U.S. Department of the Interior
U.S. Geological Survey

Methods of Analysis by the U.S. Geological Survey Organic Geochemistry Research Group—Determination of Chloroacetanilide Herbicide Metabolites in Water Using High-Performance Liquid Chromatography-Diode Array Detection and High-Performance Liquid Chromatography/Mass Spectrometry

By L.R. ZIMMERMAN, K.A. HOSTETLER, and E.M. THURMAN

Open-File Report 00–182

Lawrence, Kansas
2000

U.S. Department of the Interior

Bruce Babbitt, Secretary

U.S. Geological Survey

Charles G. Groat, Director

For additional information write to:

District Chief
U.S. Geological Survey
4821 Quail Crest Place
Lawrence, KS 66049–3839

Copies of this report can be purchased from:

U.S. Geological Survey
Information Services
Building 810, Federal Center
Box 25286
Denver, CO 80225–0286

CONTENTS

FIGURES

TABLES

CONVERSION FACTORS, MISCELLANEOUS ABBREVIATIONS, AND ABBREVIATED WATER-QUALITY UNITS

Conversion Factors

Multiply	By	To obtain
ounce (oz)	0.02957	liter
pound (lb)	453.6	gram
pound per square inch (lb/in^2)	6.895	kilopascal

Temperature can be converted to degrees Celsius (^oC) or degrees Fahrenheit (^oF) by the equations:

$$^oC = 5/9\ (^oF - 32)$$
$$^oF = 9/5\ (^oC) + 32.$$

Miscellaneous Abbreviations

cubic centimeter (cm^3)
gram (g)
mass-to-charge ratio (m/z)
meter (m)
micrometer (μm)
milliabsorbance units (mAU)
milligram (mg)
millimeter (mm)
millimole (mM)
millisecond (ms)
minute (min)
nanogram (ng)
nanometer (nm)
volt (V)

Abbreviated Water-Quality Units

liter (L)
liter per minute (L/min)
microgram per liter (μg/L)
microliter (μL)
milligram per milliliter (mg/mL)
milliliter (mL)
milliliter per minute (mL/min)
nanogram per microliter (ng/μL)

Methods of Analysis by the U.S. Geological Survey Organic Geochemistry Research Group—Determination of Chloroacetanilide Herbicide Metabolites in Water Using High-Performance Liquid Chromatography-Diode Array Detection and High-Performance Liquid Chromatography/Mass Spectrometry

By L.R. Zimmerman[1], Kenneth A. Hostetler[2], *and* E.M. Thurman[3]

Abstract

Analytical methods using high-performance liquid chromatography-diode array detection (HPLC-DAD) and high-performance liquid chromatography/mass spectrometry (HPLC/MS) were developed for the analysis of the following chloroacetanilide herbicide metabolites in water: acetochlor ethanesulfonic acid (ESA), acetochlor oxanilic acid (OXA), alachlor ESA, alachlor OXA, metolachlor ESA, and metolachlor OXA. Good precision and accuracy were demonstrated for both the HPLC-DAD and HPLC/MS methods in reagent water, surface water, and ground water. The mean HPLC-DAD recoveries of the chloroacetanilide herbicide metabolites from water samples spiked at 0.25, 0.50, and 2.0 µg/L (micrograms per liter) ranged from 84 to 112 percent, with relative standard deviations of 18 percent or less. The mean HPLC/MS recoveries of the metabolites from water samples spiked at 0.05, 0.20, and 2.0 µg/L ranged from 81 to 125 percent, with relative standard deviations of 20 percent or less. The limit of quantitation (LOQ) for all metabolites using the HPLC-DAD method was 0.20 µg/L, whereas the LOQ using the HPLC/MS method was 0.05 µg/L. These metabolite-determination methods are valuable for acquiring information about water quality and the fate and transport of the parent chloroacetanilide herbicides in water.

INTRODUCTION

The chloroacetanilide herbicides—acetochlor, alachlor, and metolachlor—are an important class of herbicides in the United States. Together with the triazine compounds, chloroacetanilide herbicides compose the majority of pesticides applied in the Midwestern United States for control of weeds in corn, soybeans, and other row crops (Gianessi and Anderson, 1995). Alachlor and metolachlor have been used extensively for more than 20 years, whereas acetochlor application is relatively recent, having been applied extensively since March 1994 (Kolpin, Nations, and others, 1996). Chloroacetanilide herbicides have been shown to degrade more rapidly in soil than other herbicides, with half-lives from 15 to 30 days, whereas triazine half-lives are typically 30 to 60 days (Leonard, 1988).

Recent studies have reported the occurrence of chloroacetanilide metabolites in surface and ground

[1]University of Kansas, Center for Research, Inc., Lawrence, Kansas.
[2]Formerly at University of Kansas, Center for Research, Inc., Lawrence, Kansas.
[3]U.S. Geological Survey, Lawrence, Kansas.

water (Aga and others, 1996; Kolpin, Thurman, and Goolsby, 1996; Thurman and others, 1996; Kolpin and others, 1998). Kolpin and others (1998) found that metabolite concentrations in ground water may be at similar or even higher concentrations than the parent compounds, whereas in surface water the parent compounds are more abundant in the spring after application and are replaced gradually by metabolites during the remaining growing season. In understanding the fate and transport of parent compounds, reliable methods for the analysis of metabolites are vital. These methods also are important for analytical verification of the metabolites in toxicological studies.

High-performance liquid chromatography (HPLC) is needed for the analysis of chloroacetanilide herbicide metabolites because they are ionic compounds and are not sufficiently volatile for analysis by gas chromatography. HPLC-diode array detection (DAD) may be used in determining metabolite concentrations, especially when the water sample is relatively free of humic materials and ionic surfactants that can cause chromatographic interference. Coupling HPLC with mass spectrometry (MS) yields more qualitative data and lower detection limits than HPLC-DAD analysis alone.

This report describes the development of reliable HPLC-DAD and HPLC/MS methods for the analysis of ethanesulfonic acid (ESA) and oxanilic acid (OXA) metabolites of acetochlor, alachlor, and metolachlor in surface water and ground water. The HPLC-DAD method was derived from an analytical method for the analysis of alachlor ESA and alachlor OXA as reported by Macomber (1992). For application to the acetochlor and metolachlor metabolites, several modifications to that method were necessary to achieve chromatographic separation of metabolite peaks. The HPLC/MS method was derived from Ferrer and others (1997), with a minor modification to resolve co-eluting peaks on the chromatogram, and was reported in Hostetler and Thurman (1999). Similarly, the analysis of the ESA and OXA metabolites of the newly registered acetanilide herbicide dimethenamid could be added to these methods of analysis, but performance data for these compounds are not included in this report. These methods supplement other methods of the U.S. Geological Survey (USGS) and have been implemented by the USGS Organic Geochemistry Research Group in Lawrence, Kansas.

The HPLC-DAD method of analysis described in this report has been assigned the method number "O–2133–00." This unique code represents the HPLC-DAD automated method of analysis for organic compounds as described in this report and can be used to identify the method.

The HPLC/MS method of analysis described in this report has been assigned the method number "O–2134–00." This unique code represents the HPLC/MS automated method of analysis for organic compounds as described in this report and can be used to identify the method.

This report provides a detailed description of the methods, including the apparatus, reagents, instrument calibration, and the solid-phase extraction (SPE) procedure required for sample analysis. Estimated method detection limits, recoveries, and relative standard deviations for six chloroacetanilide herbicide metabolites for the HPLC-DAD and the HPLC/MS methods are presented.

DETERMINATION OF CHLOROACETANILIDE HERBICIDE METABOLITES IN WATER USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY-DIODE ARRAY DETECTION

Method of Analysis (0–2133–00)

Scope and Application

HPLC-DAD is suitable for the determination of low concentrations (in micrograms per liter) of chloroacetanilide metabolites in natural water samples (table 1). Suspended particulate matter is removed from the samples by filtration, so this method is suitable only for dissolved-phase metabolites.

Metabolites were selected for analysis because of the extensive use of their parent herbicides in the United States and their importance to current (1999) studies being conducted by the USGS. The HPLC-DAD method is suitable for concentrations ranging from 0.2 to 3.0 µg/L without dilution.

Summary of Method

Water samples are filtered at the collection site using baked, glass-fiber filters with 0.7-µm pore diameter to remove suspended particulate matter. In the

Table 1. Chloroacetanilide herbicide metabolite compounds suitable for determination using methods described and molecular weights

[ESA, ethanesulfonic acid; OXA, oxanilic acid]

Metabolite	Molecular weight (atomic mass units)
Acetochlor ESA	317.4
Acetochlor OXA	268.3
Alachlor ESA	317.4
Alachlor OXA	268.3
Metolachlor ESA	331.4
Metolachlor OXA	281.3

laboratory, filtered water samples are passed through a preconditioned C-18 column. The C-18 column is rinsed with ethyl acetate to remove interfering compounds. The adsorbed chloroacetanilide metabolites are removed from the C-18 with methanol. The eluted solution is spiked with an internal standard, evaporated under nitrogen, and reconstituted. The sample components are separated, identified, and measured by injecting an aliquot of the concentrated extract into an HPLC equipped with a DAD. Compounds eluting from the LC columns are identified by comparing their retention times obtained by the measurement of control samples under the same conditions used for the collected samples. The concentration of each identified compound is measured by relating the DAD response produced by that compound to the DAD response produced by the internal standard.

Interferences

Samples with high concentrations of humic materials and ionic surfactants may cause chromatographic interference. Compounds that elute from the LC at the same time and are detected at the same wavelengths as the metabolites of interest also may interfere.

