Bull Environ Contam Toxicol (2008) 80:455–460
DOI 10.1007/s00128-008-9382-0

Extraction and Analysis Methods for the Determination of Pyrethroid Insecticides in Surface Water, Sediments and Biological Tissues at Environmentally Relevant Concentrations

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Received: 17 July 2007 / Accepted: 25 February 2008 / Published online: 28 March 2008

Abstract The aim of this study was to develop and validate chemical methods for measuring pyrethroid insecticides at environmentally relevant concentrations in different matrices. The analytes included six synthetic pyrethroids with the highest agricultural and commercial structural uses in California: bifenthrin, cyfluthrin, cypermethrin, esfenvalerate/fenvalerate, lambda-cyhalothrin, permethrin, and their corresponding stereoisomers, which includes enantiomers, diastereomers and racemic mixtures. Fortified water samples were extracted for analysis of synthetic pyrethroids using liquid–liquid extraction, while fortified sediment and fish tissue samples were extracted using pressurized fluid extraction followed by gel permeation chromatography (GPC) to remove matrix interferences. A florisil column was used for additional cleanup and fractionation of sediment and tissue extracts. Extracts were analyzed using dual column high resolution gas chromatography with electron capture detection (GC/ECD) and confirmation was obtained with gas chromatography mass spectrometry using a quadrupole ion trap detector in MS-MS mode. Method detection limits (MDLs) have been established for water (1–3 ng/L), sediment (0.5–4 ng/g dry weight) and tissue (1–3 ng/g fresh weight). Mean percent recoveries of fortified blanks and samples ranged from 75 to 115% with relative standard deviation (RSD) values less than 20% for all target compounds.

Keywords Pyrethroids · Insecticides · *Chrysanthemum cinerariaefolium* · Gas chromatography

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The US EPA's decision to phase out/eliminate certain uses of the organophosphate insecticides because of their potential for causing toxicity in humans has led to their replacement with another class of insecticides, the pyrethroids. Pyrethroids are synthetic derivatives of pyrethrins, which are natural insecticides that are produced by certain species of chrysanthemum (*Chrysanthemum cinerariaefolium*). Pyrethroid primary mode of action in insects is as a neurotoxin. Pyrethroids of greatest interest to water quality include bifenthrin, cyfluthrin, cypermethrin, esfenvalerate, *lambda*-cyhalothrin, and permethrin. Pyrethroids contain human made, or xenoestrogens, which can increase the amount of estrogen in the body (Garey and Wolf 1998). When tested, certain pyrethroids demonstrate significant estrogenicity in breast cancer cells (Go et al. 1999). Pyrethroids are extremely toxic to aquatic organisms, including fish such as bluegill and lake trout, with LC50 values less than 1.0 part per billion (ppb) (TDC 2003). These levels are similar to those for mosquito, blackfly and fly larvae, often the actual target of the pyrethroid application. Lobster, shrimp mayfly and zooplankton are the most susceptible non-target aquatic organisms (Mueller-Beilschmidt 1990). Pyrethroids are generally a complex mixture of stereoisomers or compounds that have very small differences in chemical structures, rather than one single pure compound, which is one reason for the wide variation in reported toxicities of these compounds (TDC 2003). They are

applied in urban areas primarily for structural pest control, in agricultural areas on crops such as almonds, alfalfa, cotton, lettuce, pistachios, and peaches, and in the home in pet sprays and shampoos. In 2003, permethrin was ranked the 43rd most used pesticide in California: 443,676 pounds applied over 755,978 acres (California Pesticide Use Reporting database: www.cdpr.ca.gov). Some of the new pyrethroids such as cypermethrin, which is used in much lower amounts, could be up to 20 times more toxic than permethrin (Amweg et al. 2005).

