

Utilization of Solid-Phase Extraction and Reversed-Phase and Ion-Pair Chromatography in the Analysis of Seven Agrochemicals in Water

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Six herbicides (2,4-D, 2,4-DP, 2,4-DIOE, 2,4-DPBEE, dicamba, pendimethalin) and the insecticide chlorpyrifos were solid-phase (C_8) extracted from 100 mL of water (adjusted to pH 2.2) that was collected from turfgrass run-off test plots. 2,4-D, 2,4-DP, and dicamba were analyzed by ion-pair HPLC utilizing a C_{18} column, a mobile phase of methanol-water (0.005 M octyltriethylammonium phosphate) (55:45) and UV detection at 230 nm. Pendimethalin, chlorpyrifos, and the two esters 2,4-DIOE and 2,4-DPBEE were quantified with HPLC utilizing a C_{18} column, methanol-water (82:18) mobile phase, and UV detection at 230 nm. The minimum detection limit per 25- μ L injection was 4-33.3 ng of chemical, which corresponds to a concentration of 2.4-20.0 ppb. Average overall recoveries: dicamba, 82.2%; 2,4-D, 93.8%; 2,4-DP, 103.0%; chlorpyrifos, 93.7%; 2,4-DPBEE, 100.0%; 2,4-DIOE, 92.0%; pendimethalin, 91.5%.

Increasing public concern for agrochemicals and their potential movement in the ecosystem demands that we more effectively document agrochemical residues in our environment and especially in our surface water and groundwater. To increase our basic data set, we must significantly increase the number of samples analyzed. Unfortunately, classical analytical procedures that use solvent partitions are time-consuming and costly. We must develop less costly alternative procedures to handle the future demands. One such alternative is the increased use of solid-phase extractions (SPE) to replace the time-consuming liquid-liquid extractions performed in separatory funnels, which often require substantial amounts of costly, highly pure, environmentally sensitive solvents. SPE columns are now receiving considerable acceptance (Anonymous, 1983; Bogus et al., 1985; Arjmand et al., 1988), and they will probably see an expanded use considering the wide variety of solid-phase supports becoming available. We now report the development of an analytical method utilizing C_8 SPE and C_{18} reversed-phase and ion-pair (Perry et al., 1984) high-performance liquid chromatography in the analysis of seven agrochemicals in surface and leached water derived from commercial application of pesticides to turfgrass run-off test plots.

EXPERIMENTAL SECTION

Chemicals. (2,4-Dichlorophenoxy)acetic acid (2,4-D), 2-(2,4-dichlorophenoxy)propionic acid (2,4-DP), the isooctyl ester of 2,4-D (2,4-DIOE), and *O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate (chlorpyrifos) were obtained from EPA analytical reference standards; 3,6-dichloro-2-methoxybenzoic acid (dicamba) was from Velsicol Chemical Co., Chicago, IL; the butoxyethanol ester of 2,4-DP (2,4-DPBEE) was from Union Carbide, Research Triangle Park, NC; and *N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitroaniline (pendimethalin) was from American Cyanamid Co., Princeton, NJ. Hexyl-, heptyl-, octyl-, and dodecyltriethylammonium phosphates (Q6, Q7, Q8, and Q12 reagents) and tetrabutylammonium phosphate were obtained from Regis Chemical Co.

HPLC Conditions. A Waters Associates, Inc., Model ALC/GPC 244 high-performance liquid chromatograph equipped with

Table I. Percent Elution from C_{18} and C_8 SPE Columns

chemical	av % elution from SPE columns ^a	
	C_{18}	C_8
2,4-D	95 ± 5.3	95 ± 9.4
2,4-DP	98 ± 5.6	95 ± 8.9
2,4-DIOE	18 ± 18.9	87 ± 16.7
2,4-DP BEE	22 ± 22.0	93 ± 15.0
dicamba	72 ± 3.3	74 ± 11.4
pendimethalin	95 ± 8.9	95 ± 8.5
chlorpyrifos	95 ± 13.3	85 ± 10.8

^a Average of five repetitions ± SD.

