Determination of Chloroacetanilide Herbicide Metabolites in Water Using High-Performance Liquid Chromatography-Diode Array Detection and High-Performance Liquid Chromatography/Mass Spectrometry

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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: alachlor ethanesulfonic acid (ESA), alachlor oxanilic acid, acetochlor ESA, acetochlor oxanilic acid, metolachlor ESA, and metolachlor oxanilic acid. Good precision and accuracy were demonstrated for both the HPLC-DAD and HPLC/MS methods in reagent water, surface water, and ground water. The average HPLC-DAD recoveries of the chloroacetanilide herbicide metabolites from water samples spiked at 0.25, 0.5, and 2.0 µg/L (micrograms per liter) ranged from 84 to 112 percent, with relative standard deviations of 18 percent or less. The average HPLC/MS recoveries of the metabolites from water samples spiked at 0.05, 0.2, and 2.0 µg/L ranged from 81 to 118 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 at 0.05 µg/L. These metabolite-determination methods are valuable for aquiring information about water quality and the fate and transport of the parent chloroacetanilide herbicides in water.

INTRODUCTION

The chloroacetanilide herbicides—alachlor, acetochlor, 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 Puffer, 1985). Alachlor and metolachlor have been used extensively for more than 20 years, whereas acetochlor application is relatively recent, applied extensively since March 1994 (Kolpin 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 water (Aga and others, 1996; Kolpin and others, 1996; Thurman and others, 1996). Kolpin and others (1996) 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, methodologies for the analysis of metabolites are crucial. These methods also are important for analytical verification of the metabolites in toxicological studies. Therefore, reliable methodologies for the analysis of chloroacetanilide metabolites are vital.

As with many other pesticide metabolites, 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) is very useful 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 paper addresses the development of reliable HPLC-DAD and HPLC/MS methods for the analysis of ethanesulfonic acid (ESA) and oxanilic acid metabolites of alachlor, acetochlor, 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 oxanilic acid 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.

MATERIALS AND METHODS

HPLC-grade acetonitrile, methanol, and water, along with acetic acid, was obtained from Fisher Scientific (Pittsburg, PA). The analytical standard for acetochlor ESA was obtained from Zeneca Agrochemicals (Fernhurst, Haslemere Surrey, UK). Standards for alachlor oxanilic acid and acetochlor oxanilic acid were obtained from Monsanto Chemical Co. (St. Louis, MO), and the standard for alachlor ESA was obtained from the U.S. Environmental Protection Agency Repository (Cincinnati, OH). Metolachlor ESA was synthesized in the U.S. Geological Survey laboratory in Lawrence, KS, by Diana Aga (currently with University of Nebraska-Kearney, Kearney, NE). Metolachlor oxanilic acid was obtained from Robert Zablotowitcz of the Agricultural Research Service (Stoneville, MS). Standards solutions were prepared in methanol. The solid-phase extraction (SPE) cartridges (Sep-Pak) used to extract samples were obtained from Waters-Millipore (Milford, MA). These cartridges contained 360 mg (milligrams) of 40-µm (micrometer) C18- ($C_{18}H_{37}$) bonded silica.

Control surface-water samples were collected from Poison Creek in Valley County, Idaho. Control ground-water samples were collected from a well in Valley County, Idaho. Prior to extraction, all samples were filtered through 1-µm-pore glass-fiber filters into clean 4-oz (ounce) amber bottles. Before and between sampling, all sampling equipment was cleaned with detergent and thoroughly rinsed with tap water, then distilled water, then a 50:50 distilled water/methanol rinse.

