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ARTICLE

Detecting fludioxonil residues in brown rice and rice straw using gas chromatography-nitrogen phosphorus detector

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Abstract In the present study, brown rice was steeped in 20 % wettable powder fludioxonil for 24 h, subsequently germinated, and transplanted in paddy fields. The harvested rice was tested at 156 days to detect residue levels using gas chromatography-nitrogen phosphorus detector. Validation was carried out to assess the following parameters: linearity, limit of detection and limit of quantitation (LOQ), recovery, and storage stability. Using matrix-matched calibrations, the determination coefficients were >0.999 in both matrices. Mean recoveries were 73.5–101.0 % with relative standard deviations <10 % in both matrices. The LOQ (0.006 mg/kg) was lower than the maximum residue limit (MRL = 0.02 mg/kg) set by the Ministry of Food and Drug Safety, Republic of Korea. The developed method was applied successfully, and no residues were detected in field-incurred rice and/or rice straw samples.

Keywords Analysis · Fungicide · Rice · Rice straw · Gas chromatography · Contaminant

Ah-Young Ko and Jin Jang have contributed equally to this study.

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Introduction

Rice is an important cereal crop and is extensively consumed worldwide in various forms. As rice consumption has increased (in accordance with population growth), pesticides, including herbicides (applied before rice transplantation), fungicides, and insecticides have been used (depending upon the conditions, rain, or insect attack) to improve productivity (Lee et al. 2014). Rice production in the Republic of Korea is affected by fungi, such as *Fusarium* species, and spoiled by *Aspergillus* and *Penicillium* during storage (Park et al. 2005), resulting in reduced yield. These genera can produce various mycotoxins, including aflatoxin B_1 (AFB_1), fumonisin B_1 (FB_1), ochratoxin A (OTA), trichothecenes, and zearalenone (ZEN), which are toxic to vertebrates when introduced via a natural route in small concentrations (Park et al. 2005). Therefore, fungicides are needed to control fungi. Fludioxonil is a non-systemic fungicide used as a seed treatment to control *Fusarium*, *Microdochium*, *Rhizoctonia*, *Tilletia*, *Pyrenophora*, and *Septoria* spp. in both cereal and non-cereal crops (MacBean 2012). Fludioxonil has been used in the Republic of Korea against the seedborne fungi *Fusarium fujikuroi and Gibberella fujikuroi,* which cause wintering bakanae disease in rice seed. This disease leads to abnormal plant growth (due to the production of gibberellic acid, a plant growth hormone) and has become a problem

in agriculture because of the significant reduction in rice productivity it causes (Park et al. 2009).

Due to their physicochemical properties, pesticides not only remain in treated crops but can also leach into soil and water. Residues have been linked to a wide spectrum of human health hazards, ranging from short-term impacts such as headaches and nausea to chronic impacts, including cancer, reproductive disorders, and endocrine disruption (Blasco et al. 2006). Despite the reduced toxicity of fludioxonil to humans, the proximity between application and consumption makes exposure risk a concern (Abad-Fuentes et al. 2014) and necessitates a residue analysis.

Two controversial trends currently exist for pesticide use. New laws were approved in the European Union, USA, and Canada to restrain use of agrochemicals. This legislation is aimed at protecting consumers through more thorough toxicological testing of compounds and enforcement of lower concentration limits for residues tolerated in food and water (Harris and Tomerlin 2002; Carvalho 2009). In contrast, use of crop protection chemicals seems to be a simple way to meet agricultural productivity targets in developing countries. Consequently, it is important to establish maximum residue limits (MRLs) for pesticides to produce safe and healthy agricultural products. The MRL, a regulatory limit for agricultural product safety, is established by the Ministry of Food and Drug Safety (Republic of Korea) based on results of pesticide residue trials and risk assessment as well as toxicology data.