Apparatus and Instrumentation

- *Analytical balances*—Capable of accurately weighing 0.0100 g ±0.0001 g.
- *Autopipettes*—20- to 200-µL, variable-volume autopipettes with disposable tips (Rainin, Woburn, Massachusetts, or equivalent).
- *Millilab 1A workstation*—Automated SPE workstation with an online computer. The two syringe pumps on the fluidics module are equipped with a 5- and a 1-mL syringe (Waters, Milford, Massachusetts).
  - Multiple intake accessories (MIAs): Two MIAs are attached to the 5-mL syringe to increase sample capacity from 3 to 14 samples.
  - Software: Millilab 1A software, version 3.0 (Waters, Milford, Massachusetts).

  or

  Tekmar six-position AutoTrace—Automated SPE workstation, (Tekmar-Dohrmann, Cincinnati, Ohio).
  - Software: Tekmar AutoTrace Extraction software, version 1.33 (Tekmar-Dohrmann, Cincinnati, Ohio).
- *Automated solvent evaporator*—The heated bath temperature needs to be maintained at 50 °C, and the nitrogen gas pressure at 15 lb/in^2 (TurboVap LV Zymark Inc., Hopkinton, Massachusetts, or equivalent).
- *Mechanical vortex*
- *Analytical columns*—Phenomenex (Torrance, California) 5-µm, 250- x 3-mm C-18 column coupled to a Keystone (Bellefonte, Pennsylvania) 3-µm, 250- x 4.6-mm C-18 column.
- *HPLC benchtop system*—Hewlett Packard (Wilmington, Delaware), model 1090 with autoinjector and DAD detector.
  - LC oven conditions: constant 60 °C.
  - LC mobile phase: 60 percent, pH 7.0, 25-mm phosphate buffer, 35 percent methanol, and 5 percent acetonitrile solution with a flow rate of 0.6 mL/min.
  - DAD conditions: Acquiring wavelengths 190 to 300 nm.
- *Data system*—Computer and printer compatible with the HPLC system.
- *Software*—HPLC 3D ChemStation rev.A.03.03 (Hewlett Packard, Wilmington, Delaware) is used to acquire and store data and for peak integration.

Reagents and Consumable Materials

- *Sample bottles*—Baked 4-oz amber glass bottles (Boston round) with Teflon-lined lids.
- *Sample filters*—0.7-µm glass-fiber filters (Gilson, Middleton, Wisconsin, or equivalent).
- *Reagent water*—Generated by purification of tap water through activated charcoal filtration and deionization with a high-purity, mixed-bed resin, followed by another activated charcoal filtration,

and finally distillation in an autostill (Wheaton, Millville, New Jersey, or equivalent).

- *Analytical standards*—Standards of the chloroacetanilide herbicide metabolites and the internal standard.
- *SPE columns*—C-18 Sep-Pak Plus, containing 360 mg of 40-µm C-18 bonded-silica packing (Waters, Milford, Massachusetts).
  or
  SPE columns—C-18 Sep-Pak Vac 6 cm^3, containing 500 mg of 50- to 105-µm C-18 bonded-silica packing (Waters, Milford, Massachusetts).
- *Disposable snap-cap finish centrifuge tubes*—10 mL (Kimble, Vineland, New Jersey, or equivalent).
- *Solvents*—
  - Acetonitrile, American Chemical Society (ACS) and HPLC grade.
  - Ethyl acetate, HPLC grade.
  - Methanol, ACS and HPLC grade.
  - Sodium phosphate, dibasic anhydrous, ACS grade.
- *Gas for evaporation*—Nitrogen, ultrapure grade.
- *Pasteur pipettes*—Kimble, (Vineland, New Jersey), or equivalent.
- *0.1-mL autosampler vials*—Plastic vial with glass cone insert and cap (Wheaton, Millville, New Jersey).
- Gases—
  - LC *solvent degasser*—Helium, zero grade.
  - LC *autosampler gas*—Air, zero grade.

Sampling Methods

Following USGS protocol, sampling methods capable of collecting water samples that accurately represent the water-quality characteristics of the surface water or ground water at a given time or location are used. Detailed descriptions of sampling methods used by the USGS for obtaining depth- and width-integrated surface-water samples are given in Edwards and Glysson (1988) and Ward and Harr (1990). Similar descriptions of sampling methods for obtaining ground-water samples are given in Hardy and others (1989).

Briefly, sample-collection equipment are free of tubing, gaskets, and other components made of nonfluorinated plastic material that might leach interferences into water samples or sorb the pesticides and metabolites from the water. The water samples from each site are composited in a single container and filtered through a 0.7-µm glass-fiber filter using a peristaltic pump. Filters are leached with about 200 mL of sample prior to filtration of the sample. The filtrate for analysis is collected in baked 125-mL amber glass bottles with Teflon-lined lids. Samples are chilled immediately and shipped to the laboratory within 3 days of collection. At the laboratory, samples are logged in, assigned identification numbers, and refrigerated at 4 ± 2 °C until extracted and analyzed.

Standards

- *Stock standard solutions*—Obtain chloroacetanilide herbicide metabolites and internal standard as pure materials from commercial vendors or chemical manufacturers. Prepare solutions of 1.00 mg/mL (corrected for purity) by accurately weighing, to the nearest 0.001 g, 50 mg of the pure material in a 50-mL volumetric flask and dilute with methanol. Store at less than 0 °C. This solution is stable for about 24 months.
- *Primary fortification standard*—Prepare a 1.23-ng/µL concentration, primary fortification standard by combining appropriate volumes of the individual chloroacetanilide herbicide metabolites stock solutions in a 1-L volumetric flask. Dilute with methanol. Store at less than 0 °C. This solution is stable for about 24 months.
- *Internal standard solution*—Prepare a solution of 2,4-dichlorophenoxyacetic acid in methanol at a concentration of 2.0 ng/µL. Store at less than 0 °C. This solution is stable for about 12 months.
- *Calibration solutions*—Prepare a series of calibration solutions using the primary fortification standard in reagent water (at concentrations ranging from 0.2 to 2.0 µg/L (0.2, 0.5, 1.0, and 2.0 µg/L). These will be processed through the extraction procedure (described in the "Procedure" section).

Evaluation of Liquid Chromatograph and Diode Array Detector Performance

LC performance is evaluated by background absorbance readings, peak shape, and pressure. Background absorbance signals should remain balanced and low and indicate that the columns have equilibrated with the mobile-phase flow and the absence of interferents. If peak shape deteriorates, the columns may need to be replaced. If the pressure reading is high, there may be a clog in the mobile-phase flow path, or the column compartment thermostat may not

have reached the required temperature yet. A variable DAD background signal indicates that the lamp may need replacement.

Calibration

- Acquire initial calibration data by using new columns, a new lamp, and freshly prepared calibration solutions. Use these data in the subsequent evaluation of HPLC-DAD performance.
- Acquire data for each calibration point by injecting 50 µL of each extracted calibration solution into the HPLC according to the conditions already described. Calculate the relative retention time (RRT_c) for each selected compound in the calibration solution or in a sample as follows:

$$RRT_c = \frac{RT_c}{RT_i}, \quad (1)$$

where RT_c = uncorrected retention time of the selected compound, and
RT_i = uncorrected retention time of the internal standard (2,4-dichlorophenoxyacetic acid).

See table 2 for an example of retention times and relative retention times.

- Initial calibration data are entered into a computer spreadsheet (Microsoft Excel, Microsoft, Inc., Seattle, Washington), and ratios of the peak heights to the internal standard peak height are calculated for each compound. The spreadsheet determines the slopes and y intercepts for each compound on the basis of plotting a correlation curve, with the internal-standard ratio for the compound on the x axis and the concentration of the standard used on the y axis. The spreadsheet also determines the correlation coefficient (r^2) values.
- Initial calibration data acquired using new columns, possibly a new lamp, and freshly prepared calibration solutions are acceptable if the r^2 values for all curves are greater than or equal to 0.990 for all compounds and the apexes of adjacent compound peaks are separated.
- Subsequent daily laboratory control standards need to agree within ±20 percent of the actual concentration for the selected compound of interest. At least two laboratory control standards are analyzed with each sample set, one high calibration standard ranging from 0.50 to 2.0 µg/L and one low standard ranging from 0.20 to 0.50 µg/L, to verify instrument response in each range.

Table 2. Retention times and relative retention times for chloroacetanilide herbicide metabolites and an internal standard analyzed using high-performance liquid chromatography-diode array detection
[min, minute; ESA, ethanesulfonic acid; OXA, oxanilic acid]

Compound	Retention time (min)	Relative retention time
Metabolites (in order of increasing retention time)		
Alachlor OXA	24.756	1.393
Acetochlor OXA	25.702	1.446
Alachlor ESA	30.014	1.688
Metolachlor OXA	30.629	1.723
Acetochlor ESA	31.854	1.792
Metolachlor ESA	34.158	1.921
Internal standard		
2,4-dichlorophenoxyacetic acid	17.778	1.000

Procedure

Two automated extraction systems are used in the laboratory. One method uses an automated Millilab 1A workstation (Waters, Milford, Massachusetts), and the SPE cartridges (Sep-Pak) are obtained from Waters (Milford, Massachusetts). The SPE cartridges contain 360 mg of 40-µm C-18- ($C_{18}H_{37}$) bonded silica.

An alternate SPE procedure used to extract samples is a Tekmar six-position AutoTrace (Tekmar-Dohrmann, Cincinnati, Ohio) and SPE cartridges (Vac C-18 6 mL) obtained from Waters Corporation (Milford, Massachusetts). These vacuum cartridges contain 500 mg of 55- to 105-µm C-18- ($C_{18}H_{37}$) bonded silica. The same manufacturer of C-18-bonded silica is used for both SPE procedures, but the different automation systems require different cartridge formats. The differing quantities of C-18-bonded silica and the different automation systems require minor differences in solvent volumes used in the extraction. The data in this report were produced using the automated Millilab 1A workstation, but the Tekmar six-position AutoTrace procedure is listed in Appendix 1.

- *Sample preparation*—In the automation of sample extraction, the workstation (Waters Millilab, Milford, Massachusetts) requires 23 mL of sample to prime the pumps and rinse the tubing. Therefore, each sample must be 123 mL, although only 100 mL passes through the SPE column. Conveniently, 123 mL is the exact volume that

fits in the body of a 4-oz Boston round bottle. Should an environmental sample contain less than 123 mL, distilled water is added to bring the volume to the required 123 mL. Any volume added is recorded. An extraction sample set consists of nine samples, one duplicate sample, two standard control samples (one high concentration and one low concentration), and two blank control samples.

