U.S. Department of the Interior
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# Methods of Analysis by the U.S. Geological Survey Organic Geochemistry Research Group—Update and Additions to the Determination of Chloroacetanilide Herbicide Degradation Compounds in Water Using High-Performance Liquid Chromatography/Mass Spectrometry

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

**Conversion Factors**

| Multiply | By | To obtain |
|---|---|---|
| gram (g) | $2.205 \times 10^{-3}$ | pound |
| liter (L) | $2.642 \times 10^{-1}$ | gallon |
| meter (m) | 3.281 | foot |
| microliter (µL) | $2.642 \times 10^{-7}$ | gallon |
| micrometer (µm) | $3.937 \times 10^{-5}$ | inch |
| milligram (mg) | $3.53 \times 10^{-5}$ | ounce |
| millimeter (mm) | $3.937 \times 10^{-2}$ | inch |
| ounce (oz) | 0.02957 | liter |
| pound per acre per year [(lb/acre)/yr] | 1.121 | kilogram per acre per year |
| pound per square inch ($lb/in^2$) | 6.895 | kilopascal |

Temperature can be converted to degrees Celsius (°C) or degrees Fahrenheit (°F) by the equations:

$$°C = 5/9 (°F - 32)$$
$$°F = 9/5 (°C) + 32.$$

### Miscellaneous Abbreviations

mass to charge (m/z)
volt (V)
cubic centimeter ($cm^3$)

### Abbreviated Water-Quality Units

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

## IV Update and Additions to the Determination of Chloroacetanilide Herbicide Degradation Compounds in Water Using High-Performance Liquid Chromatography/Mass Spectrometry

# Methods of Analysis by the U.S. Geological Survey Organic Geochemistry Research Group—Update and Additions to the Determination of Chloroacetanilide Herbicide Degradation Compounds in Water Using High-Performance Liquid Chromatography/Mass Spectrometry

*By* E.A. Lee[1], J.L. Kish[1], L.R. Zimmerman[2], and E.M. Thurman[1]

**Abstract**

An analytical method using high-performance liquid chromatography/mass spectrometry (HPLC/MS) was developed by the U.S. Geological Survey in 1999 for the analysis of selected chloroacetanilide herbicide degradation compounds in water. These compounds were acetochlor ethane sulfonic acid (ESA), acetochlor oxanilic acid (OXA), alachlor ESA, alachlor OXA, metolachlor ESA, and metolachlor OXA. The HPLC/MS method was updated in 2000, and the method detection limits were modified accordingly. Four other degradation compounds also were added to the list of compounds that can be analyzed using HPLC/MS; these compounds were dimethenamid ESA, dimethenamid OXA, flufenacet ESA, and flufenacet OXA.

Except for flufenacet OXA, good precision and accuracy were demonstrated for the updated HPLC/MS method in buffered reagent water, surface water, and ground water. The mean HPLC/MS recoveries of the degradation compounds from water samples spiked at 0.20 and 1.0 µg/L (microgram per liter) ranged from 75 to 114 percent, with relative standard deviations of 15.8 percent or less for all compounds except flufenacet OXA, which had relative standard deviations ranging from 11.3 to 48.9 percent. Method detection levels (MDL's) using the updated HPLC/MS method varied from 0.009 to 0.045 µg/L, with the flufenacet OXA MDL at 0.072 µg/L. The updated HPLC/MS method is valuable for acquiring information about the fate and transport of the parent chloroacetanilide herbicides in water.

## INTRODUCTION

The chloroacetanilide herbicides—acetochlor, alachlor, dimethenamid, flufenacet, 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

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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).

Recent studies have reported the occurrence of chloroacetanilide degradation compounds in surface and ground 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 compound 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 compounds during the remaining growing season.

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

This report provides a description of a reliable, previously published method (O–2134–00) for the analysis of ethane sulfonic acid (ESA) and oxanilic acid (OXA) degradation compounds of acetochlor, alachlor, and metolachlor found in surface water and ground water using high-performance liquid chromatography/mass spectrometry (HPLC/MS) (Zimmerman and others, 2000). Since publication of the original method, several modifications have been made to achieve chromatographic separation of alachlor and acetochlor peaks. Moreover, dimethenamid ESA and OXA and flufenacet ESA and OXA have been added to the list of chloroacetanalide degradation compounds suitable for determination using the HPLC/MS method.

The original HPLC/MS method was derived from Ferrer and others (1997), with minor modification to resolve co-eluting peaks on the chromatogram as reported in Hostetler and Thurman (1999). The updated method supplements other methods of the U.S. Geological Survey (USGS) and has been implemented by the USGS Organic Geochemistry Research Group in Lawrence, Kansas.

The updated HPLC/MS method of analysis described in this report has also 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 method, including the apparatus, reagents, instrument calibration, and the solid-phase extraction (SPE) procedure required for sample analysis. Estimated method detection limits, mean recoveries, and relative standard deviations for the six original and four additional chloroacetanilide herbicide degradation compounds determined using HPLC/MS are presented. The USGS parameter and method codes for these compounds are also given.

