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Comparison of cleanup methods for fipronil and its degradation products in sediment extracts

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ABSTRACT

Gel permeation chromatography (GPC) and solid phase extraction (SPE) were compared for cleaning extracts containing fipronil, fipronil-sulfide, and fipronil-sulfone at sub-ppb concentrations in sediment. With both methods, analytes were extracted using accelerated solvent extraction, and analyzed with gas chromatography equipped with an electron capture detector. The GPC was performed with a Waters Envirogel GPC column with dichloromethane as the mobile phase, while SPE was conducted with dual-layer cartridges containing graphitized carbon black and primary and secondary amines with a mixture of acetone and hexane as the eluting solvent. Method detection limits for fipronil, fipronil-sulfide, and fipronil-sulfone from three sediments with varying organic carbon content ranged from 0.12 to 0.52 μg/kg dry weight, while percent recoveries were 72–119% from sediment aged from 0.24 to 14 d. Although both methods were effective at analyzing fipronil and its degradation products, SPE was the less expensive and less labor-intensive method.

1. Introduction

The application of the phenylpyrazole insecticide, fipronil most notably includes Frontline®, Maxforce FC®, and Icon® for the eradication of fleas and ticks, fire ants, and rice pests, respectively. In aquatic environments fipronil sorbs to sediments [1], allowing for potential exposure and toxicity to those organisms that burrow or feed upon sediment-sorbed contaminants. Fipronil is highly toxic to aquatic species, and interestingly, its degradation products, fipronil-sulfide and fipronil-sulfone are reported as having equal or greater toxicity to aquatic invertebrates than the parent fipronil [2–4]. Maul et al. [2] reported that the median lethal concentrations (LC_{50}) in sediment for fipronil, fipronil-sulfide, and fipronil-sulfone were statistically similar with values of 0.88, 1.1 and 0.89 μg/kg dry weight for the benthic invertebrate, *Chironomus dilutus* (formerly *Chironomus tentans*). Mesléard et al. [5] identified fipronil as the pesticide mainly responsible for the significant decrease in invertebrate abundance in rice fields in Camargue, France. Fipronil has been detected in sediments from rivers and lakes receiving runoff from similar rice fields and agricultural areas at concentrations ranging from 1.7 to 5.5 μg/kg [3,6]. Both fipronil-sulfide and fipronil-sulfone have been detected at concentrations ranging from 0.64 to 25 μg/kg and 1.6 to 11 μg/kg, respectively [3,6–8]. In order to connect the environmental prevalence of fipronil, fipronil-sulfide, and fipronil-sulfone to toxicity of aquatic benthic invertebrates at these sub-ppb concentrations, a sensitive analytical method is needed.

Since the introduction of fipronil to the market in the U.S. in 1996, most of the field and laboratory studies of its environmental fate and toxicity have been in water and soils, with few being reported for aquatic sediments. These analytical methods have used time-consuming extraction techniques, including sonication [9,10], Soxhlet [11], and vigorous shaking and stirring [12–17]. These methods also used silica or florisil cartridges for removal of interferences or no cleanup was used. However, techniques without cleanup of extracts have substantial co-extracted interferences for samples in which a large mass of sediment must be extracted to obtain quantifiable results. In addition to higher errors in quantification, samples without a cleanup step also can require more frequent maintenance and replacement of instrumental components. Instrumentation for detection of fipronil and its degradation products commonly includes gas chromatography (GC) with electron capture detection (ECD) or mass spectrometry.

In order to reduce the lengthy and laborious sample preparation procedure prior to quantification, while still removing interfering compounds necessary for trace analysis, accelerated solvent extraction (ASE), and solid phase extraction (SPE) and gel permeation chromatography (GPC) were investigated in the current study as extraction and cleanup techniques, respectively. The ASE is advantageous compared to the traditional extraction methods, such as Soxhlet and sonication extraction, because elevated temperature and pressure results in reduced solvent and decreased extraction

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time requirements. In addition, the automation of this extraction technique produces better precision and reproducibility amongst samples as well as increased sample throughput. The objectives of the current study were to compare cleanup methods for analyzing fipronil and its degradation products in sediment at a sub-ppb level, and to investigate the influence of aging and total organic carbon on sediment extraction.

