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Analytical Methods

Simultaneous determination of plant growth regulator and pesticides in bean sprouts by liquid chromatography–tandem mass spectrometry

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ABSTRACT

A simple and sensitive analytical method based on QuEChERS approach using liquid chromatography tandem mass spectrometry was developed and validated for the determination of 6-benzylaminopurine, carbendazim and thiabendazole in bean sprouts. Sodium chloride and sodium acetate were used for salting-out step and magnesium sulfate for clean-up. The validation of optimized method was satisfactory with recoveries, between 89.5% and 103.2% for the three compounds, and relative standard deviation (RSD) values less than 3.3% at 20 and 40 ng/g fortification levels (n = 5). Limit of detection (LOD) and limit of quantification (LOQ) was 2.1–3.7 ng/g and 6.3–11.1 ng/g, respectively. Monitoring of 126 bean sprout samples collected from local markets was performed to verify the adaptability in real samples. No pesticides were detected but 6-benzylaminopurine was found in 3 samples at the level of 15–20 ng/g. The optimized method should be applicable for monitoring of 6-benzylaminopurine, carbendazim and thiabendazole in bean sprouts in short time.

1. Introduction

Bean sprouts are common ingredients in Asian cuisine. One of them, *kongnamul*, is made from the soy-beans (*Glycine max*), other common bean sprout is *Sukjunamul* which is originated from the mung-beans (*Vigna radiata*), Because of its high asparagine contents (10.0∼15.8% in the roots part), bean sprout soup is famous for hangover soup to Koreans. Bean sprouts are also rich in vitamin C (up to 275%), phenolic components (about 24%), and free amino acids (up to 800%) compared to the original raw bean (Dikshit & Ghadle, 2003; Jeong, Shon, Dhakal, Lee, & Whang, 2008). Every year, about 60,000 tons of bean sprouts are consumed in Korea but 80% of them are imported from United States, China, etc (Korea Rural Economic Institute, 2014).

Synthetic cytokinin such as 6-benzylaminopurine (6-BA) has been used by farmers to increase the marketability of bean sprouts by suppressing the growth of rootlets and promoting the growth in volume. It is a type of Plant Growth Regulators (PGRs) that promote cell division, enhance the growth of lateral buds and give resistance to high temperature (Skoog & Armstrong, 1970). The PGRs are known as naturally occurring or synthetic substances regulating plant growth and death (Shi et al., 2012). According to the chemical properties and biological features, PGRs can be divided into six classes: Gibberellins, Auxins, Ethylene, Cytokinins, Abscisic acid and Brassinosteroids (Rao, Vardhini, Sujatha, & Anuradha, 2002). Most PGRs are considered to have low toxicity (Xue et al., 2011) but they have been the subject of health concern as previous studies have shown that some of PGRs inactivate antioxidant defense systems or have teratogenic effects *in vitro* (Aire, 2005). Many countries have regulated the maximum residual limits (MRLs) for some PGRs including 6-BA (Australian Government, 2015; European Commission, 2015). Last year, the Ministry of Food and Drug Safety (MFDS) of the Republic of Korea also issued an administrative notice of a new regulation of 6-BA in bean sprouts.

Meanwhile, bean plants are susceptible to soil-borne fungal pathogens like *Phakopsora pachyrhizi* (Schneider et al., 2005).

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Benzimidazoles, such as carbendazim, benomyl and thiabendazole, are commonly used fungicides for protection of fruits and vegetable crops form fungi as well as seed treatment (Blažková, Rauch, & Fukal, 2010). In that 80% of bean sprouts consumed in Korea are imported from outside the country, continuous monitoring of pesticides is critical control point. As cultivation period of bean sprouts is short, there are much more chances of the persistence of benzimidazoles which are posing a health risks to humans (Blasco, Font, & Picó, 2006).

As many government agencies have set MRLs on some chemicals in bean products, the MFDS of the Republic of Korea have established MRLs on six substances in bean sprouts. These are carbendazim, thiabendazole, thiram, captan, sulfur dioxide and 6-BA (The Ministry of Food and drug Safety, 2014). However, the conventional analytical methods for these compounds are very time-, hazardous solvent- and labor-consuming procedure.

In recent years, researchers have developed various rapid multiresidue analysis methods. Especially, QuEChERS approach introduced by Anastassiades, Lehotay, Štajnbaher, and Schenck (2003) have been a revolutional tool to develop analytical method for many kind of chemicals including pesticides, their metabolites in fruits and vegetables (Golge & Kabak, 2015; Koesukwiwat, Lehotay, Mastovska, Dorweiler, & Leepipatpiboon, 2009), veterinary drugs (Choi, Kim, Shin, Kim, & Kim, 2015), polycyclic aromatic hydrocarbons (Sadowska-Rociek, Surma, & Cieslik, 2013), miticides and agrochemicals in honey products (Tomasini et al., 2012) since these methods have advantages such as reduced number of steps and low consumption of organic solvents. And these QuEChERS approach still continue to undergo modifications for improved sample preparation.

