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Development and optimization of a solid-phase extraction scheme for determination of the pesticides metribuzin, atrazine, metolachlor and esfenvalerate in agricultural runoff water

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ABSTRACT

Concomitant determination of the pesticides metribuzin, atrazine, metolachlor and esfenvalerate in agricultural runoff water was developed utilizing solid-phase extraction (SPE). A 2^5 factorial experimental design compared relative importance for extraction efficiency of the five variables sample pH, elution solvent strength, ionic strength of the sample, addition of organic modifier to the sample, and elution by gravity or vacuum. The protocol was further optimized with respect to sorbent mass, sample volume, elution volume and concentration. The approach offers optimal recoveries, low detection limits, rapid extraction, and final determination by either gas or high-performance liquid chromatography.

INTRODUCTION

Investigation of the impact of agricultural non-point source contamination necessitates the development of a rapid and accurate multi-residue, multi-class protocol for the determination of pesticides. A method utilizing solid-phase extraction (SPE) was developed for the determination of metribuzin, atrazine, metolachlor and esfenvalerate in agricultural runoff water. Concomitant analysis of these multi-class pesticides was desired as they may occur simultaneously in environmental matrices.

SPE is a chromatographic sample preparation technology applicable to the separation, purification and concentration of chemicals of environmental interest. SPE combines non-linear modes of chromatography; the sample loading or retention step is frontal chromatography, and the sample desorption or elution step is accomplished by stepwise (or gradient) desorption or displacement development [1–3]. SPE is an attractive alternative to traditional methods of extracting and concentrating organics from aqueous solutions. Chromatographic extractions are often less labor-intensive, use smaller volumes of organic solvents, and alleviate problems associated with the formation of emulsions as compared to liquid–liquid extractions.

Previous research on the development of SPE methods for pesticides [4] utilized an iterative approach to protocol development. Retention was first controlled while elution was optimized; subsequently, the variables affecting retention were optimized. In this research an alternative approach, a 2^5 statistical factorial design (5 variables at 2 levels), was utilized to quickly determine and optimize variables important to the SPE of metribuzin, atrazine, metolachlor and esfenvalerate. Application of the factorial ex-

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perimental design to optimization of recovery by SPE was recently published by Hannah *et al.* [5]. They used a 2^4 statistical factorial design (4 variables at 2 levels) requiring sixteen runs to optimize recoveries for a 27-component mixture of organic compounds. Hannah *et al.* studied the experimental variables sample pH (2 or 8), non-polar SPE strength (octyl or octadecyl bonded phases), polar SPE strength (cyano or diol bonded phases), and conditioning solvent concentration (0 or 500 ppm methanol). This research emulates their approach. For optimization of the recovery of pesticides, five variables were selected. Three factors related to sample modification included pH, ionic strength, and the addition of an organic modifier. The remaining two factors, related to elution, were eluotropic strength of the desorption solvent and mode of elution (by vacuum or gravity). In addition to providing a rapid screening tool for method development, the factorial approach increases understanding of the mechanisms of extraction and recovery during SPE by testing selected variables for significance.

EXPERIMENTAL

Chemicals and reagents

Methanol (HPLC or Optima grade), water (HPLC grade), ethyl acetate (Optima grade), phosphoric acid (HPLC grade), and potassium phosphate dibasic, potassium phosphate monobasic and sodium chloride (certified ACS grades) were obtained from Fisher Scientific, Fair Lawn, NJ, USA. Sodium chloride was baked at 400°C for 4 h before use.

Metribuzin [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5-(4H-one)], atrazine (2-chloro-4-ethylamino-6-isopropylamino-1, 3, 5-triazine) and metolachlor (2-chloro-N-(2-ethyl-6-methylphenyl) - N - (2-methoxy-1-methylethyl)-acetamide) were obtained from Fisher Scientific (PESTANAL, Riedel-de Haen). Esfenvalerate [(*S*)-α-cyano-3-phenoxybenzyl(*S*)-2-(4-chlorophenyl)-3-methyl butylate] was provided by DuPont (Wilmington, DE, USA). The chemical abstracts numbers for metribuzin, atrazine, metolachlor and esfenvalerate, are 21087-64-9, 1912-24-9, 51218-45-2 and 66230-04-4, respectively.

Sample preparation

Spiked samples were prepared in amber glass bottles with PTFE-lined caps. For the factorial design experiment, 100 ml of the appropriate combinations of phosphate buffer for pH control (0.1 *M*), ionic strength modifier (sodium chloride), and organic modifier (methanol) were spiked with a 100 $\mu g\ ml^{-1}$ methanolic stock solution of the four pesticides to produce a sample concentration of 1.00 $\mu g\ ml^{-1}$ in each compound. For further optimization studies, samples of variable concentration and variable volume were prepared in a similar manner. Authentic agricultural runoff samples, potentially containing the pesticides of interest, were provided by the University of Tennessee Plateau Experiment Station in Crossville, TN, USA.

