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Methods of Analysis by the U.S. Geological Survey Organic Geochemistry Research Group—Determination of Acetamide Herbicides and Their Degradation Products in Water Using Online Solid-Phase Extraction and Liquid Chromatography/Mass Spectrometry

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By E.A. LEE and A.P. STRAHAN

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

Conversion Factors

Multiply	By	To obtain
gram (g)	2.205×10^{-3}	pound (lb)
meter (m)	3.281	foot (ft)
microgram per liter (µg/L)	1.0	part per billion (ppb)
microliter (µL)	2.642×10^{-7}	gallon (gal)
micrometer (µm)	3.937×10^{-5}	inch (in.)
micron (µ)	3.937×10^{-5}	inch (in.)
millimeter (mm)	3.937×10^{-2}	inch (in.)
ounce (oz)	0.02957	liter (L)
pound per acre (lb/acre)	1.121	kilogram per hectare (kg/ha)
pound per acre per year [(lb/acre)/yr]	1.121	kilogram per hectare per year [(kg/ha)/yr]
pound per square inch (lb/in^2)	6.895	kilopascal (kPa)

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

$^oC = 5/9 (^oF - 32)$,

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

Abbreviated Water-Quality Units

liter per minute (L/min)
microgram per liter (µg/L)
microgram per milliliter (µg/mL)
milligram per liter (mg/L)
milligram per milliliter (mg/mL)
milliliter (mL)
milliliter per minute (mL/min)
molar (M)

Miscellaneous Abbreviations and Acronyms

American Chemical Society (ACS)
Chemical Abstracts Registry (CAS)
diode array detector (DAD)
ethanesulfonic acid (ESA)
internal standard (ISTD)
mass spectrometer (MS)
method detection limit (MDL)
millisecond (ms)
mole (M)
octadecylsilane (C–18)
relative retention time (RRT)
retention time (RT)
solid-phase extraction (SPE)
U.S. Geological Survey (USGS)
volume per volume (v/v)
Ångstrom (Å)
dilution factor (DF)
electrospray (ES)
high-performance liquid chromatograph (HPLC)
liquid chromatograph (LC)
mass to charge (m/z)
method reporting limit (MRL)
minute (min)
nuclear magnetic resonance (NMR)
oxanilic acid (OXA)
response factor (RF)
seconds (s)
sulfanylacetic acid (SAA)
volt (V)

Methods of Analysis by the U.S. Geological Survey Organic Geochemistry Research Group—Determination of Acetamide Herbicides and Their Degradation Products in Water Using Online Solid-Phase Extraction and Liquid Chromatography/Mass Spectrometry

By E.A. Lee *and* A.P. Strahan

Abstract

An analytical method for the determination of 6 acetamide herbicides (acetochlor, alachlor, dimethenamid, flufenacet, metolachlor, and propachlor) and 16 of their degradation products in natural water samples using solid-phase extraction and liquid chromatography/mass spectrometry is described in this report. Special consideration was given during the development of the method to prevent the formation of degradation products during the analysis. Filtered water samples were analyzed using octadecylsilane as the solid-phase extraction media on online automated equipment followed by liquid chromatography/mass spectrometry. The method uses only 10 milliliters of sample per injection. Three different water-sample matrices, a reagent-water, a ground-water, and a surface-water sample spiked at 0.10 and 1.0 microgram per liter, were analyzed to determine method performance.

Method detection limits ranged from 0.004 to 0.051 microgram per liter for the parent acetamide herbicides and their degradation products. Mean recoveries for the acetamide compounds in the ground- and surface-water samples ranged from 62.3 to 117.4 percent. The secondary amide of acetochlor/metolachlor ethanesulfonic acid (ESA) was recovered at an average rate of 43.5 percent. The mean recoveries for propachlor and propachlor oxanilic acid (OXA) were next lowest, ranging from 62.3 to 95.5 percent. Mean recoveries from reagent-water samples ranged from 90.3 to 118.3 percent for all compounds. Overall the mean of the mean recoveries of all compounds in the three matrices spiked at 0.10 and 1.0 microgram per liter ranged from 89.9 to 100.7 percent, including the secondary amide of acetochlor/metolachlor ESA and the propachlor compounds. The acetamide herbicides and their degradation products are reported in concentrations ranging from 0.05 to 2.0 micrograms per liter. The upper concentration limit is 2.0 micrograms per liter for all compounds without dilution.

With the exception of the secondary amide of acetochlor/metolachlor ESA, good precision and accuracy for the chloroacetanalide herbicides and their degradation compounds were demonstrated for the method in buffered reagent water, ground water, and surface water. The extraction method as used did not optimize the recovery of the secondary amide of acetochlor/metolachlor ESA.

INTRODUCTION

The acetamide herbicides—acetochlor, alachlor, dimethenamid, flufenacet, metolachlor, and propachlor—are an important class of herbicides in the United States. Together with the triazine compounds, acetamide 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). Acetamide herbicides have been shown to degrade more rapidly in soil than other herbicides, with half-lives from 15 to 30 days. Triazine half-lives are typically 30 to 60 days (Leonard, 1988).

The herbicide dimethenamid was registered with the U.S. Environmental Protection Agency in 1993. It has a recommended maximum application rate of 1.5 (lb/acre)/yr on corn and was ranked sixth in herbicide usage during 1998 (U.S. Department of Agriculture, Agricultural Chemical Usage, 1999). It is used most extensively in Northern States, particularly Wisconsin where it was applied to 28 percent of the corn acreage in 1998 (U.S. Department of Agriculture, Agricultural Chemical Usage, 1999). The herbicide flufenacet is used to control certain annual grasses and broadleaf weeds. It has a recommended application rate of 0.78 (lb/acre)/yr (U.S. Department of Agriculture, Agricultural Chemical Usage, 1999). Propachlor was introduced by Monsanto in 1965 and can be applied as a pre-emergent herbicide at 2.5 to 6.0 lb/acre of active ingredient (Ahrens, 1994). It controls many annual grass weeds such as barnyard grass, crabgrass, foxtail and fall panicum, and certain annual broadleaf weeds such as pigweed and carpetweed.

Recent studies have reported the occurrence of acetamide degradation products in ground and surface water (Aga and others, 1996; Kolpin, Thurman, and Goolsby, 1996; Thurman and others, 1996; Kolpin and others, 1998). Kolpin and others (1998) found that degradation product 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 degradation products during the remaining growing season.

In understanding the fate and transport of parent herbicides, reliable methods for the analysis of degradation products are vital. Reliable methods also are important for analytical verification of the degradation compounds in toxicological studies.

The online solid-phase extraction (SPE) liquid chromatography/mass spectrometry (LC/MS) method of analysis described in this report was developed by the U.S. Geological Survey (USGS) Organic Geochemistry Research Group in Lawrence, Kansas, and has been assigned the USGS method number "O–2139–03." The unique code represents the online SPE LC/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 method, including the apparatus, reagents, instrument calibration, and the SPE equipment required for sample analysis. Estimated method detection limits (MDLs), mean recoveries, and relative standard deviations for 6 acetamide herbicides and 16 of their degradation products determined using online SPE LC/MS are presented. The USGS parameter and method codes for these compounds also are given.