In a literature survey, fludioxonil has not been analyzed in a single or multiple residue analysis in grains. In the present study, rice seeds were soaked in 20 % water dispersible granule (WG) fludioxonil for 24 h before seeding. An acetate-buffered quick, easy, cheap, effective, rugged, and safe (QuEChERS) method and gas chromatography-nitrogen phosphorus detection (GC–NPD) were used to determine brown rice and rice straw residue levels after harvesting at 156 days.

Materials and methods

Chemicals and reagents

Fludioxonil (purity: 99.9 %) and its formulation (20 % WG) were donated by Dongbufarm-Hannong (Seoul, Republic of Korea). Acetonitrile, acetone, ethyl acetate, and high performance liquid chromatography-grade *n*-hexane were purchased from Burdick and Jackson (Ulsan, Republic of Korea). Magnesium sulfate, sodium acetate, and Celite545 were obtained from Junsei Chemicals Co., Ltd. (Chuo-ku, Japan). Primary secondary amine (PSA) and C18 were supplied by Agilent Technologies (Palo Alto, CA, USA). Silica solid-phase extraction (SPE) cartridges (1,000 mg, 6 mL) were provided by Phenomenex (Torrance, CA, USA).

The fludioxonil standard (0.01 g) was dissolved in 100 mL acetone to obtain a 100 mg/L stock solution. The stock solution was serially diluted with extracts of blank brown rice or rice straw to prepare working concentrations of 0.01, 0.05 0.1 0.5, 1.0, and 2.0 mg/kg. All standard solutions were stored at 4 °C pending analysis.

Field trial

Experimental trials were carried out in a paddy field located at Chonnam National University, Gwangju, Republic of Korea. Rice seeds were soaked in a single (1:4,000 dilution) or double dose (1:2,000 dilution) of 20 % WG fludioxonil for 24 h. The treated rice seeds were germinated and transplanted in paddy fields. Three experimental plots (one used as a control) were prepared on the outdoor paddy fields, and approximately 240 rice plant bundles were planted. The rice was cultivated for 156 days according to conventional culture methods. Approximately, 100 bundles were harvested from each experimental plot. After threshing, the grains and rice straw were dried in a drying room. All samples were stored at −20 °C until analyses.

Sample preparation

Brown rice

Although the QuEChERS method is an attractive sample preparation method, detection with a universal detector, such as NPD, could create complex chromatograms due to sample matrix interference. Therefore, a modification during purification is necessary when such a detector is used (Jang et al. 2014). Sample preparation was based on the acetate-buffering QuEChERS method (Lehotay et al. 2005) with modifications. At no point were the extraction conditions optimized. Rather, the experimental variables including solvents, salting-out agents, and cleanup procedure were predicated based on our experience. 10 g of frozen ground sample was placed into a centrifuge tube to which 20 mL of acetonitrile and 10 mL of distilled water were added and vortex-mixed for 1 min. 6 g of magnesium sulfate and 1.5 g of sodium acetate were added and vigorously shaken for 1 min followed by centrifugation at 5,000 rpm for 5 min. 15 mL of the supernatant was mixed with 1.2 g magnesium sulfate, 0.5 g PSA, and 0.5 g C18 and shaken for 1 min followed by centrifugation at 5,000 rpm for 5 min. A 10 mL aliquot of the supernatant was evaporated using a rotary vacuum evaporator and re-dissolved in 1 mL of acetone followed by GC-NPD analysis.

Rice straw

5 g of frozen ground sample was weighed into an Erlenmeyer flask, and 50 mL distilled water was added and sonicated for 20 min. The samples were mixed with 100 mL acetonitrile and shaken for 30 min. The samples were filtered through filter paper (Whatman No. 6) and Celite 545 under reduced pressure. The filtrates were mixed with 12 g magnesium sulfate and 3 g sodium acetate and vigorously shaken for 1 min followed by centrifugation at 3,000 rpm for 5 min. A 50 mL aliquot of the supernatant was concentrated using a rotary vacuum evaporator and re-dissolved in 10 mL *n*-hexane for purification.

