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A novel approach for simultaneous determination of E/Z-fluoxastrobins in vegetables and fruits by UHPLC-DAD

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ABSTRACT

In this work, a novel analytical method was established for simultaneous determination of E-fluoxastrobin and Z-fluoxastrobin in vegetables and fruits. After extracted by acetonitrile, the samples were cleansing with dispersive solid liquid extraction (d-SLE) process, and analyzed by ultra high performance liquid chromatography coupled to diode array detection (UHPLC-DAD). The effectiveness of the optimized method was validated by determining the linearity ($R^2 > 0.99$), sensitivity (limits of quantification of 30 μg/kg), recovery (71.3–113.2%), and precision (relative standard deviations, RSDs ≤ 13.9%) in eight vegetable and fruit samples. The results suggested that this method was simple, reliable, and feasible to determine the residues of E-fluoxastrobin and Z-fluoxastrobin in vegetable and fruit samples, which could be further applied to 160 various vegetable and fruit samples.

1. Introduction

The application of pesticide has played an irreplaceable role on crop protection, ensuring food supply, and agricultural products storage (Hong et al., 2016; Jiang et al., 2016). In the meanwhile, pesticide residue has become a main concern in environment and food safety due to its toxicity and potential hazard to ecosystem and human beings (David et al., 2016; Farajzadeh, Feriduni, & Mogaddam, 2016). Recently, the residue analysis on pesticide isomers also attracted great attentions due to their different toxicities and activities, such as hexachlorocyclohexane (Wittsiepe, Nestola, Kohne, Zinn, & Wilhelm, 2014), dichlorodiphenyltrichloroethane (DDT) (Bosch, Grimalt, & Fernandez, 2015) and chiral pesticides (Celis, Gamiz, Facenda, & Hermosin, 2015; Lv et al., 2016; Tian et al., 2015). Among them, as one class of the isomers, the separation, analysis and evaluation of E/Z-isomers are quite necessary and important for enhancing the food safety due to its existence in many pesticides and possible hazard to environment and human.

Fluoxastrobin (E/Z isomers, CAS 193740-76-0), developed by Bayer CropScience, is a new type of broad-spectrum fungicide to cure symptoms, i. e. early blight, late blight, leaf spots, leaf rust, *Rhizoctonia solani* on oil crops, fruits, cereals, and vegetables (U.S. EPA, 2005). Fluoxastrobin, as a systemic adsorption stem and leaf treatment fungicide, is classified to dihydro-dioxazines fungicide (U.S. EPA, 2005). The subchronic and chronic toxicity tests of fluoxastrobin have shown it's highly toxic to dog (U.S. National Library of Medicine, 2011). While in the acute toxicity study, fluoxastrobin is moderately toxic to estuarine/marine fish; highly toxic to freshwater fish and invertebrates; and very highly toxic to estuarine/marine invertebrates (U.S. National Library of Medicine, 2011). Therefore, the U. S. EPA and European Union (EU) had set 295 the maximum residue limits (MRLs) on fluoxastrobin in various foods and forage, ranged from 0.01 to 17 mg/kg. According to the U.S. EPA, the MRLs of fluoxastrobin (sum of E/Z-fluoxastrobins) are: 0.5 mg/kg for cucumber, 1.0 mg/kg for vegetables (fruiting group)

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and 0.05 mg/kg for vegetable (foliage of legume) (U.S. National Library of Medicine, 2011). Moreover, the EU set more rigorous and same MRLs for fluoxastrobin in vegetables and fruits as 0.05 mg/kg, which equaled to the limits of quantification (LOQs) for fluoxastrobin (EU Commission Pesticides database., 2016). For China, no MRLs of fluoxastrobin had set up, but the registers of fluoxastrobin on cucumber and tomato had been realized and the other registers had been underway (China Pesticide Information Network, 2016).

To date, several papers had shown for analysis of E-fluoxastrobin in pollen, fruit juices, vegetables, and fruits, using the gel permeation chromatography (GPC) (Bo, Wang, Guo, Qin, & Lu, 2009), enzyme-linked immunosorbent assay (ELISA) (Watanabe & Miyake, 2013), and the QuEChERS (quick, easy, cheap, effective, rugged, and safe) method (David, Botias, Abdul-Sada, Goulson, & Hill, 2015; Walorczyk & Gnusowski, 2009). While very few analytical methods have been introduced to simultaneously detect E/Z-fluoxastrobins. Only one of the isomers, E-fluoxastrobin, was the targeted analyte, without the detection of Z-fluoxastrobin, which was inconsistent with the definition residue of fluoxastrobins (U.S. National Library of Medicine, 2011). Although the degradation in crops of these two compounds was not clear, it was confirmed that E/Z-Fluoxastrobins were found to be major residues in the metabolism test (U.S. National Library of Medicine, 2011). Thus, considering its application objects (vegetables and fruits), coexisting in the pesticide formulations, and the residual risk in foods, it was necessary to develop a simple, effective, and reliable analytical method for simultaneous determination of both E/Z-fluoxastrob in vegetables and fruits.