Extraction apparatus/procedures

All solid-phase extractions were conducted with C_{18} Mega Bond Elut columns (1.0 g sorbent) and a Vac Elut extraction manifold (Varian Sample Preparation Products, Harbor City, CA, USA). PTFE tubing (1/16 in. inside diameter; 1 in. = 2.54 cm) was connected to the columns through reservoir adapters to transfer the conditioning solvents and samples. The extraction columns were conditioned by passing 10 ml of methanol through the column followed by 10 ml of the appropriate phosphate buffer (0.1 *M*, pH 2 or 7) according to the pH of the sample to be analyzed. Sample loading was performed under vacuum (380 Torr; 1 Torr = 133.322 Pa). The sorbent was never allowed to dry during the conditioning and sample loading procedures. For the factorial design experiment, sample desorption was performed with vacuum (380 Torr) or by gravity (740 Torr). The first fraction was eluted with 9.5 ml of methanol or ethyl acetate according to the factorial design, and the second fraction was eluted (by vacuum) with 9.5 ml of ethyl acetate. The sample container was rinsed with the first fraction of the elution solvent prior to desorption. The elution solvent for the second fraction was added directly to the sorbent. For further optimization studies, sorbent mass, sam-

ple volume and elution volume were varied to assess optimal conditions. In addition, independence to sample component concentration in the range of 10 to 1000 ppb was established.

Instrumentation

Analysis of the sample extracts was completed by HPLC–diode array detection (DAD) for the methanol extracts and GC–electron-capture detection (ECD) for the ethyl acetate extracts. The liquid chromatograph consisted of a Hewlett-Packard 1090M HPLC–DAD system and ChemStation data processing software, a Hypersil ODS (250 mm × 4 mm I.D., 5 μm) analytical column and a Hypersil ODS (20 mm × 4 mm I.D., 5 μm) guard column (Hewlett-Packard, Avondale, PA, USA). The column was maintained at 40°C, the mobile phase flow-rate was 1.5 ml min^{-1}, and the injection volume was 25.0 μl. Methanol–phosphate buffer (pH 2) mobile phase was delivered as a solvent gradient consisting of methanol–0.1 *M* phosphate buffer (40:60) for 6 min followed by a linear ramp to methanol–0.1 *M* phosphate buffer (95:5) at 23 min. The total run time of 25 min was followed by a 3-min post run equilibration to initial conditions. DAD was used to simultaneously monitor the absorbance maximum for each compound investigated: atrazine (221 nm), esfenvalerate (210 nm), metolachlor (204 nm) and metribuzin (204 and 298 nm) at a bandwidth of 4 nm. The reference wavelength and bandwidth were 450 nm and 50 nm, respectively. The detection limits by HPLC–DAD analysis, with 95% confidence intervals in parentheses, were metribuzin, 1.3 (0.5) ng; atrazine, 1.3 (0.4) ng; metolachlor, 5.0 (1.0) ng; and esfenvalerate, 2.5 (0.6) ng.

For gas chromatography a Hewlett-Packard 5890 GC–ECD system was used, with a 7673A automatic sampler, ChemStation data processing software, and a 15 m × 0.52 mm I.D. (0.5 μm film thickness) SPB-5 column (Supelco, Bellefonte, PA, USA). The direct injection volume was 1.0 μl. Injector and detector temperatures were maintained at 320°C. The carrier gas was helium at a flow-rate of 10 ml min^{-1}. Nitrogen was used as the ECD makeup gas to produce a total flow of 60 ml min^{-1} through the detector. The gradient temperature program consisted of 150°C for 2 min followed by a temperature ramp to 275°C at 15°C min^{-1} with a total run time of 15 min. The column was re-equilibrated to initial conditions for 2 min between samples. The detection limits by GC–ECD analysis, with 95% confidence intervals in parentheses, were metribuzin, 1.0 (0.1) pg; atrazine, 50.0 (7.0) pg; metolachlor, 49.0 (1.0) pg; and esfenvalerate, 1.02 (0.03) pg.

Data reduction

Statistical analysis of the data was conducted on a microcomputer with SAS (SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

Analytical determination by HPLC and GC

Before SPE studies began, methods for the final analytical determination of the analytes were developed. Metribuzin and atrazine are triazine herbicides, metolachlor is an acetanilide herbicide and esfenvalerate is a pyrethroid insecticide (Fig. 1). Although fortuitous for the purposes of this study, it is unusual that this group of four pesticides is amenable to analysis by both HPLC (Fig. 2) and GC (Fig. 3). Metribuzin and esfenvalerate were approximately 50 times more sensitive than atrazine or metolachlor to detection by GC–ECD. Sensitivity of the four pesticides by HPLC–DAD was within the same order of magnitude.

