RESIDUES AND TRACE ELEMENTS

Determination of Low Concentrations of Acetochlor in Water by Automated Solid-Phase Extraction and Gas Chromatography with Mass-Selective Detection

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A sensitive and reliable gas chromatographic/mass spectrometric (GC/MS) method for determining acetochlor in environmental water samples was developed. The method involves automated extraction of the herbicide from a filtered 1 L water sample through a C_{18} solid-phase extraction column, elution from the column with hexane–isopropyl alcohol (3 + 1), and concentration of the extract with nitrogen gas. The herbicide is quantitated by capillary/column GC/MS with selected-ion monitoring of 3 characteristic ions. The single-operator method detection limit for reagent water samples is 0.0015 μg/L. Mean recoveries ranged from about 92 to 115% for 3 water matrixes fortified at 0.05 and 0.5 μg/L. Average single-operator precision, over the course of 1 week, was better than 5%.

Pesticides used on agricultural crops for control of weeds and insects can migrate to surface water and groundwater after application to crops or soil. Sensitive analytical methods are needed to determine these pesticides at low concentrations in environmental water samples to aid in the early detection of surface water and groundwater contamination. As part of a national survey of pesticides and other contaminants in water, the U.S. Geological Survey (USGS) has been analyzing pesticides in surface water and groundwater by using a multiresidue solid-phase extraction (SPE) method (1). Recently, USGS considered adding the herbicide acetochlor (2-chloro-*N*-ethoxymethyl-*N*-[2-ethyl-6-methylphenyl]acetamide) to its multiresidue analytical method.

Acetochlor is a selective preemergent herbicide used to control broadleaf weeds and annual grasses in corn. It was registered for use in the United States in March 1994 to replace the more widely used corn herbicides alachlor, atrazine, butylate, EPTC, 2,4-D, and metolachlor (2). Registration of acetochlor was contingent on the reduction of the total applied kilograms of these 6 herbicides; as a consequence, use of acetochlor in the United States is expected to increase.

Acetochlor has a water solubility of 223 mg/L at 25°C and about the same vapor pressure (3.2 mPa) as alachlor (3). The U.S. Environmental Protection Agency (EPA) registration document indicates that acetochlor is moderately persistent in the environment and is moderately to very mobile in soil. As a result, acetochlor residues are very likely to reach groundwater and surface water (2). During the first season it was used, acetochlor was detected in rain and surface water in Minnesota at concentrations comparable with those of other chloroacetanilide herbicides (10–250 ng/L; 4).

Acetochlor's registration will be canceled if certain concentration limits in surface water or groundwater samples are exceeded. A provision of the registration agreement requires development of groundwater-monitoring programs. The EPA registration document states that effective methods for detecting residues of acetochlor are critical to the effectiveness of monitoring programs. However, a literature search identified only one gas chromatographic (GC) method for acetochlor in water (5). A recent communication (4), however, demonstrated that acetochlor could be incorporated into multiresidue GC or liquid chromatographic (LC) methods (4).

Acetochlor is a structural analog of the chloroacetanilide herbicides alachlor and metolachlor (Figure 1) and could be expected to show similar analytical behavior. Chloroacetanilide herbicides in water have been effectively determined by SPE combined with immunoassay (6, 7), LC (8), GC (9, 10), and GC/mass spectrometry (GC/MS; 1, 11–18). Because SPE can be easily automated for water analyses, SPE methods would be most efficient for groundwater-monitoring programs.

USGS uses an automated SPE technique combined with GC/MS to determine low concentrations of pesticides in water samples. Automated SPE has been an essential part of the USGS quality-control effort, because it permits a mostly automatic processing of a large number of samples while keeping any variations that operators might introduce to a minimum. This reduction in variability allows very low detection limits to be achieved in routine operation.

We describe method-performance data for determination of acetochlor with an automated SPE GC/MS method. Recovery

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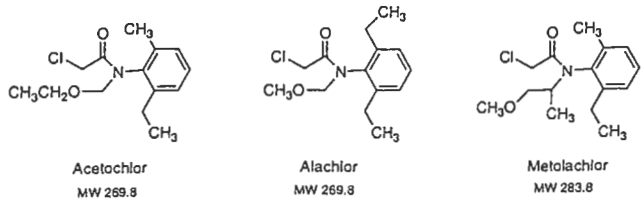

Figure 1. Structures of acetochlor, alachlor, and metolachlor.

of acetochlor at 2 concentrations in reagent water, surface water, and groundwater; variability of analyses; and determination of method detection limit (MDL) are described. Application of the method to environmental samples demonstrates that acetochlor can be added to a multiresidue method suitable for monitoring surveys. The method offers advantages over existing methods by providing the lower MDLs that are required for surface water and groundwater monitoring.