DETERMINATION OF ACETAMIDE HERBICIDES AND THEIR DEGRADATION PRODUCTS IN WATER

Method of Analysis

Application

Method O–2139–03 is suitable for the determination of low concentrations (in micrograms per liter) of the compounds listed in table 1 in ground- and surface-water samples. The degradation product listed as the secondary amide of acetochlor/metolachlor ESA has the chemical name 2-[(2-ethyl-6-methylphenol) amino]-2-oxoethanesulfonic acid (parameter code 62850). Acetochlor ESA and metolachlor ESA degrade to the same compound due to their very similar base chemical structures (fig. 1).

Because suspended particulate matter is removed from the samples by filtration, the method is suitable only for dissolved-phase compounds. The method may be suitable for other types of liquid samples such as wastewater and others matrices if they have been filtered; however, consideration should be given to the fact that performance characteristics have not been assessed for these other liquid samples and that results for these matrices have not been validated.

Summary of Method

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

Table 1. Molecular weights and U.S. Geological Survey (USGS) parameter and method codes for acetamide herbicides and their degradation products suitable for determination using method O–2139–03

[CAS, Chemical Abstracts Registry; ESA, ethanesulfonic acid; OXA, oxanilic acid; SAA, sufinylacetic acid; --, not applicable]

Compound	CAS number	Molecular weight	USGS parameter codes	USGS method codes
Acetochlor	34256–82–1	269.8	49260	U
Acetochlor ESA	--	315.4	61029	U
Acetochlor OXA	--	265.1	61030	U
[1]Acetochlor/metolachlor ESA—secondary amide	--	257.3	62850	U
Acetochlor SAA		341.3	62847	U
Alachlor	15972–60–8	269.8	46342	U
Alachlor ESA	--	315.4	50009	U
Alachlor OXA	--	265.3	61031	U
Alachlor ESA—secondary amide	--	271.3	62849	U
Alachlor SAA	140939–16–8	341.3	62848	U
Dimethenamid	87674–68–8	275.8	61588	U
Dimethenamid ESA	--	321.4	61951	U
Dimethenamid OXA	--	271.3	62482	U
Flufenacet	142459–58–3	363.3	62481	U
Flufenacet ESA	--	275.3	61952	U
Flufenacet OXA	--	225.2	62483	U
Metolachlor	51218–45–2	283.8	39415	U
Metolachlor ESA	--	329.4	61043	U
Metolachlor OXA	--	279.3	61044	U
Propachlor	1918–16–7	211.7	04024	U
Propachlor ESA	--	257.3	62766	U
Propachlor OXA	--	207.2	62767	U
Internal standard				
2,4-Dichlorophenoxyacetic acid	94–75–7	220.0	--	--
$^{13}C_6$ Metolachlor	51218–45–2	289.8	--	--
Surrogate				
D-5 Alachlor ESA	--	320.4	--	--

[1]2-[(2-ethyl-6-methylphenol)amino]-2-oxoethanesulfonic acid.

the laboratory, 10 mL of the filtered water samples were measured into vials and were spiked with 0.5 mL of the working surrogate compound. Then each sample was acidified with acetic acid. The sample components were isolated and concentrated using automated online SPE, then separated, identified, and measured by injecting an aliquot into a high-performance liquid chromatograph (HPLC) equipped with a diode array detector (DAD) and a mass spectrometer (MS) detector operated in selected-ion monitoring mode. Compounds eluting from the liquid chromatograph (LC) were identified by comparing the retention times of the mass spectral signals against the retention times of standards analyzed under the same conditions used for the samples. Compounds were identified further by selected fragment ions or isotope ions that are characteristic for each compound. The concentration of each identified compound was calculated by determining the ratio of the MS response produced by that compound to the MS response produced by the internal standard, which was injected into the sample, to the ratio of the MS responses of the primary standard analyzed using the same method. The molecular weights

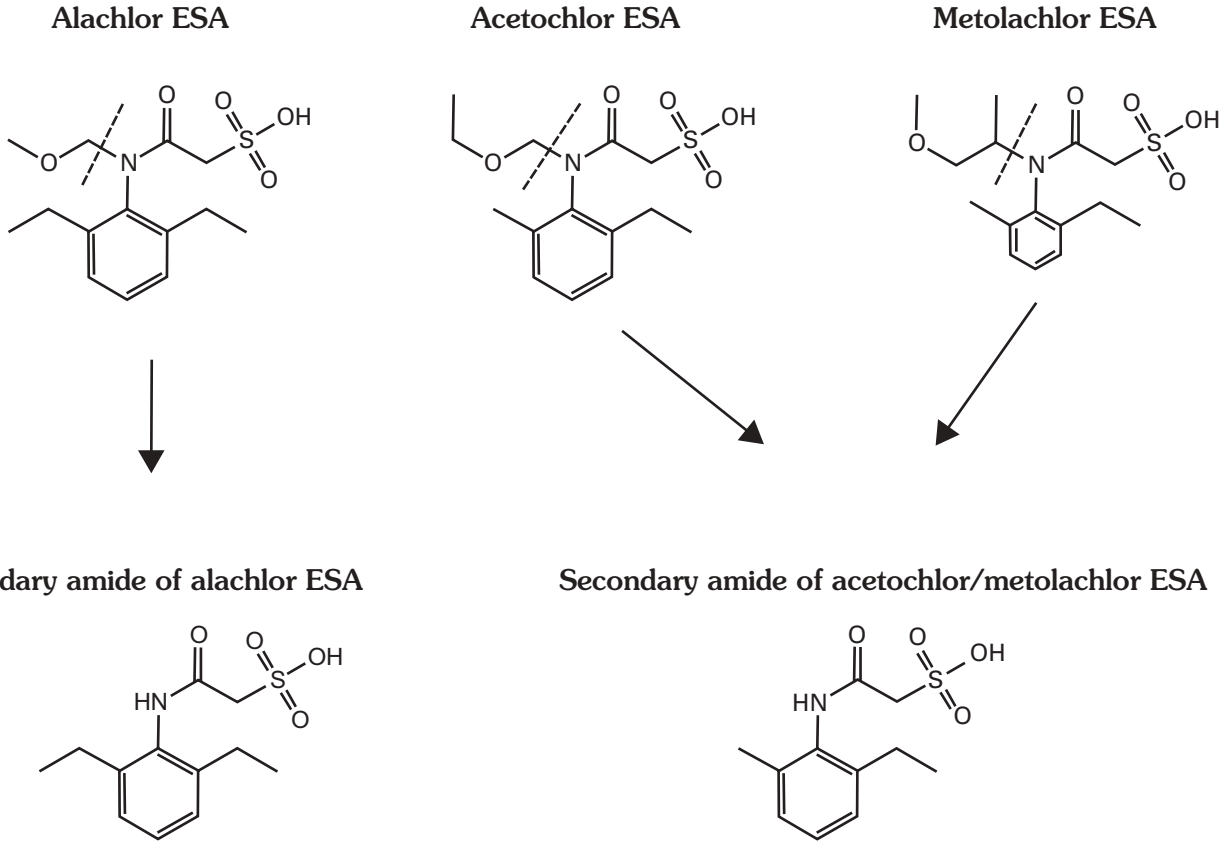

Figure 1. Chemical structures of secondary amides of alachlor ethanesulfonic acid (ESA), acetochlor ESA, and metolachlor ESA.

and USGS parameter codes for the compounds analyzed using method O–2139–03 are listed in table 1.

