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Response surface methodology for the enantioseparation of dinotefuran and its chiral metabolite in bee products and environmental samples by supercritical fluid chromatography/tandem mass spectrometry

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ABSTRACT

Tracing the enantiomers of dinotefuran and its metabolite in bee products and relevant environmental matrices is vital because of the high toxicity of their racemates to bees. In this study, a statistical optimization strategy using three-dimensional response surface methodology for the enantioseparation of dinotefuran and its metabolite UF was developed by a novel supercritical fluid chromatography/tandem mass spectrometry (SFC–MS/MS) technique. After direct evaluation of the chromatographic variables – co-solvent content, mobile phase flow rate, automated backpressure regulator pressure (ABPR), and column temperature – involved in the separation mechanism and assessment of the interactions among these variables, the optimal SFC–MS/MS working conditions were selected as a CO_2/2% formic acid-methanol mobile phase, 1.9 mL/min flow rate, 2009.8 psi ABPR, and 26.0 °C column temperature using an amylose tris-(3,5-dimethylphenylcarbamate) chiral stationary phase under electrospray ionization positive mode. Baseline resolution, favorable retention, and high sensitivity of the two pairs of enantiomers were achieved in pollen, honey, water, and soil matrices within 4.5 min. Additionally, the parameters affecting the dispersive solid-phase extraction procedure, such as the type and content of extractant or purification sorbents, were systematically screened to obtain better extraction yields of the enantiomers. Mean recoveries were between 78.3% and 100.2% with relative standard deviations lower than 8.0% in all matrices. The limits of quantification ranged from 1.0 μg/kg to 12.5 μg/kg for the dinotefuran and UF enantiomers. Furthermore, the developed method was effectively applied to authentic samples from a market, an irrigation canal, and a trial field, and the enantioselective dissipation of dinotefuran and UF in soil was demonstrated.

1. Introduction

There is growing evidence for declines in bee diversity and distribution due to extensive exposure to neonicotinoid insecticides, which has aroused great concern worldwide [1–3]. Dinotefuran, (EZ)-(*RS*)-1-methyl-2-nitro-3-(tetrahydro-3-furylmethyl) guanidine, is a typical third generation neonicotinoid. It has been increasingly utilized in more than 20 countries, such as Japan, the United States, Korea, the European Union, and China [4]. The extraordinary insecticidal potency of this compound has been demonstrated toward both sucking pests, such as whiteflies, mealybugs, and plant hoppers in vegetables, fruit, turf, and rice crops, and disease vectors, especially in areas where mosquitoes are resistant to insecticides [5,6]. However, dinotefuran is highly toxic to bees with a contact acute LD_{50} at 48 h of more than 0.023 μg/bee and is highly potential to bioconcentration [7]. The European Union has issued a temporary ban on neonicotinoid insecticides [8], and the United States has stated that new bee data must be provided for outdoor usage of neonicotinoids [9]. Oregon further restricted the

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application of dinotefuran to prevent a large killing of bees in July 2013 [10].

More interestingly, the molecular structure of dinotefuran contains an asymmetrically stereogenic center leading to a pair of enantiomers, (+)-dinotefuran and (−)-dinotefuran (Fig. 1A). It is the first chiral neonicotinoid insecticide and the existing toxicological characteristics only reflect the characteristics of the racemate. Accordingly, a solution to the issue of high toxicity to bees may lie at the enantiomer level because significant differences in biological activity have been verified between (+)-dinotefuran and (−)-dinotefuran [9,10]. Furthermore, its metabolite UF ((*RS*)-1-methyl-3-(tetrahydro-3-furylmethyl) urea, Fig. 1B), which consists of a pair of enantiomers, (+)-UF and (−)-UF, also have chronic toxicity to bees [11]. The sum of dinotefuran and UF was also recommended for the estimation of dietary intake and for compliance with the maximum residue limits in animals by the joint FAO/WHO meeting on pesticide residues [12]. It is generally known that bee products (e.g., pollen and honey) are important parts of our daily diet, and their safety is closely related to surrounding environmental matrices (e.g., water and soil). Until now, the detectability and residue levels of the two pairs of enantiomers in pollen, honey, water, and soil were unknown. Hardly any work has been performed on the chiral profiles of the dinotefuran and UF enantiomers likely due to the lack of the efficient and sensitive enantioseparation methods. A high performance liquid chromatographic method coupled with diode array detection was developed for the chiral separation and degradation of the parent in greenhouse vegetables in our earlier study [13,14]. However, it still did not meet the requirements of fast separations, high resolution, high sensitivity and low solvent load for modern analytical chemistry.

Here, dinotefuran and its chiral metabolite UF were chosen as "chiral probes" to develop a green, rapid and sensitive enantioseparation method in pollen, honey, water, and soil by eco-friendly SFC–MS/MS based on a three-dimensional response surface methodology. The supercritical fluid CO_2, which is nontoxic and has low viscosity and high diffusivity, contributed greatly to improving the separation efficiency and reducing the organic solvent usage. The tandem MS spectrometer overcame the traditional incompatibility, offering high resolution and narrow peaks. A central composite design for the SFC–MS/MS working parameters took the place of the conventional one-variable-at-a-time (OVAT) process, which invariably failed to obtain exact conclusions because the interactions between factors were not taken into account [15,16]. The optimum experimental conditions were finally identified using response surface profiles with Derringer's desirability function given from mathematical models. As a consequence, the developed method was applied to authentic samples from a market, an irrigation canal, and our trial field. The current report challengingly applied the response surface methodology to develop an SFC–MS/MS method for the chiral analysis of dinotefuran and UF in pollen, honey, water, and soil; this approach not only provided a novel reference for the development of chiral resolution but also offered basic data on potential solutions to the high toxicity of racemic dinotefuran to pollinators.