# DETERMINATION OF CHLOROACETANILIDE HERBICIDE DEGRADATION COMPOUNDS IN WATER USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY

## Method of Analysis (O–2134–00)

### Scope and Application

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

Degradation compounds were selected for analysis because of the extensive use of the parent herbicides in the United States and their importance to current (2000) studies being conducted by the USGS.

**Table 1.** Chloroacetanilide herbicide degradation compounds suitable for determination using method O–2134–00 and associated molecular weights

[ESA, ethane sulfonic acid; OXA, oxanilic acid]

| Degradation compound | Molecular weight (atomic mass units) |
|---|---|
| Acetochlor ESA | 315.4 |
| Acetochlor OXA | 265.3 |
| Alachlor ESA | 315.4 |
| Alachlor OXA | 265.3 |
| Dimethenamid ESA | 321.4 |
| Dimethenamid OXA | 271.4 |
| Flufenacet ESA | 275.3 |
| Flufenacet OXA | 225.3 |
| Metolachlor ESA | 329.4 |
| Metolachlor OXA | 279.3 |

This method is applicable to concentrations from 0.05 to 5.0 µg/L without dilution.

## Summary of Method

Water samples are filtered at the collection site using glass-fiber filters with nominal 0.7-µm pore diameter to remove suspended particulate matter. In the laboratory, the filtered water sample is passed through a preconditioned C-18 ($C_{18}H_{37}$) column. The C-18 column is rinsed with ethyl acetate to remove interfering compounds. The adsorbed chloroacetanilide degradation compounds are eluted from the C-18 with methanol. The 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 diode array detector (DAD) and a mass spectrometer (MS) detector. Compounds eluting from the liquid chromatograph (LC) are identified by comparing the retention times of the mass spectral signals against the measurement of standards analyzed using the same conditions used for the samples. Compounds are identified further by selected fragment ions for compounds that produce fragment ions. The concentration of each identified compound is 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 primary standards analyzed using the same method. The USGS parameter and method codes for the degradation compounds analyzed using method O–2134–00 are listed in table 2.

**Table 2.** U.S. Geological Survey parameter and method codes for 10 chloroacetanilide herbicide degradation compounds suitable for determination using method 0–2143–00

[ESA, ethane sulfonic acid; OXA, oxanilic acid]

| Degradation compound | Parameter code | Method code |
|---|---|---|
| Acetochlor ESA | 61029 | X |
| Acetochlor OXA | 61030 | X |
| Alachlor ESA | 50009 | X |
| Alachlor OXA | 61031 | X |
| Dimethenamid ESA | 61951 | X |
| Dimethenamid OXA | 62482 | X |
| Flufenacet ESA | 61592 | X |
| Flufenacet OXA | 62483 | X |
| Metolachlor ESA | 61043 | X |
| Metolachlor OXA | 61044 | X |

## Interferences

Compounds that elute from the LC at the same time and have mass similar to the degradation compounds may interfere. Samples with high concentrations of humic materials may cause interference with the ionization of the internal standard 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 200-µL, variable-volume autopipettes with disposable tips (Rainin, Woburn, MA, or equivalent).
- *Tekmar six-position AutoTrace*—automated SPE workstation (Tekmar-Dohrmann, Cincinnati, OH).
  - Software: Tekmar AutoTrace Extraction software, version 1.33 (Tekmar-Dohrmann, Cincinnati, OH).
- *Automated solvent evaporator*—Zymark Inc., Hopkinton, MA. The heated bath temperature needs to be maintained at 50 °C and the nitrogen gas pressure at 15 lb/in$^2$.
- *Mechanical vortex mixer.*
- *Analytical columns*—two Phenomenex 5-µm, 250- x 3-mm C-18 columns coupled to one Phenomenex 3-µm, 150- x 2.0-mm C-18 column.
- *HPLC/MS benchtop system*—Hewlett Packard (Wilmington, DE), model 1100 HPLC with autoinjector and MS detector.

  - LC oven conditions: constant 65 °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 0.37 mL/min.
  - MS detector mode: electrospray in negative-ion mode.
  - Drying gas: flow was set at 9 L/min.
  - Nebulizer gas pressure was set at 30 lb/in$^2$.
  - Fragmentor voltage was set at 70 V.
  - Drying gas temperature was set at 300 °C.
  - Capillary voltage was set at 3,100 V.
- *Data acqusition system*—computer and printer compatible with the HPLC system.
- *Software*—HP LC/MSD ChemStation rev.A.06.03 (Hewlett Packard, Wilmington, DE) was used to acquire and store data, for peak integration, and for quantitation of 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, WI, or equivalent).
- *Reagent water*—generated by purification of tap water 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, NJ, or equivalent).
- *Analytical standards*—standards of the chloroacetanilide herbicide degradation compounds and the internal standard.
- *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, MA).
- *Disposable centrifuge tubes*—10 mL (Kimble, Vineland, NJ, or equivalent).
- Solvents—
  - Acetonitrile, ACS (American Chemical Society) and HPLC grade.
  - Ethyl acetate, HPLC grade.
  - Methanol, ACS and HPLC grade.
- *Acetic acid, glacial*—ACS grade.
- *0.1 M phosphate buffer*, pH 7.0 ($Na_2HPO_4$).
- *Gas for evaporation*—nitrogen.
- *Pasteur pipettes*—(Kimble, Vineland, NJ, or equivalent).
- *0.1-mL autosampler vials*—plastic vial with glass-cone insert and cap (Wheaton, Millville, NJ).
- *Nebulizer gas*—nitrogen.