The primary transport pathway for pyrethroids entering receiving waters from agricultural and urban applications is through runoff. Pyrethroids are persistent compounds, are not very soluble in water, and have a tendency to concentrate in aquatic organisms. These compounds are extremely hydrophobic with very high Koc values which make them absorb rapidly and completely to sediment particles and the octanol-water partition coefficients (Kow) in the range of 10^6–10^7 and water solubilities of a few μg/L are typical (Laskowski 2002). Due to the aquatic toxicity of pyrethroids, offsite movement of these insecticides into surface water is of major concern. Although known to be highly immobile in soil, synthetic pyrethroids can enter surface water via run-off after adsorption to soil or sediment particulates. Several recent monitoring studies in California have reported synthetic pyrethroid insecticide contamination of both surface waters and sediments (Hengel et al. 1997; Weston et al. 2004; Gan et al. 2005).

Currently, there is no US EPA approved chemical analysis methods for synthetic pyrethroids that can attain the low concentrations at which toxicity has been observed for these compounds in environmental matrices. US EPA Method 1660 does not provide the sensitivity needed to determine environmentally relevant concentrations. Solid phase extraction (SPE) is not an effective extraction technique for pyrethroids due to their loss from the pre-filtering of suspended particulate matter from the water samples. These chemicals have a strong tendency to sorb to virtually all surfaces they contact, including filtration apparatus. Pyrethroid water samples concentrations are therefore determined using liquid–liquid extraction of unfiltered samples (water and suspended sediment). As a result of the heightened interest in this class of chemicals, new rapid, sensitive, and selective methods of analysis are needed for their measurement in environmental matrices. Many pyrethroids possess one or more halogenated atoms, which makes them sensitive to gas chromatography (GC) combined with electron capture detection (GC/ECD). The aim of this study was to develop an extraction and GC/ECD analytical method for measuring synthetic pyrethroids in water, sediment and tissue samples at environmentally relevant concentrations.

Materials and Methods

All certified pyrethroid standards were obtained from Chem Service, Inc. (West Chester, PA). Second source standards of each analyte were obtained from AccuStandard, (New Haven, CT) and Ultra Scientific (North Kingstown, RI) for verification of calibration standards. The surrogate for water samples was dibromooctafluorobiphenyl (DBOB) from Restek Corp (Bellefonte, PA) and for sediment and tissue samples, the surrogate was dibutylchlorendate (DBCE), obtained from AccuStandard. Pesticide residue or HRGC grade solvents (Burdick and Jackson) were obtained from VWR (West Chester, PA) as well as sodium sulfate, anhydrous granular reagent grade. Other chemicals used were GPC Calibration Standard Solution from Ultra Scientific and Florisil, 60/100 mesh, PR grade obtained from Floridin Corp (Berkeley Spring, WV).

Sample extracts were analyzed using dual column high resolution gas chromatography (GC) with electron capture detectors (GC/ECD). An Agilent 6890 GC equipped with two ^{63}Ni micro electron capture detectors, an autosampler and two (DB5 and DB17MS) 60 m length, 0.25 mm I.D., 0.25 μm film thickness fused silica GC columns (J&W Scientific) was used. A splitless injection using electronic pressure control (EPC) was used. The injector and detectors were operated at 240 and 310°C, respectively. The GC oven conditions were as follows: initial temperature 80°C, hold for 1 min, ramp at 15°C degrees/min to 210°C C and hold for 10 min, ramp at 2°C/min to 290°C C and hold for 14 min. The carrier gas was helium with a flow rate of 1 mL/min and the makeup gas was nitrogen with a flow rate of 30 mL/min. each. The final injection volume was 3 μL. Target analyte confirmation was done by gas chromatography-tandem mass spectrometry with an ion trap detector (GC/MS/MS-ITD).

Sediment and tissue samples were extracted by automated Pressurized Fluid Extraction (PFE) with a Dionex Accelerated Solvent Extractor (ASE 200, Salt Lake City, UT) equipped with 33 mL extraction cells and cellulose filters. Cleanup of extracts was accomplished using automated Gel Permeation (size exclusion) Chromatography (GPC), (J2 Scientific, AccuPrep 170, Columbia, MO) equipped. The GPC column was an OI Analytical (ABC model), glass, 46 cm length × 2.5 cm I.D. column packed with 65 g BioBeads of 200–400 mesh size. The mobile phase was DCM at a flow rate of 5 mL/min. The GPC program was as follows: dump for 19 min, collect for 24 min, and then rinse for 10 min. The sample size was a 10 mL DCM extract using a 5 mL sample loop.