2. Experimental

2.1. Chemicals and reagents

Standards of dinotefuran racemate (100% purity) and UF (99.5% purity) were obtained from Mitsui Chemicals Agro (Tokyo, Japan). Single enantiomers of dinotefuran and UF (>97.9% optical purity) were prepared in our laboratory. Analytical grade acetonitrile, acetic acid, triethylamine, ammonium formate, ammonium acetate, ammonium hydroxide, sodium acetate and anhydrous magnesium sulfate were purchased from Beihua Fine Chemicals (Beijing, China). Chromatographic grade methanol, acetonitrile, iso-propanol, ethanol, *n*-butanol, and *n*-hexane were obtained from Honeywell International (Morristown, NJ, USA). Mass grade formic acid was obtained from Thermo Fisher Scientific (Waltham, MA, USA). Ultrapure water was prepared from a Milli-Q system (Bedford, MA, USA). CO_2 (99.999% purity), N_2 (99.95% purity) and Ar (99.999% purity) were acquired from Haike Yuanchang Gas (Beijing, China). Primary/secondary amines (PSA, 40–60 μm), C18 (40–60 μm), and graphitized carbon black (GCB, 120–400 Mesh) were purchased from Bonna-Agela Technologies (Tianjin, China). Multi-walled carbon nanotubes (MWCNTs) with mean external diameters of <8 nm, 10–20 nm, and 20–30 nm were purchased from Boyu Technologies (Beijing, China).

2.2. Instrumentation and calculation

An ACQUITY UltraPerformance Convergence Chromatography (UPC^2) system (Waters, Milford, MA, USA) equipped with an ACQUITY UPC^2 convergence manager, an ACQUITY UPC^2 binary solvent manager, an ACQUITY UPC^2 sample manager-FL, and an ACQUITY UPC^2 column manager was used for the enantioseparation of the dinotefuran and UF enantiomers. Amylose-based chiral Trefoil AMY1_2.1 (150 mm × 2.1 mm, 2.5 μm, Waters) and AMY1_3.0 (150 mm × 3.0 mm, 2.5 μm, Waters) columns; Chiralpak IA-3 (150 mm × 4.6 mm, 3 μm, Daicel, Tokyo, Japan), IA-5 (150 mm × 4.6 mm, 5 μm, Daicel), ID-3 (150 mm × 4.6 mm, 3 μm, Daicel), IE-3 (150 mm × 4.6 mm, 3 μm, Daicel), and IF-3 (150 mm × 4.6 mm, 3 μm, Daicel) columns; a Lux Amylose-2 (150 mm × 2 mm, 3 μm, Phenomenex, Torrance, CA, USA) column; cellulose-based chiral Trefoil CEL1_2.1 (150 mm × 4.6 mm, 2.1 μm, Daicel), CEL1_3.0 (150 mm × 4.6 mm, 3.0 μm, Daicel), CEL2_2.1 (150 mm × 2.1 mm, 2.5 μm, Waters), and CEL2_3.0 (150 mm × 3.0 mm, 2.5 μm, Waters) columns; Chiralpak IB-3 (150 mm × 4.6 mm, 3 μm, Daicel) and IC-3 (150 mm × 4.6 mm, 3 μm, Daicel) columns; and Lux cellulose-1 (150 mm × 2 mm, 3 μm, Phenomenex) and cellulose-2 (150 mm × 2 mm, 3 μm, Phenomenex) columns were each evaluated individually in different types of specifications. The two pairs of enantiomers were ultimately separated on Trefoil AMY1_3.0 chiral column coated with amylose tris-(3,5-dimethylphenylcarbamate) with a 3 mm particle size. Gradient elution was used from 2% to 11.9% methanol/formic acid (88/2, *v*/*v*, co-solvent B) in CO_2 (primary solvent A) over 0.2 min, held at 11.9% B for 3.3 min, and then equilibrated by 2% B for 1 min, providing a total analysis time of 4.5 min. The pressure of the automated backpressure regulator (ABPR), the column temperature, the sample manager temperature, and the injection volume were set at 2009.8 psi, 26 °C, 4 °C, and 1 μL, respectively.

A triple quadrupole Xevo-TQD mass spectrometer (Waters) equipped with an electrospray ionization source was adopted to quantify the enantiomers of dinotefuran and UF. Higher sensitivity was obtained in positive mode with a 3500 V capillary voltage, 150 °C source temperature, and 500 °C desolvation temperature. The nebulizer gas was N_2, and the collision gas was Ar with 2×10^{-3} mbar in the T-wave cell. Cone and desolvation flows (N_2) of 50 and 1000 L/h were applied, respectively. MS detection was conducted in multiple reactions monitoring (MRM) mode by measuring the transition of the precursor to product ions for the dinotefuran and UF enantiomers. A dwell time of 163 ms per ion pair was selected to maintain the high sensitivity. For dinotefuran, the cone voltage was 16 V. The transitions *m/z* 203.2 → 129.1 and *m/z* 203.2 → 157.1 were used for quantification and confirmation when the collision energies were set at 10 and 9 V, respectively. The ion ratio was 1.5. For UF, and the cone voltage, quantitative and qualitative transitions, and ion ratio were 24 V, *m/z* 159.1 → 102.0 (collision energy, 10 V) and *m/z* 159.1 → 67.1 (collision energy,