Nowadays, various sample preparation approaches have been employed in pesticide residue analysis, known as solid phase extraction (Bonansea, Ame, & Wunderlin, 2013), dispersive-solid phase extraction (Dong, Nie, Tang, Rao, Qu, Wang, et al., 2015; Han, Sapozhnikova, & Lehotay, 2016), solid-phase microextraction (Bonansea et al., 2013), dispersive liquid–liquid microextraction (Farajzadeh et al., 2016), and magnetic solid-phase extraction (Herrero-Latorre, Barciela-Garcia, Garcia-Martin, Pena-Crecente, & Otarola-Jimenez, 2015). All the time, the trend of analytical method is a rapid, highly sensitive, and highly accurate methodology with less cost (maintenance of analytical instruments and reagents) (Watanabe, Kobara, Baba, & Eun, 2014). In this paper, an environmentally friendly sample preparation method, the dispersive solid liquid extraction (d-SLE) pretreatment, had been introduced. The d-SLE pretreatment contained two steps: 1) fixed the target compounds by adsorbing material in the extraction solvent; 2) after discarding the extraction solvent, eluted the target compounds from adsorbing material. For d-SLE, there were more steps in the sample pretreatment comparing to the d-SPE, but more impurity was gotten rid of at the same time, which was confirmed in the paper. Moreover, in the d-SLE process, sample concentration was simply and effectively realized without nitrogen blowing or rotary evaporation. And for quantification, though GC-MS/MS and LC-MS/MS had been reported to determine the residues of E-fluoxastrobin (Bo et al., 2009; David et al., 2015; Walorczyk & Gnusowski, 2009), the ultra high performance liquid chromatography coupled to diode array detection (UHPLC-DAD) was development for simultaneous analysis of E/Z-fluoxastrobins with its advantages of low cost, low matrix effect and simple procedure.

This study aims to develop a rapid, less solvent involved and reliable technique to simultaneously determine the residues of E/Z-fluoxastrobins in common vegetables and fruits (cabbage, cucumber, grape, orange, potato, spinach, strawberry, and tomato). In order to validate the effectiveness of this method, the present method had been applied in 160 real samples to monitor the residue levels of E/Z-fluoxastrobins.

2. Experimental

2.1. Materials

The standards of E-fluoxastrobin (99.5%) and Z-fluoxastrobin (99.1%) were obtained from Arysta Lifescience Corp (Japan). The acetonitrile and methanol (HPLC grade), were obtained from Merck (Darmstadt, Germany). The purified water was prepared by the Milli-Q quality water system (Millipore, Billerica, MA, USA). C_{18} (40–60 μm) was purchased from Agela Technologies, Inc (Tianjin, China).

The standard solutions of E-fluoxastrobin and Z-fluoxastrobin (1000 mg/L) were prepared by dissolving the accurately weighted solid in methanol, respectively, and were stored at −20 °C in darkness.

2.2. Instrument

An UHPLC-DAD instrument (Shimadzu Corp., Tokyo, Japan), including two LC-30AD pumps, a Sil-20AC autosampler, a CTO-20A column oven and a DAD detection, was used for analysis. The separation of the targeted analytes was performed on a Shim-pack XR-ODS III column (75 × 2.0 mm, 1.6 μm) (Shimadzu Corp., Tokyo, Japan) at 40 °C, and the flow rate was 0.35 mL/min. The injection volume was 10 μL. The gradient elution procedure was set as follows: initial mobile phase, 20% B; 0–1.0 min, 50% B; 1.0–5.0 min, 50% B; 5.0–5.1 min, 55% B; 5.1–7.5 min, 55% B; 7.5–8.0 min, 20% B; 8.0–11.0 min, 20% B (The mobile A and B were water and acetonitrile, respectively).

2.3. Sample preparation

2.5 g sample was accurately weighted into a 15 mL centrifuge tube. 2.5 mL acetonitrile was added into the tube, and the tube was vortexed vigorously for 3 min and ultrasonicated for 5 min. After centrifuged at 3000 rpm for 5 min, the supernatant was transferred into another 15 mL centrifuge tube. Then 8 mL water had been added into the tube to rinse the residues by vortexed vigorously for 1 min, and the mixture was filtered with the filter paper and combined with the sample extract. Then, 150 mg of C_{18} was added into the mixed solution and the tube was vortexed for 3 min and centrifuged at 3000 rpm for 5 min. The supernatant was given up. Then, 50 mg NaCl and 1 mL acetonitrile were added into the tube, and the tube was vortex for about 3 min and centrifuged at 3000 rpm for 5 min. Finally, the upper layer (acetonitrile part) was mixed with water as 1:1 (v:v), and the mixture was passed through 0.22 μm nylon filter, and was ready for UHPLC-DAD analysis.

2.4. Optimization of the sample preparation

In the pilot test, the extraction and clean-up approaches were optimized to reduce the solvent, simplified the experimental procedure and ensured the accuracy of the present method.

Acetonitrile is one of the most frequently used extraction solvents in pesticide analysis, and has been successfully used to extract various pesticide residues in vegetables (Zhu, Liu, Xu, Dong, Liang, Li, et al., 2013), fruits (Wang, Cang, Qi, Zhao, Xu, Wang, et al., 2015), cereals (Dong, Nie, Tang, Rao, Qu, Wang, et al., 2015), meat (Han et al., 2016) and other samples (Farajzadeh et al., 2016; Oellig, 2016). In our paper, the effect of acetonitrile volume (1–4 mL) on extraction efficiency had been first time estimated in cucumber, orange, spinach, and tomato, in which the E/Z-fluoxastrobins were spiked at 100 μg/kg into the 2.5 g cucumber, orange, spinach, and tomato samples, respectively, and the samples were settled for 30 min before the extraction.