Model 6000A pumps, a WISP 710B automatic injector (250- μ L loop), a Lambda Max 481 variable-wavelength UV detector, and a Shimadzu Model 3A integrator-recorder were used. The metal column (4.6 mm × 25 cm) contained a 5- μ m C_{18} -bonded phase (Supelco, Inc., Bellefonte, PA) and was preceded with a 2-cm guard column packed with 37-75- μ m Porasil B (Waters Associates). The mobile phase, for separation of the neutral molecules, was methanol-water (82:18) and for separation of the acids was methanol-0.005 M Q8 in water (55:45) at a flow rate of 1 mL/min. The detector was operated at 230 nm, and typically a 20-25- μ L aliquot of a 2-mL sample was used for analysis.

Sample Extraction. C_8 -SPE columns were activated by first passing 10 mL of methanol and then 10 mL of water through the column. Acidified (12 N HCl, pH 2.2) and 0.45- μ m membrane filtered water samples (100 mL) were placed in 125-mL Erlenmeyer flasks, and the water was then suction-filtered (5-6 mL/min) through the activated C_8 -SPE column with a vacuum manifold (Model 5-7030, Supelco) (Bogus et al., 1985). The SPE columns were then removed from the extraction apparatus and dried by positioning them vertically, with the outlet end down, on a vacuum source (20 mmHg) and by passing air through them for 30 min. The SPE columns were then eluted four times with 0.5 mL (5-6 mL/min) of methanol into a 10-mL centrifuge tube. The final volume was adjusted to 2 mL of methanol and transferred to a 4-mL vial prior to analysis.

For adsorption and elution studies, standard solutions (10 and 100 ppb) of each agrochemical in water were prepared. Initially, the agrochemicals were dissolved in methanol, and the appropriate aliquots were introduced to deionized water and to field-collected water to give the desired concentration. Field samples were collected from treated turfgrass run-off plots after irrigation.

RESULTS AND DISCUSSION

C_{18} SPE columns were first examined for their capability to extract the seven agrochemicals from 100 mL of

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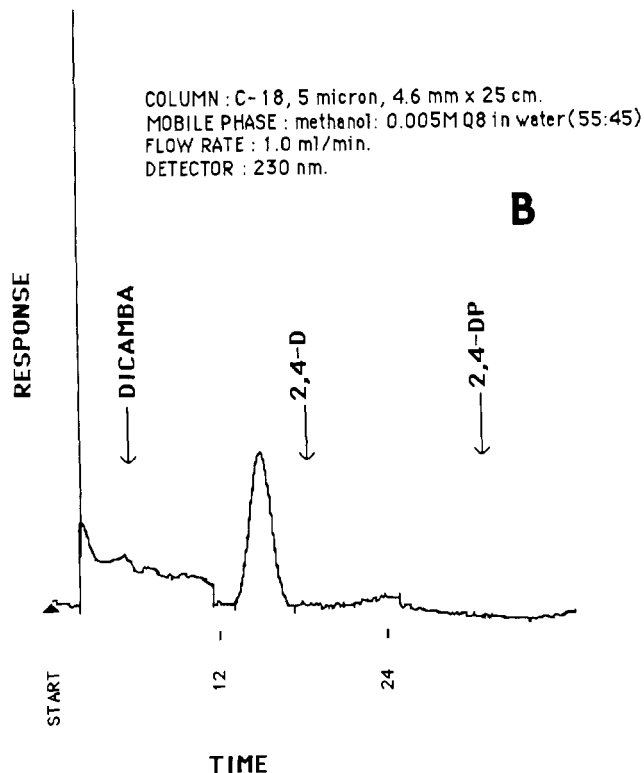
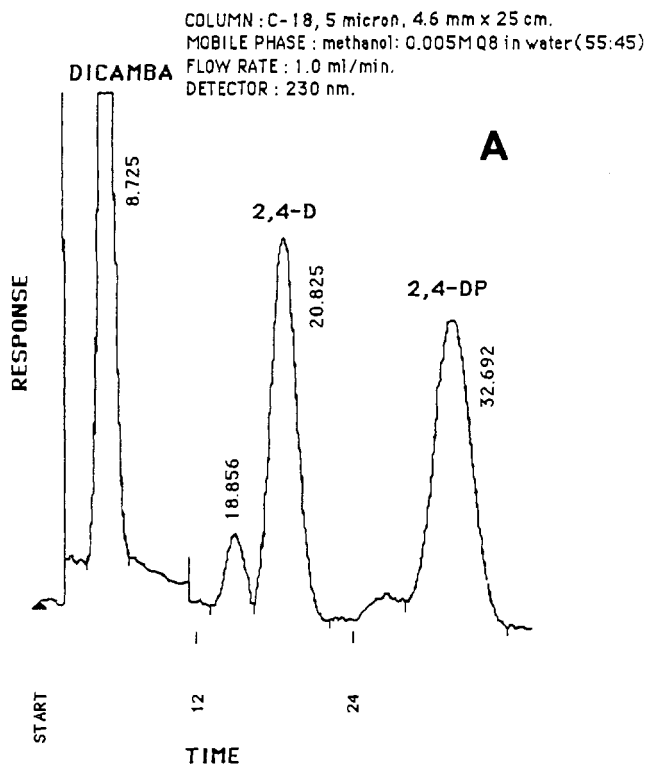