A SPE silica cartridge (1,000 mg, 6 mL) was conditioned with 10 mL *n*-hexane, and 10 mL of the extract was loaded. The cartridges were washed with 20 mL *n*-hexane–ethyl acetate (85:15, v/v), and the residues were eluted with 20 mL *n*-hexane–ethyl acetate (80:20, v/v). The eluant was evaporated to dryness and re-dissolved in 1 mL of acetone and analyzed by GC-NPD.

Instrumental analysis

An Agilent Technologies 6,890 N Network GC system equipped with NPD and an Agilent Technologies 7683 B autosampler were used to detect the sample residues. Chromatographic separation was conducted using a DB-5 capillary column (30 × 0.25 mm, 0.25 μm film thickness, Agilent Technologies). Nitrogen was used as the carrier gas at a flow rate of 10 mL/min. The detector was operated at 280 °C (brown rice) or 300 °C (rice straw) with He make-up gas flowing at 5 mL/min (brown rice) or 7 mL/min (rice straw). Oven temperatures were controlled as follows: initial temperature was 120 °C, increased to 280 °C at 10 °C/min, and held for 2 min. The oven was kept at 280 °C (brown rice) or 300 °C (rice straw) for 3 min for the post-run analysis. A 2 μL aliquot of standard solution or sample extract was injected in splitless mode. Retention times were 12.4 min for rice straw and 12.5 min for brown rice under these conditions.

Method validation

Validation was carried out to assess the following criteria: linearity, limit of detection (LOD) and limit of quantitation (LOQ), recovery, and storage stability. Matrix-matched calibration was used due to the significant matrix-induced enhancement effect of fludioxonil. Calibration curves were constructed at the following levels: 0.01, 0.05, 0.1, 0.5, 1.0, and 2.0 mg/kg, and the responses were plotted versus concentration. Linearity was assessed by the determination coefficient (R^2).

Matrix-matched standard, blank, and spiked blank extracts were injected to determine retention times and interfering peaks for selectivity and specificity, respectively.

LOD and LOQ were determined considering signals 3 and 10 times higher than baseline noise, respectively.

Recovery was determined using blank samples fortified with a standard solution at two different levels in triplicate. Extraction was carried out as described above.

Results and discussion

Specificity

Specificity was tested by analyzing blank samples to assure the absence of potential interfering components at the fludioxonil retention time. No interfering peaks were observed at the retention time, as shown in Figs. 1 and 2.

Specificity

Linearity. Linearity was evaluated by R^2 of the matrix-matched calibration curve in the range of 0.01–2.0 mg/kg (Table 1). The linear equations were $y = 6881550.6x - 213025.8$ with $R^2 = 0.9999$ and $y = 4188408.59x + 40427.35$ with $R^2 = 0.9994$ for brown rice and rice straw, respectively. The high R^2 values demonstrated good linearity for fludioxonil in both matrices within the tested range.

LOD, LOQ, and recovery

LOD, LOQ, and recovery of fludioxonil in brown rice and rice straw are shown in Table 1. The LODs were 0.002 and 0.004 mg/kg, and the LOQs were 0.006 and 0.013 mg/kg, respectively. The LOQ was three times lower than the respective MRL (0.02 mg/kg) established by the Ministry of Food and Drug Safety (MFDS 2013). Average recoveries from brown rice were 89.8–101.0 % with a relative standard deviation (RSD) <10 %. Average recoveries in rice straw were 73.7–83.4 % with a RSD <10 %. Our findings were supported by the guidelines set by the Food and Drug Safety Department and SANCO, indicating that mean recovery should be 70–120 % (SANCO 2009). (Table 1).

