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Effective liquid–liquid extraction method for analysis of pyrethroid and phenylpyrazole pesticides in emulsion-prone surface water samples

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ABSTRACT

The distribution of pyrethroid and phenylpyrazole pesticides in the water environment has raised public concerns because of their potential risks to ecosystem and human health. However, co-extraction of emulsifier type compounds (by liquid–liquid extraction, LLE) present in environmental samples can present a challenge for quantifying typically low concentrations of pesticides. Several methods were evaluated for breaking emulsions in problematic environmental surface water samples extracted by LLE using methylene chloride. Target pesticides included 11 typical pyrethroid and phenylpyrazole pesticides commonly used in agricultural and landscape insect pest control. The most effective method was selected for validation in fortification studies with GC-ECD analysis. The average recoveries of spiked pyrethroid and phenylpyrazole pesticides were 88.2–123.4% for water samples with moderate emulsions and 93.0–117.4% for water samples with severe emulsions. Recoveries of the pesticides ranged 81.0–126.4% (water samples with moderate emulsions) and 95.9–110.6% (water samples with severe emulsions) for lowest fortification level (5–20 ng L^{-1}), 88.2–123.4% (water samples with moderate emulsions) and 93.0–117.4% (water samples with severe emulsions) for middle fortification level (10–40 ng L^{-1}), and 90.2–119.9% (water samples with moderate emulsions) and 91.2–105.9% (water samples with severe emulsions) for highest fortification level (50–200 ng L^{-1}). Relative standard deviations of pesticide recoveries were usually <10%. Results indicate that this method is a robust and reproducible option for LLE of pyrethroid and phenylpyrazole pesticides from emulsion-prone surface water samples.

1. Introduction

In recent years, a wide variety of pesticides have been used in agriculture and landscape maintenance for controlling insect, bacterial, and fungal pests, and for reducing competition from weeds. Although the soil is often the principal sink for these environmental pollutants, some pesticide residues and corresponding metabolites can be transported into surface water bodies through storm water drainage [1], and runoff and leaching processes [2,3] depending on the properties of the chemical and environmental conditions. Transport into and the presence of pesticide residues in non-target water sources is a public concern due to the possibility of causing adverse impacts to non-target environmental resources and human health. Monitoring for pesticide residues in surface water bodies is necessary to evaluate potential exposures of non-target aquatic organisms [4,5]. Due to the high toxicity and tendency to accumulate in living organisms, organophosphate, organonitrogen, and organochlorine pesticides have increasingly been replaced by pyrethroid and phenylpyrazole pesticides. Advantages of these pesticide families include: lower mammalian toxicity, selective insecticidal activity, and lower environmental persistence [6–10]. Pyrethroids, with structures typically containing 2–3 asymmetric carbon atoms (chiral centers), are synthetic insecticides originally derived from pyrethrins that are produced by certain species of chrysanthemum [6,11]. The phenylpyrazoles constitute a newly developed class of chemicals with insecticidal and herbicidal properties [12,13]. A common phenylpyrazole insecticide is fipronil, which can be transformed into the relatively toxic metabolites fipronil sulfide and fipronil sulfone [14,15]. Previous studies have shown that some pyrethroids, including permethrin and bifenthrin, are possible human carcinogens [16,17]. Risks to ecosystems are uncertain for the phenylpyrazoles due to their recent introduction [9]. However, many of these compounds have been detected in non-target surface water systems [6,9], and in many cases have been found to be responsible for negative impacts on aquatic resources such as macroinvertebrate communities [9]. Due to their widespread usage and chemical properties, occurrence of many of the pyrethroids and

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phenylpyrazoles in different non-target environments is expected [14,18,19].

With high hydrophobicity and low water solubility, pyrethroid and phenylpyrazole pesticides are easily adsorbed to sediment particles, resulting in typically low concentrations present in water [6,9]. The low concentrations of these pesticides subsequently increase the difficulty in monitoring them in water samples due to the high sensitivity required. To make reliable risk assessments for these pesticides, effective and convenient water sample pre-treatment methods for monitoring them in water samples are needed. Several pre-treatment approaches including liquid–liquid extraction (LLE), solid-phase extraction (SPE), and solid-phase microextraction (SPME) for monitoring pesticides in water have been widely used [20–28]. Despite its disadvantage of requiring large volumes of organic solvents and having poor potential of automation [29,30], liquid–liquid extraction (LLE) methods are the most widely used for whole water extractions [20–22] due to fast and effective separation of two phases and satisfactory extraction results. However, frequent occurrence of emulsions is a significant obstacle for the wide application of LLE [31,32], especially when working with environmental water samples. Emulsions occur when the aqueous and organic solvent phases disperse throughout one another as microscopic/macroscopic droplets, preventing the clear phase separation needed for efficient LLE. While it is impossible to form stable emulsions with two pure liquids, addition of emulsifying agents that break the surface tension of the two phases promotes emulsion formation. Unfortunately, water samples collected under natural or anthropogenically impacted environmental conditions often contain a variety of dissolved compounds (i.e. proteins, natural surfactants, synthetic surfactants from pesticide applications, organic acids, etc.) that may act as emulsifiers, complicating LLE extraction procedures. Adoption of methods for routinely, effectively, and repeatably breaking emulsions is needed for efficiently extracting and analyzing trace concentrations of pesticides in emulsion-prone water samples. Several methods have been previously described for LLE of many different compounds in applications ranging from peptide titrations to fruit, bovine milk and tissue analysis, and others. These methods include: centrifugation [33,34], adding sodium chloride [35,36], adding ammonium sulfate [37], adding organic solvent [38], adding acid [39], and mechanical filtration [40]. These methods may not be applicable under all situations due to analyte interactions or differing emulsion properties (i.e. resistance to breaking). Studies are needed to evaluate potential impacts on analyte recoveries and effectiveness of breaking emulsions in emulsion-prone environmental samples.