Figure 1. HPLC utilizing ion-pair techniques: A, separation of dicamba, 2,4-D, and 2,4-DP when spiked in field-collected water; B, HPLC analysis of field-collected water.

Table II. Overall Recoveries from Analysis of Spiked Field-Collected Water

chemical	no. of rep	av rec, ^a %	detection limit (230 nm), ppb	detector sensitivity, ng
2,4-D	14	93.8 ± 10.2	6.0	10.0
2,4-DP	14	103.0 ± 10.2	6.0	10.0
2,4-D IOE	14	92.0 ± 17.9	20.0	33.3
2,4-DP BEE	11	100.0 ± 10.9	10.0	16.7
dicamba	14	82.2 ± 11.6	2.4	4.0
pendimethalin	10	91.5 ± 11.6	5.0	8.0
chlorpyrifos	14	93.7 ± 13.6	5.0	8.0

^a ±SD.

Table III. Selected Analysis of Field-Collected Water from Turfgrass Plots^a

sample source	ppb						
	dicamba	2,4-D	2,4-DP	Chlor	Pendi	BEE	IOE
	Run-Off						
Sept 8	7.0	56.0	47.3	<	<	<	< ^b
Oct 24	40.3	353.8	217.4	<	<	<	<
Dec 4	<	<	<	<	<	<	<
	Lysimeter						
Sept 8	9.6	50.0	58.3	<	<	<	<
Oct 24	27.0	226.9	264.0	<	<	<	<
Nov 10	11.3	<	<	<	<	<	<
Dec 5	<	<	<	<	<	<	<

^a Application of pesticides on Aug 2 and Oct 21, 1986. In order to obtain water samples, plots were irrigated with 6 in. of water/h.
^b Less than the limits of detection.

water. As expected, the acids would not adsorb unless they were protonated, so all subsequent experiments were conducted with water acidified to pH 2.2 with concentrated hydrochloric acid. Table I shows the relative recoveries (adsorption, elution) for the seven agrochemicals with the C₁₈ SPE. Note that with both esters, 2,4-DIOE and 2,4-DPBEE, poor recoveries were obtained, 18 and 22%, respectively. These esters did adsorb but did not