Experimental

Reagents

(**a**) *Solvents.*—Distilled-in-glass grade hexane, toluene, isopropyl alcohol, methylene chloride, and methanol (Burdick & Jackson, Muskegon, MI). Water was obtained from a Solution 2000 Model 2003AL (Solution Consultants, Alpharetta, GA) water purification system.

(**b**) *Standards.*—A 200 µg/mL stock solution of deuterated polycyclic aromatic hydrocarbon (PAH), consisting of [$^2H_{12}$]chrysene, [$^2H_{10}$]phenanthrene, and [$^2H_{10}$]acenaphthene in toluene, was purchased from Absolute Standards, Inc. (New Haven, CT). A custom stock solution containing acetochlor (and other organic compounds) at 50 µg/mL in methanol was purchased from Supelco (Bellefonte, PA).

High-purity Pestanal-brand terbuthylazine was purchased from Crescent Chemical Co. (Hauppauge, NY). [$^2H_{10}$]Diazinon and [$^2H_6$]α-HCH were purchased neat from Cambridge Isotope Laboratories (Woburn, MA). Stock solutions (2 mg/mL) of each of these compounds were prepared by dissolving the standard materials in methanol. These internal standards and surrogates are used as part of the broad-spectrum method that includes 47 pesticides.

(**c**) *PAH internal standard solution.*—A 1 ng/µL solution of deuterated PAH in toluene was prepared by appropriate dilution of the stock solution.

(**d**) *Calibration solutions.*—Solutions were prepared by appropriate dilution of the standard stock solution with toluene to yield solutions containing pesticides at concentrations ranging from 0.01 to 40 ng/µL and the PAH internal standard at a constant concentration of 1.0 ng/µL.

(**e**) *Spiking solution.*—Solutions of acetochlor (and the other selected compounds) at 5.0 and 0.5 ng/µL were prepared by diluting the stock solution with methanol.

(**f**) *Surrogate solution.*—[$^2H_{10}$]Diazinon, [$^2H_6$]α-HCH, and terbuthylazine in methanol at 1 ng/µL.

(**g**) *Cleaning solution.*—Toluene–methylene chloride–isopropyl alcohol (10 + 20 + 70).

(**h**) *Gases.*—Helium carrier gas, 99.999% purity; nitrogen gas, ultrapure.

Apparatus

(**a**) *Sample extraction.*—Zymark AutoTrace SPE workstation Model AT6-6, configured for 3 mL SPE columns, was used for automated extraction. The workstation simultaneously extracts up to 6 samples.

(**b**) *Sample evaporation.*—Zymark TurboVap LV was used to concentrate sample extracts. The TurboVap water bath was set to 30°C, and nitrogen gas pressure was adjusted to 34.5 kPa (5 lb/in.^{2}).

(**c**) *GC/MS instrument.*—Hewlett-Packard Model 5890 Series II gas chromatograph, connected via capillary direct interface to a Hewlett-Packard 5971 mass-selective detector.

(**d**) *GC/MS operating conditions.*—GC conditions: oven set at 100°C with a 5 min hold, then raised to 300°C at 6°C/min and held for 5 min. The injection block was set at 250°C and the injection volume was 1 µL, with splitless injection. Helium was used as the carrier gas at a flow rate of 1 mL/min. MS conditions: GC/MS interface was set at 290°C, analyzer temperature was 180°C, and the ion dwell time was 20 ms. The detector and filament were turned on at 14 min and turned off at 33 min.

(**e**) *GC capillary column.*—Fused-silica capillary column (25 m × 0.20 mm) coated with a 0.33 µm bonded cross-linked 5% phenyl methyl silicone film, Hewlett-Packard Ultra II or equivalent.

(**f**) *Glass fiber filters.*—0.7 µm nominal pore diameter (GF/F grade), baked at 450°C (Whatman, Inc., Maidstone, UK).