The SPE procedure was performed using an automated Millipore Workstation (Waters, Milford, MA) as described by Thurman and others (1990). Each C18 cartridge was preconditioned as follows: 2 mL (milliliters) methanol, 2 mL ethyl acetate, 2 mL methanol, followed by 2 mL distilled water. A 100-mL sample was passed through the cartridge at a flow rate of 10 mL/min (milliliters per minute), and the cartridge was purged with air to remove excess water. The cartridge was eluted with 3 mL ethyl acetate, followed by a transfer step to remove the ethyl acetate (top layer, which contains the parent herbicides) from the residual water (bottom layer) in the ethyl acetate fraction. The cartridge then was eluted with 3 mL methanol, which removed the herbicide metabolites. The methanolic extract was spiked with 1 µg (microgram) 2,4-D (internal standard) then evaporated to dryness under a stream of nitrogen at 45 ^{o}C (degrees Celsius) using a Turbovap (Zymark, Palo Alto, CA). Because 2,4-D will not isolate using this SPE procedure, it is spiked into the methanol extract instead of the water sample and is used to normalize injection-volume variation and as a retention-time reference. For HPLC-DAD, the extract was reconstituted in 75 µL (microliter) of a solution containing 60 percent, pH 7.0, 25-mM (millimole) phoshate buffer and 40 percent methanol . For HPLC/MS, the extract was reconstituted in 75 µL of a solution containing 0.3 percent acetic acid, 24 percent ethanol, 35.7 percent water, and 40 percent acetonitrile.

For HPLC-DAD, 50 µL of extract was injected into a Hewlett-Packard 1090 HPLC (Hewlett-Packard, Palo Alto, CA) equipped with a DAD. The mobile phase consisted of 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. The analytical columns consisted of a Phenomenex (Torrance, CA) 5-µm

(micrometer), 250- x 3-mm C18 column coupled to a Keystone (Bellefonte, PA) 3-µm, 250- x 4.6-mm C18 column. Column temperatures were set at 60 °C to achieve better separation and peak shapes for the metabolites. A Hewlett-Packard HP Chemstation (Rev. A.03.03) was the application software used for instrument control and data processing.

For HPLC/MS, the 50-µL extract was injected into a Hewlett-Packard 1100 HPLC coupled to a Hewlett-Packard 1100 Mass Selective Detector (MSD). The mobile phase consisted of 0.3 percent acetic acid, 24 percent ethanol, 35.7 percent water, and 40 percent acetonitrile solution with a flow rate of 0.3 to 0.4 mL/min, depending on the backpressure (maximum of 400 bar). The analytical columns consisted of two Phenomenex 5-µm, 250- x 3-mm C18 columns coupled to one (or two, if within backpressure limitations) Phenomenex 3-µm, 150- x 2.0-mm C18 column. Using an extra column yields better resolution of the metabolite peaks. Column temperatures were set at 70 °C to achieve better separation and peak shapes. The MSD was operated using the electrospray chamber in negative ion mode. Prior to sample analysis, the MSD was tuned using a proprietary solution obtained from Hewlett-Packard. The drying gas flow was set at 6 L/min (liters per minute), the nebulizer pressure was 25 psi (pounds per square inch), the drying gas temperature was 300 °C, the capillary voltage was 3,100 V (volts), and the fragmenter voltage was 70 V. Table 1 summarizes the HPLC/MSD acquisition parameters. A Hewlett-Packard LCMS Chemstation (Rev. A.05.04[273]) was the application software used for instrument control and data processing.

DETERMINATION OF CHLOROACETANILIDE HERBICIDE METABOLITES

SPE and recovery for chloroacetanilide metabolites have been discussed in previous work (Thurman and others, 1990; Aga and others, 1994; Ferrer and others, 1997). In those studies, chromatographic separation was achieved only for a few of the herbicide metabolites specified in this paper. In the study described in this paper, 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.