- *Workstation preparation*—Before a sample set is extracted on the workstation, each port is flushed with 15 mL of methanol:water (1:1) and then again with distilled water. All SPE columns, test tubes, solvents, internal standard solution, and samples then are loaded onto the instrument.
- *Conditioning SPE columns*—The workstation conditions each SPE column by sequentially passing 1 mL methanol, 1 mL ethyl acetate, 1 mL methanol, and 3 mL distilled water through each column at a flow rate of 20 mL/min by positive pressure.
- *Loading sample*—Each sample port is flushed with 23 mL of sample, then 100 mL of sample are passed through the SPE column at a flow rate of 20 mL/min.
- *Eluting interferring compounds from SPE column*—Each SPE column is eluted with 3.5 mL ethyl acetate at a flow rate of 4 mL/min to remove the parent herbicides and other potentially interfering compounds.
- *Eluting metabolites from SPE column*—Each SPE column is eluted with 3.5 mL methanol at a flow rate of 4 mL/min to remove the chloroacetanalide herbicide metabolites into a 10-mL glass centrifuge tube.
- *Spiking of internal standard*—After all the samples in a set have been loaded and eluted, each methanol eluate is spiked with 500 µL of 0.2-ng/µL 2,4-dichlorophenoxyacetic acid solution. The internal standard is used to normalize injection-volume variation, as a retention-time reference, and for quantitation.
- *Evaporation*—The spiked solution then is evaporated under nitrogen in a water bath at 50 °C.
- *Reconstitution*—The extracts are reconstituted with 75 µL of a solution containing 60 percent, pH 7.0, 25-mM phosphate buffer and 40 percent methanol, and then are vortexed.
- *Transfer to vials*—Using a disposable Pasteur pipette, the eluted solution from the 10-mL glass centrifuge tube is transferred to an appropriately labeled autosampler vial containing a 0.1-mL insert for HPLC-DAD analysis. The autosampler vial is capped and stored at less than 0 °C until analysis by HPLC-DAD.
- *Sample analysis and data evaluation*—Ensure that HPLC-DAD conditions for the analysis of the selected metabolites in sample extracts are the same as those used in the analysis of the calibration solutions. Prior to the analysis of any sample extracts the HPLC-DAD must meet the performance criteria and the selected-metabolite calibration data must conform to the criteria set forth. Fifty (50) µL of the sample extract is injected, and data are acquired using the HPLC-DAD conditions described.

Calculation of Results

Qualitative Identification

- The expected retention time (RT) of the peak of the selected metabolite of interest needs to be within ±2 percent of the expected retention time on the basis of the RRT_c obtained from the internal-standard analysis. The expected retention time is calculated as follows:

$$RT = (RRT_c)(RT_i) \quad , \tag{2}$$

where RT = expected retention time of the selected metabolite,
RRT_c = relative retention time of the selected metabolite, and
RT_i = uncorrected retention time of the internal standard.

- If there are multiple peaks that agree in retention time to a metabolite, then additional qualitative indentification can be made using spectral information obtained by the 3D ChemStation software on the HPLC-DAD instrument.

Quantitation

- The dilution factor is calculated as follows:

$$DF = \left(\frac{123}{123 - V_{np}}\right)\left(\frac{123}{123 + V_a}\right), \tag{3}$$

where DF = dilution factor,
V_{np} = volume not pumped = volume, in mL, not pumped through the SPE column, and

V_a = volume added = volume, in mL, of distilled water added to a sample that contains less than 123 mL.

The dilution factor is incorporated into the calculation for determining the final concentration in the sample.

- If a selected metabolite has passed the aforementioned qualitative identification criteria, the concentration in the sample is calculated as follows:

$$C = \left(\left(\frac{H_c}{H_i}\right)(m) + y\right)(DF) \quad , \tag{4}$$

where C = concentration of the selected metabolite in the sample, in micrograms per liter;

H_c = peak height, in milliabsorbance units, for the selected metabolite identified;

H_i = peak height, in milliabsorbance units, for the internal standard;

m = slope of correlation curve between the selected metabolite and the internal standard from the original calibration data;

y = y intercept of correlation curve between the selected metabolite and the internal standard from the original calibration data; and

DF = dilution factor as calculated in equation 3.

Reporting of Results

Chloroacetanalide herbicide metabolites are reported in concentrations ranging from 0.2 to 3.0 µg/L. If the concentration is greater than 3.0 µg/L, the sample is re-extracted with a 1:10 (sample:distilled water) dilution and re-analyzed for those compounds that were greater than 3.0 µg/L. Linearity experiments have been conducted showing linear response curves from 0.2 to 10 µg/L.

Method Performance

A reagent-water sample, a surface-water sample collected from Poison Creek in Valley County, Idaho, and a ground-water sample collected from a well in Valley County, Idaho, were used to test the method performance. The surface- and ground-water samples were collected in 45-L carboys and were split into twenty-one 123-mL samples. One set of seven samples was spiked with 0.25 µg/L of each chloroacetanalide metabolite of interest, one set with 0.50 µg/L, and the other set of seven samples was spiked with 2.0 µg/L. In addition, unspiked samples of surface and ground water were extracted and analyzed to determine background concentrations of the pesticides. All subsamples were analyzed in one laboratory (the USGS Organic Geochemistry Research Laboratory in Lawrence, Kansas) using one HPLC-DAD system. Each sample set was extracted and analyzed on different days from November 1998 through January 1999, so comparison of different matrices and concentrations included bias from day-to-day variation. Method recoveries from the analyses are listed in tables 3, 4, and 5.

Corrections for background concentrations: Neither surface- nor ground-water samples required correction for background concentrations of metabolites. All unspiked reagent-water samples also had no detections of metabolites.

Method detection limits (MDL's): An MDL is defined as the minimum concentration of a substance that can be identified, measured, and reported with a 99-percent confidence that the compound concentration is greater than zero. MDL's were determined according to procedures outlined by the U.S. Environmental Protection Agency (1992). Seven replicate samples of reagent water were spiked with 0.25 µg/L of each of the metabolites of interest and analyzed to determine MDL's (table 6). Each sample was analyzed on different days during November 1998 through January 1999, so day-to-day variation is included in the results.

The MDL was calculated using the following equation:

$$MDL = (S)(t_{(n-1,\ 1\text{-}\alpha=0.99)}) \quad , \tag{5}$$

where S = standard deviation of replicate analysis, in micrograms per liter, at the spiked concentration;

$t_{(n\text{-}1,\ 1\text{-}\alpha=0.99)}$ = Student's t-value for the 99-percent confidence level with n-1 degrees of freedom (U.S. Environmental Protection Agency, 1992); and

n = number of replicate analyses.

The estimated mean MDL's ranged from 0.09 to 0.17 µg/L (table 6). According to the U.S. Environmental Protection Agency (1992) procedure, the

Table 3. Mean recovery of chloroacetanilide metabolites in reagent-water samples using high-performance liquid chromatography-diode array detection

[µg/L, micrograms per liter; RSD, relative standard deviation; ESA, ethanesulfonic acid; OXA, oxanilic acid]

	Reagent water					
	Seven replicate samples spiked at 0.25 µg/L		Seven replicate samples spiked at 0.50 µg/L		Seven replicate samples spiked at 2.0 µg/L	
Metabolite	Mean recovery (percent)	RSD (percent)	Mean recovery (percent)	RSD (percent)	Mean recovery (percent)	RSD (percent)
Acetochlor ESA	112	16	104	9.0	105	2.3
Acetochlor OXA	88	18	94	14	95	3.1
Alachlor ESA	100	10	102	5.5	100	2.4
Alachlor OXA	84	17	92	9.6	90	2.7
Metolachlor ESA	108	10	104	7.3	105	3.2
Metolachlor OXA	108	14	102	5.3	100	3.1

Table 4. Mean recovery of chloroacetanalide herbicide metabolites in surface-water samples using high-performance liquid chromatography diode-array detection

[µg/L, micrograms per liter; RSD, relative standard deviation; ESA, ethanesulfonic acid; OXA, oxanilic acid]

	Surface water					
	Seven replicate samples spiked at 0.25 µg/L		Sevel replicate samples spiked at 0.50 µg/L		Seven replicate samples spiked at 2.0 µg/L	
Metabolite	Mean recovery (percent)	RSD (percent)	Mean recovery (percent)	RSD (percent)	Mean recovery (percent)	RSD (percent)
Acetochlor ESA	100	16	104	8.0	100	5.4
Acetochlor OXA	84	14	94	7.0	100	4.9
Alachlor ESA	104	8.9	98	3.4	105	4.4
Alachlor OXA	92	14	96	7.4	100	6.3
Metolachlor ESA	108	12	102	4.9	105	4.6
Metolachlor OXA	108	16	100	3.5	100	5.7

Table 5. Mean recovery of chloroacetanilide herbicide metabolites in ground-water samples using high-performance liquid chromatography-diode array detection

[µg/L, micrograms per liter; RSD, relative standard deviation; ESA, ethanesulfonic acid; OXA, oxanilic acid]

	Ground water					
	Seven replicate samples spiked at 0.25 µg/L		Sevel replicate samples spiked at 0.50 µg/L		Seven replicate samples spiked at 2.0 µg/L	
Metabolite	Mean recovery (percent)	RSD (percent)	Mean recovery (percent)	RSD (percent)	Mean recovery (percent)	RSD (percent)
Acetochlor ESA	105	18	107	8.4	102	3.8
Acetochlor OXA	87	15	95	7.8	99	5.3
Alachlor ESA	103	9.3	98	4.6	103	3.2
Alachlor OXA	88	13	94	6.9	94	4.8
Metolachlor ESA	102	12	103	5.0	98	4.1
Metolachlor OXA	104	16	100	4.8	100	3.5

Table 6. Mean method detection limits for seven determinations of chloroacetanilide herbicide metabolites spiked at 0.25 micrograms per liter in reagent water using high-performace liquid chromatography-diode array detection
[MDL, method detection, µg/L, micrograms per liter; ESA, ethanesulfonic acid; OXA, oxanilic acid]

Metabolite	Mean observed concentration (µg/L)	Mean standard deviation (µg/L)	Mean MDL (µg/L)
Acetochlor ESA	0.28	0.16	0.15
Acetochlor OXA	.22	.18	.17
Alachlor ESA	.25	.10	.09
Alachlor OXA	.21	.17	.16
Metolachlor ESA	.27	.10	.09
Metolachlor OXA	.27	.14	.13

spiked concentrations should be no more than five times the estimated MDL. The spiked concentrations were within five times the MDL.

Mean recovery: Mean recovery in reagent-, surface-, and ground-water samples was determined by comparing the mean calculated concentration (see "Quantitation" section) from seven replicate samples to the spiked concentration. Mean recoveries were highest in reagent water spiked at 0.25 µg/L (table 3). Acetochlor OXA and alachlor OXA generally exhibited the lowest recoveries regardless of matrix. The mean recoveries of all compounds spiked at the concentrations in table 3 were combined to calculate the mean recovery for reagent-water samples. The mean recovery in reagent water at all spiked concentrations was 99.6 percent.

