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Simultaneous determination of neonicotinoid insecticides in human serum and urine using diatomaceous earth-assisted extraction and liquid chromatography–tandem mass spectrometry

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ABSTRACT

A rapid and sensitive analytical method was developed for simultaneous determination of eight neonicotinoid insecticides (acetamiprid, clothianidin, dinotefuran, flonicamid, imidacloprid, nitenpyram, thiacloprid and thiamethoxam) and three specific metabolites of acetamiprid (*N*-desmethylacetamiprid, 5-(*N*-acetyl-*N*-methylaminomethyl)-2-chloropyridine and 5-(*N*-acetylaminomethyl)-2-chloropyridine) in human serum and urine. A diatomaceous earth-assisted extraction using Extrelut NT3 column with chloroform/2-propanol (3:1, v/v) as eluent was selected for the single step cleanup procedure for all the target compounds. Qualitative and quantitative analyses were conducted by liquid chromatography–tandem mass spectrometry with multiple reaction monitoring mode. The limits of detection and the limits of quantification of eleven compounds were in the ranges of 0.1–0.2 ng/mL and 0.5–10 ng/mL for serum, 0.1–1 ng/mL and 1–10 ng/mL for urine, respectively. The extraction recoveries were between 80.9% and 101.8% for serum samples, 91.9% and 106% for urine samples. The intra-day RSDs and the inter-day RSDs were less than 11.5% and 13.2% for serum, less than 8.3% and 8.8% for urine. The proposed procedure will be suitable for forensic investigations of human poisoning cases with neonicotinoid insecticides. This is the first report of simultaneous determination of eight neonicotinoids in serum and urine samples.

1. Introduction

Neonicotinoid insecticides are new-generation pesticides that were developed to exterminate insects resistant to conventional products such as organophosphorus pesticides. Neonicotinoids function as nicotinic acetylcholine receptor agonists, and their affinity to these receptors in mammals is weak compared with in insects, which means they present a low risk to mammals [1]. Eight neonicotinoids (Fig. 1), including acetamiprid, clothianidin, dinotefuran, flonicamid, imidacloprid, nitenpyram, thiacloprid and thiamethoxam, are currently marketed as insecticides. These compounds are low-toxic for humans, but acute poisoning cases caused by ingestion, most of which were suicide or attempted suicidal cases, have been reported [2–14].

For determination of neonicotinoids, chromatographic detection using liquid chromatography with diode-array detection (HPLC-DAD) [7], liquid chromatography–mass spectrometry (LC–MS) [6,15–17] and liquid chromatography–tandem mass spectrometry (LC–MS/MS) [18–22] has been developed. Although many analytical methods above for determination of neonicotinoids have been reported, very few studies have focused on the analysis of human biological samples for forensic investigation of poisoning cases [5–8], and most of previous analyses have targeted agricultural samples for determination of residual pesticides [15–22]. In addition, although there are many reports of simultaneous determination of four [16,17], six [18], seven [15,21,22] and eight neonicotinoids [19] in the analysis of agricultural samples, analytical methods in the toxicological reports have been limited for single analyte [6–8]. Therefore, in this paper, we investigated a simultaneous determination method for eight neonicotinoids in serum and urine.

In the previous reports, diatomaceous earth columns [15–17], Oasis HLB cartridge with activated carbon [18], QuEChERS method [19] and subcritical water extraction [22] were used for simultaneous extraction of neonicotinoids.

In consideration of the recorded human poisoning cases, it is necessary to investigate the metabolism of neonicotinoids. Metabolic pathways of neonicotinoids have been reported by Ford

