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Analysis of Pyrethroid Insecticides in *Chironomus dilutus* Using Matrix Solid Phase Dispersion Extraction

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Abstract A matrix solid phase dispersion method was developed to detect eight pyrethroid insecticides in the aquatic invertebrate, *Chironomus dilutus*. A mixture of silica gel, diatomaceous earth and primary/secondary amino solid absorbents were selected as the dispersion matrix, while 7% ethyl ether in hexane was used as the elution solvent. Method detection limits for the target pyrethroids ranged from 0.46 to 4.4 μg kg^{-1}, and recoveries were 63.5%–124.0%, 43.7%–116.0% and 53.1%–93.1% at spiked levels of 5, 20 and 50 μg kg^{-1}, respectively. The developed method was used to assess pyrethroid residues in laboratory-exposed and field-collected *C. dilutus*.

Keywords Matrix solid phase dispersion · Pyrethroids insecticides · *Chironomus dilutus* · Gas chromatography

Pyrethroid insecticides are widely used in agricultural production and urban areas, and their usage has increased dramatically in recent years (Weston et al. 2004, 2005). Although posing minimal threat to mammals and avian species, pyrethroids are highly toxic to non-target aquatic species (Kale et al. 1999). For example, the 10-d sediment median lethal concentrations of bifenthrin, lambda-cyhalothrin and permethrin are 6.2, 2.8 and 24.5 μg g^{-1} organic carbon for *Chironomus dilutus*, respectively (Maul et al. 2008). Analytical methods have been established for pyrethroids in sediment (You et al. 2008), foods (Esteve-Turrillas et al. 2004) and some biological species, such as fish, honeybees and isopods (You and Lydy 2004; Kristenson et al. 2004). Various analytical methods have been reported to quantify pesticides in tissues, such as Soxhlet extraction (Brokopp et al. 1981), sonication extraction (You and Lydy 2004), and microwave-assisted extraction (Esteve-Turrillas et al. 2004), however, those methods which processed extraction and cleanup steps separately were laborious and solvent-consuming. In order to better quantify pyrethroid residues in *C. dilutus*, it is necessary to establish an effective, simple and fast analytical method. A matrix solid phase dispersion (MSPD) method has been proposed recently to combine extraction and cleanup into a single step, and it has been used as a sensitive and reliable method for analyzing pesticides in biological tissue (Kristenson et al. 2004; Morzycka 2002). For example, Morzycka (2002) employed MSPD to analyze 12 pesticides in honeybees, and the method showed recoveries of 70%–110% and relative standard deviations (RSD) in the range of 2%–8% for pesticides spiked at 10–1,000 μg kg^{-1}.

The objective of the current study was to develop a simple and reliable method based on MSPD to simultaneously extract and cleanup eight pyrethroids from tissues taken from the aquatic invertebrate, *C. dilutus*. The extracts were analyzed with gas chromatography-micro electron capture detector (GC-μECD). The developed MSPD method was validated and used to quantify pyrethroid residues in both laboratory-exposed and field-collected midge larvae.

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Materials and Methods

Pyrethroids analyzed in the current study included bifenthrin, permethrin, fenpropathrin, lambda-cyhalothrin, cyfluthrin, cypermethrin, esfenvalerate and deltamethrin (ChemService, West Chester, PA, USA). The purities were 98.0% ~ 99.7% as certified by the manufacturer. Decachlorobiphenyl (DCBP; Supelco, Bellefonte, PA, USA) was used as a surrogate to verify the performance of the analytical process, and was added to the midge before the extraction. Anhydrous Na_2SO_4, Florisil (60 ~ 80 mesh), silica gel (60 ~ 80 mesh), 15 mL empty polypropylene SPE cartridges and various organic solvents (pesticide grade) were purchased from Fisher Scientific (Pittsburgh, PA, USA). Primary/secondary amino (PSA) absorbent and diatomaceous earth were purchased from Varian (Palo Alto, CA, USA), while alumina-*N* (50 ~ 200 mesh) was obtained from Aldrich (Wyoming, IL, USA). Silica gel, Florisil and alumina-*N* absorbents were activated at 130°C for 12 h, and anhydrous Na_2SO_4 was baked at 400°C for 4 h prior to use.