Tissue samples are homogenized using a Bucchi B-400 mixer equipped with a titanium knife assembly. Sediment samples may be homogenized using a Polytron homogenizer equipped with stainless steel generator equipped

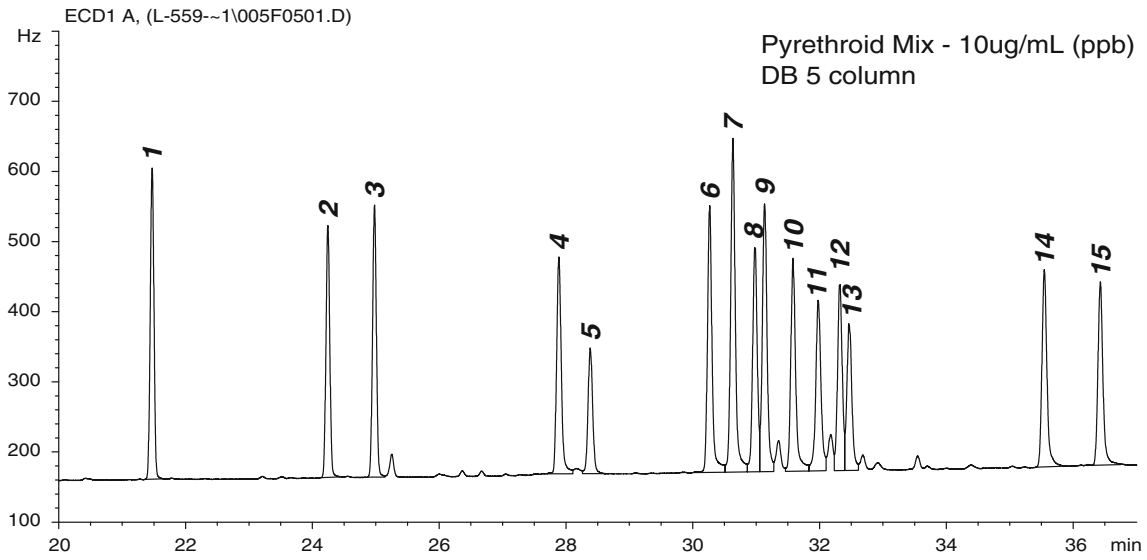

Fig. 1 Typical elution pattern of a GC-ECD chromatogram of a pyrethroid standard mixture on a DB-5 capillary column: ***1***-Bifenthrin, ***2***-lambda-Cyhalothrin-1, ***3***-lambda-Cyhalothrin-2, ***4***-Permethrin-1, ***5***-Permethrin-2, ***6***-Cyfluthrin-1, ***7***-Cyfluthrin-2, ***8***-Cyfluthrin-3, ***9***-Cyfluthrin-4, ***10***-Cypermethrin-1, ***11***-Cypermethrin-2, ***12***-Cypermethrin-3, ***13***-Cypermethrin-4, ***14***-Esfenvalerate-1, ***15***-Esfenvalerate-2

with Teflon bearings. Concentration of samples used a solvent evaporator, Organomation Assoc. Inc. (Berlin, MA).

Individual stock solutions of each pyrethroid analyte were made in acetone from neat and combined resulting in a stock mixture containing the six compounds and diluted to two parts per million (ppm) bifenthrin, es/fenvalerate and lambda-cyhalothrin, 4 ppm cyfluthrin and cypermethrin and 5 ppm permethrin. From this mixture, a matrix spiking solution used to fortify samples was made containing pyrethroid concentrations ranging from 20 to 50 ppb in acetone. The pyrethroid stock mixture was also used to make an instrument calibration mixture in iso-octane, ranging from 0.5 to 100 ppb. Figure 1 shows the response and elution pattern for a 10 ppb standard. Since individual isomer standards are not currently available, results are reported as total concentration for each analyte rather than as individual stereoisomer concentrations. External standard calibration was used to quantify the pyrethroids.

