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Determination of seven neonicotinoid insecticides in beeswax by liquid chromatography coupled to electrospray-mass spectrometry using a fused-core column☆

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ABSTRACT

A new method has been developed to measure seven neonicotinoid insecticides (acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid and thiamethoxam) in beeswax using liquid chromatography (LC) coupled to electrospray ionization mass spectrometry (ESI-MS) detection. Beeswax was melted and diluted in an *n*-hexane/isopropanol (8:2, v/v) mixture. After this, liquid extraction with water was performed followed by a clean-up on diatomaceous material based cartridges. The compounds were eluted with acetone, and the resulting solution was evaporated until dry and reconstituted with a mixture of water and acetonitrile 50:50 (v/v). The separation of all compounds was achieved in less than 15 min using a C_{18} reverse-phase fused-core column (Kinetex® C_{18}, 150 mm × 4.6 mm i.d.) and a mobile phase composed of a mixture of 0.1% formic acid in water and acetonitrile in gradient elution mode at 0.5 mL/min. This method was fully validated in terms of selectivity, linearity, precision and recovery. Low limits of detection and quantification could be achieved for all analytes ranging from 0.4 to 2.3 μg/kg, and from 1.5 to 7.0 μg/kg, respectively. Finally, the proposed method was applied to an analysis of neonicotinoid residues in beeswax samples from apiaries located close to fruit orchards.

1. Introduction

The neonicotinoid insecticides (acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid and thiamethoxam; see structures in Fig. 1), which derive from the nicotine moiety, are gaining larger shares in the global crop protection market due to: (i) a broad spectrum of efficacy; (ii) systemic and translaminar action; (iii) pronounced residual activity and a unique mode of action [1]. These compounds generally have lower toxicity to mammals (acute and chronic), birds and fish than insects, due to the low affinity of neonicotinoids for vertebrates in comparison with the insect nicotinic receptor [2]. However, they are generally less toxic than the parent compounds to insects and mammals [3]. It should also be mentioned that the potential activity of some neonicotinoids as analgesics has been investigated in mice [2,4].

Currently neonicotinoid products have become the most used botanic insecticides around the world; in fact, they have achieved annual sales of $2.5 billion [5], and imidacloprid has become the largest insecticide treatment in recent years [5,6]. These compounds are used extensively for the control of agricultural pests by spraying and are also widely used in seed dressings and soil additions [7]. Moreover, neonicotinoid treatment of plants sometimes has beneficial effects different from those of pest control, such as promoting growth and protecting against biotic factors and biotic stress [3], although in other cases they have induced peroxidative damage and foliar lesions in crops, for instance, soybeans [3]. It is possible that after field treatment, neonicotinoid insecticides may be transported into the beehive; for this reason beneficial insects such as honeybees may also be affected [1,2]. Moreover, residues of these insecticides may finally be found in bee products such as honey, pollen, royal jelly or wax [5], where it is necessary to evaluate them. The determination of neonicotinoid insecticides has been undertaken in several bee products [1,7–28], but in only a few of these publications were some of those compounds analyzed in wax [9,10,18,23,25,28], and, at best, two neonicotinoids were simultaneously determined [23]. An analysis of this group of insecticides in this matrix is of great interest when determining pesticide residues or monitoring the contamination of bee products, as well as to explain the potential relation of these compounds with the honey bee decline known as colony collapse

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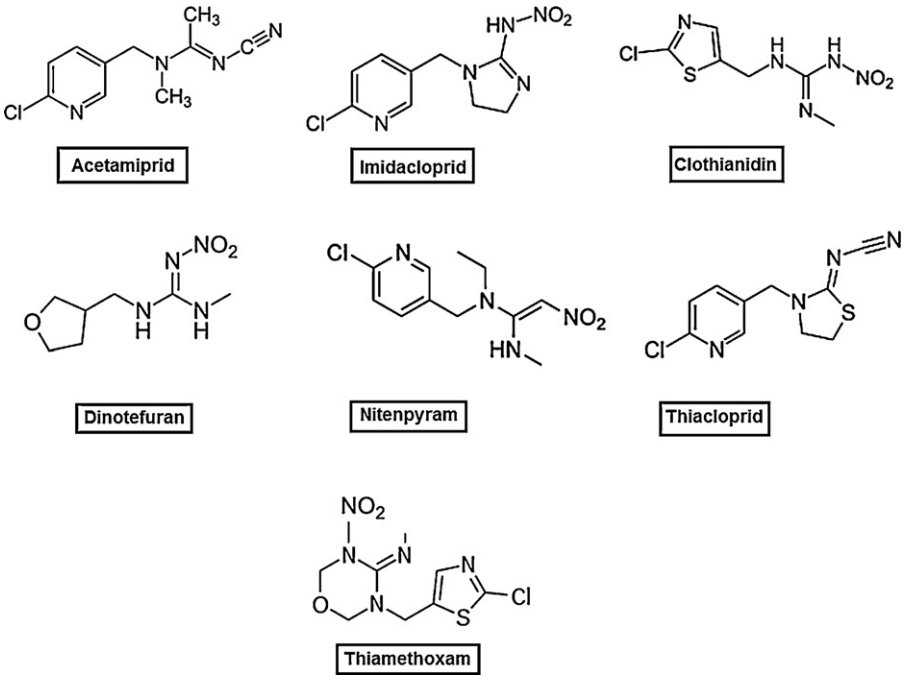