The objective of this study was to identify an effective emulsion-breaking technique for emulsion-prone water sources in support of developing a reliable method for analysis of selected pyrethroid and phenylpyrazole insecticides in native surface water samples.

2. Methods and materials

2.1. Standards, reagents, and chemicals

Eleven pyrethroids and phenylpyrazoles were chosen as target compounds for evaluation based on previous studies [9,22,28,41]. The selected pesticides and stated manufacturer purities included: cyfluthrin (also called baythroid; mixture of isomers I, II, III, and IV, purity 98%), deltamethrin (mixture of isomers, purity 99.0%), cis-permethrin (purity 99.5%), trans-permethrin (purity 91.8%), bifenthrin (purity 99.0%), lambda-cyhalothrin (purity 99.1%), esfenvalerate (purity 99.5%), fenvalerate (purity 99.2%), fipronil (purity 99%) and two of its metabolites, fipronil sulfide (purity 95%) and fipronil sulfone (purity 98.2%). As described in You and Lydy (2007), several of the pyrethroid pesticides may isomerize during extraction and analysis [41]. Results are reported for individual isomers of cyfluthrin. Results for cyhalothrin and deltamethrin are only reported for the primary peak. In the case of cyhalothrin, the first resolvable isomer was typically less than 2% of the primary peak area, and was too low to accurately quantify. The other isomers were likely unresolved within the primary peak. Likewise, the minor isomer for deltamethrin was always $\leq$2% of the primary peak, and was too low to accurately quantify. Standards were purchased from Chem Service (West Chester, PA, USA). Pesticides were individually dissolved in methyl tertiary butyl ether (MTBE) at concentrations ranging from 250 to 1000 mg L^{-1} based on analytical instrument sensitivities. Standards containing a mixture of all of the individual compounds were made by mixing appropriate amounts of each individual pesticide. The surrogate for water samples was 4,4′-dibromooctafluorobiphenyl (purity 98%), purchased from Sigma-Aldrich (St. Louis, MO, USA). Post-extraction surrogates included decachlorobiphenyl (neat chemical, purity 99%) and 1-bromo-2-nitrobenzene (neat chemical, purity 99%); and were purchased from Supelco (Bellefonte, PA, USA) and Restek (Bellefonte, PA, USA), respectively. All solvents and other reagents used were of American Chemical Society (ACS) grade or higher.

2.2. Sampling procedure

Native water samples were collected from two residential ponds (W-1 and W-2) located within the Indian River Lagoon watershed (Saint Lucie County, FL, USA). From previous experience, LLE of water samples from these two sites were always problematic due to the formation of excessive amounts of emulsions. Samples were collected by submerging amber glass bottles (1 L) below the water surface to a depth of 0.76 m. All water samples were held on ice until transported back to the laboratory where they were stored in refrigerator at 4 °C. Extraction of water samples occurred within 24 h of collection.

2.3. Extraction procedure

A liquid–liquid extraction method was used to extract pesticides from water samples. A brief description of the LLE and cleanup procedures follows. Samples were moved from the refrigerator and allowed to reach room temperature before extraction. The entire 1-L sample was next poured into a pre-cleaned 2-L Teflon separatory funnel. The sample bottle was rinsed twice with 30 mL nanopure water each time, with addition of the rinsates into the separatory funnel. Immediately 40 μL of the 0.25 mg L^{-1} 4,4′-dibromooctafluorobiphenyl surrogate solution was added to each sample. Additionally, 40 μL of pyrethroid mix (0.25–1.0 mg L^{-1}) were added to selected samples (from W-1) as matrix spikes (MS) and matrix spike duplicates (MSD). Each analysis batch always included a method blank, instrument blank, quality control check standard from a second source, MS, and MSD. Once all of the surrogates and QC additions were made, 60 mL of methylene chloride (MeCl) was added to the separatory funnel, followed by shaking for 20 min using a Glas-Col Bench Top shaker (S60012 Model, Glas-Col, Terre Haute, IN, USA). Following shaking, funnels were placed on a stationary stand and the phases were allowed to separate. Once phase separation was achieved (as much as possible), the methylene chloride extracts were collected into a 250 mL flask and the emulsions formed were collected into a safety-coated clear wide-mouth jar (7 cm in diameter, 250 mL), which was immediately closed. This extraction procedure was repeated twice more, combining the extracts and emulsions in their respective containers.

