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Optimizing recoveries of two chlorotriazine herbicide metabolites and 11 pesticides from aqueous samples using solid-phase extraction and gas chromatography–mass spectrometry

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Abstract

A method was developed for solid-phase extraction of two chlorotriazine herbicide metabolites, deethylatrazine (DEA) and deisopropylatrazine (DIA), from aqueous samples. Two C_{18} phases in cartridge format were compared and recoveries were found to be highly sensitive to sorbent amount, sample volume and presence of parent compounds. Recoveries were significantly improved using a partially non-endcapped C_{18} phase compared to the normal C_{18} phase, particularly for DIA, apparently due to polar interactions. Combinations of sample volume and sorbent amount were tested using deionized water to determine an optimal combination of 200 ml and 1.0 g, respectively. Recoveries from a variety of river, stream, runoff and ground waters averaged 105–116% and 109–117% at concentrations of 0.5–1.0 ng/ml for DIA and DEA, respectively, with minimum detection limits of 0.05 ng/ml. Other pesticides tested also have acceptable recoveries using this method.

Keywords: Water analysis; Environmental analysis; Pesticides; Triazines

1. Introduction

The triazine herbicides are among the most widely used and most frequently detected in ground and surface water in the USA [1–4]. As a result, contamination of surface and ground water by triazine herbicides has been the target of numerous investigations over the past 20 years. Methods for the extraction and detection from aqueous matrices have evolved during this period, with solid-phase extraction (SPE) and gas chromatography (GC) being the most common approach in recent years. Cartridges or disks containing octadecyl (C_{18}) sorbent have performed very well for parent compounds, such as atrazine and simazine, but poorly (<70% recovery) for their more polar metabolites, deethylatrazine (2-chloro-4-amino-6-isopropylamine-1,3,5-triazine) and deisopropylatrazine (2-chloro-4-amino-6-ethylamino-1,3,5-triazine) [5–7]. These two metabolites are the most common degradation products found in natural waters [2,8] and are of increasing interest [9].

Several attempts to circumvent this problem have met with limited success. Mills et al. [10] used a mixed-mode phase which included both octadecyl and sulfonic acid groups for a combination of nonpolar and cation-exchange interactions. Both DEA and DIA were retained well using pure water,

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but underwent early breakthrough when they were mixed in tap water due to competition from dissolved cations. Another approach involves isotope dilution [11] and mass spectrometry (MS), but this requires acquisition or synthesis of compounds labeled with isotopes such as ^{13}C, which is difficult and expensive. Liquid–liquid approaches have acceptable recovery rates [12], but this negates the advantages of SPE. A number of studies have used conventional C_{18} columns or disks and just accepted low recoveries of DIA, DEA or both [13–15]. A relatively new adsorbent, graphitized carbon black, recovers DIA and DEA from water quite well (>90% recoveries) but requires extensive column preparation or centrifugation steps [16–19]. Pichon et al. [19] obtained good recoveries of DIA and DEA using either styrene–divinylbenzene (PS–DVB) or porous graphitic carbon (PGC) but elution of the parent compounds required up to 25 ml of a mixed solvent. Nouri et al. [20] also found that PS–DVB cartridges worked well for DIA and DEA, but backpressure prevented them from exceeding a 1 ml/min flow-rate, which would be far too slow for manual extraction.

As part of our water quality programs, we compared a relatively new modification of the C_{18} phase in which silanol groups are only partially end-capped to the widely used, fully end-capped C_{18} phase for recovery of several triazines and metabolites. Our goal was to develop a simple, inexpensive, one-step extraction procedure for water samples from a variety of environments which would retain parent triazines as well as chlorinated metabolites. We used a factorial design similar to that of Wan et al. [21] in which we conducted progressively narrow experiments to optimize column size and sample volume for both recovery and detection sensitivity.

2. Experimental

2.1. Chemicals

Ethyl acetate (Fisher, pesticide grade) and methanol (Fisher, ACS grade) were used as solvents. Analytical grade (>98%) atrazine, deethylatrazine, deisopropylatrazine and diaminochlorotriazine were obtained from Ciba-Geigy (Greensboro, NC, USA). Simazine was obtained from Hewlett-Packard (Wilmington, DE, USA). Bakerbond spe* Polar Plus C_{18} (octadecyl) 6 ml extraction columns (0.5, 1.0 and 2.0 g sorbent) and Bakerbond spe* C_{18} (octadecyl) 6 ml extraction columns (0.5 and 1.0 g sorbent) were obtained from JT Baker (Phillipsburg, NJ, USA). According to information shipped with the 0.5 g cartridges, carbon loading was 17.9% for the C_{18} packing and 16.8% for the Polar Plus packing. Standard solutions were prepared in ethyl acetate and stored under refrigeration at 4°C.

