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Development and validation of ultra high performance-liquid chromatography–tandem mass spectrometry based methods for the determination of neonicotinoid insecticides in honey

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ABSTRACT

In this study, the feasibility of two sample treatments has been evaluated for the determination of seven neonicotinoid insecticides in honey from different botanical origins using ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC–MS/MS). A solid phase extraction with a polymeric sorbent (Strata® X) is proposed for analyzing dark honeys, while a QuEChERS (quick, easy, cheap, effective, rugged and safe) approach is recommended for light honeys. Chromatographic analysis (6 min) was performed on a core-shell column (Kinetex® EVO C_{18}). The proposed methods were fully validated using two different MS/MS systems: quadrupole-time-of-flight and triple quadrupole. The results showed that the best overall analytical performance was achieved using triple quadrupole, mainly due to its better sensitivity and the reduced influence of the matrix onto the analyte signals. The methods developed were applied to the analysis of commercial honey samples from different regions of Spain, as well as from experimental apiaries.

1. Introduction

Honey, one of the most used products of the hive, is a natural, unprocessed and easily digested food that has been part of the human diet since ancient times (Ares et al., 2017), and it is mainly composed of glucose, fructose and sucrose (Dong, Xiao, Xian, & Wu, 2018). It is a highly valuable natural food product due to its characteristic flavor, nutritional value and therapeutic applications; this has led to a significant increase in its consumption in the last years (Juan-Borrás, Domenech, & Escriche, 2016). However, food alerts caused by the detection of contaminants, e.g. insecticides such as the family of neonicotinoids, have recently affected its healthy image, as they could represent a potential risk for consumers (Ares et al., 2017; Tette et al., 2016). Neonicotinoids are among the most widely used insecticides in the world due to their broad spectrum of efficacy, their systemic and translaminar action, and their pronounced residual activity and unique mode of action (Valverde, Bernal, Martín, Nozal & Bernal, 2016). However, concerns regarding the side effects on health and the environment of this family of insecticides continue increasing, since they can be transferred to the environment and the food chain, with potential adverse consequences for biodiversity, and for example non-target organisms, such as honeybees. As a consequence of those negative effects associated with the use of neonicotinoid insecticides, International institutions, such as the European Union, have established stringent maximum residue levels (MRLs) for these substances in honey (50–200 μg/kg; European Union Pesticide Database, 2017). Therefore, efficient, selective and sensitive methods are needed for the simultaneous determination of these pesticides in honey.

In order to achieve accurate and reliable analytical data, an efficient pre-concentration/separation step is usually required prior to the determination of neonicotinoid residues in honey (see Supplementary Material, Table S1), even using sensitive detection systems, such as tandem mass spectrometry (MS/MS). After dilution with an aqueous solution honey can be extracted using protocols similar to those applied to water samples, as solid phase extraction (SPE) (Calatayud-Vernich, Calatayud, Simó, & Picó, 2016; Campillo, Viñas, Férez-Melgarejo, & Hernández-Córdoba, 2013; Gblylik-Sikorska, Sniegocki, & Posyniak, 2015; Sánchez-Hernández et al., 2016; Tanner & Czerwenka, 2011). Current trends in sample preparation techniques are focused on the simplification of this step in order to reduce costs, the amount of reagents and time spent, which are some of the principles of green analytical chemistry (Calatayud-Vernich et al., 2016; Gałuszka, Migaszewski, & Namieśnik, 2015). In recent years, (QuEChERS; quick, easy, cheap, effective, rugged and safe) based procedures have been

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predominately used for the extraction of pesticides in food matrices, and in particular of neonicotinoids from honey (Codling, Al Naggar, Giesy, & Robertson, 2016; Galeano et al., 2013; Jovanov et al., 2015; Laaniste et al., 2017; Shendy, Al-Ghobashy, Mohammed, Alla, & Lofty, 2016; Tanner & Czerwenka, 2011; Tette et al., 2016; Tomasini et al., 2012). The simple steps involved and the relatively low cost of reagents and equipment allow its application in most laboratories. Another possibility is the employ of liquid-liquid microextraction (LLME), which overcomes some of the problems of conventional liquid-liquid extraction (large volumes of organic solvents, time and steps) (Campillo et al., 2013 Jovanov et al., 2013; Rezaee, Yamini, & Faraji, 2010; Vichapong, Burakham, Santaladchaiyakit, & Srijanarai, 2016; Vichapong, Burakham, & Srijaranai, 2015).

Due to their thermolability, low volatility and high polarity, neonicotinoid residues in honey have usually been determined by high-performance liquid chromatography (HPLC) in reverse phase mode with C_{18} columns. HPLC coupled with tandem mass spectrometry (MS/MS) (see Supplementary Material, Table S1) has been predominately used due to its excellent performance in terms of sensitivity, selectivity and robustness, as well as the reliable identification and quantification of the analytes. In the last years, ultra-high performance liquid chromatography (UHPLC) has been also employed in this field because of the better resolution and sensitivity attained and shorter running times (Galeano et al., 2013; Tette et al., 2016; Sánchez-Hernández et al., 2016).

