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Analytical Methods

Simultaneous analysis of multiple pesticide residues in minor fruits by ultrahigh-performance liquid chromatography/hybrid quadrupole time-of-fight mass spectrometry

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ABSTRACT

An ultrahigh-performance liquid chromatography/hybrid quadrupole time-of-fight mass spectrometry (UPLC/QTOF-MS) method for the simultaneous identification and quantification of 50 multi-class pesticides in minor fruits is reported. The method consists of a sample extraction step, followed by analysis of the pesticides by UPLC/QTOF-MS. Satisfactory chromatographic separation was achieved over a 20 min runtime. The pesticides were identified by the accurate mass measurements of the protonated molecules ($[M+H]^+$) and their main fragment ions, isotopic pattern analysis and retention time matching. The mass accuracy obtained was below 2 ppm error for all the pesticides analysed. The method was validated by spiking starfruit with the 50 analytes. Satisfactory results regarding sensitivity and linearity were obtained. The method was successfully applied to the analysis of 87 real-world starfruit and Indian jujube samples, demonstrating its applicability for the routine analysis of multiple pesticide residues in minor tropical fruits.

1. Introduction

Tropical fruits are noted for their unique flavours, wide-ranging functions and high commercial value in domestic and international markets (Clerici & Carvalho-Silva, 2011; Panda, Panda, Pramanik, & Mondal, 2014). Most tropical fruits are grown on a small scale with low total yields. In this sense, such tropical fruits are termed “minor fruits” (IUPAC, 2010). Although tropical fruits are of relatively minor economic importance, they do contribute significantly to the global horticulture.

The production of tropical fruits is seriously affected by pests and diseases owing to the uniquely higher temperature and humidity of tropical regions (Froes, Santos, & Navickiene, 2013). Pesticides are commonly used in the production and post-harvest treatment of minor tropical fruits for the control of undesirable pathogens, pests and weeds. Recent reports from China, Brazil, Southeast Asia and other countries and regions have demonstrated that few pesticides have been registered for use on minor fruits and a wide variety of illicit pesticides are detected in these commodities (Jardim & Caldas, 2012; Skretteberg et al., 2015; Vidal, Moreno, Liebanas, & Garrido, 2007; Yang, Luo, Li, & Liu, 2016). Owing to their potential adverse effects on human health and the environment, the presence of pesticide residues in food commodities has always been a matter of serious concern. From an analytical point of view, minor tropical fruits have generally been far less studied than other fruits frequently consumed in the worldwide range. Furthermore, the list of banned and newly authorized pesticides for use on minor fruits is continually changing. Therefore, it is necessary to develop sensitive and precise methods to cover all the pesticides for the control purpose of multi-residues.

Sample preparation is necessary for routine multi-residue analysis and usually involves solvent extraction and clean-up steps. However, in most cases, sample preparation is time-consuming. “Quick, Easy, Cheap, Effective, Rugged, and Safe” (QuEChERS) approaches have been developed and widely applied in pesticide residues analytical field. These procedures typically involve extraction with acetonitrile, removal of water from the acetonitrile by addition of anhydrous $MgSO_4$ and NaCl, and subsequent clean-up using dispersive solid-phase extraction (d-SPE) with a primary secondary amine (PSA) (Botero-Coy, Marin, Serrano, Sancho, & Hernandez, 2015). Over time, this QuEChERS approach

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has been modified and a combination of different sorbents can be used in d-SPE to improve the clean-up step.

Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is widely used as an effective technique for routine multiple residue analysis nowadays (Botero-Coy et al., 2015; Yang et al., 2016). The main drawback of LC-MS/MS method is that the specific masses of the analytes must be predefined before performing analysis. With the advances in analytical technology, the more powerful liquid chromatography coupled to time-of-fight mass spectrometry (LC/TOF-MS) method has gained increasing acceptance owing to its excellent applicability to multi-residue analysis (Bueno, Ulaszewska, Gomez, Hernando, & Fernandez-Alba, 2012; Ferrer & Thurman, 2007; Garcia-Reyes, Hernando, Molina-Diaz, & Fernandez-Alba, 2007; Gilbert-Lopez et al., 2012; Ulaszewska et al., 2013). Compared with LC-MS/MS, TOF-MS combines the high resolution of TOF analyzers with the capability to perform MS/MS experiments, thus overcoming the weaknesses of the former (Lacina, Urbanova, Poustka, & Hajslova, 2010; Sivaperumal, Anand, & Riddhi, 2015). TOF-MS offers excellent functionality including accurate mass analysis, enhanced selectivity and high sensitivity in full scan acquisition mode (Gilbert-Lopez et al., 2010). TOF-MS also offers the ability to accurately confirm the identity of the analytes detected, effectively avoiding false positives (Herrera-Lopez, Hernando, Garcia-Calvo, Fernandez-Alba, & Ulaszewska, 2014; Grimalt, Pozo, Sancho, & Hernandez, 2007). The use of TOF-MS for the screening, identification and conformation of target, non-target and unknown pesticides in vegetables and fruits is becoming increasingly common (Gomez-Ramos, Ferrer, Malato, Aguera, & Fernandez-Alba, 2013; Lopez et al., 2014).