Design of the factorial screening study

A factorial experimental design (2^5) was employed as a screening device to statistically identify variables that would influence recovery efficiency by SPE of metribuzin, atrazine, metolachlor and esfenvalerate. In a 2^n factorial design, the value of each variable is restricted to only two levels. The factorial approach (5 variables at 2 levels) resulted in a design matrix of 32 sets of experimental conditions (Table I). The five variables studied consisted of sample pH (2 or 7); elution solvent strength [methanol (MeOH) or ethyl acetate (EtOAc)]; sample ionic strength [no sodium chloride added or 17.4% (w/v) added sodium chloride]; addition of

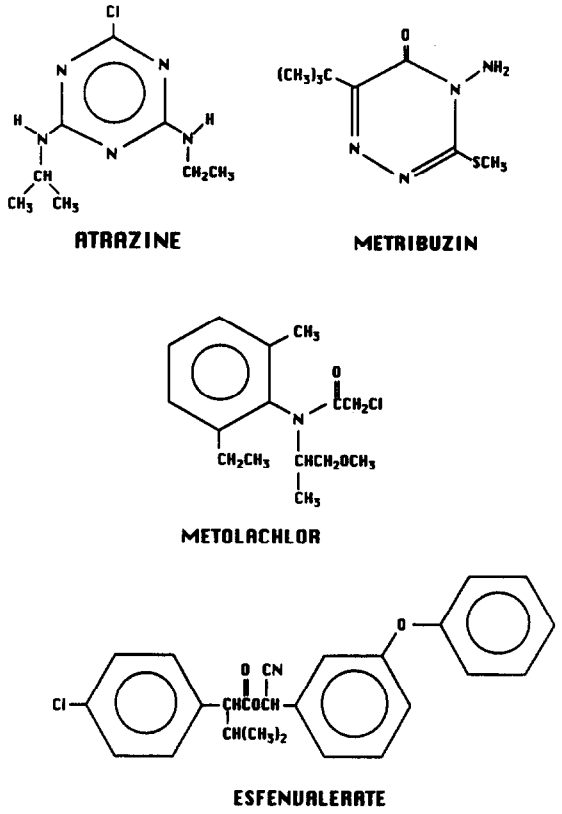

Fig. 1. Chemical structures of the herbicides included in this study.

organic modifier to the sample [no methanol added or 20% (v/v) methanol added]; and elution by vacuum (380 Torr) or gravity (740 Torr). The variables screened for an effect on recovery efficiency, and the levels at which they were tested, were arbitrarily chosen based on published literature and past experience with SPE.

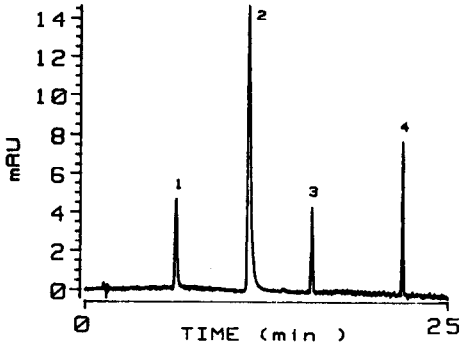

Fig. 2. High-performance liquid chromatogram of a standard mixture of (1) metribuzin, (2) atrazine, (3) metolachlor and (4) esfenvalerate at 221 nm.

Although the SPE of many compounds by octadecyl sorbents is pH dependent [4], in this case the levels selected for sample pH (*i.e.*, 2 and 7) were chosen mainly with the background matrix in mind. For this and future studies, it was desired to optimize recovery of these pesticides at each of these pH values in order to study the effects of concurrent recovery of macromolecular fulvic and humic acids potentially present as interferents in agriculturally derived sample matrices. (The pK_a of humic acids is estimated to be approximately 5.5.) Possible interferences from dissolved organic material during the SPE of pesticides from water were recently examined by Johnson *et al.* [6].

Adding solutes that increase the ionic strength of the sample has been used to improve extraction and recovery of analytes by SPE [7]. As in liquid–liquid extraction, it is assumed that the role of additional electrolyte is to enhance the salting-out effect on organic analytes in aqueous solution in contact with a hydrophobic phase. The addition of salt may also counteract secondary interferences from negatively charged silanol groups present on reversed-phase surfaces. The level of added ionic modifier used in this study (17.4%) is the same as that published by Schuette *et al.* [8] for the SPE determination of herbicides including atrazine and metolachlor.

