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Simultaneous determination of nine neonicotinoids in human urine using isotope-dilution ultra-performance liquid chromatography–tandem mass spectrometry☆

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ABSTRACT

Neonicotinoids (neonics), a class of systemic insecticides, have been frequently detected in pollen, vegetables, and fruits. Recently, an increasing concern has been aroused for human exposure to neonics. However, biological monitoring for quantifying body burden of neonics has rarely been reported. In this study, we developed an isotope-dilution ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS) method to simultaneously quantify nine neonics, including acetamiprid (ACE), thiamethoxam (THIAM), imidacloprid (IMIP), clothianidin (CLO), flonicamid (FLO), thiacloprid (THIAC), dinotefuran (DIN), nitenpyram (NIT), and imidaclothiz (IMIT) in urine. The limits of quantification were 0.1 μg/L for ACE, FLO, DIN, NIT and IMIT, and 0.2 μg/L for THIAM, IMIP, CLO, and THIAC. The overall recoveries were 80.8–103%, 81.5–91.7% and 83.0–92.3% for QA/QC samples fortifying at 1, 25, and 100 μg/L levels, respectively. UPLC/MS/MS method was used to analyze urine samples obtained from 10 children in Hangzhou, China. The detection frequencies were 80% for ACE and IMIP, 70% for THIAM and CLO, 20% for DIN and IMIT and 10% for THIAC. FLO and NIT were not detected in those urine samples. The data provided here will be helpful for conducting biological monitoring of neonics exposure in the future.

1. Introduction

Neonicotinoids (neonics) have been one of the most widely used insecticides around the world since they were introduced to the pesticide market in early 1990s (Yamamoto, 1999). Neonics are highly soluble and prone to be absorbed and then distributed to all parts of the plants (Sanchez-Bayo and Goka, 2014). Therefore, neonics have been detected in water, soil, pollen, honey, vegetables, or fruits. Previous studies have revealed non-target organisms, such as humans, that have been exposed to neonics through inhalation, ingestion, or direct contact (Chen et al., 2013; Ueyama et al., 2014; Lu et al., 2018; Zhang et al., 2018).

Recently, the potential ecological risk of neonics has been reported as well. The deadly impacts were observed by neonics on the ecosystem (Hallmann et al., 2014; Di Prisco et al., 2013; Sanchez-Bayo, 2014). Under a non-acute lethal exposure scenario, imidacloprid and thiamethoxam have led to a delayed onset of increased mortality in honey bees (Henry et al., 2012). After 8 years of field monitoring, it was determined that low concentration of imidacloprid (0.1 $\mu g\,L^{-1}$) resulted in a notable reduction of macroinvertebrates in surface waters (Van Dijk et al., 2013). Furthermore, some studies have shown that neonics are also potentially toxic to mammals. Imidacloprid (2 and 8 mg/kg/day) could cause several adverse effects, including male reproductive system in rats, DNA fragmentation, antioxidant imbalance, and apoptosis (Bal et al., 2012). After 30-day exposure to thiacloprid (112.5 mg/kg), the levels of free thyroxine and triiodothyronine in serum were increased in rats (Sekeroglu et al., 2014), indicating potential endocrine disruption by thiacloprid. Data published by the European Food Safety Authority (EFSA) have shown the potential

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neurotoxicity caused by acetamiprid and imidacloprids (EFSA, 2013). In addition, a few studies have reported acute neonics toxicity in kidneys (Vinod et al., 2015; Yeh et al., 2010).

Considering the potential adverse effects on human and the character of ubiquity, it is vitally important to assess neonics exposure to human (Yamamuro et al., 2014). In previous studies, the Oasis HLB (Hydrophile-Lipophile Balance) cartridge (Xie et al., 2011), diatomaceous material based cartridges (Yanez et al., 2013), the QuEChERS method (Chen et al., 2013), and subcritical water extraction (Xiao et al., 2013) have been used for extracting neonics from various sample media. However, those analytical methods are restricted by the number of simultaneous detection of neonics and the analytical sensitivity. Therefore, it is imperative to develop an efficient LC-MS/MS method with a relatively lower limit of quantification (LOQ) for simultaneous monitoring nine frequently used neonics in human (Arroyo-Manzanares et al., 2013).

In the present study, we reported the development of an isotope-dilution ultra-high performance LC (UPLC) coupled with the MS/MS method which shows a conspicuous advantage over GC–MS/MS methods due to the convenient sample preparation without derivatization (Chen et al., 2012). Additionally, we employed this developed UPLC/MS/MS method to analyze urine samples collected from 10 children in primary school in Hangzhou, China in order to determine the sensitivity and specificity of quantifying those nine neonics.

