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Alternatives to methanol–water elution of solid-phase extraction columns for the fractionation of high log K_{ow} organic compounds in aqueous environmental samples

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ABSTRACT

A toxicity-directed method for fractionating non-polar organic toxicants using solid-phase extraction (SPE) is described in phase II of EPA's "Methods for aquatic toxicity identification evaluations". This method has been used very successfully to extract and fractionate acutely and chronically toxic complex effluents and ambient waters. However, when fractionating samples that contain very hydrophobic (high log K_{ow}) toxicants the methanol–water elution sequence requires modification for optimum results. An elution modification has been made to the phase II SPE fractionation method for use with aqueous samples which contain such compounds (*e.g.* sediment pore water). The modified elution and fractionation method has been found to be effective for the separation and isolation of a mixture of compounds with log K_{ow} values ranging from 2.5 to 7 from aqueous solution and for toxicants from a sediment pore water sample.

INTRODUCTION

It is a difficult task to identify the contaminants which cause toxicity in complex environmental samples. A chemical screening method such as priority pollutant analysis may fail to identify the toxicants because the sample may contain thousands of compounds and there is no assurance that those measured are responsible for the toxicity. One approach for separating toxic from non-toxic components is the use of toxicity guided fractionation [1–4]. This approach uses organism response as the "toxicity detector". The US Environmental Protection Agency (USEPA) has developed a method to identify acutely toxic compounds in aqueous environmental samples using freshwater fish or invertebrates as the toxicity detector. This method, entitled "Methods for aquatic toxicity identification evaluations" (TIE), uses a three phase approach: phase I, toxicity characterization [5]; phase II, toxicity identification [6]; and phase III, toxicant confirmation [7]. These procedures have been used successfully to identify toxicants in wastewater effluents [1,8,9], ambient water [10], and sediment pore water [11,12]. Typical toxicants identified were cationic metals, ammonia, chlorine, pesticides, and non-polar organics. The identification procedure for non-polar organic toxicants described in phase II of the TIE methods relies on reversed-phase chromatography to separate toxic from non-toxic sample components [13–15]. The reversed-phase chromatography is achieved with C_{18} solid-phase

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extraction (SPE) and a methanol–water solvent system. This method has been used very successfully to fractionate and isolate non-polar organic toxicants from complex effluents [1,2,8]. However, other aqueous samples, such as sediment pore water, often contain toxicants not typically found in effluents, which are not effectively fractionated by the phase II method. For example, compounds such as polyaromatic hydrocarbons, polychlorinated biphenyls and long chain aliphatic hydrocarbons have been implicated in the toxicity of sediment pore waters [16]. These compounds are highly hydrophobic, as is evidenced by their high log K_{ow} values. Such hydrophobic compounds are not well fractionated by the phase II method. To overcome this limitation a study was conducted which evaluated alternative solvent systems and alternate sorbents, to achieve optimum toxicant separation and recovery over a broad range of log K_{ow} values. One major limitation of the solvent system choice is that the fractionation method is toxicity directed, therefore all SPE fractions must be tested with aquatic organisms to track the toxicity. Methanol is one of the very few solvents that these organisms can tolerate at the percentage levels used in the aqueous exposure tests. When solvents were evaluated for their efficacy in eluting highly hydrophobic compounds we were left with very few choices that met our toxicity testing requirements. Problems such as high toxicity, the formation of azeotropes or immiscibility with methanol eliminated the use of solvents such as acetonitrile, acetone, diethyl ether and hexane. Methylene chloride was selected because of its solvent strength and because it can be removed from a methanol–methylene chloride mixture leaving a methanol fraction that can be tested for toxicity.

EXPERIMENTAL

Instrumentation

GC–MS. A Hewlett-Packard (Palo Alto, CA, USA) Model 5970B mass selective detector with a Model 5980A gas chromatograph, Model 7673A automatic liquid sampler and HP-UX series Chemstation.

HPLC. A Hewlett-Packard Model 1090 liquid chromatograph with a diode array detector and an Isco (Lincoln, NE, USA) Foxy fraction collector were used for HPLC analyses.

Materials

SPE. The two SPE sorbents evaluated were octadecylsiloxane (C_{18}) and octylsiloxane (C_8) bonded to silica. The SPE columns were obtained from J.T.Baker (Phillipsburg, NJ, USA) and contained 1 g of sorbent.