Extraction absolute recovery: Absolute recovery of each chloroacetanalide metabolite was determined by comparing samples processed using the aforementioned procedure versus solvent spiked with the metabolites injected directly into the HPLC-DAD. Metabolite peak heights were compared to internal-standard peak heights. Absolute recoveries are listed in table 7. Absolute recovery is different than mean recovery listed in tables 3–5 in that mean recovery is calculated from an initial calibration curve that is processed in the same manner as the samples, thus correcting for routine analyte losses.

Method discussion: SPE and recovery for chloroacetanilide metabolites have been discussed in

Table 7. Absolute recovery for 10 determinations of chloroacetanilide herbicide metabolites in reagent water using high-performance liquid chromatography-diode array detection
[ESA, ethanesulfonic acid; OXA, oxanilic acid]

Metabolite	Absolute recovery (percent)
Acetochlor ESA	71
Acetochlor OXA	110
Alachlor ESA	76
Alachlor OXA	110
Metolachlor ESA	72
Metolachlor OXA	106

previous work (Aga and others, 1994; Thurman and others, 1996; Ferrer and others, 1997). In those studies, chromatographic separation was achieved only for a few of the herbicide metabolites specified in this report. In the work described in this report, each control surface- and ground-water sample was spiked with a standard containing all the ionic chloroacetanilide metabolites of interest. For purposes of accuracy and precision, chromatographic separation of the metabolites was essential.

The phosphate buffer supplied sodium as a counter ion to the anionic metabolites, creating neutral species that interact with the column. Coupling two columns and maintaining the columns at 60 °C yielded enough metabolite-peak resolution for peak-height quantitation. In this case, it is not known exactly why using two columns with different particle diameters (5 µm and 3 µm) and column diameters (3 mm and 4.6 mm) gave better metabolite separation than using two identical columns. One hypothesis is that water capacity of the column is related to the particle diameter, giving rise to subtle differences in ionic interactions. The columns were configured so that the larger particle column was positioned before the smaller particle column for effective backpressure regulation (smaller phase thickness gives higher backpressure). The analytical wavelength was set at 210 nm, and DAD spectra were stored for every integrated peak with a peak height greater than 0.5 mAU. Figure 1 shows a typical HPLC-DAD chromatogram of a 2.0-µg/L control reagent-water sample.

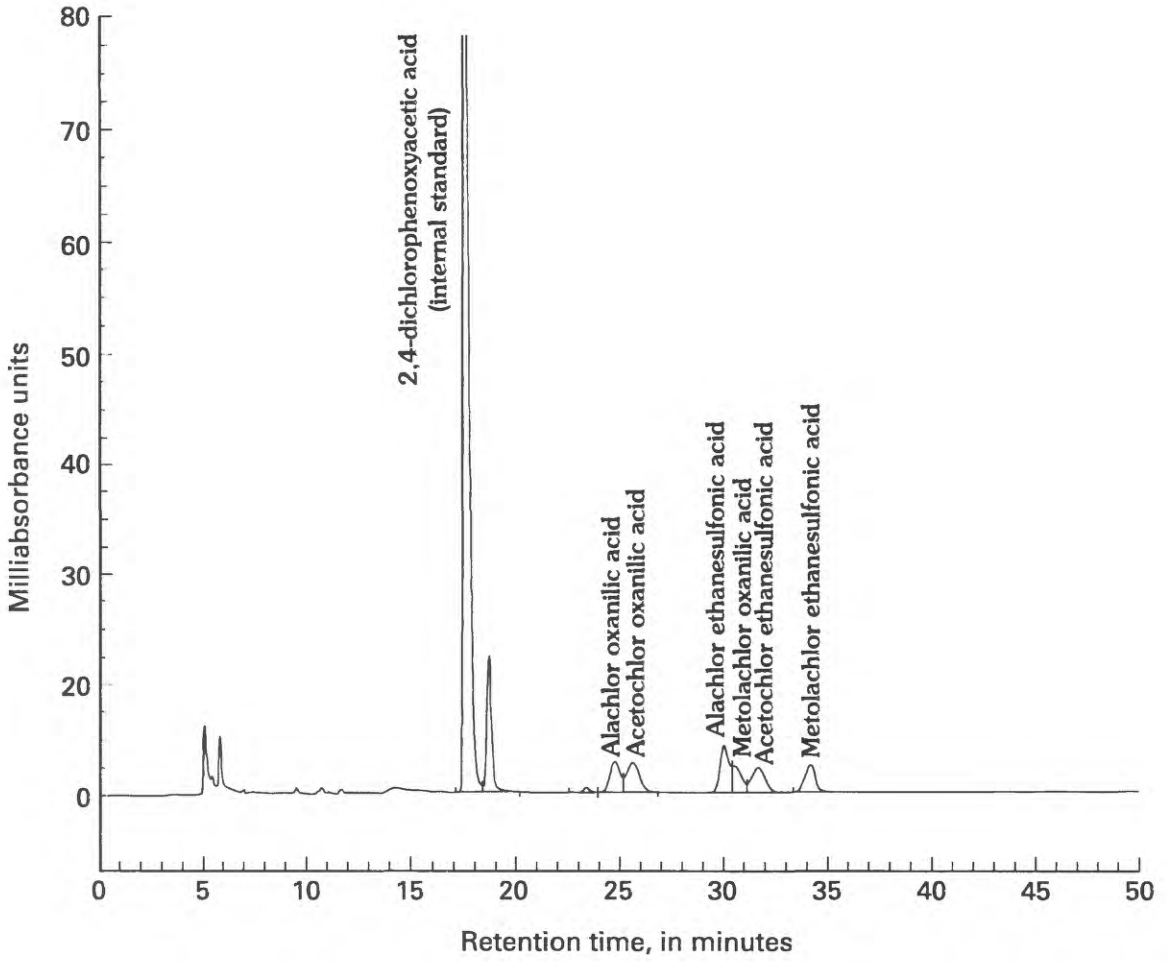

Figure 1. Typical chromatogram of a 2.0-microgram-per-liter control reagent-water sample using high-performance liquid chromatography-diode array detection.

DETERMINATION OF CHLOROACETANILIDE HERBICIDE METABOLITES IN WATER USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY

Method of Analysis (0–2134–00)

Scope and Application

The HPLC/MS is suitable for the determination of low concentrations (in micrograms per liter) of chloroacetanilide metabolites in water samples (table 1). Suspended particulate matter is removed from the samples by filtration, so this method is suitable only for dissolved-phase metabolites.

Metabolites were selected for analyses because of the extensive use of their parent herbicides in the United States and their importance to current (1999) studies being conducted by the USGS. The HPLC/MS method is suitable for concentrations ranging from 0.05 to 5.0 µg/L without dilution.

Summary of Method

Water samples are filtered at the collection site using baked, glass-fiber filters with 0.7-µm pore diameter to remove suspended particulate matter. In the laboratory, filtered water samples are passed through a preconditioned C-18 column. The C-18 column is rinsed with ethyl acetate to remove interferring compounds. The adsorbed chloroacetanilide metabolites are removed from the C-18 with methanol. The eluted solution is spiked with an internal standard, evaporated under nitrogen, and reconstituted. The sample components are separated, identified, and measured by

injecting an aliquot of the concentrated extract into an HPLC equipped with a DAD and a mass spectrometer detector. Compounds eluting from the LC columns are identified by comparing their retention times obtained by the measurement of control samples under the same conditions used for the collected samples. Compounds are identified further by selected fragment ions for compounds that can produce fragment ions. The concentration of each identified compound is measured by relating the MS response produced by that compound to the MS response produced by the internal standard.

Interferences

Compounds that elute from the LC at the same time and have identical ions as the metabolites of interest may interfere. Samples with considerable humic materials can cause interference with the ionization of the internal standard if they are eluting from the LC column at the same time.

Apparatus and Instrumentation

- *Analytical balances*—Capable of accurately weighing 0.0100 g ± 0.0001 g.
- *Autopipettes*—5- to 200-µL, variable-volume autopipettes with disposable tips (Rainin, Woburn, Massachusetts, or equivalent).
- *Millilab 1A workstation*—Automated SPE workstation with an online computer. The two syringe pumps on the fluidics module are equipped with a 5- and a 1-mL syringe (Waters, Milford, Massachusetts).
  - Multiple intake accessories (MIAs): Two MIAs are attached to the 5-mL syringe to increase sample capacity from 3 to 14.
  - Software: Millilab 1A software, version 3.0 (Waters, Milford, Massachusetts).

  or

  Tekmar six-position AutoTrace—Automated SPE workstation (Tekmar-Dohrmann, Cincinnati, Ohio).
  - Software: Tekmar AutoTrace Extraction software, version 1.33 (Tekmar-Dohrmann, Cincinnati, Ohio).
- *Automated solvent evaporator*—The heated bath temperature needs to be maintained at 50 °C, and the nitrogen gas pressure at 15 lb/in^2 (TurboVap LV, Zymark Inc., Hopkinton, Massachusetts, or equivalent).
- *Mechanical vortex*
- *Analytical columns*—two Phenomenex 5-µm, 250- x 3-mm C-18 columns coupled to one (or two, if within backpressure limitations) Phenomenex 3-m, 150- x 2.0-mm C-18 column.
- *HPLC/MS benchtop system*—Hewlett Packard (Wilmington, Delaware), model 1100 HPLC with autoinjector and MS detector.
  - LC oven conditions: constant 70 °C .
  - LC mobile phase: 0.3 percent acetic acid, 24 percent methanol, 35.7 percent distilled water, and 40 percent acetonitrile solution with a flow rate of 0.3 to 0.4 mL/min.
  - MS detector mode: electrospray in negative ion mode.
  - Drying gas: flow was set at 6 L/min.
  - Nebulizer pressure was 25 lbs/in^2, the drying gas temperature was 300 °C, the capillary voltage was 3,100 V, and the fragmenter voltage was 70 V.
- *Data system*—Computer and printer compatible with the HPLC system.
- *Software*—HP LC/MSD ChemStation rev.A.06.03 (Hewlett Packard, Wilmington, Delaware) is used to acquire and store data and for peak integration.