HPLC-DAD Results

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 is positioned before the smaller particle column for effective backpressure regulation

Table 1. Typical liquid chromatography/mass selective detector acquisition parameters

Parameter	Settings
Ions monitored	146, 160, 206, 264, 278, 314, 328 mass-to-charge ratio
Spray chamber mode	electrospray, negative mode
Drying gas temperature	300 degrees Celsius
Drying gas flow rate	6 liters per minute
Nebulizer pressure	25 pounds per square inch
Capillary voltage	3,100 volts
Fragmenter	70 volts
Analytical columns	two 5-micrometer, 250- x 3.0-millimeter C18 columns coupled to a 3-micrometer, 150- x 2.0-millimeter C18 column
Mobil phase	0.3 percent acetic acid in 24:36:40 methanol/water/acetonitrile
Flow rate	0.35 millimeter per minute

(smaller phase thickness gives higher backpressure). The analytical wavelength was set at 210 nm (nanometers), and DAD spectra were stored for every integrated peak with a peak height greater than 0.5 milliabsorbance units (mAU). Figure 1 shows a typical HPLC-DAD chromatogram of a 2.0-µg/L control reagent water sample.

Aliquots (123 mL) of reagent water (distilled), metabolite-free/interference-free surface water, and metabolite-free/interference-free ground water were spiked at 0.25, 0.5, and 2.0 µg/L with chloroacetanilide metabolites, making seven replicate samples at each concentration level. Each sample was extracted using the previously described automated SPE procedure. The average HPLC-DAD recoveries of the metabolites from the spiked water samples ranged from 84 to 112 percent, with relative standard deviations of 18 percent or less (tables 2–4). The limit of quantitation (LOQ) for this method was 0.2 µg/L.

HPLC/MS Results

A HPLC/MS method for the analysis of ethanesulfonic acids and oxanilic acids of alachlor, acetochlor, and metolachlor was reported by Ferrer and others (1997). The described HPLC system used an 5-µm, 250- x 3.0-mm C18 column, with a mobile phase consisting of 0.3 percent acetic acid in 24 percent methanol, 36 percent water, and 40 percent acetonitrilem solution. With this configuration, peak resolution was not achieved for alachlor ESA and acetochlor ESA, which have the same molecular ion (table 5). Thus, accurate quantitation of these metabolites was not possible. Chromatographic separation of alachlor ESA and acetochlor ESA was achieved with the same mobile phase by coupling two 5-µm, 250- x 3.0-mm C18 columns to one (or two, if backpressure permits) 3-µm, 150- x 2.0-mm C18 column. Figure 2 shows a total ion chromatogram (TIC) of a 0.05-µg/L control water