Storage stability

Stability of fludioxonil in/on rice (fortified with 0.1 mg/kg) and rice straw (fortified with 0.2 mg/kg) was tested after 55 and 60 days of storage at −20 °C, respectively. Results are expressed as recovery rates of 89.5 ± 2.2 % (rice) and 84.1 ± 4.5 % (rice straw) and were acceptable, indicating that the analyte did not decompose under the tested conditions.

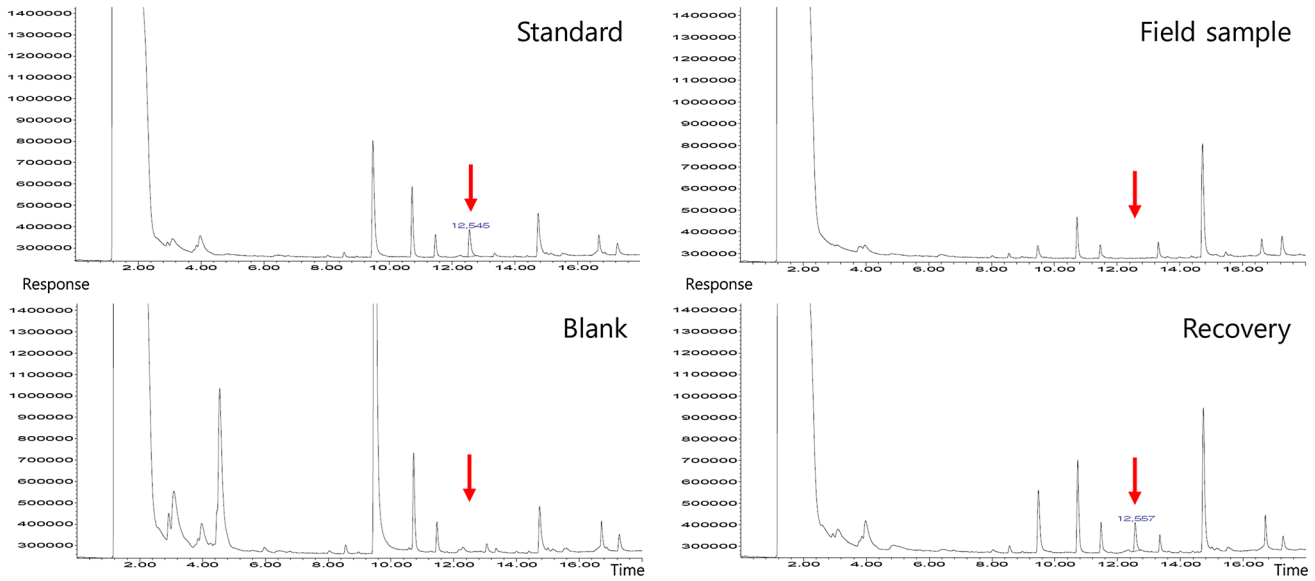

Fig. 1 Representative gas chromatography-nitrogen phosphorus detection chromatograms of fludioxonil in rice containing no detectable residue in blank or field-incurred samples compared to fludioxonil standard (in solvent) or spiked concentration to blank samples (recovery)