Regarding bean sprout samples, very little has been published on analytical methodology for 6-BA, carbendazim and thiabendazole. Cho et al. (2013) developed multi-residue analytical method based on low temperature partitioning (LTP). The samples were extracted with acetonitrile followed by partitioning at −80 °C without salting-out agents. The recovery for 6-BA and carbendazim were around 80% and limit of detection (LOD) ranged from 2 to 3 ng/g, respectively.

In this regard, we sought to propose new QuEChERS approach for the determination of residues of 6-BA, carbendazim and thiabendazole in bean sprouts using LC–MS-MS by comparing the effectiveness of different kinds of extraction salts and sorbents. The developed method was applied to the analysis of 126 bean sprout samples from local markets in Gwang-ju city.

2. Experimental

2.1. Chemicals and reagents

Carbendazim and thiabendazole were obtained from Dr. Ehrenstorfer (Augsburg, Germany), and 6-benzylaminopurine was from Sigma-aldrich (Steinheim, Germany), and their chemical structures and physicochemical properties are shown in Table 1. Formic acid was from Sigma-aldrich (Steinheim, Germany). HPLC-grade acetonitrile, sodium chloride were obtained from Merck (Darmstadt, Germany). Magnesium sulfate, sodium acetate, primary secondary amine (PSA), octadecylsilyl (C18), sodium citrate tribasic dehydrate and sodium citrate dibasic sesquihydrate were obtained from Agilent technologies (Santa Clara, CA, USA). Deionized water was purified with DE/Genpure standard systems (Niederelbert, Germany).

Stock standard solutions of each compound were prepared in acetonitrile at concentration of 200 μg/mL and store at −20 °C. A mixture of these standards at 10 μg/mL, prepared in acetonitrile, was used to prepare the standard solutions in solvent and in matrix and spiking solutions (20 ng/g, 40 ng/g). Calibration standard solutions, at the concentration of 0.002, 0.01, 0.02, 0.05, 0.1 and 0.2 μg/mL, in solvent and in matrix were prepared just before the analysis.

2.2. Instrumentation

Chromatographic determination was performed with an Acquity H-class ultra-performance liquid chromatography (Waters, Milford, MA, USA) connected to TSQ Quantum Ultra triple stage quadrupole mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) with a electrospray ionization (ESI) probe. The LC was operated under gradient conditions with mobile phase A {water/methanol (95:5) + 0.1% formic acid} and B {water/methanol (50:50)} at a flow rate of 0.4 mL/min. The linear gradient program was set as follow: 95% of A for 0.2 min, 100% B (0.2–4 min, hold for 0.5 min) and 95% A (4.5–6 min, for re-equilibration). The injection volume was 1uL. The analytic column was 50 mm × 2.1 mm (i.d), 1.7 um particle size Acquity UPLC® BEH C18 column (Waters, USA) which was kept for 40 °C during analysis.

The MS conditions were: ion source polarity: positive mode, spray voltage: 3.5 kV, vaporizer temperature: 200 °C, sheath gas pressure (N_2): 45 units, auxillay gas pressure (N_2): 15 units, capillary temperature: 300 °C, Collision cell pressure: 1.5 mTorr. Optimized values of cone voltages and collision energies for each analyte were obtained by infusing 1000 ng/g of each standard at a rate of 10 μL/min, which was tuned by Quantum Tune Master software.

2.3. Sample collection

Blank samples were obtained by firstly purchasing the bean seed from local market and then cultivating it for a long time (over 10 days) because of the difficulty in finding bean sprouts confirmed not containing the target compounds.

The applicability of optimized method was evaluated by analyzing 126 samples purchased from local traditional markets and supermarkets in Gwang-ju city. The samples were stored at 10 °C until analysis, which does not exceed 2 days.

2.4. Sample preparation

Approximately 50 g of representative portion of bean sprouts was homogenized in a blender. Ten g of homogenized sample was weighted into 50 mL centrifuge tube. Ten mL of acetonitrile was added and the mixture was shaken by hand for 1 min. After adding 3 g sodium chloride and 1 g sodium acetate, tubes were shaken vigorously for 1 min and centrifuged for 5 min at 3000 RPM using Allegra X-15R (Beckman coulter, CA, USA). Then, 5 mL of supernatant were transferred to 15 mL centrifuge tube containing magnesium sulfate. Next, the tubes were shaken by hand for 1 min and centrifuged again for 5 min at 3000 RPM. After centrifugation, 200 μL of upper layer extract diluted with 100 μL acetonitrile and 700 μL water were injected to the LC–MS/MS system. The mixture was filtered through a 0.2 μm PTFE syringe filter (Whatman, USA) before analysis. We chose this method after testing 6 different combinations of salting-out agents and 4 types of sorbents for clean-up. All experiments were performed in quintuplicate and the data were analyzed with IBM SPSS statictics 20, using analysis of variance (ANOVA) at 95% confidence interval, to see if there were the difference between combinations.

2.5. Method validation and matrix-effect

The method validation was performed on the basis of SANCO/2013/12571 (SANCO, 2013) and ICH/2005/Q2/R1 (ICH, 2005). Linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) were evaluated.