2. Experimental

2.1. Chemicals

Fipronil and its degradation products, fipronil-sulfide and fipronil-sulfone, were purchased from ChemService Inc. (West Chester, PA, USA) and Accustandard (New Haven, CT, USA), respectively. Bifenthrin was purchased from ChemService Inc. and used as a surrogate for the GPC method, while two surrogate standards 4,4′-dibromooctafluoro-biphenyl (DBOFB) and decachlorobiphenyl (DCBP) were obtained from Supelco (Bellefonte, PA, USA) and were used with the SPE method. Anhydrous Na_2SO_4 and all pesticide grade solvents were obtained from Fisher Scientific (Pittsburgh, PA, USA), while diatomaceous earth (DE) was obtained from Dionex (Sunnyvale, CA, USA). The 500 mg graphitized carbon black (GCB) and 1000 mg florisil cartridges were purchased from Restek (Bellefonte, PA, USA), while Supelco (Bellefonte, PA, USA) supplied the GCB/PSA (polymerically bonded, ethylenediamine-N-propyl phases containing primary and secondary amines) (300/600 mg) cartridges.

2.2. Sediment

Spiked sediments were prepared from three uncontaminated reference sediments collected at Touch of Nature (TON), Carbondale, IL, American River (AR), Folsom, CA, and Bearskin Lake (BS), Grand Marais, MN. The sediments had different total organic carbon (TOC) levels of 0.98 ± 0.025 (measured by Midwest Laboratories, Omaha, NE), 1.1 ± 0.07 and $7.85 \pm 0.18\%$ (measured on an EA 1110 CHN analyzer, CE Instruments, Milan, Italy) for TON, AR, and BS sediments, respectively. Prior to homogenization, TON soil was hydrated with moderately hard water [18] to achieve a sediment slurry with a dry:wet ratio of approximately 0.40, while AR and BS sediments already had a dry: wet ratio of 0.30 and 0.80, respectively. Sediments were spiked to achieve concentrations of 0.5, 1, 5 or 10 μg/kg dry weight of fipronil, fipronil-sulfide, and fipronil-sulfone to develop and validate extraction and cleanup methods. Sediments spiked with 10 μg/kg dry weight of target compounds were also analyzed with the optimized method to test the influence of aging time (0.24, 1, 4, 7 and 14 d) on recoveries. Stock solutions carried in acetone were added drop-wise to the sediment slurry to achieve the target concentrations; the slurry was stirred for 1 h using a stainless steel paddle stirrer powered by an overhead motor.

2.3. Accelerated solvent extraction

A previously established ASE method using the Dionex ASE 200 was employed using 33 ml stainless steel cells and 60 ml glass collection vials [19]. Briefly, samples were extracted by filling the cells with dichloromethane (DCM): acetone (1:1, v/v) and heating at 100 °C and 1500 pounds per square inch (psi) for two 5 min static cycles. The cells were flushed with 60% solvent for 60 s. Prior to extraction, two techniques were compared to remove water from the sediment, including the use of DE as a drying agent and freeze-drying the samples. Statistical differences in the two methods were analyzed with a *t*-test. For the drying technique using DE, 10 g sediment wet weight (ww) was centrifuged at $3300 \times g$ to initially remove excess water. After centrifugation, $5 \cdot g$ DE was added to the sample and thoroughly homogenized, and transferred to the ASE cell, where the appropriate surrogate was added. Extracts were collected in the 60 ml glass collection vials and residual water was removed with the addition of 12 g anhydrous Na_2SO_4. The Na_2SO_4 was then washed three times with 10 ml hexane, and the extracts and washes were combined and evaporated to 5 ml under nitrogen gas at 30 °C with a Zymark TurboVap II Evaporator (Hopkinton, MA, USA). The extracts were solvent exchanged with hexane and further reduced to 1 ml prior to cleanup.

