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Analytical Methods

Pesticide determination in sweet peppers using QuEChERS and LC–MS/MS

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ABSTRACT

In this work, a rapid, effective, and safe method, generating only a small amount of waste, based on the citrate version of QuEChERS was optimized and validated for multiresidue determination of pesticides of different classes in sweet green peppers, determined by liquid chromatography coupled with tandem mass spectrometry. The matrix components influenced the measurement of the pesticides by the developed analysis technique, so that, analytical curves were prepared using pesticide-free matrix extracts for quantification of the analytes. The method provides satisfactory accuracy verified by recoveries of 70–120%, and good precision (coefficients of variation ≤20%). It also showed selectivity, linearity of response, and lower limits of quantification than the maximum limit of residue for each compound, as established by ANVISA and Codex Alimentarius.

1. Introduction

Due to population growth and the consequent demand for food, agriculture has increased intensely in productivity, using lesser acreage. However, due to the undesired incidence of diseases of bacterial, fungal, nematological and viral origin, to arthropods that cause disturbances and to weed seeds, with consequent environmental imbalance, there has been an increase in the use of pesticides, often applied inappropriately and indiscriminately, which causes contamination of crops and, consequently, adverse health effects for human and animals. In addition, the use of pesticides can cause contamination of surface water, groundwater and soil and lead to animal mortality (Ahmed, Randhawa, Yusuf, & Khalid, 2011).

In order to control the use of pesticides and limit concentrations of residues in foods, many agencies, such as the Brazilian Health Surveillance Agency (ANVISA) and Codex Alimentarius, have established maximum residue limits (MRL) for pesticides.

Sweet peppers (*Capsicum annuum* L.) (Buckler, Pearsall, & Holtsiord, 1998), whose world production is approximately 32 million tonnes, according to the latest survey conducted by the Food and Agriculture Organization of the United Nations (FAOSTAT, 2017), are consumed due their taste and to the presence of compounds that prevent some diseases (Collera-Zúñiga, Jiménez, & Gordillo, 2005; Nishino, Murakoshi, Tokuda, & Satomi, 2009; UNICAMP, 2011) and deserve attention due to possible irregularities in control of pesticide residues (ANVISA, 2014; FDA, 2017). According to a report of activities released by ANVISA in 2014 regarding the Program for the Analysis of Pesticide Residues in Food (PARA), 89% of the sweet peppers samples grown in Brazil were deemed inadequate, i.e., contained residues of pesticides not allowed or at levels above the MRL. The Pesticide Residue Monitoring Program 2015, published in 2017 and conducted by the U.S. Food and Drug Administration (FDA), revealed that 9.0% of samples obtained from several Brazilian states contained irregularities.

Due to negligence and the adverse effects of pesticides, there is increased interest in conducting research addressing the development and validation of methods to monitor the presence of multiresidues of pesticides in food matrices, such as sweet peppers. In this context, one of the steps for the determination of pesticide residues in food is the preparation of the sample for extraction and concentration of the analytes, as well as for the clean-up of the samples. However, some techniques have limitations and drawbacks, such as not providing high recovery of the compounds of interest, efficient clean-up of the samples and sufficient accuracy of results, and often because they are time consuming, costly and difficult to apply.

The QuEChERS (quick, easy, cheap, effective, rugged, safe) technique of sample preparation was introduced in 2003 by Anastassiades, Lehotay, Štajnbaher and Schenck, in order to overcome the limitations and disadvantages of some traditional extraction techniques for multiresidues of pesticides. The method has been widely used in the determination of pesticide residues in various matrices, being a fast, easy, economical, effective, rugged and safe method. Moreover, the application of this method, including the acetate buffer (Lehotay, Maštovská, & Lightfield, 2005) and citrate buffer (Anastassiades, Scherbaum, Taşdelen, & Štajnbaher, 2007) versions, enables the extraction of acidic, basic and neutral compounds, obtaining precise and accurate results due to high recoveries of the analytes.

