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SHORT COMMUNICATION

Residue analysis of orthosulfamuron herbicide in fatty rice using liquid chromatography–tandem mass spectrometry

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ABSTRACT

In the present study, orthosulfamuron residues were extracted from fatty (unpolished) rice and rice straw using a modified QuEChERS method and analyzed using liquid chromatography–tandem mass spectrometry. The matrix-matched calibration was linear over the concentration ranges of 0.01–2.0 mg/kg with determination coefficient (R^2) $\geqslant$ 0.997. The recovery rates at two fortification levels (0.1 and 0.5 mg/kg) were satisfactory and ranged between 88.1% and 100.6%, with relative standard deviation (RSD) $<$8%. The limit of quantitation, 0.03 mg/kg, was lower than the maximum residue limit, 0.05 mg/kg, set by the Ministry of Food and Drug Safety in the Republic of Korea. The developed method was applied successfully to field samples harvested at 116 days and none of the samples were positive for the residue.

Introduction

Rice is one of the most consumed grains in the world. As its consumption has been increased in accordance with population growth, the use of pesticides, including pre- and post-emergence herbicides, insecticides, and fungicides, raised consequently to improve its production during the various stages of cultivation [1]. In the Republic of Korea, the main pesticides employed are herbicides (before rice transplantation) and fungicides or insecticides, depending upon the conditions (rain or insect attack). After harvesting, several steps are

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needed to produce the final marketing products; including paddy, brown, and white rice [2]. As the nutritional components are mainly exit in the germ and bran layers, the nutritional quality of various rice forms is diverse. In rice, the high and low molecular weight components are either enhancing the response or interfering with compound identification and quantitation in chromatographic analysis [3]. Rice straw, which is separated from rice grains by a combine tractor, was used as a feed for livestock production in the Republic of Korea. So far, little is known on which proportion of the pesticide originally applied in the field could be found in various types of rice and rice straw, which necessitates residue analysis.

Orthosulfamuron, one of the sulfonylureas (SUs), is a selective and systemic early post-emergence herbicide, which is absorbed by foliage and root and translocated apoplastically and symplastically into the plants. It inhibits acetolactate synthase (ALS) enzyme, which catalyzes the first committed step in the branched-chain amino acids (valine, isoleucine, and leucine) biosynthetic pathway and hence stop cell division and plant growth [4]. ALS-inhibiting herbicides are important in all crops due to their efficiency at low rates, flexibility of use, favorable environmental profile, and low mammalian toxicity [5] compared to other alternative herbicides [6]. Orthosulfamuron controls the post-emergence of annual and perennial broad leaves weeds, sedges and barnyard grass, in dry and water-seeded and transplanted rice [4]. It is therefore, possible that the residues of this herbicide may contaminate and be accumulated in grains, including barely, wheat, rice, and soybeans [7]. Because of their low application rates and thermal instability, the determination of SU residues continues to present an analytical challenge, which promotes the development of sample pre-treatment and analytical detection [8,9].

High-performance liquid chromatography (HPLC) with an UV or diode array, mass spectrometry (MS) or tandem mass spectrometry (MS/MS) detection system was the most common approach for the determination of SUs in grains because of their polar characteristic, low volatility, and thermal instability [8,10–12]. In the literature survey, orthosulfamuron has been analyzed neither as a single nor among multiple residue analysis in grains. In this study, a simple liquid chromatography–tandem mass spectrometry (LC/MS/MS) method was established to detect the residues of orthosulfamuron in brown fatty (unpolished rice) and rice straw using the QuEChERS as an extraction method.

Experimental

Chemicals and reagents

Orthosulfamuron of purity 99.34% was kindly donated from KYUNG NONG CO. LTD. (Seoul, Republic of Korea). HPLC-grade acetonitrile (MeCN) was supplied by Burdick and Jackson (Ulsan, Republic of Korea). Sodium acetate (NaOAc, purity 98.0%) and anhydrous magnesium sulfate ($MgSO_4$, purity 99.5%) were provided by Junsei Chemical Co. Ltd. (Kyoto, Japan). Sodium chloride (NaCl, purity 99.5%) was obtained from Merck (Darmstadt, Germany). Primary secondary amine (PSA) and C_{18} were supplied by Agilent Technologies (Palo Alto, CA, USA). All other chemicals were of analytical and/or HPLC grade.