### Sampling Methods

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).

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 degradation compounds 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 ±2 °C until extracted and analyzed.

### Standards

- *Primary standard solutions*—Chloroacetanilide herbicide degradation compounds and internal standard are obtained as pure materials from commercial vendors or chemical manufacturers (acetochlor products—Zeneca Ag, Wilmington, DE; alachlor products—Monsanto, St. Louis, MO; dimethenamid products—BASF, Research Triangle Park, NC; flufenacet products—Bayer, Stillwell, KS; metolachlor products—Novartis, Greensboro, NC). A solution of 1 mg/mL (corrected for purity) is prepared by accurately weighing, to the nearest 0.001 g, 50 mg of the pure material into a 50-mL volumetric flask and then diluting with methanol. The solution is stored at less than 0 °C. This solution is stable for 24 months if protected from evaporation losses.
- *Intermediate composite standard*—A 1.23-ng/µL composite standard is prepared by combining in a

1-L volumetric flask appropriate volumes of the stock solutions of the individual chloroacetanilide herbicide degradation compounds. This composite solution is diluted with methanol and stored at less than 0 °C. The solution is stable for 24 months if protected from evaporation losses.

- *Internal standard solution*—A solution of 2,4-dichlorophenoxyacetic acid (2,4-D) in methanol is prepared at a concentration of 2.0 ng/µL and stored at less than 0 °C. This solution is stable for 12 months if protected from evaporation losses.
- *Calibration solutions (standards)*—At concentrations of 0.05, 0.10, 0.20, 0.50, 1.0, 2.0, and 5.0 µg/L, a series of calibration solutions is prepared in buffered reagent water (0.5 mL of 0.1 M phosphate buffer, pH 7.0, per 123 mL of distilled deionized water) using the intermediate composite standard solution.

## Evaluation of High-Performance Liquid Chromatograph/Mass Spectrometer Performance

### Evaluation of Liquid Chromatograph and Diode Array Detector Performance

- HPLC performance is evaluated by background absorbance readings, peak shape, and system pressure. Background absorbance signals should remain stable 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. A variable DAD background signal indicates that the lamp may need to be replaced.

### Evaluation of Mass Spectrometer Performance

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

### Calibration

A calibration table and calibration curve from the analyzed extracted standards are prepared using the HP LC/MSD Chemstation software (Hewlett Packard, Wilmington, DE). Manufacture's instructions are followed for using the internal standard as a time reference and for quantitation.

### Alternate Calibration

- Data for each calibration point are acquired by injecting 10 µL of each extracted calibration solution into the HPLC/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:

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

where

$RT_c$ = uncorrected retention time of the selected compound, and

$RT_i$ = uncorrected retention time of the internal standard (2,4-D).

See table 3 for retention times, relative retention times, and confirming ions.

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

$$RT = (RRT_c)(RT_i), \qquad (2)$$

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 of the processed sample is calculated as follows:

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

where

$DF$ = dilution factor,

**Table 3.** Retention times, relative retention times, and ions for chloroacetanilide herbicide degradation compounds analyzed using method 0–2134–00

[m/z, mass-to-charge ratio; ESA, ethane sulfonic acid; OXA, oxanilic acid; --, not determined]

| Degradation compound | Retention time (minutes) | Relative retention time | Molecular ion (m/z) | Fragment ion (m/z) |
|---|---|---|---|---|
| **Chloroacetanilide degradation compounds (in order of increasing retention time)** | | | | |
| Flufenacet OXA | 35.749 | 1.680 | 224 | 152 |
| Dimethenamide OXA | 37.841 | 1.779 | 270 | 198 |
| Metolachlor OXA | 45.012 | 2.116 | 278 | 206 |
| Alachlor OXA | 57.764 | 2.715 | 264 | 160 |
| Acetochlor OXA | 57.865 | 2.720 | 264 | 146 |
| Flufenacet ESA | 60.173 | 2.828 | 274 | -- |
| Dimethenamid ESA | 63.224 | 2.972 | 320 | -- |
| Alachlor ESA | 77.870 | 3.660 | 314 | -- |
| Metolachlor ESA | 79.269 | 3.726 | 328 | -- |
| Acetochlor ESA | 79.855 | 3.753 | 314 | -- |
| **Internal standard** | | | | |
| 2,4-dichlorophenoxyacetic acid | 21.275 | 1.000 | 219 | 161 |

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

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

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

- Initial calibration data using extracted standards are entered into a computer spreadsheet (Microsoft Excel, Microsoft, Inc., Seattle, WA), 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 if the apex of adjacent compound peaks is separated.
- At least two laboratory standards are analyzed with each extraction sample set, one high calibration standard ranging from 0.50 to 5.0 µg/L and one low standard ranging from 0.05 to 0.20 µg/L, to verify instrument response in each range. All seven standards must be included within each HPLC/MS run to prepare the calibration curve.