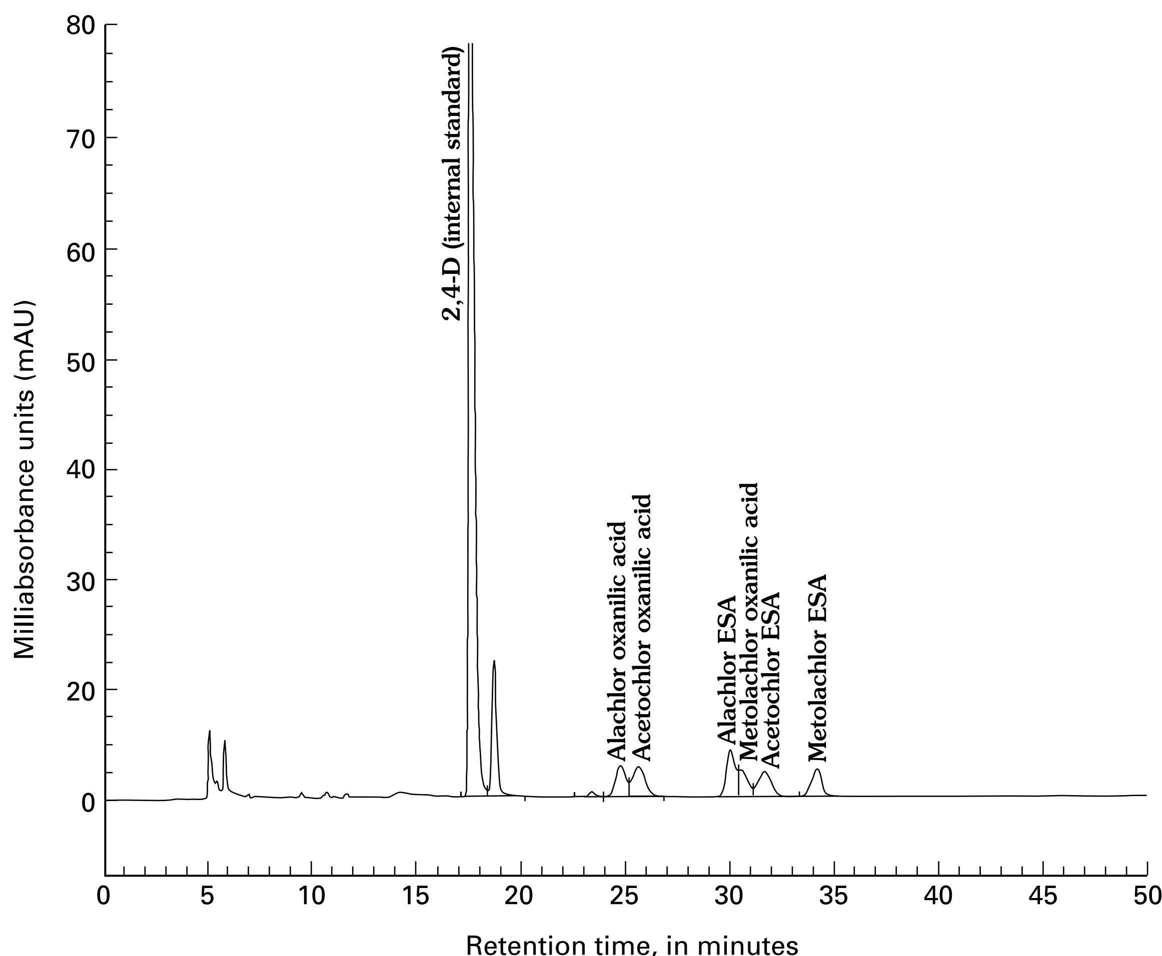

Figure 1. Typical chromatogram of a 2.0-microgram-per-liter control water sample using high-performance liquid chromatography-diode array detection.

Table 2. High-performance liquid chromatography-diode array detection method recoveries in reagent water

[µg/L, micrograms per liter; RSD, relative standard deviation]

	Reagent water					
	Seven replicate samples at 0.25 µg/L		Seven replicate samples at 0.5 µg/L		Seven replicate samples at 2.0 µg/L	
Metabolite	Average recovery (percent)	RSD (percent)	Average recovery (percent)	RSD (percent)	Average recovery (percent)	RSD (percent)
Alachlor ESA	100	10	102	5.5	100	2.4
Alachlor oxanilic acid	84	17	92	9.6	90	2.7
Acetochlor ESA	112	16	104	9.0	105	2.3
Acetochlor oxanilic acid	88	18	94	14	95	3.1
Metolachlor ESA	108	10	104	7.3	105	3.2
Metolachlor oxanilic acid	108	14	102	5.3	100	3.4

Table 3. High-performance liquid chromatography-diode array detection method recoveries in surface water

[µg/L, micrograms per liter; RSD, relative standard deviation]

	Surface water					
	Seven replicate samples at 0.25 µg/L		Seven replicate samples at 0.5 µg/L		Seven replicate samples at 2.0 µg/L	
Metabolite	Average recovery (percent)	RSD (percent)	Average recovery (percent)	RSD (percent)	Average recovery (percent)	RSD (percent)
Alachlor ESA	104	8.9	98	3.4	105	4.4
Alachlor oxanilic acid	92	14	96	7.4	100	6.3
Acetochlor ESA	100	16	104	8.0	100	5.4
Acetochlor oxanilic acid	84	14	94	7.0	100	4.9
Metolachlor ESA	108	12	102	4.9	105	4.6
Metolachlor oxanilic acid	108	16	100	3.5	100	5.7

Table 4. High-performance liquid chromatography-diode array detection method recoveries in ground water

