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Highly sensitive quantification of pyrethroid insecticide etofenprox in vegetables with high-performance liquid chromatography and fluorescence detection

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ABSTRACT

This paper describes a highly sensitive analytical method using high-performance liquid chromatography and fluorescence detection (HPLC–FLD) capable of quantifying trace amounts of synthetic pyrethroid insecticide etofenprox residue in six vegetable samples: bell pepper, cucumber, eggplant, Japanese mustard spinach, spinach, and tomato. After extraction with acetonitrile, the crude sample extract was cleaned up with a solid-phase extraction cartridge. The matrix interference derived from the tested vegetable samples was evaluated. Quantification was conducted using external calibrators prepared in pure acetonitrile. The limits of quantification for etofenprox in each sample were 1.87–3.87 ng/g. Recoveries obtained by application of the proposed analytical method of vegetable samples spiked at the considerably low levels (5–100 ng/g) were 85–111% with relative standard deviations of less than 12%. The proposed method using the HPLC–FLD was applied for trace analysis of the insecticide residue in vegetable samples.

1. Introduction

Etofenprox (2-(4-ethoxyphenyl)-2-methylpropyl-3-phenoxybenzyl ether), which acts immediately on various pests by the highly insecticidal activity based on inhibition of normal function of the sodium channel on nerve axons, was introduced in Japan in 1987 by Mitsui Chemicals, Inc. [1]. Especially, it is effective for lepidoptera and hemiptera at a low dosage. The feature on the chemical structures of the insecticide is to possess not an ester linkage that conventional synthetic pyrethroid insecticides possess but an ether linkage. An important difference from existent pyrethroid insecticides is the different chemical structure: it is beneficial that etofenprox has weaker toxicity for fish (LC_{50} values (96 h): 0.0027 mg/L (rainbow trout, *Oncorhynchus mykiss*), 0.013 mg/L (bluegill, *Lepomis macrochirus*), 0.141 mg/L (carp, *Cyprinus carpio*)) than other pyrethroid insecticides [1,2]. Based on its superior insecticidal activity, it is used for the extermination of pests from various agricultural products such as rice, beans, vegetables, and fruits as a less-toxic and safe insecticide. The United States Department of Agriculture (USDA), the European Union (EU), and the Japanese Ministry of Health, Labour and Welfare (JMHLW) have set maximum residue limits (MRLs) of etofenprox for various agricultural products [3].

Because of the widespread application of etofenprox, appropriate analytical methods for detection and quantification of the residues are required for use with agricultural products. With regard to analytical methods particularly addressing the insecticide residues, several related papers have been published. Specifically, analytical methods have been proposed mainly for chromatographic techniques such as gas chromatography (GC) coupled with mass spectrometry (MS) [4], and high-performance liquid chromatography (HPLC) coupled with UV or diode array detection (DAD) [5–9], tandem MS [10–12], and time-of-flight MS [12]. Immunoassay for the preliminary screening method of the insecticide has also been reported as a non-chromatographic technique [13].

Recent demands for a new analytical method for pesticide residues strongly emphasize several needs: (1) detection of analyte(s) at lower levels and with greater precision, (2) reduction of overall analytical turnaround times (from sample preparation to data processing), (3) reduction of usage of hazardous organic solvents, (4) reliability of the validity of analytical results, and (5) determination of numerous pesticides simultaneously using a single analysis (for the circulation food). To acquire highly reliable results obtained with chromatographic methods, sample preparation is normally necessary to isolate and concentrate analytes from the sample matrices before their determination. Although

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traditional methods such as liquid–liquid extraction and clean-up with Florisil column chromatography have been used as sample preparation for etofenprox [5], such methods are time-consuming. They require large amounts of organic solvents. Therefore, solid-phase extraction (SPE) [4] and dispersive-SPE [9,12] are used frequently to remove matrix components effectively. Furthermore, dispersive liquid–liquid microextraction for extraction with extremely small amounts of organic solvents has been proposed for etofenprox residue analysis [8].

It is particularly interesting that etofenprox shows a natively strong fluorescence property in the UV region [14]. Because few pesticides show native fluorescence, as do benzimidazole fungicides [15,16], the applicable range of the method to pesticide residue analysis is restrictive. However, the performance of fluorescence detection (FLD) in a highly sensitive and selective system is attractive. Therefore, the determination of an insecticide using HPLC–FLD can be accomplished precisely without any effects by matrix components other than the above-described analytical methods, such as GC–MS, HPLC–UV or DAD, and LC–MS/MS methods.

This work was undertaken to establish a sensitive analytical method with HPLC–FLD for the determination of etofenprox in vegetable samples of six kinds: bell pepper, cucumber, eggplant, Japanese mustard spinach, spinach, and tomato. For achieving the requirements for development of analytical method for pesticide residues described above to the greatest extent possible, a user-friendly sample preparation method is investigated for removal of matrix components using small amounts of solvents with a versatile SPE method.

