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Analytical Methods

Validation of quantitative method for azoxystrobin residues in green beans and peas

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ABSTRACT

This study presents a method validation for extraction and quantitative analysis of azoxystrobin residues in green beans and peas using HPLC-UV and the results confirmed by GC–MS. The employed method involved initial extraction with acetonitrile after the addition of salts (magnesium sulfate and sodium chloride), followed by a cleanup step by activated neutral carbon. Validation parameters; linearity, matrix effect, LOQ, specificity, trueness and repeatability precision were attained. The spiking levels for the trueness and the precision experiments were (0.1, 0.5, 3 mg/kg). For HPLC-UV analysis, mean recoveries ranged between 83.69% to 91.58% and 81.99% to 107.85% for green beans and peas, respectively. For GC–MS analysis, mean recoveries ranged from 76.29% to 94.56% and 80.77% to 100.91% for green beans and peas, respectively. According to these results, the method has been proven to be efficient for extraction and determination of azoxystrobin residues in green beans and peas.

1. Introduction

According to UN's Food and Agriculture Organization (FAO), Egypt occupies the sixth position in the production world ranking of green beans and peas. The production quantities (tons) of Egypt in 2012 are 251.279 and 180.631 (FAO, 2014).

Azoxystrobin (Fig. 1) is a systemic, broad-spectrum fungicide belonging to the class of methoxyacrylates, which are derived from the naturally-occurring strobilurins. It exerts its fungicidal activity by inhibiting mitochondrial respiration in fungi. It is absorbed through the roots and translocated in the xylem to the stems and leaves, or through leaf surfaces to the leaf tips and growing edges. Azoxystrobin Controls foliar and soil-borne diseases including downy and powdery mildew, early and late blight, and pathogens *Sclerotinia*, *Alternaria*, *Ascochyta*, *Pythium*, and *Rhizoctonia* on many crops (PMRA, 2009). Several studies focused on the analytical methods of azoxystrobin in different matrices. In these studies, gas chromatography (GC) was the most commonly used method to determine azoxystrobin residues in different fruits and vegetables (Aguilera, Valverde, Camacho, Boulaid, & García-Fuentes, 2012; Bo, Bi, & Chen, 2005; Bo & Sun, 2008; Bo, Wang, Guo, Qin, & Lu, 2008; Ding et al., 2006; Gajbhiye et al., 2011; Han, Yao, Wu, Wang, & Qin, 2009; Huan, Xu, Lv, Xie, & Luo, 2013; Li et al., 2008; Liu, Sun, Zeng, & Liu, 2010; Sun, Bo, & Han, 2007; Wang, Hou, Zou, & Lu, 2010; Wang, Sun, & Liu, 2013; Wei, Lu, He, & Zuo, 2011; Wu, Wang, Wu, Zhao, & Yang, 2010; Yin et al., 2011; Zhang, Zhang, Lu, & Li, 2008). High-performance liquid chromatography (Abreu, Caboni, Cabras, Garau, & Alves, 2006; Polati et al., 2006; Shi, Zhao, Che, & Huang, 2010) and gas chromatography–mass spectrometry (GC–MS) (Bo, 2007; Bo et al., 2008; Melo et al., 2012) have also been introduced in the analysis of azoxystrobin residues. Moreover, liquid chromatography-tandem mass spectrometry was used to determine azoxystrobin residue (Itoiz, Fantke, Juraske, Kounina, & Vallejo, 2012; Polati et al., 2006; Wu et al., 2009). Photochemically induced fluorescence was developed to determine the presence of azoxystrobin (Javier, Antonio, & Maria, 2007). In this work, we aimed to develop and validate an effective method for extraction and quantitative determination of azoxystrobin in green beans and peas using high performance liquid chromatography with UV-detection; the results were confirmed by GC–MS.

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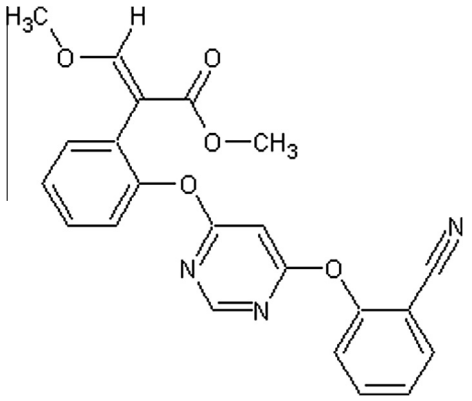

Fig. 1. The chemical structure of azoxystrobin.

2. Material and methods

2.1. Chemicals and reagents

Acetonitrile, isopropanol and methanol of HPLC quality were from J.T.Baker, USA. Double de-ionized water obtained by using an EasyPure LF (Compact Ultrapure Water System), USA. Anhydrous magnesium sulfate and sodium chloride were purchased from J.T.Baker, USA. Neutral Decolorizing Carbon from Fisher Scientific, USA. Azoxystrobin standard 1000 μg/mL ampoules (obtained from Absolute Standards, Inc., USA) were serially diluted in methanol to prepare stock standard of 100 μg/mL and other spiking concentrations. Pyrene D10 (obtained from Sigma–Aldrich, USA) was used as internal standard (ISTD) at 50 μg/mL in methanol.