XAD. Two resins were evaluated: prepurified XAD-4 and XAD-7 obtained from Alltech Associates, Inc. (Deerfield, IL, USA).

High-purity water was obtained from a SuperQ system of Millipore (Bedford, MA, USA). High purity Burdick & Jackson (Muskegon, MI, USA) methanol, methylene chloride and high-purity water were used for all fractionations.

A test mixture containing compounds with a range of log K_{ow} values of 2.5 to 7.0 was used to evaluate chromatographic separations of the SPE and XAD resins. Chemicals in the test mixture and their estimated log K_{ow} are listed in Table I. The stock solutions of the test mixture compounds were prepared in acetone, and the sorbent loading solution contained approximately 10 μg/l of each compound in high-purity water with 5% methanol.

Methods

SPE loading and fractionation. A 1-g SPE column was conditioned by pumping 25 ml methanol through the column, followed by 25 ml high-purity water. A 950-ml volume of loading solution (unfiltered) or sediment pore water (filtered through a 0.45-μm nylon filter) was then passed through the column at a rate of 5 ml/min. The post-column so-

TABLE I

TEST MIXTURE COMPOSITION

Compound	Abbreviation	Estimated Log K_{ow}[a]
Diethyl phthalate	DEP	2.57
Naphthalene	NAPH	3.32
Phenanthrene	PHEN	4.49
Chrysene	CHRY	5.66
Hexachlorobenzene	HCB	6.42
p,p'-DDE	DDE	6.94

[a] Calculated using the CLOGP program of A. J. Leo, Pomona College, Claremont, CA, Medchem Project, CLOG-3.3 computer program, 1985.

TABLE II

COMPOSITION OF 11 RECOMMENDED FRACTIONS IN MODIFIED ELUTION SCHEME

Fraction	Composition of eluting solvents (%, v/v)		
	Water (%)	Methanol (%)	Methylene chloride (%)
1	75%	25	0
2	50	50	0
3	25	75	0
4	20	80	0
5	15	85	0
6	10	90	0
7	5	95	0
8	0	50	50
9	0	0	100
10	0	0	100
11	0	0	100

lution was collected and extracted with hexane. The hexane extract was concentrated and analyzed by GC–MS for breakthrough. The column was then eluted with 2 × 1.5 ml volumes of solvents such as those described in Table II. The resulting fractions were diluted to approximately 475 ml with high-purity water, extracted with hexane, concentrated, and analyzed by GC–MS.

XAD loading and fractionation. A glass chromatography column was packed with 5 ml XAD prepurified resin as a methanol slurry. The resin bed was then washed with 100 ml methanol, followed by 100 ml high-purity water. A 950-ml volume of loading solution was then passed through the column at a rate of 5 ml/min. The post-column solution was collected, extracted into hexane, concentrated, and analyzed by GC–MS for breakthrough. The column was then fractionated with 15-ml volumes of the methanol and methylene chloride solutions listed in Table II. These fractions were then diluted to approximately 475 ml with high-purity water, extracted into hexane, concentrated, and analyzed by GC–MS.

Original elution method. The 1-g C_{18} SPE column was eluted with two successive 1.5-ml aliquots of each of the following methanol–water mixtures: 25, 50, 75, 80, 85, 90, 95 and 100% (v/v) methanol in water. This resulted in eight 3-ml fractions.

Modified elution method. The modified elution scheme eliminates the 100% methanol fraction used in the original method, and adds four fractions, one 50%, and three 100% (v/v) methylene chloride in methanol fractions. The composition of the resulting eleven 3-ml fractions is shown in Table II.

Solvent exchange. Methylene chloride is toxic to aquatic organisms, even at very low concentrations. As a consequence, it must be removed from SPE fractions before the fraction can be tested for toxicity. This should be accomplished without risking the loss of fraction toxicants, and can be achieved by exchanging the methylene chloride fraction into methanol. Exchanging methylene chloride into methanol is relatively easy because of its volatility. The fractions to be exchanged (12 ml) are placed in a centrifuge tube with a PTFE stir bar and an additional 12 ml of methanol. The tube is placed in a 30°C water bath and stirred while a gentle stream of nitrogen is passed over the solution surface. When the volume of the solution reaches 3-ml, the sides are carefully rinsed with 3 additional ml of methanol, and the solution is reduced again to a final 3 ml volume. The recoveries of the test mixture chemicals using this method of solvent exchange are shown in Table III.