2. Experimental

2.1. Chemicals

Etofenprox reference material (traceable reference material) (purity 99.5%) was provided by Wako Pure Chemical Industries Ltd. (Osaka, Japan). HPLC-grade acetonitrile, pesticide residue analysis-grade acetonitrile and toluene, and analytical grade sodium chloride (NaCl) and anhydrous magnesium sulfate ($MgSO_4$) were also obtained from Wako Pure Chemical Industries Ltd. Trebon® (emulsifiable concentrate) containing 20.0% etofenprox was obtained from Mitsui Chemicals Agro, Inc. (Tokyo, Japan).

The pesticide stock solution (1 mg/mL) was prepared by dissolving about 10 mg of analyte in 10 mL of HPLC-grade acetonitrile, and was stored in the dark at 4 °C. Under these conditions, the stock solution was stable for at least six months. A working standard solution (10 μg/mL) was prepared daily, diluting the primary stock solution with acetonitrile. It was used for spiking agricultural matrices and for preparing calibration standards. Water used for HPLC was prepared directly in the laboratory using a Milli-Q water purification system (Millipore Corp., Bedford, MA).

2.2. Sample

About 1 kg portions of bell pepper, cucumber, eggplant, Japanese mustard spinach, spinach, and tomato samples (all labeled as organic) were used as blanks and for the preparation of fortified samples and matrix-matched calibration standards for validation experiments. Actual samples (five vegetables, except for Japanese mustard spinach) were grown in a vinyl greenhouse or open fields on the National Institute for Agro-Environmental Sciences, and were sprayed with Trebon® diluted with water by 1000 times at the harvesting stage. The samples (about 1 kg) were harvested at 1, 3, 7, and 10 days (bell pepper, cucumber, eggplant, and tomato), and 4, 7, and 11 days (spinach) after spraying. Blank and etofenprox-incurred samples were homogenized thoroughly using a blender (7011HB; Waring, Torrington, CT). In addition, although etofenprox has no pesticide registration for spinach in Japan, incurred spinach samples were prepared experimentally to evaluate the validity of the proposed method in this work.

2.3. Sample preparation procedure

Flowchart of sample preparation procedure for the proposed and the authorized reference official methods is given in Fig. 1.

2.3.1. Proposed extraction and clean-up method

Homogenized sample (5 g) was weighed into a 100 mL of an Erlenmeyer flask. A volume of 10 mL of acetonitrile was added and shaken mechanically for 30 min. Later, 1 g of NaCl and 4 g of anhydrous $MgSO_4$ were added. The sample was shaken gently by hand for 1 min. Then 1.2 mL of toluene was added to 4 mL aliquots of the extract, equivalent to 2 g of sample. Subsequently, the sample extract was applied to a Supelclean ENVI-Carb-II/PSA SPE cartridge (500 mg + 500 mg/6 mL; Supelco, Bellefonte, PA) equilibrated with 4 mL of acetonitrile/toluene (3:1). Then the analyte was eluted with the same solvent (8 mL). The eluate was concentrated with a vacuum rotary evaporator and evaporated under a gentle nitrogen stream at 50 °C. The dried residue was dissolved by sonication in 0.2 mL of acetonitrile. Then the final solution was syringe-filtered using a 0.45 μm PTFE filter (Millipore Corp., Billerica, MA) into an autosampler vial (2 mL capacity) that put a glass insert (0.1 mL capacity).

2.3.2. Japanese authorized official method (reference method)

To verify the applicability of the proposed analytical method for etofenprox residue, we used the Japanese authorized official method composed of (1) extraction with acetonitrile, (2) separation of organic (acetonitrile) and aqueous phases (salting-out) with phosphate buffer and NaCl, and (3) clean-up with a Supelclean ENVI-Carb/LC-NH_2 SPE cartridge (500 mg + 500 mg/6 mL; Supelco) [17] as a reference method.

2.4. Instrumentation

The HPLC system used for these experiments consisted of a quaternary pump (G1311A; Agilent Technologies Inc., Waldbronn, Germany) operating at a flow rate of 0.8 mL/min. Mobile phases were degassed using an online membrane system (G1322A; Agilent Technologies Inc.). The column was maintained in a thermostated compartment (G1316A; Agilent Technologies Inc.). Separation was done using an Atlantis dC18 column (150 mm × 4.6 mm, 5 μm) (Waters Corp., Milford, MA) in combination with an Atlantis dC18 guard column (20 mm × 4.6 mm, 5 μm) (Waters Corp.). Injection (20 μL) was performed using an autosampler (G1313A; Agilent Technologies Inc.). The fluorescence detector (G1321A; Agilent Technologies Inc.) was operated at 277 and 298 nm, respectively, as excitation and emission wavelengths. Under the analytical conditions described, typical HPLC–FLD chromatogram of etofenprox reference standard dissolved in pure acetonitrile is shown in Fig. 2. Repeatability and reproducibility studies at 100 ng/mL in acetonitrile with five consecutive injections for same day ($n = 5$) in five different days ($n = 25$) were carried out. The relative standard deviations (RSDs) obtained for both retention time (t_R) and peak area of the insecticide were acceptable precision in all cases (intraday RSD < 2%, interday RSD < 5%).

2.5. Quantification

The etofenprox concentration in samples was estimated based on the calibration curve using seven levels (5, 10, 25, 50, 100, 500,