(**g**) *SPE columns.*—Isolute C_{18} (EC) 3 mL columns, packed with 500 mg C_{18} hydrocarbon phase chemically bonded to silica (International Sorbent Technology Ltd., Mid Glamorgan, UK). The C_{18} phase was endcapped to reduce polar secondary interactions with surficial silanol groups. Stainless steel fritted discs were used on the columns to keep the sorbent phase in place.

(**h**) *Amber glass bottles.*—1000 mL, baked at 450°C for 2 h, and fitted with Teflon-lined screw caps.

(**i**) *Disposable culture tubes.*—Borosilicate glass, 16 mm × 100 mm, baked at 450°C for 2 h prior to use.

Sample Preparation

Filter water samples through 0.7 µm glass-fiber filters (5) and store at 4°C until use.

Preclean SPE columns with 3 mL elution solvent (3 + 1, hexane–isopropyl alcohol), dry with nitrogen, weigh, and store in glass beakers covered with aluminum foil until use. Weigh each water sample before extraction. Prepare water samples by adding the equivalent of 1% of the sample volume (assume 1 g is equivalent to 1 mL) of methanol to each sample (e.g., 10 mL/10 g for a 1 L/1 kg sample) to maintain column conditioning during extraction. Before extraction, add 100 µL of a 1 ng/µL surrogate solution to each water sample. Add 100 µL

spiking solution to the water samples to be fortified. Extract each sample set within an hour of preparation and fortification.

Automated Sample Extraction

Place precleaned SPE columns on the AutoTrace System, insert intake lines into sample bottles, and begin automated SPE procedure. The procedure conditions the SPE column with 3 mL methanol, followed by 6 mL water. Then the sample is pumped through the column at 25 mL/min. After sample extraction, the AutoTrace tubing and valves are cleaned with toluene–methylene chloride–isopropyl alcohol, followed by methanol, and then water. The automated extraction procedure takes 58 min to simultaneously process 6 environmental or quality-control samples.

Elution and Evaporation of Sample Extract

Dry SPE columns with a positive pressure (138 kPa or 20 lb/in.^{2} for 5 to 20 min) of nitrogen. Verify complete removal of water by comparing the weight of columns after drying to the tare weight (should be within 0.001 g). Add 100 µL PAH internal standard solution to a culture tube and elute compounds from the column into the culture tube with 3 mL hexane–isopropanol (3 + 1). Place sample extract in TurboVap evaporator for about 15 min and concentrate extract to ca 100 µL under a steady stream of nitrogen. To avoid loss of compounds, do not allow the extract to evaporate completely.

GC/MS Procedure

At the beginning of analysis, generate a calibration curve from a minimum of 5 calibration solutions with concentrations ranging from 0.01 to 40 ng/µL. Because the extract volume is about 100 µL, these "on-column" concentrations correspond to actual sample concentrations of 0.001 to 4.0 µg/L, respectively. Calibration is based on a quadratic fit without fixed origin. Analyze a calibration solution every 10th sample to check instrument consistency.

Quantitation of acetochlor is based on the electron-ionization base peak (*m/z* 146), with confirmation ions at *m/z* 162 and 223, as shown in Figure 2A. The qualifying ions will have the same retention time as the quantitation ion, and the relative ratios of the 3 ions must be within ± 20% of the ratios obtained for the calibration solution. The response factor for acetochlor is calculated by comparing the peak area of the acetochlor quantitation ion with the peak area of the quantitation ion of the PAH internal standard [$^2H_{10}$]phenanthrene at *m/z* 188. Under these conditions, the retention time of acetochlor is ca 26.1 min. Retention times, quantitation ions, and confirmation ions for acetochlor and for alachlor (which may interfere with acetochlor) are listed in Table 1.