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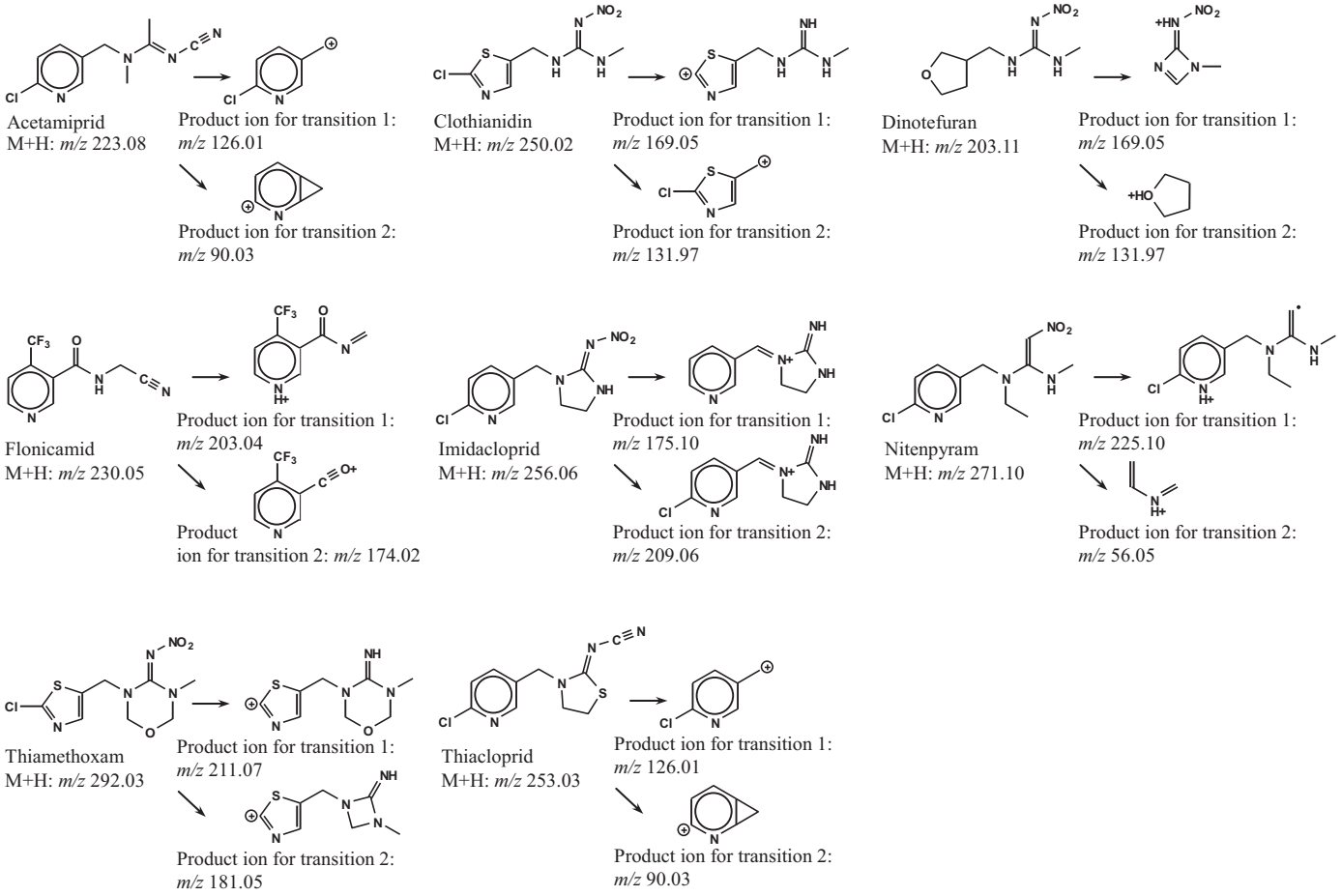

Fig. 1. The neonicotinoid insecticides and the proposed structures of the product ions for transition 1 and 2.

and Casida [23,24]. Some compounds such as 6-chloronicotinic acid and 2-chloro-1,3-thiazole-5-carboxylic acid were reported as common metabolites of neonicotinoids [23–26]. However, in the field of forensic investigation and emergency medicine, detection of more unique metabolites is required for identification of ingested insecticides in human poisoning cases.

For acetamiprid, it is known that the metabolism is fast compared to other neonicotinoids, and *N*-desmethylated substance is known as a major metabolite in mice, rat and human [5,23,25,27]. *N*-desmethylacetamiprid was also detected from human urine sample exposed to acetamiprid through the consumption of contaminated foods [5]. Therefore, in the acetamiprid poisoning case, *N*-desmethylacetaimprid is the most important analytical target. In the low-level exposure poisoning or in the case in which the biological samples were taken long time after poisoning, usually only *N*-desmethylated substance was barely detected or no specific compounds were not detected [3]. 5-(*N*-acetyl-*N*-methylaminomethyl)-2-chloropyridine (5-AMAM-2-CP), hydrolyzed product of acetamiprid and 5-(*N*-acetylaminomethyl)-2-chloropyridine (5-AAM-2-CP), hydrolyzed product of *N*-desmethylacetamiprid are also known as top 3 metabolites of acetamiprid [5,23,25,27] (Fig. 2). We also have previously investigated a suicide case caused by massive ingestion of acetamiprid [8]. In this case, the patient dissolved Mospiran Wettable Powder (20% acetamiprid, 100 g) in 500 mL of water, drank it up and died. The patient vomited most of the solution out, so the actual dosage was unknown. After autopsy, heart blood and urine were taken and analyzed. In both of blood and urine, acetamiprid, *N*-desmethylacetamiprid and 5-AAM-2-CP were detected [8]. From these reports [5,8,23,25,27], acetamiprid and its metabolites should be analyzed simultaneously in acetamiprid poisoning cases (Fig. 2).

Concerning thiacloprid, it is reported that thiacloprid was metabolized at moderate rate in mice [28]. In the report, unchanged thiacloprid and 14 metabolites were detected from the urine of mouse after oral and intravenous administration [28], and there was no significant difference between concentration of unchanged thiacloprid and its metabolites in urine at large amount oral administration (100 mg/kg) to mouse. Thus, unchanged thiacloprid was selected as an analytical target in this study. For other neonicotinoids, metabolic rates were not so rapid and unchanged substances were sufficiently excreted in mice or rats [23,24,29–33], thus, for those neonicotinoids, unchanged compounds were selected as analytical targets in this study.

The aim of the present study was to develop a simple and quick method for simultaneous quantitative analysis of eight neonicotinoids and three metabolites of acetamiprid in human serum and urine simulating actual poisoning cases. For rapid cleanup of the samples, extraction with a diatomaceous earth column was selected as it has been widely used for toxicological analysis of biological specimens [15–17]. For sensitive detection and quantitative analysis of neonicotinoids, liquid chromatography–tandem mass spectrometry (LC–MS/MS) using multiple reaction monitoring (MRM) mode was chosen [18–22]. Four deuterated neonicotinoids, Acetamiprid-d_3, clothianidin-d_3, thiamethoxam-d_3 and imidacloprid-d_4 were used as internal standards for quantification. The proposed method was validated by measuring the recovery,