Rapid Method for Analysis of Atrazine and Acetanilide Herbicides in Groundwater by Micro Liquid/Liquid Extraction

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Performance of a technique combining micro liquid/liquid extraction and gas chromatography with nitrogen-selective detection (thermionic ionization detector) is reported. Compounds investigated included atrazine, propachlor, butachlor, metolachlor, alachlor, and two alachlor degradation products, 2′,6′-diethylaniline and 2-chloro-2′,6′-diethylacetanilide. The method was found to be rapid, inexpensive, sensitive, and reproducible. It was applied to groundwater samples collected from monitoring wells installed in an agricultural field. Alachlor, metolachlor, and atrazine were detected in the 0.1–2.5 μg/L range. The concentrations of target alachlor degradation products were below detectable levels of 0.1 μg/L.

INTRODUCTION

The herbicides atrazine, alachlor, metolachlor, butachlor, and propachlor are widely used for pre-emergent weed control in corn, soybeans, and other field crops. In recent years, concern has arisen regarding groundwater contamination from their normal agricultural use. Alachlor, in particular, has been targeted. The parent compound has been detected in groundwater and is classed as a probable human carcinogen by the U.S. EPA (U.S. EPA, 1990; Ritter, 1990). On the basis of the recently completed National Survey of Pesticides in Drinking Water Wells (NPS), the agency concluded that alachlor may be detected above an analytical detection limit of 0.5 μg/L in 0.03% of rural domestic wells (U.S. EPA, 1990).

Various alachlor environmental degradation products including 2′,6′-diethylaniline (2′,6′-DEA), 2-chloro-2′,6′-diethylacetanilide, and 2-hydroxy-2′,6′-diethylacetanilide can also be expected to leach to groundwater. For example, Alhajjar et al. (1990) reported detection of 8–12 alachlor degradation products in leachate collected from soil microcosm lysimeters. Specific compounds were not identified, although reported degradation pathways indicate that the presence of 2′,6′-DEA in leachate was likely (Tiedje and Hagedorn, 1975). This compound has been shown to be a promutagen in animal and microbial based bioassays (Kimmel et al., 1986; Brown et al., 1988). To date, there has been only one reported effort to monitor 2′,6′-DEA in groundwater (Pereira et al., 1990).

The later investigation, like most which have involved monitoring of herbicide residues in groundwater, used a traditional analytical approach, namely macro liquid/liquid extraction (MLLE). It was combined with gas chromatography using elemental specific or mass spectrometric detection. Use of MLLE techniques in the analysis of herbicides and related degradation products in water has been shown to be effective. For example, Munch et al. (1990), in their summary of performance of MLLE-based methods used in the NPS, reported recoveries of atrazine, alachlor, butachlor, metolachlor, and propachlor from fortified reagent water samples in the 79–120% range and minimum detection limits of 0.2–0.4 μg/L. This level of performance is generally acceptable in light of drinking water standards and other considerations (U.S. EPA, 1990).

MLLE methods, however, have many limitations. They include high manpower and equipment requirements, susceptibility to interferences from the concentration of large volumes of solvent, the potential for analyte loss during solvent concentration, and relatively high cost per unit analysis. Solvent contamination of laboratory atmospheres and analyst exposure to halogenated solvents also present problems.

Given these limitations, many investigators have turned to "solid-phase" extraction (SPE) methods. Reported recoveries and detection limits for alachlor, metolachlor, and related compounds are generally comparable to MLLE methods (Bagnati et al., 1988; Junk and Richard, 1988; Brooks et al., 1989; Nash, 1990). Advantages of SPE include its speed, lower requirements for solvents and related equipment, and lower cost per unit analysis (Wells and Michael, 1987; Nash, 1990). Nash (1990) has reported that a single analyst may process over 48 water samples a day for the analysis of residues of five nitrogen-containing pesticides and herbicides. This is well in excess of the number of samples that can be processed by MLLE. A potentially serious limitation of SPE is that interferences may be derived from sorbents or sorbent containers (Junk et al., 1988; Brooks et al., 1989).

In our research, which addresses the fate and transport of acetanilide herbicides in soil and groundwater, we chose to evaluate an alternative technique, "micro liquid/liquid extraction" (MCLLE). Grob et al. (1975), Blanchet (1979), Murray (1979), Glaze and Lin (1984), and Thielen et al. (1987) have reported successful applications of this approach to the gas chromatographic analysis of a wide range of organic compounds in water. It is also the basis of EPA drinking water analysis Methods 505 and 504 (U.S. EPA, 1988) and NPS Method 7 (Munch et al., 1990). Method 505 targets alachlor and atrazine.

