JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Development of an Analytical Scheme for Simazine and 2,4-D in Soil and Water Runoff from Ornamental Plant Nursery Plots

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The transport and fate of pesticides applied to ornamental plant nursery crops are not well documented. Methodology for analysis of soil and water runoff samples concomitantly containing the herbicides simazine (1-chloro-4,6-bis(ethylamino)-*s*-triazine) and 2,4-D ((2,4-dichlorophenoxy)acetic acid) was developed in this research to investigate the potential for runoff and leaching from ornamental nursery plots. Solid-phase extraction was used prior to analysis by gas chromatography and liquid chromatography. Chromatographic results were compared with determination by enzyme-linked immunoassay analysis. The significant analytical contributions of this research include (1) the development of a scheme using chromatographic mode sequencing for the fractionation of simazine and 2,4-D, (2) optimization of the homogeneous derivatization of 2,4-D using the methylating agent boron trifluoride in methanol as an alternative to in situ generation of diazomethane, and (3) the practical application of these techniques to field samples.

KEYWORDS: Ornamental plant nursery industry; herbicide; simazine; (2,4-dichlorophenoxy)acetic acid; 2,4-D; gas chromatography; high-performance liquid chromatography; solid-phase extraction; chromatographic mode sequencing; enzyme-linked immunoassay

INTRODUCTION

Middle Tennessee has one of the largest ornamental plant nursery businesses in the United States. Nurseries in this geographic area traditionally apply large amounts of pesticides to sloped terrains that are highly susceptible to erosion (*1*). Significant amounts of pesticides may be present in soil and water runoff from nursery operations, potentially having detrimental effects on nontarget organisms. The transport and fate of pesticides applied to nursery crops are not well documented.

Simazine (1-chloro-4,6-bis(ethylamino)-*s*-triazine), a triazine herbicide, and 2,4-D ((2,4-dichlorophenoxy)acetic acid), a chlorophenoxy class acidic herbicide, are widely used in the nursery industry. This research compares several methods of sample preparation and final determination for the cleanup, isolation, and concentration of the pesticides simazine and 2,4-D that exist concomitantly in runoff water, soil, and runoff sediment. Either no preliminary extraction or solid-phase extraction (SPE) was used for runoff water samples, while liquid vortex extraction and/or SPE with chromatographic mode sequencing (CMS) were used for recovery from soil and runoff sediment sample matrices. High-performance liquid chromatography (HPLC) with diode array detection (DAD) and gas chromatography (GC) using an electron capture detector (ECD) for 2,4-D or a nitrogen−phosphorus detector (NPD) for simazine were compared with enzyme-linked immunoassay analysis (EIA) for quantitative determination. SPE-CMS is a multiple-mode extraction scheme applied to enhance selective isolation of these compounds by chemical compound class. The analytical techniques applied here to simazine should be generally applicable to other triazine compounds. Likewise, procedures used in this research for 2,4-D should also apply to other acidic herbicides (*2*).

Field-scale experiments often require analysis of a large number of soil, runoff, and sediment samples for investigation of pesticide fate and transport (*3*); therefore, rapid sample processing and establishment of adequate detection limits are important. The analytical methodology generated in this study will improve implementation of management practices that reduce pesticide runoff and pesticide waste in the ornamental nursery industry.

MATERIALS AND METHODS

Field Application. In field studies, 4.49 kg/ha of both simazine and 2,4-D (**Figure 1**) were applied in compliance with the manufacturer's recommendations to nursery plots located at the Shipley Farm Agricultural Facilities of Tennessee Technological University (*4*). Simazine was sprayed as Princep 80 wettable powder, and 2,4-D was applied in the ester form. Water and sediment runoff samples were preferentially collected after rainfall events. To gain a representation of pesticide distribution, soil samples were taken at varying depths

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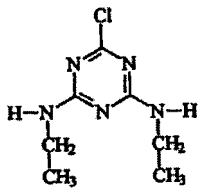
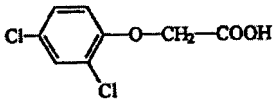
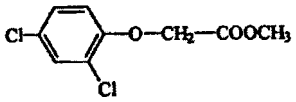
10.1021/jf025661p CCC: $25.00
Published on Web 11/26/2002



SIMAZINE



2,4-D



2,4-D METHYL ESTER

Figure 1. Structures of simazine, 2,4-D, and 2,4-D methyl ester.

(0−90 cm) periodically after pesticide application. Subsequent sample processing varied according to the matrix of the sample, i.e., runoff water, sediment, or soil. Pretreatment samples were used to establish recovery and matrix background interferences.

Soil Extraction. Soil extraction procedures followed those of Stearman and Adams (*5*). Soil (12.5 g) was weighed into a 50-mL glass centrifuge tube. Twenty-five milliliters of acetonitrile/water/acetic acid (80:20:2.5 v:v:v) was added, and the soil solution was vortexed for 2 min, three times. The soil solution was equilibrated overnight at room temperature. The following morning, the sample was vortexed four times for 10 s and was centrifuged for 5 min or until clear. The supernatant was transferred to a glass vial by pipet.