Reagents and Consumable Materials

- *Sample bottles*—Baked 4-oz amber glass bottles (Boston round) with Teflon-lined lids.
- *Sample filters*—0.7-µm glass-fiber filters (Gilson, Middleton, Wisconsin, or equivalent).
- *Reagent water*—generated by purification of tap water through activated charcoal filtration and deionization with a high-purity, mixed-bed resin, followed by another activated charcoal filtration, and finally distillation in an autostill (Wheaton, Millville, New Jersey, or equivalent).
- *Analytical standards*—Standards of the chloroacetanilide herbicide metabolites and the internal standard.
- *SPE columns*—C-18 Sep-Pak Plus, containing 360 mg of 40-µm C-18 bonded-silica packing (Waters, Milford, Massachusetts).

  or

  SPE columns—C-18 Sep-Pak Vac 6 cm^3, containing 500 mg of 50- to 105-µm C-18

bonded-silica packing (Waters, Milford, Massachusetts).

- *Disposable snap-cap finish centrifuge tubes*—10 mL (Kimble, Vineland, New Jersey, or equivalent).
- *Solvents*—
  - Acetonitrile, ACS and HPLC grade.
  - Ethyl acetate, HPLC grade.
  - Methanol, ACS and HPLC grade.
- *Acetic acid, glacial*—ACS grade.
- *Gas for evaporation*—nitrogen, ultrapure grade.
- *Pasteur pipettes*—(Kimble, Vineland, New Jersey, or equivalent).
- *0.1-mL autosampler vials*—Plastic vial with glass cone insert and cap (Wheaton, Millville, New Jersey).
- Nebulizer gas—*nitrogen, ultrapure grade.*

Sampling Methods

Following USGS protocol, sampling methods capable of collecting water samples that accurately represent the water-quality characteristics of the surface water or ground water at a given time or location are used. Detailed descriptions of sampling methods used by the USGS for obtaining depth- and width-integrated surface-water samples are given in Edwards and Glysson (1988) and Ward and Harr (1990). Similar descriptions of sampling methods for obtaining ground-water samples are given in Hardy and others (1989).

Briefly, sample-collection equipment are free of tubing, gaskets, and other components made of nonfluorinated plastic material that might leach interferences into water samples or sorb the pesticides and metabolites from the water. The water samples from each site are composited in a single container and filtered through a 0.7-µm glass-fiber filter using a peristaltic pump. Filters are leached with about 200 mL of sample prior to filtration of the sample. The filtrate for analysis is collected in baked 125-mL amber glass bottles with Teflon-lined lids. Samples are chilled immediately and shipped to the laboratory within 3 days of collection. At the laboratory, samples are logged in, assigned identification numbers, and refrigerated at 4 ±2 °C until extracted and analyzed.

Standards

- *Stock standard solutions*—Obtain chloroacetanilide herbicide metabolites and internal standard as pure materials from commercial vendors or chemical manufacturers. Prepare solutions of 1.00 mg/mL (corrected for purity) by accurately weighing, to the nearest 0.001 g, 50 mg of the pure material in a 50-mL volumetric flask and dilute with methanol. Store at less than 0 °C. This solution is stable for about 24 months.
- *Primary fortification standard*—Prepare a 1.23-ng/µL concentration, primary fortification standard by combining appropriate volumes of the individual chloroacetanilide herbicide metabolites stock solutions in a 1-L volumetric flask. Dilute with methanol. Store at less than 0 °C. This solution is stable for about 24 months.
- *Internal standard solution*—Prepare a solution of 2,4-dichlorophenoxyacetic acid in methanol at a concentration of 2.0 ng/µL. Store at less than 0 °C. This solution is stable for about 12 months.
- *Calibration solutions*—Prepare a series of calibration solutions using the primary fortification standard in reagent water at concentrations ranging from 0.05 to 2.0 µg/L (0.05, 0.10, 0.20, 0.50, 1.0, and 2.0 µg/L).

Evaluation of High-Performance Liquid Chromatography/Mass Spectrometry Performance

Evaluation of Liquid Chromatograph and Diode Array Detector Performance

LC performance is evaluated by background absorbance readings, peak shape, and pressure. Background absorbance signals should remain balanced and low and indicate that the columns have equilibrated with the mobile-phase flow. If peak shape deteriorates, the columns may need to be replaced. If the pressure reading is high, there may be a clog in the mobile-phase flow path, or the column compartment thermostat may not have reached the required temperature yet. A variable DAD background signal indicates that the lamp may need replacement.

Evaluation of Mass Spectrometer Performance

- Tune the MS in electrospray in negative-ion mode before each HPLC/MS sample set (approximately 45 injections) using the procedure and software supplied by the manufacturer.
- For the first sample of a sample set, inject a solution of 0.3 percent acetic acid, 24 percent methanol,

35.7 percent distilled water, and 40 percent acetonitrile solution to check for contamination.

Calibration

- Acquire initial calibration data by using new columns, a clean MS source, and freshly prepared calibration solutions. Use these data in the subsequent evaluation of HPLC/MS performance.
- Acquire data for each calibration solution by injecting 50 µL of each solution into the HPLC/MS according to the conditions already described. Calculate the relative retention time for each selected compound (RRT_c) in the calibration solution or in a sample using equation 1. See table 8 for an example of retention times, relative retention times, and confirmation ions.
- Initial calibration data are entered into a computer spreadsheet (Microsoft Excel, Microsoft, Inc., Seattle, Washington), and ratios of the quantitation ion peak areas to the internal standard quantitation ion peak area are calculated for each compound. The spreadsheet determines the slopes and y intercepts for each compound by plotting the correlation curve with the internal standard ratio of a single compound on the x axis and the concentration of the standard used on the y axis. The spreadsheet also determines the correlation coefficient (r^2) values.
- Initial calibration data are acceptable if the r^2 value for all curves is greater than or equal to 0.990 for all compounds and the apex of adjacent compound peaks are separated.
- Subsequent daily laboratory control standards need to agree within ±20 percent of the actual concentration for the selected compound of interest. At least two laboratory control standards are analyzed with each sample set, one high calibration standard ranging from 0.50 to 2.0 µg/L and one low standard ranging from 0.05 to 0.20 µg/L to verify instrument response in each range.

Procedure

Two automated extraction systems are used in the laboratory. One method uses an automated Millilab 1A workstation (Waters, Milford, Massachusetts), and SPE cartridges (Sep-Pak) obtained from Waters (Milford, Massachusetts). The SPE cartridges contain 360 mg of 40-µm C-18- ($C_{18}H_{37}$) bonded silica.

An alternate SPE procedure used to extract samples is a Tekmar six-position AutoTrace (Tekmar-Dohrmann, Cincinnati, Ohio) and SPE cartridges (Vac C-18 6 mL) obtained from Waters Corporation (Milford, Massachusetts). These vacuum cartridges contained 500 mg of 55- to 105-µm C-18- ($C_{18}H_{37}$) bonded silica. The same manufacturer of C-18-bonded silica is used for both SPE procedures, but the different automation systems require different cartridge formats. The differing quantities of C-18-bonded silica and the different automation systems require minor differences in solvent volumes used in the extraction. The data in this report were produced using the automated Millilab 1A workstation, but the Tekmar six-position AutoTrace procedure is listed in Appendix 1.

- *Sample preparation*—In the automation of sample extraction, the workstation (Waters Millilab, Milford, Massachusetts) requires 23 mL of sample to prime the pumps and rinse the tubing. Therefore, each sample must be 123 mL, although only 100 mL passes through the SPE column.

Table 8. Retention times, relative retention times, and ions for chloroacetanilide herbicide metabolites analyzed using high-performance liquid chromatography/mass spectrometry
[min, minute; m/z, mass-to-charge ratio; OXA, oxanilic acid; ESA, ethanesulfonic acid; --, not applicable]

Compound	Retention time (min)	Relative retention time	Quantitation ion (m/z)	Fragmentation ion(s) (m/z)
Metabolites (in order of increasing retention time)				
Metolachlor OXA	37.655	1.754	278	206
Alachlor OXA	38.031	1.772	264	160, 192
Acetochlor OXA	38.258	1.782	264	146
Alachlor ESA	48.368	2.253	314	--
Metolachlor ESA	49.283	2.296	328	--
Acetochlor ESA	49.532	2.308	314	--
Internal standard				
2,4-dichlorophenoxyacetic acid	21.465	1.000	219	161

Conveniently, 123 mL is the exact volume that fits in the body of a 4-oz Boston round bottle. Should an environmental sample contain less than 123 mL, distilled water is added to bring the volume to the required 123 mL. Any volume added is recorded. An extraction sample set consists of nine samples, one duplicate sample, two standard control samples (one high concentration and one low concentration), and two blank control samples.

- *Workstation preparation*—Before a sample set is extracted on the workstation, each port is flushed with 15 mL of methanol:water (1:1) and then again with distilled water. All SPE columns, test tubes, solvents, internal standard solution, and samples then are loaded onto the instrument.
- *Conditioning SPE columns*—The workstation conditions each SPE column by sequentially passing 1 mL methanol, 1 mL ethyl acetate, 1 mL methanol, and 3 mL distilled water through each column at a flow rate of 20 mL/min by positive pressure.
- *Loading sample*—Each sample port is flushed with 23 mL of sample, then 100 mL of sample are passed through the SPE column at a flow rate of 20 mL/min.
- *Eluting potential inteferring compounds from SPE column*—Each SPE column is eluted with 3.5 mL ethyl acetate at a flow rate of 4 mL/min to remove the parent herbicides and other potentially interferring compounds.
- *Eluting metabolites from SPE column*—Each SPE column is eluted with 3.5 mL methanol at a flow rate of 4 mL/min to remove the chloroacetanalide herbicide metabolites into a 10-mL glass centrifuge tube.
- *Spiking of internal standard*—After all the samples in a set have been loaded and eluted, each methanol eluate is spiked with 500 µL of 0.2-ng/µL 2,4-dichlorophenoxyacetic acid solution. The internal standard is used to normalize injection-volume variation, as a retention-time reference, and for quantitation.
- *Evaporation*—The spiked solution then is evaporated under nitrogen in a water bath at 50 °C.
- *Reconstitution*—The extracts are reconstituted with 75 µL of a solution containing 0.3 percent acetic acid, 24 percent methanol, 35.7 percent distilled water, and 40 percent acetonitrile, and then are vortexed.
- *Transfer to vials*—Using a disposable Pasteur pipette, the eluted solution from the 10-mL glass centrifuge tube is transferred to an appropriately labeled autosampler vial containing a 0.1-mL insert for HPLC/MS analysis. The autosampler vial is capped and stored at less than 0 °C until analysis by HPLC/MS.
- *Sample analysis and data evaluation*—Ensure that HPLC/MS conditions for the analysis of the selected metabolites in sample extracts are the same as those used in the analysis of the calibration solutions. Prior to the analysis of any sample extracts, the HPLC/MS must meet the performance criteria and the selected-metabolite calibration data must conform to the criteria set forth. Fifty (50) µL of the sample extract is injected, and data are acquired using the HPLC/MS conditions described.