The second drying technique used a FreeZone 2.5 Labconco freeze drier (Kansas City, MO, USA). Samples (10 g sediment wet weight) were dried overnight at approximately −48 °C and 0.133 psi. After homogenizing the dried sediment, it was transferred to an ASE cell, and extracted as previously discussed. The final extracts were concentrated and solvent exchanged to 1 ml of hexane. Due to the enhanced drying efficiency of the freeze-drying method, no residual water was observed in the extracts, and therefore, further drying with anhydrous Na_2SO_4 was not required.

2.4. Comparison of cleanup methods

Two cleanup methods, GPC and SPE, were developed and compared for analyzing fipronil, fipronil-sulfide, and fipronil-sulfone at sub-ppb concentrations in sediment. Optimization of the SPE method included two types of cartridge combinations, namely PSA/GCB and florisil coupled with GCB. Copper was added to samples in which SPE was applied as the cleanup technique to eliminate sulfur interference during analysis. Due to differences in size, the compounds of interest were isolated from sulfur during GPC cleanup, thereby eliminating the need for removal of sulfur with copper.

Prior to GPC, the sediment extract was filtered through a 0.2 μm Whatman GD/X filter (13 mm diameter), and then concentrated to 0.4–0.5 ml with a Pierce Model 1878 Reactivap (Rockford, IL, USA) prior to injection into the GPC. The extract was injected into the GPC with a Rheodyne 7225 injector with a 0.5 ml sample loop (Cotati, CA, USA). The GPC was performed on an Agilent 1100 high-pressure liquid chromatography (HPLC) (Agilent Technologies, Palo Alto, CA, USA) equipped with a UV detector. A Foxy Jr. fraction collector (ISCO, Inc. Lincoln, NE, USA) was used to collect the fraction that eluted between 7.5 and 8.5 min, which contained fipronil and its degradation products. The separation was completed on a Waters 300 mm × 19 mm Envirogel GPC column with a 5 mm × 19 mm precolumn (Waters, Milford, MA, USA). The mobile phase (DCM) was set at a flow rate of 5 ml/min. The fractions were evaporated to near dryness and solvent exchanged to 0.5 ml of hexane for analysis using an Agilent 6890 series GC-ECD (Agilent Technologies, Palo Alto, CA, USA).

A dual-layer cartridge containing 300 mg GCB in combination with 600 mg of PSA was evaluated as a SPE cleanup technique for fipronil and its degradation products. The PSA sorbent was used to eliminate interference from fatty acids, organic acids, polar pigments, and sugars [20,21], whereas the GCB was used to remove planar pigments and sterols [21]. Anhydrous Na_2SO_4 was added on the top of the sorbent bed to remove any residual water remaining in the extracts. After conditioning the PSA/GCB cartridge with 6 ml hexane, the extract was loaded onto the cartridge, and the tube previously containing the extract was washed twice with 0.5 ml hexane and these rinses also were transferred to the cartridge. The extract and washes were passed through the cartridge at a slow drop-wise rate of 1 drop/s. Optimization of the eluting solvent included a variety of solvent combinations and volumes ranging from 7 to 10 ml of 30% DCM in hexane, 100% DCM, 50% ether in hexane, 50% acetone in hexane and 50% acetone in DCM. The eluent was evaporated and solvent exchanged to 1 ml of hexane, and further analyzed with GC-ECD.

A second SPE cleanup approach with sorbent combinations of 1000 mg florisil and 500 mg GCB also was evaluated. The procedure was analogous to the aforementioned SPE method aside from the eluting solvent. Experimentation involved the use of the following eluting solvents with volumes ranging from 7 to 10 ml: 30, 40 and 50% ether in hexane, 100% DCM as well as 50, 60 and 75% acetone in hexane.