Solid-Phase Extraction of Runoff Water Samples for HPLC Analysis. Water samples (300 mL) were acidified to pH 2 by dropwise addition of 85% o-phosphoric acid. A C_{18} column (Mega Bond Elut, 1.0 g, Varian Sample Preparation Products, Harbor City, CA) was conditioned with 10 mL of methanol, followed by 10 mL of 0.1 M H_3PO_4 (pH 2). The sample was then introduced to the column by vacuum via Teflon tubing. After sample loading was complete, the column was allowed to dry by vacuum for 15 min and eluted with 5 mL of methanol. To ensure that split peaks due to injection of pure organic solvent did not occur, the 5-mL aliquot was diluted with 3 mL of 0.1 M H_3PO_4 prior to determination by HPLC.

Solid-Phase Extraction of Soil Extracts by Chromatographic Mode Sequencing for GC Analysis. Strong cation exchange (SCX) Mega Bond Elut columns (1.0 g sorbent) were coupled above C_{18} Mega Bond Elut columns (1.0 g sorbent) via an adapter and then fitted into a Vac Elut Extraction Manifold (Varian). To accommodate large sample volumes, a 75-mL sample reservoir was connected to the SCX column. While the columns were connected in tandem, the sorbents were conditioned with 10 mL of methanol, followed by 10 mL of 0.1 M phosphate buffer (pH 2). The conditioning solvents were discarded. The samples potentially containing simazine and 2,4-D consisted of approximately 20-mL aliquots of soil extracts in a solution of acetonitrile/water/acetic acid (80:20:2.5 v:v:v). The samples were diluted to approximately 100 mL with 80 mL of 0.1 M phosphoric acid (pH 2). Without allowing the conditioned sorbent to dry, the sample was then loaded onto the tandem columns under vacuum (10−15 in. Hg). The objective of the two-column sequence was to adsorb simazine on the ion-exchange column and 2,4-D on the C_{18} column, thereby separating the compounds.

Following sample loading, the two columns were separated, and 2,4-D was eluted from the C_{18} column with 10 mL of methanol. After elution, 1.5 mL of 0.5 N NaOH (in methanol) was added to the sample aliquot. The sample was then vortexed for 15 s, placed in a 100 °C sand bath, and allowed to stand for 5 min to ensure that all of the compound was hydrolyzed to the free acid form. Upon completion of the hydrolysis, the sample was cooled to room temperature by running a gentle stream of tap water over the outside of the sample container. For gas chromatographic analysis with an ECD, 2,4-D was converted from its nonvolatile free acid form into the more volatile 2,4-D methyl ester (2,4-DME). Six milliliters of boron trifluoride in methanol (14% BF_3−MeOH, Supelco, Inc., Bellefonte, CA) was added to the sample. The sample was vortexed for 20 s and placed in a 100 °C sand bath for 20 min. At the end of the allotted time, the sample was cooled to room temperature, thus completing the derivatization. Following derivatization, the 2,4-DME fraction was diluted with 80 mL of 0.1 M phosphoric acid (pH 2). A C_{18} column, the same one used for initial separation, was conditioned with 10 mL of methanol, followed by 10 mL of 0.1 M H_3PO_4. After the sample was extracted, the column was allowed to vacuum-dry for approximately 25−30 min to remove water from the sorbent. After drying, the 2,4-DME derivative was eluted from the column with ethyl acetate (10 mL) and analyzed using GC-ECD.

With the columns separated, simazine was eluted from the SCX column with a 25-mL aliquot consisting of 0.2 M K_2HPO_4 buffer (pH 9, adjusted to pH 10.8 with KOH) and acetonitrile (60:40 v:v). The eluate was collected and further diluted with an additional 300 mL of 0.1 M K_2HPO_4/KH_2PO_4 buffer (pH 7). The same C_{18} column used for the initial separation of simazine and 2,4-D was reconditioned, and the diluted SCX extract containing simazine was introduced to the column. To accommodate the large sample volume, Teflon tubing was connected to the C_{18} column with an adapter for sample transfer. Following sample loading, the C_{18} sorbent was allowed to vacuum-dry for approximately 25−30 min. After drying, simazine was eluted from the C_{18} column with 5 mL of ethyl acetate. The sample aliquot was analyzed by gas chromatography with a NPD.

HPLC Analysis of Simazine and 2,4-D. Liquid chromatographic analysis of simazine and the free acid form of 2,4-D was conducted with a Hewlett-Packard high-performance liquid chromatograph (model 1090M). The HPLC was equipped with a DAD, 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 Co., Avondale, PA). The column was maintained at 40 °C, the mobile phase flow rate was 1.5 mL/minute, and the injection volume was 25 μL. The isocratic mobile phase consisted of acetonitrile/0.1 M phosphoric acid, pH 2 (30:70 v:v). The diode array detector was used to monitor simultaneously simazine and 2,4-D at their maximum absorbances. The absorbance maxima were determined to be 221 and 206 nm for simazine and 2,4-D, respectively, using a Perkin-Elmer Lambda 2 UV/vis scanning spectrometer.