2.4. Emulsion-breaking procedure

Emulsions always occurred during LLE of water samples from both sites. For this study, emulsion severity was quantitatively described based on the volume of methylene chloride recovered following the extraction procedure. Typically, the emulsion fraction accounted for 20 to 100 percent of the total volume of methylene chloride recovered during the extraction process. Several of the previously-mentioned methods [33–40] were evaluated for their effectiveness at breaking the emulsion and facilitating phase separation. These methods included centrifugation at 5000 rpm for 30 min; addition of 3–5 g NaCl; addition of 3–5 g $(NH_4)_2SO_4$; addition of 1 mL tetrahydrofuran; or addition of 3–5 drops of H_2SO_4 (96%, p.a.) into the emulsions; cooling of the emulsions in the refrigerator (4 °C) for 30 min; and mechanical filtration by vacuum and pressure driven methods.

Mechanical phase separation by filtration was evaluated using two different methods. The primary difference between these two methods was that one used an open system filtering by vacuum; whereas the other method used a pressure-driven syringe filter. For vacuum filtration, the emulsion extract fractions were poured onto an open vacuum filtration apparatus. For the pressure-driven filtration method, the emulsion extract fractions were filtered into a secondary 250 mL Teflon separatory funnel using a glass syringe and Whatman binder-free glass microfiber filter (GF/D, pore size: 2.7 μm, Fisher Scientific, Pittsburgh, PA, USA) placed within a Teflon filter holder. After filtration, the extract was well separated into an aqueous phase and organic solvent phase without emulsion in the secondary funnel. The organic solvent extracts from the secondary funnel were then combined with the MeCl phase from the initial extractions, which were then transferred into a flask containing 2–3 pieces of Teflon boiling stones.

2.5. Clean-up and post-treatment procedure

After breaking the emulsions, the flasks containing the combined (former emulsion + extract) methylene chloride phase was placed in a water bath set at 60 °C; where the methylene chloride was evaporated until the extracts were totally dry. Next, approximately 6 mL of MTBE was added into the flask to rinse the interior surface and redissolve the extracted pesticides. The MTBE-reconstituted extract was next passed through a Florisil cartridge (6 mL/1000 mg, Thermo Scientific, Waltham, MA, USA) by gravity. The florisil cartridge was pre-washed with 6 mL of MTBE. The extract was collected into a 25 mL glass concentrating tube (Kontes Glass Co., Vineland, NJ, USA). This procedure was repeated twice more until the apparent volume was about 16–18 mL. The extracts were next concentrated to less than 1 mL using a RapidVap system (Model 79000-02, Labconco Corporation, Kansas City, MO, USA). The post-extraction surrogates (40 μL of 0.25 mg L^{-1} 1-bromo-2-nitrobenzene and 40 μL of 0.25 mg L^{-1} decachlorobiphenyl) were next added to each extract, followed by addition of MTBE to a final volume of exactly 1 mL. The finished extract was then transferred to a 2 mL amber glass GC vial for analysis.

2.6. Instrumental analysis

A Hewlett Packard 5890 Series II gas chromatograph (Agilent Technologies, Santa Clara, CA, US) equipped with dual electron capture detectors was used for the analysis of pyrethroid/phenylpyrazole pesticides. Compounds were quantified using external standards. An Rxi-5ms (Restek, Bellefonte, PA, USA) (30 m × 0.25 mm i.d.) and a SGEPX350-25 (SGE Incorporated, Austin, TX, USA) capillary column (30 m × 0.25 mm i.d.) was used with helium as carrier gas at constant flow of 1 mL min^{-1}. The two columns were connected to a Siltek™-treated, glass capillary y-splitter (Restek, Bellefonte, PA, USA) to enable dual column confirmation from a single injection. The injector and detector temperatures were 225 °C and 300 °C, respectively. The oven temperature program was as follows: initial temperature 80 °C, hold for 2 min; increase to 180 °C at 9 °C min^{-1}, hold for 2 min; increase to 200 °C at 10 °C min^{-1}, followed by a 1 min hold; and finally increase to 270 °C at 9 °C min^{-1}, followed by a 34 min hold time. Total run time was 59.89 min. For confirmation, a compound had to appear on both analytical columns at their corresponding retention times (±0.2 min).