## Extraction Efficiency

Extraction efficiency is determined by analyzing seven standards of the same concentrations used for extraction that are prepared for direct injection into the HPLC/MS. 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.

## Procedure

The SPE procedure used a Tekmar six-position AutoTrace (Tekmar-Dohrmann, Cincinnati, OH). The SPE columns (C-18 Sep-Pak Vac 6 $cm^3$) used to extract samples were obtained from Waters Corporation (Milford, MA). These vacuum cartridges contain 500 mg of 50- to 105-µm C-18 bonded to silica. The data in this report were produced using the Tekmar six-position AutoTrace procedure as listed in Appendix 1.

- *Sample preparation*—123 mL is the volume that fits in the body of a 4-oz Boston round bottle. If an environmental sample contains 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 eight unknown samples, one duplicate sample, two

**Table 4.** Extraction efficiency of chloroacetanilide herbicide degradation compounds in buffered reagent-water samples using method 0–2134–00

[ESA, ethane sulfonic acid; OXA, oxanilic acid]

| Degradation compound | Extraction efficiency (slope as a percentage) | Standard deviation (relative percentage) |
|---|---|---|
| Acetochlor ESA | 82.8 | 13.9 |
| Acetochlor OXA | 81.2 | 13.8 |
| Alachlor ESA | 81.2 | 12.4 |
| Alachlor OXA | 81.6 | 14.3 |
| Dimethenamid ESA | 86.8 | 16.1 |
| Dimethenamid OXA | 86.5 | 21.5 |
| Flufenacet ESA | 83.2 | 13.8 |
| Flufenacet OXA | 74.6 | 11.0 |
| Metolachlor ESA | 80.2 | 13.0 |
| Metolachlor OXA | 83.2 | 13.8 |
| **Minimum** | **74.6** | **11.0** |
| **Maximum** | **86.8** | **21.5** |

standard samples (one high concentration and one low concentration), and a blank sample.

- *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, reagents, solvents, and samples then are loaded onto the instrument.
- *Conditioning SPE columns*—The workstation conditions each SPE column by sequentially passing 3 mL methanol, 3 mL ethyl acetate, 3 mL methanol, and 3 mL distilled water through each column at a flow rate of 20 mL/min by positive pressure.
- *Loading sample*—123 mL of each unknown, standard, and blank sample are passed through a SPE column at a flow rate of 20 mL/min.
- *Eluting potential interfering compounds from SPE column*—Each SPE column is eluted with 3.2 mL ethyl acetate at a flow rate of 4 mL/min to remove the parent herbicides and other interfering compounds.
- *Eluting degradation compounds 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 degradation compounds. The solution is collected in a 10-mL glass centrifuge tube.
- *Spiking of internal standard*—After all the samples in a set have been eluted, each methanol eluate is spiked with 500 µL of 2.0-ng/µL 2,4-D (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 125 µL of a solution containing 0.3 percent acetic acid, 24 percent methanol, 35.7 percent distilled water, and 40 percent acetonitrile and are mixed by vortexing.
- *Transfer to vials*—Using a disposable Pasteur pipette, the reconstituted 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*—The HPLC/MS conditions for the analysis of the degradation compounds are the same as those used in the analysis of the calibration solutions. Prior to the analysis of any sample extracts, the HPLC/MS is checked to verify that the performance criteria and the calibration data for the degradation compounds conform to the criteria described. Ten microliters of the sample extract are injected, and data are acquired using the HPLC/MS conditions described.

## Calculation of Results

### Qualitative Identification

The HP LC/MSD Chemstation software (Hewlett Packard, Wilmington, DE) is used with the previously prepared calibration table for identification of compounds.

Alternate method (manual):

- A degradation compound is not correctly identified unless it has the correct quantitation ion. If more than one ion is acquired for a degradation compound, 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 samples. The relative ratios of the

ions need to be within ±20 percent of the relative ratios of those obtained from the standards.

- The expected retention time (*RT*) of the peak of the selected degradation 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 expected retention time is calculated using equation 2.

#### Quantitation

The HP LC/MSD Chemstation software (Hewlett Packard, Wilmington, DE) is used with the previously prepared calibration table for quantification of compounds.

Alternate method (manual):

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

$$C = \left(\left(\frac{A_c}{A_i}\right)(m) + y\right)(DF), \qquad (4)$$

where

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

$A_c$ = area of peak of the quantitation ion for the selected degradation compound;

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

$m$ = slope of calibration curve using extracted standards between the selected degradation compound and the internal standard from the original calibration data;

$y$ = intercept of calibration curve between the selected degradation compound and the internal standard from the original calibration data; and

$DF$ = dilution factor calculated using equation 3.

## Reporting of Results

Chloroacetanalide herbicide degradation compounds are reported in concentrations ranging from 0.05 to 5.0 µg/L. If the concentration is greater than 5.0 µg/L, 5 µL of sample extract are reinjected and re-analyzed. If the concentration is greater than 10 µg/L, the sample is re-extracted with a 1:10 dilution (sample:distilled water) and re-analyzed for those degradation compounds that have concentrations greater than 10 µg/L.

