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Analytical Methods

Rapid determination of pesticide residues in fruits and vegetables, using ultra-high-performance liquid chromatography/time-of-flight mass spectrometry

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ABSTRACT

A multiresidue method, based on the sample preparation by solid-phase extraction cartridges and detection by ultra-high-performance liquid chromatography/time-of-flight mass spectrometry (UHPLC/TOF–MS), was used for the analysis of 60 pesticides in vegetable and fruit samples. Quantitation by UHPLC/TOF–MS is accomplished by measuring the accurate mass of the protonated molecules $[M+H]^+$. The mass accuracy typically obtained is routinely better than 2 ppm. The rates of recovery for pesticides studied were satisfactory, ranging from 74% to 111% with a relative standard deviation (RSD) of less than 13.2%, at concentrations below 10 μg kg^{-1}. The method limit of quantification (*MLOQ*) for most compounds was below the MRLs established by the Food Safety Standard Authority of India and the European Union. The uncertainty was determined using repeatability, recovery and calibration curves data for each pesticide. The method illustrated is suitable for routine quantitative analyses of pesticides in food samples.

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1. Introduction

India is the second largest producer of vegetables, having 13% of the world's total vegetable production. Several food commodities, including fruits and vegetables, are contaminated with pesticides. Therefore, monitoring of pesticide residues in food commodities is necessary, in order to assess potential health risks and to fix the maximum residue limits (*MRLs*) for safe human consumption. About 240 pesticides are registered in India for the purpose of controlling undesirable pests and weeds in food crops (Central Insecticides Board (CIB), 2012, Sinha, Vasudev, & Rao, 2012).

Pesticide residues have traditionally been monitored by GC-based multi-residue methods. However, many new polar and ionic pesticides cannot be determined directly by this method, due to their poor thermal stability or volatility (Cajka, Hajslova, Lacina, Mastovska, & Lehotay, 2008; Lacina, Urbanova, Poustka, & Hajslova, 2010; Pihlstrom, Blomkvist, Friman, Pagard, & Osterdahl, 2007). Pesticide analysis is not commonly carried out using ultra-high-performance liquid chromatography/time-of-flight mass spectrometry (LC–TOF/MS).

The presence of matrix interferences in extracts can affect analyte quantification. Sample clean-up is necessary in order to remove matrix interferences, which may impair chromatographic performance and reduce instrument sensitivity. Solid-phase extraction simplifies the purification of the initial extract, reduces the volume of solvent consumed, and improves the method sensitivity (Anastassiades, Lehotay, Stajnbaher, & Schenck, 2003; Gonzalez-Rodríguez, Rial-Otero, Cancho-Grande, & Simal-Gandara, 2008; Hernando, Agüera, Fernández-Alba, Piedra, & Contreras, 2001; Yang et al., 2011).

In recent times, liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) has become a useful technique in multiple residues analysis (Hernandez et al., 2006; Kovalczuk, Lacina, Jech, Poustka, & Hajslova, 2008; Pozo et al., 2007). High sensitivity and selectivity in detection of pesticide residues can be achieved by tandem mass analysers when operating in selective reaction monitoring mode. This approach allows optimisation of the parameters for each target analyte. However, it does not allow the identification of non-target compounds. Liquid chromatography with high-resolution time-of-flight mass spectrometry (LC–TOF/MS) can be used for target and non-target analysis of pesticide residues in food analysis (Ferrer & Thurman, 2007; Gilbert-Lopez, Garcia-Reyes, Ortega-Barrales, Molina-Diaz, & Fernández-Alba,

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2007). In quantification of pesticides in food and water, several researchers have used accurate mass identification of both target and non-target compounds by LC–TOF/MS (Gilbert-Lopez et al., 2010; Lacorte & Fernandez-Alba, 2006; Masia et al., 2013; Mezcua, Malato, Garcia-Reyes, Molina-Diaz, & Fernandez-Alba, 2009).

Ultra-high-performance liquid chromatography time-of-flight mass spectrometry (UHPLC–TOF/MS) instrumentation provides sensitive full-scan acquisition, identification and confirmation of target and non-target analytes of pesticide residues in fruits and vegetables in a short run time. The use of sub-2 μm UHPLC column provides excellent chromatographic resolution and sensitivity. In this paper we have developed and validated a method for rapid multi-residue analysis in vegetables and fruits, using solid-phase extraction (SPE) followed by UHPLC–TOF/MS analysis. This method was simple with fast analysis time using a low volume of mobile phase.

2. Materials and methods

2.1. Instrumentation

A UHPLC (Acquity; Waters Corporation, Milford, MA) system coupled with TOF/MS (Synapt; Waters) with UHPLC®BEH C_{18} column (2.1 × 50 mm; 1.7 μm particle size; Waters) was used. In addition, an analytical weighing balance (AUX 220; Shimadzu, Kyoto, Japan), homogeniser (Tulip, Japan), rotary evaporator (Heidolph Instruments, Schwabach, Germany), centrifuge (Thermo Fisher Scientific Inc., Waltham, MA), TurboVap LV Evaporator (Zymark, Hopkinton, MA), and SPE vacuum manifold (Supelco, Bellefonte, PA) were used.