Characteristics of water samples was determined as follows. Water pH and electrical conductivity (EC) were measured in the field using a YSI 650 Multiparameter Display System (YSI Incorporated, Yellow Springs, Ohio, USA). Turbidity was measured using a DRT-100B turbidity meter (HF Scientific Inc., Fort Myers, FL, USA). Total organic carbon (TOC) and total nitrogen of water samples were determined using a C/N analyzer (Vario MAX CN Macro Elemental Analyzer, Elemental Elementar Analysensysteme GmbH, Hanau, Germany) and EPA Methods 415.1, 351.2, and 353.2 [43–45].

2.7. Determination of method recovery performance

Once the most effective emulsion-breaking technique was identified, sample fortification studies were conducted to evaluate the potential impacts on recoveries and repeatability for each analyte. Initially, water samples from each site were spiked at concentrations of 10 ng L^{-1} for fipronil, fipronil sulfide and fipronil sulfone, 20 ng L^{-1} for bifenthrin, lambda-cyhalothrin, cis-permethrin, trans-permethrin, and esfenvalerate, and 40 ng L^{-1} for cyfluthrin, fenvalerate, and deltamethrin. Following fortification, water samples were incubated under ambient lab conditions for approximately 1 h, extracted, and analyzed following the above-mentioned procedures with 6 replicates.

From previous experience, it was observed that the degree of emulsion formation at these two sites also historically increased in samples collected during and shortly following rainfall events (data not shown). To further evaluate the identified emulsion-breaking method, water samples were also collected from the W-1 site 1 h after a rainfall event occurred (1.2 cm) [42]. In this case a total of 10 samples were collected, including two for background measurement, two for MS and MSD spiking, and six for evaluation of the emulsion-breaking method. These samples were extracted and analyzed as previously described to evaluate the robustness of the selected method. The method was further validated by fortifying, extracting, and analyzing 6 replicate samples at 0.5× and 5× the concentrations previously noted.

2.8. Statistical analysis and calculations

Standard errors were calculated for the EC, turbidity, total-N, and TOC measurements. In addition, differences between sites were determined by analysis of variance (ANOVA) with calculation of least significant differences (LSD, $P = 0.05$). The variability in analyte recoveries was measured as the percent relative standard deviation. Method detection limits (MDL) were determined by multiplying the standard deviation of 11 replicate spiked samples by the Student's t-value from statistical tables for 99% confidence level at $(n-1)$ degrees of freedom [46,47]. The method reporting limits (MRL) were established at 4× the MDL [46].