The remaining factors in the experimental design (*i.e.*, eluotropic strength of the elution solvent, addition of organic modifier to the sample, and elution by vacuum or gravity) were included to elucidate effects on the recovery of compounds differing in hydrophobicity. The octanol–water partition coefficients (log P) of the pesticides investigated are metribuzin (1.65), atrazine (2.68), metolachlor (2.9) and esfenvalerate (>4), a relative order of hydrophobicity also evident in the reversed-phase HPLC elution pattern (Fig. 2). Highly hydrophobic compounds have notoriously poor recovery from octadecyl sorbents by SPE. Generally, very hydrophobic compounds adsorb strongly making desorption difficult. Therefore, two desorption solvents, methanol and ethyl acetate, respectively representing low and high eluotropic strength relative

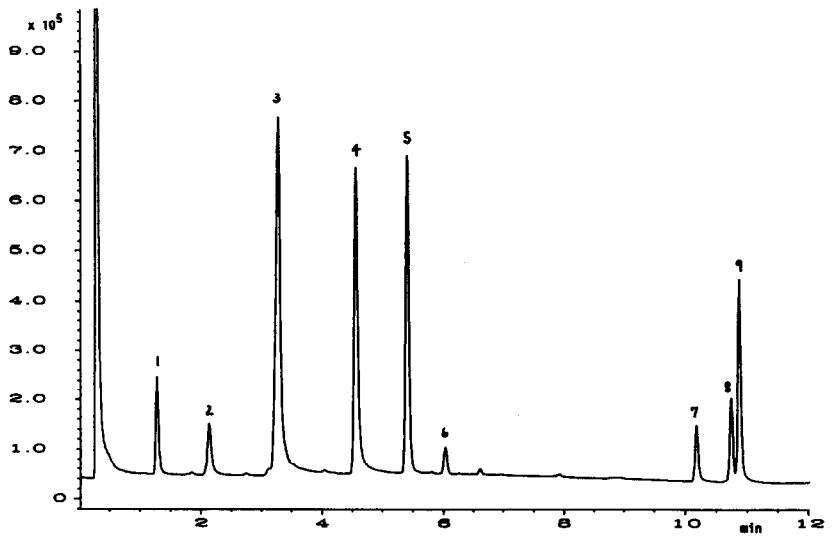

Fig. 3. Gas chromatogram of a standard mixture of (1) impurity, (2) impurity, (3) atrazine (55 ng), (4) metribuzin (376 pg), (5) metolachlor (4.9 ng), (6) impurity, (7) impurity, (8) impurity and (9) esfenvalerate (720 pg). *y*-Axis: ECD detector response.

to reversed-phase sorbents, were tested to investigate the effect of solvent strength on SPE elution.

The addition of organic modifier to the sample was examined by comparing the results from samples to which no solvent was added with those obtained from samples containing 20% methanol. Adding trace amounts of methanol to the sample may be necessary to maintain conditioning of the stationary phase throughout the extraction of large volumes of aqueous samples [7]. However, in the statistical factorial design of Hannah *et al.* [5], levels of 0 or 500 ppm methanol added to the sample were tested with inconclusive results. In this study, the primary purpose of adding methanol was not to maintain conditioning of the sorbent but to modify the retention factor of the analytes on the column. Even in a totally aqueous sample, a solute will have a finite retention factor. At some point, as the sample is continuously added to the column, a breakthrough volume will be reached. Addition of organic modifiers to the sample, such as methanol, will reduce the breakthrough volume, and may potentially improve recovery of highly hydrophobic compounds. The addition of methanol to the sample also alters the character of the hydrophobic octadecyl surface. Reversed-phase sorbents become enriched with the organic modifier, depending upon the percentage of water present [9]. The solvation layer reaches a maximum in pure methanol [10] while there is no solvation layer in pure water [11]. This effect may also influence the manner in which the analyte interacts with the stationary phase, and may ultimately influence the ease of recovery by SPE.

The mode of elution was also included as a factor in the experimental design. In unpublished research on the SPE of chlorobenzenes, it was observed that as the hydrophobicity of the solute increased, so did the importance of allowing elution to occur by gravity rather than by the normal mode of vacuum elution. The effect appears to result from slow mass transfer for very hydrophobic compounds from the stationary phase into the mobile phase.

During the screening study, certain factors were not allowed to vary. The mass of the sorbent (1.0 g), the sample volume (100 ml), and the sample concentration (1 ppm) remained constant. A 1.0-g mass of C_{18} sorbent has become a standard starting point in SPE studies [4] as a reasonable compromise for method development for groups of compounds of widely ranging hydrophobicity. A sample volume of 100 ml was