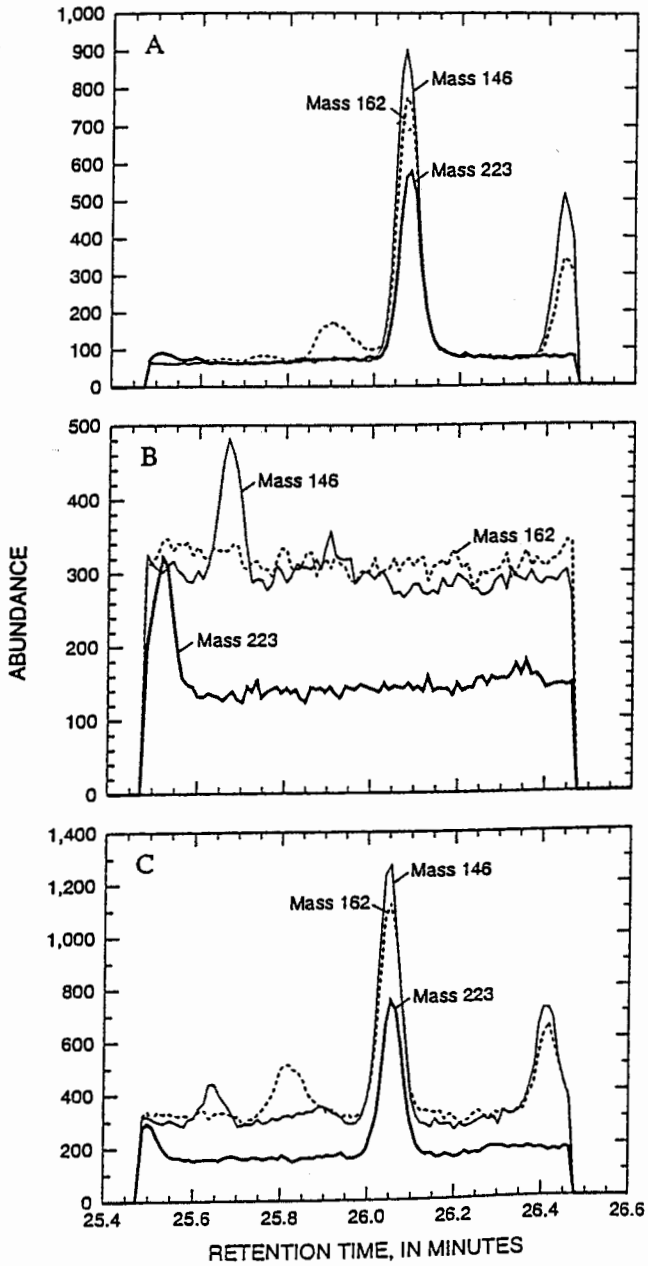

Figure 2. Mass chromatograms showing the determination of acetochlor (26.1 min) with mass-selective detection at *m/z* 146, 162, and 223: (A) acetochlor standard, 0.2 ng injected; (B) reagent water blank containing no detectable acetochlor; (C) surface water fortified with 0.05 µg/L of acetochlor, equivalent to 115% recovery. Standard and surface water also contained alachlor, which has a retention time of 26.4 min.

Results and Discussion

Method validation recovery and precision data for acetochlor were obtained by analyzing reagent water, surface water, and groundwater samples fortified at 2 concentrations. Surface water was collected from the South Platte River at Cherry Creek, CO. Groundwater was obtained from a domestic well in Jefferson County, CO. Approximately 40 L sample water was obtained from the sites and transported to the laboratory in either a 40 L stainless steel milk can or 4 L glass bottles. In the laboratory, water samples were filtered through a 0.7 µm glass-fiber filter, separated into 1 L glass bottles, and stored at 4°C for up to 7 days.

Sets of eight 1 L replicate samples were prepared at 2 concentrations in reagent water, surface water, and groundwater.

Table 1. Retention time (RT), quantitation ion, and confirmation ions for acetochlor and alachlor

Compound	RT, min	Quantitation ion, *m/z*	Confirmation ions, *m/z*	
Acetochlor	26.1	146	162	223
Alachlor	26.4	160	188	237

One set of 8 was fortified at 0.5 µg/L and the other set was fortified at 0.05 µg/L, for a total of 48 samples. One objective of this test was to eliminate experimental bias caused by sample matrix, compound concentration, or extraction set. However, equipment considerations made simultaneous extraction of all 48 samples impractical. Consequently, the samples were placed into random sets of 12. Two additional 1 L samples from the groundwater and surface water sites were extracted to check for compounds already present in the water. These samples were included in the random sets. To minimize instrumental bias, all 52 samples (48 samples plus 4 unfortified samples) were analyzed in a single analytical batch.