The autosampler and Prospekt function together as a unit to prepare the cartridge for the SPE and the loading of the sample. Both are controlled by Sparklink software on the computer that contains the HPChemstation software that controls the LC/MS. Once the sample is loaded on the cartridge, the cartridge is placed into the flow path of the LC/MS mobile phase before the column, and the analysis is initiated. The Sparklink and HPChemstation software are interconnected, and signals are sent by and received by each to coordinate the operations. The Prospekt has two clamps for cartridges, so that once the analysis has begun on the LC/MS, the next sample sequence of cartridge preparation and sample loading is performed by the autosampler-Prospekt unit to be ready for the next analysis.

Interferences

Compounds that elute from the LC at the same time and have ions similar to the targeted compounds may interfere. Samples with high concentrations of humic materials may cause interference with the ionization of the internal standards and the analyzed compounds if they elute from the LC at the same time.

Apparatus and Instrumentation

- *Analytical balances*—capable of accurately weighing 0.0100 g ±0.0001 g.
- *Autopipettes*—5 to 10,000 µL, variable-volume autopipettes with disposable tips (Rainin, Woburn, Massachusetts, or equivalent).
- *Autosampler*—Triathlon, type 900 (Spark-Holland, The Netherlands) equipped with:
  10-mL syringe
  10-mL sample loop, and
  Type C sample trays (eight each, holding four 20-mm, 10-mL vials).
- *Automated online SPE instrument*—Prospekt, type 795/796—900 (Spark-Holland, The Netherlands).
- *Analytical column*—Luna (Phenomenex, Torrance, California) 250- x 3-mm, 5-µ particulate-size packing, pore size 100 Å, octadecylsilane (C–18).

- *HPLC/MS benchtop system*—Hewlett Packard (Wilmington, Delaware), model 1100 HPLC with autosampler and MS detector.
  - LC column temperature conditions: constant 65 ^{o}C.
  - LC mobile-phase A: 0.3-percent acetic acid in 50/50 methanol/acetonitrile.
  - LC mobile-phase B: 0.3-percent acetic acid in reagent water.
  - LC flow rate: 0.650 mL/min.
  - MS detector: atmospheric pressure electrospray (ES) positive- and negative-ion modes alternating with each scan.
  - Drying gas flow: set at 9.0 L/min.
  - Nebulizer gas pressure: set at 30 lb/in^2.
  - Gas temperature: set at 350 ^{o}C.
  - Fragmentor voltage: set at variable volts.
  - Capillary voltage: set at 3,000 V.
- *Data acquisition sys*tem—computer and printer compatible with the HPLC system.
- *Software*—LC/MSD Chemstation revision 09.01 (Hewlett Packard, Wilmington, Delaware) was used to acquire and store data, for peak integration, and for quantitation of the compounds.

Reagents and Consumable Materials

- *Sample bottles*—baked 4-oz amber glass bottles (Boston round) with Teflon-lined lids.
- *Sample filters*—nominal 0.7-µm glass-fiber filters (Gilson, Middleton, Wisconsin, or equivalent).
- *10-mL autosampler vials*—glass vial with Teflon-lined cap (Chromacol, Trumbull, Connecticut).
- *SPE cartridges*—C18–HD extraction cartridges, Prospekt (10 mm x 2 mm) (Spark-Holland, The Netherlands).
- *Analytical standards*—solutions of the herbicides and degradation products, the surrogates, and the internal standards.
- *Reagent water*—generated by purification of tapwater through activated charcoal filter 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).
- Solvents—
  - Acetonitrile, American Chemical Society (ACS) and HPLC grade.
  - Methanol, ACS and HPLC grade.
- *Acetic acid, glacial*—ACS grade.
- *Acetic acid solution*—5-percent glacial acetic acid (v/v) in reagent water.
- *Nebulizer*—nitrogen.

Sampling Methods

Sampling methods used were capable of collecting water samples that accurately represented the water-quality characteristics of the ground water or surface water at a given time or location. Detailed descriptions of sampling methods for obtaining ground-water samples are given in Hardy and others (1989). 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).

Sample-collection equipment must be free of tubing, gaskets, and other components made of nonfluorinated plastic material that might leach interfering compounds into water samples or absorb the herbicides or degradation products from the water. The water samples from each site are composited in a single container and filtered through a nominal 0.7-µm glass-fiber filter using a peristaltic pump. Filters are preconditioned 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 ^{o}C until extracted and analyzed.

Standards

- *Primary standard solutions*—Herbicides, degradation products, surrogates, and internal standards were obtained as pure material from commercial vendors, chemical manufacturers, or other scientists. The secondary amide compounds were synthesized by the authors using the method described by Potter and Carpenter (1995) and further purified and isolated using flash chromatography followed by lypholization (unpublished data on file with the USGS Organic Geochemistry Research Group in Lawrence, Kansas). Identification of each compound was by its mass spectrum yielding the correct molecular weight for the molecular ion. Purity was determined by LC/MS, NMR, organic carbon content, and sodium

analyses. Each standard was prepared at the concentration and in the solution listed in table 2.

- *Intermediate composite standard*—A 1.23-µg/mL composite standard was prepared by combining in a 100-mL volumetric flask appropriate volumes of the stock solution of the individual compounds. The composite solution was diluted in methanol and stored at less than 0 °C.
- *Intermediate internal standard solution (A)*—The solution of 2,4-dichlorphenoxyacetic acid was prepared by diluting in a volumetric flask the appropriate amount to equal 16 µg/mL using methanol.
- *Intermediate internal standard solution (B)*—The solution of isotopically labeled ($^{13}C_6$) metolachlor was prepared by diluting in a volumetric flask the appropriate amount to equal 0.25 µg/mL using methanol.
- *Working internal standard solution*—Combine 0.5 mL reagent water with 0.5 mL of intermediate internal standard solution (A) with 1 mL of intermediate internal standard solution (B).
- *Intermediate surrogate solution*—The solution of D-5 alachlor ESA was prepared by diluting in a volumetric flask the appropriate amount to equal 2.41-µg/mL using methanol.
- *Working surrogate solution*—Add 200 µL of 2.41-µg/L D-5 alachlor ESA to 49.8 g of reagent water in a clean 123-mL amber bottle.
- *Calibration standards*—At concentrations of 0.020, 0.05. 0.10, 0.20, 0.50, 1.00, and 2.00 µg/L, a series of calibration standards is prepared in buffered reagent water (1.0 mL of 0.1 M phosphate buffer, pH 7.0 standard units, per 123 mL of distilled deionized water) using the intermediate composite standard solution.

Safety Precautions

- Perform all steps involving organic solvents and strong acids in a well-vented fume hood.
- Use appropriate personal protective equipment during the handling of any reagents and standards.
- The ES waste exhaust and the vacuum pump exhaust should be vented through a laboratory hood system.