Calculation of Results

Qualitative Identification

- The expected retention time (*RT*) of the peak of the quantitation ion for the selected metabolite of interest needs to be within ±2 percent of the expected retention time on the basis of the RRT_c obtained from the internal-standard analysis. The expected retention time is calculated using equation 2.
- A metabolite is not identified unless it has the correct quantitation ion. If more than one ion is acquired for a metabolite, then additional verification is done by comparing the relative integrated abundance values of the significant ions monitored with the relative integrated abundance values obtained from the standard control samples. The relative ratios of the ions need to be within ±20 percent of the relative ratios of those obtained in the absence of any obvious interferences.

Quantitation

- The dilution factor is calculated using equation 3.
- If a selected metabolite has passed the aforementioned qualitative identification criteria, the concentration in the sample is calculated as follows:

$$C = \left(\left(\frac{Ac}{Ai}\right)(m) + y\right)(DF) \quad , \qquad (6)$$

where C = concentration of the selected metabolite in the sample, in micrograms per liter;

A_c = peak area of the quantitation ion for the selected metabolite;

A_i = peak area of the quantitation ion for the internal standard;

m = slope of correlation curve between the selected metabolite and the internal standard from the original calibration data;

y = y intercept of correlation curve between the selected metabolite and the internal standard from the original calibration data; and

DF = dilution factor calculated using equation 3.

Reporting of Results

Chloroacetanalide herbicide metabolites are reported in concentrations ranging from 0.05 to 5.0 µg/L. If the concentration is greater than 5.0 µg/L, the sample extract is diluted (volume increased to approximately 150 µL with the reconstitution solution (0.3 percent acetic acid, 24 percent methanol, 35.7 percent distilled water, and 40 percent acetonitrile) and re-analyzed. If the concentration is greater than 10 µg/L, the sample is re-extracted with a 1:10 (sample:distilled water) dilution and re-analyzed for those compounds that were greater than 10 µg/L. Linearity experiments have been conducted showing linear response curves from 0.5 to 10 µg/L.

Method Performance

A reagent-water sample, a surface-water sample collected from Poison Creek in Valley County, Idaho, and a ground-water sample collected from a well in Valley County, Idaho, were used to test the method performance. The surface- and ground-water samples were collected in 45-L carboys and were split into twenty-one 123-mL samples. One set of seven samples was spiked with 0.05 µg/L of each chloroacetanalide metabolite of interest, one set with 0.20 µg/L, and the other set of seven samples was spiked with 2.0 µg/L. In addition, unspiked samples of surface and ground water were extracted and analyzed to determine background concentrations of the pesticides. All subsamples were analyzed in one laboratory (the USGS Organic Geochemistry Research Laboratory in Lawrence, Kansas) using one HPLC/MS system. Each sample set was extracted and analyzed on different days from November 1998 through January 1999, so comparison of different matrices and concentrations included bias from day-to-day variation. Method recoveries from the analyses are listed in tables 9, 10, and 11.

Corrections for background concentrations: Neither surface- nor ground-water samples required correction for background concentrations of metabolites. All unspiked reagent-water samples also had no detections of metabolites.

Method detection limits (MDL's): An MDL is defined as the minimum concentration of a substance that can be identified, measured, and reported with a 99-percent confidence that the compound concentration is greater than zero. MDL's were determined according to procedures outlined by the U.S. Environmental Protection Agency (1992). Seven replicate samples of reagent water spiked with 0.05 µg/L of each of the metabolites of interest and were analyzed to determine MDL's (table 12). Each sample was analyzed on different days during November 1998 through January 1999, so day-to-day variation is included in the results. The MDL was calculated using equation 5.

The estimated mean MDL's ranged from 0.04 to 0.05 µg/L (table 12). According to the U.S. Environmental Protection Agency (1992) procedure, the spiked concentrations should be no more than five times the estimated MDL. The spiked concentrations were within five times the MDL.

Mean recovery: Mean recovery in reagent-, surface-, and ground-water samples was determined by comparing the mean calculated concentration (see "Quantitation" section) from seven replicate samples to the spiked concentration. Mean recoveries were highest in surface water at the 2.0-µg/L concentration (table 10), except for acetochlor ESA in reagent water spiked at 0.05 and 0.20 µg/L and in surface water spiked at 0.05 µg/L and for metoalchlor ESA in reagent water spiked at 2.0 µg/L. Acetochlor OXA and alachlor OXA exhibited the lowest recoveries in reagent water, and acetochlor ESA and metolachlor ESA exhibited the highest recoveries regardless of matrix. The mean recoveries of all compounds spiked

Table 9. Mean recovery of chloroacetanilide metabolites in reagent-water samples using high-performance liquid chromatography/mass spectrometry

[µg/L, micrograms per liter; RSD, relative standard deviation; ESA, ethanesulfonic acid; OXA, oxanilic acid]

	Reagent water					
	Seven replicate samples spiked at 0.05 µg/L		Sevel replicate samples spiked at 0.20 µg/L		Seven replicate samples spiked at 2.0 µg/L	
Metabolite	Mean recovery (percent)	RSD (percent)	Mean recovery (percent)	RSD (percent)	Mean recovery (percent)	RSD (percent)
Acetochlor ESA	117	20	125	6.3	110	7.4
Acetochlor OXA	84	12	85	9.6	86	5.9
Alachlor ESA	95	19	100	5.4	100	4.9
Alachlor OXA	81	11	85	11	88	6.4
Metolachlor ESA	113	13	110	5.8	115	6.8
Metolachlor OXA	110	11	105	5.0	104	5.7

Table 10. Mean recovery of chloroacetanilide metabolites in surface-water samples using high-performance liquid chromatography/mass spectrometry

[µg/L, micrograms per liter; RSD, relative standard deviation; ESA, ethanesulfonic acid; OXA, oxanilic acid]

	Surface water					
	Seven replicate samples spiked at 0.05 µg/L		Sevel replicate samples spiked at 0.20 µg/L		Seven replicate samples spiked at 2.0 µg/L	
Metabolite	Mean recovery (percent)	RSD (percent)	Mean recovery (percent)	RSD (percent)	Mean recovery (percent)	RSD (percent)
Acetochlor ESA	118	4.3	104	7.7	104	15
Acetochlor OXA	90	8.1	101	6.1	111	11
Alachlor ESA	81	19	95	7.1	109	13
Alachlor OXA	90	10	99	6.2	109	10
Metolachlor ESA	92	11	101	6.3	114	10
Metolachlor OXA	90	9.5	100	5.9	110	12

Table 11. Mean recovery of chloroacetanilide metabolites in ground-water samples using high-performace liquid chromatography/mass spectrometry

[µg/L, micrograms per liter; RSD, relative standard deviation; ESA, ethanesulfonic acid; OXA, oxanilic acid]

	Ground water					
	Seven replicate samples spiked at 0.05 µg/L		Sevel replicate samples spiked at 0.20 µg/L		Seven replicate samples spiked at 2.0 µg/L	
Metabolite	Mean recovery (percent)	RSD (percent)	Mean recovery (percent)	RSD (percent)	Mean recovery (percent)	RSD (percent)
Acetochlor ESA	102	12	103	5.0	98	4.1
Acetochlor OXA	95	9.3	98	4.6	103	3.2
Alachlor ESA	104	16	100	4.8	100	3.5
Alachlor OXA	85	8.3	95	7.8	99	5.3
Metolachlor ESA	92	11	107	8.4	102	3.8
Metolachlor OXA	95	12	94	6.9	94	4.8

Table 12. Mean method detection limits for seven determinations of chloroacetanilide herbicide metabolites spiked at 0.05 microgram per liter in reagent water using high-performance liquid chromatography/mass spectrometry

[µg/L, micrograms per liter; MDL, method detection limit; ESA, ethanesulfonic acid; OXA, oxanilic acid]

Metabolite	Mean observed concentration (µg/L)	Mean standard deviation (µg/L)	Mean MDL (µg/L)
Acetochlor ESA	0.06	0.12	0.04
Acetochlor OXA	.04	.11	.04
Alachlor ESA	.05	.19	.05
Alachlor OXA	.04	.11	.04
Metolachlor ESA	.06	.13	.05
Metolachlor OXA	.06	.11	.04

at all concentrations in table 9 were combined to calculate the mean recovery for reagent-water samples. The mean recovery in reagent water at all spiked concentrations was 100.7 percent.

Method discussion: A HPLC/MS method for the analysis of ethanesulfonic acids and oxanilic acids of acetochlor, alachlor, and metolachlor was reported by Ferrer and others (1997). The described HPLC system used an 5-µm, 250- x 3.0-mm C-18 column, with a mobile phase consisting of 0.3 percent acetic acid in 24 percent methanol, 36 percent distilled water, and 40 percent acetonitrile solution. With this configuration, peak resolution was not achieved for acetochlor ESA and alachlor ESA, which have the same quantitation ion (table 8). Thus, accurate quantitation of these metabolites was not possible. Chromatographic separation of acetochlor ESA and alachlor ESA was achieved with the same mobile phase by coupling two 5-µm, 250- x 3.0-mm C-18 columns to one (or two, if backpressure permits) 3-µm, 150- x 2.0-mm C-18 column. Figure 2 shows a total ion chromatogram (TIC) of a 0.05-µg/L control reagent-water sample. Figure 3 shows the extracted ion chromatogram for the molecular ion (314 mass-to-charge ratio) of acetochlor ESA and alachlor ESA with near baseline separation. The