## Method Performance

A buffered 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 123-mL samples. One set of eight samples was spiked with 0.20 µg/L of each chloroacetanalide degradation compound, and the other set of eight samples was spiked with 1.0 µg/L of each degradation compound. 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 March through September 2000. Comparison of different matrices and concentrations included bias from day-to-day variation. Method recoveries from the analyses are listed in tables 5, 6. and 7.

*Corrections for background concentrations*—Neither surface- nor ground-water samples required correction for background concentrations of degradation compounds. All unspiked buffered reagent-water samples also had no detections of degradation compounds.

*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). Eight replicate samples of buffered reagent water spiked with 0.05 µg/L of each of the degradation compounds were analyzed to determine MDL's (table 8). Each sample was analyzed on different days from March through September 2000 so that day-to-day variation is included in the results.

**Table 5.** Mean recovery of chloroacetanilide herbicide degradation compounds in buffered reagent-water samples using method 0–2134–00

[µg/L, microgram per liter; ESA, ethane sulfonic acid; OXA, oxanilic acid]

| Degradation compound | Eight samples spiked at 0.2 µg/L | | | | Eight samples 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) |
| | (µg/L) | (percent) | | | (µg/L) | (percent) | | |
| Acetochlor ESA | 0.194 | 97.0 | 0.013 | 6.7 | 0.969 | 96.9 | 0.046 | 4.7 |
| Acetochlor OXA | .191 | 95.5 | .013 | 6.8 | .960 | 96.0 | .048 | 5.0 |
| Alachlor ESA | .183 | 91.5 | .015 | 8.2 | .932 | 93.2 | .050 | 5.4 |
| Alachlor OXA | .195 | 97.5 | .017 | 8.7 | .982 | 98.2 | .064 | 6.5 |
| Dimethenamid ESA | .196 | 98.0 | .018 | 9.2 | .971 | 97.1 | .069 | 7.1 |
| Dimethenamid OXA | .201 | 100.5 | .018 | 9.0 | .984 | 98.4 | .075 | 7.6 |
| Flufenacet ESA | .188 | 94.0 | .017 | 9.0 | .949 | 94.9 | .086 | 9.1 |
| Flufenacet OXA | .169 | 84.5 | .037 | 21.9 | .758 | 75.8 | .204 | 26.9 |
| Metolachlor ESA | .192 | 96.0 | .016 | 8.3 | .964 | 96.4 | .036 | 3.7 |
| Metolachlor OXA | .187 | 93.5 | .020 | 10.7 | .961 | 96.1 | .045 | 4.7 |
| **Average** | **.190** | **94.8** | **.018** | **9.9** | **.943** | **94.3** | **.072** | **8.1** |

**Table 6.** Mean recovery of chloroacetanilide herbicide degradation compounds in surface-water samples using method 0–2134–00

[µg/L, microgram per liter; ESA, ethane sulfonic acid; OXA, oxanilic acid]

| Degradation compound | Eight samples spiked at 0.2 µg/L | | | | Eight samples 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) |
| | (µg/L) | (percent) | | | (µg/L) | (percent) | | |
| Acetochlor ESA | 0.151 | 75.5 | 0.012 | 7.9 | 0.897 | 89.7 | 0.071 | 7.9 |
| Acetochlor OXA | .188 | 94.0 | .017 | 9.0 | 1.121 | 112.1 | .118 | 10.5 |
| Alachlor ESA | .150 | 75.0 | .014 | 9.3 | .898 | 89.8 | .077 | 8.6 |
| Alachlor OXA | .179 | 89.5 | .020 | 11.2 | 1.017 | 101.7 | .134 | 13.2 |
| Dimethenamid ESA | .165 | 82.5 | .013 | 7.9 | .865 | 86.5 | .051 | 5.9 |
| Dimethenamid OXA | .204 | 102.0 | .017 | 8.3 | 1.057 | 105.7 | .094 | 9.4 |
| Flufenacet ESA | .165 | 82.5 | .023 | 13.9 | 1.000 | 100.0 | .073 | 7.3 |
| Flufenacet OXA | .223 | 111.5 | .109 | 48.9 | 1.135 | 113.5 | .303 | 26.7 |
| Metolachlor ESA | .163 | 81.5 | .015 | 9.2 | .933 | 93.3 | .080 | 8.6 |
| Metolachlor OXA | .176 | 88.0 | .017 | 9.7 | .989 | 98.9 | .112 | 11.3 |
| **Average** | **.176** | **88.2** | **.026** | **13.5** | **.991** | **99.1** | **.111** | **10.9** |

**Table 7.** Mean recovery of chloroacetanilide herbicide degradation compounds in ground-water samples using method 0–2134–00

