Determination of the Ethanesulfonate Metabolite of Alachlor in Water by High-Performance Liquid Chromatography

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A high-performance liquid chromatography method for the analysis of 2-[(2,6-diethylphenyl)(methoxymethyl)amino]-2-oxoethanesulfonic acid, a major soil metabolite of alachlor, in groundwater and surface water is presented. Extraction of the metabolite from water was accomplished by passing 100 mL through a C_{18} cartridge. Separation was performed on a C_{18} column with a mobile phase of methanol–50 mM phosphate buffer, pH 7.0 (50/50). Detection was at 205 nm. Mean recoveries of the ethanesulfonate from fortified surface water and groundwater ranged from 93 to 100%. This alachlor metabolite was detected and quantified in nonfortified water from Ohio and Indiana and confirmed using ultraviolet spectra and LC/MS/MS.

INTRODUCTION

Water contamination from pesticides is not a new environmental problem. However, information regarding the nature and geographical extent of pesticide contamination is increasing rapidly (Chesters et al., 1989; Baker and Richards, 1990; Bushway et al., 1991; Thurman et al., 1991). One such pesticide is alachlor [2-chloro-2,6-diethyl-*N*-(methoxymethyl)acetanilide]. It is a chloroacetanilide herbicide, with approximately 37 million kg (Chesters et al., 1989) used annually in the United States to control annual weeds primarily in corn, soybeans, sorghum, and peanuts. Seasonal contamination of surface waters by alachlor and occasional instances of groundwater contamination have been observed throughout the alachlor use areas of the United States (Chesters et al., 1989; Feng et al., 1990; Baker and Richards, 1990; Thurman et al., 1991; Bushway et al., 1992). Furthermore, Monsanto has conducted a year-long statistically based study to determine the occurrence of alachlor in well H_2O. These data indicate that less than 1% of the 6 million wells in the target population are expected to have detectable levels of alachlor, and only an estimated 0–0.02% of the wells exceed the MCL of 2 ppb (Holden and Graham, 1992).

Although numerous laboratory experiments have shown that alachlor degrades to many compounds in water and soil, these metabolites have not been investigated under field conditions and most have not been identified (Alhajjar et al., 1990; Potter et al., 1991). Recently, several water samples being analyzed for alachlor by enzyme immunoassay at the Water Quality Laboratory at Heidelberg College were determined to be "false positives" since the presence of alachlor could not be confirmed by gas or liquid chromatography. After further immunoassay research, it was concluded that the false positives were most likely caused by the major alachlor soil metabolite [2-[(2,6-diethylphenyl)(methoxymethyl)amino]-2-oxoethanesulfonic acid] (Feng et al., 1990).

To substantiate the presence of that metabolite in these water samples, a classical analytical method was needed. However, since the metabolite has never been shown to be present in water, there was no method available. Therefore, this paper describes a simple and reproducible HPLC procedure for the analysis of ethanesulfonate (ES) analog of alachlor in water.

MATERIALS AND METHODS

Materials. All solvents were of HPLC grade obtained from VWR (Bridgeport, CT). Sodium phosphate dibasic (anhydrous) was purchased from Sigma (St. Louis, MO). Alachlor metabolites [2-[(2,6-diethylphenyl)(methoxymethyl)amino]-2-oxoethanesulfonic acid, 2-hydroxy-2′,6′-diethylacetanilide, 2-chloro-2′,6′-diethylacetanilide, and [(2,6-diethylphenyl)(methoxymethyl)amino]oxoacetic acid] were a gift from the U.S. Geological Service (Manhattan, KS), and all were 98% pure.

The C_{18} Sep-Pak plus cartridges were from Waters Associates (Milford, MA).

Water samples were obtained from Dr. David Baker (Tiffin, OH). Water samples, both ground and surface, were collected from Indiana and Ohio and sent to ImmunoSystems and the University of Maine for further investigation in glass bottles. They were stored at 4–8 °C.

Liquid Chromatography System. The HPLC consisted of a Waters 510 pump, a Valco pneumatic injector (VICI Instruments, Houston, TX) containing a 50-μL loop, and a Hewlett-Packard (Avondale, PA) 1040A photodiode array detector equipped with a Hewlett-Packard 900 computer.

LC/MS/MS System. This was a SCIEX API III model (Thornhill, ON, Canada).

Methods. *Extraction Procedure.* A 100-mL aliquot of water was allowed to come to room temperature before it was passed through a conditioned C_{18} cartridge. The conditioning steps consisted of wetting the cartridge with 5 mL of methanol followed by 10 mL of water. Once the 100-mL aliquot had been put through the cartridge, the excess water was removed from the Sep-Pak Plus by vacuum. The cartridge was then eluted with methanol with the first milliliter being collected. This methanol fraction contained the metabolite. To the 1 mL of sample was added 1 mL of sodium phosphate dibasic, pH 7.0.

Preparation of Standards. A stock solution of ES was prepared at a concentration of 1 mg/mL in methanol. From the stock standard an intermediate solution of 12.8 μg/mL in mobile phase was made. The working standards were obtained by making dilutions with the intermediate solution to yield standards of 20, 40, 80, 320, 1280, 2560, and 6400 ng/mL. These working standards were prepared in the HPLC mobile phase.