TABLE III

RECOVERIES OF TEST MIXTURE CHEMICALS USING SOLVENT EXCHANGE METHOD

Recovery data from one experiment. Abbreviations are listed in Table I.

	Test chemical					
	DEP	NAPH	PHEN	CHRY	HCB	DDE
Recovery (%)	82	69	85	108	81	100

GC–MS analyses. The quantitations of the test mixture chemicals were done using the selected ion monitoring (SIM) mode of the mass spectrometer. For each compound the two major ions and a qualifier ion were acquired. The sediment pore water fractions were analyzed using a full scan mode collecting data on 50–650 amu. Library searches of the resulting spectra were performed automatically by probability matching algorithms and by using the USEPA/NIH/NBS mass spectral library database.

RESULTS AND DISCUSSION

The original phase II TIE procedure of isolating non-polar organic compounds on a C_{18} SPE column was carried out on a sediment pore water. The toxicants were removed by the C_{18} SPE column from the pore water sample, but were not recovered in the methanol–water SPE fractions. However, when methylene chloride was added to the methanol–water elution scheme, toxicity recovered from the column was measured in the eluted fractions. With this initial success in reclaiming difficult to recover toxicity from pore water, further investigation was initiated to determine which elution mixtures of water, methanol and methylene chloride would yield the optimum recovery and separation of compounds over a log K_{ow} range of 2.5 to >5.

Original % recoveries SPE

The % recoveries from C_{18} SPE columns using the original phase II methanol–water elution scheme are listed in Table IV and Fig. 1a. The recoveries of the diethylphthalate, naphthalene and phenanthrene range from acceptable, in the case of phenanthrene, to very good. The percent recovery of the compounds with log K_{ow} greater then five drop off significantly to levels that would probably be undetectable in a toxicity test.

Methods development of elution scheme

The first change that was made to the elution scheme was to increase the number of 100% methanol elutions. When the number was increased to three there was no improvement for the higher log K_{ow} compounds. This was unexpected because these compounds could be eluted from a C_{18} HPLC analytical column using a methanol–water gradient. The next change was to add methylene chloride after the three 100% methanol fractions. The elution profile of this change is shown in Fig. 1b. The higher log K_{ow} compounds did elute in the methylene chloride fractions but there seemed to be two distinct peaks of the same compounds, one in the methanol–water elution portion followed by one in the methylene chloride–methanol fractions. This elution doublet could possibly be the result of a mixed-mode interaction (polar and non-polar) of the C_{18} SPE resin and the test compounds.

Modified % recoveries SPE

Using the modified elution scheme (Table II) the recovery of the higher log K_{ow} compounds increased while the double peak effect observed in the initial

TABLE IV

RECOVERIES OF TEST MIXTURE CHEMICALS USING ORIGINAL AND MODIFIED ELUTION SCHEMES

Recovery data from one experiment. Abbreviations are listed in Table I.

Elution scheme	Sorbent	Test chemical (total % recovery[a])					
		DEP	NAPH	PHEN	CHRY	HCB	DDE
Original	C_{18}	108	116	62	22	52	32
Modified	C_{18}	99	98	109	60	95	76
Modified	XAD-4	112	72	94	101	57	82
Modified	XAD-7	103	95	126	102	109	104
Modified	C_8	104	87	115	77	75	89

[a] Total % recovery = sum of all fraction recoveries.

scheme decreased. The double peak effect for phenanthrene could not be eliminated. The percent recoveries of the sorbent loading mixture compounds are listed in Table IV and Fig. 1c. This elution scheme was repeated using other sorbents commonly used in environmental analyses [17–19]. Two XAD resins, XAD-4 and XAD-7 were evaluated along with C_8 SPE resins. The results of these experiments can be seen in Table IV and Fig. 2. The evaluated resins could be substituted for C_{18} SPE