Choice of MCLLE was based on the need for a simple, inexpensive, yet sensitive technique for herbicide residue extraction and enrichment which was suitable for sample volumes of 100 mL or less Method performance is described in this paper. The method involves a one-step extraction of water samples (60 mL) with *n*-hexane (1 mL)

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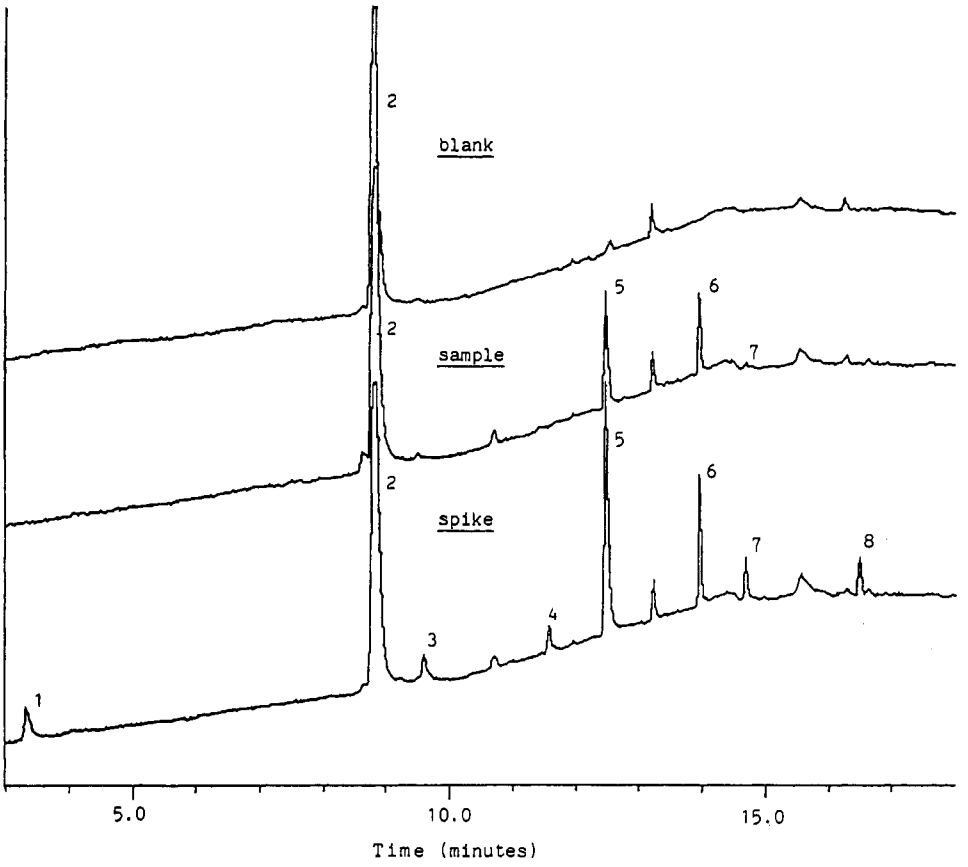

Figure 1. GC/NPD chromatograms of MCCLE extracts of a groundwater sample, 1 μg/L overspike, and a reagent blank. Peaks are labeled as follows: 1, 2′,6′-DEA; 2, 2-chlorolepidine; 3, propachlor; 4, 2-chloro-2′,6′-diethylacetanilide; 5, atrazine; 6, alachlor; 7, metolachlor; 8, butachlor.

followed by direct analysis of extracts using capillary gas chromatography with nitrogen-phosphorous detection (GC/NPD) or combined gas chromatography/mass spectrometry (GC/MS). Method detection limits, precision, and accuracy were found to be comparable to those of more time-consuming and expensive methods.

PROCEDURE

Reagents and Materials. Herbicides were obtained from the U.S. EPA Pesticides and Industrial Chemicals Repository (Research Triangle Park, NC). The 2-chlorolepidine (internal standard) and 2′,6′-DEA were purchased from Aldrich Chemical Co. (Milwaukee, WI). "Distilled in glass" hexane was from Burdick and Jackson (Muskegon, MI) and certified ACS sodium chloride from Fisher Scientific (Medford, MA). The 2-chloro-2′,6′-diethylacetanilide was prepared by hydrolysis of alachlor in 5 N HCl and purified by successive recrystallizations from acetone/water. Purity as defined by combined gas chromatography/mass spectrometry was greater than 99%.

Sample Extraction. Extraction and sample collection vessels were 60-mL serum bottles (Wheaton, Millville, NJ) which were soap and water washed and solvent rinsed with acetone and dichloromethane. Sodium chloride (14 g) was weighed into bottles prior to filling completely with soil column leachate, groundwater samples, or distilled deionized water. Crimp seals were then applied using aluminum rings and Teflon-faced silicone rubber septa (Wheaton No. 224173). Bottle volumes, determined by weighing before and after the bottles were completely filled with water, were found to be within 1% relative standard deviation of 60 mL.

