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EVALUATION OF C_{18} SOLID-PHASE EXTRACTION CARTRIDGES FOR THE ISOLATION OF SELECT PESTICIDES AND METABOLITES

Key words: solid-phase extraction, pesticides, metabolites, C_{18}

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ABSTRACT

Nine different C_{18} solid-phase extraction (SPE) cartridges were evaluated for their efficiency at extracting nine pesticides and two *s*-triazine metabolites from spiked deionized water samples. The SPE cartridges were found to contain nitrogen (N) and/or phosphorus (P) contaminants and varied in their extraction efficiency for certain pesticides and metabolites. Four of the nine SPE cartridges gave acceptable (70 to 120%) pesticide and metabolite recovery percentages, while five cartridges had marginal (50 to 70%) to poor (< 50%) recoveries. Statistical analyses showed that the poor to marginal recoveries found for three compounds could not be explained by considering several indigenous chemical and physical traits of the cartridge. It is suggested that proper SPE cartridge

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selection for pesticide recovery should be evaluated using several different cartridges.

INTRODUCTION

Concern for water quality and public health has prompted the continued development and evaluation of analytical methods for pesticide extraction and quantification. Pesticide concentrations in unextracted water samples are often below instrument detection limits. Additionally, water is not a compatible matrix with gas chromatographic (GC) analyses. Pesticide detection using GC procedures requires an extraction and concentration step such as liquid-liquid extraction (LLE) or solid-phase extraction (SPE). Although pesticide extraction using LLE from biological matrices (fruits, vegetables, and animal tissues) is still an accepted practice, application to water matrices is time consuming, requires large amounts of organic solvents, and generates waste disposal costs (Vidal et al., 1994). On the other hand, SPE requires less solvent and time, and is capable of replacing many of the tedious LLE procedures for various chemicals in water samples.

Solid-phase extraction cartridges for the isolation of nonpolar to slightly polar pesticides typically consist of a solid-phase silicon bed with attached 18-carbon chain (C_{18}) functional groups. The C_{18} SPE cartridge has been used for extraction of triazines (Nash, 1990; Thurman et al., 1990; Watts et al., 1994), chloroacetamides (Macomber et al., 1992; Aga et al., 1994), and phenoxy herbicides (Wang and Huang, 1989) from a water matrix. In most of these

studies, pesticide recovery percentages from spiked samples ranged from 80 to 100%.

Selection of an appropriate C_{18} SPE cartridge that will retain the pesticide of interest and produce quantitative recoveries is a critical analytical procedure. Several different C_{18} cartridge types are commercially available. These cartridge types vary in bed, pore, and particle sizes as well as in % C and absence or presence of end caps. In spite of their popularity, we are unaware of any studies that have rigorously compared the ability of differing C_{18} cartridges to extract certain pesticides and metabolites. Our objective was to evaluate differing C_{18} cartridges for their ability to extract several pesticides and two *s*-triazine metabolites.

MATERIALS AND METHODS

SPE Cartridge Selection, Activation, and Pesticide Extraction

The C_{18} SPE cartridges chosen for this evaluation are shown in Table 1. Cartridges were chosen to obtain variation in physical and chemical properties. Each SPE cartridge was initially cleansed with ethyl acetate (EtOAc) and activated with methanol (MeOH). Pesticide extraction efficiency was evaluated at two spiking concentrations (50 and 100 μg L^{-1}). The two pesticide spiking solutions, which contained the 11 pesticides (except TBA) listed in Table 2, were prepared in MeOH. Fifty grams of deionized water were prepared in a beaker, 1.0 mL of the pesticide spiking solution was added, and then the solution was passed through the SPE cartridge. The compounds of interest retained

TABLE 1

Manufacturer, Physical, and Chemical Characteristics of Several SPE Cartridges.

Cartridge	Abbreviation	Bed Load	C	Bed Load C	Avg. Particle Size	Pore Size
		mg	%	mg	μm	nm
Alltech Extract-Clean C18	AEC	500	6	30	50	6
Alltech Extract-Clean C18 Hi-Load	AECHL	500	17	85	50	6
Baker Bond Octadecyl C18	BBOD	500	18.5	92.5	40	6
Baker Bond Polar Plus C18	BBPP	500	16.5	82.5	40	6
Supelco ENVI-18	SENVI18	500	17	85	40	6
Supelco LC-18	SLC-18	500	10	50	40	6
Varian Bond Elut C18	VBE	500	18	90	40	6
Waters tC18+	WtC18+	360	17	61.2	45	12.5
Waters C18 ENV	WC18ENV	1000	17	170	80	12.5

on the SPE cartridge were desorbed with EtOAc on a Visiprep Solid-Phase Extraction Vacuum Manifold (Model 5-7030M; Supelco, Inc., Bellefonte, PA)[1]. Unspiked deionized water samples were also extracted in a similar manner to determine the presence of eluting contaminants. The EtOAc extract from each SPE cartridge contained some residual water, which was separated by

[1] Mention of a trade-mark, proprietary product, or vendor is for information only and does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

TABLE 2

Agricultural Pesticides and Metabolites Investigated in Study.