2.2. Chemicals and analytical standards

LC–MS grade methanol, acetonitrile, water, anhydrous Na_2SO_4 (ACS, Certified) and NaCl (ACS, Certified) were obtained from Thermo Fisher Scientific. Sodium sulphate was heated at 650 °C for 4 h and kept in a desiccator until use. The lock-mass internal calibration standard leucine-enkephalin was obtained from Ultra Scientific (Kingstown, RI). The 60 analytical standards (purity > 99.9%) were obtained from AccuStandard, Inc. (New Haven, CT). The individual stock standard solutions of 200 mg L^{-1} of pesticides were prepared from 1000 mg L^{-1} original standards in LC–MS methanol; 5 mg L^{-1} intermediate mixture solutions were prepared from stock solutions. The working standard solutions were prepared from the intermediate solutions and used for method validation, quantification and confirmation of residues (see Table 1). All standards were stored at 5 °C.

2.3. Sample preparation

The vegetable and fruit samples (n = 286) including brinjal, cabbage, cauliflower, guava, okra, onion, potato apple, banana, grape, mango orange and pomegranate were selected at random from the local markets at Ahmedabad, Anand, Surat, Navsari, Kheda, Narmada, Patan and Radhanpur, in the state of Gujarat, in the western part of India. The samples were chosen according to the consumption pattern of residents in the region, and the pesticides were selected according to the recommended use in different crops. The sample wet weight was 2 kg for small and medium sized fresh product and the unit sample weight was generally in the range of 15–250 g. The vegetable and fruit samples were prepared as an analytical sample for determination of pesticide residues according to the Codex Alimentarius (Volume 2A, Part 1: 2011) Commission (2011). A representative portion of the analytical sample was blended using a food processor and mixed thoroughly. The homogenised samples were stored at −20 °C. Before using, the samples were thawed at 5 °C overnight. The quantities of each sample are presented in Table 3.

2.3.1. Samples extraction process

The of homogenised samples (10 g) were accurately weighed into 50-mL PTFE centrifuge tubes and mixed with 25 mL of acetonitrile-methanol mixture (90:10 v/v). The mixture was vortexed for 3 min, 5 g sodium chloride was added and vortexed for 3 min again. The mixture was centrifuged for 5 min at 5000 rpm, and the supernatants were transferred into a 50-mL round bottom flask and evaporated to dryness at 35 °C using a rotary evaporator.

2.3.2. Clean-up process

Solid phase extraction (SPE) was carried out using graphitized carbon black (0.5 g) and primary secondary amine (0.5 g) (GCB/PSA) in a 3.0 mL cartridge (Supelco, Bellefonte, PA). A layer (*ca.* 1 cm) of anhydrous sodium sulphate was added to the GCB/PSA column to remove traces of water from the eluate. The columns were washed with 5 mL of acetonitrile–methanol (95:5 v/v) mixture. Utmost care was taken not to allow the sorbent to dry out during the conditioning and sample loading steps. After the conditioning step, the extracted dry samples were re-dissolved in 2 mL acetonitrile–methanol mixture (95:5 v/v) and loaded onto the columns. The extracted samples were passed through the columns at a flow rate of 1 ml min^{-1}. The retained analytes were eluted with 10 mL of acetonitrile-methanol (95:5 v/v) at a rate of 2 mL min^{-1}. This eluent was collected in a 15-mL test tube and evaporated to near dryness using a Turbovap system. Finally, the residues were re-dissolved in 1 mL of methanol and 5 μL were injected into the UHPLC–TOF/MS.

2.4. Chromatographic analysis

The analysis was performed using UHPLC–TOF/MS; the column temperature was maintained at 40 °C. The mobile phase consisted of 0.1% (v/v) formic acid in methanol (A) and 0.1% (v/v) formic acid in water (B). The initial mobile phase composition was 5% A for 0.1 min, following by a linear gradient to 100% A up to 4.29 min, and kept for 0.7 min at 95% A. The flow rate used was 0.5 mL/min and the UHPLC operating pressure was maintained at 6500 psi at initial gradient conditions, and the maximum pressure was maintained at less than 8000 psi. Only 5 μL of samples were injected during the experiments. The autosampler temperature was maintained at 8 °C.

The UHPLC system was connected to TOF/MS, as mentioned above. The instrument was operated in positive electrospray ionisation mode (ESI+) with the capillary and sampling cone voltages of 80 and 30 V, respectively. The source and desolvation temperatures were maintained at 115 and 250 °C, respectively. Nitrogen was used as desolvation and cone gas at flow rates of 600 and 50 L h^{-1}, respectively. The instrument was tuned using leucine-enkephalin to provide a resolution higher than 11,000 FWHM (m/z 556.2771) in ESI+ and the total current ion chromatogram was acquired over the mass (m/z) range of 50–1000.

The mass calibration in positive ionisation mode was performed using sodium formate solution (0.5 M). The mass accuracy was maintained within the whole acquisition period by using a lock spray with leucine-enkephalin as the internal reference compound. MassLynx 4.1 software was used for data acquisition and processing, whereas QuanLynx software was used for quantification and confirmation of the pesticide residues in the samples.

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