Fig. 1. Chemical structures of the studied neonicotenoid insecticides.

disorder (CCD) [23], due to the fact that beeswax could be considered a contaminant reservoir, and the pesticides present in wax could directly affect the bee colony or be transmitted to other bee products. Therefore, it is necessary to develop an adequate analytical methodology to determine neonicotinoid residues in this matrix.

Although certain analytical approaches, such as enzyme-linked immune-sorbent assays (ELISA) [19], electrochemical [29] and GC [18,23] methods, have been employed to analyze this group of compounds, some of them in bee products [18,19,23], liquid chromatography (LC) using C_{18} based analytical columns is the technique of choice if the physical–chemical properties of those compounds [7–28,30–32] are taken into consideration. In the last years, the coupling of LC with mass spectrometry (MS) has been widely used [1,8–11,15,17–25,28,30–32], although electrochemical (ECD) [14], fluorescence (FLD) [16] and UV/diode array (DAD) [26,27] detectors have been also employed. As has been previously mentioned, certain neonicotinoids have been analyzed in several bee products like honey [9–12,14–22,24–27], pollen [8,10,13,15,20–23,25], beeswax [9,10,18,23,25,28], propolis [26], nectar [9,15] and honey bees [9,10,20,24,25,27]. Yet to our knowledge only one study has been only published that was developed exclusively to analyze neonicotinoids (imidacloprid) in beeswax [28].

Regarding the sample treatments, it could be said that as in the case of the chromatography methods a lack of specific extraction and clean-up procedures was observed for this matrix and analytes. The extraction and clean-up procedures proposed when analyzing different beehive products implied many stages and the use of huge volumes of solvents. Meanwhile, in other cases, where neonicotinoids were analyzed in different matrices like crops [30], bovine tissues [31] and fruits or vegetables [32], different extraction procedures were employed comprising several steps as a quick easy cheap effective rugged (QuEChERS) method [30], pressurized solvent extraction [31] and solid phase extraction [32]. For these reasons we decided, due to the good results achieved, to employ initially our previously proposed extraction methodology [28] based on a liquid–liquid extraction (LLE) and a subsequent clean-up using cartridges filled with different sorbents.

Thus, we sought to develop a new, rugged and sensitive LC–ESI-MS method to detect the lowest amount possible of the seven most frequently used neonicotinoid insecticides in beeswax, as well to optimize an exclusive sample treatment for this matrix and thereby ensure good recovery and clean chromatograms. Moreover, a fused-core analytical column was used to achieve the separation of those compounds, specially relevant due to the relatively low number of reports and applications to real samples using this type of columns. Accordingly, another goal of the present study was to validate this method and apply it to the analysis of beeswax samples from apiaries located close to areas with intense fruit orchards where neonicotinoids are being used, in order to check the possible presence of residues of these insecticides as done previously [20,25].

2. Materials and methods

2.1. Materials and chemicals

Neonicotinoid insecticide standards (acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid and thiamethoxam) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Methanol, acetonitrile, isopropanol (all LC grade), *n*-hexane (95%) and acetone (99.8%) (both Pestican grade) were supplied by Lab Scan Ltd. (Dublin, Ireland). Formic acid (98–100%) was obtained from Sigma–Aldrich Chemie Gbmh (Steinheim, Germany). Isolute® HM-N, diatomaceous earth packed (10 mL sample) cartridges were obtained from Biotage (Uppsala, Sweden). Florisil® (500 mg, 3cc) and Oasis® HLB (60 mg, 3cc) solid phase extraction (SPE) cartridges were provided by Waters (Milford, MA, USA), while Strata® C_{18}-E (500 mg, 3cc) were obtained from Phenomenex (Torrance, CA, USA). A 12-port system of solid-liquid extraction vacuum manifold from Waters Corporation (Milford, MA, USA), a magnetic shaker and heater (Agimatic-N) and ultrasonic bath (Ultrasons), both supplied by J.P. Selecta S.A. (Barcelona, Spain), and an R-210/215 rotary evaporator from Buchi (Flawil, Switzerland), were used for all the extractions. Nylon syringe filters (17 mm, 0.45 μm) were from Nalgene (Rochester, NY,