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Analytical Methods

Determination of hymexazol in 26 foods of plant origin by modified QuEChERS method and liquid chromatography tandem-mass spectrometry

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ABSTRACT

A rapid and sensitive method based on modified QuEChERS for hymexazol determination in 26 plant-derived foods using liquid chromatography tandem-mass spectrometry (LC-MS/MS) was developed. Variables affecting the separation (LC column, mobile phase additives) and clean-up effects of various dispersive phases, such as PSA, C_{18}, GCB, MWCNTs, PEP-2, Al_2O_3, Florisil, and PVPP were evaluated. The method was validated using 26 matrices at spiked levels of 0.01 or 0.02, 0.05, 0.1, and 0.5 mg/kg (0.05, 0.2, 0.5, and 1.0 mg/kg for green tea). Mean recoveries were between 71.2% and 113.8%, and intra and inter-day precisions were below 14.8%. The limit of quantitation for 26 matrices ranged from 10 to 50 μg/kg. Matrix-matched calibration was used. The method was subsequently applied for real sample analysis, and hymexazol was detected in a cucumber (below the LOQ) and was not detected in any other sample. The method is simple and effective, and meets the routine monitoring requirements for hymexazol residue in foods.

1. Introduction

Pesticides play a key role in increasing the yield and quality of agro-products in modern agriculture (Jin, Wang, Shao, & Jin, 2010). However, the use of pesticides leads to residues in agro-products, and thus has negative effects on the quality and safety of agro-products (Chen, Dong, Xu, Liu, & Zheng, 2015; Jin et al., 2010). Therefore, it is necessary to develop methods to monitor pesticide residues in foods.

Hymexazol, a broad-spectrum fungicide and soil disinfectant, has been used to control various diseases caused by fungi such as *Fusaruim*, *Pythium*, or *Aphanomyces cochlioides*; it also has a

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Abbreviations: ACN, acetonitrile; Al_2O_3, neutral aluminium oxide; C_{18}, octadecylsilyl; CAD, collision gas; CE, collision energy; CUR, curtain gas; CXP, collision cell exit potential; DP, declustering potential; EP, entrance potential; EU, European Union; FA, formic acid; GCB, graphitized carbon black; GC-FPD, gas chromatography with flame photometric detector; GC–MS, gas chromatography–mass spectrometry; GC-NPD, gas chromatography with nitrogen phosphorus detector; GS1, atomization air pressure; GS2, auxiliary gas; HILIC, hydrophilic interaction liquid chromatography; IS, ion spray voltage; LC–ESI-MS/MS, liquid chromatography tandem-mass spectrometry with electrospray ionization; LOQ, limit of quantitation; ME, matrix effects; $MgSO_4$, magnesium sulfate; MRLs, Maximum residue limits; MRM, multiple reaction monitoring; MWCNTs, multi-walled carbon nanotubes; NaCl, sodium chloride; NH_4AC, ammonium acetate; PEP-2, polar enhanced polymer-2; PSA, primary secondary amine; PVPP, polyvinylpolypyrrolidone; QuEChERS, Quick Easy Cheap Effective Rugged and Safe; RSD, relative standard deviation; TEM, source temperature; UPLC-DAD, ultra-high performance liquid chromatography with diode-array detector.

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synergistic effect on endogenous auxin, which is widely used on sugar beets, rice, and vegetables (Harveson et al., 2007; Myresiotis, Karaoglanidis, Vryzas, & Papadopoulou-Mourkidou, 2012; Vera et al., 2011). As a systemic fungicide, it can be absorbed by roots and leaves and translocated to other plant tissues; thus, it may pose a potential threat to consumer health if it is present at harvest (Qian et al., 2011). Maximum residue limits (MRLs) for hymexazol in many foods have been established by the European Union (EU Pesticides Database., 2016) and Japan (Japan Pesticides Database., 2016), while provisional MRLs in a few foods have been set by China (China Pesticides Database., 2016). Thus, it is necessary to develop a sensitive analytical technique to quantify hymexazol residues. However, owing to its special physical–chemical characteristics, such as low mass, high polarity, and lack of chromophores, it is difficult to identify and quantify hymexazol at trace levels.

To date, the number of reports available regarding hymexazol determination in foods is limited. Tamura et al. (2008) analysed hymexazol in agricultural products by gas chromatography with a nitrogen phosphorus detector (GC-NPD), and Tan and Guo (2011) reported a method for detecting hymexazol in rice by GC-NPD. Sun et al. (2011) developed a method for monitoring hymexazol in cucumbers using GC with a flame photometric detector (GC-FPD), which included a time-consuming pre-column derivatization step. Moreover, the pretreatments required in GC-based techniques involve multi-stage procedures, large volumes of solvents, and are complicated, time-consuming, and laborious. Viñas, Aguinaga, Campillo, and Hernández-Córdoba (2008a) reported a method for analysing oxazole fungicides including hymexazol in wine and juice by ultra-high performance liquid chromatography with a diode-array detector (UPLC-DAD); however, this method lacks sensitivity and selectivity. Some reports (Viñas, Campillo, Aguinaga, Martínez-Castillo, & Hernández-Córdoba, 2008b; Viñas, Martínez-Castillo, Campillo, & Hernández-Córdoba, 2010) also described the use of GC-mass spectrometry (GC–MS) for the analysis of hymexazol in malt beverages, juices, and fruits. Furthermore, Martínez-Domínguez, Romero-González, and Garrido Frenich (2015) reported a LC-Orbitrap-MS method for the determination of many compounds including hymexazol in Ginkgo biloba nutraceuticals. All of the aforementioned methodologies are available for pesticide determination in one or a few kinds of food matrices; however, this does not meet the current needs in pesticide monitoring. Hence, it is imperative to develop a simple, efficient, and reliable method for the detection of hymexazol in foods.