TABLE I

2^5 FACTORIAL DESIGN MATRIX

Run type	pH	Eluotropic strength	Ionic strength	Added MeOH	Vacuum/ gravity
1	2	MeOH	–	–	Vacuum
2	7	MeOH	–	–	Vacuum
3	2	EtOAc	–	–	Vacuum
4	7	EtOAc	–	–	Vacuum
5	2	MeOH	NaCl	–	Vacuum
6	7	MeOH	NaCl	–	Vacuum
7	2	EtOAc	NaCl	–	Vacuum
8	7	EtOAc	NaCl	–	Vacuum
9	2	MeOH	–	20	Vacuum
10	7	MeOH	–	20	Vacuum
11	2	EtOAc	–	20	Vacuum
12	7	EtOAc	–	20	Vacuum
13	2	MeOH	NaCl	20	Vacuum
14	7	MeOH	NaCl	20	Vacuum
15	2	EtOAc	NaCl	20	Vacuum
16	7	EtOAc	NaCl	20	Vacuum
17	2	MeOH	–	–	Gravity
18	7	MeOH	–	–	Gravity
19	2	EtOAc	–	–	Gravity
20	7	EtOAc	–	–	Gravity
21	2	MeOH	NaCl	–	Gravity
22	7	MeOH	NaCl	–	Gravity
23	2	EtOAc	NaCl	–	Gravity
24	7	EtOAc	NaCl	–	Gravity
25	2	MeOH	–	20	Gravity
26	7	MeOH	–	20	Gravity
27	2	EtOAc	–	20	Gravity
28	7	EtOAc	–	20	Gravity
29	2	MeOH	NaCl	20	Gravity
30	7	MeOH	NaCl	20	Gravity
31	2	EtOAc	NaCl	20	Gravity
32	7	EtOAc	NaCl	20	Gravity

representative of reasonable volumes encountered in environmental applications, yet was small enough to yield quick sample throughput for screening purposes. The relatively high initial sample concentration in the screening phase of this study was designed to ensure that if fractional recovery was observed in adsorption and/or desorption stages, the partial recoveries would still be detectable. Following optimization of the five factors tested in the design matrix, the protocol was then optimized for sorbent mass and sample volume, elution volume and sample concentration.

While the clear advantage of a factorial experimental design is that it is particularly well suited to study screening for significant variables, there are two disadvantages to performing the factorial design screening test as it was conducted in this research. Recognizing the disadvantages of this approach at the outset of the project enables the researcher to determine whether it is unproductive for a given purpose. In the case of screening for the significance of five variables, 32 experiments are required. Each combination however, was tested only once. There were no replications in the screening portion of this study. Therefore, there was no direct means by which to determine the variance.

However, this was overcome by estimating the experimental error from the mean square errors of the six fourth- and fifth-order interaction terms. In non-replicated experiments, it is common to use some or all of the interaction mean squares as an estimate of the error variance [12]. The second disadvantage is the lack of experimental blanks. If a blank was generated for each situation, it would be necessary to double the number of samples. A blank would be required for each of the 32 conditions examined. In this study, replicated experiments with blanks as appropriate were conducted once the best variable combinations were selected by screening.

Statistical evaluation of the factorial screening study

By applying the factorial design outlined in Table I, a large amount of information was obtained with relatively few analyses (Table II). Evaluation of recovery data by analysis of variance (ANOVA) allowed determination of the variables and variable interactions that were

TABLE II

RECOVERIES FROM 2^5 FACTORIAL DESIGN MATRIX

Run type	Recovery (%)			
	Metribuzin	Atrazine	Metolachlor	Esfenvalerate
1	80.6[a]	95.8	99.6	99.8
2	83.3	97.0	97.2	91.7
3	50.0	43.0	73.1	55.1
4	78.1	79.9	99.6	56.3
5	87.4	98.3	94.0	0.8
6	101.0	98.0	94.7	36.2
7	57.7	55.0	85.8	52.7
8	76.9	81.4	95.2	61.6
9	56.0	97.6	98.0	91.2
10	62.8	98.7	99.7	0.8
11	39.7	42.2	104.9	107.0
12	60.3	91.2	111.6	69.3
13	77.1	100.6	114.4	78.8
14	103.6	98.9	99.3	85.7
15	40.6	50.3	95.3	75.2
16	88.1	97.3	114.5	96.2
17	91.0	95.2	97.1	86.9
18	97.4	97.5	97.8	83.6
19	49.8	75.8	87.9	70.9
20	88.2	97.2	95.5	76.9
21	78.3	90.5	98.0	90.9
22	98.0	98.4	97.6	87.1
23	83.6	84.8	93.5	85.1
24	92.8	86.0	95.3	83.0
25	56.1	95.9	97.4	84.0
26	69.6	98.6	98.1	88.8
27	83.2	71.9	94.9	75.0
28	77.8	113.2	95.5	88.3
29	48.2	87.5	98.6	91.8
30	94.7	100.1	99.7	88.8
31	88.7	84.1	94.3	90.8
32	79.5	81.0	92.6	93.6

[a] All recoveries are for the first eluted fraction.