[µg/L, micrograms per liter; RSD, relative standard deviation]

	Ground water					
	Seven replicate samples at 0.25 µg/L		Seven replicate samples at 0.5 µg/L		Seven replicate samples at 2.0 µg/L	
Metabolite	Average recovery (percent)	RSD (percent)	Average recovery (percent)	RSD (percent)	Average recovery (percent)	RSD (percent)
Alachlor ESA	103	9.3	98	4.6	103	3.2
Alachlor oxanilic acid	88	13	94	6.9	94	4.8
Acetochlor ESA	105	18	107	8.4	102	3.8
Acetochlor oxanilic acid	87	15	95	7.8	99	5.3
Metolachlor ESA	102	12	103	5.0	98	4.1
Metolachlor oxanilic acid	104	16	100	4.8	100	3.5

Table 5. Summary of typical molecular and fragmentation ions

[fragmenter voltage = 70 volts; m/z, mass-to-charge ratio]

Metabolite	Molecular ion (m/z)	Fragmentation ion(s) (m/z)
Alachlor ESA	314	314
Alachlor oxanilic acid	264	264, 160, 192
Acetochlor ESA	314	314
Acetechlor oxanilic acid	264	264, 146
Metolachlor ESA	328	328
Metolachlor oxanilic acid	278	278, 216
2,4-D (internal standard)	220	219, 161