Pesticide	Abbreviation	Chemical Name
alachlor	ALA	2-chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl)acetamide
ametryn	AMET	N-ethyl-N'-(1-methylethyl)-6-(methythio)-1,3,5-triazine-2,4-diamine
atrazine	ATR	6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine
cyanazine	CYAN	2-[[4-chloro-6-(ethylamino)-1,3,5-triazine-2yl]amino]-2-methyl-propanenitrile
deethylatrazine	DEA	6-chloro-N-(1-methylethyl)-1,3,5-triazine-2,4-diamine
deisopropylatrazine	DIA	6-chloro-N-ethyl-1,3,5-triazine-2,4-diamine
metalaxyl	METAL	N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-alanine methyl ester
metolachlor	METOL	2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide
metribuzin	METRIB	4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazine-5(4H)-one
prometon	PMT	6-methoxy-N,N'-bis(1-methylethyl)-1,3,5-triazine-2,4-diamine
prometryn	PRYN	N,N'-bis(1-methyethyl)-6-(methylthio)-1,3,5-triazine-2,4-diamine
terbuthylazine	TBA	6-chloro-N-(1,1-dimethylethyl)-N'-ethyl-1,3,5-triazine-2,4-diamine

centrifugation and removed using a small pipet and weighed. The EtOAc extract was then evaporated to < 1 mL, spiked with TBA (final concentration 100 μg L^{-1}), and the final volume adjusted to 1.0 mL with EtOAc. The pesticide extraction experiments were repeated nine times (triplicate runs performed on three different days) for each SPE cartridge. Detailed procedures of cleansing, activation, and pesticide loading are outlined by Novak and Watts (1996).

GC Conditions

All analyses were conducted on a Varian 3600 CX GC (Walnut Creek, CA) fitted with a Varian N and P detector and a Restek (Bellefonte, PA) RtX-35 (crossbond 35% diphenyl, 65% dimethyl polysiloxane), 30-m x 0.25-mm-I.D. capillary column. The GC operating conditions have been described (Novak and Watts, 1996).

Statistical Analyses

An analysis of variance (ANOVA) was used to statistically compare the mean percentage pesticide and metabolite recovery between the nine SPE cartridges. A Spearman Rank Order Correlation test was then used to evaluate relationships for compounds with poor to marginal mean recoveries vs. the % C, mg C-bed load, mg-bed load, average particle size, pore space, and residual water weight for each cartridge. All statistical tests were performed using SigmaStat software (San Rafael, CA).

RESULTS AND DISCUSSION

Cartridge Contaminants

Several researchers (Junk et al., 1988; Watts et al., 1994; Novak and

Watts, 1996) have shown that N- and/or P-containing contaminants from SPE cartridges can coelute and interfere with compound determination when using a GC with a N and P detector. These contaminants (alkanes, alkenes, plasticizers, and antioxidants) originated from compounds eluting from the C_{18} bonded porous silica, polypropylene housing, and the polyethylene frits (Junk et al., 1988). Novak and Watts (1996) noted that the contaminants were present in extracts from unspiked water samples even after extensive cartridge cleansing (3 to 6x with 1 mL portions) with EtOAc. Because of these findings, our initial concern about the different C_{18} SPE cartridges was to determine the presence of contaminants.

Extraction of an unspiked and spiked (50 $\mu g\ L^{-1}$) deionized water sample through an EtOAc-cleansed cartridge (WtC18+) showed that the extracts contained N- and/or P-containing contaminants (Fig. 1. A and B). The unspiked deionized water sample (Fig. 1A) contained a coeluting contaminant that had a similar retention time as ATR. Although all cartridges had at least one coeluting contaminant, a few of the cartridges had several (Table 3). The BBOD cartridge contained the most coeluting contaminants (seven), while two cartridges (BBPP and WtC18ENV) contained two. The presence of coeluting contaminants in extracts from these cartridges is a serious problem for pesticide monitoring at extremely low quantification levels (ρg to ηg pesticide weight on column). Our data suggest that extensive cleansing procedures or GC method modifications are necessary to correct for contaminants from the C_{18} SPE cartridges evaluated in this study.