Matrix-matched calibration

Orthosulfamuron stock solution was prepared in MeCN at a concentration of 1000 μg/mL. A working solution of 10 μg/mL was prepared by diluting the stock solution with blank rice or rice straw extracts, which were confirmed previously to be free of the target analyte. A matrix-matched calibration was prepared by mixing the working standard solution with blank sample extracts to reach a concentration range of 0.01–2 mg/kg. Stock solution was stored at −26 °C in a dark amber bottle, whereas calibration standards were kept at 4 °C.

Field trails

Experimental field trials were carried out at Chonnam National University, Gwangju, Republic of Korea. The on-farm research product, tablet for direct application (DT) of 1.5% orthosulfamuron, was applied to two paddy field plots at two different doses on fifteen days after transplanting the rice seedlings. The first plot received the herbicide at the recommended dose of 500 g/10 a (a.i. [active ingredient] 0.0075 kg/10 a) (T1) and the second one was sprayed with double the recommended dose 1000 g/10 a (a.i. 0.015 kg/10 a) (T2), along with the untreated control (T3). Representative rice (800 g) and rice straw (500 g) samples were collected at harvest (116 days) from the treated and untreated plots. The collected rice and straw were dried to approximately 12% moisture content in a drying room. Subsequently, the dried grains were incompletely husked to make unpolished rice. Unpolished rice grains and straw samples were then ground using a mechanical grinder and used for residue analysis. The samples were stored at −20 °C until analyzed.

Sample preparation

Sample preparations for fatty rice and rice straw were based on the acetate-buffering QuEChERS method [13] following minor modifications. At no point the extraction conditions were optimized. Rather, the experimental variables including solvents, salting out agents, and cleanup procedure were predicated based on our experience.

Unpolished fatty rice

Ten grams of well-ground rice sample was placed into a 50-mL Teflon centrifuge tube. Ten milliliters of distilled water was added to the tube and then vortex-mixed for 1 min. Afterward, MeCN (20 mL), NaCl (2 g), $MgSO_4$ (4 g), and NaOAc (1.5 g) were added to the mixture and shaken by a vortex-mixer for 2 min. The extract was centrifuged for 5 min at 5000 rpm and 5 °C, and the supernatant was aspirated into a 1.5-mL microcentrifuge tube that contained 0.03 g of both PSA and C_{18}. Following shaking for 1 min, the tubes were centrifuged for 5 min at 5000 rpm. The purified extract was subjected to filtration using a polytetrafluoroethylene (PTFE) membrane filter (0.2 μm, ADVANTEC®, Toyo Roshi Kaisha, Ltd., Tokyo, Japan) and 1 mL of the extract was ready for analysis using LC/MS/MS.

Rice straw

Five grams of sample was placed into a 250-mL Erlenmeyer flask, to which MeCN (100 mL), distilled water (50 mL), NaCl (10 g,) $MgSO_4$ (10 g), and NaOAc (5 g) were added. After vigorous shaking for 1 min, the mixture was kept in a shaking incubator for 30 min. The mixture was vacuum filtered through a filter paper (Whatman No. 6, GE Healthcare Co. Ltd., Buckinghamshire, UK) and Celite 545 (Junsei Chemical Co. Ltd., Kyoto, Japan), and 15 mL filtrate was transferred to a 15-mL Teflon centrifuge tube to which $MgSO_4$ (1 g), PSA (0.5 g), and C_{18} (0.5 g) were added. The tube was vigorously shaken for 1 min and centrifuged for 5 min at 5000 rpm. Ten milliliters of supernatant was vacuum evaporated under 40 °C until dryness and then redissolved with 1 mL MeCN. The extract was filtered with a membrane filter (PTFE, 0.2 μm) and 1 mL portion was prepared for LC/MS/MS analysis.