significant to the SPE of these pesticides [12]. SAS programs were written to perform the statistical evaluation for the 2^5 factorial design. In the data matrix, the low level of a variable is indicated by -1 and the high level of a variable is indicated by $+1$. The F-test was used to evaluate significance of the main effects and second- and third-order interactions by comparing to the mean square error for the fourth- and fifth-order interaction terms. Comparisons were made at the 0.025 probability level. After the analysis of variance was used to evaluate the significant effects, standardized regression coefficients were determined by linear regression analysis (Table III). The magnitude of the standardized regression coefficients indicates the relative importance of the factors found to be significant. The sign of the standardized regression coefficients shows the level of the factor (plus for the high level, minus for the low level) that produces the best SPE recovery. The interaction terms are more difficult to interpret than the main factors but can be very revealing. The signs of the standardized regression coefficients for the interaction terms follow the rules for algebraic multiplication (*i.e.*, two pluses yield a plus, two minuses yield a plus, and a plus and a minus produce a minus). The ANOVA developed for the SPE recovery data will be discussed separately for each pesticide, and for summed pesticide recoveries.

Metribuzin. The ANOVA for metribuzin demonstrated that three main effects and one interaction term were significant to the recovery by SPE. Of the significant variables, sample pH most influenced recovery of metribuzin. Metribuzin recovery was best at a sample pH of 7 with added sodium chloride. Methanol added to the sample reduced the recovery of metribuzin. The detrimental effect on metribuzin recovery of adding methanol to the sample appears to be overcome by the simultaneous addition of sodium chloride (17.4%). The variable interaction significant to the recovery of metribuzin (BE) combines the remaining two main effects, *i.e.*, elution solvent/elution by gravity or vacuum. For metribuzin, the positive coefficient for the BE interaction term indicates that if elution is done under vacuum, methanol is the preferred elution solvent. If elution is allowed to occur by gravity, ethyl acetate produces better recovery.

Atrazine. Atrazine recovery was significantly affected at the 0.025 probability level by pH, eluotropic strength, and mode of elution (Table III). Standardized regression coefficients of the significant effects indicated that increased recovery was observed for atrazine with a sample pH of 7, methanol elution solvent, and elution

TABLE III

STANDARDIZED REGRESSION COEFFICIENTS OF SIGNIFICANT FACTORS

Variable	Standardized parameter estimates				
	Metribuzin	Atrazine	Metolachlor	Esfenvalerate	Summed recoveries[b]
A: pH	0.4954	0.4478	NS[a]	NS	0.5684
B: Elution solvent	NS	−0.5724	NS	NS	−0.3992
C: Ionic strength	0.3004	NS	NS	NS	NS
D: Added MeOH	−0.2931	NS	0.4486	NS	NS
E: Vacuum/gravity	NS	0.2413	NS	NS	NS
Interaction AB	NS	0.3539	NS	NS	NS
Interaction BE	0.2976	0.3185	NS	NS	0.2691
Interaction ABE	NS	−0.2253	NS	NS	−0.2897

[a] NS = Not significant at $\alpha = 0.025$. α is the probability of making a Type I statistical error.
[b] Summed recoveries of metribuzin, atrazine and metolachlor only, excluding esfenvalerate.

by gravity. Significant two- and three-level variable interactions of the main effects were also noted. Since the sign for the standardized regression coefficients for the interaction BE term for both metribuzin and atrazine is positive, the same argument can be used for atrazine as given earlier for metribuzin for this interaction. For atrazine, a positive sign for interaction AB indicates that at pH 2 the best elution solvent is methanol; while at pH 7, ethyl acetate produces better recovery. A negative sign for the ABE interaction (*i.e.*, pH/elution solvent/mode of elution) implies that optimal results will be achieved if any two of these effects are at the high level while the third effect is at the low level.

Metolachlor. Recovery of metolachlor was significantly affected only by addition of organic modifier to the sample. The positive sign of the standardized regression coefficient for this variable indicates that 20% methanol added to the sample improves the recovery of metolachlor. This strongly contrasts with the observations for metribuzin and atrazine in which all five of the variables examined were significant either as main effects or interaction terms. The most apparent explanation is the differing chemical nature of these pesticides (Fig. 1). Metribuzin and atrazine are ionizable while metolachlor is not.

Esfenvalerate. The analysis of variance for recovery data of esfenvalerate revealed no significant variables or variable interactions at the 0.025 probability level. Two factors are believed to have contributed to the lack of significance observed. Firstly, esfenvalerate was incompletely desorbed from the sorbent by the initial elution. In virtually every sample in the factorial study, esfenvalerate, in amounts up to 15% of the sample, was detected in the second elution fraction while no concentrations of metribuzin, atrazine or metolachlor greater than 2% were detected in the second fraction. Secondly, isomerization or degradation of esfenvalerate may have occurred during sample processing, thereby confounding results of the factorial study. Fenvalerate has two chiral centers, resulting in four stereoisomers or two sets of diastereomers. The esfenvalerate analytical standard used in this research was the 2*S*,α*S* stereoisomer (99.1% purity). In effluents from some of the screening studies, a second peak near the standard esfenvalerate peak was observed. Those treatment combinations that produced low recovery of esfenvalerate in Table III may indicate conditions inappropriate for SPE, or conditions that promote isomerization or degradation. Therefore, these factors cannot be distinguished statistically from the data collected.