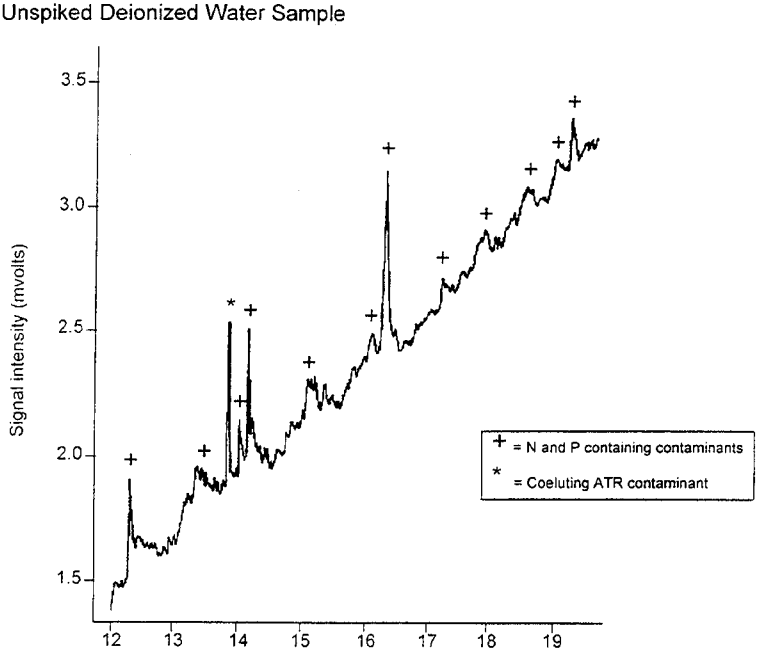


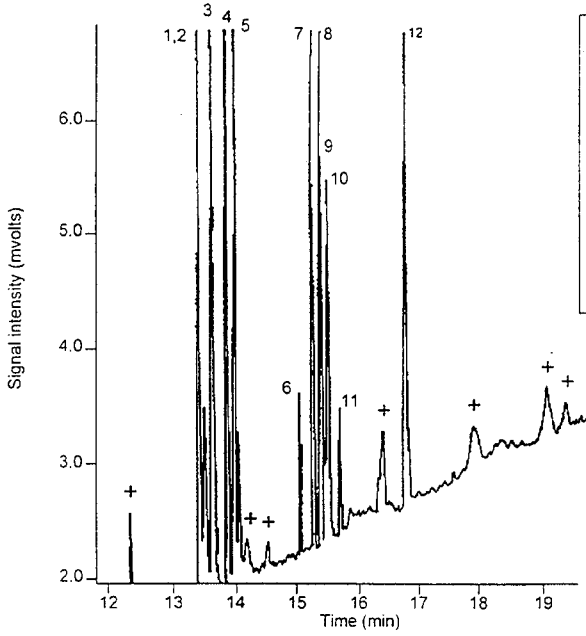
FIGURE 1

Chromatograms of (A) an unspiked deionized water sample and (B) a 50 μg L^{-1} spiked deionized water sample (both samples extracted using a WtC18+ SPE cartridge).

Pesticide and Metabolite Recovery

Mean (n = 9) SPE pesticide and metabolite recovery percentages using spiking solutions at 50 and 100 μg L^{-1} are shown in Tables 4 and 5, respectively. Recoveries were corrected for background contaminants when necessary. The overall mean recoveries ranged from 3 to 115%. The wide range of standard deviations (4 to 34%) suggests that there was also some recovery variation

(B) 50 μg L^{-1} Spiked Deionized Water Sample



Cmpd	Retention Time (min)	Peak #
DEA	12.897	1
DIA	12.980	2
PMT	13.100	3
ATR	13.348	4
TBA	13.451	5
ALA	14.516	6
PRYN	14.725	7
AMET	14.854	8
METAL	14.934	9
METRIB	14.982	10
METOL	15.172	11
CYAN	16.267	12

FIGURE 1 Continued

between extraction days. The wide range of standard deviations is not unusual because variations in cartridge packing between production lots may occur (Steve Klose, Varian Representative, 1996, personal communication).

Pesticide recovery trends, in general, were similar at the 50 and 100 μg L^{-1} spiking concentrations (Tables 4 and 5). Both tables show that pesticide and metabolite recoveries were influenced by SPE cartridges. The SPE cartridges AEC, VBE, WtC18+, and WC18ENV had significantly lower (ANOVA, $P <$ 0.05) recoveries for DEA, DIA, and PMT suggesting that these cartridges may

TABLE 3

Coeluting Contaminants From SPE Cartridges After Cleansing*.