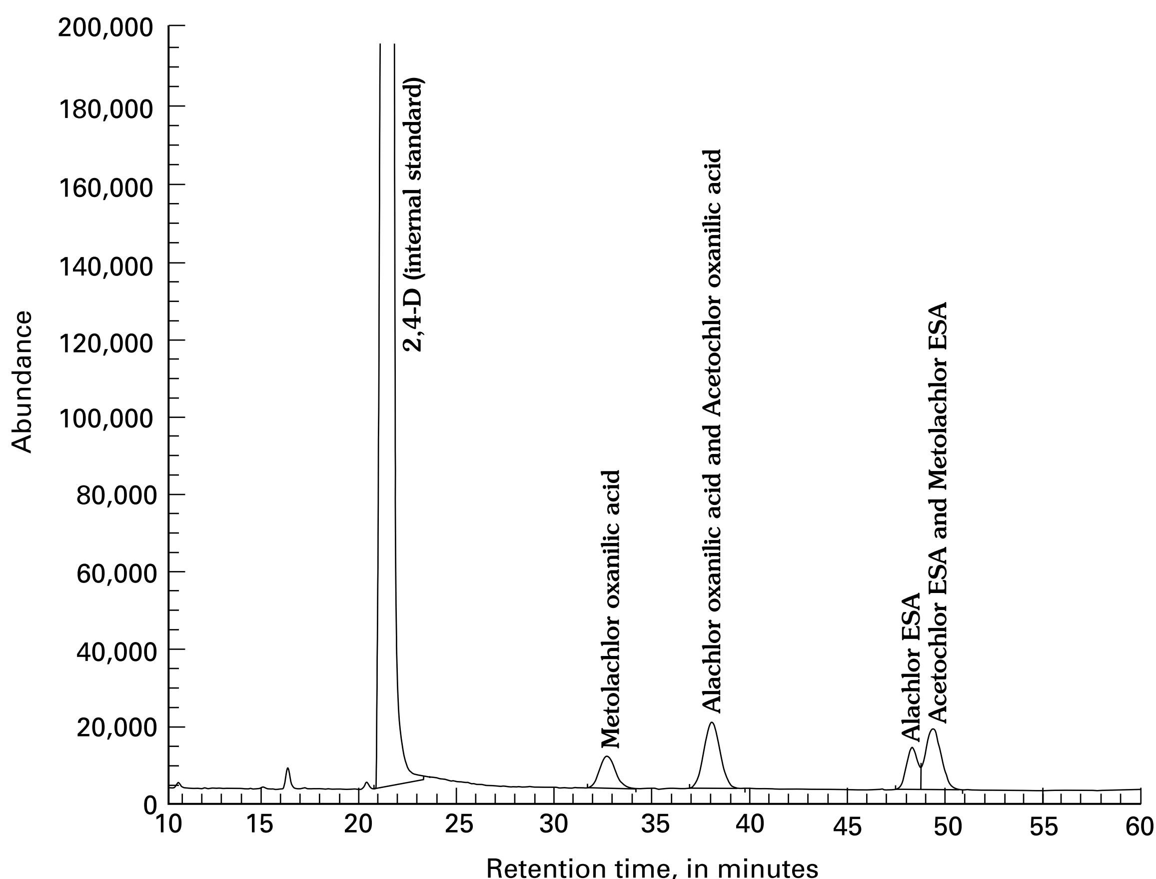

Figure 2. Total ion chromatogram (TIC) of a 0.05-microgram-per-liter control water sample using high-performance liquid chromatography/mass spectrometry.

sample. Figure 3 shows the extracted ion chromatogram for the molecular ion (314 mass-to-charge ratio) of alachlor ESA and acetochlor ESA with near baseline separation. The 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.

Aliquots (123 mL) of reagent water, (distilled), metabolite-free surface water, and metabolite-free ground water were spiked at 0.05, 0.2, and 2.0 µg/L with the metabolites, making seven replicate samples at each concentration. Each sample was extracted using the previously described automated SPE procedure. The average HPLC/MS recoveries of the metabolites from the