Table 2. Stock solution composition for determination of acetamide herbicides and their degradation products

[mg/mL, milligrams per milliliter; ESA, ethanesulfonic acid; OXA, oxanilic acid; SAA, sufinylacetic acid]

Compound	Concentration (mg/mL)
Acetochlor	1.00
Acetochlor ESA	.950
Acetochlor OXA	.630
Acetochlor/metolachlor secondary amide of ESA	.230
Acetochlor SAA	.752
Alachlor	1.000
Alachlor ESA	.900
Alachlor OXA	.750
Alachlor secondary amide of ESA	.640
Alachlor SAA	.875
Dimethenamid	1.000
Dimethenamid ESA	1.000
Dimethenamid OXA	1.000
Flufenacet	1.030
Flufenacet ESA	1.000
Flufenacet OXA	1.000
Metolachlor	1.030
Metolachlor ESA	.950
Metolachlor OXA	.960
Propachlor	1.020
Propachlor ESA	.0900
Propachlor OXA	.0900
Internal standard	
2,4-Dichlorophenoxyacetic acid	1.000
$^{13}C_6$ Metolachlor	.100
Surrogate	
D-5 Alachlor ESA	.438

Evaluation of Instrument Performance

High-Performance Liquid Chromatograph and Diode Array Detector Performance

HPLC performance is evaluated using background absorbance reading, peak shape, and system pressure. Background absorbance signals should remain stable and low and indicate that the column has equilibrated with the mobile-phase flow. If peak shape deteriorates, the column 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. A variable DAD background signal indicates that the lamp may need to be replaced.

Mass Spectrometer Performance

The MS is tuned in atmospheric pressure ES positive-ion and negative-ion mode before each HPLC/MS analysis sequence using the solutions, procedure, and software supplied by the manufacturer. With the first injection of each analysis sequence, inject a solution of the mobile-phase solution to check for contamination.

Calibration

Two calibration tables and calibration curves were prepared for the analyzed standards using the LC/MSD Chemstation software (Hewlett Packard, Wilmington, Delaware). All calibration standards were analyzed by processing through the entire method as listed in this report. One table and set of curves were for data acquired in ES negative mode, the other set for data acquired in ES positive mode. Manufacture's instructions were followed for using the internal standards as time references and for quantitation. The LC/MSD Chemstation software used the method and calculations as described in the "Alternate Calibration" section. This includes the dilution correction factors that are entered as part of the sequence table used by the instrument to label and identify each injection.

Alternate Calibration

Data for each calibration point are acquired by analyzing a mixture of each calibration solution plus the surrogate using the online SPE LC/MS according to the conditions already described. The relative retention time (RRT_c) is calculated for each selected compound in the calibration solution or in a sample as follows:

$$RRT_c = RT_c/RT_i, \quad (1)$$

where

RT_c = uncorrected retention time of the selected compound, and

RT_i = uncorrected retention time of the internal standard.

The results are presented in table 3.

- The expected retention time (RT) of the peak of the selected compound 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 RT is calculated as follows:

$$RT = (RRT_c)(RT_i) \quad (2)$$

where

RT = expected retention time of the selected compound,

RRT_c = relative retention time of the selected compound, and

RT_i = uncorrected retention time of the internal standard.

- The dilution factor (DF) of the processed sample is calculated using equation 3.

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

where

DF = dilution factor,

V_{np} = volume not pumped = milliliters not pumped through the SPE column, and

V_a = volume added = milliliters of distilled water added to a sample that contained less than 123 mL.

The DF is incorporated into the calculation for determining final concentrations of samples.

- Initial calibration data are acceptable if the correlation coefficient (r^2) value for all curves is greater than or equal to 0.980 for all compounds. A quadratic formula was used for curve fitting.
- A complete extracted calibration curve is included within each instrument sequence.

Extraction Efficiency

Extraction efficiency is determined by analyzing the extracted 0.50-, 1.0-, and 2.0-µg/L standards against standards that were prepared for direct injection into the LC/MS. Both sets of standards are quantified using the internal standard. The extraction efficiency is the slope of the line obtained by plotting the value of the extracted standards calculated from the direct injected standards. The results are tabulated in table 4.

Table 3. Retention times, relative retention times, quantitation ions, and confirmation ions for acetamide herbicides and their degradation products determined using method O–2139–03

[m/z, mass to charge; ESA, ethanesulfonic acid; OXA, oxanilic acid; SAA, sufinylacetic acid; --, not applicable]

Compound	Retention time (minutes)	Relative retention time (ratio)	Quantitationion positive mode (m/z)	Quantitationion negative mode (m/z)	Confirmationion positive mode (m/z)	Confirmation ion negative mode (m/z)
Acetochlor	24.83	1.001	224	--	270	--
Acetochlor ESA	45.73	3.679	--	314	270	--
Acetochlor OXA	33.92	2.729	--	146	148	264
Acetochlor/metolachlor secondary amide of ESA	20.71	1.666	--	256	258	--
Acetochlor SAA	18.18	.732	148	146	364	234
Alachlor	24.51	.988	238		270	--
Alachlor ESA	43.70	3.516	--	314	284	--
Alachlor OXA	33.19	2.670	--	160	162	264
Alachlor secondary amide of ESA	27.44	2.208	--	270	272	--
Alachlor SAA	17.32	.698	162	160	364	234
Dimethenamid	15.59	.628	276	--	278	--
Dimethenamid ESA	29.51	2.374	--	320	322	--
Dimethenamid OXA	20.75	1.669	--	198		270
Flufenacet	28.09	1.132	364	--	194	--
Flufenacet ESA	24.07	1.936	--	274	298	--
Flufenacet OXA	18.15	1.46	--	152	--	224
Metolachlor	25.79	1.040	284		286	--
Metolachlor ESA	45.60	3.669	--	328	298	330
Metolachlor OXA	30.66	2.467	--	278	--	206
Propachlor	9.96	.401	212	214	--	--
Propachlor ESA	21.13	.852	258	216	--	--
Propachlor OXA	15.36	1.236	--	--	206	134
Internal standard						
2,4-Dichlorophenoxyacetic acid	12.43	1.000	--	219	--	161
$^{13}C_6$ Metolachlor	24.81	1.000	290	--	258	--
Surrogate						
D-5 Alachlor ESA	43.21	3.476	--	289	319	--

Analytical Procedure

- Each sample is loaded into the sample tray of the autosampler. The SPE instrument is loaded with cartridges. The SPE instrument performs one complete cycle of a cartridge before proceeding to the next cartridge (sample). The cartridge is activated with methanol, 1 mL/min for 2 min, and conditioned with reagent water, 1 mL/min for 2 min. The autosampler adds 250 µL of the 5-percent acetic acid solution to the sample and mixes with the sample by repeated aspiration from and then dispensing back into the vial. Then 10 mL of sample are loaded onto the cartridge from the autosampler at a rate of 1.1 mL/min. The cartridge is washed with reagent water at the same rate for 15 s.
- Sample analysis—The loaded SPE cartridge is placed in the flow path of the LC/MS prior to the

Table 4. Extraction efficiency of acetamide herbicides and their degradation products in buffered reagent-water samples using method O–2139–03

[ESA, ethanesulfonic acid; OXA, oxanilic acid; SAA, sufinylacetic acid]