The aim of this study was to propose a specific analytical methodology to quantify seven of the most commonly employed neonicotinoid insecticides (dinotefuran-DN, nitenpyram-NT, thiamethoxam-TMX, clothianidin-CLO, imidacloprid-IMI, acetamiprid-ACET, and thiacloprid-THIA), with special emphasis to IMI and TMX, in honeys from three different botanical origins (multifloral, rosemary and heather) using UHPLC–MS/MS. In order to propose the most suitable sample treatment, relevant parameters (extraction efficiency, organic solvent consumption, overall time, cost and number of steps) of two of the most employed approaches (SPE and QuEChERS) were evaluated. Honey samples from different botanical origins were tested and the methodology optimized in order to evaluate matrix effects as their different chemical composition may strongly affect the insecticide determination. The final objective was the selection of the most appropriate sample treatment according to the honey botanical origin. The analytical performance of two different MS/MS systems (quadrupole-time-of-flight-QTOF; triple quadrupole-QqQ), was also evaluated. To the best of our knowledge, this is the first study in which a simultaneous comparison is made for different combinations of sample treatments and MS analyzers, considering honeys from three different botanical origins (multifloral, rosemary and heather). The proposed methods for the different honeys were validated and eventually applied to samples from different regions of Spain as well as from experimental apiaries located close to cultivars in which a TMX treatment had been applied.

2. Materials and methods

2.1. Reagents and materials

Fluka-Pestanal analytical standards of ACET (Det. Purity 99.9%), CLO (Det. Purity 99.9%), DN (Det. Purity 98.8%), IMI (Det. Purity 99.9%), NT (Det. Purity 99.8%), THIA (Det. Purity 99.9%), TMX (Det. Purity 99.6%), and TMX-d3 (Det. Purity ≥ 98%) were purchased from Sigma-Aldrich Laborchemikalien GmbH (Seelze, Germany). An isotope-labeled standard (TMX-d3) was chosen as internal standard (IS), since it has the same physical and chemical properties as the unlabeled analyte. Ethyl acetate, acetone, methanol, ethanol and acetonitrile (HPLC grade) were supplied by Lab Scan Ltd. (Dublin, Ireland). Formic acid (98–100% pure), ammonium acetate, ammonium hydroxide, and magnesium sulfate anhydrous were obtained from Sigma–Aldrich Chemie Gbmh (Steinheim, Germany). Sodium chloride, sodium acetate, trisodium citrate dihydrate, and disodium hydrogen citrate sesquihydrate were supplied by Panreac (Barcelona, Spain), while primary secondary amine (PSA) and C_{18} were provided by Supelco (Bellefonte, PA, USA). Meanwhile, Strata® X (3 mL with 600 mg of sorbent) SPE cartridges (Phenomenex, Torrance, CA, USA), and a 10-port Visiprep vacuum manifold (Supelco, Bellefonte, PA, USA), were used in the SPE procedure. A vibromatic mechanical shaker, a thermostated ultrasound system, and a drying oven, both supplied by J.P. Selecta S.A. (Barcelona, Spain), a vortex mechanical mixer from Heidolph (Schwabach, Germany), a 5810 R refrigerated bench-top Eppendorf centrifuge (Hamburg, Germany), and an R-210/215 rotary evaporator from Buchi (Flawil, Switzerland) were employed for all extractions. Nylon syringe filters (17 mm, 0.45 µm) were from Nalgene (Rochester, NY, USA), and ultrapure water was obtained using Milipore Mili-RO plus and Mili-Q systems (Bedford, MA, USA).

2.2. Standards

Standard stock solutions (~1000 mg/L) were prepared by dissolving approximately 10 mg of each neonicotinoid insecticide, accurately weighed, in 10 mL of methanol. These solutions were further diluted with a water and methanol mixture (80:20, v/v) in order to prepare the working solutions. Honey samples (5.0 g) were spiked before (BF samples) or after (AF samples) sample treatment with different amounts of the neonicotinoid insecticides and with 50 µg/kg of the IS to prepare the matrix-matched standards, as described in Section 2.3. The samples were employed for validation (quality control (QC) samples and calibration curves), matrix effect, and treatment studies. Each QC sample was prepared with 5.0 g of honey spiked with the neonicotinoids at three concentration levels within the corresponding linear range for each MS/MS (QTOF and QqQ). These were as follows: low QC-LOQ; medium QC-10 µg/kg for QqQ and 50 µg/kg for QTOF; high QC-50 µg/kg for QqQ and 300 µg/kg for QTOF. The stock solution was stored in glass containers in darkness at −20 °C; working and matrix-matched solutions were stored in glass containers and kept in the dark at 4 °C. All solutions were stable for over two weeks.

2.3. Sample procurement and treatment

Several honey types were selected according to their different color, composition and botanical origin. Samples from different regions of Spain, in which a neonicotinoid treatment had been employed in some crops, were kindly donated by the “Centro Apícola Regional-CAR” at Marchamalo (Guadalajara, Spain). Their botanical origin was confirmed by melissopalynological analysis, and corresponded to: rosemary, *Rosmarinus officinalis* (n = 6); multifloral (n = 6); and heather, *Erica* spp (n = 6). In addition, multifloral honey samples (n = 10) collected from controlled apiaries were also supplied by CAR. Apiaries were located close to experimental crops, previously treated with TMX dressed rapeseeds (1 L per 100 kg of Cruiser 350 FS (Syngenta, Madrid, Spain) containing TMX-35%, w/v. In this study, all honey samples were examined in triplicate, and also underwent a preliminary analysis by HPLC–MS/MS in order to check for the presence of neonicotinoids. Once absence was confirmed in the samples, different subsamples were generated and used to prepare matrix-matched standards for validation and sample treatment studies. The blank honey samples were stored in a fresh (4 °C) and dark place before analysis. Two different sample treatments (SPE and QuEChERS) were developed and compared. Fig. 1 outlines the steps of the selected procedures used during the present study.

2.4. UHPLC–MS/MS system

2.4.1. UHPLC conditions

The chromatographic system consisted of an Acquity™ UHPLC system (Waters, Milford, MA, USA) equipped with an online vacuum