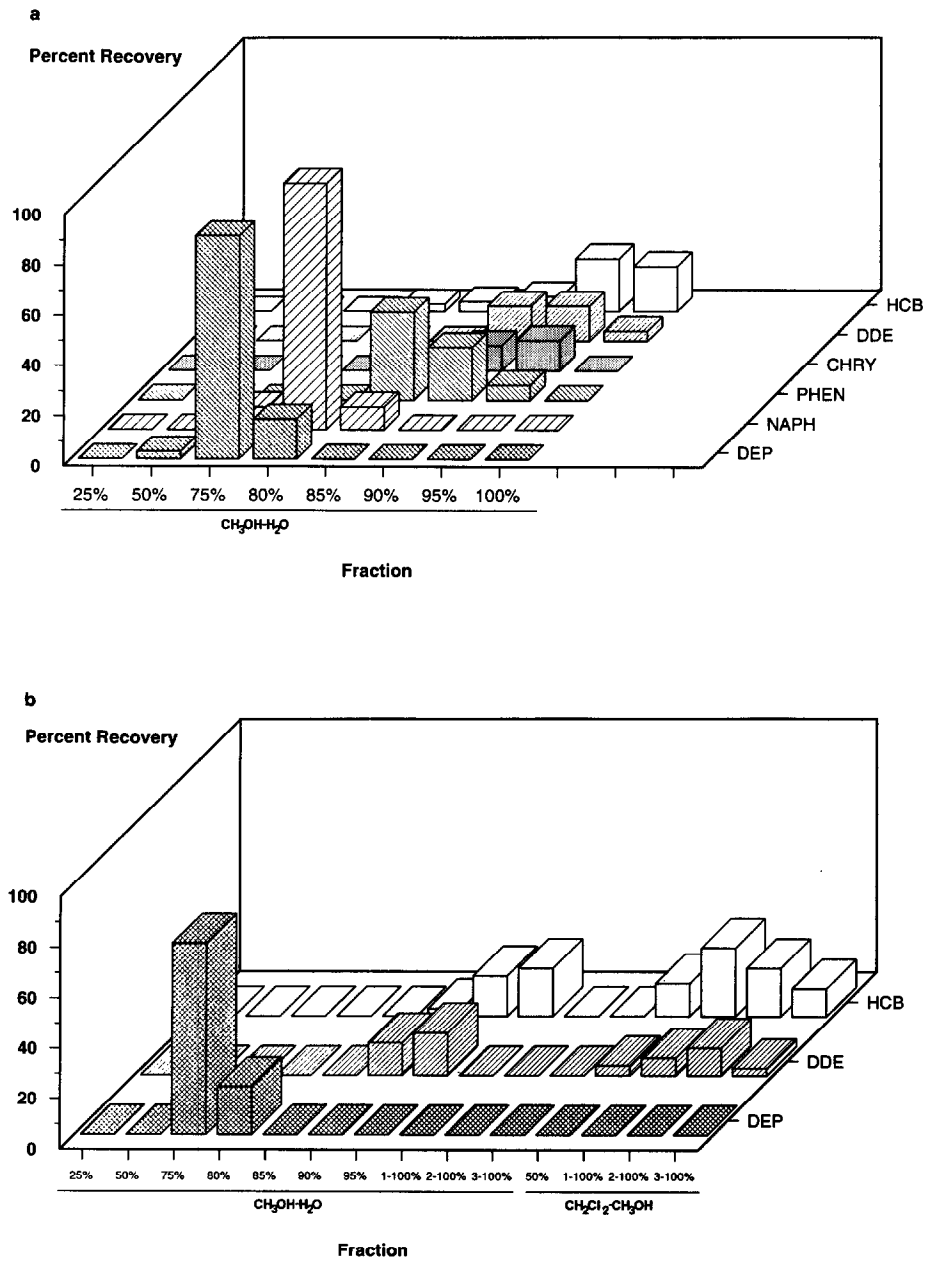

Fig. 1.

(Continued on p. 72)