Summed recoveries. In addition to optimizing the recovery of each of the pesticides examined, the ultimate goal of this research was to develop the best concomitant recovery of the pesticides. An analysis of variance was conducted on the summed recoveries of metribuzin, atrazine and metolachlor. Data for esfenvalerate recovery were omitted from the ANOVA. Sample pH was determined to be the single most significant variable. A sample pH of 7 using methanol as the elution solvent appears to be the best choice for this combination of analytes. The interaction terms BE and ABE were also significant.

The variables and levels chosen for this investigation are clearly interrelated. Variation in one can be offset by changes in another. For variable D (*e.g.*, added methanol), the sign of the standardized parameter estimate is negative for metribuzin and positive for metolachlor. Because metribuzin is less hydrophobic than metolachlor, methanol added to the sample (20%) reduces the breakthrough volume of metribuzin to the point that some of the analyte is lost during sample loading; whereas, for metolachlor, desorption is improved by reducing the retention factor of the more hydrophobic solute.

Optimization of SPE recovery data

Three of the 32, five-variable combinations in the factorial screening study (Table I) were selected for further optimization: run type 1 (pH 2, methanol elution by vacuum, no added methanol or sodium chloride); run type 2 (pH 7, methanol elution by vacuum, no added methanol or sodium chloride); and run type 16 (pH 7, ethyl acetate elution by vacuum with added sodium chloride and methanol). Sample volume,

elution volume, sorbent mass, and sample concentration were examined.

Run types 1 and 2, performed with elution by methanol, produce extracts appropriate for HPLC analysis, while run type 16 conditions yield an ethyl acetate extract that was analyzed directly by GC. Therefore, it was possible to determine the SPE conditions necessary to optimize both the HPLC and GC analyses for these compounds. Under conditions 1 and 2, recoveries are excellent for atrazine, metolachlor and esfenvalerate but less than desired for metribuzin. Since one-fifth of the sample missing from metribuzin recovery did not remain on the column (the second fraction does not account for it), it is assumed that metribuzin experienced breakthrough during sample loading. Doubling the sorbent mass used to extract the sample greatly improved the recovery of metribuzin, from 80.6 to 94.7% for run type 1 and from 83.3 to 99.3% for run type 2, and slightly improved the recovery of atrazine from 95.8 to 96.8% (run type 1) and 97.0 to 100.5% (run type 2). Predictably, the opposite effect was observed for metolachlor and esfenvalerate. Increasing the sorbent mass to 2.0 g decreased recovery of metolachlor from 99.6 to 93.7% (run type 1) and 97.2 to 93.3% (run type 2), and for esfenvalerate from 99.8 to 98.0% (run type 1) and 91.7 to 81.0% (run type 2). (Subsequently, the 2-g sorbent versions of runs 1 and 2 are denoted by addition of an asterisk.)

Replicated sample volumes of 100, 250, 500 and 1000 ml were examined for extraction efficiency. Recoveries for metribuzin, atrazine, metolachlor and esfenvalerate, respectively, from 1-l samples were 93.4, 98.0, 99.7 and 87.0% (run type 1*), 96.9, 99.3, 99.5 and 71.9% (run type 2*), and 15.5, 100.4, 95.7 and 95.7% (run type 16). Recovery is good from sample volumes as large as 1 l using 2 g of sorbent under conditions of run types 1* and 2*. In run type 16 (1 g of sorbent), metribuzin recovery decreases as sample volume increases.

Elution volumes of 3.0, 5.0, 7.0 and 9.5 ml were examined at corresponding sample volumes of 100 ml and component concentrations of 1.0 ppm. The smallest satisfactory volume for methanol elution from 2 g of sorbent (runs 1* and 2*) is 5.0 ml. As hydrophobicity increases from metribuzin to esfenvalerate, 3.0 ml is no longer adequate to completely elute the compounds from 2 g of sorbent. Elution under run 16 experimental conditions (ethyl acetate elution, 1 g sorbent) was quite good even at the 3.0 ml elution volume. The best concentration factor (*i.e.*, sample volume/elution volume ratio) achievable without evaporation by run type 1* or 2* (HPLC) is 1000/5.0 or 200-fold, and for run 16 (GC) is 100/3.0 or 33-fold.

Initial screening concentrations of 1 ppm were used in order to monitor the fractionation of the sample during SPE. To examine the variation in pesticide recovery with concentration, synthetic samples having component concentrations of 10, 25, 50, 100, 500 and 1000 ppb were extracted by methods 1*, 2* and 16, for sample volumes of 100 ml and elution volumes of 9.5 ml. Satisfactory results were obtained for pesticide recovery with the exception that metolachlor was not detectable at 10 ppb under these conditions. Further concentration can be realized by using larger sample volumes, smaller elution volumes or evaporation of the extract prior to analysis. Tabularized details of the optimization of SPE recovery data are available from the corresponding author upon request.