Liquid Chromatography Conditions. Operating conditions were as follows: injection volume, 50 μL; flow rate, 1.0 mL/min;

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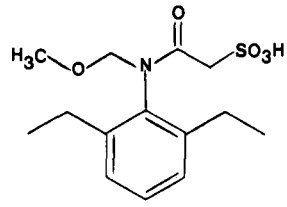

Figure 1. Structure of the alachlor metabolite 2-[(2,6-diethylphenyl)(methoxymethyl)amino]-2-oxoethanesulfonic acid.

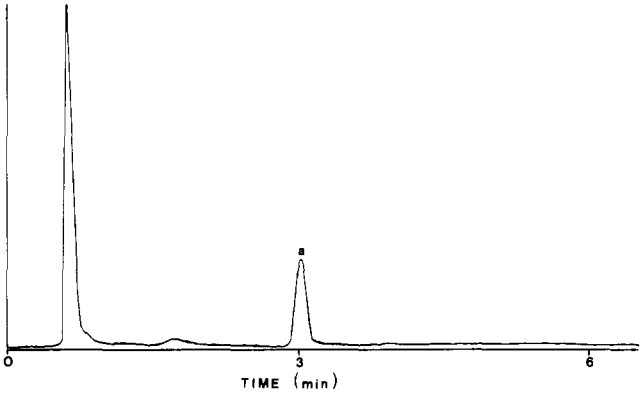

Figure 2. HPLC chromatogram of a groundwater sample. (Peak a) Alachlor metabolite ES. Fifty microliters of groundwater sample 2 containing 21 ng/mL was injected.

column, Perkin-Elmer Pecosphere STET, C_{18} 80 mm × 4.6 mm, 3-μm stainless steel cartridge with 12% carbon load not end-capped; mobile phase, 50/50 methanol–50 mM sodium phosphate dibasic buffer, pH 7.0; UV absorbance, 205 nm; absorbance range, 0.04 AUFS.

LC/MS/MS Conditions. Operating conditions were as follows: injection volume, 50 μL; flow rate, 1.0 mL/min; column, Merck C_{18}, 125 mm × 4.6 mm, 5 μm stainless steel; mobile phase, 50/50 0.5% acetic acid–methanol; split ratio, 95/5; inlet, IonSpray; mode, negative ion.

Linearity Study. Standard concentrations ranging from 20 to 6400 ng/mL were used to determine peak area response vs concentration of ES.

Recovery Study. Both groundwater and surface water free of ES were fortified with ES at the following concentrations: 1, 20, and 100 ng/mL. This study was used to ascertain the efficiency of the ES extraction procedure.

Reproducibility Study. Six water samples were analyzed several times on the same day and different days to determine the within and between days variation of the HPLC ES method.

RESULTS AND DISCUSSION

The structure of the alachlor metabolite (ES) in its pronated form is shown in Figure 1. It is a difficult compound to chromatograph. The sulfonic acid moiety requires derivatization before analysis by gas chromatography. In addition, the acid group together with the nitrogen structure makes HPLC analysis more difficult due to the polarity of the molecule. After many attempts to chromatograph ES, the best system developed required the employment of a short-length C_{18} column with a buffer–methanol system at pH 7.0. These conditions yielded complete separation of ES from interfering peaks from unknown compounds in the water samples within 3 min and excellent symmetry (Figure 2). Also, all metabolites of alachlor listed under Materials and Methods along with alachlor and metolachlor were also shown not to interfere. No metabolites of metolachlor were tried.

If possible because of sensitivity, one likes to analyze pesticides at the maximum ultraviolet (UV) absorbance, which in this instance was 205 nm. The linearity of ES at 205 nm for peak area ranged from 1 to 320 ng. These linearity ranges are more than adequate to cover most ES concentrations that might occur in water supplies. The lower limit of detection (LOD) was 0.5 ng/mL, and the lower limit of quantitation (LOQ) was 1.0 ng/mL in both surface water and groundwater as determined according to the procedure recommended by the American Chemical Society (ACS, 1980).

Table I. Recovery of ES from Fortified Groundwater and Surface Water

water sample[a]	% recoveries of ES from three fortification levels		
	1 ng/mL	20 ng/mL	100 ng/mL
1	109	92	90
2	100	88	95
3	87	96	99
4	105	95	107
av % recovery[b]	100	93	98
% CV[c]	9.6	3.8	7.3

[a] Four types of water: tap, spring, pond, and river. [b] Means of four different samples done on four different days. [c] Percent coefficients of variation.

Table II. ES Concentration in 11 Groundwater and Surface Water Samples from Ohio

water type	ES found, ng/mL	water type	ES found, ng/mL
ground 1	74	surface 1	ND[a]
ground 2	21	surface 2	1
ground 3	4	surface 3	0.6
ground 4	20	surface 4	2
ground 5	39	surface 5	ND
ground 6	44		

[a] None detected at a detection limit of 0.5 ng/mL.