LC/MS/MS

The LC/MS/MS system consisted of a Waters Alliance 2695 LC Separations Module and a Micromass Quattro Microtriple quadrupole tandem mass (Waters Crop., Milford, MA, USA). The analytes were separated on a Gemini C_{18} (2.0 mm i.d × 50 mm, 3.0 μm, Phenomenex, CA, USA). A binary solvent system containing 0.1% formic acid in MeCN (mobile phase A) and 50 mM ammonium acetate in water (mobile phase B) was run in a gradient mode to detect orthosulfamuron. A linear mobile phase gradient started at 5% A (0–1 min), increased to 50% A (1–3 min), increased to 90% A (3–6 min), maintained at 90% A (6–8 min), decreased to 5% A (8–8.1 min), and maintained at 5% A (8.1–12 min). Flow rate and injection volume were set to 0.25 mL/min and 5 μL, respectively. The MS positive electrospray ionization source conditions were as follows: capillary voltage, 3.3 kV; RF lens voltage, 0.2 V; source temperature, 150 °C; desolvation temperature, 370 °C; desolvation gas (N_2) flow, 600 L/h; cone gas (N_2) flow, 50 L/h; and collision gas (argon) 0.15 mL/min. Mass Lynx V4.1 software (Waters Corp.) was used for instrument control, data acquisition, and processing.

Method validation

Matrix effect (ME) was assessed by comparing each slope generated from calibration curves, which were created with standard working solutions prepared in a pure MeCN and in matrix extracts. ME (%) was calculated as follow: (B/A) × 100, where B is a slope of matrix-matched calibration curve, and A is a slope of non-matrix-matched calibration curve [14]. Each matrix-matched calibration curve was created at concentrations of 0.01, 0.02, 0.1, 0.2, 1, and 2 mg/kg, and determination coefficient (R^2) of the matrix-matched calibration curve was used to assess linearity. Limits of detection (LOD) and quantitation (LOQ) were determined based on a signal-to-noise ratio, and concentrations showing peak intensity of signal-to-noise ratio 3 and 10 were designated as LOD and LOQ, respectively. Recovery test was performed with blank samples at 0.1 and 0.5 mg/kg, in triplicate. Percent rate of recovery was obtained by comparing an extracted concentration with a true value. The extracted concentration was calculated by the matrix-matched calibration curve substituting orthosulfamuron peak area detected.

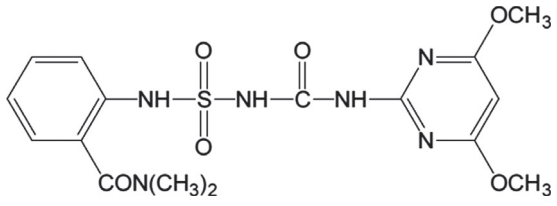
Storage stability of orthosulfamuron

Rice grains and straws have been kept under −20 °C for 97 days until the analysis since collected after drying. In order to evaluate stability of the analyte in the samples during this storage time (97 days), standard working solution was spiked onto the samples at 0.5 mg/kg, sealed, and kept under −20 °C. The stored fortified samples were extracted, and stability was expressed in terms of a percentage in the same manner as the recovery test.

Results and discussion

Sample preparation and LC/MS/MS analysis

Sulfonylurea herbicides have been traditionally extracted by liquid–liquid extraction (LLE) and solid-phase extraction (SPE); however, these extraction methods have undesirable features, such as time-consuming and multi-step procedures, and large consumption and discharge of organic solvents [8,10–12]. On the other hand, the use of the QuEChERS method has been effectively improving the demerits of traditional extraction methods because of its small scale LLE, dispersive SPE, and a combination with mass spectrometry [13,15]. Therefore, the present study first introduced the original QuEChERS method to extract orthosulfamuron from unpolished rice and straw samples. The original QuEChERS



Pesticide	Precursor ion (*m/z*)	Product ion (*m/z*)[a]		
		Quantitation	Confirmation	Ion ratio
Orthosulfamuron	425 $[M + H]^+$	199 227		0.58

[a] Both monitoring at cone voltage 24 V and collision energy 3 V

Fig. 1 Chemical structure and MRM mode of orthosulfamuron in LC/MS/MS.

method not using buffers resulted in poor recoveries ranging 62–78%. Subsequently, the AOAC QuEChERS method buffering acetate was employed with minor modifications, and successful outcomes were generated with good recoveries. Therefore, the acetate-buffering QuEChERS method was used to extract orthosulfamuron in rice and straw samples, and more volume of MeCN was needed for straw samples due to their large volume per unit mass.

