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Simultaneous determination of neonicotinoid insecticides and insect growth regulators residues in honey using LC–MS/MS with anion exchanger-disposable pipette extraction

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ABSTRACT

In this study, we developed an anion exchanger-disposable pipette extraction (DPX) method to detect the residual concentrations of eight neonicotinoid insecticides (dinotefuran, acetamiprid, clothianidin, thiacloprid, imidachloprid, imidaclothiz, nitenpyram, and thiamethoxam) and eight insect growth regulators (IGRs; triflumuron, cyromazine, buprofezin, methoxyfenozide, tebufenozide, chromafenozide, fenoxycarb, and RH 5849) in Chinese honey samples collected from different floral sources and different geographical regions using liquid chromatography tandem mass spectrometry (LC–MS/MS). QAE Sephadex A-25 was used as the anion exchanger in the DPX column for the purification and cleanup of honey samples. Analytes were eluted with a mixture of acetonitrile and 0.1 M HCl, and the elution was subjected to LC analysis. This method was thoroughly validated for its reproducibility, linearity, trueness, and recovery. Satisfactory recovery of pesticides was obtained ranging from 72% to 111% with intraday RSDs (n = 5) of 1%–10%. High linearity ($R^2 \geq 0.9987$) was observed for all 16 pesticides. Limits of detection and quantification for all 16 compounds ranged from 0.3 to 3 μg/kg and from 1 to 10 μg/kg, respectively. Pesticide residues (9–113 μg/kg) were found in Chinese honey samples. The anion exchanger-DPX method was effective for removing sugars and retaining target analytes. Moreover, this method was highly reliable and sensitive for detecting neonicotinoids and IGRs in different floral sources of honey and will be applicable to matrixes with high sugar content.

1. Introduction

Neonicotinoid insecticides, including dinotefuran, acetamiprid, clothianidin, thiacloprid, imidacloprid, imidaclothiz, nitenpyram, and thiamethoxam, act as agonists at the nicotinic acetylcholine receptors of insects [1]. They comprise a relatively new class of insecticides with novel modes of action and are used to control numerous sucking and biting insect pests [2]. These insect pests are of concern because of their high mobility in plants and environmental matrices and have been detected in surface water samples and in the vicinity of agriculture areas in different geographical regions [3–5]. Because of their widespread distribution, insect pests pose a serious risk to human health and safety [6,7]. Insect growth regulators (IGRs) are chemicals that disrupt the life cycle of insects, particularly molting and metamorphosis, by mimicking juvenile hormone activity or juvenile hormone agonists, and by antagonizing juvenile hormone activity [8–10]. Briefly, the mode of action of IGRs was that the hormone analogues have induced or activated phenoloxidase activity, and may also activate the relevant steps in the phenoloxidase activation system, thus promoting the activity of phenoloxidase [8]. Also, IGRs hinder the biosynthesis and deposition of chitin, reduces the hardness of new epidermis, and hinders the growth and development of chitin [10]. Because of their mode of action, IGRs result in insect mortality before reaching adulthood. IGRs are advantageous over conventional insecticides [11], as they are more potent, less toxic to mammals, and biodegradable in soil and water [12]. Examples of IGRs include triflumuron, cyromazine, buprofezin, methoxyfenozide, tebufenozide, chromafenozide, fenoxycarb, and RH 5849.

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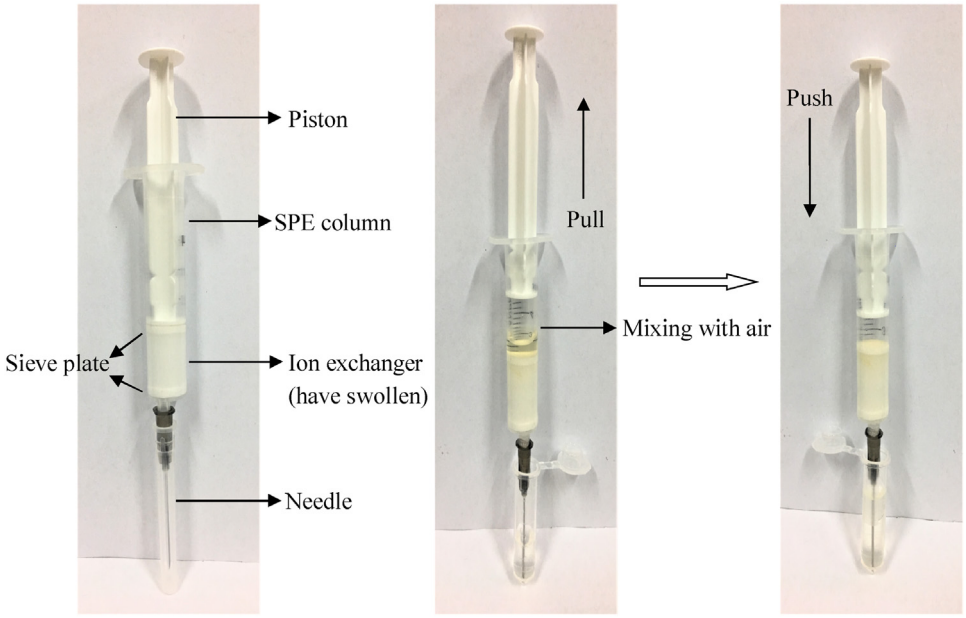

Fig. 1. Step-by-step working of the DPX column.