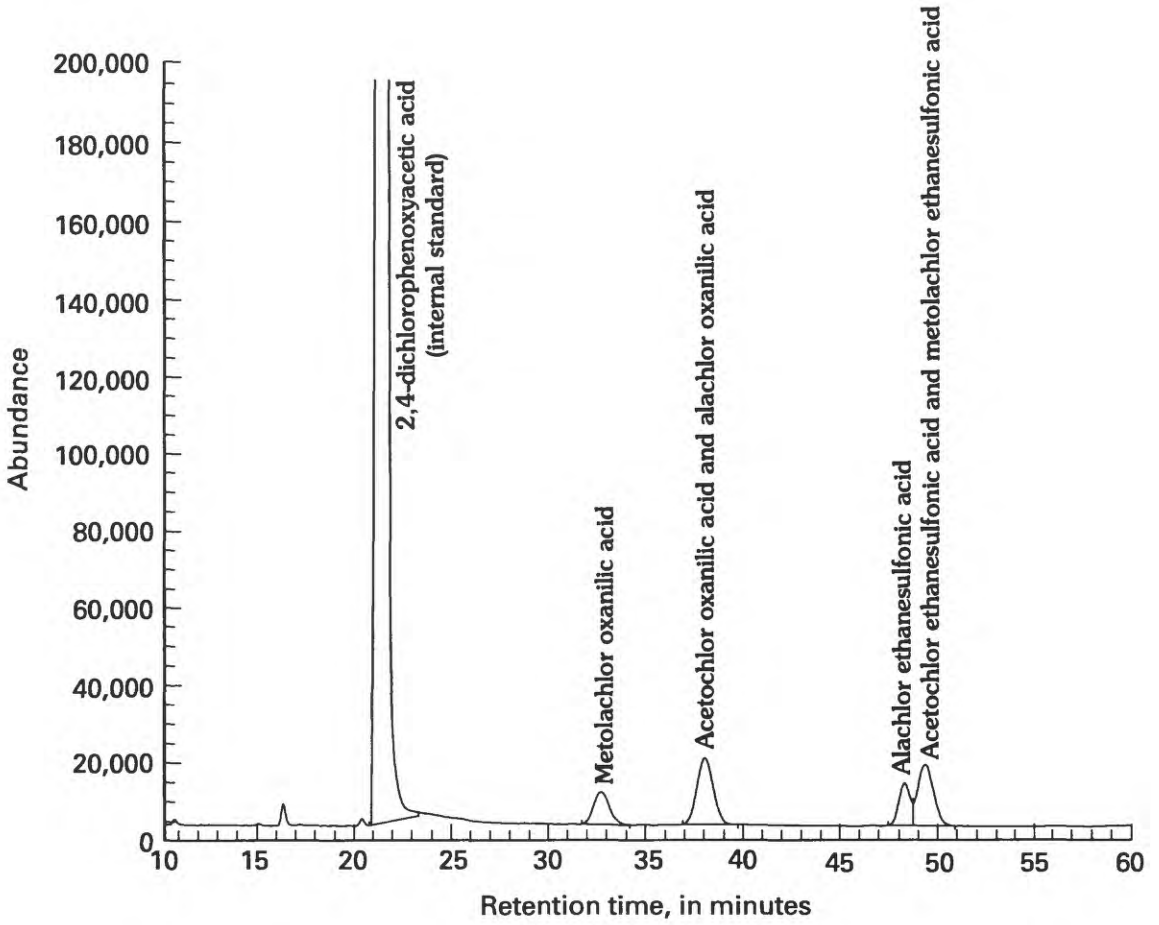

Figure 2. Total ion chromatogram (TIC) of a 0.05-microgram-per-liter control reagent-water sample using high-performance liquid chromatography/mass spectrometry.

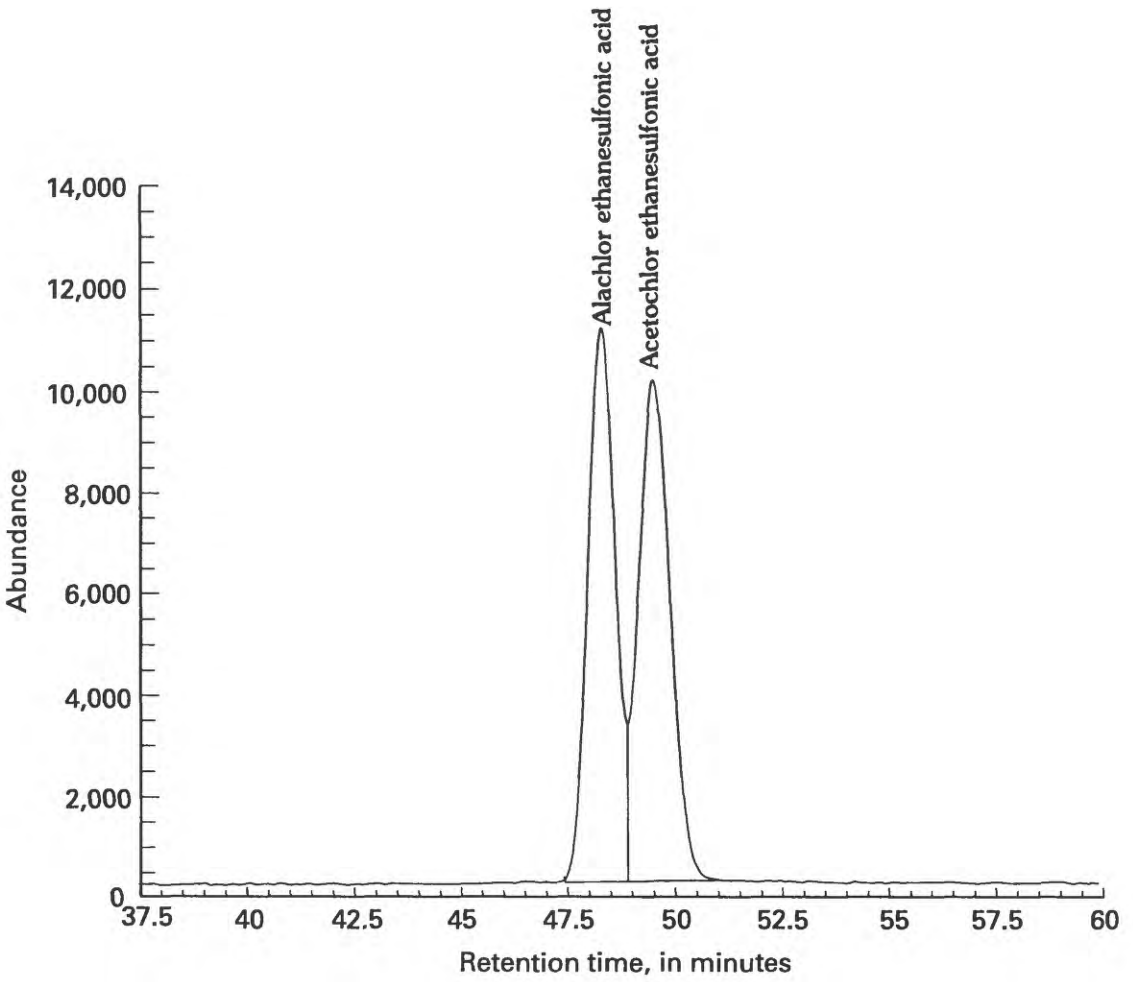

Figure 3. Selected ion chromatogram of a 0.05-microgram-per-liter control reagent-water sample for molecular ion 314 mass-to-charge ratio using high-performance liquid chromatography/mass spectrometry.

elution order of the metabolites using the HPLC/MS method differs from that of the HPLC-DAD method because the pH of the respective mobile phases are different. They are different because the HPLC buffer is nonvolatile and not compatible with mass spectrometry.

2,4-dichlorophenoxy acid was used as the internal standard because it is amenable to negative ion electrospray and is readily available as a commercial standard. The use of deuterated surrogate standards is being investigated.

CONCLUSIONS

This report presents two methods for routine analysis of six chloracetanalide herbicide metabolites in natural water samples. From the data presented in this report, solid-phase extraction and determination by high-performance liquid chromatography-diode array detection (HPLC-DAD) or high-performance liquid chromatography/mass spectrometry (HPLC/MS) are shown to be sensitive and reliable methods for the determination of low concentrations. Good precision and accuracy were demonstrated for both the HPLC-DAD and HPLC/MS methods in reagent water, surface water, and ground water. Method detection limits for the HPLC-DAD method ranged from 0.09 to 0.17 µg/L. Method detection limits for the HPLC/MS method ranged from 0.04 to 0.05 µg/L. The mean HPLC-DAD recoveries of the chloroacetanilide herbicide metabolites from water samples spiked at 0.25, 0.50, and 2.0 µg/L ranged from 84 to 112 percent, with relative standard deviations of 18 percent or less. The mean HPLC/MS recoveries of the metabolites from water samples spiked at 0.05, 0.20, and 2.0 µg/L ranged from 81 to 125 percent, with relative standard deviations of 20 percent or less. The limit of quanti-

tation (LOQ) for all metabolites using the HPLC-DAD method was 0.20 µg/L, whereas the LOQ using the HPLC/MS method was 0.05 µg/L. Information about the fate and transport of the chloroacetanilide herbicides—acetochlor, alachlor, and metolachlor—in water can be acquired from the analysis of surface-water runoff and ground water from wells. These methods also can be useful for water-quality determinations and analytical verification in toxicological studies.

REFERENCES CITED

Aga, D.S., Thurman, E.M., and Pomes, M.L., 1994, Determination of alachlor and its sulfonic acid metabolite in water by solid-phase extraction and enzyme-linked immunosorbent assay: Journal of Analytical Chemistry, v. 66, p. 1495–1499.

Aga, D.S., Thurman, E.M., Yockel, M.E., Zimmerman, L.R., and Williams, T.D., 1996, Identification of a new sulfonic acid metabolite of metolachlor in soil: Environmental Science and Technology, v. 30, p. 592–597.

Edwards, T.K., and Glysson, G.D., 1988, Field methods for measurement of fluvial sediment: U.S. Geological Survey Open-File Report 86–531, 118 p.

Ferrer, Imma, Thurman, E.M., and Barcelo, Damia, 1997, Identification of ionic chloroacetanilide herbicide metabolites in surface water and groundwater by HPLC/MS using negative ion spray: Journal of Analytical Chemistry, v. 69, p. 4547–4553.

Gianessi, L.P., and Anderson, J.E., 1995, Pesticide use in U.S. crop production—national data report: Washington, D.C., National Center for Food and Agricultural Policy, unpaginated.

Hardy, M.A., Leahy, P.P., and Alley, W.M., 1989, Well installation documentation and ground-water sampling protocols for the pilot National Water-Quality Assessment Program: U.S. Geological Survey Open-File Report 89–396, 36 p.