Compound	Extraction efficiency (slope as a percentage)	Standard deviation (relative percentage)
Acetochlor	88.6	5.4
Acetochlor ESA	86.1	5.9
Acetochlor OXA	93.6	11.5
Acetochlor/metolachlor secondary amide of ESA	96.0	7.3
Acetochlor SAA	84.8	8.6
Alachlor	88.6	6.3
Alachlor ESA	86.8	6.5
Alachlor OXA	92.4	12.5
Alachlor secondary amide of ESA	89.2	7.0
Alachlor SAA	96.2	22.2
Dimethenamid	83.1	8.6
Dimethenamid ESA	88.2	3.6
Dimethenamid OXA	86.2	2.9
Flufenacet	85.8	16.8
Flufenacet ESA	88.7	5.1
Flufenacet OXA	89.9	8.3
Metolachlor	85.9	6.4
Metolachlor ESA	88.5	7.6
Metolachlor OXA	85.9	5.6
Propachlor	89.0	7.9
Propachlor ESA	100.2	21.3
Propachlor OXA	90.7	7.5
Surrogate		
D-5 Alachlor ESA	88.3	7.7

column (using the conditions previously listed). The compounds are eluted using the mobile phase consisting of a gradient beginning with 55-percent mobile-phase A and 45-percent mobile-phase B to 45-percent mobile-phase A and 55-percent mobile-phase B over 30 min then remaining unchanged over the remaining 45 min. At 30 min the flow rate of the LC is increased from 0.65 to 0.75 mL/min. The cartridge remains in the flow path for the first 9 min.

- *Spiking of internal standard*—Twenty microliters of the ISTD are injected at the beginning of each instrument analyses using the autosampler of the LC/MS. The ISTD is used to normalize, as a time reference, and for quantitation of the compounds being analyzed.
- *Sample analysis*—The online SPE LC/MS conditions for the analysis of the herbicides and their degradation products are the same as those used in the analysis of the calibration solutions. Prior to the analysis of any samples, the LC/MS is checked to verify that the performance criteria and the calibration data for herbicides and their degradation products conform to the criteria described.
- *Data acquisition*—The data are acquired using the Chemstation software.

Calculation of Results

Qualitative Identification

The LC/MSD Chemstation software (Hewlett Packard, Wilmington, Delaware) is used with the previously prepared calibration table (table 3) for identification of compounds. A compound is not correctly identified unless it has the correct quantitation ion. Additional verification is done by comparing the relative integrated abundance values of the significant ions monitored with relative integrated abundance values obtained from the standard samples. The relative ratios of the ions need to be within ± 20 percent of the relative ratios of those obtained from the standards. A compound is not correctly identified unless it has the correct retention time. The relative retention times of the compounds should be within ± 2 percent of those obtained from the standards.

Quantitation

The LC/MSD Chemstation software (Hewlett Packard, Wilmington, Delaware) is used with the previously prepared calibration table (table 3) for quantification of the compound. This software allows for dilution factors to be entered and uses the ISTD for quantitation. Calibration curve fitting is by quadratic equation. Correlation coefficients should be 0.95 or greater.

Alternate Quantitation

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

$$C = \left(\left(\frac{Ac}{Ai} \right)(m) + y \right)(DF), \quad (4)$$

where

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

Ac = area of peak of the quantitation ion for the selected compound;

Ai = area of peak of the quantitation ion for the ISTD;

m = slope of calibration curve using extracted standards between the selected compound and the ISTD from the original calibration data;

y = intercept of calibration curve between the selected compound and the ISTD from the original calibration data; and

DF = dilution factor calculated using equation 3.

Reporting of Results

The acetamide herbicides and their degradation products are reported in concentrations ranging from 0.05 to 2.0 µg/L. If the concentration is greater than 2.0 µg/L, the sample is re-analyzed with a 1:10 dilution or greater (sample:buffered reagent water) and re-analyzed for those compounds that have concentrations greater than 2.0 µg/L.

Method Performance

A reagent-water sample, a ground-water sample collected from a well in Sedgwick County, Kansas, and a surface-water sample from the Kisco River below Mt. Kisco, New York, were used to test the performance of method O–2139–03. All samples were filtered through a nominal 0.7-µm glass-fiber filter and stored at 4 ^{o}C.

Subsamples of each matrix were spiked with the herbicides and degradation products listed in table 1 at concentrations of 0.10 and 1.0 µg/L and analyzed on different days from June 2001 through September 2002. In addition, unspiked subsamples of each matrix were analyzed. Comparisons of the different matrices and concentrations included bias from day-to-day variations. Method recoveries and standard deviations from the analyses are included in tables 5–7.

Corrections for Background Concentrations

The unspiked subsamples of ground water from Sedgwick County, Kansas, and surface water from the Kisco River, New York, did require correction for background concentrations. The uncorrected concentrations are listed in tables 6 and 7.

Method Detection Limits

A method detection limit (MDL) is defined as the minimum concentration of a substance that can be identified, measured, and reported with a 99-percent

Table 5. Mean recovery and standard deviations for acetamide herbicides and their degradation products in buffered reagent water analyzed using method O–2139–03

[µg/L, micrograms per liter; ESA, ethanesulfonic acid; OXA, oxanilic acid; SAA, sufinylacetic acid]

Compound	Eight subsamples spiked at 0.10 µg/L				Eight subsamples spiked at 1.0 µg/L			
	Mean recovery of spiked subsample		Standard deviation (µg/L)	Relative standard deviation (percent)	Mean recovery of spiked subsample		Standard deviation (µg/L)	Relative standard deviation (percent)
	(µg/L)	(percent)			(µg/L)	(percent)		
Acetochlor	0.097	96.5	0.009	9.4	1.011	101.1	0.045	4.5
Acetochlor ESA	.100	100.0	.022	22.1	1.009	100.9	.068	6.7
Acetochlor OXA	.095	95.0	.007	7.1	.986	98.6	.046	4.7
Acetochlor/metolachlor secondary amide of ESA	.091	91.4	.004	4.0	.985	98.5	.018	1.8
Acetochlor SAA	.101	101.3	.015	14.5	1.056	105.6	.105	9.9
Alachlor	.103	102.6	.013	12.7	1.005	100.5	.046	4.6
Alachlor ESA	.096	95.6	.014	15.1	1.005	100.5	.045	4.4
Alachlor OXA	.094	93.8	.005	5.5	.992	99.2	.041	4.1
Alachlor secondary amide of ESA	.095	95.1	.004	4.3	.992	99.2	.045	4.5
Alachlor SAA	.096	96.3	.009	9.7	1.053	105.3	.101	9.6
Dimethenamid	.098	97.9	.007	7.4	1.014	101.4	.062	6.2
Dimethenamid ESA	.094	93.6	.007	7.7	.993	99.3	.066	6.6
Dimethenamnid OXA	.094	93.9	.006	6.7	1.009	100.9	.046	4.5
Flufenacet	.099	98.9	.008	7.8	1.002	100.2	.052	5.2
Flufenacet ESA	.095	94.5	.003	3.0	1.015	101.5	.048	4.7
Flufenacet OXA	.095	95.1	.006	5.8	1.011	101.1	.048	4.8
Metolachlor	.095	95.1	.013	13.6	1.010	101.0	.026	2.6
Metolachlor ESA	.090	90.3	.009	10.3	1.002	100.2	.042	4.2
Metolachlor OXA	.094	93.6	.006	6.7	1.010	101.0	.046	4.6
Propachlor	.092	92.1	.006	6.4	.999	99.9	.062	6.2
Propachlor ESA	.118	118.3	.047	39.3	.992	99.2	.109	11.0
Propachlor OXA	.094	94.3	.004	4.6	.998	98.8	.043	4.3
Mean	.097	96.6	.010	10.2	1.007	100.7	.055	5.4
Minimum	.090	90.3	.003	3.0	.985	98.5	.018	1.8
Maximum	.118	118.3	.047	39.3	1.056	105.6	.109	11.0
Surrogates								
D-5 Alachlor ESA—negative mode	.986	98.600	.101	10.251	1.003	100.325	.036	3.6
D-5 Alachlor ESA—positive mode	1.065	106.488	.091	8.554	1.068	106.838	.080	7.5