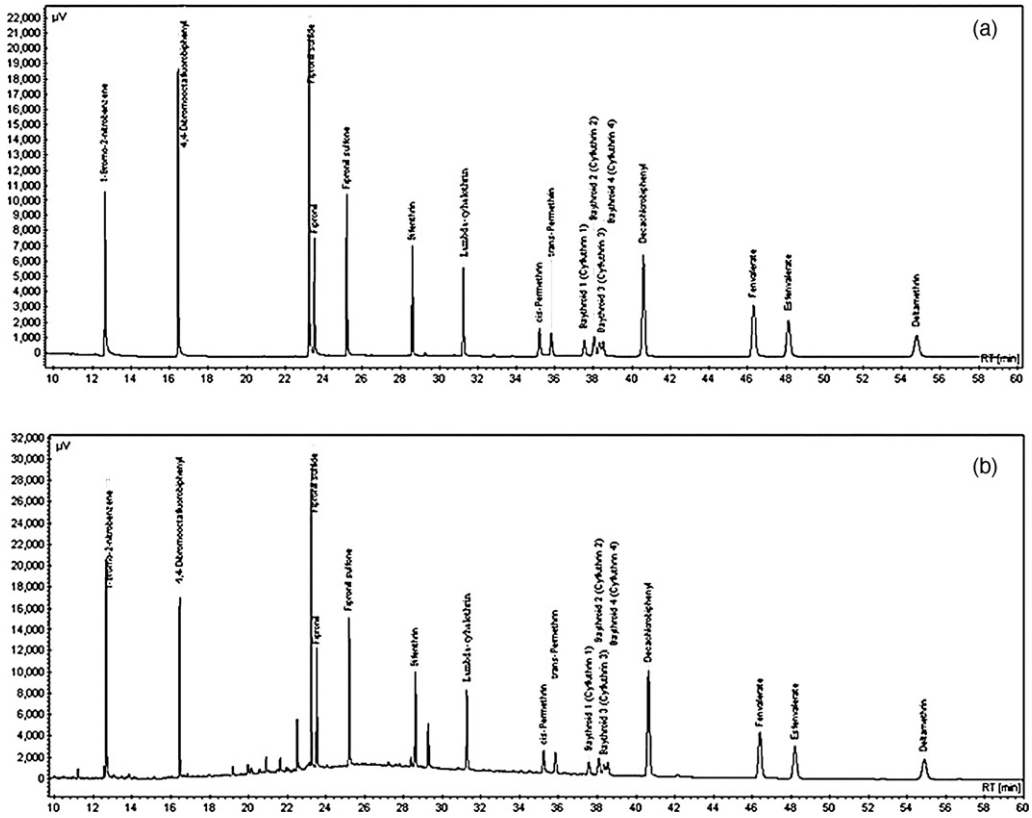

Fig. 1. GC-ECD chromatogram of pyrethroids and phenylpyrazoles (column: SGEPX350-25 capillary column). (a) Analytical standard GC-ECD chromatogram of pyrethroids and phenylpyrazoles; (b) example of GC-ECD chromatogram of pyrethroids and phenylpyrazoles spiked in water sample collected from W-1. Liquid–liquid extraction with filtration for breaking emulsion was employed.

3. Results

3.1. Physical characteristics of water sample and emulsion-breaking performances

A summary of the water characteristics is listed in Table 1. Water from both sites differed significantly in pH, EC, turbidity, total-N, and total organic carbon (Table 1). The largest differences were for EC and turbidity, where each at the W-2 site was 2 and 1.6× that measured at the W-1 site, respectively. Following extraction, samples collected from W-1 showed a moderate degree of emulsion formation, while samples collected from W-2 showed severe emulsion formation. The degree of emulsion formation is described as the percentage of methylene chloride recovered during the extractions (i.e. less MeCl recovered as emulsion formation increases). Moderate emulsions accounted for 20–50% of the MeCl used in the extractions; whereas severe emulsions accounted for 51–100% of the MeCl added. Based on experience, these types of emulsions are commonly encountered for surface waters collected in canals and lakes, especially darker-colored ones and those with abundant floating aquatic plants. Without an effective emulsion-breaking procedure, accurate and repeatable analysis of these pesticides by LLE is impossible.

None of the chemical-based (including centrifugation; addition of sodium chloride, ammonium sulfate, H_2SO_4, and tetrahydrofuran; or cooling) or cooling emulsion breaking approaches evaluated were effective for breaking these moderate and severe emulsions, contrary to previous reports [33–39]. However, the filtration methods, particularly pressurized filtration, proved to be a very effective method for addressing this problem. Filtration by vacuum had the disadvantages of using a bulky filtering pump and apparatus, and the immediate freezing of the emulsion making this method tedious. On the other hand, pressure-driven filtration using the glass syringe was very effective with few disadvantages. The emulsion mixture could be filtered within 1 min without any loss using the binder-free glass microfiber filter placed within the Teflon filter holder. Pressure-driven filtration was the most effective and efficient method for breaking the emulsions.

3.2. Method recovery performance

The following results focus only on the method performance using pressurized filtration as the only method for breaking emulsions. A sample chromatogram (peaks of individual pesticides were symmetrical and well separated) and summary of the pyrethroids and phenylpyrazoles recoveries ($n = 6$) are shown in Figs. 1 and 2. Except for cis-permethrin, whose average recovery was 88.2%, the average recoveries of the other pesticides ranged from 101.1% to 123.4% for water samples collected from W-1. Furthermore, with the exception of trans-permethrin, the %RSD values for the other pyrethroids were less than 10%, indicating good reproducibility for each compound in samples with moderate emulsions. Percent recoveries for the pesticides in fortified samples with severe emulsion formation (from the W-2 site) were similar to those from the W-1 site. Except for lambda-cyhalothrin (average percent recovery = 94.1%) and cis-permethrin (average percent recovery = 93.0%), the average recoveries of the other pesticides ranged from 100.4% to 117.4%. Moreover, with the exception of cyfluthrin 3, the percent RSD values were all less than 10%. These results suggest that filtration is a robust and universal method for breaking emulsions (without reducing recoveries or recovery reproducibility) formed during LLE of these compounds in environmental water samples.

Table 1
Characteristics (±standard error) of water samples.

Site	pH[a]	EC (μS cm^{-1})[a]	Turbidity (NTU)[b]	Total-N (mg L^{-1})[b]	TOC (mg L^{-1})[b]
W-1	7.8 ± 0.06*	473 ± 8*	2.8 ± 0.04*	1.17 ± 0.03*	13.88 ± 0.29*
W-2	6.7 ± 0.03*	953 ± 20*	4.5 ± 0.08*	1.41 ± 0.04*	12.69 ± 0.09*

[a] $n = 6$.
[b] $n = 3$.
* Statistically significant difference between sites, ANOVA ($P = 0.05$).