[µg/L, microgram per liter; ESA, ethane sulfonic acid, OXA; oxanilic acid]

| Degradation compound | Eight samples spiked at 0.2 µg/L | | | | Eight samples 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) |
| | (µg/L) | (percent) | | | (µg/L) | (percent) | | |
| Acetochlor ESA | 0.169 | 84.5 | 0.023 | 13.6 | 0.830 | 83.0 | 0.095 | 11.4 |
| Acetochlor OXA | 0.185 | 92.5 | .013 | 7.0 | 1.072 | 107.2 | .134 | 12.5 |
| Alachlor ESA | 0.155 | 77.5 | .012 | 7.7 | .831 | 83.1 | .098 | 11.8 |
| Alachlor OXA | 0.187 | 93.5 | .014 | 7.5 | 1.000 | 100.0 | .156 | 15.6 |
| Dimethenamid ESA | 0.170 | 85.0 | .019 | 11.2 | .839 | 83.9 | .072 | 8.6 |
| Dimethenamid OXA | 0.198 | 99.0 | .011 | 5.6 | 1.076 | 107.6 | .126 | 11.7 |
| Flufenacet ESA | 0.164 | 82.0 | .011 | 6.7 | 963 | 96.3 | .103 | 10.7 |
| Flufenacet OXA | 0.159 | 79.5 | .018 | 11.3 | .980 | 98.0 | .172 | 17.6 |
| Metolachlor ESA | 0.173 | 86.5 | .016 | 9.2 | .879 | 87.9 | .113 | 11.3 |
| Metolachlor OXA | 0.186 | 93.0 | .014 | 7.5 | .957 | 95.7 | .151 | 15.8 |
| **Average** | **0.175** | **87.3** | **.015** | **8.7** | **.943** | **94.3** | **.122** | **12.7** |

**Table 8.** Mean concentrations and method detection limits for eight determinations of chloroacetanilide herbicide degradation compounds spiked at 0.05 microgram per liter in eight samples of buffered reagent water using method 0–2134–00

[µg/L, microgram per liter; ESA, ethane sulfonic acid; OXA, oxanilic acid]

| | Eight samples spiked at 0.05 µg/L | | |
|---|---|---|---|
| Degradation compound | Mean concentrations (µg/L) | Standard deviation (µg/L) | Method detection limit (µg/L) |
| Acetochlor ESA | 0.053 | 0.012 | 0.036 |
| Acetochlor OXA | .052 | .010 | .030 |
| Alachlor ESA | .052 | .011 | .033 |
| Alachlor OXA | .053 | .009 | .027 |
| Dimethenamid ESA | .051 | .011 | .033 |
| Dimethenamid OXA | .056 | .006 | .018 |
| Flufenacet ESA | .055 | .003 | .009 |
| Flufenacet OXA | .049 | .024 | .072 |
| Metolachlor ESA | .052 | .011 | 033 |
| Metolachlor OXA | .052 | .015 | 045 |
| **Minimum** | | | **.009** |
| **Maximum** | | | **.072** |

The MDL was calculated using the following equation:

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

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's ranged from 0.009 to 0.045 µg/L (table 8) for 9 of the 10 compounds, with flufenacet OXA being 0.072 µg/L (table 8). This may make low-concentration determinations of flufenacet OXA somewhat more variable than for the other nine compounds. 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 buffered reagent-, surface-, and ground-water samples was determined by comparing the mean analyzed concentration (see "Quantitation" section) from eight replicate samples to the spiked concentration. Mean recoveries were highest overall in surface water at the 1.0-µg/L concentration (table 6) and lowest overall in ground water at 0.2 µg/L (table 7). Flufenacet OXA exhibited the greatest inconsistencies and lowest recoveries. This would indicate the extraction method is not optimized for flufenacet OXA. Alachlor ESA exhibited the lowest recoveries in all three matrices, with the lowest, 75 percent (table 6) at the 0.2-µg/L concentration, in surface water. Dimethenamid OXA exhibited consistently high recoveries in all three matrices. Relative standard deviations of the recoveries, excluding flufenacet OXA, ranged from a low of 3.7 percent to a high of 15.8 percent. Relative standard deviations for flufenacet OXA ranged from 11.3 to 48.9 percent.

## Discussion

An HPLC/MS method for the analysis of ethane sulfonic acids and oxanilic acids of acetochlor, alachlor, and metolachlor was reported by Ferrer and others (1997). The HPLC system described by Ferrer and others (1997) used a 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 3). Thus, accurate quantitation of these degradation compounds was not possible. However, 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 3-µm, 150- x 2.0-mm C-18 column. The separation of the acetochlor ESA and the alachlor ESA allows quantitation of these degradation compounds. 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. Figure 1 shows a total ion chromatogram of a 1.0-µg/L standard in a buffered reagent-water sample. Figure 2 shows the extracted ion chromatogram for the molecular ion (314 mass-to-charge ratio) of acetochlor ESA and alachlor ESA with near baseline separation.