Cartridge	ALA	AMET	ATR	CYAN	DEA	DIA	METAL	METOL	METRIB	PMT	PRYN	TBA
AEC	--	--	X	--	--	--	--	--	--	--	--	--
AECHL	--	--	X	--	--	--	--	--	--	--	--	--
BBOD	X	X	X	X	--	--	--	--	--	--	--	X
BBPP	--	--	X	--	X	--	--	--	--	--	--	--
SENVI18	--	--	X	--	--	--	X	X	--	X	--	--
SLC18	--	--	X	--	--	--	--	--	--	--	--	--
VBE	--	--	X	--	--	--	--	--	--	--	--	--
WtC18+	--	--	X	--	--	--	--	--	--	--	--	--
WC18ENV	--	X	X	--	--	--	--	--	--	--	--	--

* X indicates a coeluting contaminant.

TABLE 4

Mean and Standard Deviation for the Percentage Pesticide and Metabolite Recoveries from 50 $\mu g\ L^{-1}$ Spiked Deionized Water Samples Using Several Types of C_{18} Cartridges.

	AEC		AECHL		BBOD		BBPP		SENVI18		SLC18		VBE		WtC18+		WC18ENV	
Cmpd	$\bar{x}$†	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd
ALA	104	12	90	12	98	21	97	17	91	14	101	25	92	12	110	23	115	30
AMET	101	10	82	10	88	16	77	13	85	9	91	15	71	18	102	12	98	15
ATR	82	16	72	9	82	15	80	12	72	8	93	21	77	15	79	15	89	14
CYAN	96	10	88	7	91	15	99	13	95	10	101	21	87	8	101	10	114*	16
DEA	61*	13	89	11	92	19	97	15	90	10	94	17	92	12	99	11	99	18
DIA	26*	13	84	9	88	16	96	13	85	9	74	23	87	12	44*	13	90	14
METAL	108*	10	86	11	93	18	88	10	91	8	93	17	87	20	107*	14	100	20
METOL	81	31	71	9	104*	29	94	34	67	9	94	23	96	8	99	25	101	30
METRIB	104	9	87	12	93	29	88	11	93	8	89	17	91	18	102	8	95	19
PMT	82	23	74	14	78	19	21*	11	84	10	85	15	23*	34	90	9	87	14
PRYN	100	10	84	8	97	15	86	10	86	7	94	19	82	17	112*	22	99	19

† $\bar{x}$ = mean and sd = standard deviation

* Significant at the 0.05 level of rejection

TABLE 5

Mean and Standard Deviation for the Percentage Pesticide and Metabolite Recoveries from 100 $\mu g\ L^{-1}$ Spiked Deionized Water Samples Using Several Types of C_{18} Cartridges.

	AEC		AECHL		BBOD		BBPP		SENVI18		SLC18		VBE		WtC18+		WC18ENV	
Cmpd	$\bar{x}$†	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd
ALA	84	8	93	20	86	8	86	13	84	14	95	14	80	11	91	12	95	10
AMET	83	11	78	15	75	13	81	10	73	13	90	17	62	27	88	16	87	9
ATR	96	11	92	11	98	19	99	13	94	17	100	18	94	10	95	13	103	12
CYAN	88	11	86	11	88	14	82	12	83	11	101	21	88	13	90	10	83	9
DEA	52*	18	89	16	89	20	93	17	85	16	95	16	83	11	90	18	89	13
DIA	3*	4	88	14	89	24	97	18	83	16	69	18	90	14	10*	12	41*	18
METAL	85	13	82	27	85	18	83	15	76	15	107	27	81	14	83	14	84	13
METOL	81	14	98	27	87	14	92	21	79	8	96	17	77	12	90	13	93	14
METRIB	79	10	81	16	76	6	85	10	76	12	86	14	79	12	82	8	80	11
PMT	65	8	76	15	74	23	65	23	75	16	74	12	19*	21	73	14	79	12
PRYN	80	11	79	16	72	10	80	8	71	13	86	13	68	18	82	9	84	9

† $\bar{x}$ = mean and sd = standard deviation

* Significant at the 0.05 level of rejection

not be ideally suitable for isolation of these compounds from water matrices. Low recoveries of DEA using the WtC18+ cartridge were also reported by Nash (1990), who recovered only 26% DEA from spiked samples. Novak and Watts (1996) also reported low recoveries for DEA and DIA of 60 and 19%, respectively, using the WtC18+ cartridge, and attributed the low recoveries to an increase in their hydrophilic nature because of a loss of the functional groups. Some significant pesticide recoveries of >100% for four of the cartridges were noted (Table 4). These high recoveries were attributed to analytical error during GC analyses.