Water samples are collected in one liter amber glass bottles and iced or refrigerated at 4°C and are extracted as soon as possible. One study shows bifenthrin and permethrin degrade up to 41% within 3 days (Lee 2002). Sediment samples are collected in wide-mouth glass jars and must be frozen if not extracted within 14 days. Fish should be double wrapped in aluminum foil and immediately frozen with dry ice in a covered ice chest. Following dissection and homogenization, tissue samples are re-frozen if not extracted within 14 days.

Deionized water was used for initial method development and unfiltered American River water was used for method validation. All water samples (1 L) were fortified with pyrethroid pesticide spiking solution and DBOB surrogate directly into the sample bottles. The fortified water samples were manually agitated before transferring samples to 2 L separatory funnels which were extracted twice with 120 mL of dichloromethane (DCM) (original sample bottles were extracted first) using liquid–liquid technique. The extracts were dried using sodium sulfate and evaporated to approximately 2–5 mL using K-D glassware. The sample was then solvent exchanged by adding petroleum ether (PE) (~50 mL three times). The extracts were evaporated to 5 mL and further concentrated to less than 1 mL and brought to a final volume of 2 mL with iso-octane for GC-ECD analysis.

Frozen homogenized sediment or tissue samples were removed from the freezer and allowed to thaw. Excess water was decanted from the sediment container. Sediment samples were thoroughly mixed by hand using a clean glass rod. A 10 g sample of homogenized sediment or tissue was mixed with 7 g of pre-extracted Hydromatrix® (Varian Corp) in a 250 mL wide mouth jar. The mixture was then poured into a 33 mL stainless steel Dionex Accelerated Solvent Extractor (ASE 200) cell. A surrogate standard solution (20 µg/L DBCE in acetone) was then added to the extractor cell, which was then extracted twice using a solvent mixture of acetone/DCM (1:1, v/v) at 100°C and 1,500 psi. The extracts were combined, dried using sodium sulfate, evaporated to approximately 0.5 mL and diluted to 10 mL with DCM. The extracts were then cleaned by GPC. The cleaned extracts were solvent exchanged from DCM to PE. The extracts were then fractionated using 25 g of Florisil (60/100 mesh) in an 11 mm × 300 mm column equipped with a 250 mL reservoir. The Florisil column was eluted with PE (fraction 1,

no target analytes elute in this fraction) followed by 50% diethyl ether/PE (fraction 2, all pyrethroids elute in this fraction). The fractions were then concentrated and reconstituted with isooctane to a final volume of 2 ml for GC-ECD analysis.

Results and Discussion

The primary goals of this paper are to develop, test and validate this method for analysis of six or more pyrethroid insecticides in water, sediment and biota.

Initially the method was tested using several replicates of deionized water fortified at five times the method detection level (MDL). The mean recovery was 80–100% for all pyrethroids tested following the extraction and analytical procedure discussed in this paper.

Surface water commonly differs from DI water in that the former contains certain amounts of suspended solids (Lee et al. 2002). Since our facility is within walking distance to the American River, the method was validated using fortified American River water. River water samples, collected from control sites, were determined in advance to be pesticide free. Unspiked control site samples were extracted and analyzed with each set of fortified samples to ensure there was no background contribution to pyrethroid recoveries. Replicate water samples were fortified at 1xMDL level (n = 16), 2×MDL level (n = 7) and 10×MDL level (n = 8), extracted and analyzed. Recoveries ranged from 92.5 to 103% for trace level (1×MDL), from 88.1 to 103% for 2×MDL and from 76.5 to 83.8% for 10×MDL (Table 1). There were no residues of pyrethroids found in the unspiked control samples. Detection limits were calculated using USEPA procedures found in Title 40 Code of Federal Regulations Part 136 (40CFR 136, Appendix B, revision 1.11). Overall, the recoveries were excellent; however, the mean recovery slightly decreased as the pyrethroid concentration increased in the water.