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Among the modern analysis techniques, liquid chromatography (LC) coupled to different detectors stands out, due to its facility in effecting separations, identifications and quantification the species present in a sample (Braga et al., 2007; Collins, Braga, & Bonato, 2006). In recent years, liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) has shown great progress in terms of technological development and application (Kmellár, Pareja, Ferrer, Fodor, & Fernández-Alba, 2011). Satisfactory results have been obtained when this technique is used, since it combines the high selectivity and efficiency of separation by liquid chromatography with obtaining identification information of the separated compounds, due to the high detectability and increased selectivity of mass spectrometry, allowing the determination of low concentrations of mixtures of pesticide residues belonging to different chemical groups in complex matrices in a single analysis (Vékey, 2001; Niessen, 2006).

Based on this, in the present study a method was optimized, validated and applied to samples of commercially available sweet green peppers (*Capsicum annuum* L.) for the determination of residues of pesticide that are permitted to be applied to the crop, as well as some that are not allowed, but were found, according to the PARA report. This work used the techniques of QuEChERS for sample preparation and LC–MS/MS with electrospray ionization and triple quadrupole detection in the multiple reaction monitoring (MRM) mode for the development and validation of a method for multiresidue determination of pesticides in sweet green pepper.

2. Materials and methods

2.1. Reagents and solvents

The analytical standards of pesticides, with their respective purity and suppliers, for: clomazone (98.1% w/w), difenoconazole (97.0% w/w), ethion (97.8% w/w), methamidophos (98.5% w/w), methomyl (99.9% w/w), pyraclostrobin (99.9% w/w), pyriproxyfen (99.1% w/w), thiabendazole (99.8% w/w), tiacloprid (99.9% w/w) and thiamethoxam (99.7% w/w) were from Fluka (Madrid, Spain); those for acephate (97.2% w/w), azoxystrobin (99.9% w/w), carbofuran (99% w/w), fenarimol (99.8% w/w), iprodione (99.3% w/w), metconazole (99.5% w/w) and tebuconazole (99.8% w/w) were from Pestanal, Riedel-de Häen (Seelze, Germany); carbendazim (99.1% w/w) and methiocarb (98.5% w/w) were from Dr. Ehrenstorfer GmbH (Augsburg, Germany); carbaryl (99.5% w/w) was from Chem Service (West Chester, PA, U.S.A.); and imidacloprid (99.9% w/w) was from Riedel-de Häen (Seelze, Germany). Polyethylene membranes of 0.45 μm porosity (Millipore – Milli-Q, Bedford, MA, U.S.A.), formic acid (p.a., Synth, São Paulo, SP, Brazil), water from a Millipore – Milli-Q (Bedford, MA, U.S.A.) and methanol (chromatographic grade, J.T. Baker, Phillipsburg, NJ, U.S.A.) were used to prepare the mobile phases, the latter was also used in the preparation of standard solutions. Acetonitrile and methanol (chromatographic grade, J.T. Baker, Phillipsburg, NJ, U.S.A.), acetone and formic acid (p.a., Synth, São Paulo, SP, Brazil), ethyl acetate (Mallinckrodt Chemicals, Saint Louis, MO, U.S.A.), magnesium sulfate, (p.a., Vetec, Rio de Janeiro, RJ, Brazil), sodium chloride, (p.a., ECIBRA, São Paulo, SP, Brazil), disodium hydrogen citrate sesquihydrate, and trisodium citrate dihydrate (Sigma Aldrich, Madrid, Spain), primary-secondary amine, PSA (Varian, Harbor City, CA, U.S.A.) and graphitized carbon (Hexis, São Paulo, SP, Brazil) were used in sample preparation.

2.2. Stock solutions of pesticides

The standard stock solutions of each pesticide in concentrations of 1000 mg L^{-1} were prepared by solubilizing each analytical standard in methanol. The working solutions were prepared by diluting the stock standard solutions with the same solvent. All were stored at a refrigerator temperature of approximately 4 °C.