Recovery tests were run using deionized laboratory water spiked with each compound. All test solutions were stored under refrigeration at 4°C for no more than one week. Unspiked water from the same source as the experiments was also extracted to check for background interferences. Natural water samples were collected in amber glass bottles, stored under refrigeration and then spiked and extracted within a week of collection. In routine use the method includes an internal standard of terbutylazine (2-*tert*.-butylamino-4-chloro-6-ethylamino-1,3,5-triazine) spiked into all samples at a concentration of 1.0 ng/ml.

2.2. Solid-phase extraction procedures

The columns were prepared by washing under vacuum with 10 ml of methanol followed by 10 ml of deionized water. Samples were extracted in groups of six using a vacuum manifold (Waters, Milford, MA, USA) at the rate of 4–5 ml/min. After the sample was passed through the column, the columns were dried with air for approximately 30 min to remove residual water and the analytes were eluted with two 5 ml aliquots of ethyl acetate. The ethyl acetate was evaporated under nitrogen at 30°C to a volume that gave a one hundred-fold concentration factor for each sample (1, 2 or 5 ml). River, stream and runoff waters were filtered through a 47 mm, 0.45 μm, borosilicate fiber filter prior to extraction.

2.3. Factors tested

Our objective was to devise a rapid method for extracting both parent and metabolite triazines, so we tested the most important factors relating to speed

Table 1
Factors tested during the study

Factor	Experiment 1	Experiment 2	Experiment 3
Column size	0.5 g	1.0 g	2.0 g
Solid phase sorbent	Normal C_{18}, Polar C_{18}	Normal C_{18}, Polar C_{18}	Polar C_{18}
Sample volume	100, 200, 500 ml	200, 500 ml	500 ml
pH	Ambient (~6)	6, 10	Ambient
Metabolite/parent concentrations (ng/ml)	0.3:0, 3.0:0, 0:3.0, 0.3:3.0, 3.0:3.0	3.0:0, 0.3:3.0	3.0:0, 0.3:3.0

and accuracy of an extraction procedure (Table 1). We also tested interactions between metabolites and parent compounds for effects on recoveries of each, since environmental samples often contain both. Extractions were conducted in groups of six in treatment combinations chosen at random.

2.4. Gas chromatography–mass spectrometry

GC–MS analyses of the eluates were performed on a Hewlett-Packard Model G1800A GCD (Palo Alto, CA, USA). The GCD is an HP5890 Series II GC with electronic pressure control (EPC) coupled with an electron ionization mass selective detector (MSD). Operating conditions were as follows: ionization voltage, 70 eV; detector interface temperature of 290°C; electron multiplier voltage determined by autotune (1400–1600 V); daily tuning with perfluorotributylamine (PFTBA). For sample analysis the filament and electron multiplier were not turned on until 4 min into the run. Data were collected in the selected ion monitoring mode (SIM) with two ions monitored per compound (Table 2). Diaminochlorotriazine, deethylatrazine and deisopropylatrazine had dwell times of 200 ms. Atrazine and simazine had dwell times of 100 ms. Quantitation was performed using an external standard calibration curve for the selected quantitation ion. A seven-point calibration curve was used with a range of 0.1–5 ng/ml equivalent in the samples or 0.02–1.0 ng injected. Confirmation was based upon the presence of a qualifier ion with abundance ratios of ±20% and a retention time of ±0.05 min compared to the standards. Despite efforts to alter chromatographic conditions to separate the four triazine analytes, overlapping retention times resulted in a reduction in dwell times and sensitivity. Therefore, separate injections were made for the determination of either DIA and atrazine or DEA and simazine.