GC Analysis of Simazine and 2,4-DME. A GC analysis was developed that allowed determination of both simazine and 2,4-DME using an ECD. An additional GC analysis using a NPD was developed for determination of simazine. Simazine can be analyzed using either the ECD or NPD method; however, optimum results were obtained by using the ECD for quantitation of 2,4-DME and the NPD for simazine, following separation by SPE.

The GC-ECD used in the analysis of simazine and 2,4-DME consisted of a Hewlett-Packard 5880 GC, a 7673A autosampler, an ECD, a 5880A series GC Terminal Integrator Level Four, and a 30-m × 0.53-mm-i.d. (0.5-μm film thickness) SPB-5 column (Supelco, Inc.). The injection volume was 1.0 μL. The temperature program developed allowed for separation of simazine and 2,4-DME. An initial oven temperature of 160 °C was maintained for 0.5 min. A temperature gradient of 3 °C/min was then initiated until the final temperature of 193 °C was obtained. The temperature was maintained for an additional 0.5 min. A postrun oven temperature of 220 °C was maintained for 2 min to elute impurities from the column that could be retained from the sample. The entire analysis required a run time of 14 min. Injector

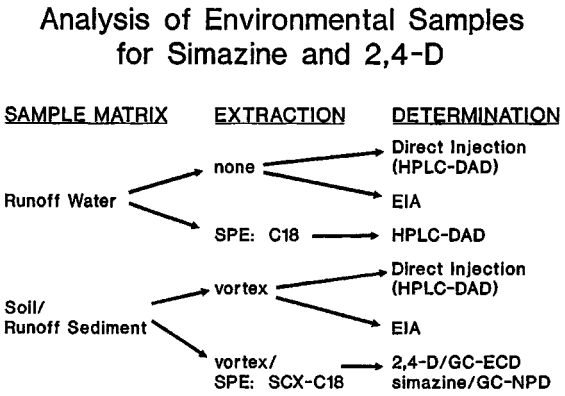


Figure 2. Schematic representation of the extraction and analysis of various sample matrices encountered in this research.

and detector temperatures were maintained at 250 and 275 °C, respectively. The carrier gas was helium at a flow rate of 10 mL/min. Nitrogen was used as the makeup gas for the ECD, to produce a total flow rate of 60 mL/min through the detector.

The GC-NPD used for the analysis of simazine extracts consisted of a Hewlett-Packard 5890 GC, a 7673 autosampler, a NPD, Hewlett-Packard computer software, and a 30-m × 0.32-mm-i.d. (0.25-μm film thickness) HP-5 capillary column. A splitless injection was first performed, followed by a septum purge at 1 min. The purge was necessary for the volatilization of the 1.0-μL injection volume and subsequent removal of nonvolatile species that remained at the head of the column after injection. An initial oven temperature of 60 °C was maintained for 0.5 min. A temperature gradient of 20 °C/min was initiated until a final temperature of 270 °C was reached and maintained for an additional 0.5 min. The entire analysis required a run time of 13 min. Injector and detector temperatures were maintained at 250 and 275 °C, respectively. The carrier gas, helium, was maintained at a flow rate of 1.5 mL/min. Helium was also used as the makeup gas, which was set at a flow rate of 10−15 mL/min. To activate the element in the NPD, hydrogen and air were introduced at flow rates of 3.5 and 100−120 mL/min, respectively. The total gas flow was thus between 120 and 130 mL/min through the detector.

EIA Analysis of Simazine and 2,4-D. EIA procedures were developed by Stearman et al. (*6*, *7*). Appropriate dilutions of aqueous samples or soil extracts (25−100:1) were prepared, and 80 μL of the solution was added to an antibody-coated microtiter well to determine simazine or 2,4-D. Standards were included on the same microtiter plate. The EIA kits (RES-I-Quant, ImmunoSystems, Inc., Scarborough, ME, or AgriDiagnostics, Cinnaminson, NJ) consisted of 96 antibody-coated wells in a microtiter plate, solutions of enzyme conjugate, color reagents, and stop solution. An 80-μL aliquot of the sample containing pesticide was incubated in the well with 80 μL of pesticide−enzyme conjugate for 60 min while the covered plate was stirred on an orbital shaker at 200 rpm. After incubation, the unreacted molecules were washed away with water. Eighty microliters each of substrate and chromogen were added to each well. The plate was covered and shaken on the orbital shaker for 30 min. The reaction was stopped by adding 40 μL of 2.5 N H_2SO_4, which changed the blue color in the wells to yellow. The color in the plate was read on a microtiter plate reader at 450 nm.

RESULTS AND DISCUSSION

Various analytical techniques (**Figure 2**), including solid-phase extraction, gas chromatography, high-performance liquid chromatography, and enzyme immunoassay analysis, were utilized for determination of simazine and 2,4-D runoff and leaching from nursery plots. The environmentally contaminated samples obtained in this research contained simazine and 2,4-D in the same sample matrix. Therefore, methods for the simultaneous determination of these analytes, as well as means of fractionating the analytes before analysis, were pursued.