The benthic invertebrate, *C. dilutus* (4th instar larvae) was obtained from the culture in the Fisheries and Illinois Aquaculture Center at Southern Illinois University, Carbondale, IL, USA, where the organisms were cultured in accordance with U.S. EPA standard protocols (U.S. EPA 2000). The lipid content of the midge larvae was 1.1 ± 0.1% wet weight (ww) measured with a spectrometric method after acid digestion (Van Handel 1985).

For developing the MSPD method to analyze pyrethroid residues in *C. dilutus*, parameters including the types of the dispersion absorbents (Florisil, silica gel or alumina-*N*), the types of elution solvents (3%, 5% and 7% ethyl ether in hexane, 50% dichloromethane in hexane and 100% ethyl acetate) and the volume of elution solvents, were optimized. The MSPD parameters were optimized to achieve the highest recoveries for the target pyrethroids with low lipid breakthrough. To validate the developed method, spiked control midges were prepared at three levels including 5, 20 and 50 μg kg^{-1} by adding an appropriate amount of a standard mixture of pyrethroids and DCBP to 0.5 g of homogenized wet midge tissue. After a 1-h holding period, *C. dilutus* were homogenized with 0.7 g diatomaceous earth and 2 mL of acetone to obtain a powerlike matrix. After acetone was evaporated, 1.0 g silica gel and 30 mg PSA absorbents were gently mixed with the matrix, and then the homogeneous dispersed matrix was transferred onto the top of 1 g of silica gel and 0.5 g of anhydrous Na_2SO_4 which were pre-packed inside of an empty SPE cartridge. Optimized solvents were then used to elute the target pyrethroids at a rate of ~1 drop/s. The eluent was evaporated to near dryness under a gentle flow of nitrogen using a ReactiVap (Rockford, IL, USA), and 0.1 mL of 0.1% acetic acid acidified hexane was added to avoid isomerization of pyrethroids during GC analysis (You and Lydy 2007).

Analysis of the extracts was performed on an Agilent 6890 series GC-μECD following the method described in You et al. (2008). Two columns, a HP-5MS (30 m × 0.25 mm × 0.25 μm film thickness) and a DB-608 (30 m × 0.32 mm × 0.25 μm film thickness) were used to confirm the analytical results. The oven temperature for the HP-5MS column was set at 100°C, heated to 180°C at 10°C min^{-1}, then heated to 205°C at 3°C min^{-1} and held at 205°C for 4 min, then heated to 280°C at 20°C min^{-1} and held at 280°C for 10.92 min, while the oven temperature for DB-608 was set at 100°C, heated to 250°C at 10°C min^{-1}, then heated to 280°C at 3°C min^{-1} and held at 280°C for 20 min. The flow rates of carrier gas were 3.8 and 1.8 mL min^{-1} for the HP-5MS and DB-608 columns, respectively. Qualitative identity was established using a retention window of 1%, and quantitative analysis was based on peak area using six external standards of each pyrethroid and surrogate in a range of 1 to 250 μg L^{-1}. The calibration curve was linear within this concentration range with linear regression coefficients $r^2 > 0.995$.

Recovery was calculated as the ratio between the measured and spiked concentrations, and precision was determined as the RSD of the replicates. The method detection limits (MDL) were computed from seven replicates of the *C. dilutus* samples spiked at 5.0 μg kg^{-1} ww, and were calculated as follows: MDL = $s\, t_{(0.99,\ n-1)}$, where, s is a standard deviation of the seven measurements and $t_{(0.99,\ n-1)} = 3.14$ is a *t*-distribution value taken at a confidence level of 0.99 ($\alpha = 0.01$) and six degrees of freedom for a one-tailed hypothesis (You and Lydy 2004).

The MSPD method was applied to analyze surviving midges after a laboratory exposure study and a group of field-collected midges. In the laboratory exposure test, sixty 4th instar midge larvae were exposed in 1 L of moderately hard water (U.S. Environmental Protection Agency 2000) spiked with 100 ng L^{-1} of permethrin. The test was conducted at 23°C for 9 h, and then the organisms were sieved through a 500 μm sieve, blotted dry, and weighed to the nearest 0.01 mg on a Mettler-Toledo analytical balance (Schwiez, Switzerland). Pyrethroid residues were detected using the MSPD procedure described above with three replicates. In August 2008, midges were collected from Scattering Fork creek in Douglas County, IL, which was surrounded by cornfields. Approximately 0.4 g midge (ww) was used to analyze pyrethroid residues using the newly developed MSPD method.