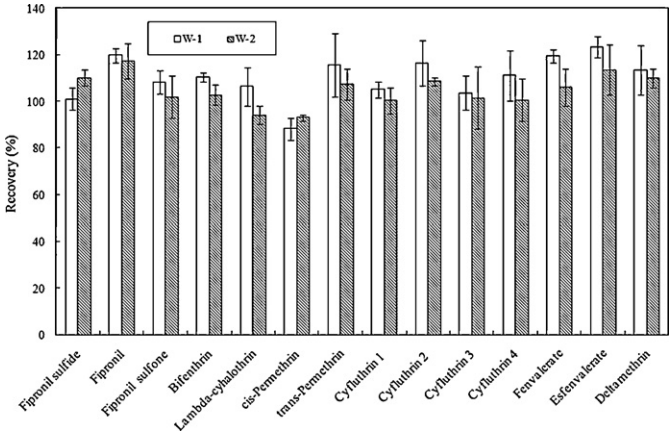

Fig. 2. Percent recoveries (±standard deviation) for pyrethroids and phenylpyrazoles in water samples forming moderate (W-1) and severe (W-2) emulsions ($n = 6$).

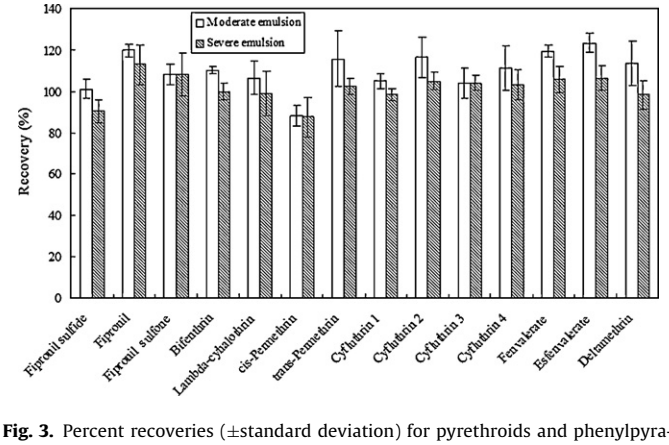

Fig. 3. Percent recoveries (±standard deviation) for pyrethroids and phenylpyrazoles in water samples collected from W-1 with different emulsion degrees before and after a rainfall event ($n = 6$).

Following the rainfall event at W-1, emulsion formation became very severe. However, as illustrated in Fig. 3, the percent recoveries for the pesticides were still high ($n = 6$). Except for cis-permethrin, whose average recovery was 87.5%, the recoveries of the other pesticides ranged from 90.4% to 112.8%. The high recoveries of pyrethroids and phenylpyrazoles observed in all of the samples with emulsions further suggested that the pressurized filtration method was effective without reducing reproducibility or recoveries.

3.3. Method validation

Method accuracy and precision were determined by spiking water samples with three different concentration levels of pyrethroids and phenylpyrazoles. Results for the percent recoveries of target pyrethroids and phenylpyrazoles at the three fortification levels are shown in Fig. 4. The recoveries at the lowest level (spiking level: 5 ng L^{-1} for fipronil, fipronil sulfide and fipronil sulfone, 10 ng L^{-1} for bifenthrin, lambda-cyhalothrin, cis-permethrin, trans-permethrin, and esfenvalerate, and 20 ng L^{-1} for cyfluthrin, fenvalerate, and deltamethrin) ranged from 81.0% (cis-permethrin) to 126.4% (esfenvalerate) for water samples with moderate emulsion formation collected from W-1 and from 95.9% (fipronil sulfone) to 110.6% (cyfluthrin 2) for water samples with severe emulsion formation collected from W-2. Recoveries ranged from 88.2% (cis-permethrin) to 123.4% (esfenvalerate) for water samples collected from W-1 and from 93.0% (cis-permethrin) to 117.4% (fipronil) for water samples collected from W-2 at the middle fortification level (spiking level: 10 ng L^{-1} for fipronil, fipronil sulfide and fipronil sulfone, 20 ng L^{-1} for bifenthrin, lambda-cyhalothrin, cis-permethrin, trans-permethrin, and esfenvalerate, and 40 ng L^{-1} for cyfluthrin, fenvalerate, and deltamethrin), and from 90.2% (cis-permethrin) to 119.9% (fipronil) for water samples collected from W-1 and from 91.2% (cis-permethrin) to 105.9% (fipronil) for water samples collected from W-2 at the highest fortification level (spiking level: 50 ng L^{-1} for fipronil, fipronil sulfide and fipronil sulfone, 100 ng L^{-1} for bifenthrin, lambda-cyhalothrin, cis-permethrin, trans-permethrin, and esfenvalerate, and 200 ng L^{-1} for cyfluthrin, fenvalerate, and deltamethrin). The percent relative standard deviations ($n = 6$) for all of the compounds at all three fortification levels were less than 10% (most <8%), except for trans-permethrin and deltamethrin at the lowest level and trans-

Table 2
Method detection limits (MDLs), method reporting limits (MRLs), and recoveries for pyrethroids and phenylpyrazoles ($n = 11$).

