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Analytical Methods

Multiclass method for pesticides quantification in honey by means of modified QuEChERS and UHPLC–MS/MS

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ABSTRACT

Bee products can be produced in an environment contaminated by pesticides that can be transported by honey bees to the hive and incorporated into the honey. Therefore, rapid and modern methods to determine pesticide residues in honey samples are essential to guarantee consumers' health. In this study, a simple multiresidue method for the quantification of 116 pesticides in honey is proposed. It involves the use of a modified QuEChERS procedure followed by UHPLC–MS/MS analysis. The method was validated according to the European Union SANCO/12571/2013 guidelines. Acceptable values were obtained for the following parameters: linearity, limit of detection (0.005 mg/kg) and limit of quantification (0.010 and 0.025 mg/kg), trueness (for the four tested levels the recovery assays values were between 70 and 120%), intermediate precision (RSD < 20.0%) and measurement uncertainty tests (<50.0%). The validated method was applied for determination of 100 honey samples from five states of Brazil.

1. Introduction

Honey is one of the most used products of the hive, both naturally and in several industrialized forms (Komatsu, Marchini, & Moreti, 2002). Known since ancient times, honey has always attracted the attention of man, especially because of its sweet taste (Bera & Almeida-Muradian, 2007; Rossi, Martinelli, Lacerda, Camargo, & Victória, 1999). Furthermore, several hive products have been appreciated due to their antimicrobial and antiseptic properties. However, in recent years, the pesticide monitoring in honey has become a public health issue in view of the growth of the levels of these chemicals in bee products (Li et al., 2013; Rial-Otero, Gaspar, Moura, & Capelo, 2007). Therefore, the monitoring of pesticide residues in honey is important to evaluate the potential risk of these products to consumers' health. Also, such monitoring can provide information about the use of pesticides in crop fields around the hives and in their neighborhoods. In this case, honey can be used as a bio-indicator for the evaluation of environmental impact (Rissato, Galhiane, Knoll, Andrade, & Almeida, 2006).

In this context, analytical methods for the determination of pesticides in honey must be available for routine analysis. The determination of pesticide residues in foods requires a prior step of sample preparation due to the low concentrations of the analytes in the sample, the distinct chemical properties of the analytes and the complexity of the matrices (Prestes, Friggi, Adaime, & Zanella, 2009). Although most of these procedures are carried out by conventional techniques, such methods are generally not applicable to all food matrices, do not produce clean extracts and generate low recovery. These disadvantages have led to the development of new approaches with an emphasis on the practicality of implementation, the use of significantly lower amounts of organic solvents, and the ability to detect analytes in very low concentrations. In recent years, efforts in the field of analytical chemistry focused on the miniaturization of sample preparation associated with improvement in selectivity and sensitivity (Melwanki & Fuh, 2008). However, these efforts are far from being considered ideal, due to the limitation of application, quickness, sensitivity and reliability of the results (Martínez-Vidal, Liébanas, Rodríguez, Frenich, & Moreno, 2005). In this context, QuEChERS

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(an acronym for quick, easy, cheap, effective, rugged, and safe), developed by Anastassiades, Mastovska, and Lehotay (2003), is an appropriate alternative. This technique, which has the advantages of being fast, easy, economical, effective, robust and secure, can be applied in any laboratory, due to the simplification of the steps (Prestes et al., 2009). This approach has become popular for sample preparation at international level (Cieslik, Sadowska-Rociek, Ruiz, & Surma-Zadora, 2011).

Besides the extraction and purification procedures, the choice of appropriate separation and detection techniques is a step of fundamental importance. Technological advances in mass spectrometry technique allow meeting the criteria of sensitivity and selectivity (Chiaradia, Collins, & Jardim, 2008). Accordingly, the performance of liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) has shown great success in multiresidue pesticide analysis in complex food matrices such as honey (Barganska, Slebioda, & Namiesnik, 2013; Jovanov et al., 2013; Lopez, Pettis, Smith, & Chu, 2008; Orso et al., 2016; Tomasini et al., 2012; Wiest et al., 2011). This technique provides information regarding the characteristic ion of each analyte as well as two or more transitions of these ions, useful to quantify and confirm the analytes at concentrations consistent with maximum residue levels (MRLs) established (Martins Júnior, Bustillos, & Pires, 2006).

Several studies on multiresidue determination of pesticides in honey have been reported in the literature. Kasiotis, Anagnostopoulos, Anastasiadou, and Machera (2014) developed a method to investigate the occurrence of 115 pesticides of different chemical classes such as neonicotinoids, organophosphates, triazoles, carbamates, dicarboximides and dinitroanilines in honey from different areas of Greece using modifications of the QuEChERS technique and LC–MS/MS. The total chromatographic run time was 35 min. Similarly, the method developed by Cotton et al. (2014) evaluated the occurrence of 83 pesticides and antibiotics of different classes in honey from France using QuEChERS and LC–MS/MS in a run time of 30 min. Kujawski et al. (2014) determined pesticides in honey after 14 min run using two extraction techniques, QuEChERS and extraction on a diatomaceous earth support (SLE). However, the developed method was applied to only 30 pesticides including acaricides, insecticides, herbicides and fungicides. Rapid methods for multiresidue analysis of pesticides in honey have not been described in the literature. Gómez-Pérez, Plaza-Bolanosa, Romero-Gonzáleza, Martínez-Vidala, and Garrido-Frenicha (2012) created a method for the simultaneous analysis of more than 350 pesticides and veterinary drugs in honey using ultra-high performance liquid chromatography coupled to high resolution Orbitrap mass spectrometry (UHPLC-Orbitrap-MS) in a run time of 14 min, but the liquid liquid extraction was time consuming, due to the 1 h agitation required for the extraction of the compounds.