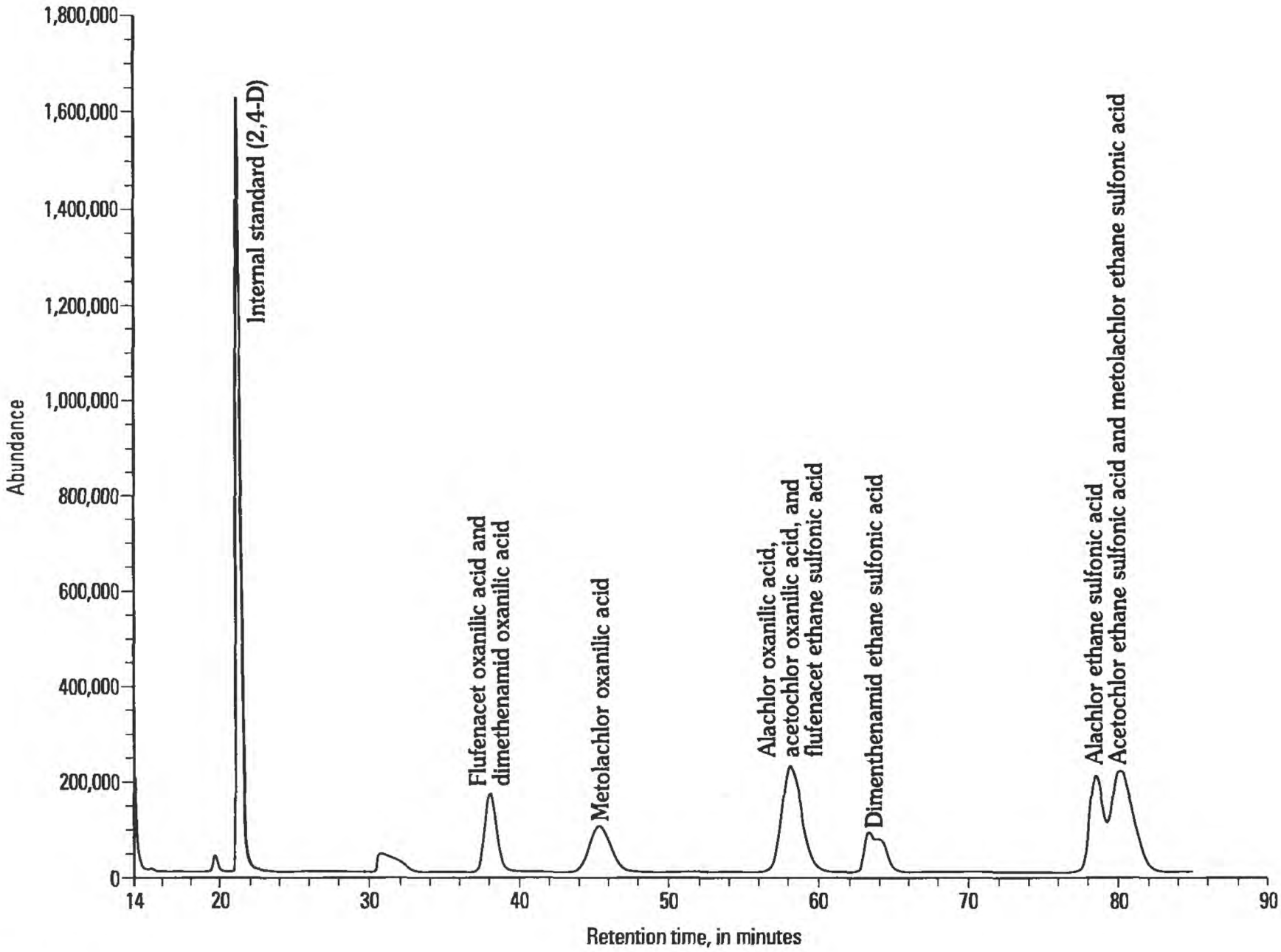


**Figure 1.** Total ion chromatogram of 1.0-microgram-per-liter standard in buffered reagent-water sample using method 0–2134–00.

## CONCLUSIONS

This report presents a method for routine analysis of 10 chloroacetanalide herbicide degradation compounds in environmental water samples. The degradation compounds are acetochlor ESA, acetochlor OXA, alachlor ESA, alachlor OXA, dimethenamid ESA, dimethenamid OXA, flufenacet ESA, flufencet OXA, metolachlor ESA, and metolachlor OXA. From the data presented in this report, solid-phase extraction and analysis using high-performance liquid chromatography/mass spectrometry (HPLC/MS) are shown to be sensitive and reliable for the determination of degradation compounds at low concentrations.

Except for flufenacet OXA, good precision and accuracy for the degradation compounds were demonstrated for the HPLC/MS method in buffered reagent water, surface water, and ground water. The extraction method as used did not optimize the recovery of flufenacet OXA. Method detection limits (MDL's) for the HPLC/MS method ranged from 0.009 to 0.045 µg/L, with the flufenacet OXA MDL at 0.072 µg/L. The mean HPLC/MS recoveries of degradation compounds from water samples spiked at 0.2 and 1.0 µg/L ranged from 75 to 114 percent, with relative standard deviations of 15.8 percent or less for all compounds except flufenacet OXA which had relative standard deviations ranging from 11.3 to 48.9 percent. The MDL for the HPLC/MS method was established at 0.05 µg/L.

