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Analytical Methods

Simple multiresidue extraction method for the determination of fungicides and plant growth regulator in bean sprouts using low temperature partitioning and tandem mass spectrometry

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ABSTRACT

A simple multiresidue analytical method is developed for the simultaneous determination of carbendazim (CB), thiabendazole (TB), and 6-benzyl aminopurine (6-BA) in bean sprouts. The samples were extracted with acetonitrile followed by partitioning at −80 °C for 5–10 min. A YMC C_8 column was used to separate the analytes before being qualitatively and quantitatively determined by liquid chromatography–electrospray ionization tandem mass spectrometry (LC–ESI-MS/MS) in positive ion mode using multiple reaction monitoring (MRM). The matrix-matched calibration curves showed good linearity in the range 0.01–1.0 mg/kg with correlation coefficients in excess of 0.998. The mean recoveries were in the range of 80.4–96.3% at 0.1 and 0.5 spiked levels, and the relative standard deviations (RSDs) were in the range of 0.5–7.6%. The limits of quantifications (LOQ) were in the range of 0.005–0.01 mg/kg. The method was successfully applied to 90 samples (among which 45 were organic) collected from a commercial bean sprout production house throughout the city. Except for 6-BA, the rest of the analytes had values lower than their LOQs. In sum, carbendazim, thiabendazole, and 6-BA were extracted in a single step, and no steps for clean-up or concentration of the extracts were needed. The current method can be used for sensitive and accurate determination and confirmation of residues in bean sprout samples.

1. Introduction

In agricultural practice, the use of insecticides, herbicides, and fungicides provides an unquestionable benefit for crop protection. However, residues may remain at the harvest stage, causing hazardous effects to both humans and environment (Jiang, Li, Jiang, Li, & Pan, 2012). Therefore, it is necessary to monitor such residues regularly through multiresidue analytical methods that combine short analysis time, sufficient selectivity, and sensitivity.

Plant growth regulators (PGRs, among which 6-benzal aminopurine) are a class of physiological and biological substances that have effects similar to their endogenous counterparts. They are used to improve fruit set and development in many crops overall when pollination and fertilization conditions are unfavorable (Yu, Li, Qian, & Zhu, 2001). Farmers often apply various PGRs either independently or in various combinations to produce crops of desirable quality (Oulkar, Banerjee, & Kulkarni, 2011). The increased application of PGRs has led to more concerns about their presence in different commodities. Although most PGRs are considered to have low toxicity, they might pose potential risks to the consumers (Xue et al., 2011). The strong polarity of most PGRs causes them to be retained weakly in reversed-phase LC systems and makes their separation in traditional analytical methods more difficult (Xue et al., 2011).

Benzimidazolic fungicides (BFs), including carbendazim and thiabendazole, are extensively applied pre- and post-harvest to prevent fungal deterioration, and persist in farming products for a long time (Blazková, Rauch, & Fukal, 2010; Su, Mitchell, & Mac AntSaoir, 2003). Methods used for the determination of these polar and thermally labile benzimidazolic fungicides in fruits and vegetables consist of extraction in organic solvents (frequently ethyl acetate), clean-up by liquid–liquid partitioning or SPE using silica-C_{18} or cation exchange resins, and subsequent determination by LC with UV or fluorescence detection (Moral, Sicilia, & Rubio, 2009). As such, a rapid and convenient multi-residue analysis

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method for PGRs and benzimidazolic fungicides for pursuing routine analysis is clearly needed.

Sprouts are produced (within 2–7 days) in hydroponic culture by soaking the seeds in water followed by incubation in a warm (temp. 20–30 °C), humid environment to optimize germination and sprout growth. Germinated bean sprouts are served as staple vegetables and are eaten raw as components of salads or slightly cooked in various side dishes in many Asian countries. In the natural environment, seed sprouts survive during germination by enhancing their defensive responses through phenolic biosynthesis (Randhir, Lin, & Shetty, 2004). Germination may cause changes in the nutrients, including functional substances, through aerobic respiration and biochemical metabolism. Sprouting also removes antinutrients such as enzyme inhibitors in the seeds, thus making sprouts safe for the diet (Mwikya, Camp, Rodriguez, & Huyghebaert, 2001). The health benefits of bean sprouts mainly depend on the amount of biologically active compounds present in the bean (Chun, Chang, Choi, Kim, & Ku, 2005). Sprouts contain a wide variety of antioxidant compounds, which in turn provide protection against oxidative damage (Kestwal, Bagal-Kestwal, & Chiang, 2012). Sprouts are low in calories (14 kcal/100 g) and fat and provide substantial amounts of key nutrients such as vitamin C, folate, and fibre (U.S. Food and Drug Administration, 1999). The above mentioned germination conditions are optimum for the growth of fungi and hence may deteriorate the seeds. BFs might be effective for treatment and suppressing the growth of these microorganisms.