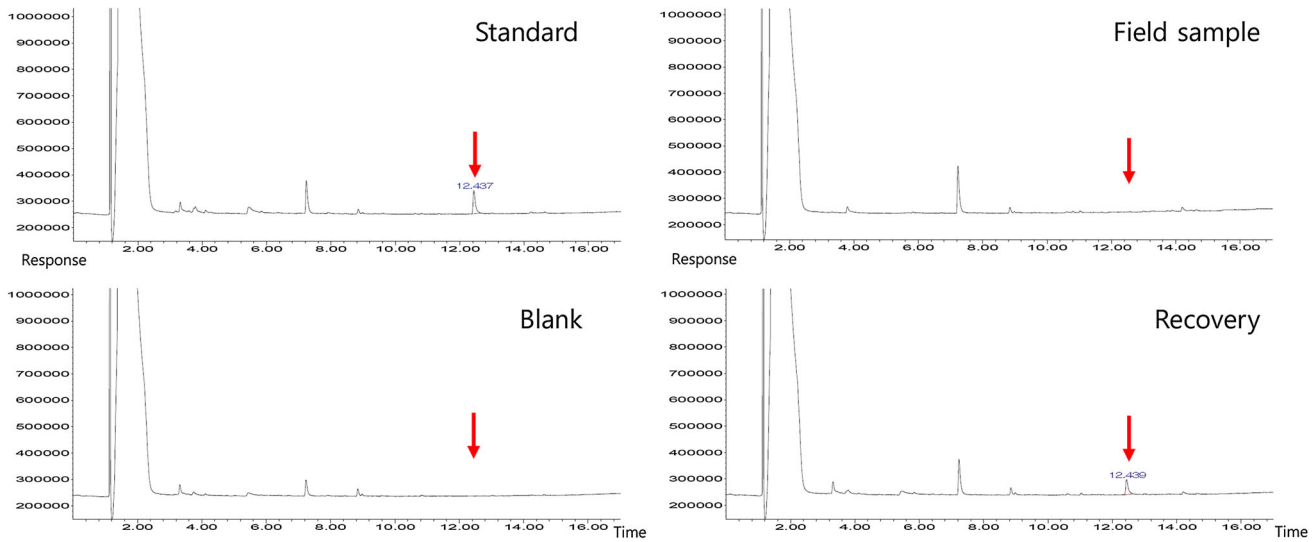

Fig. 2 Representative gas chromatography-nitrogen phosphorus detection chromatograms of fludioxonil in rice straw containing no detectable residues in blank or field-incurred samples compared to fludioxonil standard (in solvent) or spiked concentration to blank samples (recovery)

Table 1 Linearity, fortification level, recovery, limit of detection (LOD), and limit of quantification (LOQ) of fludioxonil in brown rice and rice straw

Sample	R^2	Fortification level (mg/kg)	Recovery (%)				LOD (mg/kg)	LOQ (mg/kg)
			Replicate			Mean (RSD)		
			1	2	3			
Brown rice	0.9999	0.02	91.8	109.3	101.8	101.0 (8.7)	0.002	0.006
		0.1	92.5	86.0	90.7	89.7 (3.8)		
Rice straw	0.9994	0.04	72.9	74.2	73.6	73.5 (0.9)	0.004	0.013
		0.2	74.3	86.6	89.4	83.4 (9.7)		

Table 2 Fludioxonil residues in field-incurred rice and rice straw

Treatment	Elapsed days until harvest	Residue (mg/kg)				MRL (mg/kg)
		Replicate			Average	
		1	2	3		
Untreated	–	<0.002	<0.002	<0.002	<0.002	0.02*
Single dose	156 days	<0.002	<0.002	<0.002	<0.002	
Double dose	156 days	<0.002	<0.002	<0.002	<0.002	
Rice straw						
Untreated	–	<0.004	<0.004	<0.004	<0.004	–
Single dose	156 days	<0.004	<0.004	<0.004	<0.004	
Double dose	156 days	<0.004	<0.004	<0.004	<0.004	

* Ministry of Food and Drug Safety (2013)

Fludioxonil residue analyses

The harvested brown rice and straw were tested to detect residues following method development. None of the samples contained residues higher than the LOD (Table 2 and Figs. 1, 2).

In conclusion, the acetate-buffered QuEChERs method was successfully modified and validated to determine fludioxonil residues in brown rice and rice straw. All LOQ values were lower than the established MLR, indicating that the method was sensitive and reliable to detect lower fungicide concentrations in rice. Recovery was independent of matrix type and the fortified level. A residue level higher than the LOD was not detected in any of the tested samples. This validation suggests that the method developed and the outcomes can be used as a basic data for a safety pesticide evaluation and to establish the MRL.

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