2.3. Equipment for sample preparation

A multiprocessor (Model Faciclic – Arno, São Paulo, SP, Brazil), an analytical balance with accuracy of 5 decimal places (Model CP225 D – Sartorius, Göttingen, Germany), micropipettes of 0.5–10 μL, 10–100 μL and 100–1000 μL (Eppendorf Research, Hamburg, Germany), a glass vacuum filtration system, with vacuum pump (Model WP6111560 – Millipore, Billerica, MA, U.S.A.), a vortex (Model GENIUS 3 – IKA Vortex ®, Staufen, Germany) and a centrifuge (Model Rotofix 32 – Analytical, Hettich, Germany) were used.

2.4. Liquid chromatography–tandem mass spectrometry

For the chromatographic analysis an Alliance 2695 liquid chromatograph (Waters, Milford, MA, U.S.A.) was used. The chromatographic separations were carried out with a Nova-Pak C18 analytical chromatographic column (150 mm × 3.9 mm i.d., 4 μm) (Waters, Milford, MA, U.S.A.) and a Nova-Pak C18 guard column (20 mm × 3.9 mm i.d., 4 μm) (Waters, Milford, MA, U.S.A.) with a flow rate of 0.3 mL min^{-1}. The column was kept at (25 ± 2) °C and the sample injection volume was 17 μL. Before chromatographic analyses, all the samples were filtered through 0.2 μm PTFE membranes.

The mobile phase used was 0.1% aqueous formic acid (A) and methanol (B). Gradient elution was used and the amount of methanol was changed as follows: 0 min – 50%, 12 min – 50%, 13 min – 75%, 30 min – 90%, 33 min – 90%; 35 min – 50%, 43 min – 50%.

A tandem mass spectrometer with triple quadrupole and Z-spray interface for electrospray (ESI) (Micromass Quattro Micro™ API spectrometer, Waters, Milford, MA, U.S.A.), operating in the positive mode with MRM acquisition was used. The parameters of the mass spectrometer for analysis were: capillary voltage – 2 kV, cone extractor voltage – 3 V, RF lens voltage – 0.2 V, source temperature – 120 °C, desolvation gas temperature – 400 °C, desolvation gas flow rate – 500 L h^{-1}, cone gas flow – 50 L h^{-1}. Nitrogen was used as the cone and desolvation gas and argon as the collision gas at a constant pressure of 2.45×10^{-3} mbar. The data acquisition and processing were performed using Mass Lynx v. 4.1 software from Waters (Milford, MA, U.S.A.).

2.5. Technique for QuEChERS sample preparation

2.5.1. Samples preparation and fortification of sweet pepper

In the optimization of the QuEChERS sample preparation technique, organic sweet green peppers obtained in the Campinas, SP, region (Brazil) were used, from which extracts were prepared and analyzed by LC–MS/MS to confirm the absence of pesticides studied. Sweet peppers, free of the pesticides studied, were chopped to pieces and ground in a household multiprocessor until full homogenization. To a falcon tube of 50 mL capacity were added 10.00000 g of sweet pepper pulp, which was then fortified with 100 μL of a working solution (concentrations of 50 mg L^{-1}) containing the pesticides studied. The sample was left standing for about 30 min and then subjected to the QuEChERS method in the citrate buffer version.

2.5.2. Extraction of pesticides from samples of sweet pepper

After 30 min, the falcon tube containing the crushed and fortified sample sweet pepper, with added solvent was vortexed. After vortexing, the partitioning salts were added. The mixture was stirred using the vortex and then centrifuged. Then, 7 mL of extract were transferred to another falcon tube containing the salts for the clean-up step. After vortexing and centrifugation, 5 mL of supernatant was transferred to a flask containing 50 μL of 5% formic acid (v/v) in acetonitrile, kept under a flow of nitrogen gas to dryness, resuspended in 1.0 mL of methanol and stored in a glass tube in the freezer until the time of analysis by LC–MS/MS. The tests and injections were each performed in triplicate. Table 1 shows the salts, their amounts, and the times of agitation used in the steps of the citrate buffer QuEChERS method.