Also because of the structure of ES, solid-phase extraction was used to extract and concentrate the metabolite from the water samples. Liquid/liquid extraction gave a recovery of 10–20% whether or not the water was acidified prior to extraction. The results are not unanticipated since the compound is so polar. However, with solid-phase extraction the mean recoveries ranged from 93 to 100% with no pH adjustment needed. In fact, adjusting the pH to acid or basic pH did not change the percent recovery. Results of a recovery study are shown in Table I. Both groundwater and surface water were spiked at three levels (1, 20, 100 ng/mL) on four different days. In addition to obtaining excellent percent recoveries, the percent coefficients of variation were good, varying from 3.9 to 9.6%.

Eleven water samples (ground and surface) collected from Indiana and Ohio were analyzed for ES. The results are given in Table II. Of these 11 samples, 9 (including all 6 immunoassay false positive groundwater samples) were shown by HPLC to contain ES varying in concentration from 0.6 to 74 ng/mL. The groundwater samples were shown to have the highest ES concentrations. To our knowledge this is the first time that ES has been demonstrated as a groundwater and surface water contaminant; since the complete toxicological effects are unknown, it is difficult to expound on the significance of these concentrations. However, according to Monsanto registration data on file with the U.S. EPA, ES is nonmutagenic, does note bioconcentrate, and does not undergo significant metabolic transformations when fed to animals. A structural isomer of ES has an LD_{50} of >5000 mg/kg in short-term animal tests (Monsanto Co., unpublished data). Furthermore, Alhajjar et al. (1990) found 12 metabolites of alachlor from a laboratory experiment but did not identify them.

Confirmation of the presence of ES was performed using two techniques. First, UV scans from 190 to 350 nm were

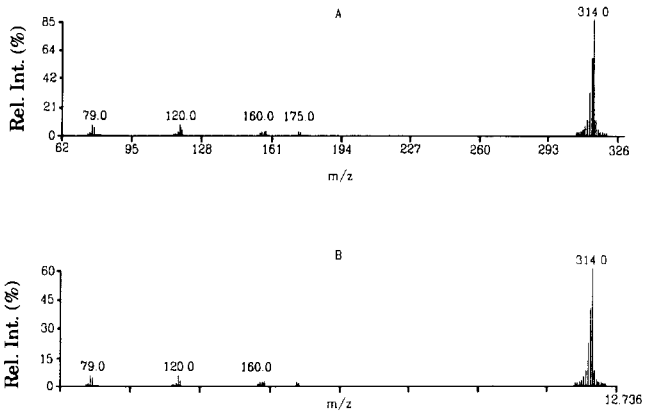

Figure 3. Mass spectra of (A) standard of ES and (B) a groundwater sample.

Table III. Reproducibility of the ES HPLC Method for Six Groundwater Samples

groundwater sample	mean values, ng/mL		% CV	% CV
	ES (intra)[a]	ES (inter)[b]	(intra)[a]	(inter)[b]
1	74	70	7.8	10
2	21	23	3.4	10
3	4	5	6.3	22
4	20	19	9.6	8.2
5	39	41	3.9	9.2
6	44	40	4.3	9.3

[a] Means and percent coefficients of variation of the intraassay were based on five determinations done the same day. [b] Means and percent coefficients of variation of the interassay were based on six determinations done on six different days.

taken of each ES peak at the up slope, pinnacle, and down slope using a photodiode array system. All spectra—with the exception of the two negative surface water samples—indicated that the remaining nine samples contained ES, and each peak was free of interfering substances. Second, as ultimate confirmation, LC/MS/MS analysis was done on 6 of the 11 samples (all of the groundwater samples). These six samples were shown to contain a molecular ion at 314 *m/e* in the negative mode with fragment ions at 160, 120, and 79 *m/e*, which was expected for ES. Typical MS/MS scans of the ES standard and a water sample are shown in Figure 3.

To determine if the reproducibility of the method was adequate, the six groundwater samples were analyzed five times in a single day and six times in 6 days. Results are given in Table III. The intraassay data yielded % CVs from 3.4 to 9.6 and interassay values from 8.2 to 22. The interassay data were not as consistent as the intraassay results, but the precision was good, especially if one considers that the 4 ng/mL sample had the highest % CV (22) and that five of the six samples analyzed by this technique had combined inter- and intraassay % CVs of 10% or less.

Other metabolites of alachlor [2-hydroxy-2′,6′-diethylacetanilide, 2-chloro-2′,6′-diethylacetanilide, and [(2,6-diethylphenyl)(methoxymethyl)amino]oxoacetic acid] were chromatographed on the HPLC system. All of these other alachlor metabolites had retention times either shorter or longer than ES. Thus, it would be possible to look for other metabolites in water using this HPLC system. Alachlor elutes from this system at a retention time of 26 min as a broad peak with poor symmetry.

Further immunoassay and HPLC data are being collected on these and additional water samples from the midwest. Preliminary correlation work suggests good agreement between both methods when ES is used as the standard for immunoassay analysis in place of alachlor. The immunoassay results will be detailed in another paper.

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