Therefore, the aim of this study was to develop and validate a rapid, sensitive and selective method for determination of 116 pesticide residues from 35 different classes (acylamino acid, anilinopyrimidine, aryloxyphenoxypropionate, benzimidazole, benzofuran, carbamate, carbanilate, carboxamide, chloroacetamide, cyanoimidazole, diacylhydrazine, dicarboximide, dinitroaniline, hydroxyanilide, imidazole, morpholine, neonicotinoid, organophosphate, oxadiazine, phenylamide, phenylpyrazole, phenylurea, phosphorothiolate, pyrazole, pyrethroid, pyridazinone, pyridine, pyrimidine, strobilurin, sulphite ester, tetrazine, tetronic acid, triazine, triazole, urea and other pesticides unclassified) in honey using QuEChERS and ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC–MS/MS). The developed method was validated according to European Union SANCO/12571/2013 guideline (SANCO, 2013). Also, measurement uncertainty was evaluated as well as method performance by means of participation in a proficiency test. Finally, the method was used to evaluate the quality of the honey produced in five states from Brazil.

2. Experimental

2.1. Material

2.1.1. Honey samples

Honey samples were purchased from consumer stores or provided by honey producers or cooperatives: 66 from the state of Minas Gerais (49 wild flower honey, 4 from eucalyptus, 1 from *Vernonia polyanthes* and 12 without flower type), 9 samples from São Paulo (1 wild flower honey and 8 without flower type), 18 samples from Santa Catarina (all wild flower honey), 2 samples from Espírito Santo (all wild flower honey) and 5 from Pará (all wild flower honey). All collected samples were produced by *Apis mellifera* honey bees except one sample from Pará, which was produced by *Melipona scutellaris*. The blank honey samples were acquired from the consumer market. The samples were stored at ambient temperature (20 °C) until analysis. Honey sample from the provider BIPEA, code 18-3619-0038, analyzed in the proficiency test, was maintained under refrigeration (5 °C) until analysis.

2.1.2. Chemicals and reagents

Acetonitrile and glacial acetic acid were supplied by Merck (Darmstadt, Germany), methanol, ethyl acetate and formic acid were obtained from Tedia (Ohio, USA), all HPLC grade. Polymerically bonded ethylenediamine-*N*-propyl phase (PSA) (Varian, Palo Alto, CA, USA), anhydrous magnesium sulfate (purity ⩾ 97%-Sigma-Aldrich, Saint Louis, MO, USA), Florisil (Mallinckrodt, St. Louis, USA), and anhydrous ammonium acetate and sodium acetate (Vetec-Rio de Janeiro, RJ, Brazil) were of analytical grade. The solutions were prepared with ultra pure-water (Milli-Q Plus system; Millipore Corp., Billerica, MA, USA). All reference standards were of high purity grade (>98.0%) and were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Individual stock solutions were prepared at 1000 mg/L in acetonitrile or methanol and stored in a freezer at −18 °C. The working solutions were prepared through appropriate dilutions of the stock solutions.

2.2. Apparatus

2.2.1. Chromatography parameters

The UHPLC system (Shimadzu LC20ADXR) comprised a binary pump (Shimadzu LC20ADXR), an auto sampler (Shimadzu SIL20ACXR) and a column oven (Shimadzu CTO20AC). Chromatography was carried out using a Shim-pack XR-ODSII column (2.0 × 100 mm, 2.2 μm particle size) with a mobile phase consisting of ammonium acetate (10 mmol/L) (phase A) and methanol (phase B) both acidified with 0.1% formic acid at a flow rate of 0.5 mL/min. The gradient elution program was as follows: 0 min, 50% B; 6 min, 80% B; 10 min, 90% B; 10.5 min, 50% B; 10.5–13 min, 50% B. The total chromatographic run time was 13 min. Injection volume was 5 μL and the column temperature was set at 60 °C. The chromatographic method was previously developed by Madureira et al. (2012) and was adapted for the UHPLC system.

2.2.2. Mass spectrometry parameters

Mass spectrometry analysis was performed using a 5500 Triple Quadrupole mass spectrometer (Applied Biosystems, MDS SCIEX, Ontario, Canada). The instrument was operated using electrospray ionization (ESI) in the positive ion mode. Instrument settings, data acquisition and processing were controlled by the software Analyst (Version 1.5.1, Applied Biosystems). Source parameters were optimized as follows: ion spray voltage 4.5 kV for ESI (+), curtain gas 20 psi, collision gas 8 psi, nebulizer gas and auxiliary gas 30 psi and ion source temperature 500 °C. Retention time, precursor