According to European Decision 657/2002/EC [16], confirmation of organic residues using tandem mass spectrometry is based on identification points (IPs), ion ratio, and retention time. The requested IPs are three or four (if a substance is banned), and each precursor and product ion records IP 1 and 1.5, respectively. In this respect, the MRM mode using two transitions is required, resulting in four IPs and an ion ratio of signals detected between MRM 1 and MRM 2. All necessary ions of orthosulfamuron were identified by direct infusion of a working standard solution (0.5 mg/L). As shown in Fig. 1, the protonated ion of orthosulfamuron was m/z 425 of $[M+H]^+$, and the product ions were m/z 199 (quantitative) and m/z 227 (qualitative). The ion ratio comparing MRM 1 (m/z 425 $\rightarrow$ 199) and MRM 2 (m/z 425 $\rightarrow$ 227) was 0.58 that should match that of the sample within 1.7% tolerance.

Method performance and storage stability of orthosulfamuron

All validation results are noted in Fig. 2 and Table 1. The calculated ME was 79.4% and 40.9% for unpolished rice and straw, respectively, implying a severe suppression effect caused by matrices on positive electrospray ionization of orthosulfamuron. Hence, matrix-matched calibration was essential to minimize quantitative errors in the present study.

Specificity was tested by analyzing blank samples to ascertain the absence of potential interfering compounds at the retention time of orthosulfamuron. No interfering peaks were observed at the retention time as shown in Fig. 2.

