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A Multi-residue Method for the Analysis of Pesticides and Pesticide Degradates in Water Using HLB Solid-phase Extraction and Gas Chromatography–Ion Trap Mass Spectrometry

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Abstract A method was developed for the analysis of over 60 pesticides and degradates in water by HLB solid-phase extraction and gas-chromatography/mass spectrometry. Method recoveries and detection limits were determined using two surface waters with different dissolved organic carbon (DOC) concentrations. In the lower DOC water, recoveries and detection limits were 80%–108% and 1–12 ng/L, respectively. In the higher DOC water, the detection limits were slightly higher (1–15 ng/L). Additionally, surface water samples from four sites were analyzed and 14 pesticides were detected with concentrations ranging from 4 to 1,200 ng/L.

Keywords Pesticides · Water · Solid-phase extraction · GC–MS

Analytical methods are needed to measure pesticides in water to determine their fate and transport in the environment. Currently, there are hundreds of pesticides registered for use in the United States (PAN 2007). Multiple pesticides can be applied to one crop (separately or co-formulated) and areas with a diverse array of crops, such as California or Florida, have many different pesticides applied throughout the year. Frequently, multiple pesticides have been detected during routine monitoring. Water samples collected from 1999 to 2001 as part of the U.S. Geological Survey's National Water-quality Assessment Program detected five or more pesticides or degradates in 70% of the samples (Gilliom et al. 2006).

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Solid-phase extraction (SPE) is an efficient method for the concentration and cleanup of organic contaminants in water (Pichon 2000). Several types of cartridges have been used in prior studies (e.g., C8, C18); C8 cartridges are capable of extracting multiple pesticide classes (Crepeau et al. 2000) but suffered from low recoveries if the cartridge went dry during conditioning or extraction. Recently the Waters Oasis® HLB has become a preferred sorbent because recoveries are still high even if the cartridge goes dry. A previous study found the HLB sorbent to be one of the most effective at extracting a limited number of pesticides (16) that covered multiple chemical classes (D'Archivio et al. 2007).

The following study presents a sensitive method for the simultaneous analysis of 62 pesticides and degradates in water using HLB SPE with gas chromatography (GC) and ion trap mass spectrometry (MS) detection. The effectiveness of the method was tested by analyzing surface waters from four different sampling locations in Northern California with inputs from agricultural runoff.

Materials and Methods

Pesticide reference materials were purchased from Chem Service (West Chester, PA), Riedel-de Haën (Seelze, Germany) and Ultra Scientific (North Kingstown, RI) or were donated by the U.S. Environmental Protection Agency National Pesticide Repository (Ft. Meade, MD). Purities ranged from 95% to 99%. Internal standards (d_{10}-acenaphthene, d_{10}-phenanthrene and d_{10}-pyrene) and surrogates (ring-$^{13}C_3$-atrazine and diethyl-d_{10} diazinon) were purchased from Cambridge Isotope Labs (Andover, MA). Neat pesticides were dissolved individually in acetone or methanol for an initial concentration of 1 mg/mL.

Standard calibration curves were made with concentrations ranging from 0.025 to 2.5 ng/μL in ethyl acetate and stored in a freezer at −20°C. All solvents and other reagents used were ACS grade or better (Fisher Scientific, Waltham, MA).

Two surface waters were used for determining initial recoveries and method detection limits (MDLs). The first water sample was from the Sacramento River at Miller Park (Sacramento, CA), a large river with multiple land-uses including forests, agriculture, and urban. The second water sample was from Colusa Basin Drain (near Knight's Landing, CA), a drainage canal with predominately agricultural input. Each water sample was analyzed for background pesticide concentrations before spiking. An additional sample was collected at each site in a 125-mL baked amber glass bottle for analysis of dissolved organic carbon (DOC) analysis (Bird et al. 2003).

The final method was validated using water samples from two agricultural watersheds collected under low- and high-flow conditions. The first set of samples was collected from Gilsizer Slough (Sutter County, CA) weekly over 3 months of a low-flow period in summer of 2005. This site has been channelized and receives urban storm-water and agricultural runoff. The second set of samples was collected from three inputs to Yolo Bypass (a flood plain for the Sacramento River) during winter (December) 2005 to quantify pesticides present during the first flush (high-flow) event of the season. Surface water samples were collected from Knights Landing Ridge Cut, Willow Slough and Colusa Basin Drain 3 at Tule Road near College City (for more details on sampling see Smalling et al. 2006).