Results and Discussion

The principle of MSPD is to disperse a lipid-rich sample over absorbents with large surface areas to retain the lipids

on the solid phase material, and then to desorb the target analytes with small amounts of organic solvents. By combining extraction and cleanup into a single step, MSPD provides a simple and quick method for tissue sample preparation. In addition, it is ideal for preparing samples with small mass limitations. Thus, MSPD was selected in the present study to assess pyrethroid residues in midge tissues.

A drying agent, diatomaceous earth, was used as the solid supports for the matrix dispersion, and it removed the moisture from organisms to obtain a powder-like homogeneous matrix. Previous studies (Lehotay and Lee 1997; You et al. 2008) showed that the ratio of the amounts of drying agent and sample did not affect recoveries of non-polar pesticides. Therefore, 0.7 g of diatomaceous earth was used, which represented the smallest amount of diatomaceous earth needed to dry 0.5 g of tissue. Three normal phase absorbents including Florisil, silica gel and alumina-*N* were tested for their suitability as the dispersion absorbent. The solid phase activity should be great enough to retain interference in the sample, while allowing the target compounds to be eluted. Pyrethroids were eluted from the absorbents with 3 mL of 5% ethyl ether in hexane and the results are shown in Fig. 1. Recoveries of pyrethroids from alumina-*N* were significantly lower than those from the other absorbents, and no deltamethrin was eluted from the alumina-*N*. This indicated that the activation sites for alumina-*N* were too strong, and overly retained the pyrethroids. Though no significant difference was found between the peak areas of most pyrethroids eluted from Florisil and silica gel, silica gel provided the highest peak areas of all the target pyrethroids using the mean value of three replicates. Therefore, silica gel was chosen as the matrix dispersion absorbent for further study. As an anion exchange absorbent, PSA extensively removed the matrix interferences in sediment extracts, thereby resulting in better pyrethroid recoveries (You et al. 2008). Therefore, 30 mg PSA was added as the additional dispersion absorbent.

Due to the high lipid content of the tissue samples, an additional cleanup step was needed prior to GC analysis in order to extend the life of the chromatographic column and to improve detection sensitivity (Valsamaki et al. 2006). In order to more effectively remove lipids in the samples, an SPE column could be coupled with the packed MSPD column or additional SPE absorbents could be packed at the bottom of the MSPD column. In the current study, 1 g of silica gel, and 0.5 g of anhydrous Na_2SO_4 was packed at the bottom of the MSPD column as an inline SPE to trap residue lipids and water. Lipid content of the final extract was also analyzed (Van Handel 1985), and results showed $\sim$90% of tissue lipid was removed when an additional inline SPE was coupled with the MSPD.

A mixture of solvents with various polarities, including 3%, 5% and 7% ethyl ether in hexane, 50% DCM in hexane and 100% ethyl acetate were evaluated as elution solvents to elute the target pyrethroids from the MSPD column. As shown in Fig. 2, bifenthrin, fenpropathrin and permethrin were quantitatively recovered using all of the tested solvents. The least polar solvent (3% ethyl ether in hexane), however, provided low recoveries for the remaining analytes (<40%). With an increase in solvent polarity, recoveries of pyrethroids dramatically increased, with recoveries of most pyrethroids reaching a 100% when 7% ethyl ether in hexane was used as the elution solvent. Although the other two solvents (50% DCM in hexane and 100% ethyl acetate) could also fully recover the pyrethroids, recoveries were not uniformly good for all target analytes. Therefore, 7% ethyl ether in hexane was selected as the elution solvent to recover the target pyrethroids.

Different volumes of the 7% ethyl ether in hexane solution were used to elute pyrethroids from the MSPD column and results are detailed in Fig. 3. Elution profiles for the majority of the pyrethroids were similar. Pyrethroids recoveries increased as the amount of solvent increased until constant values were reached. Permethrin was the first eluted pyrethroid and over 70% of it was recovered in the first 3 mL fraction, while the recoveries of bifenthrin and fenpropathrin increased in the 3–6 mL fraction and then continued to increase at a very low rate in the later fractions. Additional elution solvent was needed for the remaining five pyrethroids including lambda-cyhalothrin, cyfluthrin, cypermethrin, esfenvalerate, and deltamethrin. Recoveries of most pyrethroids reached constant values when 15 mL of elution solvent was applied except for deltamethrin. Only half of the deltamethrin was recovered even with an increase in the solvent usage to 21 mL, and this may be the result of the strong binding of this compound to the dispersion matrix. Therefore, 15 mL of 7% ethyl ether in hexane was chosen to recover the target pesticides.