An HP-5MS (crosslinked, 5% phenyl methylsilicone) capillary column (Hewlett-Packard) of 30 m×0.25 mm I.D., with a film thickness of 0.25 μm was used. Helium was used as the carrier gas at a flow-rate of 1 ml/min. Splitless injections of 2 μl were made using an autosampler. A 4 mm I.D. tapered, deactivated injection port liner was used and the injection port temperature was 280°C. The initial column temperature of 100°C was held for 1 min then programmed to 170°C at 40°C/min, then to 200°C at 3°C/min, and then to a final temperature of

Table 2
Selected ions for mass spectrometry of triazine metabolite and parent compounds

Compound	Mass/charge (m/z)			
	Retention time (min)	Quantitation ion	Qualifier ion	MDL (ng/ml)[a]
Diaminochlorotriazine	4.90	144.95	146.95	nd
Deisopropylatrazine	5.61	173.05	158.05	0.06
Deethylatrazine	5.76	172.05	174.05	0.03
Simazine	6.90	186.05	201.00	0.04
Atrazine	7.04	200.05	215.05	0.05

[a] Assuming 200 ml sample volume.

Table 3
Factor analysis in 0.5 g column test

Factor	Description	Significant $F(0.05)$		Recovery effect
		DIA	DEA	
Concentration	0.3, 3.0 ng/ml	No	No	None
Phase	C_{18}, Polar C_{18}	Yes	Yes	Polar C_{18} > C_{18}
Volume	100, 200, 500 ml	Yes	Yes	Higher volume = lower recovery
Parent	None, 3.0 ng/ml both atrazine and simazine	Yes	Yes	Parent reduces recovery of metabolite

250°C at 40°C/min with a final hold time of 4 min. Minimum detection limits (Table 2) were determined using EPA Method 525.2 [21].

3. Results and discussion

Atrazine and simazine were unaffected by any of the factors we tested, with average recoveries of 105% and 101%, respectively. Therefore, no further discussion of these recoveries will be included. Recoveries of <8% were observed for diaminochlorotriazine, so none of the extraction procedures we tested are appropriate for this metabolite. The lack of either alkyl group appears to reduce its hydrophobic interactions with the sorbent. Recoveries of any of the triazines tested were not affected by raising the pH to 10.

Recoveries of DIA and DEA were significantly affected by sorbent phase, extraction volume and presence of parent triazines (Table 3). Interactions between the factors were not significant at the $F(0.05)$ level. The effects of phase, volume, parent presence are evident when the results are broken out into the various combinations for the 0.5 g columns (Figs. 1 and 2). DIA recovery was low (<70%) for the conventional C_{18} phase in any combination of factors, but it was >80% for the polar C_{18} phase for the 100 ml extraction volume except when DIA, atrazine and simazine were all present at 3.0 ng/ml. DEA recoveries were higher than those for DIA for any combination of factors, and again the polar C_{18}

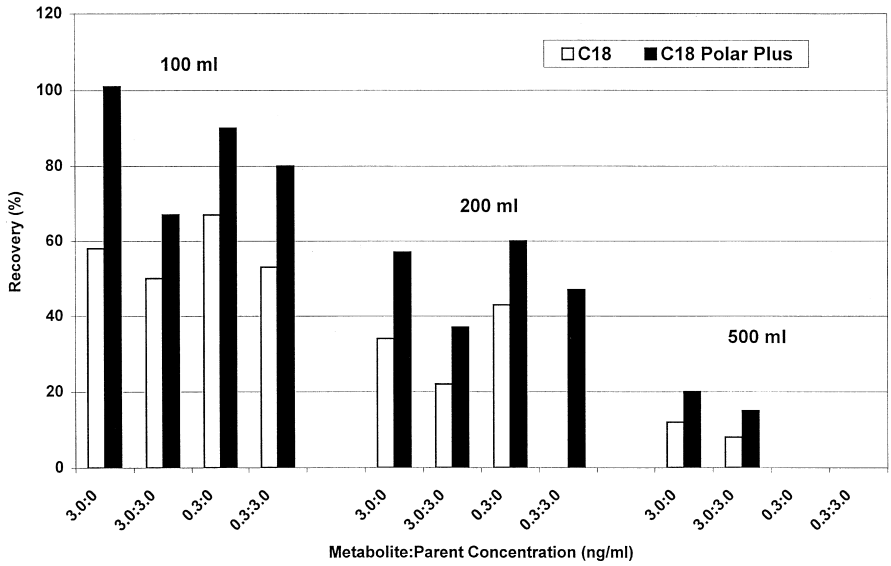

Fig. 1. Effects of sample volume, sorbent phase and metabolite/parent ratio on recovery of DIA from laboratory water using 0.5 g C_{18} SPE columns.