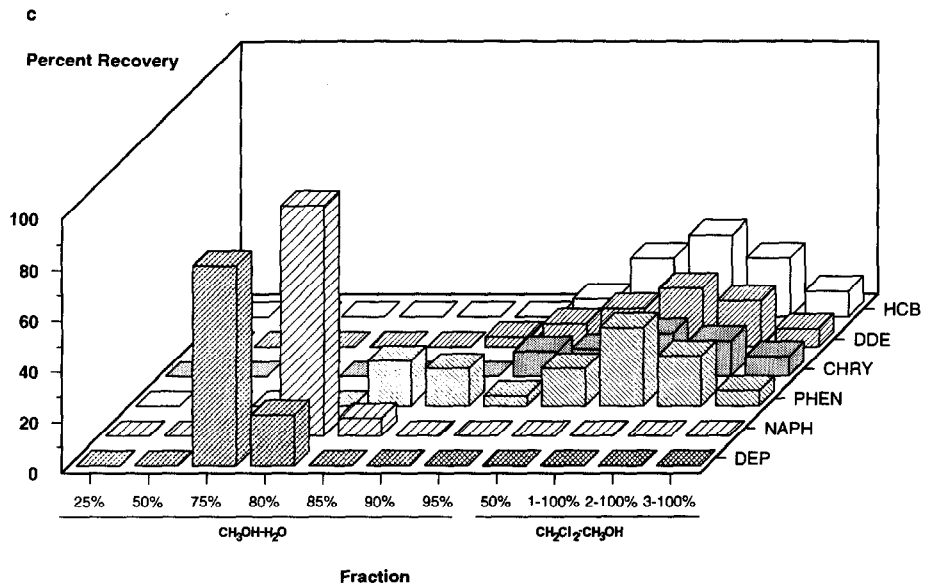

Fig. 1. (a) C_{18} SPE original elution scheme; (b) C_{18} SPE methanol extended elution scheme; (c) C_{18} SPE modified elution scheme.

and reasonable recovery results could be expected. Even though the total recoveries for the test compounds were very good for XAD-7 there was significant compound coelution and each compound eluted in three to five fractions. The ideal resin, and elution scheme would separate each compound from the others, and each chemical would elute in only one fraction.

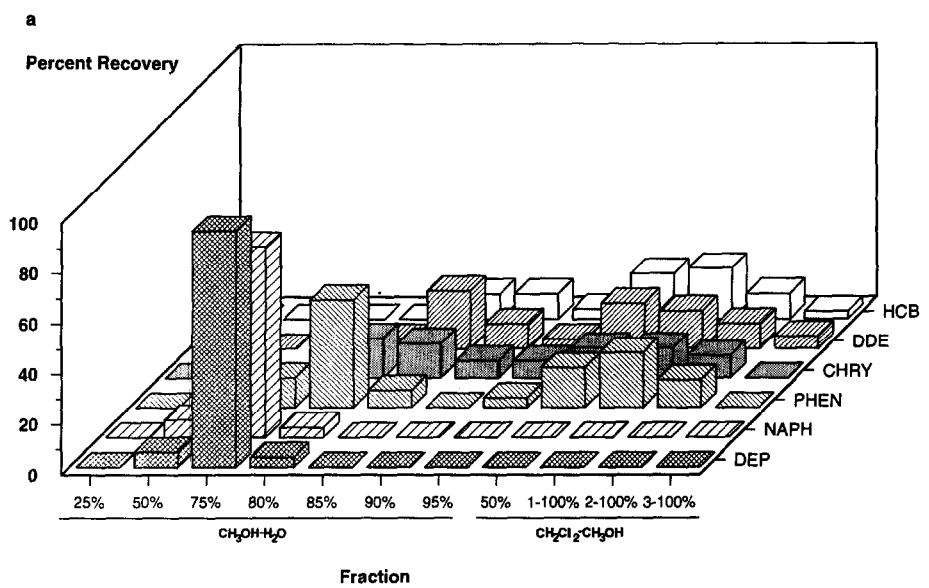

Fig. 2.