To evaluate the performance of the newly developed MSPD procedure, method accuracy, precision and MDL were measured using *C. dilutus* spiked with known concentrations of target pyrethroids (Table 1). The accuracy of the method was expressed as mean recoveries for the eight pyrethroids, and they were 63.5%–124%, 43.7%–116% and 53.1%–93.1% at 5, 20 and 50 μg kg^{-1}, respectively. Precision was characterized by calculating the RSD of the replicated samples. The RSD were less than 21.0% for all the pyrethroids at the three spiked levels, which is considered acceptable given the difficulty in analyzing trace pesticides in small size invertebrate samples. Recoveries of the surrogate DCBP were from 50.4% to 83.0% with a RSD <15.2%. The MDL of the eight pyrethroids were reported in Table 1, and ranged from 0.46 to 4.4 μg kg^{-1} ww.

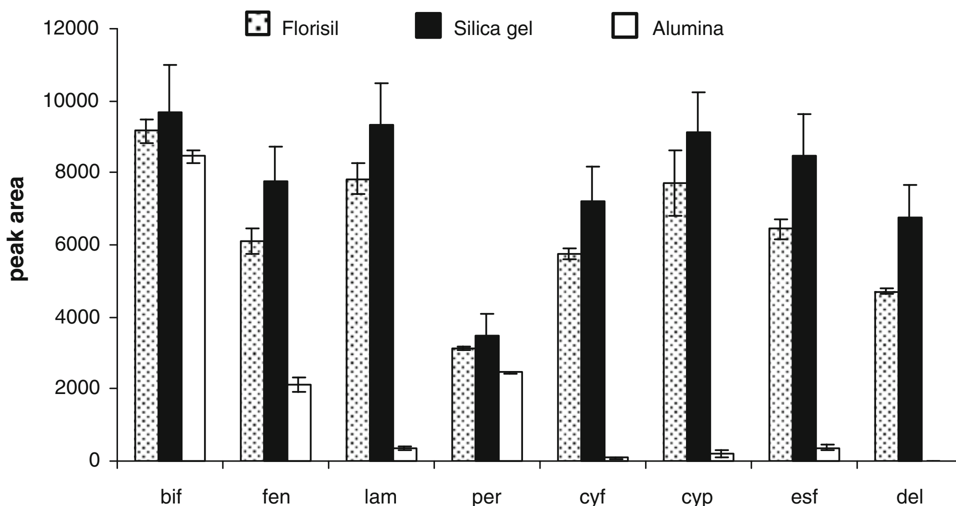

Fig. 1 Influence of dispersion absorbent on pyrethroid extraction efficiency (n = 3). *Bif* Bifenthrin, *Per* permethrin, *Fen* fenpropathrin, *Lam* lambda-cyhalothrin, *Cyf* cyfluthrin, *Cyp* cypermethrin, *Esf* esfenvalerate, *Del* deltamethrin

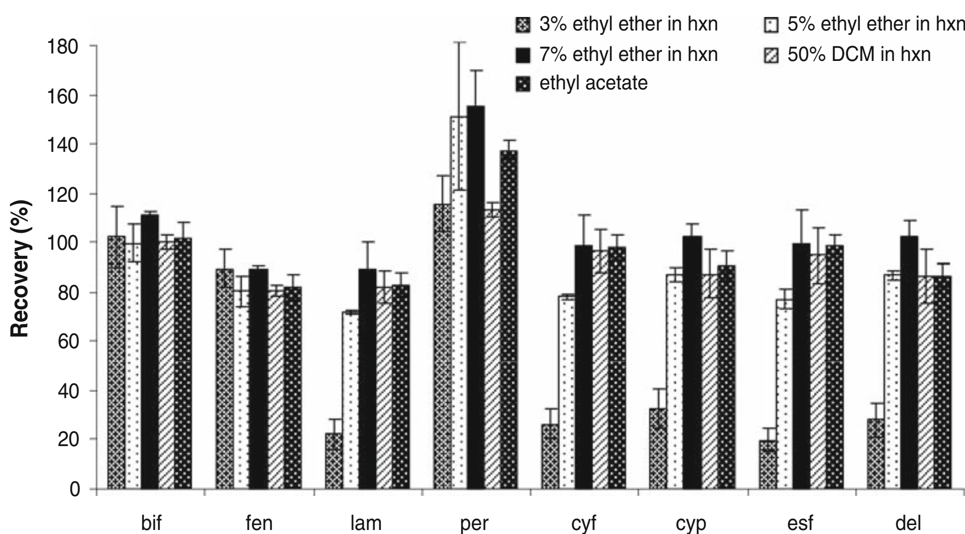

Fig. 2 Influence of elution solvent on pyrethroid extraction efficiency (n = 3). *Bif* Bifenthrin, *Per* permethrin, *Fen* fenpropathrin, *Lam* lambda-cyhalothrin, *Cyf* cyfluthrin, *Cyp* cypermethrin, *Esf* esfenvalerate, *Del* deltamethrin