In this paper we developed and validated a multi-residue method for the analysis of 50 pesticides using TOF-MS. In order to cover a wide range of chemicals used in minor fruits production, representative pesticides belonging to different chemical families (e.g., organophosphorus pesticides, organochlorine pesticides, carbamates, and chloronicotinoids) and with various chemical activities (e.g., insecticides, fungicides, nematicides, herbicides, and plant growth regulators) were selected. All the pesticides analysed are subject to the routine monitoring and risk assessment program for vegetables and fruits as enacted by the Ministry of Agriculture (MOA), China. The results comprise detailed information on the accurate masses and errors for the protonated molecules ($[M+H]^+$), fragment ions, retention times and limits of detection. The method comprises a sample extraction step based on QuEChERS methodology and an analysis step of the target pesticides by UPLC/QTOF-MS. The method is simple and sensitive enough to detect the 50 pesticides at levels below the general maximum residue limits (MRLs) (0.01–10 mg/kg) as set in the European Food Regulations (EU, 2012). The method was applied to the analysis of starfruit and Indian jujube samples, and satisfactory results were obtained.

2. Materials and methods

2.1. Instrumentation

The identification and quantification of the pesticides analysed was performed using a UPLC System consisting of a vacuum degasser, an autosampler, and a binary pump (Agilent 1290 Infinity, Agilent Technologies, Santa Clara, CA, USA) coupled to a quadrupole time-of-flight mass spectrometer equipped with a DuoSpray™ ion source comprising an electrospray interface and an atmospheric pressure chemical ionization interface (AB SCIEX TripleTOF™ 5600$^+$, USA). Chromatographic separation was performed on an Agilent Eclipse Plus C_{18} RRHD column (50 mm × 2.1 mm; 1.8 μm) (Agilent, USA).

2.2. Chemicals and analytical standards

Certified pesticide standards were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany) or the Environmental Quality Supervision and Testing Center, Ministry of Agriculture (Tianjin, China). High-performance liquid chromatography (HPLC) grade acetonitrile and methanol were obtained from Fisher Scientific (USA). Analytical grade ammonium acetate (CH_3COONH_4), magnesium sulfate ($MgSO_4$) and sodium chloride (NaCl) were purchased from the Beijing Reagent Company (Beijing, China). Deionized water (18.2 MΩ.cm resistivity) was prepared using a Milli-Q® Integral Water Purification system (Millipore Corporation, USA). For filtration of the extracts, 0.22-μm polytetrafluoroethylene (PTFE) filters were obtained from ANPEL Scientific Instrument Co., Ltd. (Shanghai, China). Primary secondary amine (PSA) was obtained from Varian (Oxfordshire, UK).

Individual standard solutions of 50 pesticides (1.0 mg/mL in HPLC grade acetonitrile, hexane or acetone, with the exception of carbendazim, which was prepared at 0.5 mg/mL in ethanol) were prepared and used as stock standard solutions. The mixture working solutions of different concentrations were prepared by dilution. The stock standard and working solutions were stored at −18 °C and 4 °C, respectively.

2.3. Sample preparation

A total of 87 minor fruit samples (44 starfruits and 43 Indian jujubes) were collected from fields, local retail markets and supermarkets in Hainan. The samples were stored between 0 and 4 °C until arrival at the laboratory. Subsequently, the samples were chopped and homogenized in a commercial blender at high speed. Prepared samples were stored at −20 °C until analysis.

Samples were extracted using a modified QuEChERS method (Ferrer & Thurman, 2007). A blended samples (10 ± 0.01 g) was weighed into a 50-mL PTFE centrifuge tube and mixed thoroughly with 10 mL of acetonitrile using a high-speed homogenizer (18,000 rpm). The mixture was then passed through a filter paper into another 50-mL PTFE centrifuge tube containing 2.5*g* of NaCl. Each mixture was shaken vigorously by hand for 2 min and then centrifuged for 2 min at 4000 rpm. A 5-mL aliquot of the supernatant was transferred into a 15-mL graduated centrifuge tube containing 0.25*g* of PSA and 0.75*g* of $MgSO_4$ and the tube was shaken vigorously for 1 min. The mixture was then centrifuged for 2 min at 4000 rpm again. All the supernatant was transferred to a 50-mL evaporation flask and evaporated to dryness using a vacuum rotary evaporator at 36 °C. The dry residue from each sample was re-dissolved in 2.5 mL of mobile phase, filtered through a 0.22 μm PTFE membrane and transferred into an autosampler vial for UPLC/QTOF-MS analysis.

2.4. UPLC/QTOF-MS analysis

Sample analysis was performed using UPLC/QTOF-MS. The chromatographic conditions were optimized to achieve good separation. Mobile phase A and B were an aqueous solution containing 1 mM of ammonium acetate and methanol, respectively. The gradient was initiated at 0 min with 5% B followed by a linear increase to 15% B over 3 min and another linear increase to 95% B over 10.5 min. This composition was maintained for 2.5 min.