Water was filtered through baked 0.7 μm glass fiber filters (GF/F) (Whatman; Florham Park, NJ) prior to spiking and extraction. Samples were spiked, as appropriate, with pesticides or surrogates from 2 ng/μL stock solutions (final concentrations were 20–50 ng/L for pesticides and 200 ng/L for surrogates). A 1-L water sample was pumped through an Oasis® HLB extraction cartridge (6 cm^3, 500 mg, 60 m; Waters Corporation, Milford, MA) that had been preconditioned with 10 mL of ethyl acetate, 10 mL methanol and 5 mL of water. The sample was pumped at a flow rate of 10 mL/min. The cartridge was dried under carbon dioxide for 1 h and then eluted with 12 mL of ethyl acetate. Carbon dioxide was used as the drying gas as it was found to be cleaner and remove water from the SPE cartridge more effectively than nitrogen. After the extraction, ~1 g of sodium sulfate was added to the sample bottle to remove any residual water and the bottle was rinsed three times with approximately 4 mL of dichloromethane. The bottle rinse was reduced to 1 mL under a gentle stream of nitrogen (N-evap; Organomation Associates, Berlin, MA) and then added to the ethyl acetate fraction. The sample was reduced to 200 μL under nitrogen and 40 μL of deuterated internal standards were added (10 ng/μL). Injections (1 μL) were made onto the GC/MS with conditions listed in Table 1; the fungicides were run separately using the short GC program for greater ease in setting selection ion scanning (SIS) windows.

Results and Discussion

The response of compounds was linear over the calibration range (0.025–2.5 ng/μL). Each compound was quantified using a calibration curve with a minimum of six points. Linear regression analysis by the least-squares method of peak area ratio of analyte/internal standard against different analyte concentrations for each compound gave R^2 values >0.999.

A rinse of the sample bottle was necessary after SPE extraction to remove pesticides that were loosely associated with the bottle wall. Previous studies to quantifying the bottle rinse from the SPE extract showed that significant amounts (10%–40%) of most pyrethroids and methoprene sorbed to the bottle wall (data not shown). A rinse of the bottle with dichloromethane was sufficient to remove any residual pesticides. Bottles were not silanized as this has been shown to be ineffective at reducing pyrethroid sorption to bottle walls (Wheelock et al. 2005).

All compounds had acceptable recoveries (80%–110%; Table 2) on the HLB cartridges when spiked at 20–50 ng/L into Sacramento River water (DOC = 1.7 mg/L). Relative standard deviations (RSD) were 2%–13%. The Colusa Basin Drain water with higher DOC (6.6 mg/L) also had acceptable recoveries (72%–123%), although there were slightly more variable (RSD ranged from 3% to 20%) than the Sacramento River water.

Table 1 Conditions for the Varian Saturn 2000 GC/MS

Injector	Splitless, 275°C; pressure pulse of 50 psi for 0.5 min
Column	Agilent DB-5MS; 30 m length × 0.25 mm ID × 0.25 μm; He carrier gas at 1 mL/min
Oven program	Long: 80°C for 0.5 min, 10°C/min to 120°C, 3°C/min to 200°C, hold for 5 min, 3°C/min to 219°C, 10°C/min to 300°C, hold for 10 min (total runtime 61 min)
	Short: 80°C for 1 min, 10°C/min to 300°C, hold for 10 min (total runtime 33 min)
MS	Transfer line 280°C; ion trap 220°C; electron ionization (EI) mode; full scan emission current, 15 μA, no offset; SIS emission current, 45 μA, 300 V multiplier offset