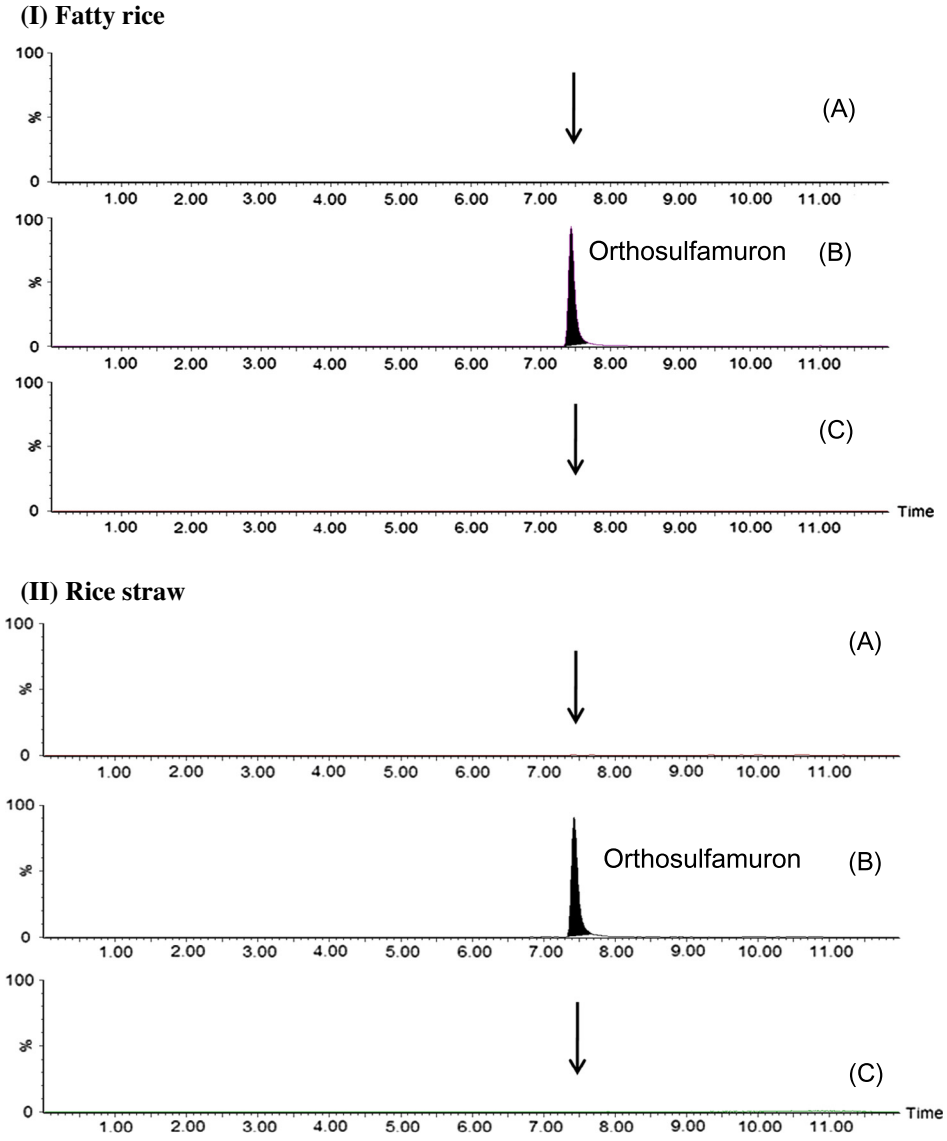


Fig. 2 Typical LC/MS/MS chromatograms of orthosulfamuron in unpolished rice (I) and rice straw (II) containing no detectable residue in untreated rice or rice straw (A); blank rice or rice straw fortified at 0.5 mg/kg of orthosulfamuron (B); and 116 days incurred samples after treatment at the recommended dose (a.i. 0.0075 kg/10 a) (C).

Table 1 Matrix effect (ME), determination coefficient (R^2), spiking levels (mg/kg), recovery (%), relative standard deviations (RSD%), limit of detection (LOD, mg/kg), limit of quantification (LOQ, mg/kg), and storage stability of orthosulfamuron in unpolished rice and straw using a QuEChERS–LC/MS/MS method (n = 3).

Sample	ME (%)	R^2	Recovery (RSD, %)		LOD (mg/kg)	LOQ (mg/kg)	Storage stability
			0.1 mg/kg	0.5 mg/kg			0.5 mg/kg
Unpolished rice	79.4	0.999	92.7 (5.4)	88.8 (5.6)	0.01	0.03	92.7 (5.3)
Rice straw	40.9	0.997	100.6 (4.2)	88.1 (7.7)	0.01	0.03	103.3 (4.7)

Each linearity for orthosulfamuron in unpolished rice and rice straw was validated with the determination coefficient (R^2) resulted from a calibration curve in a range of 0.01–2 mg/kg. All the determination coefficients were higher than 0.997 and linearity was good and reliable.

LODs and LOQs were measured to evaluate sensitivity of the present study. Each LOD and LOQ values were 0.01 and 0.03 mg/kg both in unpolished rice and rice straw. The LOQ was low enough compared with the maximum residue level (MRL) (0.05 mg/kg) of orthosulfamuron in rice grains set by the Ministry of Food and Drug Safety [17].

Recovery tests were carried out at two different concentrations (0.1 and 0.5 mg/kg) in three replicates. The recoveries of the orthosulfamuron were good and ranged between 88.1% and 100.6% with RSD values $\leqslant$8%. The current results were consistent with the acceptable range specified by SANCO Guidelines [18].

The fortified (0.5 mg/kg) and stored (−20 °C, 97 d) unpolished rice and rice straw samples were extracted and detected, and recovery rates were from 92.7% to 103.3% with RSDs $\leqslant$6%. The recovery values of the stored samples were approximately similar to those of freshly prepared samples. We could imply that orthosulfamuron was not degraded under storage conditions.

Determination of orthosulfamuron in field-incurred samples

The developed method was applied for the detection of orthosulfamuron in paddy field following single and double dose application. Neither rice nor rice straw, harvested after 116 days following rice transplantation, contains orthosulfamuron residue levels (Fig. 2).

Conclusions

The method developed was simple and reliable and was used to accurately detect orthosulfamuron residues in a rice paddy field. Orthosulfamuron applied at the recommended dose or up to double the recommended dose was not detected in rice grains or rice straw.

Conflict of interest

The authors have declared no conflict of interest.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

Acknowledgment

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