Compound	MDL (ng L^{-1})	Standard error (ng L^{-1})	MRL (ng L^{-1})	Recovery (%)	RSD (%)	Calibration range (μg L^{-1})	Linearity (R^2)
Fipronil sulfide	0.36	0.04	1.42	82.8	1.55	2.5–40	0.9987
Fipronil	0.31	0.03	1.23	115.5	0.96	2.5–40	0.9997
Fipronil sulfone	0.29	0.03	1.16	103.5	1.01	2.5–40	0.9999
Bifenthrin	0.56	0.06	2.26	104.6	0.98	5–80	0.9996
Lambda-cyhalothrin	0.78	0.09	3.13	107.8	1.31	5–80	0.9993
Cis-permethrin	1.07	0.12	4.27	98.5	1.96	5–80	0.9990
Trans-permethrin	0.83	0.09	3.30	106.7	1.40	5–80	0.9996
Cyfluthrin-1	2.25	0.25	8.99	106.8	1.90	10–160	0.9996
Cyfluthrin-2	1.77	0.19	7.06	109.9	1.45	10–160	0.9998
Cyfluthrin-3	2.26	0.25	9.02	107.9	1.89	10–160	0.9999
Cyfluthrin-4	2.29	0.25	9.16	107.9	1.92	10–160	0.9997
Fenvalerate	1.81	0.20	7.23	104.5	1.56	10–160	0.9995
Esfenvalerate	1.28	0.14	5.12	105.6	2.19	5–80	0.9995
Deltamethrin	1.17	0.13	4.70	112.5	0.94	10–160	0.9996

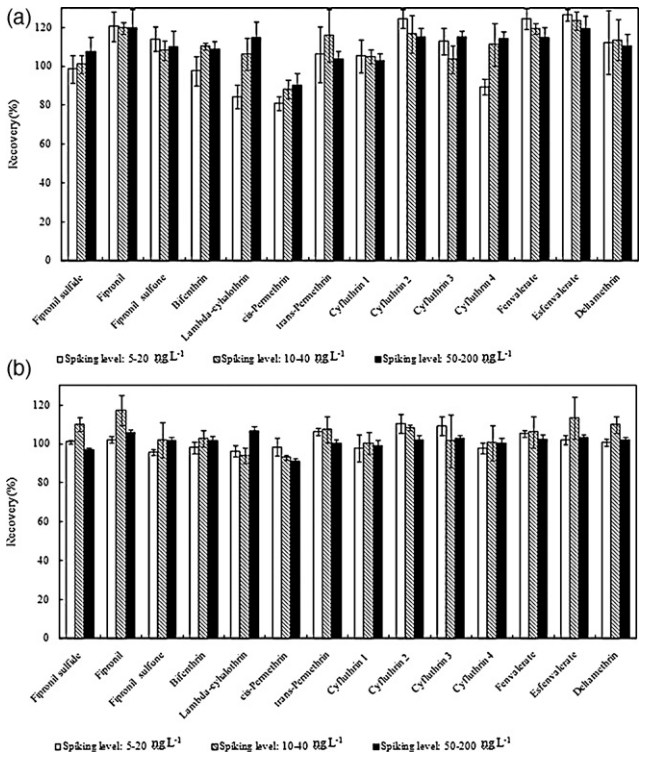

Fig. 4. Percent recoveries (±standard deviation) for pyrethroids and phenylpyrazoles in water samples collected from W-1 (a) and W-2 (b) at three spiking levels (n = 6).

permethrin at the middle level for water samples collected from W-1. These results indicate that the accuracy and reproducibility of this method was very good at all three fortification levels as illustrated 81.0–126.4% recoveries of all analytes and RSDs typically less than 10 percent. These results further illustrate the utility of this LLE-emulsion-breaking-analytical method.

3.4. Method detection limit

The MDLs for individual pesticides are shown in Table 2. MDLs of target pesticides ranged from 0.29 ng L^{-1} to 2.29 ng L^{-1}. The MDL of fipronil was 0.31 ng L^{-1} while that of its metabolite, fipronil sulfone, has the lowest MDL (0.29 ng L^{-1}). Cyfluthrin and fenvalerate had much higher MDLs (>1.70 ng L^{-1}) than that of fipronil. Average recoveries of pesticides ranged from 98.5% to 115.5%, with the exception of fipronil sulfide whose recovery was 82.8%. Each pesticide possessed a relatively low MDL. These low MDLs allow measurement of environmentally relevant concentrations of these pesticides in the environment.