Four cartridges (AECHL, BBOD, SENVI18, and SLC18) had mean pesticide and metabolite recoveries between 70 and 107%, indicating that these cartridges should provide acceptable recoveries of both parent compounds and *s*-triazine metabolites from water matrices. Although the BBPP cartridge showed acceptable pesticide recoveries for most compounds, poor to marginal PMT recovery occurred at both spiking concentrations (Tables 4 and 5). The recovery of PMT may be improved with extraction method modification (i.e., a longer cartridge drying time to remove entrapped water). Although the BBOD cartridge had acceptable recoveries for all the compounds, the large amount of eluting contaminants (Table 3) may limit application, especially with sensitive (ρg to ηg range) GC instruments.

Since some monitoring studies may target specific pesticides and metabolites, an arbitrary rating of SPE cartridge extraction performance by

TABLE 6

Application Ratings† for the Nine SPE Cartridges Using Mean Pesticide and Metabolite Recovery Percentages.

Compound	Rating
ALA	nine cartridges acceptable
AMET	eight cartridges acceptable; one marginal (VBE)
ATR	nine cartridges acceptable
CYAN	nine cartridges acceptable
DEA	eight cartridges acceptable; one marginal (AEC)
DIA	five cartridges acceptable; one marginal (ALC18), and three poor (AEC, WtC18+, and WC18ENV)
METAL	nine cartridges acceptable
METOL	eight cartridges acceptable; one marginal (SENVI18)
METRIB	nine cartridges acceptable
PMT	six cartridges acceptable; two marginal (AEC, BBPP) and one poor (VBE)
PRYN	eight cartridges acceptable; one marginal (VBE)

† Where acceptable (70-120%), marginal (50-70%), and poor (<50%).

compound is presented in Table 6. An acceptable level (> 70%) of recovery was found for all SPE cartridges for the isolation of ALA, ATR, CYAN, METAL, and METRIB in spiked deionized water. An acceptable level (> 70%) of recovery for AMET, DEA, METOL, and PRYN was found using eight of the cartridges. Proper SPE cartridge selection is critical for the isolation of DIA and PMT from water matrices because of the large number of poor to marginal cartridge recoveries for these compounds.

Statistical Analyses

The recovery data presented in Tables 4 and 5 indicate that several different SPE cartridges are suitable for isolation of certain pesticides and metabolites from water matrices. However, poor to marginal recoveries with respect to DEA, DIA, and PMT often occurred. The efficiency of extraction for these compounds may be strongly influenced by the physical and chemical traits of the cartridges. Poor extraction may occur if the conditions necessary to facilitate partitioning between the compound of interest and the C_{18} functional groups are not optimized.

Cartridge pore spaces, average particle size, bed weight, and % C in the functional groups are conditions that will affect the partition process. Therefore, it was speculated that some physical (mg-bed load, residual water, particle and pore size) or chemical (% C-bed and mg C-bed) cartridge trait may explain the low mean DEA, DIA, and PMT recoveries. The Spearman Rank Order Correlation test showed that there was no relationship between cartridge traits mentioned above vs. the mean DEA, DIA, and PMT recovery (r range 0.09 to 0.45 and *P* range of 0.14 to 0.81). This is an interesting finding considering that the cartridges varied in their chemical and physical properties (Table 2). There appears to be some other cartridge trait regulating the extraction efficiency for these compounds. This finding implies that proper selection of a SPE cartridge for the isolation of DEA, DIA, and PMT should be made on trial experimentation and not based upon the considered physical and chemical properties of the SPE cartridge.

CONCLUSIONS

The data demonstrated that pesticide and metabolite extraction efficiencies were influenced by the SPE cartridge. Four of the C_{18} SPE cartridges had acceptable recoveries for pesticides and metabolites in spiked deionized water samples. Two SPE cartridges may provide acceptable recoveries if the extraction and/or GC procedures were optimized for these cartridges. Three of the cartridges had poor metabolite recoveries, indicating that these three may be unsuitable for *s*-triazine metabolite monitoring studies. All of the SPE cartridges contained some eluting N- and/or P-containing contaminants that could potentially coelute with the compound of interest. It is recommended that for the extraction of pesticides from water samples, an extensive cartridge cleansing step be incorporated into the isolation method. The poor DEA, DIA, and PMT recoveries were found not to be related to the physical and chemical traits for each cartridge. The presence of eluting contaminants and the lack of a relationship between the cartridge chemical and physical properties suggest that proper column selection should be based on trial experimentation of several types of C_{18} SPE cartridges.

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