2.5. Instrumentation and chemical analyses

Chemical analysis of the final extracts was performed on a GC-ECD with a HP-5 column (30 m × 0.25 μm, film thickness 0.25 μm). Helium and nitrogen were employed as the carrier and make-up gas, respectively, with the flow rate of the carrier gas being 3.5 ml/min. A 2 μl sample was injected into the GC using pulsed split-less mode. The oven was set at 100 °C, heated to 180 °C at 10 °C/min increments, then to 205 °C at 3 °C/min increments and held for 4 min and then heated to 280 °C at 20 °C/min increments and held for 7 min. Seven external standards in hexane were used for linear calibration of all analytes at concentrations of 500, 250, 100, 50, 10, 5 and 1 μg/l. Qualitative identity of analytes was established using a retention window of 1%.

2.6. Lipid-like compound analysis

Analyses of lipid-like compounds were performed for sediment extracts (approximately 10 g sediment dry weight) with (i.e., GPC or SPE) and without cleanup for the TON, AR, and BS sediments to determine the removal efficiency of the lipid-like matrix by the methods following the method of van Handel [22]. An aliquot of 25 and 50 μl of sediment extracts without and with cleanup, respectively, were placed in test tubes. Chloroform: methanol (1:1, v/v) (500 μl) was added to each test tube and evaporated in a water bath. Next, 200 μl concentrated sulfuric acid was added to each test tube and heated in the water bath for 10 min. The test tubes were removed and cooled prior to the addition of 4.8 ml vanillin-phosphoric acid reagent. After 5 min of development, transmittance of the solutions was measured in a Spectronic 20 Genesys spectrophotometer (Sigma–Aldrich, St. Louis, MO) at 525 nm. A vanillin-phosphoric acid reagent blank was used for instrumental zero initially and every five samples to calibrate the reading. Calibration standards were made using 10, 50, 100, 200 and 400 μl of the 1 mg/ml vegetable oil standard and were ran on the spectrophotometer before and after the samples and averaged for the calibration curve. All standards were prepared in the same manner and at the same time as the samples.

3. Results and discussion

3.1. Optimization of the drying procedure

To remove water while minimizing the loss of analytes, the use of DE and freeze-drying were compared as drying techniques prior to extraction with ASE. Diatomaceous earth does not affect recoveries of non-polar pesticides [19,23]; however, a low DE: sediment ratio of 1:2 was used to maximize the mass of sediment extracted during ASE [19]. Therefore, 5 g of DE was used for approximately 10 g (wet weight) sediment. The use of both DE and freeze-drying produced acceptable recoveries that were not significantly different from one another for fipronil and its degradation products and ranged from 101 to 116 and 86.7 to 97.2%, respectively. While freeze-drying required up to 24 h to remove water for sediments with high moisture content, it provided higher water removal efficiency and no residual water was present in the sediment extracts, thereby eliminating the need for further drying extracts with anhydrous Na_2SO_4. In addition, less labor was needed for the drying procedure as the samples could run unattended overnight. Thus, freeze-drying was selected as the optimized drying technique prior to ASE extraction.

Table 1
Percent recoveries and corresponding relative standard deviations of seven replicates for fipronil, fipronil-sulfide, and fipronil-sulfone spiked at 0.5 and 1 μg/kg dry weight for the methods using gel permeation chromatography (GPC) and primary secondary amines/ graphitized carbon black (PSA/GCB), respectively, for Touch of Nature, IL (TON), American River, CA (AR) and Bear Skin Lake, MN (BS) sediments.