Table 1. Solid-Phase Extraction (C_{18}) of Synthetically Spiked Water Samples (300 mL) for Determination by HPLC-DAD

water samples concn (ng/mL)	recovery (%)[a]	
	simazine	2,4-D
5	103 ± 9.5	128 ± 26
25	101 ± 11.7	107 ± 5.3
50	101 ± 3.4	104 ± 3.8

[a] Mean ± SD; $n = 3$.

Simultaneous Determination of Simazine and 2,4-D by HPLC. Depending upon the concentration of analytes in the aqueous runoff samples and the soil or sediment extracts, some samples, particularly those collected soon after field treatment, were analyzed by direct injection HPLC. The samples were analyzed on an octadecyl (C_{18}) reversed-phase column using a mobile phase comparable to the sample extract matrix. Derivatization of 2,4-D to the methyl ester is unnecessary for analysis by HPLC. The detection limits for each pesticide by direct injection were limited to simazine concentrations of greater than or equal to 75 ng/mL, and concentrations of 2,4-D that were greater than or equal to 100 ng/mL.

For samples having concentrations of simazine and 2,4-D below the detection limits of HPLC by direct sample injection, a concentration step was developed using SPE. Recovery data for synthetically spiked samples (300 mL) from a single column (C_{18}, 1.0 g) are given in **Table 1**. By eluting the sample in a 5-mL aliquot of methanol, the sample was subsequently concentrated to 60 times its initial concentration. Due to the large concentration factor, the detection limit of the water runoff samples is approximately 2 ng/mL for each analyte.

Fractionation of Simazine and 2,4-D by Chromatographic Mode Sequencing with Subsequent Determination by GC. Various mixed-mode/multiple-mode approaches are used in SPE to improve recovery and achieve very selective fractionation of analytes. Mixed-mode sorbents can be used that are chemically designed to have multiple retentive sites on an individual particle. Mechanical approaches to achieving multiple-mode retention include homogeneously "blending" sorbents that exhibit separate mechanisms of retention or "layering" them into the same column by packing one phase over another. Alternatively, multiple phases can be "stacked" by arranging in tandem series sorbents of different retention mechanisms contained in different columns. The latter technique, the use of tandem SPE columns of differing sorbents, is termed "chromatographic mode sequencing" (CMS) (*8*) and is the multiple-mode approach used in this research. Increased selectivity is attained by coupling two or more cartridges containing different sorbents in series. CMS may be applied to compounds differing in hydrophobicity, charge, and structure. CMS is not limited to herbicide analysis and should find application to various multiresidue samples.

Chromatographic fractionation of simazine and 2,4-D prior to derivatization was desirable, because of potentially adverse effects upon simazine during the alkaline hydrolysis and derivatization procedures to which 2,4-D is subjected. In addition, by separating the herbicides into separate fractions, all subsequent chromatographic analyses can be optimized for each compound.

A CMS procedure (**Figure 3**) in which a strong cation-exchange column was placed above a hydrophobic column was developed for the separation of simazine and 2,4-D (*9*). The procedure was developed on the premise that, at pH 2, 2,4-D should pass through the SCX column and be retained on a C_{18}

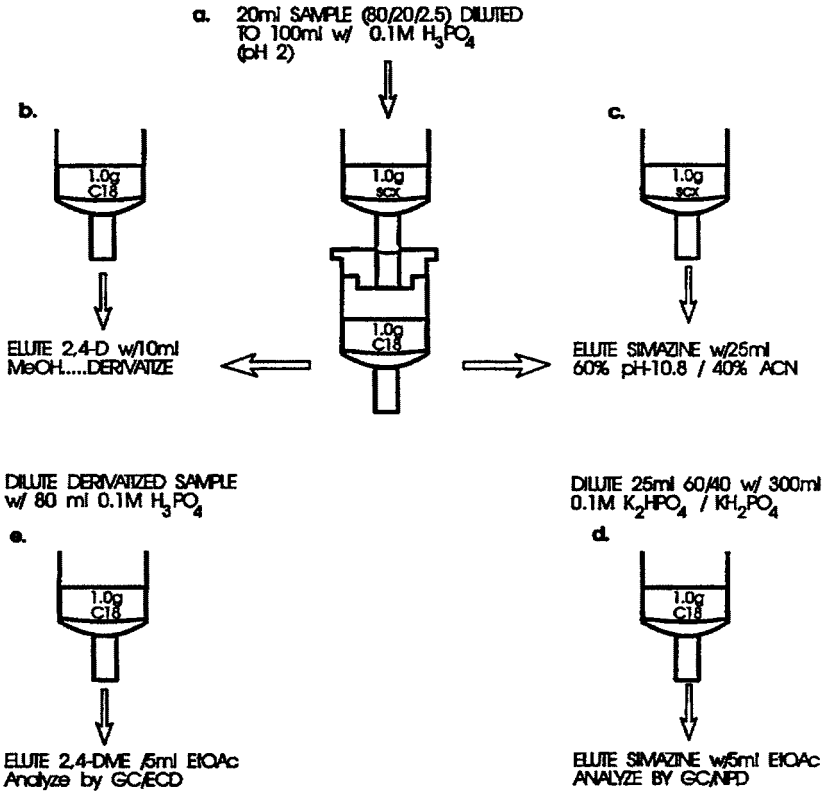


Figure 3. Chromatographic mode sequencing parameters for the fractionation of simazine and 2,4-D. Reprinted with permission from ref *9*. Copyright 1996 American Chemical Society.