Representative mass chromatograms of quantitation and confirmation ions of acetochlor in a low-concentration standard, a reagent water blank, and surface water fortified at 0.05 µg/L are shown in Figure 2. The SPE GC/MS selected-ion monitoring technique provides low background noise and offers excellent sensitivity. Separation of acetochlor and alachlor is typically about 0.3 min, with sample resolution as shown in Figure 2A.

Data for recovery and precision (relative standard deviation) are listed in Table 2. Recoveries from reagent water fortified at 0.5 µg/L averaged 97.8%, with precision of 2.1%; recoveries from reagent water fortified at 0.05 µg/L averaged 91.6%, with precision of 4.2% (Table 2). Recoveries of acetochlor from surface water fortified at 0.5 µg/L averaged 101%, with precision of 3.7%; recoveries from surface water fortified at 0.05 µg/L averaged 115%, with precision of 2.8%. Recoveries from groundwater fortified at 0.5 µg/L averaged 101%, with precision of 3.4%; recoveries from groundwater fortified at 0.05 µg/L averaged 102%, with precision of 4.4%. The mean recovery of acetochlor from 97 routine laboratory reagent water samples fortified at 0.1 µg/L and analyzed in 1995 was 97.19%, with precision of 13.79%.

The surrogates were used to monitor performance of the broad-spectrum analytical method. Typical recoveries from reagent water were 100% for terbuthylazine, 88% for [$^2H_{10}$]diazinon, and 90% for [$^2H_6$]α-HCH.

Method Detection Limit

A preliminary estimated MDL for acetochlor was calculated for a set of 8 replicates (Table 2). The EPA procedure for determination of MDLs (6) was used. The preliminary estimated MDL calculated for reagent water fortified at 0.01 µg/L was 0.0015 µg/L. According to the EPA procedure, the fortified concentration must be no more than 5 times the MDL. Because the fortified concentration (0.01 µg/L) was slightly more than 5 times the determined MDL, the real MDL for this procedure may be lower. The determined MDL is greater than the lowest calibration standard, which is equivalent to 0.001 µg/L. This MDL is about an order of magnitude lower than those of comparable SPE procedures for determination of herbicides in water (18).

Application to Environmental Samples

USGS determined acetochlor in samples submitted to the National Water Quality Laboratory from October 1994 to August 1995. Acetochlor was detected in 15 of 1100 samples (1.4%). Eleven of these samples had concentrations less than 0.01 µg/L. The lowest concentration was 0.004 µg/L; the highest was 0.177 µg/L. Also analyzed were selected sample extracts received before October 1994, which had not been previously analyzed for acetochlor. Forty-one of 798 extracts contained acetochlor at concentrations ranging from 0.005 to 0.429 µg/L, with a median value of 0.023 µg/L. Analysis of these extracts was requested specifically for samples that could contain acetochlor, so the percentage of detection was higher than that for samples not analyzed specifically for acetochlor.

Table 2. Mean concentration, standard deviation (SD), relative standard deviation (RSD), recovery, and calculated method detection limit (MDL) of acetochlor added at selected concentrations to samples of reagent water, surface water, and groundwater[a]

Sample	Fortification concentration, µg/L	Mean concentration determined, µg/L	SD, µg/L	RSD, %	Mean recovery, %	MDL, µg/L
Reagent water	0.50	0.489	0.010	2.1	97.8	—[b]
Reagent water	0.05	0.046	0.002	4.2	91.6	—
Reagent water	0.01	0.011	0.001	4.6	111	0.0015
Surface water	0.5	0.505	0.019	3.7	101	—
Surface water	0.05	0.058	0.002	2.8	115	—
Groundwater	0.5	0.504	0.017	3.4	101	—
Groundwater	0.05	0.051	0.002	4.4	102	—

[a] Data are means of 8 replicate samples for each water type and fortification concentration.
[b] —, not calculated.

Conclusions

Use of C_{18} SPE columns to extract acetochlor from water samples and analysis by GC/MS operated in the selected-ion mode is a sensitive and reliable method for determining the herbicide in surface water and groundwater. The automated SPE system permits reproducible sample extraction and consistent compound recovery and, thereby, aids in achieving the low MDLs needed for environmental monitoring programs. The C_{18} SPE and GC/MS method for detecting acetochlor can be included easily in a broader, multiresidue method for pesticide determination.

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