Compound	GPC	PSA/GCB		
	TON	TON	AR	BS
Fipronil	106 (8.70)	97.1 (11.3)	114 (8.84)	87.5 (18.9)
Fipronil-sulfide	114 (9.80)	98.6 (8.98)	116 (6.45)	108 (9.45)
Fipronil-sulfone	108 (8.40)	121 (3.34)	143 (9.09)	147 (9.94)

3.2. Optimization of the cleanup procedure

Gel permeation chromatography, the first cleanup technique tested, effectively removed fipronil, fipronil-sulfide, and fipronil-sulfone from co-extracted sediment interferences with analyte recoveries from 106 to 114% (Table 1). High molecular weight lipid-like compounds in the sediment extracts could deposit on the GC inlet and interfere with the GC analysis; therefore, a good cleanup method is desirable to remove those compounds from the extracts. Lipid-like compounds were analyzed before and after cleanup to verify the cleanup efficiency. The GPC procedure reduced 96% of the lipid-like compounds in TON sediment extracts; however, the sample still exhibited a yellow hue upon concentration of the collected fraction (7.5–8.5 min) to 0.5 ml. Sulfur, a potential interference in GC-ECD analysis, eluted after 14 min during GPC, and thus the use of copper to remove sulfur was not required when GPC was used as a cleanup technique. However, due to fraction collecting with manual start as well as manual injection, cleanup with GPC was time-consuming and labor-intensive. In addition, only one sample could be run at a time using GPC, and injection and run-time for the 12 samples took approximately 4 h. In addition, this technique used a large amount of solvent (over 1 l of DCM for 12 samples).

In an attempt to minimize sample cleanup time and solvent usage, while still producing satisfactory recoveries, SPE was evaluated as an alternative technique. Florisil and PSA in conjunction with GCB were tested as SPE sorbents. Various organic solvent combinations were evaluated as eluting solvents for both SPE cartridge combinations (Fig. 1). Solvent mixtures of acetone and hexane (1:1, v/v) produced the most satisfactory recoveries of the studied analytes for florisil and PSA. When florisil was used, recoveries were 96 ± 13, 88 ± 11 and 95 ± 11% for fipronil, fipronil-sulfide, and fipronil-sulfone, respectively, whereas the PSA cartridge recovered 101 ± 14, 98 ± 14, and 99 ± 13 of fipronil, fipronil-sulfide, and fipronil-sulfone, respectively. Thus, both sorbents potentially could be used to clean sediment extracts for analysis of fipronil and its degradation products. The cartridge containing PSA/GCB was chosen for two reasons, including its wide applicability for various matrices and pesticides [19–21,24], and slightly lower costs (~$5 versus $7/cartridge). Previous studies showed that PSA and other sorbents that contain amide functional groups are very effective at reducing or eliminating matrix interference compared to C-18 and strong-anion exchange (SAX) sorbents [25,26]. Elution solvents of 30% DCM in hexane have been validated for cleanup of sediment extracts containing pyrethroid, organochlorine and organophosphate insecticides [19]. However, because of the more polar nature of fipronil and its degradation products, the solvents were not adequate to elute these compounds. Therefore, solvent combinations with a higher polarity, such as ethyl ether or acetone in hexane were required for qualitative recoveries of the analytes. On the