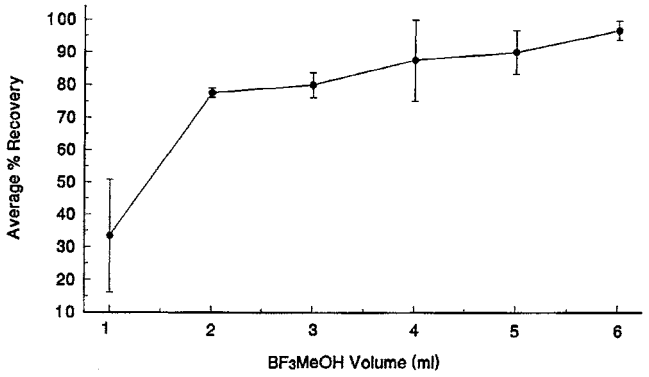


Figure 4. Optimization of the BF_3–methanol derivatization of 2,4-D to its methyl ester.

column, while simazine is retained on the SCX column. At pH 2, the carboxylic acid group of 2,4-D is protonated and uncharged, while simazine has a positive charge.

To develop the CMS procedure, solutions (20 mL) composed of acetonitrile, water, and acetic acid (80/20/2.5 v:v:v), representing the soil extraction solution, were spiked with known amounts of 2,4-D and simazine. The spiked samples were diluted with 80 mL of 0.1 M H_3PO_4 (pH 2). The dilution resulted in a final acetonitrile concentration of approximately 16%. Dilution of the sample to reduce the percentage of acetonitrile present was necessary because concentrations of 20% acetonitrile or greater resulted in sample breakthrough. Following column conditioning and sample loading (**Figure 3a**), the columns were separated (**Figure 3b**,**c**). The herbicide 2,4-D was then eluted from the C_{18} column and collected in a sample flask (**Figure 3b**). Subsequent sample processing of the 2,4-D extract is discussed later.

Simazine was retained on the SCX column (**Figure 3c**) following initial sample loading from a low-pH solution. Simazine adsorbed onto the benzenesulfonic acid SCX sorbent not only by a primary mechanism of ionic (electrostatic) bonding but also through a secondary mechanism of van der Waals (nonpolar) interactions. This was evident because little recovery of simazine was attained by elution with either acetonitrile or high-pH phosphate buffer individually.

To overcome the combined forces retaining simazine on the sorbent, it was necessary to elute the compound with a high-pH aqueous solution combined with an organic solvent. Recovery was optimized from the SCX sorbent by varying the percentage of acetonitrile, the molar concentration and pH of the buffered portion of the eluting solvent, and the total volume of eluting solvent. Sample aliquots were analyzed by HPLC before and after solid-phase extraction to directly monitor adsorption and desorption of simazine. HPLC analysis of the solutions could be done without solvent exchange. The HPLC analyses indicated that all of the simazine was being successfully loaded on the SCX column. Simazine was successfully eluted from the SCX sorbent (**Figure 3c**) with a 25-mL aliquot consisting of 0.2 M K_2HPO_4 buffer (pH 9, adjusted to pH 10.8 with KOH) and acetonitrile (60:40 v:v). Therefore, desorption directly from the SCX column with a GC-compatible solvent could not be accomplished.

Elution from the SCX column directly onto the sorbent of a coupled C_{18} column was desired to effect a solvent exchange appropriate for analysis by GC and to provide additional sample purification. However, when a C_{18} column (1.0 g) was attached to the bottom of the SCX column with a column adaptor, and the eluting solution (60% acetonitrile/40% pH 10.8) was added to the SCX column, allowing the simazine to elute directly onto the C_{18} column, it was determined that over 70% of the analyte was unretained by the C_{18} column. Of the potential approaches to overcome the problem, such as increasing the mass of sorbent or diluting the sample, the latter was chosen because it was