4. Discussion

The robustness of an extraction and analytical method depends on its effectiveness, accuracy, and precision. A good extraction method should yield high recoveries and low MDLs for subject chemicals. Traditionally, LLE methods have offered many of these features, despite several disadvantages including large volume requirements for organic solvent, personnel safety issues, and poor potential of automation [29,30]. From an analytical standpoint, co-extraction of emulsifiers (natural and synthetic) also complicates the extraction procedure, possibly reducing efficiencies and recoveries. Frequent occurrence of emulsions is a significant obstacle for application of LLE with environmental water samples. There are many reported approaches for breaking emulsions formed during LLE procedures, including centrifugation [33,34], adding sodium chloride [35,36], adding ammonium sulfate [37], adding organic solvent [38], and adding acid [39]. However, none of these approaches could successfully break the emulsions formed during LLE procedure of natural environmental water samples with moderate and severe emulsions. While the severity of emulsion formation was not reported in the previous references, the degree of emulsion formation may significantly impact the applicability of the above-mentioned methods which may be more suitable for breaking slight emulsions. Those methods also focused on extractions from specific types of matrices (i.e. wine, bovine milk and tissues, etc.). Within the environment, there are many different potential factors potentially contributing emulsifiers, possibly contributing to the more complex nature of these samples. These diverse factors include: surfactants from pesticide applications in the well maintained surrounding landscapes, proteins from plant and animal degradation, naturally occurring amphipathic biomolecules, exudates from aquatic plants, etc. Given that the only requirement for a candidate emulsifier is to decrease the surface tension of water, any of these factors (and others) could have possibly contributed amphipathic substances capable of acting as emulsifiers. Additionally, extraction solvent may also significantly influence the applicability of these methods for breaking emulsions. Interestingly, the greater emulsion-forming potential in samples from the W-2 site may have been grossly associated with the higher levels of EC, turbidity, and total-N measured at that site. These parameters are often used to characterize water quality impacts.

The most effective and efficient method for breaking emulsions found in these studies was pressurized filtration using a syringe to pass the emulsion through the filter. This emulsion-breaking procedure did not impact the recoveries of the target pyrethroids and phenylpyrazoles, allowing very low concentrations to be detectable. The >100% recoveries were probably due to matrix effects for some of the compounds based on comparisons between nanopure water-spiked samples and matrix spikes. Other biases may also be present within the extraction and analytical systems that contribute to these recoveries. However, the results were always consistent, indicating a fixed bias. For environmental monitoring studies, recoveries between 80% and 120% are widely acceptable because that range represents a compromise between the cumulative errors associated with the spiking experiment, the noises associated with extraction from complex media, and the more precise analytical procedure for a pure substance. The LLE method used for analyzing pesticides in water samples during these experiments employed the optimal clean-up procedures based on experience, providing consistent results. The average percent recoveries of individual pyrethroids and phenylpyrazoles for different water samples were similar to other pretreatment and analytical methods, including SPE/GC-MS [24], SPE/HPLC–MS [25], SPME/GC-μECD [27], SPME/HPLC-FD [28], and SPE/GC-ECD [48]. MDLs of individual pesticides ranged from 0.29 ng L^{-1} to 2.29 ng L^{-1} with high average recoveries of pesticides ranging from 82.8% to 115.5%, indicating that this method is suitable for water sample analysis with low pyrethroid and phenylpyrazole pesticide concentrations. Compared with the related EPA LLE Method [49], this LLE and analytical method simplified the emulsion-breaking procedure using pressure-filtration, hastened the dryness process of MeCl extracts, and simplified solvent exchange procedures. However, some caution must be exercised if accurate quantitation of specific isomers for cyhalothrin and deltamethrin are needed since these were not separately quantifiable using this method.

The results suggest that this improved LLE emulsion-breaking method is a robust and reliable (i.e. high recoveries and reproducibility) method for determining pyrethroids and phenylpyrazoles in water samples at environmentally relevant concentrations. These characteristics may make this method attractive for a wider

range of environmental contaminants in water. It is also important to point out that this emulsion-breaking procedure is simple, easy to conduct, and time saving, making it a laboratory friendly technique.

5. Conclusions

This study evaluated several techniques for breaking emulsions in problematic surface water samples collected in the environment. Pressurized filtration was the only effective method. Fortification studies indicate that the method is accurate (recoveries 81.0–126.4%) and reproducible (%RSD < 10%) for most of the pyrethroid and phenylpyrazole pesticides. This method provides an excellent opportunity for simultaneous determination of pyrethroid and phenylpyrazole pesticides in problematic, emulsion-prone environmental water samples. The analytical method as a whole (extraction and GC-ECD) also produces low detection limits necessary for quantifying environmentally relevant concentrations of these pesticides. This emulsion-breaking procedure is also easy to execute and time saving, making it very laboratory friendly for personnel.

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