As a result of the findings in Table 1, we decided to evaluate the method with environmental water containing higher levels of sediment and presumably, higher total organic carbon (TOC) content. Sediment (0.5 g) was added to several replicates of American River water samples. The samples were fortified with increasing amounts of pyrethroids. The sample bottles were then agitated and left to rest for 1–2 h before extraction. After transferring the water samples to the 2 L separatory funnels, the sample bottles were rinsed with DCM and the rinsate was added to the separatory funnels. The mean recovery for low level (10×MDL) was 80.2–88.6%. Mean recoveries for 100×MDL and 500×MDL were 68.3–96.6% and 64.9–100%, respectively. The higher level (1000×MDL) resulted in recoveries from 60 to 87.9%. Overall, the percent recoveries were good but the study showed that the concentration of pyrethroids increased in the sample with high TOC, the bifenthrin recovery decreased, as shown in Table 2.

The question was posed: what happens if the particulate matter did not get extracted as part of the total sample? The fortified samples were agitated and the samples were decanted into separatory funnels leaving the solid particulates

Table 1 Method validation results for pyrethroids in American River water

Spike level		1 × MDL		2 × MDL		10 × MDL	
Pyrethroids by GC/ECD	MDL (µg/L)	Mean %Rec	%RSD n = 16	Mean %Rec	%RSD n = 7	Mean %Rec	%RSD n = 8
Bifenthrin	0.001	95.8	12.0	88.1	17.7	82.0	9.86
Cyfluthrin	0.002	96.5	13.4	103	13.3	83.8	7.62
Cypermethrin	0.002	96.6	15.4	101	8.64	83.0	9.33
Es/Fenvalerate	0.001	103	12.6	101	13.6	78.2	8.68
Lambda-cyhalothrin	0.001	99.4	12.2	96.2	13.3	76.5	7.30
Permethrin	0.003	92.5	11.8	102	5.64	83.7	8.35

Table 2 American River water containing high levels of suspended solids with increasing amounts of pyrethroids

Spike level	10 × MDL		100 × MDL		500 × MDL		1000 × MDL	
Pyrethroids by GC/ECD	Mean %Rec	%RSD n = 4	Mean %Rec	%RSD n = 4	Mean %Rec	%RSD n = 4	Mean %Rec	%RSD n = 4
Bifenthrin	84.5	10.3	68.3	5.21	64.9	3.14	60.0	4.09
Cyfluthrin	85.0	8.64	96.6	3.12	100	1.92	87.9	4.09
Cypermethrin	88.6	4.80	88.1	3.22	93.2	1.71	84.8	3.48
Es/Fenvalerate	82.3	7.55	89.0	5.05	94.0	3.44	79.8	4.38
Lambda-cyhalothrin	80.2	7.55	93.4	2.83	94.5	1.06	74.7	4.80
Permethrin	87.9	8.38	83.3	11.6	75.7	1.03	79.9	3.02

Table 3 Comparison of pyrethroid recovery from total extraction (A) (water plus suspended solids) vs. water only extracted (B), n = 4 for all treatments

Spike level	10 × MDL		100 × MDL		500 × MDL		1000 × MDL	
Pyrethroids by GC/ECD	A Mean % Rec	B Mean % Rec	A Mean % Rec	B Mean % Rec	A Mean % Rec	B Mean % Rec	A Mean % Rec	B Mean % Rec
Bifenthrin	84.5	79.4	68.3	67.3	83.7	52.4	61.7	34.2
Cyfluthrin	85.0	82.6	96.6	84.7	93.9	57.0	98.3	32.8
Cypermethrin	88.6	77.4	88.1	79.1	91.9	56.6	97.0	25.4
Es/Fenvalerate	82.3	74.1	89.0	80.2	87.2	50.4	91.3	23.6
Lambda-cyhalothrin	80.2	72.9	93.4	73.5	81.3	55.7	79.8	32.4
Permethrin	87.9	79.6	83.3	77.8	88.5	60.9	87.4	40.1