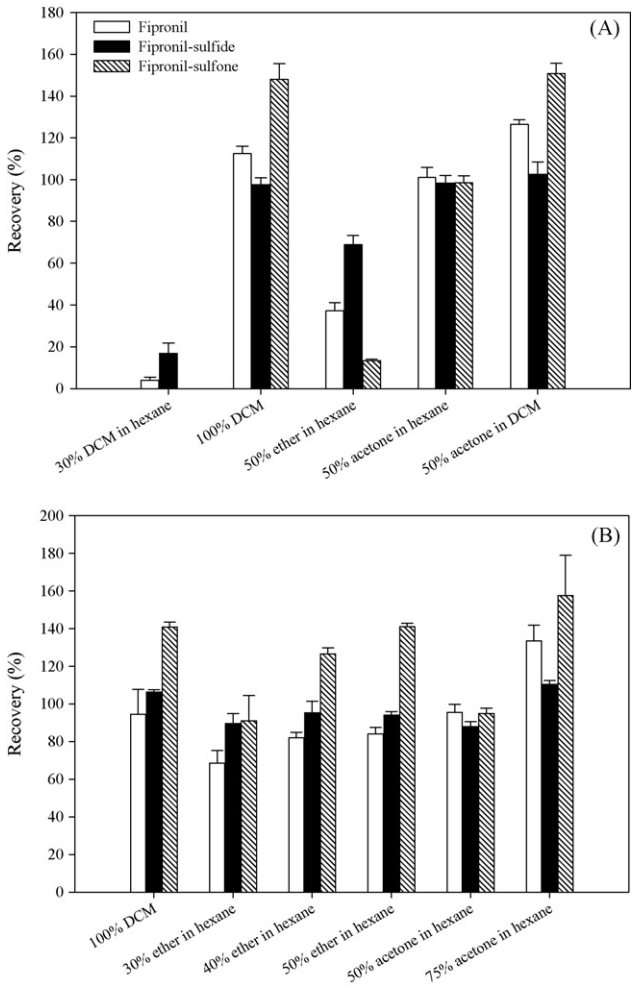

Fig. 1. Percent recoveries and corresponding relative standard deviations for fipronil, fipronil-sulfide, and fipronil-sulfone using different solvent combinations with primary and secondary amines/graphitized carbon black (A) and florisil (B) solid phase extraction absorbents. DCM = dichloromethane.

other hand, increasing the polarity of the eluting solvents increased the amount of fatty acid breakthrough from the cartridges [21,24]. Shimelis [21] stated that the use of GCB in conjunction with PSA slightly increased the retention of fatty acids in addition to removing planar pigments and sterols; however, 36% of oleic acid passed through a PSA/GCB (500 mg/500 mg) cartridge using 6 ml acetone: hexane (1:1, v/v) as the elution solvents. In the current study, 53% of the lipid-like compounds in TON sediment passed through a PSA/GCB cartridge using 10 ml of an acetone: hexane (1:1, v/v) solution. Although breakthrough of lipid-like compounds occurred, little to no interference with analytes was observed during GC-ECD analysis (Fig. 2).

3.3. Method validation

Method validation included accuracy and precision estimates, and method detection limit (MDL) determination. Accuracy and precision of SPE with PSA/GCB was determined by spiking three sediments with different total organic carbon (TOC) levels at varying analyte concentrations and aging periods. As shown in Table 2, recoveries of fipronil, fipronil-sulfide, and fipronil-sulfone ranged from 72 to 119%. Precision was determined by relative standard deviations (RSD) of three replicates, and these values ranged from 0.5 to 8.4% with most less than 5% at a spiked concentration of 10 μg/kg dry weight. The low RSD values demonstrated applicability of the established methods amongst sediments with different TOC levels and across different aging periods. Precision decreased for sediments spiked at lower concentrations of 1 μg/kg or less, with RSD values ranging from 3.3 to 19% (Table 1). In addition to increased variability, recoveries also were higher, especially for fipronil-sulfone, which ranged from 121 to 147%. The higher recoveries at lower concentrations suggested the existence of co-eluted interference and most notably the difficulty in quantifying concentrations approaching the detection limit. The presence of co-extracted interferences also can explain the variability amongst replicates. The lower sensitivity (smaller response factor) of fipronil-sulfone than those of the other analytes increased the difficulty in quantifying this compound at lower concentrations, contributing to the higher recoveries and variability. Amongst the three sediment types, the compounds spiked into the AR sediment had higher recoveries than the other two sediments at the 1 μg/kg dry weight concentration only, possibly resulting from increased interference.