2. Materials and methods

2.1. Chemicals and reagents

Analytical standards of nine neonicotinoids, including acetamiprid (98.1%), thiamethoxam (99.0%), imidacloprid (99.0%), clothianidin (99.9%), flonicamid (99.0%), thiacloprid (98.5%), dinotefuran (98.0%), nitenpyram (98.6%), and imidaclothiz (96%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Three surrogates for imidacloprid-d_4 (99.9%), thimethoxam-d_3 (99.9%) and clothianidin-d_3 (99.9%) were obtained from C/D/N Isotopes Inc (Quebec, Canada). We used HPLC grade acetonitrile, dichloromethane, and formic acid from Merck (Rahway, NJ) and the ultrapure water using a Milli-Q system (Merck Millipore, MA, USA). A Waters Oasis HLB cartridge (Waters, USA) was used in the solid-phase extraction (SPE) procedure. Artificial urine was purchased from Chaoyan Biotechnology Co. (Shanghai, China).

2.2. Sample collection and preparation

Spot urine samples were collected from 10 children aged from 8 to 10 (5 boys and 5 girls) and stored at −20 °C until analysis. Information regarding age, gender, and body weight was collected using questionnaires. All subjects provided written informed consent prior to their participation.

2.3. Preparation of standard and extraction procedure

Neat neonics were dissolved in acetonitrile and further diluted at concentrations of 0.1, 0.5, 1, 5, 10, 25, 50, 100 and 200 μg/L. The standard solutions were stored at −20 °C and prepared within 2 weeks.

The artificial urine was used to minimize the background contamination of neonics in urine blanks. Firstly, the artificial urine was thawed to room temperature and 2 mL of urine was transferred to a centrifuge tube and mixed with nine neonics and three surrogates at different fortifying levels. The centrifuge tube was allowed to precipitation for 5 min after gentle shaking. Then the SPE cartridge was conditioned by 2 mL of each dichloromethane and water in sequence. For testing the efficiencies of different SPE cartridge, urine samples were loaded to the cartridges and directly eluted with dichloromethane. The different mixtures (v:v) of water and formic acid were examined in the washing steps. At last, the prepared solutions (acetonitrile, dichloromethane, dichloromethane: acetonitrile (50:50, V/V), dichloromethane: acetonitrile (70:30, V/V)) were tested for elution efficiency. The eluent was collected and concentrated to dryness under a gentle nitrogen stream. Extraction procedures were repeated with three times at least.

2.4. Instrumental conditions

Separation and detection of nine neonics were achieved using the UPLC–MS/MS (Waters Corporation, Milford, MA) interfaced with a triple quadrupole mass spectrometer Xevo TQ-S (Waters Corporation) using a YMC ODS-AQ column (Octadecylsilyl) (100 mm × 2.1 mm, 3 μm, YMC, Allentown, PA, USA). The system was run in the isocratic mode with the mobile phase consisting of acetonitrile and Milli-Q water (95:5, V/V) acidified with 0.01% formic acid. The flow rate of mobile phase was 0.2 mL/min and the injection volume was 6 μL.

The MS/MS was conducted using an electrospray ionization (ESI) source in the positive ion mode with multiple reactions monitoring (MRM). The transitions for quantification were 223.1/126.1, 292.0/211.0, 256.0/209.1, 250.0/169.0, 230.0/203.0, 253.0/126.1, 203.1/129.1, 271.0/225.1, and 262.0/181.0 for acetamiprid, thiamethoxam, imidacloprid, clothianidin, flonicamid, thiacloprid, dinotefuran, nitenpyram, and imidaclothiz, respectively. The *m/z* of the internal standards (IS, imidacloprid-d_4, thiamethoxam-d_3 and clothianidine-d_3) were 260.0/213.0, 253.0/172.1 and 295.0/214.1, respectively.

2.5. Validation procedure

The method performance including the recovery, accuracy, precision, and matrix effect was examined. Calibration curves of those nine neonics were prepared by fortifying blank urine with the standard mixture at nine levels from 0.1 to 200 μg/L (Chen et al., 2014). The calibration curve was calculated by linear regression analysis. QC samples at concentrations of 1, 10 and 100 μg/L ($n = 5$ for each) were used to assess the precision and accuracy, and to determine the intra- and inter-day validation on three consecutive days (data not shown). Precision was shown as the relative standard deviation (RSD) for variability and accuracy. Precision was acceptable under an RSD <15% for all standards (Chen et al., 2012). Accuracy was acceptable if the deviation of standards was <15% compared to the nominal values.

The limit of detection (LOD) was defined as the concentration at a signal-to-noise (S/N) ratio of 3. Similarly, the limit of quantification (LOQ) was calculated as the lowest concentration at an S/N ratio higher than 10 and the percent of relative standard deviation (RSD) was <20%. The recoveries of extraction optimizing procedure were calculated at three fortified concentration levels (1, 25 and 100 μg/L of nine neonics) and three isotope-labeled neonics.

The matrix effect (ME) could either attenuate or enhance the response of analytes during quantitation. In order to determine the matrix effect of urine on the quantitation procedure, artificial urine and blank solvent were spiked with nine neonics at nine concentration levels. Two standard curves were generated and the slopes were used to calculate the matrix effect by the following equation:

ME% = (The slope of matrix-matched calibration curve / The slope of solvent calibration curve) × 100%