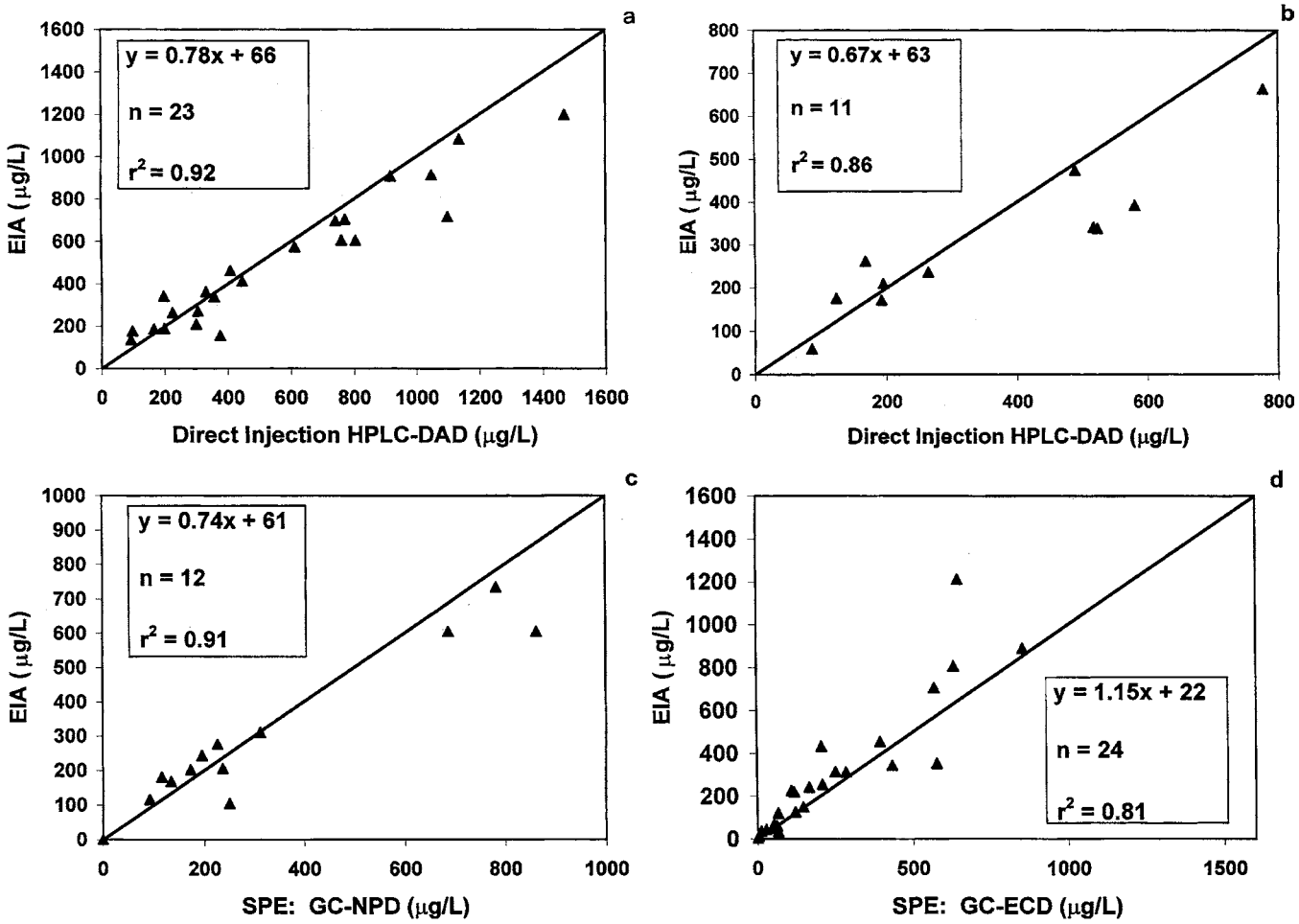


Figure 5. Comparison of EIA and HPLC-DAD analyses of field soil and sediment extracts for (a) simazine and (b) 2,4-D. Comparison of EIA and GC-NPD analyses of field soil extracts for (c) simazine and (d) 2,4-D.

less expensive, even though it meant that the simazine would have to be sorbed on the hydrophobic sorbent with the SCX and C_{18} phases decoupled (**Figure 3d**).

Derivatization of 2,4-D (**Figure 3b**) to the methyl ester, 2,4-DME (**Figure 1**), was necessary for analysis by GC-ECD. Thus, optimization of derivatization following solid-phase extraction was investigated. A hydrolysis and homogeneous derivatization method described by Jahncke et al. (*10*) was adapted for use in this research. The methylating agent BF_3−methanol (14% BF_3−MeOH) was used as a safer alternative than diazomethane to convert 2,4-D to 2,4-DME. Diazomethane is toxic, highly flammable, and explosive (*11*). Initially only 77% recovery of 2,4-DME was achieved using a ratio of 2 mL of BF_3−MeOH to 10 mL of sample extract (**Figure 4**). The volume ratio of derivatizing reagent to sample was examined to determine if poor recovery was due to incomplete derivatization of the compound. A series of samples (10 mL) containing approximately 1 ppm 2,4-D were prepared, and increasingly larger volumes of BF_3−MeOH were added. The optimum volume of BF_3−MeOH that must be added to a 10-mL aliquot of 2,4-D in MeOH collected by elution from C_{18} was 6 mL. The derivatizing reagent, BF_3−MeOH, is subject to hydrolysis by water, so the increased amount of reagent necessary for complete derivatization may be due to water that is extracted from the C_{18} cartridge with MeOH.

Solvent exchange of the 2,4-DME from methanol to ethyl acetate was effected via a C_{18} column (**Figure 3e**). Phosphoric acid solution (0.1 M, 80 mL) was added to the derivatized sample to simultaneously quench the derivatizing reaction and dilute the sample for loading onto the C_{18} sorbent.