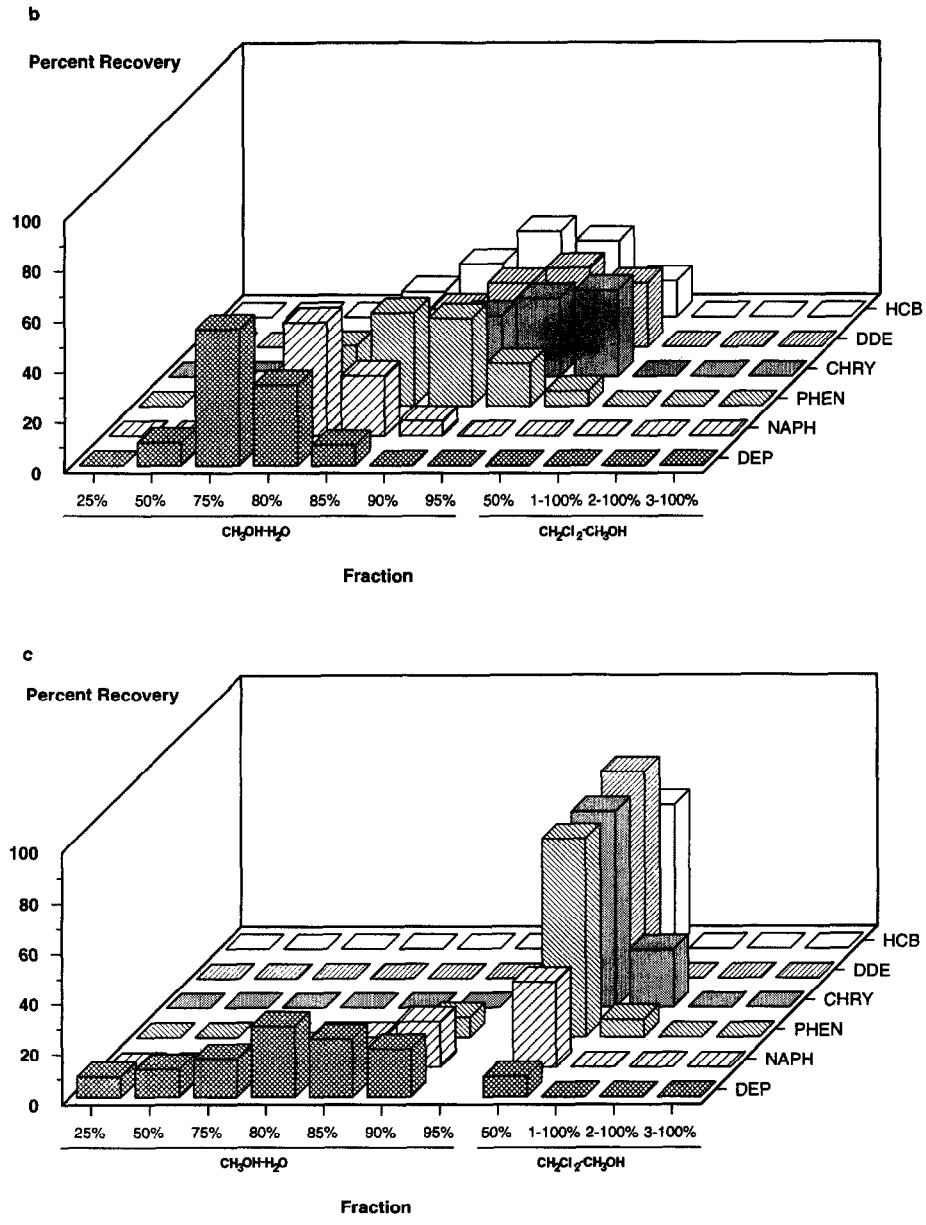

Fig. 2. (a) C_8 SPE modified elution scheme; (b) XAD-7 modified elution scheme; (c) XAD-4 modified elution scheme.

GC–MS identification of fractions of sediment pore water

The fractions collected from the pore water C_{18} SPE fractionation were analyzed using GC–MS. The resulting chromatograms were library searched and a list of tentatively identified compounds was compiled. In general, the results indicate that there are compounds (eleven in this case) that will be eluted from the C_{18} SPE column that cannot be removed by methanol–water elutions. These com-

TABLE V

AVERAGE LOG K_{ow} OF COMPOUNDS IDENTIFIED BY GC–MS IN SEDIMENT PORE WATER C_{18} SPE FRACTIONS

C_{18} SPE fraction	No. of compounds identified	Average log K_{ow}[a]
Methanol–water (25:75)	2	2.2
Methanol–water (50:50)	3	1.3
Methanol–water (75:25)	5	2.1
Methanol–water (80:20)	2	3.4
Methanol–water (85:15)	4	3.7
Methanol–water (90:10)	2	4.2
Methanol–water (95:5)	1	6.1
All methylene chloride fractions	11	7.0

[a] Calculated using the CLOGP program of A. J. Leo, Pomona College, Claremont, CA, Medchem Project, CLOG-3.3 computer program, 1985.

pounds generally have higher log K_{ow} values than the compounds in the methanol–water fractions. A list of all the fractions and the average log K_{ow} of the identified compounds can be seen in Table V.

CONCLUSIONS

The current phase II method for fractionating non-polar organic toxicants in aqueous samples does not effectively fractionate compounds that are highly hydrophobic. Modifications made to the SPE method have been successful in overcoming this limitation. An elution scheme incorporating water, methanol and methylene chloride has been designed that effectively elutes and fractionates compounds over a log K_{ow} range from 2.5 to 6.9. The higher log K_{ow} compounds, however, elute in the same set of fractions. Further fractionation by HPLC is needed to achieve better resolution of these kinds of compounds. Substituting other sorbents for the currently used C_{18} SPE resin has shown that both C_8 SPE and XAD-7 sorbents can be effective for the fractionation of particular kinds of toxicants.

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