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Original Research Article

Development of HPLC-DAD method for determination of neonicotinoids in honey

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ABSTRACT

After the EU banned the use of the neonicotinoids in flowering crops that honeybees might visit, there has been an increased interest in determining the neonicotinoid residues in honeybee products such as honey. The aim of this study was to develop and optimize an HPLC-DAD analytical method with dispersive liquid–liquid microextraction (DLLME) and QuEChERS sample preparation procedures for the simultaneous analysis of seven neonicotinoids (dinotefuran, nitenpyram, thiamethoxam, clothianidin, imidacloprid, acetamiprid and thiacloprid) in honey samples. The liquid chromatographic conditions were optimized by Response surface methodology with Box–Behnken design and Derringer's desirability. The optimized method was validated to fulfill the requirements of SANCO/12571/2013 standard for both sample pretreatment procedures providing results for accuracy (73.1–118.3%), repeatability (3.28–10.40%) and within-laboratory reproducibility (6.45–17.70%), limits of detection (1.5–2.5 μg kg^{-1}) and quantification (5.0–10.0 μg kg^{-1}) with the use of matrix-matched calibration to compensate the matrix effects. For the first time 104 honey samples from Vojvodina were analyzed. The presence of thiacloprid, imidacloprid and thiametoxam was found in a small number of samples implicating the usefulness of ongoing control of honey. Residues were confirmed by LC–MS/MS. Q2

1. Introduction

Q3 Neonicotinoids are a relatively new class of insecticides chemically related to nicotine. In less than 20 years, neonicotinoids have become the most widely used class of insecticides. Their presence now accounts for at least one quarter of the world's insecticide market (Agropages, 2013). This group of insecticides includes nitro-substituted (dinotefuran, nitenpyram, thiamethoxam, imidacloprid and clothianidin) and cyano-substituted (acetamiprid and thiacloprid) compounds. They are intended for the treatment of a wide range of plants including sunflower, corn, canola, cotton, potato, rice, sugar beets, oil rapeseed, soy, ornamental plants, tree nursery, and fruits (Biever et al., 2003).

Neonicotinoids are nicotinic acetylcholine receptor agonists interfering with the transmission of neural messages in insects more efficiently than in mammals and vertebrates (Decourtye and Devillers, 2010; Tomizawa and Casida, 2005). Being systematic insecticides translocated to the whole plant (flowers, pollen and

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nectar), they can even reach the leaves through guttation when applied to seeds, revealing ways by which some honey bees and other beneficial pollinators can be exposed to these compounds (Van der Sluijs et al., 2013; Van Dijk et al., 2013). Different studies in Europe and the USA have demonstrated that sublethal amounts of neonicotinoids alone or combined with other pesticides, such as fungicides (Iwasa et al., 2004) may cause disorientation, reduced communication, impaired learning and memory, reduced longevity and disruption of honeybee brood cycles (Farooqui, 2013). Moreover, residues of these insecticides may be found in bee products such as honey, pollen, beeswax, and propolis (Kasiotis et al., 2014; Tanner and Czerwenka, 2011).

The European Commission has banned the use of imidacloprid, thiamethoxam and clothianidin in crops attractive to pollinators in the next two years emphasizing the awareness of the potential harmful impact of the neonicotinoids on honeybees and their products (Commission, 2013; EFSA, 2013; Gross, 2013). Therefore, monitoring and determination of trace levels of neonicotinoids in honey are necessary and demand highly efficient, selective and sensitive analytical techniques.

Neonicotinoids are usually determined by liquid chromatography (LC) coupled to diode array detector (DAD) (Campillo et al., 2013; Vichapong et al., 2013; Wang et al., 2012; Watanabe et al., 2015), ultraviolet (Rahman et al., 2013), fluorescence (García et al., 2007), mass spectrometric (MS or MS/MS) (Campillo et al., 2013; Fidente et al., 2005; Jovanov et al., 2013, 2014; Yáñez et al., 2013), and electrochemical detectors. Application of post-column photochemical reactor (Rancan et al., 2006) or even detectors based on thermal lens spectrometry (Franko, 2008; Guzsvány et al., 2007) was also reported. Gas chromatographic analysis (Ko et al., 2014; Rossi et al., 2004) is more complex due to neonicotinoids' low volatility and relatively high polarity. In addition, different pretreatment procedures of honey samples were developed for the liquid chromatographic analysis of neonicotinoid residues (Campillo et al., 2013; Fidente et al., 2005; Kamel, 2010; Schöning, 2001; Tanner and Czerwenka, 2011). Commonly used techniques as pretreatment procedures included the traditional liquid–liquid extraction (LLE) (Fidente et al., 2005; Liu et al., 2010), modified QuEChERS method (Kamel, 2010; Proietto Galeano et al., 2013) or dispersive liquid–liquid microextraction (DLLME) (Campillo et al., 2013; Jovanov et al., 2013; Viñas et al., 2014).