Table 4 Method validation results for pyrethroids in American River sediment

Spike level		1 × MDL		2 × MDL		4 × MDL		10 × MDL	
Pyrethroids by GC/ECD	MDL ng/g dry wt	Mean %Rec	%RSD n = 8	Mean %Rec	%RSD n = 7	Mean %Rec	%RSD n = 7	Mean %Rec	%RSD n = 7
Bifenthrin	0.50	106	2.70	96.4	4.89	99.2	8.58	96.7	5.74
Cyfluthrin	2.00	108	8.77	92.5	14.7	91.1	10.6	84.2	8.31
Cypermethrin	2.00	108	7.54	97.7	12.3	95.4	16.1	86.8	14.8
Es/Fenvalerate	1.00	107	5.83	84.5	13.5	102	8.43	87.5	8.99
Lambda-cyhalothrin	1.00	104	6.22	90.8	11.6	100	13.6	87.8	7.99
Permethrin	4.00	99.0	15.1	99.5	13.3	83.8	14.1	98.3	12.9

at the bottom of the bottle behind. The results showed significant decreases in the recovery for all compounds (Table 3). This was very noticeable in 1,000×MDL.

The water study results showed that pyrethroids absorb to particulates due to their extremely hydrophobic characteristics. For this reason, the second phase of this study focused on sediments. Sediment method validation was done on fortified sediments from three sites that were previously tested to be pesticide free: Sacramento River, San Mateo Creek and American River. The sediment samples were extracted according to procedures detailed above. Mean percent recoveries of fortified sediments ranged from 83.8% to 108% with relative standard deviation (RSD) values <16.1% for all target compounds at the levels listed in Table 4.

Homogenized Rainbow Trout tissue from the California Department of Fish and Game's American River Hatchery (eight replicates) were fortified at the MDL level and subjected to same extraction and analysis method as sediment samples. Mean percent recoveries ranged from 74.3 to 98.7%, except for the esfenvalerate/fenvalerate recovery which was 52.8% (Table 5).

Table 5 Method validation results for pyrethroids in American River Hatchery rainbow trout

Spike level		1 × MDL	
Pyrethroids by GC/ECD	MDL (ng/g, fresh weight)	Mean %Rec	%RSD n = 8
Bifenthrin	1.00	88.1	5.89
Cyfluthrin	3.00	98.7	5.48
Cypermethrin	2.00	74.3	8.89
Es/ Fenvalerate	2.00	52.8	25.3
Lambda-cyhalothrin	2.00	89.1	13.3
Permethrin	3.00	97.2	6.07

Table 6 Results of additional synthetic pyrethroids utilizing these methods

Extraction method	Liquid/Liquid		PFE	
Pyrethroids by GC/ECD	Mean %Rec	%RSD n = 3	Mean %Rec	%RSD n = 3
Allethrin	105	5.57	112	11.3
Deltamethrin	92.3	7.04	76.9	8.84
Fenpropathrin	97.4	5.11	85.0	3.40
Flucythrinate	85.3	3.11	109	3.71
Phenothrin	90.5	4.82	92.6	14.0
Prallethrin	91.4	4.47	109	10.1
Tetramethin	114	1.75	118	9.07
Tralomethrin*	50.3	12.7	93.0	1.56

* Tralomethrin is transformed into deltamethrin in the injector port of the GC system

These extraction and analysis methods have been used extensively and successfully by our laboratory for surface water and sediment monitoring programs throughout California for the past 2 years. We have recently extended the method to include additional synthetic pyrethroid pesticides (Table 6). Future research is recommended to attempt to decrease reporting limits and reduce matrix interferences, especially for sediment samples.

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