Table 6. Mean recovery and standard deviations for acetamide herbicides and their degradation products in ground water from Sedgwick County, Kansas, analyzed using method 0–2139–03

[µg/L, micrograms per liter, ESA; ethanesulfonic acid; OXA, oxanilic acid; SAA, sufinylacetic acid; --, not detected]

Compound	Eight subsamples spiked at 0.10 µg/L					Eight subsamples spiked at 1.0 µg/L				
	Mean recovery			Standard deviation (µg/L)	Relative standard deviation (percent)	Mean recovery			Standard deviation (µg/L)	Relative standard deviation (percent)
	Spiked subsamples (µg/L)	Unspiked subsample (µg/L)	Unspiked subsample (percent)			Spiked subsamples (µg/L)	Unspiked subsample (µg/L)	Unspiked subsample (percent)		
Acetochlor	0.100	--	99.9	0.011	11.3	0.957	--	95.7	0.056	5.8
Acetochlor ESA	.173	0.071	101.8	.026	15.2	1.053	0.071	98.1	.107	10.2
Acetochlor OXA	.226	.134	91.6	.016	7.2	1.034	.134	90.0	.105	10.2
Acetochlor/metolachlor secondary amide of ESA	.076	.028	48.1	.013	17.5	.442	.028	41.4	.142	32.2
Acetochlor SAA	.133	.046	87.3	.028	20.9	.998	.046	95.2	.189	19.0
Alachlor	.097	--	97.4	.010	10.3	.980	--	98.0	.053	5.4
Alachlor ESA	.163	.064	99.3	.012	7.3	1.093	.064	102.9	.071	6.5
Alachlor OXA	.205	.103	101.4	.020	10.0	1.039	.103	93.5	.074	7.2
Alachlor secondary amide of ESA	.121	.004	117.4	.016	13.0	1.088	.004	108.4	.063	5.8
Alachlor SAA	.122	.033	89.9	.017	14.1	.992	.033	96.0	.180	18.2
Dimethenamid	.088	--	88.3	.008	9.1	.878	--	87.8	.069	7.8
Dimethenamid ESA	.098	--	97.8	.017	17.9	1.020	--	102.0	.033	3.3
Dimethenamnid OXA	.103	--	102.5	.033	32.3	1.003	--	100.3	.056	5.6
Flufenacet	.087	--	87.3	.010	11.3	.889	--	88.9	.089	10.0
Flufenacet ESA	.100	--	100.4	.016	15.6	1.020	--	102.0	.057	5.6
Flufenacet OXA	.099		98.9	.008	8.3	.992	--	99.2	.056	5.6
Metolachlor	.147	.051	96.3	.009	6.4	.974	.051	92.3	.055	5.6
Metolachlor ESA	.309	.225	83.6	.026	8.3	1.093	.225	86.8	.113	10.4
Metolachlor OXA	.346	.264	81.6	.037	10.7	1.047	.264	78.2	.123	11.7
Propachlor	.074	.012	62.3	.011	14.8	.839	.012	82.7	.091	10.9
Propachlor ESA	.090	--	90.1	.029	31.9	.849	--	84.9	.108	12.7
Propachlor OXA	.096	--	95.5	.010	10.3	.680	--	68.0	.099	14.6
Mean	.092	--	91.7	.017	13.8	.916	--	90.5	.090	10.2
Minimum	.048	--	48.1	.008	6.4	.414	--	41.4	.033	3.3
Maximum	.117	--	117.4	.037	32.3	1.084	--	108.4	.189	32.2
Surrogates										
D-5 Alachlor ESA—negative mode	1.04875	--	104.9	.092	8.8	1.028375	--	102.8	.088	8.6
D-5 Alachlor ESA—positive mode	.892	--	89.2	.091	10.2	.900	--	90.0	.086	9.5

Table 7. Mean recovery and standard deviations for acetamide herbicides and their degradation products in surface water from Kisco River below Mt. Kisco, New York, analyzed using method 0–2139–03

[µg/L, micrograms per liter, ESA; ethanesulfonic acid; OXA, oxanilic acid; SAA, sufinylacetic acid; --, not detected]

Compound	Eight subsamples spiked at 0.10 µg/L					Eight subsamples spiked at 1.0 µg/L				
	Mean recovery			Standard deviation (µg/L)	Relative standard deviation (percent)	Mean recovery			Standard deviation (µg/L)	Relative standard deviation (percent)
	Spiked subsamples (µg/L)	Unspiked subsample (µg/L)	Unspiked subsample (percent)			Spiked subsamples (µg/L)	Unspiked subsample (µg/L)	Unspiked subsample (percent)		
Acetochlor	0.093	--	92.5	0.014	15.1	0.957	--	95.7	0.111	11.6
Acetochlor ESA	.096	0.015	81.0	.016	17.1	1.053	0.015	103.8	.086	8.2
Acetochlor OXA	.095	--	95.4	.006	6.3	1.034	--	103.4	.075	7.2
Acetochlor/metolachlor secondary amide of ESA	.040	--	40.1	.009	22.9	.442	--	44.2	.130	29.5
Acetochlor SAA	.092	--	92.4	.019	20.8	.998	--	99.8	.226	22.7
Alachlor	.097	--	97.1	.013	13.8	.980	--	98.0	.095	9.7
Alachlor ESA	.146	.053	93.1	.007	5.0	1.093	.053	104.0	.066	6.0
Alachlor OXA	.098	--	97.8	.007	7.1	1.039	--	103.9	.059	5.7
Alachlor secondary amide of ESA	.105	.001	104.1	.005	4.6	1.088	.001	108.7	.052	4.8
Alachlor SAA	.090	--	90.4	.018	19.8	.992	--	99.2	.204	20.5
Dimethenamid	.092	--	92.4	.013	14.2	.878	--	87.8	.137	15.6
Dimethenamid ESA	.102	--	101.8	.007	7.3	1.020	--	102.0	.069	6.8
Dimethenamnid OXA	.087	--	87.3	.013	15.1	1.003	--	100.3	.049	4.9
Flufenacet	.086	--	86.1	.014	16.7	.889	--	88.9	.133	14.9
Flufenacet ESA	.097	--	97.0	.015	15.9	1.020	--	102.0	.156	15.3
Flufenacet OXA	.094	--	93.8	.012	12.5	.992	--	99.2	.085	8.5
Metolachlor	.097	--	96.5	.011	10.9	.974	--	97.4	.083	8.6
Metolachlor ESA	.139	.052	87.4	.008	5.6	1.093	.052	104.1	.079	7.3
Metolachlor OXA	.102	--	102.0	.009	8.6	1.047	--	104.7	.094	9.0
Propachlor	.078	--	77.5	.014	18.4	.839	--	83.9	.130	15.5
Propachlor ESA	.110	--	109.9	.028	25.4	.849	--	84.9	.169	19.9
Propachlor OXA	.063	--	63.0	.006	8.7	.680	--	68.0	.110	16.2
		--								
Mean	.091	--	89.9	.012	13.3	.960	--	94.7	.109	12.2
Minimum	.040	--	40.1	.005	4.6	.442	--	44.2	.049	4.8
Maximum	.110	--	109.9	.028	25.4	1.087	--	108.7	.226	29.5
Surrogates										
D-5 Alachlor ESA—negative mode	1.030	--	103.0	.087	8.5	1.028	--	102.8	.071	6.9
D-5 Alachlor ESA—positive mode	.868	--	86.8	.138	15.9	.900	--	90.0	.110	12.2