Table 2. Solid-Phase Extraction (SCX/C_{18}) of Representative Solvent Matrix and Soil Extract Spikes for Determination of 2,4-D by GC-ECD

2,4-D concn (ng/mL)	recovery (%)[a]	
	solvent matrix spikes	soil extract spikes
1	111 ± 4.2	105 ± 6.4
25	103 ± 3.5	101 ± 5.2
100	104 ± 7.2	99.2 ± 6.7
300	98.7 ± 9.4	90.9 ± 8.3
500	90.7 ± 18	84.2 ± 15

[a] Mean ± SD; $n = 3$.

Once successful derivatization of 2,4-D was established, spiked samples of varying concentration were prepared to verify the chromatographic mode sequencing method and subsequent derivatization (**Table 2**). Samples varying from 1 to 500 ng/mL were prepared by spiking 20 mL of acetonitrile/water/acetic acid (80/20/2.5 v:v:v) with a known concentration of 2,4-D. The sample was then diluted with 80 mL of 0.1 M H_3PO_4, extracted, and derivatized as previously described (**Figure 3**). To determine if dissolved organic matter in soil samples has adverse effects on the extraction and derivatization of 2,4-D, 20-mL extracts of field soil collected prior to herbicide application were spiked, extracted, and derivatized. No differences were apparent between the recovery from the solvent matrix spikes and the soil extract spikes.

Table 3. Solid-Phase Extraction (SCX/C_{18}) of Representative Solvent Matrix and Soil Extract Spikes for Determination of Simazine by GC-NPD

simazine concn (ng/mL)	recovery (%)[a]	
	solvent matrix spikes	soil extract spikes
6	134 ± 5.8	134 ± 2.8
66	94.5 ± 7.0	103 ± 13
146	93.6 ± 8.8	109 ± 8.1
485	94.5 ± 18	95.6 ± 18

[a] Mean ± SD; $n = 3$.

Synthetically spiked samples of simazine (**Table 3**) were prepared in triplicate to verify the procedure over concentrations ranging from 6 to 485 ng/mL. The spiked samples were diluted appropriately and loaded onto SCX/C_{18} columns in tandem (**Figure 3a**). Simazine was eluted with 5 mL of ethyl acetate and analyzed by GC-NPD. Spiked extracts of field soil collected prior to herbicide application demonstrated no effect on the solid-phase extraction of simazine in the presence of co-extracted dissolved organic matter.

Variable Extraction Efficiency of Simazine Resulting from Preferred Sample Loading pH. An interesting anomaly was observed when simazine was loaded and eluted from the C_{18} sorbent. Simazine-containing samples that were buffered with phosphate buffer at pH 2 and loaded onto the C_{18} sorbent were recovered with nearly 100% efficiency when eluted with methanol. However, when the same samples were loaded at pH 2 and eluted with ethyl acetate, recoveries were highly variable, averaging about 70%. Poor recovery was also observed with other GC-compatible solvents, including methyl *tert*-butyl ether (MTBE), hexane, and acetone. Acceptable recovery (95−100%) of simazine from the C_{18} sorbent by elution with ethyl acetate was finally achieved when the sample was prepared in a pH 7 phosphate buffer. The anomalous behavior has been observed previously for a related triazine compound, atrazine (*12*). In a factorial optimization of SPE for analysis of atrazine, it was found that at pH 2, the best elution solvent was methanol, while at pH 7, ethyl acetate produced better recovery. Wells et al. (*12*) also reported that a solution containing 20% methanol in pH 7 buffer slightly improved recovery of atrazine when eluting the compound with ethyl acetate from a C_{18} sorbent. The unusual behavior of simazine and atrazine eluting easily from the octadecyl sorbent with methanol when loaded at pH 2, and eluting with less water-miscible organic solvents only when loaded at pH 7, doubtless reveals a difference in the nature of the sorption of the compounds at different pHs. The observed behavior could also reveal a pH-dependent difference in the structural characteristics of the sorbent itself.

Analysis of Field Soil Samples. Once successful procedures were developed and acceptable recoveries of simazine and 2,4-D were achieved with spiked samples, runoff water samples and extracts of soil and runoff sediment samples were analyzed by direct injection HPLC, by C_{18} solid-phase extraction for HPLC analysis, or by the SCX/C_{18} CMS procedure for GC analysis, as outlined in **Figure 2**. The field samples were also analyzed by EIA, and the results were compared to those obtained chromatographically, as illustrated for selected samples in **Figure 5**. In these graphs, the least-squares fit to the data is presented in the box inset on the figure, while the line shown represents a 1:1 correlation. The line indicates the values at which a perfect correlation between the methods could be obtained. Concentrations obtained from the methods developed in this research compared well with those obtained from EIA. Correlation of the concentrations obtained is best below 500 μg/L. EIA exhibited deviations in response as the concentration increased because of significant dilutions required for analysis. Conversely, EIA results are more accurate than the chromatographic results at very low concentrations when minimal dilution is necessary.

The concentrations encountered in field samples ranged from 0.1 to 3253 ng/mL for simazine in runoff water and from 0.1 to 4180 ng/mL for 2,4-D in runoff water. In soils, simazine ranged from 5 to 1200 ng/g and 2,4-D from 2 to 680 ng/g.