Since there are only few publications on the use of DLLME as a sample pretreatment procedure in honey analysis (Campillo et al., 2013; Jovanov et al., 2013; Wang et al., 2010), the first goal of our investigation was to employ our proposed extraction methodology based on DLLME (Jovanov et al., 2013) and to compare it with previously used QuEChERS pretreatment procedure (Jovanov et al., 2014). Although the MS/MS detector provides higher sensitivity and selectivity than DAD for determination of neonicotinoids in complex matrices, it is a very expensive and complex instrument not affordable to every control laboratory. For these reasons the HPLC-DAD method was chosen to be developed and optimized for a simultaneous analysis of 7 neonicotinoids in honey samples. Previously developed HPLC-DAD methods were focused on investigation of other matrices, such as grains (Wang et al., 2012), water and fruit juices (Vichapong et al., 2013), cucumber and eggplant (Watanabe et al., 2015) or on determination of fewer neonicotinoids in a single run in honey (Campillo et al., 2013). Finally, the proposed method was validated and used for analyzing the presence of the selected neonicotinoids in more than 100 honey samples which were systematically collected from the Autonomous Province of Vojvodina.

2. Materials and methods

2.1. Chemicals and reagents

Standards of neonicotinoids (certified purity > 99%) and formic acid (purity 98%, w/w) were obtained from Sigma–Aldrich (Steinheim, Germany), while dichloromethane and acetonitrile of HPLC grade were purchased from Merck (Darmstadt, Germany). The ultrapure water was produced by a Simplicity UV system from Millipore (Bedford, MA, USA). Stock solutions of neonicotinoid standards (100.0 mg L^{-1}) were prepared in water and stored in a freezer at −10 °C, and were stable over a period of at least three months. Multicomponent standard solution (100.0 μg L^{-1}) was prepared by mixing and properly diluting the calculated amounts of each standard stock solution with water. The obtained multicomponent solution was used for spiking honey samples, matrix-matched calibration (MMC), and solvent based calibration (SC). The MMC standards were prepared by spiking of blank honey samples with multicomponent stock solution at the final reconstitution step, over the range from the limit of quantification (LOQ) to 100.0 μg kg^{-1} for all analyzed neonicotinoids. The standard solutions were hermetically sealed and stored in the refrigerator (at 4 °C) protected from light. Under these conditions the standard solutions were stable for at least one month. Kits for QuEChERS sample preparation (buffered extraction kits; part no ECQUEU750CT and general fruits and vegetables sample cleanup kits; part no ECMPS15CT) were purchased as ready to use from United Chemical Technologies (UCT Inc., Bristol, USA).

2.2. Sample collection and preparation

The 104 honey samples of different floral origin (sunflower, wildflower, linden, and acacia) were collected from different locations in 7 regions of the Autonomous Province of Vojvodina, Republic of Serbia as shown in Fig. 1. All samples were kept in their original packaging at ambient temperature as in everyday use. A blank sample ($n = 5$) was prepared by weighing 10.0 g of multifloral honey from a known location with no neonicotinoid contaminations. A 50.0 g L^{-1} honey solution was prepared in

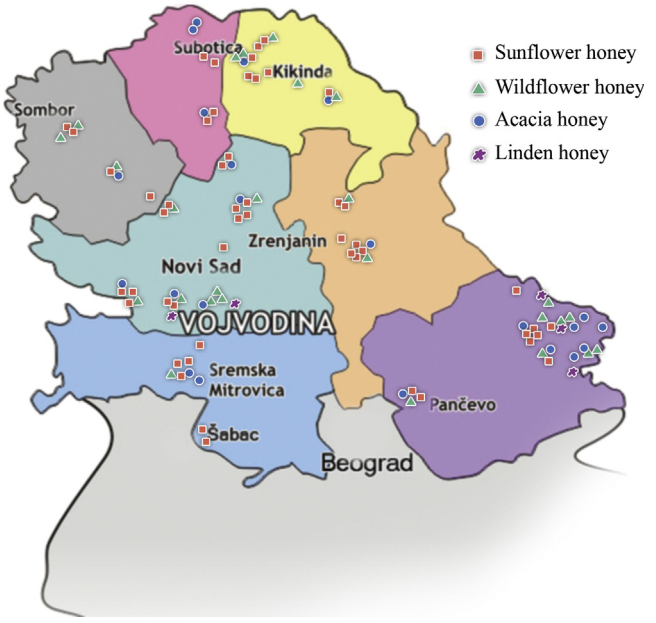

Fig. 1. Sampling sites of different floral origin (51 sunflower, 26 wildflower, 22 acacia and 5 linden) honey samples in Autonomous Province of Vojvodina, Republic of Serbia.

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