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Analytical Methods

Analysis of benzobicyclon and its metabolite in brown rice and rice straw after field application using liquid chromatography–tandem mass spectrometry

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ABSTRACT

This study was carried out to develop an extraction as well as an analytical method for detecting benzobicyclon and its amino-substituted metabolite (1315P-570) in brown rice and rice straw using liquid chromatography–tandem mass spectrometry (LC/MS/MS) in positive ion mode with multiple reaction monitoring (MRM). The parent as well as the metabolite in rice and rice straw were extracted and analysed under the same conditions. A correlation coefficient (R^2) of >0.994 was obtained for matrix-matched calibration curves constructed in various concentration ranges. Recoveries at two fortification levels were satisfactory and ranged between 75.4% and 118.9% with relative standard deviations (RSDs) < 13%. Under storage conditions (−20 °C), the analyte and its metabolite were stable for up to 92 days. The limits of quantitation (LOQ) were lower than the maximum residue limit (MRL) (0.1 mg/kg) set by the Korea Food and Drug Administration for brown rice. Field trials with recommended or double the recommended dose revealed that the herbicide could safely be applied to rice and rice straw, as no residues were detected in the harvested samples. The sensitivity of the developed method was sufficient to ensure reliable determination of benzobicyclon and its metabolite in rice grain and rice straw.

1. Introduction

Rice is a staple of the Asian diet, and is also produced and eaten worldwide. As the nutritional components mainly exit in the germ and bran layers, unpolished brown rice (BR) has higher nutritional quality than that of polished white rice (Monks et al., 2013). Replacing the common cereal staple white rice (WR) with BR could have beneficial effects for reducing the risk for diabetes and related complications (Mohan et al., 2014). These protective health effects have been attributed to bioactive compounds, including polyphenols, γ-aminobutyric acid (GABA), acylated steryl β-glucoside, and γ-oryzanol (Goffman, & Bergman, 2004; Kim et al., 2012). BR contains not only non-volatile high molecular weight components such as lipids but also low molecular weight components (Zhang, Chu, Cai, An, & Li, 2006). The high molecular weight components condense in the injection port of the gas chromatograph or in front of the capillary column, resulting in reduced chromatographic efficiency and the chromatographic response enhancement effect (Hajšlová & Zrostlíková, 2003; Schenck & Lehotay, 2000), whereas the low molecular weight components generate interference peaks in the chromatogram and affect identification and quantification (Zhang et al., 2006).

Rice straw is the principal crop residue fed to ruminant livestock in the Republic of Korea. Its chemical and mineral composition varies between varieties and growing seasons, with higher nitrogen and cellulose contents in early season rice compared to that of other times (Shen, Ni, & Sundstøl, 1998). Several methods, including chemical, fungal, and enzyme treatments have been introduced to improve utilisation of rice straw by ruminants (Sarnklong, Cone, Pellikaan, & Hendriks, 2010). Therefore, rice straw should be analysed to determine that it does not contain contaminants that might be transmitted to consumers (via animal products) at levels that exceed the maximum residue limit (MRL).

Benzobicyclon [3-(2-chloro-4-mesylbenzoyl)-2-phenylthiobicyclo[3.2.1]oct-2-en-4-one] (Fig. 1) is a newly developed compound

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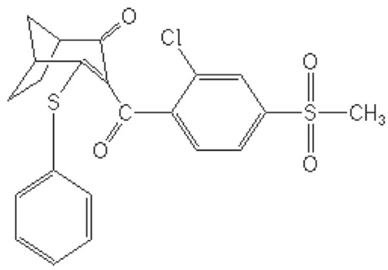
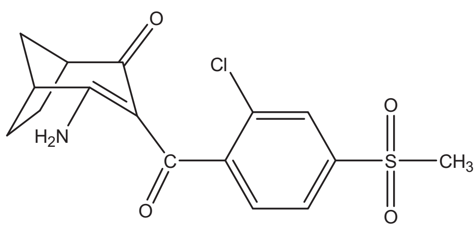


Benzobicyclon



1315P-570

Fig. 1. Chemical structure of benzobicyclon and its amino metabolite (1315P-570).

with potent herbicidal activity against *Scirpus juncoides*, one of the major troublesome weeds in paddy fields (Komatsubara, Sekino, Yamada, Koyanagi, & Nakahara, 2009). After being absorbed through root and basal stems of weeds, it is translocated through the entire plant and causes bleaching of newly developing leaves, followed by necrosis, and death (Komatsubara et al., 2009). Prolonged exposure studies have demonstrated that benzobicyclon exhibits no carcinogenicity, teratogenicity, or reproductive toxicity (Komatsubara et al., 2009). The compound can be used alone or in a combined formulation with other herbicides such as cyclosulfamuron or penoxsulam. In plants, benzobicyclon is metabolized into a large number of metabolites, including hydrolysate, amino-, and hydroxyethylamine-substituted compounds (Komatsubara et al., 2009). Benzobicyclon hydrolysate is a potent *p*-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor that inhibits carotenoid biosynthesis in weeds (Komatsubara et al., 2009). The MRL for benzobicyclon and its metabolite is 0.1 mg/kg, as established by the Korea Food and Drug Administration (KFDA, 2011).