Honey is a complex matrix, composed largely of fructose and glucose [13]. It has become a wholesome natural product and is consumed worldwide because of its health benefits, such as high antioxidant activity and high amounts of minerals, vitamins, and enzymes [14]. Therefore, the nutritional and quality aspects of honey are important, and it must be free from chemical contamination and safe for human consumption. Neonicotinoid insecticides and IGRs directly impact honey production and are toxic to honeybees, thus, posing a threat to the biodiversity of ecosystems [15–18]. According to Woodcock et al. [19], exposure to low levels of neonicotinoid insecticides (1.5 μg/kg) reduces hive fitness and capacity of honeybees to establish new populations. Moreover, the coexistence of pesticides (i.e., neonicotinoid insecticides and IGRs) is harmful to bees and other pollinators [20]. Varying levels of the neonicotinoid insecticide clothianidin (0.1–912 μg/kg) have been found on honeybees in treated fields, which may influence on deflower and the physiological behavior of honeybees [21]. Coating the seeds with neonicotinoid insecticides reduces the density of wild bees in flowering oilseed rape fields and adjacent uncultivated field borders [22]. Rundlof et al. showed that a clothianidin seed coating in oilseed rape has negative effects on wild bee populations [22]. Furthermore, maximum residue limits (MRLs) of neonicotinoid insecticides in vegetable and fruit ranged of 0.02–2 mg/kg established by Chinese government [23]. Whereas MRLs of IGRs in vegetable and fruit ranged of 0.05–10 mg/kg, except for chromafenozide, fenoxycarb, and RH 5849 [23]. Therefore, it is highly important to determine the level of neonicotinoid insecticides and IGRs residues in honey and its products.

Sample preparation and chromatographic methods have been developed to determine the level of pesticide (neonicotinoids and IGRs) residues in honey and its products. Liquid–liquid extraction (LLE) [24] and solid-phase extraction (SPE) [25,26] are the most common sample preparation methods. However, LLE and SPE are time consuming, tedious, and require large volumes of organic (toxic) solvents. To overcome these limitations, LLE and low-temperature purification (LLE-LTP) [27]; dispersive liquid–liquid microextraction (DLLME) [5,14]; ionic liquid-based cold-induced aggregation microextraction [6]; supercritical fluid extraction (SFE) [28]; solid-phase microextraction (SPME) [29]; and quick, easy, cheap, rugged, effective, and safe (QuEChERS) extraction [7] have been developed. These methods use different techniques, such as high-pressure liquid chromatography (HPLC) with ultraviolet detector (HPLC-UV) [30] or diode array detector (HPLC-DAD) [1], liquid chromatography tandem mass spectrometry (LC–MS/MS) [3,7], gas chromatography tandem mass spectrometry (GC–MS/MS) [15,31], and GC with electron capture detector (GC–ECD) [27] or nitrogen phosphorus detector (GC–ECD) [14]. Furthermore, enzyme-linked immunosorbent assays (ELISAs) [32,33] and strip-based immunoassay [4] have been developed for the determination of pesticide residues in different matrices.

Disposable pipette extraction (DPX) is a novel approach for determining pesticide residues; application of DPX has not been previously reported in honey. DPX mixes solutions with the sorbent in a dynamic dispersive manner to provide rapid equilibration partitioning, and enhance contact between analytes and solid-phase sorbent [34]. In this method, the sample is thoroughly mixed with a loose absorbent in the DPX pipette or column (Fig. 1). The sample and adsorbent form a homogenizing gel, which is extracted and eluted. The elution is subjected to LC or GC for analysis. This method did not need additional vortexing or centrifugation steps and not requires lots of organic or toxic solvent. Also, the interferents are concentrated on the sorbent and a clean extract can be dispensed, thus reducing the need for solvent evaporation [35]. The main advantage of DPX approach is that dynamic mixing uses less sorbent and provides faster extractions compared to SPE and SPME [25,29]. Furthermore, the extraction of a few compounds that are problematic with LLE-LTP, DLLME, SFE and QuEChERS [7,14,27,28], but shown to be easily detected and analyzed using DPX approach [36]. QAE Sephadex A-25 is a strong anion exchanger, which remains charged and maintains consistently high capacity over a wide pH range of 2–9. Moreover, it has a strong adsorption capacity for anions, such as Cl^-, Br^-, and SO_4^{2-}, which could be effective for adsorbing sugars, such as fructose and glucose from the complex matrix of honey.

In this study, we investigated the anion exchanger-DPX method to determine the level of eight neonicotinoid insecticides and eight IGRs in honey samples produced from different flora in different regions in China using LC–MS/MS. We also compared the DPX method with the QuEChERS method for the multianalysis of both categories of pesticides in honey.

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