The choice of analytical technique is dependent on the method of sample preparation. Extraction and cleanup are the most challenging parts for analysis in foods and are often the critical steps in deciding the levels of detection limits of the overall methods. Methods applied to determine pesticide residues in fatty and non-fatty foods often require many steps and are very time-consuming. Also, crop varieties and different physicochemical properties of the target compounds make it difficult to develop analytical methodologies that could cover them in one method (Jiang et al., 2012). For this reason, highly selective and sensitive methodologies are required. Liquid chromatography coupled to tandem mass spectrometry with electrospray ionization (LC–ESI-MS/MS) is the most powerful qualitative and quantitative analytical technique that has increasingly been chosen as the preferred tool for pesticide residue analysis due to its undeniable capabilities (Picó & Barceló, 2008). Precision, robustness and high sensitivity and selectivity make it the perfect choice for multiresidue food analysis. Despite all these advantages, one important drawback of electrospray ionization that should be taken into consideration is matrix effect.

The method developed involves simple solvent extraction followed by storage at low temperature. The extract is then applied to LC–MS/MS for quantitative and qualitative analysis of carbendazim, thiabendazole, and 6-benzal aminopurine (6-BA). To our knowledge, this method is the first application for the simultaneous determination of residue levels of the selected analytes in bean sprouts. 6-BA was tested among the 12 plant growth regulators analyzed in bud sprouts and grapes (Oulkar et al., 2011).

2. Materials and methods

2.1. Chemicals and Reagents

Carbendazim (98.5%, purity) and thiabendazole (98.5%, purity) were purchased from Dr. Ehrenstorfer (Augsburg, Germany), and 6-benzyl aminopurine (6-BA, 96% purity) was supplied by Sigma–Aldrich (St. Louis, MO, USA). Analytical-grade acetonitrile, methanol, and water were obtained from Merck KGaA (Darmstadt, Germany). All solvents and reagents used were of high performance liquid chromatography or analytical grades.

2.2. Standard solutions

Stock standard solutions (100 mg/L) of carbendazim, thiabendazole, and 6-BA were prepared in acetonitrile. The individual stock solutions were stored in dark vials in a refrigerator at 4 °C. An intermediate stock standard mixture of 10 mg/L was prepared by mixing appropriate quantities of the individual stock solution in acetonitrile. Matrix-matched multi-level calibration standard solutions were prepared in sample extracts obtained from fresh organic bean sprout purchased from a local market (Gwangju, Republic of Korea). The final concentrations were 0.01, 0.05, 0.1, 0.3, 0.5, 0.8, and 1.0 mg/kg.

2.3. Sample preparation

Ten grams of homogenized bean sprout samples were placed in a 50 mL conical tube to which 40 mL acetonitrile was added. An internal standard (triphenyl phosphate, 50 μg/L) was added and the contents were vigorously shaken for 5 min followed by centrifugation for 5 min at 966*g*. Afterwards, the tubes were stored at low temperature (−80 °C) for 5–10 min to separate the acetonitrile and water layers. Two millilitre portions of the acetonitrile layer were filtered through 0.2 μm membrane filters (Smartpor, Woongki Science Co., USA) prior to analysis by LC–ESI-MS/MS.

2.4. LC-electrospray ionization MS/MS

An MS/MS detector was equipped with an Agilent 1200 Series Rapid Resolution LC System (CA, USA), which consisted of a binary pump, autosampler, vacuum degasser, thermostated column compartment, and a diode array detector. The analytes were separated on a YMC Pack Pro C_8 column (150 × 4.6 mm i.d., 3 μm, Kyoto, Japan) kept in an oven at 40 °C. The binary solvent system consisted of 10 mM ammonium formate in water (A) and methanol (B), with a linear gradient. The linear mobile phase gradient started at 5% B (0 min), increased to 60% B (0–3 min), increased again to 99% B (3–5 min), then ramped back to 60% B (5–8 min) and 5% B (8–12 min), and maintained at 5% B (12–15 min). The flow rate was 0.5 mL/min, and the injection volume was 10 μL. MS/MS detection using an Agilent 6410 Triple Quadrupole LC/MS (QQQ) was conducted in the positive electrospray ionization mode using multiple reaction monitoring (MRM) with two mass transitions. In the two mass transitions, one product ion with the most intensity and the other lower intensity were used as quantifier and qualifier ions, respectively (Fig. 1). Analyte standard solutions were directly infused into the QQQ for the optimal MS instrument parameters. Nitrogen was employed as nebulizer and drying gas at 45 psi and 350 °C. The ionspray voltage was set to 4000 V. All dwell times for MRM transitions of the analytes were set to 200 ms, and other conditions are presented in Table 1. Both MS1 and MS2 quadrupoles were maintained at unit resolution. Mass Hunter Workstation Software (B.01.03) controlled the LC–ESI-MS/MS system and processed the data.

2.5. Method validation

The linearity of the calibration curve, obtained by plotting the peak area against concentration of the corresponding calibration standard, was maintained in the concentration range of 0.01–1.0 mg/kg.

Untreated control samples were selected for the method validation experiments. Recovery studies were carried out (in triplicate) via spiking bean sprouts with a mixture of the 3 analytes at levels of 0.1 and 0.5 mg/kg. The spiked samples were permitted to equilibrate for 1 h before extraction to allow the spiked solution to penetrate the matrix, and then processed according to the above procedures. The recovery was calculated from the ratio of the peak