Hostetler, K.A., and Thurman, E.M., 1999, Determination of chloroacetanilide herbicide metabolites in water using high-performance liquid chromatography-diode array detection and high-performance liquid chromatography/mass spectrometry, *in* Morganwalp, D.W., and Buxton, H.T., eds., U.S. Geological Survey Toxic Substances Hydrology Program—Proceedings of the Technical Meeting, Charleston, South Carolina, March 8–12, 1999, volume 2 of 3—Contamination of hydrologic systems and related ecosystems: U.S. Geological Survey Water-Resources Investigations Report 99–4018B, p. 345–353.

Kolpin, D.W., Nations, B.K., Goolsby, D.A., and Thurman, E.M., 1996, Acetochlor in the hydrogeologic system in the Midwestern United States, 1994: Environmental Science and Technology, v. 30, p. 1459–1464.

Kolpin, D.W., Thurman, E.M., and Goolsby, D.A., 1996, Occurrence of selected pesticides and their metabolites in near-surface aquifers of the Midwestern U.S.: Environmental Science and Technology, v. 30, no. 5, p. 335–340.

Kolpin, D.W., Thurman, E.M., and Linhart, S.M., 1998, The environmental occurrence of herbicides—the importance of degradates in ground water: Archives of Environmental Contamination and Toxicology, v. 35, p. 1–6.

Leonard, R.A., 1988, Herbicides in surface water, *in* Grover, R., ed., Environmental chemistry of herbicides—volume I: Boca Raton, Fla., CRC Press, p. 45–88.

Macomber, C., 1992, Determination of the ethane sulfonate metabolite of alachlor in water by high performance liquid chromatography: Journal of Agricultural and Food Chemistry, v. 40, p. 1450–1452.

Thurman, E.M., Goolsby, D.A., Aga, D.S., Pomes, M.L., and Meyer, M.T., 1996, Occurrence of alachlor and its sulfonated metabolite in rivers and reservoirs of the Midwestern U.S.: Environmental Science and Technology, v. 30, p. 569–574.

U.S. Environmental Protection Agency, 1992, Guidelines establishing test procedures for the analysis of pollutants (App. B, Part 136, Definition and procedures for the determination of the method detection limit): U.S. Code of Federal Regulations, Title 40, Revised as of July 1, 1992, p. 565–567.

Ward, J.R., and Harr, C.A., 1990, Methods for collection and processing of surface-water and bed-material samples for physical and chemical analyses: U.S. Geological Survey Open-File Report 90–140, 71 p.

APPENDICES

APPENDIX 1. AUTOMATED SOLID-PHASE EXTRACTION PROCEDURE USING AUTOTRACE WORKSTATION

Tekmar AutoTrace Extraction Workstation 1.33
[mL, milliliter; min, minute]

AutoTrace Extraction Procedure : JK.123.MEOH

Estimated time for samples : 49.1 minutes

Date : December 12, 1999

Step 1 : Process six samples using the following procedure:

Step 2 : Condition column with 3 mL methanol into SOLVENT WASTE

Step 3 : Condition column with 3 mL ethyl acetate into SOLVENT WASTE

Step 4 : Condition column with 3 mL methanol into SOLVENT WASTE

Step 5 : Condition column with 3 mL distilled water into AQUEOUS WASTE

Step 6 : Wash syringe with 5 mL ethyl acetate

Step 7 : Load 125 mL of sample onto column

Step 8 : Dry column with gas for 0.5 min

Step 9 : Condition column with 3.5 mL ethyl acetate into SOLVENT WASTE

Step 10 : Collect 3.5 mL fraction into sample tube using methanol

Step 11 : Dry column with gas for 3 minutes

Step 12 : END

Setup Parameters

[mL/min, milliliters per minute; mL, milliliter]

AutoTrace Extraction Workstation

FLOW RATES (mL/min)			
Condition flow:	10.0	Rinse flow:	20.0
Load flow:	10.0	Elute flow:	5.0
		Condition air push:	15.0
		Rinse air push:	20.0

Elute air push:	5.0	Autowash volume:	1.00 mL

WORKSTATION PARAMETERS

SOLID-PHASE EXTRACTION		Maximum elution volume:	12.0 mL
PARAMETERS		Exhaust fan on:	Yes
Push delay:	5 seconds	Beeper on:	Yes
Air factory:	1.0		

Name Solvents

Solvent 1 : Ethyl acetate

Solvent 2 : Methanol

Solvent 3 : Distilled water

Solvent 4 : not used

Solvent 5 : not used

APPENDIX 2. AUTOMATED SOLID-PHASE EXTRACTION PROCEDURE USING MILLILAB 1A WORKSTATION

Millilab 1A Solid-Phase Extraction Procedure
[mL, millileters; mL/min: milliliters per minute]

Estimated time for samples : 11 hours

Date : December 1, 1998

Tube name	**Tube type**
sample	PORT
elution	TUBE
splspike	TUBE
washprobe	TUBE
methelute	TUBE
Organic_waste	TUBE

Element name	**Element type**
Seppak+	CARTRIDGE

Port name	**Liquid name**
Syr1v1m1	sample
Syr1v1m2	sample
Syr1v1m3	sample
Syr1v1m4	sample
Syr1v2m1	sample
Syr1v2m2	sample
Syr1v2m3	sample
Syr1v2m4	sample

Syr1v3m1	sample
Syr1v3m2	sample
Syr1v3m3	sample
Syr1v3m4	sample
Syr1v4m1	sample
Syr1v4m2	sample
Syr1v4m3	distilled1
Syr2v1	distilled2
Syr2v2	ethyl acetate
Syr2v3	methanol

Pump name	**Syringe size**
Pump 1	5.0 mL
Pump 2	1.0 mL

Reagent name	**Liquid name**
Reagent_1	methanol
Reagent_2	d10-phenanthrene
Reagent_3	Not used
Reagent_4	ethyl acetate

Loop size

4 mL

Technique name		**Technique parameters**
(1)	WASH PROBE	Solvent = distilled1 Fill_Rate = 60 mL/min Empty_Rate = 60 mL/min Volume = 20 mL Strokes = 4
(2)	SPE SELECT	Cartridge = Seppak+

	Technique name	Technique parameters
(3)	SPE LOAD	Working_solvent = distilled1 Rate = 30 mL/min Empty_rate = 20 mL/minVolume = 1 mL Level = 0 From = methanol To = Organic_waste Gap = 0.1 mL
(4)	SPE LOAD	Working_solvent = distilled1 Rate = 30 mL/min Empty_rate = 20 mL/minVolume = 1 mL Level = 0 From = ethyl acetate To = Organic_waste Gap = 0.1 mL
(5)	SPE LOAD	Working_solvent = distilled1 Rate = 30 mL/min Empty_rate = 20 mL/minVolume = 1 mL Level = 0 From = methanol To = Organic_waste Gap = 0.1 mL
(6)	SPE WASH	Solvent = distilled1 Rate = 30 mL/min Empty_rate = 20 mL/min Volume = 3 mL To = Waste
(7)	WASH PROBE	Solvent = sample Fill Rate = 60 mL/min Empty_rate = 60 mL/min Volume = 15 mL Strokes = 3
(8)	SPE WASH	Solvent = sample Rate = 30 mL/min Empty_rate = 20 mL/min Volume = 100 mL To = Waste

Technique name		Technique parameters
(9)	WASH PROBE	Solvent = distilled1 Fill Rate = 60 mL/min Empty_rate = 60 mL/min Volume = 10 mL Strokes = 2
(10)	ELEMENT PURGE	Element = Seppak+ Dispose = No Gas = Purge 6 Level = 0 Clear_time = 0.2 min Purge_time = 1 min To = Organic_waste
(11)	SPE LOAD	Working_solvent = ethyl acetate Rate = 4.0 mL/min Empty_rate = 4.0 mL/min Volume = 3.5 mL Level = 0 From = ethyl acetate To = elution Gap = 0.1 mL
(12)	GAS PURGE	Gas = Purge 6 To = Organic_waste Level = 900 Clear_time = 0 min Purge_time = 0.4 min
(13)	ELEMENT PURGE	Element = Seppak+ Dispose = No Gas = Purge 6 Level = 0 Clear_time = 0 min Purge_time = 0.3 min To = elution
(14)	SPE LOAD	Working_solvent = ethyl acetate Rate = 4.0 mL/min Empty_rate = 4.0 mL/min Volume = 3.5 mL Level = 0 From = methanol To = methelut Gap = 0.1 mL

Technique name		Technique parameters
(15)	BATCH+PIPETTE	Working_solvent = ethyl acetate Fill_rate = 4 mL/min Empty_Rate = 4 mL/min Asperate_level = 60 Dispense_level = 560 Volume = 0.5 mL Gap = 0.1 mL From = d10-phenan To = elution Sample_count = All
(16)	WASH PROBE	Solvent = ethyl acetate Fill_Rate = 6.0 mL/min Empty_Rate = 6.0 mL/min Volume = 2 mL Strokes = 4
(17)	MIX	Working_solvent = ethyl acetate Fill_Rate = 6.0 mL/min Empty_Rate = 6.0 mL/min Asperate_level = 250 Dispense_level = 300 Volume = 2.5 mL Gap = 0.1 mL Count = 2 To = elution
(18)	WASH PROBE	Solvent = ethyl acetate Fill_Rate = 6.0 mL/min Empty_Rate = 6.0 mL/min Volume = 2 mL Strokes = 4
(19)	PIPETTE	Working_solvent = ethyl acetate Fill_Rate = 4.0 mL/min Empty_Rate = 4.0 mL/min Asperate_level = 270 Dispense_level = 550 Volume = 4 mL Gap = 0.2 mL From = elution To = Splspike

Technique name		Technique parameters
(20)	MIX	Working_solvent = ethyl acetate Fill_Rate = 6.0 mL/min Empty_Rate = 6.0 mL/min Asperate_level = 150 Dispense_level = 150 Volume = 3 mL Gap = 0.1 mL Count = 1 To = Washprobe
(21)	GAS PURGE	Gas = Purge 6 To = Organic_waste Level = 900 Clear_time = 0 min Purge_time = 0.3 min
(22)	BUBBLE MIX	Gas = Purge 6 To = Washprobe Level = 0 Clear_time = 0 min Purge_time = 0.3 min
(23)	WASH PROBE	Solvent = ethyl acetate Fill_Rate = 6.0 mL/min Empty_Rate = 6.0 mL/min Volume = 1 mL Strokes = 2
(24)	SPE DONE	Dispose = No