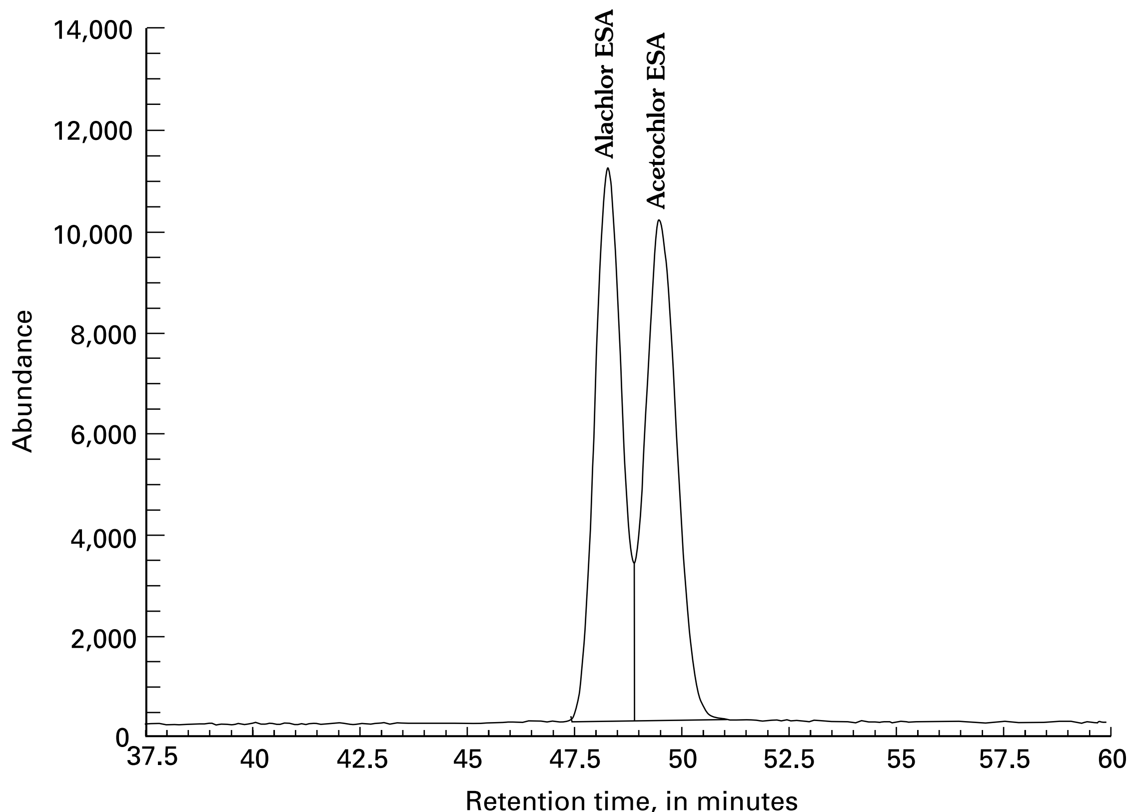

Figure 3. Selected ion chromatogram of a 0.05-microgram-per-liter control water sample for molecular ion 314 mass-to-charge ratio using high-performance liquid chromatography/mass spectrometry.

spiked water samples ranged from 81 to 118 percent, with relative standard deviations of 20 percent or less (tables 6–8). The limit of quantitation (LOQ) for this method was 0.05 µg/L.

DISCUSSION

Accurate and precise measurements of metabolite concentrations in surface water and ground water are obtained using the HPLC-DAD and HPLC/MS methods specified in this paper. Information about the fate and transport of the chloroacetanilide herbicides—alachlor, acetochlor, and metolachlor—in water can be acquired from the analysis of field-runoff water and ground water from nearby wells. These methods also can be useful for water-quality determinations and analytical verification in toxicological studies.

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Table 6. High-performance liquid chromatography/mass spectrometry method recoveries in reagent water

[µg/L, micrograms per liter; RSD, relative standard deviation]

Metabolite	Reagent water					
	Seven replicate samples at 0.05 µg/L		Seven replicate samples at 0.2 µg/L		Seven replicate samples at 2.0 µg/L	
	Average recovery (percent)	RSD (percent)	Average recovery (percent)	RSD (percent)	Average recovery (percent)	RSD (percent)
Alachlor ESA	95	19	100	5.4	100	4.9
Alachlor oxanilic acid	81	11	85	11	88	6.4
Acetolachlor ESA	117	20	125	6.3	110	7.4
Acetolachlor oxanilic acid	84	12	85	9.6	86	5.9
Metolachlor ESA	113	13	110	5.8	115	6.8
Metolachlor oxanilic acid	110	11	105	5	104	5.7

Table 7. High-performance liquid chromatography/mass spectrometry method recoveries in surface water

[µg/L, micrograms per liter; RSD, relative standard deviation]

Metabolite	Surface water					
	Seven replicate samples at 0.05 µg/L		Seven replicate samples at 0.2 µg/L		Seven replicate samples at 2.0 µg/L	
	Average recovery (percent)	RSD (percent)	Average recovery (percent)	RSD (percent)	Average recovery (percent)	RSD (percent)
Alachlor ESA	81	19	95	7.1	109	13
Alachlor oxanilic acid	90	10	99	6.2	109	10
Acetolachlor ESA	118	4.3	104	7.7	104	15
Acetolachlor oxanilic acid	90	8.1	101	6.1	111	11
Metolachlor ESA	92	11	101	6.3	114	10
Metolachlor oxanilic acid	90	9.5	100	5.9	110	12

Table 8. High-performance liquid chromatography/mass spectrometry method recoveries in ground water

[µg/L, micrograms per liter; RSD, relative standard deviation]

Metabolite	Ground water					
	Seven replicate samples at 0.05 µg/L		Seven replicate samples at 0.2 µg/L		Seven replicate samples at 2.0 µg/L	
	Average recovery (percent)	RSD (percent)	Average recovery (percent)	RSD (percent)	Average recovery (percent)	RSD (percent)
Alachlor ESA	104	16	100	4.8	100	3.5
Alachlor oxanilic acid	85	8.3	95	7.8	99	5.3
Acetolachlor ESA	102	12	103	5.0	98	4.1
Acetolachlor oxanilic acid	95	9.3	98	4.6	103	3.2
Metolachlor ESA	92	11	107	8.4	102	3.8
Metolachlor oxanilic acid	95	12	94	6.9	94	4.8

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