Table 2 Compounds, mean recoveries and MDLs for two surface waters

Compound	Type[a]	Sacramento River		Colusa Basin Drain	
		Mean % recovery (% RSD)	MDL (ng/L)	Mean % recovery (% RSD)	MDL (ng/L)
Anilines					
3,4-Dichloroaniline	D	110 (12)	8.3	115 (16)	11.7[b]
Ethalfluralin	H	97 (6)	3.0	86 (6)	5.2
Pendimethalin	H	99 (4)	2.3	107 (16)	11.3[b]
Trifluralin	H	99 (4)	2.1	86 (6)	3.2
Carbamates					
Carbaryl	I	106 (10)	6.5	98 (16)	9.8
Carbofuran	I	93 (10)	3.1[b]	108 (13)	8.8
Chloroacetanilides					
Alachlor	H	97 (3)	1.7	111 (7)	4.9
Metolachlor	H	95 (2)	1.5	108 (8)	5.4[b]
Organochlorines					
Pentachloroanisole	D	80 (10)	4.7	94 (12)	7.0
Pentachloronitrobenzene	F	85 (13)	3.1	76 (12)	5.7
p,p′ DDD	D	81 (7)	3.6	81 (4)	2.0
p,p′ DDE	D	83 (8)	4.1	73 (7)	3.3
p,p′ DDT	I	85 (8)	4.0	77 (9)	4.3
Organophosphates					
Chlorpyrifos	I	84 (4)	2.1	89 (13)	7.1
Diazinon	I	98 (1)	0.9[b]	109 (7)	4.5[b]
Malathion	I	105 (6)	3.7	116 (13)	9.3
Methidathion	I	99 (12)	7.2	103 (13)	14.9
Methylparathion	I	94 (6)	3.4	80 (12)	6.0
Phosmet	I	94 (8)	4.4	78 (5)	2.3
Pyrethroids					
Allethrin	I	107 (9)	6.0	84 (15)	8.0
Bifenthrin	I	93 (8)	4.7	70 (5)	2.4
Cyfluthrin	I	98 (8)	5.2	97 (19)	11.3
λ-Cyhalothrin	I	85 (7)	2.0	73 (10)	4.4
Cypermethrin	I	85 (10)	5.6	99 (16)	10.0
Deltamethrin	I	97 (6)	3.5	87 (12)	6.5
Esfenvalerate	I	91 (7)	3.9	92 (8)	4.7
Fenpropathrin	I	85 (8)	4.1	107 (13)	9.3
T-Fluvalinate	I	86 (10)	5.3	72 (11)	4.7
Permethrin	I	99 (11)	3.4	86 (14)	7.5
Resmethrin	I	92 (10)	5.7	74 (20)	11.7
Sumithrin (phenothrin)	I	99 (8)	5.1	108 (12)	8.5
Tetramethrin	I	95 (5)	2.9	87 (4)	2.1
Thiocarbamates					
Butylate	H	86 (2)	1.8	101 (9)	5.7
Cycloate	H	90 (2)	1.1	78 (11)	5.2
Eptam (EPTC)	H	89 (3)	1.5	74 (12)	5.7
Molinate	H	87 (6)	3.2	80 (4)	2.0
Pebulate	H	97 (4)	2.3	74 (15)	7.1
Thiobencarb	H	99 (3)	1.9	107 (15)	10.0[b]
Triazines/Triazones					
Atrazine	H	106 (4)	2.3	108 (8)	5.7

Table 2 continued

Compound	Type[a]	Sacramento River		Colusa Basin Drain	
		Mean % recovery (% RSD)	MDL (ng/L)	Mean % recovery (% RSD)	MDL (ng/L)
Hexazinone	H	112 (12)	8.4[b]	119 (17)	12.6[b]
Prometryn	H	109 (3)	1.8	123 (9)	7.0
Simazine	H	109 (7)	5.0	115 (20)	15.6[b]
Terbuthylazine	H	99 (3)	1.6	113 (6)	4.1
Triazoles					
Cyproconazole	F	90 (7)	11.2	85 (12)	13.2
Metconazole	F	99 (6)	11.4	74 (13)	14.7
Myclobutanil	F	100 (5)	9.2	73 (13)	14.7
Propiconazole	F	85 (6)	8.8	84 (10)	12.8
Tebuconazole	F	93 (6)	10.2	93 (11)	15.4
Tetraconazole	F	103 (4)	8.2	88 (8)	11.2
Strobilurins					
Azoxystrobin	F	97 (5)	9.3	81 (8)	10.4
Trifloxystrobin	F	92 (2)	3.9	100 (8)	11.8
Miscellaneous					
Chlorothalonil	F	85 (8)	12.1	73 (11)	11.2
Dacthal (DCPA)	H	108 (3)	2.0	115 (11)	7.9
Fipronil	I	102 (4)	2.9	110 (3)	2.0
Fipronil desulfinyl	D	91 (3)	1.6	102 (4)	2.7
Fipronil sulfide	D	98 (3)	1.8	106 (3)	2.2
Fipronil sulfone	D	98 (6)	3.5	73 (15)	7.0
Iprodione	F	95 (11)	6.5	92 (7)	4.0
Methoprene	I	91 (9)	6.4	119 (11)	8.4
Napropamide	H	107 (12)	8.2	107 (14)	9.2
Oxyfluorfen	H	98 (5)	3.1	117 (4)	3.2[b]
Piperonyl butoxide	S	100 (4)	2.3	106 (8)	5.5

Samples were spiked at 20–50 ng/L; n = 7

[a] D = degradate; F = fungicide; H = herbicide; I = insecticide; S = synergist

[b] Compound detected in unspiked water

Method detection limits (MDLs) were determined using seven replicates of filtered water for the two different surface waters, Sacramento River and Colusa Basin Drain. Deionized water was not used as it does not have the same characteristics as environmental water samples that are analyzed for pesticides and gives unrealistic results. Prior to determining MDLs, the waters used were measured for background concentrations of pesticides (pesticides detected are noted in Table 2). Concentrations were low for all compounds detected (<50 ng/L). Water samples were spiked at 20 or 50 ng/L depending on the response of the compound on the GC/MS. For example, the fungicides were spiked at a higher concentration because they typically have a lower response compared to the other compounds in the method. MDLs were calculated using a U.S. EPA method based on the standard deviation (USEPA 1992).

