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Determination of neonicotinoid insecticides and their metabolites in honey bee and honey by liquid chromatography tandem mass spectrometry

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ABSTRACT

The original analytical method for the simultaneous determination and confirmation of neonicotinoids insecticides (imidacloprid, clothianidin, acetamiprid, thiametoxam, thiacloprid, nitenpyram, dinotefuran) and some of their metabolites (imidacloprid guanidine, imidacloprid olefin, imidacloprid urea, desnitro-imidacloprid hydrochloride, thiacloprid-amid and acetamiprid-N-desmethyl) in honey bee and honey was developed. Preparation of honey bee samples involves the extraction with mixture of acetonitrile and ethyl acetate followed by cleaned up using the Sep-Pak Alumina N Plus Long cartridges. Honey samples were dissolved in 1% mixture of acetonitrile and ethyl acetate with addition of TEA, then extracts were cleaned up with Strata X-CW cartridges. The identity of analytes was confirmed using liquid chromatography tandem mass spectrometry. All compounds were separated on a Luna C18 column with gradient elution. The whole procedure was validated according to the requirements of SANCO 12571/2013. The average recoveries of the analytes ranged from 85.3% to 112.0%, repeatabilities were in the range of 2.8–11.2%, within-laboratory reproducibility was in the range of 3.3–14.6%, the limits of quantitation were in the range of 0.1–0.5 $\mu g\,kg^{-1}$, depending of analyte and matrices. The validated method was successfully applied for the determination of clothianidin, imidacloprid and imidacloprid urea in real incurred honey bee samples and clothianidin in honey.

1. Introduction

The functioning of agriculture and world wild plants without bees is possible, but necessary to this effort is unimaginable, because of the role of insect in the world plants ecosystems as the pollinators is irreplaceable. Moreover bees are one of the most economically important insects because of high production of honey, beeswax, royal jelly, pollen and propolis. All of these products are widely used in food, cosmetic industry, medicine, especially bee venom is increasingly used in alternative medicine—apitherapy. In last few decades it is observed colony collapse disorder (CCD) is still increasing. This state of affairs is influenced by many factors (pollutants, climate changes, parasites, pathogens, plant protection products, especially insecticides).

This study focuses specifically on the determination and confirmation of neonicotinoid insecticides and some of their metabolites in honey bee and honey samples, which are one of the most potentially a threat to bee health. Neonicotinoids are one of the synthetic insecticides which are the fastest growing class in crop protection against sucking insects, moths, butterflies, various species of beetles and other pest herbivores in last 20 years [1,2]. This success is due to the molecular structure (Fig. 1). Commercialised neonicotinoids can be divided into two categories of compounds which have open-chain structure (acetamiprid, clothianidin dinotefuran and nitenpyram) or five/six-member heterocyclic ring structure (imidacloprid, thiacloprid and thiamethoxam) [3]. Neonicotinoids have a part of a molecular structure that is responsible for a particular biological or pharmacological interaction (pharmacophores). In general they are represented by nitro or cyano substitute and also NH, N-Me, S or Me group in their structure (Fig. 1). These types of pharmacophores influenced on neonicotinoids insecticides activity, toxicity and some physicochemical properties which determined the analytical approach [4–6]. The neonicotinoid activity is based on their interaction with the nicotinic acetylocholine receptors (nAChRs), the membrane proteins which are responsible for inducing membrane depolarization in nerve synapses situated in the insect central nervous system (CNS). Neonicotinoids are high selectivity to the insect nAChRs compared to the mammalian nAChRs, which makes them very desirable insecticides in the agricultural industry [1,7,8]. Unfortunately, their main advantage