2.2. Instruments and apparatus

A centrifuge (IEC/Centa GP8) was used for phase separation. Weighing was performed using a PG503 DeltaRange scale for masses above 0.1 g and an AG104 scale for masses below 0.1 g. Sample homogenization was conducting using a Cole Parmer, Analytical Mill. An ultrasonic bath (FS110H-Fisher Scientific) used for sonication during the extraction step. Chromatography consumables, certified glassware and personal protective equipment were used during all the analysis steps.

HPLC analysis of azoxystrobin was performed with Shimadzu HPLC system consisting of LC-10AS pump, SIL-9A auto injector and SPD-6AV UV–VIS spectrophotometric detector. A Biphenyl 100A Kinetex column (250 mm × 4.6 mm × 5 μm) from Phenomenex was kept at room temperature. Separation of azoxystrobin was done with isocratic elution using the mobile phase (50% acetonitrile: 50% water: 0.4% acetic acid). The flow rate was 0.8 mL/min and the injection volume was 40 μL. The detection wavelength set at 255 nm. The azoxystrobin residues identified by comparing the retention time of the sample peak with the retention time of the standards. The retention time of azoxystrobin was14.55 min. The GC–MS analysis of azoxystrobin was performed with gas chromatograph (HP6890 Series GC system) coupled to 5973 mass selective detector (Agilent Technologies, Inc., CA, USA) with detection system in the selective ion-monitoring mode (SIM). Sample ionization was achieved by electron impact at 70 k eV. The column used was an HP-5, 5% phenyl methyl siloxane (30 mm × 0.25 mm × 0.25 μm, Agilent Technologies). The oven was programed to start at 80 °C for 2 min, ramp at 20 °C/min until 180 °C, and a second ramp at 5 °C/min until 300 °C. The volume injected was 2 μL at 0 psi, the optimum vent pressure for each condition, calculated automatically by GC–MS software. Other operating conditions were explored by means of a simplex experimental design. Helium was used as carrier gas (1 mL/min). The transfer line was held at 280 °C. The retention time of Azoxystrobin was 30.10 min.

2.3. Sample preparation

The green beans and peas samples was purchased from the market in Athens, GA, USA, and analyzed before the validation to ensure that it was free from any pesticide residues. All required materials for the experiment were organized and labeled. Samples were roughly cut with a knife into small portions and homogenized for at least 30 s, the homogeneous matrix was stored in sealable plastic bag at −18 °C until the preparation day.

2.4. Extraction and cleanup

10 g (±0.1 g) of frozen sample homogenate were weighed into 50-mL centrifuge tubes. The samples were spiked with azoxystrobin standard solution while frozen to reach 3 concentration levels (0.1, 0.5, 3 mg/kg), 5 replicates for each level. The extraction involved the addition of 10 mL of acetonitrile followed by 100 μL of the internal standard solution (ISTD) containing 10 μg/mL of Pyrene D10. The tubes were closed and vigorously shaken by hand for 1 min. To induce separation and partitioning, salt mixture of 4 g of anhydrous magnesium sulfate and 1 g of sodium chloride was added. The tubes were re-closed, vigorously shaken by hand for 1 min, placed for 15 min in ultrasonic bath then centrifuged for 5 min at 4500 rpm. The extracts were subjected to freeze-out/-cleanup (aliquot placed in freezer for >2 h before cleanup). The cleanup was carried out by transferring 1 mL of the acetonitrile phase into 15 mL centrifuge tubes containing 0.002 g activated decolorizing neutral carbon. The tubes were closed, vigorously shaken by hand for 1 min followed by 5 min of centrifugation at 4500 rpm. The supernates were filtered using 0.2 μm nylon syringe filter (Chrom Tech Inc., Apple Valley, MN, USA) into auto-sampler vials which was tightly closed using a cap and septum and stored at −18 °C until the day of analysis.

2.5. Preparation of matrix-matched calibration solutions

Matrix-matched calibration was used to compensate for the matrix effects. Unspiked samples (blank) of green beans and peas were treated like the spiked samples without adding internal standard. One mL aliquots of the blank extracts were spiked by 100 μL of azoxystrobin at a concentration level corresponding to 120% of the spiking level using appropriately diluted working standard solutions. 100 μL of ISTD solution (1 μg/mL) (corresponding to one-tenth of the amount of ISTD added to the samples during the procedure) were also added to each of the matrix-matched calibration solution. Bracketing calibration was attained in which the matrix-matched calibration solutions were injected at the beginning and at the end of each sequence to insure that the determination system was free from any significant drift. According to document SANCO/12571/2013 (SANCO, 2013), the acceptable drift between two bracketing injections of the same calibration standard should not exceed 30%.

2.6. Method validation

According to Document SANCO/12571/2013 (SANCO, 2013), a within-laboratory method validation was performed to provide evidence that the method is fit for the extraction and quantitative determination of azoxystrobin in green beans and peas. Method validation is a requirement of accreditation bodies, and must be