Method detection limits for all compounds in the Sacramento River water ranged from 1 to 12 ng/L (Table 2) and are similar to other MDLs (3–40 ng/L) for others pesticides in water using HLB cartridges (D'Archivio et al. 2007). In contrast, the overall MDLs in water from the Colusa Basin Drain were slightly higher, ranging from 1 to 15 ng/L (Table 2). About 26 of the 62 MDLs (42%) calculated from the Colusa Basin Drain water were similar to those for the Sacramento River and 47 (76%) were within a factor of two. Water characteristics other than the DOC were similar between the two waters; conductivities were low (200–400 μS/cm) and the pH was circum-neutral (6.8–7.5). The higher DOC in the Colusa Basin Drain water caused a noisier baseline on the GC/MS making low-level concentrations harder to quantify and possibly competing for sorption sites on the HLB cartridge making recoveries more variable, thus increasing the MDLs. Using water that

Table 3 Concentrations of pesticides detected at Gilsizer Slough

Date (mm/dd/yy)	Carbaryl	Chlorpyrifos	Diazinon	Malathion	Methyl-parathion	Molinate	Simazine	Piperonyl butoxide
04/26/05	nd	nd	nd	nd	nd	nd	120	nd
05/03/05	nd	nd	21	nd	nd	nd	85	nd
05/10/05	91	nd	nd	58	nd	28	200	nd
05/17/05	nd	nd	13	nd	nd	28	81	nd
05/24/05	nd	nd	14	nd	nd	13	nd	130
05/31/05	nd	nd	nd	nd	nd	180	nd	nd
06/07/05	nd	11	nd	nd	nd	32	nd	nd
06/14/05	nd	49	nd	nd	130	19	73	nd
06/21/05	nd	4.4	64	nd	nd	nd	83	nd
06/28/05	nd	nd	nd	nd	nd	nd	53	10
07/05/05	nd	15	nd	nd	nd	22	nd	nd

Concentrations are in ng/L. nd = not detected

Table 4 Concentrations of pesticides detected at three inputs to the Yolo Bypass

Site	Date (mm/dd/yy)	Atrazine	Diazinon	3,4 Dichloro-aniline	Hexazinone	Metolachor	Molinate	Oxyfluorfen	Simazine	Trifluralin
KL Ridge Cut	12/30/05	nd	14	110	63	28	6.0	14	nd	nd
Willow Slough	12/28/05	nd	15	nd	1,200	280	nd	41	nd	nd
CBD 3 at Tule Road	12/29/05	nd	14	110	180	37	7.7	19	nd	nd

Concentrations are in ng/L. nd = not detected

is characteristic of typical environmental samples is key when conducting MDL studies. Higher DOC will ultimately affect sample extraction as well as analysis and MDLs should reflect field collected samples.

In the 14 field samples analyzed, 14 different pesticides were detected. At Gilsizer Slough, samples were collected over 11 weeks and five insecticides, two herbicides and one synergist were detected (Table 3). The insecticides (and synergist) were detected relatively infrequently over the sampling period and their concentrations ranged from 13 to 130 ng/L. The herbicides were detected more frequently with concentrations ranging from 28 to 200 ng/L. Five herbicides, one insecticide and one herbicide degradate were detected in samples collected at the three other field sites (Table 4). Concentrations for the herbicides in general were higher than the insecticide (6–1,200 ng/L versus 14–15 ng/L). Quality control samples comprised 10% of the samples. Matrix spikes ranged from 75% to 108% and surrogate recoveries ranged from 80% to 100%. Replicates were within 20%.

This method was validated using water samples collected during different times of the year under varying environmental conditions (e.g., high- and low-flow). A variety of pesticides were detected in the field samples and concentrations were all above calculated MDLs. This is a robust method yielding MDLs in the low parts per trillion levels for 56 pesticides and seven degradates that encompass a variety of different classes of pesticides with varying physical–chemical properties.

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