Fortunately, liquid chromatography tandem-mass spectrometry with electrospray ionization (LC-ESI-MS/MS) in multiple reaction monitoring (MRM) mode is an effective and powerful tool for monitoring pesticides in various food matrices due to its high selectivity and sensitivity (Stachniuk & Fornal, 2016; Wong et al., 2010). The QuEChERS methodology, developed by Anastassiades, Lehotay, Stajnbaher, and Schenck (2003), has many merits over classical methods such as flexibility (modification of sorbents depending on analyte properties and matrix composition), simplicity, rapidity, low-solvent consumption, and wide analytical scope (González-Curbelo et al., 2015; Payá et al., 2007; Wilkowska & Biziuk, 2011), thus making it an attractive alternative sample preparation procedure for monitoring pesticides in various food matrices (Choi, Kim, Shin, Kim, & Kim, 2015; Sinha, Vasudev, & Vishnu.Vardhana.Rao, 2012).

Here, we describe a sensitive and reliable LC-ESI-MS/MS method using a modified QuEChERS approach for monitoring hymexazol residues in 26 foods of plant origin. In the present study, various experimental parameters such as MS/MS conditions, LC column, mobile phase additives, and clean-up effects of different sorbents were compared and optimized in order to obtain sensitive and reliable results. The matrix effects and performance of the developed method were also evaluated. The method was successfully applied in the analysis of real samples.

2. Materials and methods

2.1. Reagents and chemicals

Hymexazol (3-hydroxy-5-methylisoxazole, 90%) was purchased from Sigma (St. Louis, MO, USA). LC-MS grade formic acid (FA) and ammonium acetate (NH_4AC) were purchased from Sigma-Aldrich (Steinheim, Germany), and LC grade acetonitrile (ACN) was purchased from Fisher Scientific (Pittsburgh, PA, USA). Graphitized carbon black (GCB, 120–400 mesh), primary secondary amine (PSA, 40–60 μm), octadecylsilyl (C_{18}, 40–60 μm), florisil (100–120 mesh), multi-walled carbon nanotubes (MWCNTs, 10–20 nm), and polar enhanced polymer-2 (PEP-2, 40–60 μm) sorbents were obtained from Agela (Tianjin, China). Neutral aluminium oxide (Al_2O_3, 100–200 mesh) was obtained from J&K (Beijing, China) and polyvinylpolypyrrolidone (PVPP) was obtained from Solarbio (Beijing, China). Analytical grade anhydrous magnesium sulfate ($MgSO_4$) and sodium chloride (NaCl) were obtained from Sinopharm Chemical Reagent Co. (Shanghai, China). Anhydrous $MgSO_4$ was dried at 500 °C for at least 5 h, cooled naturally, and stored in a desiccator. Ultra-pure water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Sample preparation

Six fruit samples (apple, grape, orange, peach, pear, watermelon), 11 vegetable samples (beetroot, cabbage, celery, rape, cucumber, eggplant, leek, kidney bean, potato, tomato, turnip), 3 cereal samples (maize, rice, wheat), 2 oilseed samples (peanut, soybean), 1 nut sample (almond), 1 tea sample (green tea), 1 edible fungi sample (mushroom), and 1 plant oil sample (soybean oil) were purchased from local super markets. The blank samples for the calibration and spiked experiments were prescreened to confirm that they were residue-free. Only the edible parts were used for analysis. All samples were chopped into small pieces and homogenized using a Multiquick-3 food processor (Braun, Neu-Isenburg, Germany), and then stored in a deep freezer at −20 °C until analysis.

The QuEChERS methodology developed by Anastassiades et al. (2003) was used with slight modifications. Representative portions (10 g for fruits, vegetables, edible fungi, and plant oil; 5 g for cereals, oilseeds and nuts; 2 g for tea) of well-homogenized samples were weighed into a 50-mL plastic centrifuge tube. Recovery assays were carried out by adding appropriate volumes of the working standard solution to blank samples. Then, the spiked samples were vortexed for 30 s and equilibrated for at least 30 min at room temperature for even hymexazol distribution and interaction with the sample matrix. Afterwards, water (according to the indications in Table 1) was added and the sample was soaked for 15 min before 10 mL ACN was added. The tube was shaken vigorously for 3 min. Then, 4 g $MgSO_4$ and 1 g NaCl were added to the tube and the samples were shaken for 2 min. After centrifuging for 5 min at 5000 rpm, 1.0 mL of the extract was transferred into a 1.5-mL centrifuge tube containing 150 mg $MgSO_4$ and the sorbents (see Table 1). The tube was shaken vigorously for 1 min and centrifuged for 5 min at 6000 rpm. The resulting supernatant was filtered through a 0.22-μm membrane into a glass autosampler vial for LC-MS/MS analysis.

2.3. LC-MS/MS analysis

Chromatographic analyses were carried out using an Agilent 1200SL Series HPLC system (Agilent, Waldbronn, Germany)