Prior to extraction, bottles were brought to room temperature (ca. 25 °C). Subsequent operations performed through the septa using gastight syringes included removal of 1.5 mL of water and injection of 1 mL of hexane containing 0.5 μg of the internal standard. Simultaneously puncturing septa with a 20-gauge syringe needle allowed maintenance of atmospheric pressure in the bottles.

Following hexane addition, bottles were mixed for 1 min using a vortex mixer (VWR Model K-550-G) operated at its highest setting. After mixing, they were stored at room temperature in the dark until the hexane had separated. Typically, this occurred in less than 1 h. Crimp seals were then pried off with a screwdriver and hexane was transferred to 1-mL autosampler vials containing 100-μL glass inserts (Sun Brokers, Wilmington, NC) with Pasteur pipets. Vials were crimp-sealed with Teflon-faced red rubber septa (Hewlett-Packard, Avondale, PA).

GC/NPD Analysis. Extracts were analyzed using a Hewlett-Packard Model 5890 gas chromatograph, equipped with a "nitrogen–phosphorus" detector, a Model 7673A autosampler and a 15 m × 0.25 mm (i.d.) DB-17 (0.25-μm film) fused silica capillary column (J&W Scientific, Folsom, CA). Helium carrier gas was maintained at 125 kPa at the column inlet. Three-microliter splitless injections were made with the injector maintained at 250 °C. The oven temperature program was 1-min initial hold at 120 °C, 4 °C/min to 156 °C, 12 °C/min to 205 °C, final hold at 205 °C for 4 min. Gas flows to the detector were hydrogen at 3.5, air at 126, and nitrogen makeup at 30 mL/min. Detector output was monitored and data were processed with a Spectra Physics Model 4290 integrator and Chromstation AT Autolab Software operated on an IBM personal computer. The gas chromatographic conditions reported yield "base line" separation of each compound (see Figure 1).

GC/MS Analysis. Analyte identity in groundwater samples was confirmed by gas chromatography/mass spectrometry with a Hewlett-Packard Model 5985B GC/MS system. Chromatographic conditions were a 60 m × 0.32 mm (i.d.) DB-5 (0.25-μm film) fused silica capillary column programmed linearly from 80 to 260 °C at 8 °C/min with a 4-min final hold at 260 °C. Helium carrier gas was maintained at a head pressure of 101 kPa and 3-μL splitless injections were made at 250 °C. The capillary column was interfaced to the mass spectrometer with an SGE open/split interface (Scientific Glass and Engineering, Austin, TX). Electron impact ionization was at 70 eV with selected ion monitoring of ions 134.1 (2′,6′-diethylaniline), 177.1 (2-chlorolepidine), 120.1 (propachlor), 160.2 (alachlor), 162.2 (metolachlor),

Table I. Linear Regression Parameters: Internal vs External Standardization[a-c]

compd	std type	r^2	SE of concn estimate
2',6'-diethylaniline	int[d]	0.999	0.74
	ext[e]	0.997	1.93
propachlor	int	0.999	0.66
	ext	0.997	1.89
2-chloro-2',6'-diethylacetanilide	int	0.999	0.47
	ext	0.996	2.11
atrazine	int	0.999	0.78
	ext	0.996	2.17
alachlor	int	0.999	0.59
	ext	0.997	1.79
metolachlor	int	0.999	0.74
	ext	0.997	1.76
butachlor	int	0.999	1.00
	ext	0.997	1.73

[a] Linear least-squares regression, concentration vs detector response. [b] "Internal" standard computations used the relative detector response of the compound and the internal standard. [c] "External" standard computations used the raw intergrated area counts for each peak. [d] int, internal. [e] ext, external.

and 176.2 (butachlor, 2-chloro-2',6'-diethylacetanilide). Pairs of ions were monitored sequentially at 5 scans/s according to elution sequence. Mass spectrometer tune parameters were optimized with perfluorotributylamine (PFTBA).

RESULTS AND DISCUSSION

Method Calibration. Four replicates each of fortified distilled deionized water at 0.5, 1, 5, 10, 50, and 100 μg/L per component were prepared and analyzed. The herbicides were spiked into the water using acetone as a "carrier". The final concentration of the acetone in the water was 0.07% v/v. Corresponding "standards" to each nominal herbicide concentration in water were prepared by spiking 1 mL of the hexane extracting solution with an equivalent mass of the analytes dissolved in acetone.

Partitioning of each analyte into the hexane was evaluated by computing the ratio of the normalized (to 2-chlorolepidine) detector response obtained for a water extract and its corresponding standard. Values obtained translated to recovery of 45.7–73.8% of the compounds in the extracting solvent. Relative standard deviations of these values were 4.7–9.3% over the entire concentration range.