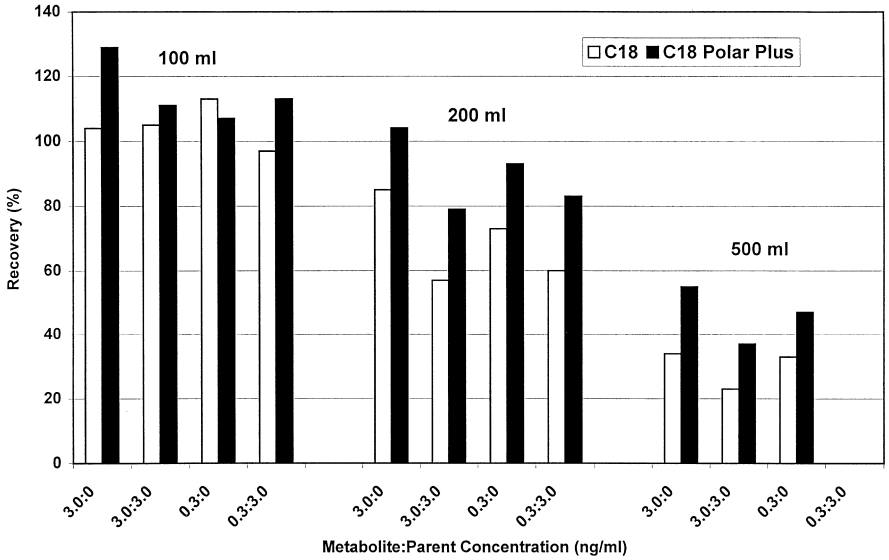

Fig. 2. Effects of sample volume, sorbent phase and metabolite/parent ratio on recovery of DEA from laboratory water using 0.5 g C_{18} SPE columns.

phase outperformed the conventional C_{18}. Increasing extraction volumes decreased recoveries and were $<$60% above 100 ml for DIA and above 200 ml for DEA, as would be expected for weakly sorbed analytes [22–25]. The presence of atrazine and simazine at 3.0 ng/ml also reduced recoveries of both metabolites.

The results of experiment 1 indicated that the presence of the free silanols in the adsorbent provided a polar binding mechanism for DIA and DEA which improved recoveries significantly. This is apparently the intent of the manufacturer, which actually uses frontal chromatography with DIA as an indicator of quality control for batches of PolarPlus C_{18} packing (Maria Bacolod, Mallinckrodt Baker, personal communication). However, using the 0.5 g cartridges would reduce our extraction volume to 100 ml for acceptable recoveries of DIA. This would restrict us to a detection limit of 0.2 ng/ml, which would prevent us from detecting the levels of residues often found in natural waters and above the level set by the European Economic Community for regulatory action [26]. This led us to try increasing the sorbent amount for increased capacity, which would allow us to extract at least 200 ml in order to reduce our minimum detection limits to 0.1 ng/ml or lower.

The larger column size increased recoveries sub-

Table 4
Recovery of DIA and DEA from laboratory water using two sizes of polar C_{18} SPE columns

Column size	Volume, metabolite:parent ratio	Recovery (%)	
		DIA	DEA
1.0 g	200, 1:0	100 (10)	111 (3)
	200, 0.1:1	90 (12)	117 (0)
	500, 1:0	61 (38)	101 (8)
	500, 0.1:1	56 (10)	91 (17)
2.0 g	500, 1:0	78 (3)	99 (1)
	500, 0.1:1	82 (14)	106 (5)

Standard deviations given in parentheses.

stantially for both compounds (Table 4). DIA recoveries were still affected by volume and cartridge size but averaged 90% or better at 200 ml for the 1.0 g size. DEA recovery from 500 ml extraction volumes increased from <60% in the 0.5 g columns to >90% in the 1.0 g columns, and there was apparently more capacity than needed at the 2.0 g size. While the maximum sensitivity is achieved at 500 ml for the 2.0 g columns, the extraction takes well over 2 h compared to 30–45 min with the 200 ml and 1.0 g combination.