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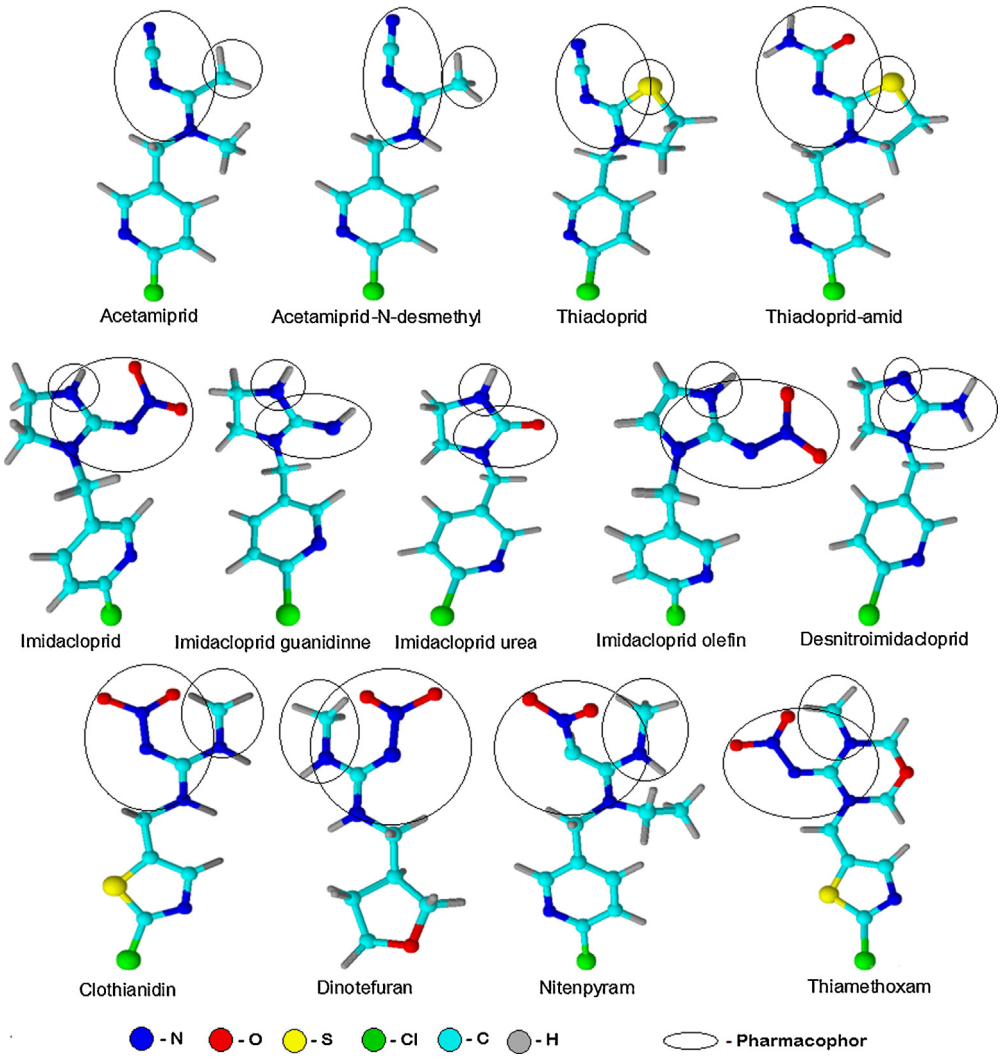

Fig. 1. Chemical structure of neonicotinoids and their metabolites, including pharmacophores indication.

proved to be deadly to honey bees and pollinators because of their toxic and neurotoxic properties.

The honey bee exposition to neonicotinoids can lead to contamination of apiarian products, especially honey, which is the most commonly consumed bee product and because of the potential threat to human health, the European Union established maximum residue limits (MRLs) for acetamiprid, clothianidin, imidacloprid, thiacloprid and thiamethoxam in the range of 10–200 $\mu g\,kg^{-1}$ [9].

Different approach for the neonicotinoids and their metabolites in environmental samples (soil, river water) [10–12], animal tissues [13,14], agricultural (chestnut, shallot, tea, ginger, amaranth, sedum, anion, pepper, lettuce) samples [15–18], alcohol industry products (honey liqueur) [19] and honey bee [20–22] and bees products (honey, bee pollen, beeswax) [22–25] were proposed. These methods used different techniques LC-MS/MS [11,13–16,18–24], GC-MS/MS [17], UPLC-UV [10], LC-*hυ*-ED [27], UPLC-DAD [20], CE [28] and LC-amperometric detector [12]). Most of the methods described the determination of several neonicotinoids [10,11,13,14,18,19,25,26,28] or only one neonicotinoid with its metabolites, in different biological matrices [15–17,27]. Only a few methods demonstrated the determination of neonicotinoids or neonicotinoids and their metabolites in honey bee [20,21,23,24] and bee products such as bee pollen [12,22]. However, the reported method for determination neonicotinoids in honey did not include their metabolites. Several sample preparation techniques, liquid–liquid extraction [23], solid phase extraction [24], QuEChERS [20,22] and combination of them [21], for the sample preparation in honey bee and honey were reported.

The presented study reports the development and validation of the analytical method for the determination of 7 neonicotinoids and 6 of their metabolites in honey bee and honey. Proposed method include some of the novelties such as usage of the Sep-Pak Alumina N Plus Long cartridges as the filter for the honey bee supernatants clean-up step and the Strata X-CW cartridges usage for the honey SPE process. To the best of our knowledge and the available literature data, this is the first time that 13 analytes from neonicotinoid group are determined in one analytical protocol.

2. Material and methods

2.1. Reagents

All reagents used were of analytical grade, >95% purity. Acetonitrile and ethyl acetate were purchased from J.T. Baker (Deventer, the Netherlands). Triethylamine (TEA) was purchased from Sigma-Aldrich (Steinhiem, Germany). Water was deionised (>18 $M\Omega\,cm^{-1}$) by the Millipore system. Certified standards of neonicotinoids, acetamiprid, acetamiprid-*N*-desmethyl, clothianidin, dinotefuran, imidacloprid, desnitro imidacloprid