deionized water (8:2, v/v) was employed as the mobile phase for the separation of chlorfluazuron in the peaches using an Apollo C_{18} (250 × 4.6 mm i.d., 5 μm, Alltech, IL, USA) column at a wave length of 254 nm at a flow rate of 1.0 mL min^{-1}.

Triplicate recovery analyses were carried out at two different levels of fortification (0.04 and 0.2 mg kg^{-1}) by spiking appropriate concentrations of chlorfluazuron working solutions to uncontaminated blank peach samples (50 g). The fortified samples were thoroughly mixed and permitted to stand for 30 min prior to extraction. The samples were then extracted, purified, and analyzed via the sample preparation procedures described above in this paper. The recovery rate was calculated by comparison of the peak areas of the fortified samples with those of the standard solutions. Recoveries with standard deviations (SDs) are provided in Table 2.

The chemical stability of the analyte in samples was assessed via recovery experiments. When the field-incurred peach samples were mixed homogeneously and maintained in a refrigerator, the fortified blank peach samples at 0.2 mg kg^{-1} were also maintained, and lasted until the other whole experiments were completed. In the final step of this study, the fortified samples were pulled from the refrigerator and examined to determine any differences in their concentrations.

Results and Discussion

Selectivity was evaluated via the analysis of blank samples extracted in accordance with the same preparation procedures as were utilized in this study. The absence of any peak above the signal-to-noise ratio of 3 at the retention time of chlorfluazuron indicated that no interference had occurred (Fig. 2).

Calibration curve was obtained by plotting peak areas in 'y' axis against concentrations of chlorfluazuron in 'x' axis within the investigated range (0.02–0.4 mg kg^{-1}) of concentrations. The linearity were good with an excellent correlation coefficient of $r^2 = 0.9999$. The residual concentrations of chlorfluazuron in the field-incurred peach samples were determined via the calibration curve developed herein.

The LOQ was determined on the basis of a signal-to-noise ratio of 10 (S/N 10) which was performed by comparison of the measured signals from the samples spiked with known low concentrations of chlorfluazuron with those of blank samples. The LOQ of chlorfluazuron in this study was measured to be 0.02 mg kg^{-1}, which was substantially lower than the MRL of chlorfluazuron in the peach (0.5 mg kg^{-1}, Republic of Korea).

The extraction efficiency of the analytical procedure was evaluated via recovery experiments conducted in triplicate using the fortified blank peach samples at two different concentration levels. The average percent recoveries obtained were between 87.8 ± 9.7% and 93.6 ± 9.2%, with standard deviations of less than 10% (Table 2).

The residue analyses of the field samples were implemented at the end of the total experiments. As the

Table 2 Recovery, storage stability, and limit of quantification (LOQ) of the analytical method conducted in this study, and the MRL of chlorfluazuron in the peach

Pesticide	Recovery (mean ± SD, %)			LOQ (mg kg^{-1})	MRL[a] (mg kg^{-1})
	0.04 mg kg^{-1}	0.2 mg kg^{-1}	Storage stability (0.2 mg kg^{-1})		
Chlorfluazuron	93.6 ± 9.2	87.8 ± 9.7	95.4 ± 3.3	0.02	0.5

[a] Korea Food and Drug Administration, Republic of Korea

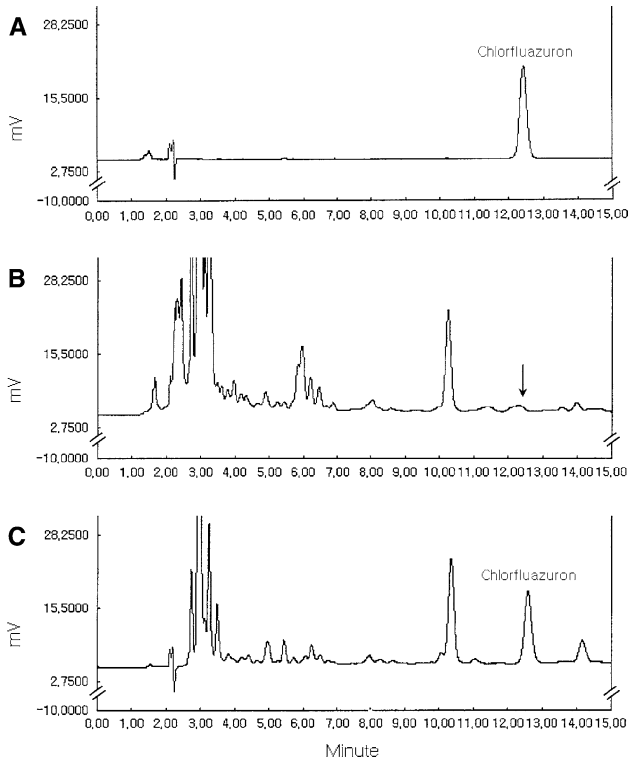

Fig. 2 Typical chromatograms of chlorfluazuron standard in solvent at 5.0 mg L^{-1} (**a**); blank peach sample (**b**); recovery peach sample at 0.2 mg kg^{-1} (**c**)

determination of the field-incurred chlorfluazuron residues could be delayed due to unexpected systematic errors and/or poor preliminary experimental results, the actual residues could be altered by chemical or metabolic reactions with sample matrices during storage. Therefore, it is necessary that the stability of the analyte be assessed in samples stored for experimental periods of time. Triplicate recovery experiments were conducted at 0.2 mg kg^{-1} in this study. The mean recovery rate for storage stability was 95.4 ± 3.3%, which indicates that chlorfluazuron is relatively stable in samples under storage conditions during the experimental period of time in this study.

Peaches were applied with chlorfluazuron 10% SC at different days and frequencies in the orchard, and were harvested on the same day. Table 3 shows the residual concentration of chlorfluazuron in the incurred peach samples. The maximum residues were detected in the samples sprayed 6 days before harvest, regardless of the treatment frequencies of 2 and 3 times (0.15 and 0.27 mg kg^{-1}, respectively). In the samples collected with a longer pre-harvest interval, the chlorfluazuron residues were lower. Additionally, the residues of the peach samples sprayed 3 times were relatively higher than those of the samples sprayed 2 times at identical pre-harvest intervals. However, the residual concentrations of all treatments including those sprayed 6 days prior to harvest were below its MRL (0.5 mg kg^{-1}). These study results make it possible to predict that spraying with chlorfluazuron 10% SC three times until 6 days before harvest should be permitted at the recommended dose, as evidenced by the detection in this study of residues below the MRL of chlorfluazuron. The results demonstrated that the treatment schedules of chlorfluazuron 10% SC in the peach were not harmful to consumers, according to the measured residue levels and established MRL values.

Table 3 Field-incurred residues of chlorfluazuron in peach samples

Treatment frequency	Elapsed days after final treatment	Residue of chlorfluazuron (mg kg^{-1})			
		Replicate-1	Replicate-2	Replicate-3	Maximum
0	–	–	–	–	–
2	14	0.06	0.05	0.07	0.07
2	6	0.14	0.15	0.15	0.15
3	21	0.08	0.06	0.07	0.08
3	14	0.15	0.14	0.13	0.15
3	6	0.27	0.26	0.24	0.27

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