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Case Report

Detection of imidacloprid in biological fluids in a case of fatal insecticide intoxication

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ABSTRACT

Here, we describe a high-performance liquid chromatography/photodiode array detector method for the detection of imidacloprid in biological fluids in a case of suicide by ingestion of liquor mixed with Admire® Flowable insecticide (containing 20% imidacloprid). A plastic bottle containing a cloudy liquid (concentration of ethanol in the liquid was 150 mg/ml and that of imidacloprid was 50 mg/ml) was found near the decedent. The biological fluids collected at autopsy were prepared by deproteinization with acetonitrile. Zolpidem was used as an internal standard. The concentrations of imidacloprid in femoral blood and cerebrospinal fluid were 105 and 58.5 μg/ml, respectively. Ethanol was also detected in the samples, with concentrations of 1.0 mg/ml in femoral blood and 1.4 mg/ml in cerebrospinal fluid.

1. Introduction

Imidacloprid [1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylideneamine] is a neonicotinoid insecticide in the chloronicotinyl nitroguanidine chemical family (Fig. 1). It was introduced in the Japanese market in 1992. Trade names for Bayer CropScience's imidacloprid products include Admire® and Hachikusan®.

Imidacloprid acts on several types of postsynaptic nicotinic acetylcholine receptors (nAChRs) in the nervous system. In insects, these receptors are located exclusively within the central nervous system. Following the binding of imidacloprid to the nicotinic receptor, nerve impulses are spontaneously discharged at first; however, subsequently, the neurons fail to propagate any signal [1].

There are various subtypes of mammalian nAChRs, all of which have a lower binding affinity for imidacloprid than that observed in insects [1,2]. Therefore, the acute oral toxicity of imidacloprid in mammals is considered moderate [1]. For example, the oral lethal dosage (LD_{50}) in male rats is 420 mg/kg [3]. In mammals, imidacloprid is mainly metabolized to 6-chloronicotinic acid and its glycine conjugate, and 5-hydroxyimidacloprid [1,3].

Insecticide products containing imidacloprid are used worldwide; therefore, occasionally, accidental intoxication or intentional self-intoxication with imidacloprid occurs throughout the world [4–15]. Some fatal intoxication cases have been reported, despite the relatively low toxicity of imidacloprid in mammals [6,7,9,13,14]. In this report, we describe a high-performance liquid chromatography/photodiode array detector (HPLC/PDA) detection method for the identification of imidacloprid in biological fluids in a fatal intoxication case.

2. Case report

A man in his 70s, who was missing after a quarrel with his family, was found dead near his ancestor's grave. A plastic bottle containing a white liquid with an alcoholic odor was found near the decedent. A medico-legal autopsy was performed 2 days after the death. The man's height was 154 cm and he weighed 56 kg. His face showed congestion, and there were some viscous fluid in his mouth. There were no signs of serious injury on the body. The heart, which weighed 430 g, showed left ventricular hypertrophy without myocardial ischemic change or coronary artery stenosis. The left and right lungs showed congestion and weighed 420 and 610 g, respectively. Gastric contents were a white viscous fluid (70 ml) with an unpleasant odor. Substantial petechial bleeding was observed in the mucosa of the stomach and duodenum. The abdomen had a surgical scar, and there were indications that the man had undergone right hemicolectomy with an ileotransverse anastomosis in the abdominal cavity. There was no evidence of a lethal disease.

3. Materials and methods

3.1. Chemicals

Imidacloprid and 6-chloronicotinic acid were purchased from Wako (Osaka, Japan). Zolpidem, used as an internal standard (IS),

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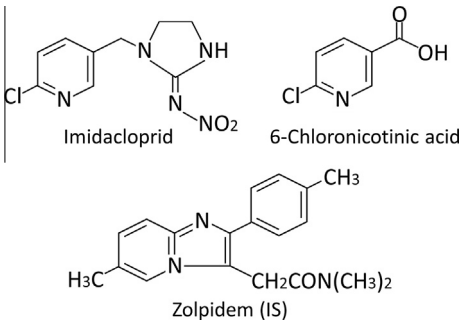

Fig. 1. Structures of imidacloprid, 6-chloronicotinic acid, and zolpidem (internal standard).

was purchased from Sigma–Aldrich, Japan (Tokyo, Japan). Other chemicals used were HPLC grade or analytical grade. Water was purified using a Milli-Q A10 system (Millipore, MA, USA).