The MDL is the minimum concentration of a substance that can be measured and reported with 99% confidence that the concentration is greater than zero [27]. As proposed by the U.S. EPA [27,28] the MDL was determined by multiplying the standard deviation of seven replicate samples by the Student's t value from statistical tables for a 99% confidence level and $(n-1)$ degrees of freedom. Table 3 lists the MDLs for each compound for both cleanup tech-

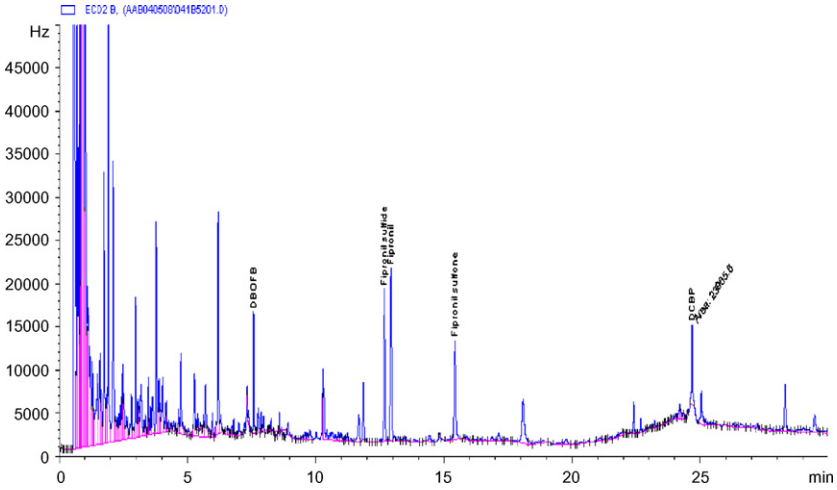

Fig. 2. Example of GC-ECD chromatogram of sample extract cleaned with primary and secondary amines/graphitized carbon black. The analytes and surrogates were spiked at 10 ng/g.

Table 2
Percent recoveries and corresponding relative standard deviations of three replicates for fipronil, fipronil-sulfide, and fipronil-sulfone at time points (0.24, 1, 4, 7, and 14 d) during sediment aging. Sediment extracts were cleaned with solid phase extraction. Sediment from Touch of Nature in IL (TON), American River, CA (AR) and Bear Skin Lake, MN (BS) was spiked at 10 μg/kg dry weight.

Sediment	Compound	Aging time (d)				
		0.24-d	1-d	4-d	7-d	14-d
TON	Fipronil	96.9 (3.20)	98.9 (6.33)	88.6 (1.25)	96.0 (4.96)	91.7 (1.29)
	Fipronil-sulfide	101 (5.54)	103 (5.71)	89.3 (2.26)	95.9 (4.43)	96.1 (1.53)
	Fipronil-sulfone	84.8 (5.58)	82.7 (8.01)	76.8 (0.510)	80.9 (5.36)	75.4 (1.45)
AR	Fipronil	83.3[a]	94.5 (6.70)	99.2 (4.71)	91.4 (8.13)	80.5 (6.82)
	Fipronil-sulfide	103.3[a]	105 (8.43)	97.1 (8.21)	89.6 (5.91)	85.0 (1.47)
	Fipronil-sulfone	94.9[a]	96.6 (6.38)	93.7 (6.21)	83.6 (6.20)	71.6 (6.63)
BS	Fipronil	94.6 (5.24)	93.7 (1.19)	109 (1.55)	102 (1.08)	119 (4.77)
	Fipronil-sulfide	99.7 (6.27)	98.9 (1.38)	101 (1.79)	97.1 (0.946)	111 (5.25)
	Fipronil-sulfone	81.7 (7.99)	84.5 (3.31)	88.6 (1.91)	85.0 (1.78)	87.8 (5.84)

[a] Unable to determine relative standard deviations since only 1 replicate was used.