Application of methods developed

The purification achieved by SPE for authentic agricultural runoff water samples is illustrated in chromatograms generated by HPLC–DAD (Fig. 4) and GC–ECD (Fig. 5). Table IV compares

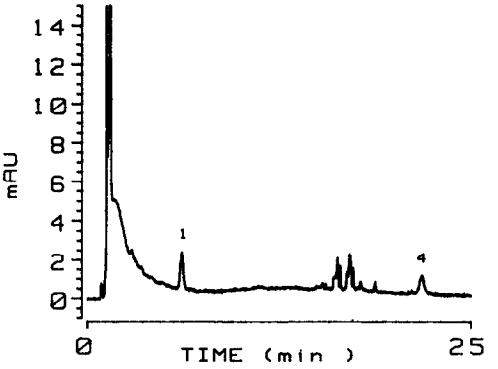

Fig. 4. High-performance liquid chromatogram of an authentic agricultural runoff sample in which (1) metribuzin and (4) esfenvalerate were detected at 221 nm.

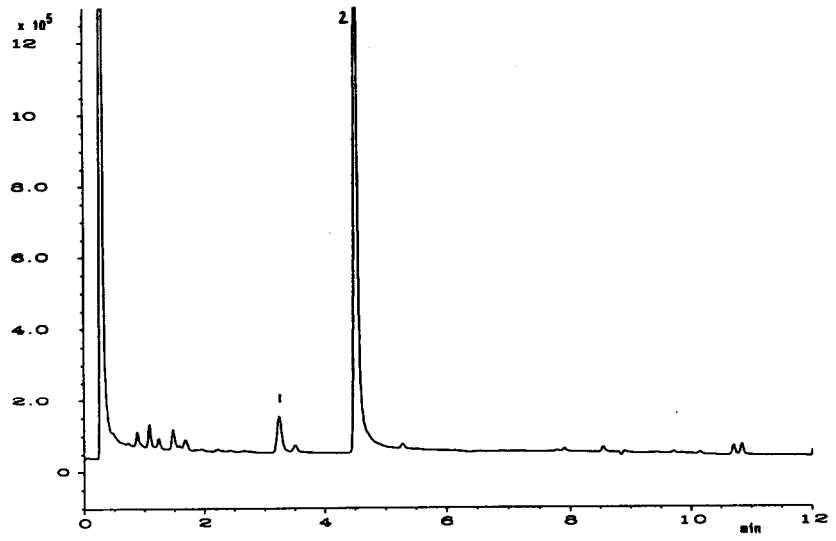

Fig. 5. Gas chromatogram of an authentic agricultural runoff sample in which (1) atrazine and (2) metribuzin were detected. *y*-Axis: ECD detector response.

analyses for metribuzin in authentic agricultural runoff water samples (100 ml) by SPE methods 1*, 2* and 16. The data were corrected for the recovery efficiency appropriate to each SPE method used and are the average of duplicate extractions. A two-tailed paired-sample *t*-test detects no mean population differences at $\alpha = 0.005$. The correlation coefficients for regressions between run types 1* and 2*, 16 and 1*, and 16 and 2* are 0.982, 0.984 and 0.994, respectively.

TABLE IV

COMPARISON OF THREE SPE APPROACHES TO THE ANALYSIS OF AUTHENTIC SAMPLES FOR METRIBUZIN CONTENT

Sample identity	Run type[a]		
	1* (HPLC) (μg/ml)	2* (HPLC) (μg/ml)	16 (GC) (μg/ml)
211.2	0.098	0.106	0.096
221.2	0.130	0.140	0.132
231.2	0.133	0.140	0.142
311.2	0.136	0.144	0.140
312.2	0.284	0.305	0.294
321.2	0.136	0.168	0.158
331.2	0.142	0.154	0.142

[a] Conditions given in Table I. Asterisk refers to extraction with 2 g sorbent.

CONCLUSIONS

In order to keep pace with the increasing analytical demands of monitoring non-point source contamination of pesticides in agricultural runoff water, multi-residue, multi-class analytical procedures must be developed. The factorial experimental design is demonstrated to be useful for method development for SPE protocol. Additionally, this statistical approach reveals significant factors in the mechanism of extraction and recovery of pesticides by SPE. Procedures for the gas or liquid chromatographic determination of metribuzin, atrazine, metolachlor and esfenvalerate were developed. The approach adopted in this research, statistical optimization of variables affecting the concomitant analysis of pesticides having diverse chemical and biological activities, is generally applicable to pesticides other than those studied here.

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