We selected a 200 ml sample size and a 1.0 g polar C_{18} column as the optimum for routine extraction of aqueous samples for pesticides and the two chlorotriazine metabolites. We have been using this method for the analysis of water samples from a mountain river, streams including agricultural drainage ditches, runoff from a golf course and ground water in shallow monitoring wells. For quality control, each round of samples collected involves a matrix spike of pesticides of interest in that project, usually including DIA, DEA, atrazine and simazine. All studies involve matrix spikes made in the field and in the laboratory. As a result, we have data collected over the last year which indicates the actual performance of this method.

The two chlorotriazine metabolites have been recovered consistently and reliably regardless of matrix (Table 5). It should be noted that the stream, river and runoff water varied considerably in the amount and composition of solids in the samples, depending on storm events and time of the year. The chromatography is very good for these compounds in the environmental samples tested, as shown in a spiked river sample in Fig. 3. Other compounds have also had acceptable recoveries in these samples (Table 5), with the exception of ametryn, which tends to have recoveries below 70% in monitoring well water samples. Although the atrazine and simazine reduced DIA and DEA recoveries in laboratory water, no reduction in DIA or DEA recoveries has been found in environmental samples due to the addition of parent or other pesticides. Since those samples were analyzed after the SPE testing, it is possible that our technique has improved with experience. Another explanation is that natural compounds which usually discolor the cartridges are binding to or lodging in the adsorbent and creating additional capacity.

Table 5
Recoveries of DIA, DEA and various pesticides from natural waters using the 200 ml and 1.0 g polar C_{18} SPE column combination optimized in our tests

Compound	Matrix	*n*	Mean recovery
DIA	Ground water	33	111 (10)
	Streams	10	105 (13)
	River	8	116 (11)
DEA	Ground water	33	113 (7)
	Streams	10	109 (10)
	River	8	117 (8)
Atrazine	Ground water	33	114 (7)
	Streams	10	116 (16)
	River	8	119 (8)
Simazine	Ground water	33	110 (6)
	Streams	10	115 (8)
	River	8	90 (21)
Lindane	River	9	105 (7)
Alachlor	Ground water	33	113 (11)
	Streams	10	115 (10)
Ametryn	Ground water	33	66 (36)
	Streams	10	107 (31)
Chlorothalonil	Runoff	6	122 (17)
Chlorpyrifos	Runoff	6	96 (14)
Diazinon	Runoff	6	103 (7)
Metalaxyl	Ground water	33	112 (23)
	Streams	10	112 (8)
	Runoff	6	132 (18)
Metolachlor	Ground water	33	125 (10)
	Streams	10	126 (11)
Terbuthylazine	Ground water	56	113 (1.4)
	Streams	69	109 (8)

Spiking levels varied from 0.5 ng/ml to 1.0 ng/ml. Standard deviations are shown in parentheses.

4. Conclusions

The polar C_{18} adsorbent appeared to have significant advantages over conventional C_{18} material in retaining the two major chlorotriazine metabolites DIA and DEA. Using a rapid, factorial analysis, we

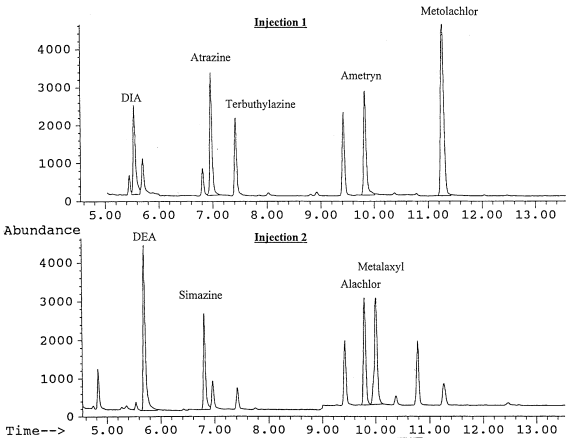

Fig. 3. Example total ion chromatograms of DEA, atrazine, DIA and simazine extracted from a spiked (1.0 ng/ml per compound) river water sample using a 200 ml sample size and a 1.0 g polar C_{18} SPE column. Two injections per sample are required and both are shown.

have determined that a 200 ml sample volume and a 1.0 g column size produced recoveries >90% for these analytes. This combination of sample and column size has been demonstrated to perform well for DIA and DEA as well as a number of pesticides in a wide range of water matrices.

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