niques. The MDL values for the three compounds ranged from 0.12 to 0.30, 0.22 to 0.32, and 0.32 to 0.52 μg/kg dry weight for TON, AR, and BS sediments, respectively. In comparing the MDL values for both methods in TON sediment, the SPE method had slightly lower MDL values except for fipronil, which were 0.30 and 0.17 μg/kg dry weight for SPE and GPC, respectively. For the optimized SPE method, MDLs were higher for all compounds in BS sediment, most likely due to the low mass of sediment extracted compared to the other two sediments. Since BS sediment contained 80% moisture, a total of approximately 3–4 g dry weight was extracted as opposed to approximately 10 g dry weight for TON and AR sediment. Although the spiked concentration was 1 μg/kg dry weight in all sediments, the overall extracted amount was lower for BS sediment; therefore, any loss during extraction or cleanup will be more evident. In addition, quantifying lower extract concentrations, especially those approaching detection limits, typically increases variability amongst replicates. Since the MDL calculation takes into consideration the standard deviation or variability amongst replicates, the MDL will consequently also increase with increasing variability, thus the higher MDL values for BS sediment. Again, fipronil-sulfone in BS sediment had the highest MDL value of 0.52 μg/kg dry weight, which could be a result of the lower response factor of the compounds and difficulty with quantification. The MDL values ranging from 0.12 to 0.52 μg/kg dry weight in the current study are in accord with the lower range of published MDL values for fipronil, fipronil-sulfide, and fipronil-sulfone in soils and sediments of 0.13–9.0 μg/kg dry weight [11,13–15,29].

The optimized SPE method was also used to analyze field samples from urban sites in central Texas, which potentially contain fipronil and its degradation products. Out of the 10 sediments, fipronil, fipronil-sulfide, and fipronil-sulfone were detected in all of the sediment samples. However, only half of the sediments contained fipronil, fipronil-sulfide, or fipronil-sulfone above reporting limits, with all three compounds detected in only one sediment sample (Table 4). The reporting limit was calculated as three times the MDL, which was the average of all three sediment types.

Table 3
Method detection limits (MDL) for fipronil, fipronil-sulfide, and fipronil-sulfone for the cleanup methods using gel permeation chromatography (GPC) and solid phase extraction (SPE) with primary secondary amines/ graphitized carbon black cartridge in sediment from Touch of Nature, IL (TON), American River, CA (AR) and Bear Skin Lake, MN (BS).

Compound	MDL (μg/kg dry weight)				
	GPC	SPE			
	TON	TON	AR	BS	Method[a]
Fipronil	0.17	0.3	0.32	0.44	0.44
Fipronil-sulfide	0.21	0.12	0.23	0.32	0.32
Fipronil-sulfone	0.18	0.16	0.22	0.52	0.52

[a] The MDL for the SPE cleanup method across sediments. It was the maximum MDL amongst sediments.

Table 4
Field validation of the optimized method. All values are μg/kg dry weight with reporting limits being calculated as three times the average method detection limit (of three sediment types).

Sample	Fipronil	Fipronil-sulfide	Fipronil-sulfone
1	<RL	<RL	<RL
2	<RL	<RL	<RL
3	<RL	1.78	<RL
4	<RL	1.20	1.51
5	<RL	0.824	<RL
6	1.33	3.52	0.997
7	<RL	0.903	<RL
8	<RL	<RL	<RL
9	<RL	<RL	<RL
10	<RL	<RL	<RL

<RL = below reporting limits.
Reporting limits for fipronil, fipronil-sulfone, and fipronil-sulfide are 1.05, 0.674, and 0.885 μg/kg.

4. Conclusions

Cleanup methods using GPC and SPE have been optimized and comparatively evaluated for analyzing fipronil, fipronil-sulfide, and fipronil-sulfone at sub-ppb concentrations in sediment. The MDL values ranged from 0.12 to 0.52 μg/kg dry weight. Both methods resulted in MDL values similar to the lowest reported values for fipronil and its degradation products, with decreased sample preparation times and solvent usage for the SPE cleanup. The labor-intensive cleanup and cost associated with GPC minimizes its appeal compared to SPE. Automation of GPC and fraction collecting would reduce hands-on sample cleanup, but it would still require substantial volumes of solvent that would have to be disposed of as waste. Ultimately, both cleanup techniques were shown to be effective for analyzing fipronil and its degradation products; however, preference is given to SPE.

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