Table 8. Mean concentrations and estimated mean method detection limits for nine determinations of acetamide herbicides and their degradation products in eight samples of buffered reagent water analyzed using method O–2139–03

[µg/L, micrograms per liter; ESA, ethanesulfonic acid; OXA, oxanilic acid; SAA, sufinylacetic acid]

Compound	Spiked level (µg/L)	Mean concentration (µg/L)	Standard deviation (µg/L)	Estimated mean method detection limit (µg/L)
Acetochlor	0.020	0.027	0.007	0.021
Acetochlor ESA	.020	.026	.017	.051
Acetochlor OXA	.020	.024	.006	.017
Acetochlor/metolachlor secondary amide of ESA	.020	.022	.007	.022
Acetochlor SAA	.020	.031	.007	.020
Alachlor	.020	.021	.006	.019
Alachlor ESA	.020	.028	.010	.030
Alachlor OXA	.020	.023	.008	.024
Alachlor secondary amide of ESA	.020	.020	.003	.010
Alachlor SAA	.020	.031	.006	.017
Dimethenamid	.020	.024	.006	.018
Dimethenamid ESA	.020	.023	.008	.024
Dimethenamid OXA	.020	.022	.008	.024
Flufenacet	.020	.023	.004	.011
Flufenacet ESA	.020	.025	.006	.019
Flufenacet OXA	.020	.025	.005	.016
Metolachlor	.020	.023	.001	.004
Metolachlor ESA	.020	.025	.008	.023
Metolachlor OXA	.020	.021	.006	.017
Propachlor	.020	.022	.003	.008
Propachlor ESA	.020	.014	.017	.051
Propachlor OXA	.020	.024	.006	.019

confidence that the compound concentration is greater than zero. MDLs were determined according to procedures outlined by the U.S. Environmental Protection Agency (1992). Eight replicate samples of buffered reagent water spiked with 0.020 µg/L of each of the acetamide herbicides and their degradation products were analyzed to determine MDLs (table 8). Each sample set was analyzed on different days from June 2002 through September 2002 so that day-to-day variation is included in the results.

The MDL was calculated using the following equation:

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

where

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

$t_{(n-1,\ 1-\alpha,\ =\ 0.99)}$ = 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 for each compound is listed in table 8. MDLs ranged from 0.004 to 0.051 µg/L for the acetamide herbicides and their degradation products. 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 recoveries for all of the acetamide herbicides and their degradation products except one in the

ground- and surface-water samples ranged from 62.3 to 117.4 percent (tables 6 and 7). The secondary amide of acetochlor/metolachlor ESA was recovered at an average rate of 43.5 percent. The mean recoveries for propachlor and propachlor OXA were next lowest, ranging from 62.3 to 95.5 percent. Mean recoveries from reagent-water samples ranged from 90.3 to 118.3 percent for all compounds (table 5). Overall the mean of the mean recoveries for all compounds, by group, in the three matrices spiked at 0.10 and 1.0 µg/L ranged from 89.9 to 100.7 percent, including the secondary amide of acetochlor/metolachlor ESA and the propachlor compounds.

DISCUSSION

An LC/MS method for the analysis of ethanesulfonic acids and oxanilic acids of acetochlor, alachlor, dimethanmid, flufenacet, and metolachlor was reported in Lee and others (2001). That method is identified by USGS method code O–2134–00. The method described in this report includes the ESA and OXA degradates of a sixth acetamide herbicide, propachlor, the sulfanylacetic acid (SAA) degradates of acetochlor and alachlor, and the secondary amide degradates of acetochlor ESA, alachlor ESA, and metolachlor ESA. Acetochlor ESA and metolachlor ESA degrade to yield the same secondary amide compound. The method described in this report also incorporates the analyses of the parent herbicides, acetochlor, alachlor, dimethenamid, flufenacet, metholachlor, and propachlor. The parent herbicides do not give a signal in the MS using ES negative mode; they do, however, give excellent signals in positive ES mode. The analyses of parent compounds and their degradation products are accomplished in one injection using an MS capable of measuring positive and negative ions, alternating with each scan. Cycle time is set to 50 percent for positive scan and 50 percent for negative scan. Total cycle time is 580 ms for a 0.1 min peak width.

The addition of ES positive mode to the method also allows for the determiniation of confirmation ions of the ESA compounds (table 3). Confirmation ions are lacking when using ES negative mode. The signal yielding the largest response for each compound that gives responses in both ES positive and negative modes is used for quantitation. Using the largest signal allows for maximum sensitivity of MDLs.

Figure 2 is a total ion chromatogram of a 1.0-µg/L standard in a buffered reagent-water sample analyzed in ES positive mode. Figure 3 is a total ion chromatogram of a 1.0-µg/L standard in a buffered reagent-water sample analyzed in ES negative mode.

Changes in the concentrations of the mobile phase plus the addition of a small gradient with increased flow rate allow for the easy separation of the compounds with one column. The actual time required for each injection decreases from 80 to 45 min when compared to method O–2134–00 (Lee and others, 2001). The same type of column, a 5-µm, 250- x 3.0-mm