Reliable analytical methods are required for a variety of compounds, including parent pesticides as well as their metabolites that must be determined at very low concentrations in complex matrices (Abd El-Aty et al., 2008). Only a few methods have been reported to determine the herbicide residues in BR (Shin et al., 2011). This is probably due to their low residue levels in food crops. Moreover, the matrices in food crops include both polar (carbohydrate and sugar) and non-polar (starch, macromolecule, and pigment) compounds, all of which seriously complicate extraction (Koesukwiwat, Sanguankaew, & Leepipatpiboon, 2008). Thus, highly selective and sensitive methodologies are required. LC/MS/MS with electrospray ionisation is the most powerful qualitative and quantitative analytical technique and has been increasingly chosen as the preferred tool for residue analysis of pesticides and their nonvolatile, (polar) metabolites due to its undeniable capabilities (Maurer, 1998; Pico, & Barcelo, 2008). Therefore, the aim of the present study was to develop an extraction method to detect benzobicyclon and its amino-substituted metabolite (1315P-570), 2-amino-3-[2-chloro-4-(methylsulfonyl)benzoyl] bicycle[3.2.1]-oct-2-en-4-one (Fig. 1), in BR and rice straw using liquid chromatography–tandem mass spectrometry (LC/MS/MS). So far, there were no previous articles regarding that topic in the literature survey.

2. Experimental

2.1. Chemicals and reagents

Standard benzobicyclon (purity 99.2%) and its metabolite 1315P-570 (purity 99.6%) were a generous gift from Kyung Nong Co. Ltd. (Seoul, Republic of Korea). High performance liquid chromatography-grade acetonitrile (ACN) was supplied by Burdick and Jackson (Ulsan, Republic of Korea). Analytical grade sodium chloride (NaCl) was obtained from Merck (Darmstadt, Germany). Primary secondary amine (PSA) and C_{18} were supplied by Agilent Technologies (Santa, Clara, CA, USA). Formic acid was obtained from Daejung Chemicals & Materials (Siheung, Republic of Korea), and Celite 545 was from Junsei Chemical Co., Ltd. (Tokyo, Japan).

2.2. Standard solution

Standard stock solutions of benzobicyclon and 1315P-570 (100 mg/L) were individually prepared in ACN and stored at −20 °C. Calibration curves were constructed by mixing benzobicyclon and 1315P-570 in appropriate dilutions with matrix extract at concentrations of 0.005, 0.01, 0.05, 0.25, 0.5, 1.0, and 2.0 mg/kg for rice and 0.01, 0.05, 0.1, 0.2, 0.5, 1.0, and 2.0 mg/kg for rice straw. Pooled blank sample extracts was used as solvent to avoid the quantitative matrix effect error during LC/MS/MS analysis.

2.3. Field experiment

BR was grown in an open experimental field at Chonnam National University, Gwangju, Republic of Korea. Fifteen days after transplanting the rice seedlings, the on-farm research product was applied to two different paddy field plots at two different doses on two different plots on June 19, 2013. The first plot received the herbicide at a recommended dose of 3 kg/10 a (a.i. [active ingredient] 0.018 kg/10 a) (T1) and the second one was sprayed with double the recommended dose 6 kg/10 a (a.i. 0.036 kg/10 a) (T2), along with the untreated control (T3). At harvest (116 days), 800 g of representative rice grains and 500 g of straw samples were collected from the herbicide-treated and untreated plots. The straw samples were cut into small pieces and air-dried. The 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 analysis.

2.4. Sample preparation

The original QuEChERS method (Anastassiades, Lehotay, Štajnbaher, & Schenck, 2003) with a slight modification was used to extract benzobicyclon and its amino-substituted metabolite (1315P-570) from rice, however, the method used in rice straw was developed by our laboratory. At no point the extraction conditions were optimised. Rather, the experimental variables including solvents, salting out agents, and cleanup procedure were predicated based on our experience.

2.4.1. Rice

Ten grams of well-ground rice samples were placed in a 50 mL Teflon centrifuge tube. Ten millilitre of distilled water was added to the tube, and it was shaken by vortex mixer for 2 min. Then, 20 mL ACN and 10 g NaCl were added and vortex mixed for an additional 2 min. The extract was centrifuged for 5 min at 5000 rpm, and 1.5 mL of the supernatant was transferred to a