CONCLUSIONS

A sample preparation method was developed using solid-phase extraction and final determination by HPLC and GC for the pesticides simazine and 2,4-D in runoff water, soil, and runoff sediment samples. Several extraction and determination procedures were implemented for the analysis of various sample matrices encountered.

Vortex extraction of pesticides from soil samples, followed with separation by centrifugation, produced a matrix that could be directly injected in HPLC. Filtered runoff water samples could also be injected directly. Analysis by direct injection is an efficient way of obtaining quick quantitative results of soil and water samples potentially containing relatively large amounts of pesticide residues, especially those collected soon after field treatment.

Runoff water samples with pesticide concentrations that were undetectable by direct injection HPLC were concentrated using C_{18} solid-phase extraction. Using SPE, aqueous samples (300 mL) were loaded onto a C_{18} column and subsequently concentrated 60 times their initial concentration.

Chromatographic mode sequencing for simultaneous separation of simazine and 2,4-D from a single matrix, followed with analysis by GC, was successful. The fractionation procedure developed in this research for two distinct classes of compounds, triazine and phenoxy acid herbicides, should also apply to other compounds in these representative classes.

Optimization of the homogeneous derivatization of 2,4-D to the methyl ester using boron trifluoride in methanol as a safer alternative methylating agent compared to diazomethane was accomplished. The ratio of the derivatizing reagent to the sample size was investigated. The presence of excess derivatizing reagent was necessary because it was hydrolyzed by water that was invariably present in the sample following SPE.

ACKNOWLEDGMENT

The assistance of Binney Stumpf, Scott Adkisson, and Tad Ridgill is gratefully acknowledged.

LITERATURE CITED

(1) Cripps, R. W.; Bates, H. K. Effect of Aisle Cover Crops on Surface Runoff Quantity and Quality. *Proceedings of Southern Nurseryman's Association Research Conference*, 36th Annual Report, 1991; pp 151−154.

(2) Wells, M. J. M.; Yu, L. Z. Solid-Phase Extraction of Acidic Herbicides. *J. Chromatogr. A* **2000**, *885*, 237−250.

(3) Turin, H. J.; Bowman, R. S. A Solid-Phase Extraction Based Soil Extraction Method for Pesticides of Varying Polarity. *J. Environ. Qual.* **1993**, *22*, 332−334.

(4) Stearman, G. K.; Wells, M. J. M.; Cripps, R. W. Leaching and Runoff of Simazine, 2,4-D, and Bromide from Nursery Plots. *J. Soil Water Conserv.* **1997**, *52* (2), 137−144.

(5) Stearman, G. K.; Adams, V. D. Atrazine Soil Extraction Techniques for Enzyme Immunoassay Microtiter Plate Analysis. *Bull. Environ. Contam. Toxicol*. **1992**, *48*, 144−151.

(6) Stearman, G. K.; Wells, M. J. M.; Adkisson, S. M.; Ridgill, T. E. Supercritical Fluid Extraction Coupled with Enzyme Immunoassay Analysis of Soil Herbicides. *Analyst* **1995**, *120*, 2617−2621.

(7) Stearman, G. K.; Wells, M. J. M.; Adkisson, S. M.; Ridgill, T. E. Enzyme Immunoassay Analysis Coupled with Supercritical Fluid Extraction of Soil Herbicides. In *Environmental Immunochemical Methods Perspectives and Applications*; Van Emon, J. M., Gerlach, C. L., Johnson, J. C., Eds.; American Chemical Society: Washington, DC, 1996; pp 56−64.

(8) *Anal. Int. Curr. Newsl.* **1982**, *1* (4).

(9) Wells, M. J. M.; Stearman, G. K. Coordinating Supercritical Fluid and Solid-Phase Extraction with Chromatographic and Immunoassay Analysis of Herbicides. In *Herbicide Metabolites in Surface Water and Groundwater*; Meyer, M. T., Thurman, E. M., Eds.: American Chemical Society: Washington, DC, 1996; pp 18−33.

(10) Jahncke, M. L.; Smith, T.; Seaborn, G. *Marine Forensics Program: A Biochemical Method to Distinguish Wild from Cultured Fish: Final Report*; NOAA Technology Memo NMFS-SEFSC-277, 1991.

(11) OSHA. Occupational Safety and Health Guideline for Diazomethane. Available online: http://www.osha-slc.gov/SLTC/healthguidelines/diazomethane/recognition.html.

(12) Wells, M. J. M.; Riemer, D. D.; Wells-Knecht, M. C. Development and Optimization of a Solid-Phase Extraction Scheme for Determination of the Pesticides Metribuzin, Atrazine, Metolachlor and Esfenvalerate in Agricultural Runoff Water. *J Chromatogr. A* **1994**, *659* (2), 337−348.

Received for review May 9, 2002. Revised manuscript received October 11, 2002. Accepted October 16, 2002. Financial support was provided by the U.S. Geological Survey through the University of Tennessee−Knoxville Water Resource Research Center

JF025661P