Partition coefficients computed from these data ranged from 49.3 to 165. Estimated method detection limits based on these values and an instrumental detection limit of 20 pg/component were 0.1–0.2 μg/L.

In general, partitioning into the hexane increased with decreasing aqueous solubility of the compounds with the exception of atrazine and butachlor. This observation is explainable for the atrazine due to its relatively poor solubility in hexane. A possible explanation of the butachlor behavior is "salting-in", i.e., lower partition coefficient at higher ionic strength. In companion experiments, partitioning of butachlor from distilled deionized water into hexane was observed to be slightly greater than from 4 M NaCl.

Method linearity was demonstrated by regression parameters shown in Table I. The data also showed that enhanced precision was obtained with the internal standard approach. For example, the 95% confidence interval of the regression line at the mean detector response for standards at 1.0 μg/L spanned 0.6–1.4 μg/L for internal and 0–2 μg/L for external standards.

Relative response factors (RRF) to the internal standard, computed as the ratios of detector responses per unit mass of compounds injected, were 0.54–0.81 for the acetanilides and 2',6'-DEA. The RRF for atrazine was 3.08, reflecting its increased nitrogen content.

Table II. Recovery of Herbicides and Degradation Products from Fortified Groundwater Samples[a,b]

	MCLLE[c]		SPE[d]		MLLE[e]	
compd	mean	RSD	mean	RSD	mean	RSD
2',6'-diethylaniline	92	8.1			40.2	11.2
propachlor	116	7.4				
2-chloro-2',6'-diethylacetanilide	94	8.2				
atrazine	89	5.7	91.1	7.3	117	11.9
alachlor	98	9.5	89.9	7.8	68.1	7.9
metolachlor	96	33			93.5	8.4
butachlor	92	14				

[a] Mean percent recovery and percent relative standard deviation (RSD). [b] Groundwater samples fortified at the 0.5–1 part per billion level. [c] MCLLE (micro liquid/liquid extraction) from the current work. [d] SPE (solid-phase extraction data from Nash (1990). [e] MLLE [macro liquid/liquid extraction data from Pereira et al. (1990)].

Method Application. The method was applied to the analysis of five groundwater samples collected in September 1990 from monitoring wells installed in an agricultural field located in the Connecticut River Valley region of Massachusetts. Site conditions and sample collection techniques have been described by Jenkins et al. (1988). Corresponding aliquots of each well sample with and without fortification at the 1 μg/L level with each compound were analyzed. Note that although some of the samples analyzed did contain suspended particulates in the form of fine sand and silt, emulsion formation during extraction was not a problem.

The GC/NPD chromatograms obtained from one of the samples, an overspike at 1 μg/L per component, and a reagent blank are shown in Figure 1. Table II provides summary statistics for results. In this case, percent recovery was computed on the basis of the difference in measured concentration of each analyte in each sample and corresponding "spikes". Values are expressed as relative percent.

Accuracy of the MCLLE method was reflected in the nearly quantitative spike recoveries (89–116%) obtained for all compounds. In addition, the relatively low RSD values indicated excellent precision. The exception was metolachlor. Its RSD was nearly 3 times the values found for other compounds. The result is attributable to data obtained for two of the five samples studied. One sample gave 40% while the other 139% recovery. Others were in the 90–110% range. We do not have an explanation for the metolachlor behavior and therefore attribute it to a "matrix" effect.

The analytes detected in the groundwater samples included alachlor (0.1–1.2 μg/L), atrazine (0.1–0.6 μg/L), and metolachlor (0.1–2.5 μg/L). The target alachlor degradation products were not detected in these analyses at an estimated detection limit of 0.1 μg/L. All results were confirmed by GC/MS analysis.

CONCLUSIONS

Results have shown that one-step MCLLE of 60-mL water samples with 1 mL of hexane combined with GC/NPD or GC/MS is a rapid, simple, and inexpensive method for herbicide residue analysis in water. As indicated by the Table II data, accuracy and precision of this MCLLE method were found to compare favorably with data reported for SPE-based (Nash, 1990) and MLLE-based methods (Pereira et al., 1990).

Significant advantages of the MCLLE method are that inexpensive glass serum bottles can be used for sample collection and extraction, reagents and disposable materials cost less than $2.00 per analysis, and in excess of 40 samples can be processed in a single day by an experienced analyst. Another feature of the method is that it can be applied under field conditions and addition of salt and/or complete field extraction will preserve samples for subsequent laboratory analysis (Blanchet, 1979).

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Registry No. Water, 7732-18-5; atrazine, 1912-24-9; propachlor, 1918-16-7; butachlor, 23184-66-9; metolachlor, 51218-45-2; alachlor, 15972-60-8; 2′,6′-diethylaniline, 579-66-8; 2-chloro-2′,6′-diethylacetanilide, 6967-29-9.