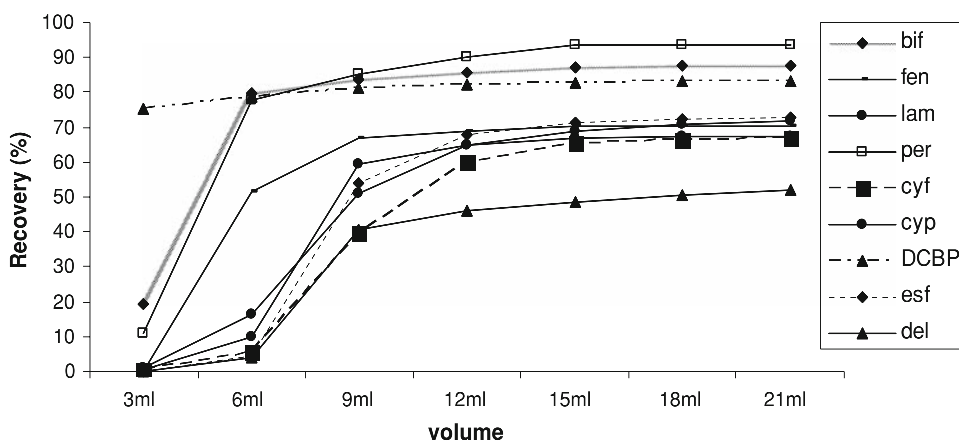

Fig. 3 Influence of volume of elution solvents on pyrethroid extraction efficiency. Ethyl ether in hexane (7%, v/v) was used as an elution solvent. *Bif* Bifenthrin, *Per* permethrin, *Fen* fenpropathrin, *Lam* lambda-cyhalothrin, *Cyf* cyfluthrin, *Cyp* cypermethrin, *Esf* esfenvalerate, *Del* deltamethrin, *DCBP* decachlorobiphenyl

The developed MSPD procedure was applied to determine pyrethroid residues in both laboratory-exposed and field-collected *C. dilutus* samples. The mean body residue of laboratory-exposed organism was 19.5 μg kg^{-1} ww with a RSD of 21.1% (n = 3), and the mean recovery and RSD of the surrogate DCBP were 81.9% and 17.1%, respectively. No pyrethroid residues, at concentrations above the reporting limits, which equaled to three times of MDL, were detected in the field-collected midges from Scattering Fork Creek in Douglas County, IL, USA. These

Table 1 Method detection limits (MDL, μg kg^{-1}), recovery (%) and relative standard deviations (RSD, %) of pyrethroid residues in *Chironomus dilutus* at different spiking levels

Pyrethroid	MDL	5 μg kg^{-1} (n = 7)		20 μg kg^{-1} (n = 4)		50 μg kg^{-1} (n = 3)	
		Recovery	RSD	Recovery	RSD	Recovery	RSD
Bif	2.4	68.9	19.7	62.4	9.9	77.5	10.3
Fen	0.46	68.3	6.7	116	13.6	93.1	8.7
Lam	2.3	77.2	11.1	55.0	10.0	79.3	14.8
Per	4.4	124	19.9	86.9	16.2	92.2	11.4
Cyf	1.0	63.5	20.9	62.4	3.8	86.2	15.2
Cyp	1.6	73.4	19.6	67.4	8.7	89.1	12.1
Esf	1.6	73.8	16.9	71.9	19.9	91.6	12.8
Del	1.3	74.5	10.4	43.7	6.7	53.1	10.2

Bif Bifenthrin, *Per* permethrin, *Fen* fenpropathrin, *Lam* lambda-cyhalothrin, *Cyf* cyfluthrin, *Cyp* cypermethrin, *Esf* esfenvalerate, *Del* deltamethrin

results are consistent with the toxicity study results, in which no toxicity was observed for the sediment samples collected in the same location.

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