Information about the fate and transport of the chloroacetanilide herbicides, acetochlor, alachlor, dimethenamid, flufenacet, and metolachlor, and their degradation compounds in water can be acquired from the analysis of surface water and ground water using the HPLC/MS method. This method also can be

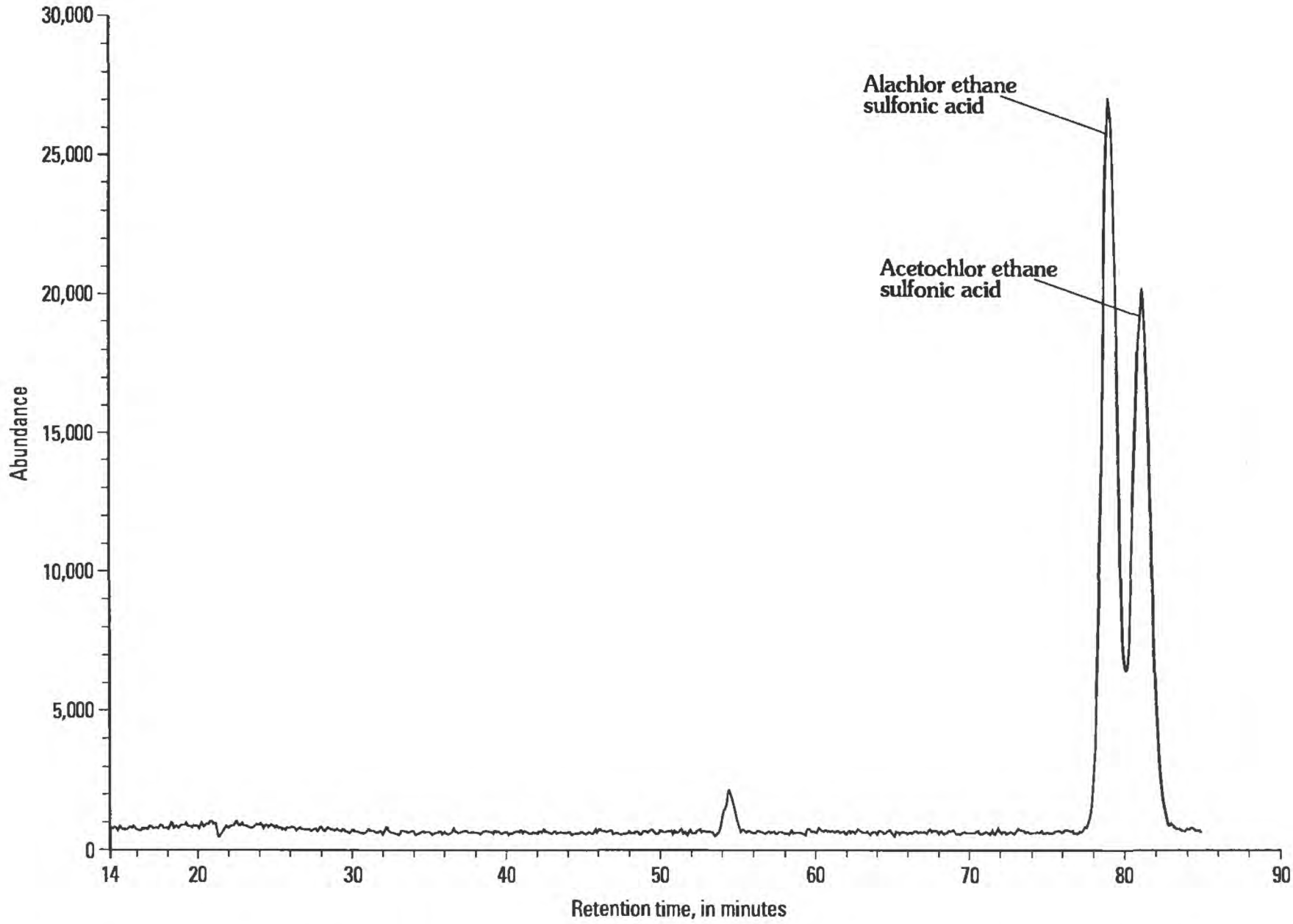


**Figure 2.** Selected ion chromatogram of 0.2-microgram-per-liter standard in buffered reagent-water sample for molecular ion 314 mass-to-charge ratio using method 0–2134–00.

useful for water-quality determinations and analytical verification in toxicological studies.

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# APPENDIX

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

[mL, milliliters; mL/min, milliliters per 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 123 mL of sample onto column
Step 8 : Dry column with gas for 0.5 minute
Step 9 : Condition column with 3.2 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

### FLOW RATES (mL/min)

| | |
|---|---|
| Condition flow: | 10.0 |
| Load flow: | 10.0 |
| Rinse flow: | 20.0 |
| Elute flow: | 5.0 |
| Condition air push: | 15.0 |
| Rinse air push: | 20.0 |
| Elute air push: | 5.0 |

### SOLID-PHASE EXTRACTION PARAMETERS

| | |
|---|---|
| Push delay: | 5 seconds |
| Air factory: | 1.0 |
| Autowash volume: | 1.00 mL |

### WORKSTATION PARAMETERS

| | |
|---|---|
| Maximum elution volume: | 12.0 mL |
| Exhaust fan on: | Yes |
| Beeper on: | Yes |

### Name solvents

Solvent 1 : Ethyl acetate
Solvent 2 : Methanol
Solvent 3 : Distilled water
Solvent 4 : Not used
Solvent 5 : Not used