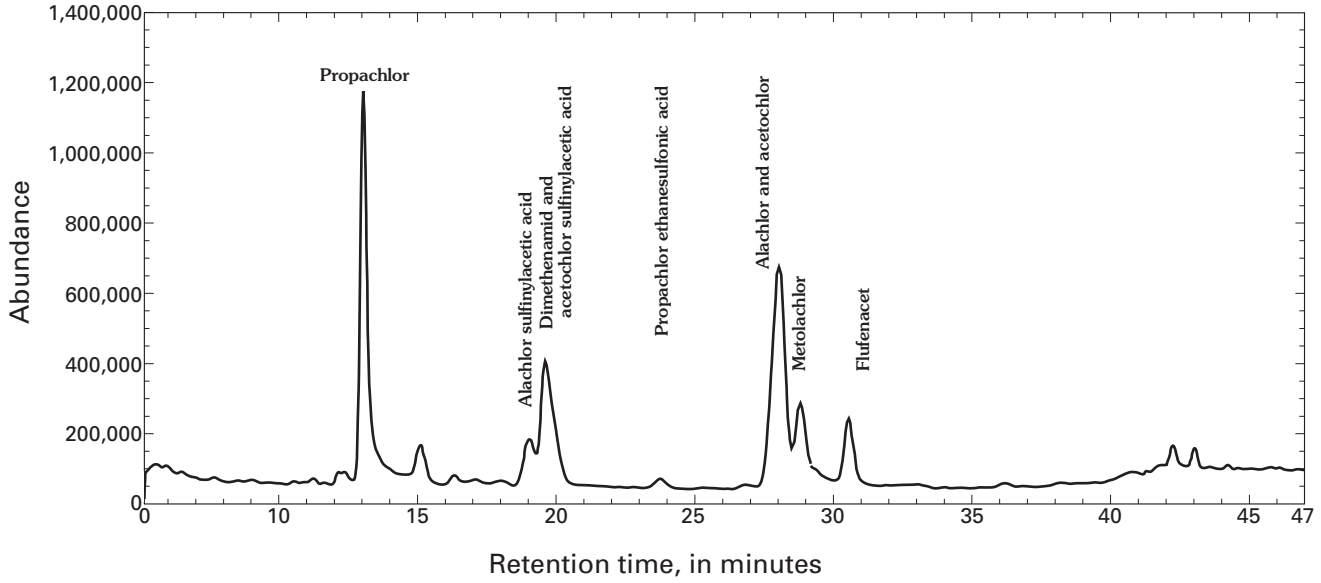

Figure 2. Total ion chromatograph of a 1.0-microgram-per-liter standard in a buffered reagent-water sample analyzed using electrospray positive mode.

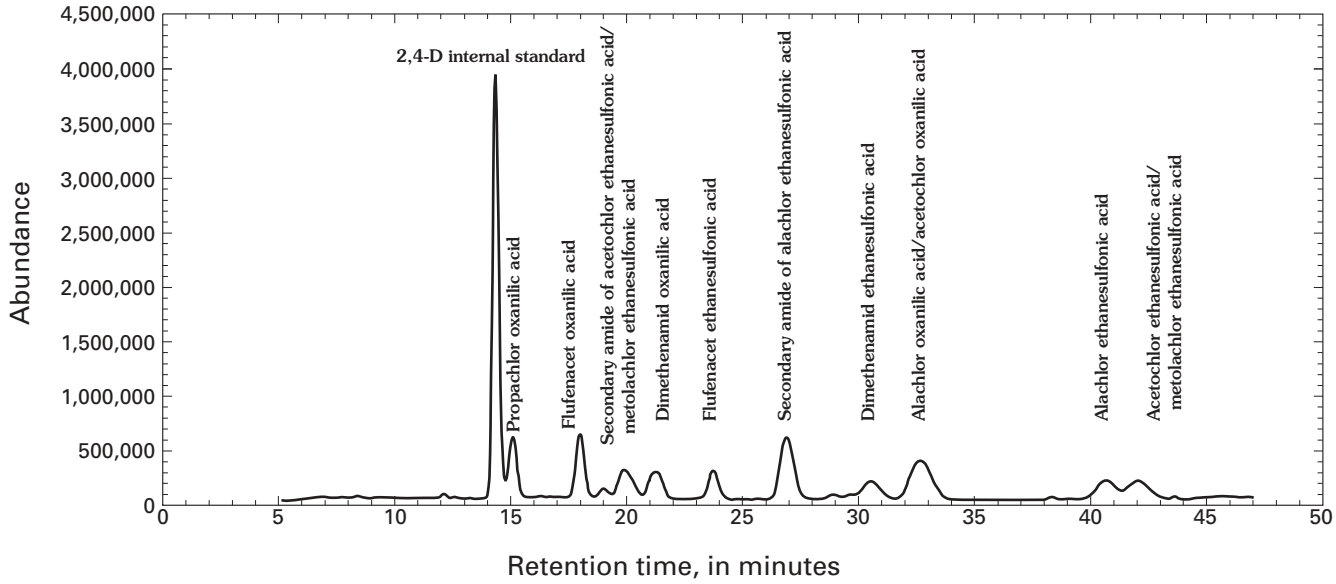

Figure 3. Total ion chromatograph of a 1.0-microgram-per-liter standard in a buffered reagent-water sample analyzed using electrospray negative mode.

C–18, is used for method O–2139–03 and for method O–2134–00.

The utilization of online SPE for the concentration and isolation of the compounds from the sample matrices greatly decreases the amount of sample used, from 123 to 10 mL. This also allows for the automation of the SPE process and eliminates the labor and errors associated with manual evaporation, reconstitution, and transferring into vials of the concentrated samples. The use of the Triathlon autosampler allows for acidification of the sample and the immediate SPE of the sample online. These two functions yield better extraction efficiencies than method O–2134–00 for the flufenacet and propachlor degradation compounds (unpublished data on file with USGS Organic Geochemistry Research Group in Lawrence, Kansas). Method O–2139–03 improves the extraction of the secondary amide of acetochlor/metolachlor ESA but does not optimize the extraction for this compound. The secondary amide of acetochlor/metolachlor ESA was recovered at an average rate of 43.5 percent.

CONCLUSIONS

This report presents a method for routine analysis of 6 acetamide herbicides and 16 of their degradation products in natural water samples. The acetamide herbicides are acetochlor, alachlor, dimethenamid, flufenacet, metolachlor, and propachlor. The degradation products are acetochlor ESA, acetochlor OXA, secondary amide of acetochlor/metolachlor ESA, acetochlor SAA, alachlor ESA, alachlor OXA, secondary amide of alachlor ESA, alachlor SAA, dimethenamid ESA, dimethenamid OXA, flufenacet ESA, flufenacet OXA, metolachlor ESA, metolachlor OXA, propachlor ESA, and propachlor OXA. From the data presented in this report, online solid-phase extraction (SPE) and analysis using high-performance liquid chromatography/mass spectrometry (LC/MS) are shown to be sensitive and reliable for the determination of nearly all acetamide degradation products at low concentrations. With the exception of the secondary amide of acetochlor/metolachlor ESA, good precision and accuracy for the determination of acetamide herbicides and their degradation compounds were demonstrated for the LC/MS method in buffered reagent water, ground water, and surface water. The extraction method as used did not optimize the recovery of the secondary amide of acetochlor/metolachlor ESA.

Method detection limits (MDLs) for the LC/MS method ranged from 0.004 to 0.051 µg/L. Mean recoveries for the acetamide compounds in the ground- and surface-water samples ranged from 62.3 to 117.4 percent. The secondary amide of acetochlor/metolachlor ESA was recovered at an average of rate 43.5 percent. The mean recoveries for propachlor and propachlor OXA were next lowest, ranging from 62.3 to 95.5 percent. Mean recoveries from

reagent-water samples ranged from 90.3 to 118.3 percent for all compounds. Overall, the mean of the mean recoveries for all compounds in the three matrices spiked at 0.10 and 1.0 µg/L ranged from 89.9 to 100.7 percent, including the secondary amide of acetochlor/metolachlor ESA and the propachlor compounds. The lower method reporting limit for the online SPE LC/MS method was set at 0.05 to 2.0 µg/L.

Information about the fate and transport of the acetamide herbicides, alachlor, acetochlor, dimethenamid, flufenacet, metolachlor, propachlor, and their degradation compounds in water can be acquired from the analysis of ground water and surface water using the online SPE LC/MS method. This method also can be useful for water-quality determinations and analytical verification in toxicological studies.

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