3.2. Biological samples

Samples (whole blood from the heart and femoral vein, cerebrospinal fluid, vitreous humor, and urine) for analysis were collected at the time of autopsy. Frozen human whole blood was purchased from KAC Co. Ltd. (Kyoto, Japan). All samples were stored at −30 °C prior to analysis.

3.3. Stock solutions

Stock solutions of imidacloprid and 6-chloronicotinic acid were prepared in methanol at 1.0 mg/ml. The IS working solution (1.0 mg/ml zolpidem) was also prepared in methanol.

3.4. Sample preparation

Ten microliters of the IS working solution were placed into a microtube (1.5 ml) and evaporated to dryness under a stream of nitrogen gas. An aliquot (100 μl) of the liquid specimen was added to the tube and vortex-mixed. Two hundred microliters of acetonitrile were added to the tube while vortex-mixing. The mixture was centrifuged at 12 000*g* for 5 min. The supernatant was transferred to another microtube and evaporated to dryness under a stream of nitrogen gas at 40 °C. The residue was dissolved in 100 μl of 15% acetonitrile–water (v/v) and centrifuged at 12 000*g* for 5 min. The supernatant was then transferred to the appropriate glass autosampler vial insert and injected into the HPLC system.

3.5. HPLC conditions for the analysis of imidacloprid

The HPLC system consisted of an Alliance 2695 separation module and 2996 photodiode array detector (Waters, MA, USA). The analytical column used was an XTerra® MS C_{18} 2.1 × 150 mm, 3.5 μm (Waters, MA, USA). Column temperature was set at 40 °C. Chromatograms were monitored at 270 nm, and injection volume

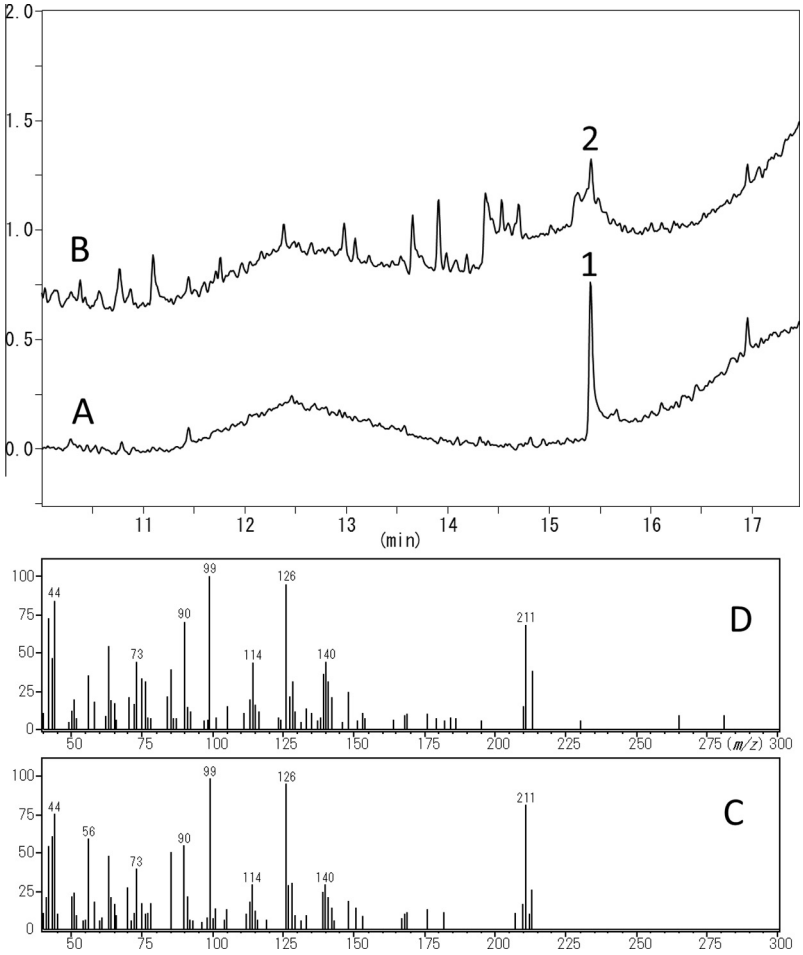

Fig. 2. Total ion chromatograms of GC/MS analysis of 100.0 μg/ml imidacloprid (A) and ethylacetate extract of the heart blood (B), and the mass spectra of peak 1 (C) and peak 2 (D).