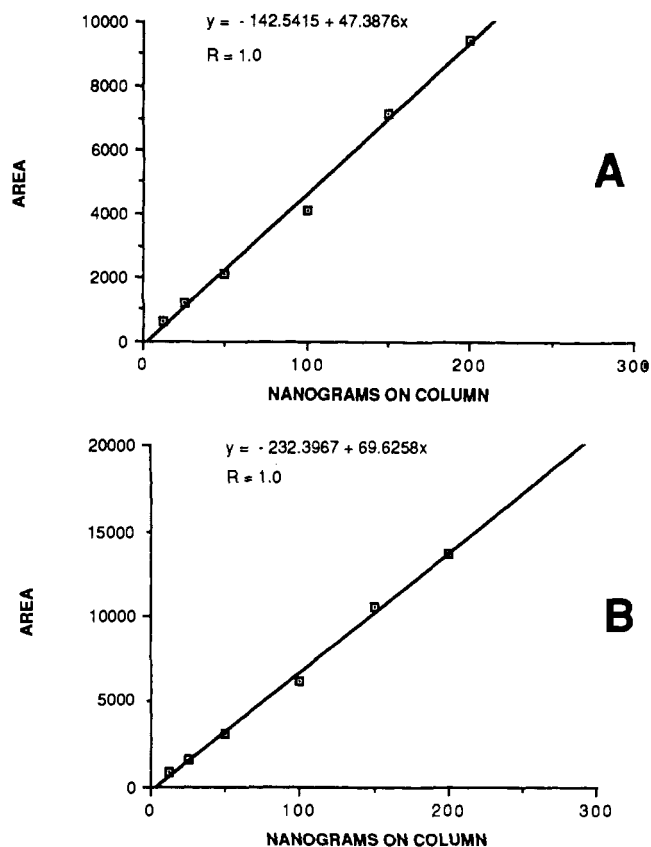


Figure 2. Standard curve for 2,4-D (A) and dicamba (B).

elute from the C₁₈ SPE columns. We evaluated three different C₁₈ SPE columns (Waters Associates, Supelco, and J. T. Baker Chemical Co.), and all three behaved in the same manner. Realizing that the esters were too strongly adsorbed, we evaluated the use of C₈ SPE columns and the results are also shown in Table I. All seven agrochemicals gave good overall recoveries for the adsorp-

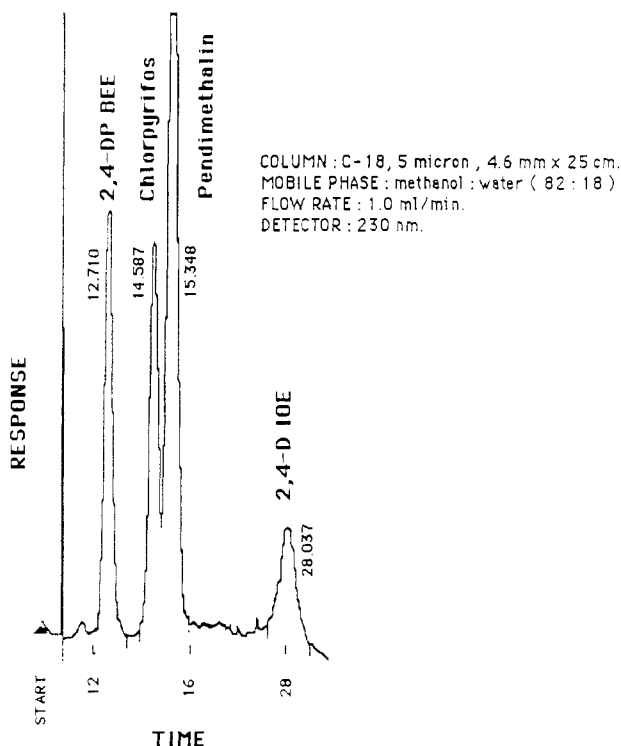


Figure 3. HPLC of 2,4-DPBEE, chlorpyrifos, pendimethalin, and 2,4-DIOE.

tion and elution experiment. The esters were readily removed from the C_8 SPE columns with methanol, in contrast to the results obtained with the C_{18} SPE support. Dicamba recoveries are a little low (74%), but we did not want to make the aqueous solution any more acidic for fear of hydrolysis of the esters and alteration of the adsorptive properties of the SPE columns.

We were not able to separate all seven agrochemicals utilizing one HPLC column and an isocratic solvent system. When ion-pair reversed-phase conditions were utilized, however, the acids separated nicely. Figure 1A shows the separation of the three acids when spiked in field-collected water. The sample contains an unknown peak at 18.8 min, but this material was also in the field-collected water from untreated plots (Figure 1B). Tetrabutylammonium phosphate and Q6 and Q7 reagents did not have dicamba far enough away from the origin. Field samples often contained interfering substances eluting close to the origin. A mobile phase containing Q12 gave good separations, but dicamba took nearly 1 h to elute; consequently, the Q8 ion-pair reagent gave the best separation of those ion-pair reagents tested. Figure 2 shows the standard curves for 2,4-D and dicamba when measured at 230 nm. The two curves are typical of the standard curves for all seven pesticides, which all possessed a coefficient of regression (R) of >0.98 .

2,4-DIOE, 2,4-DPBEE, pendimethalin, and chlorpyrifos separated nicely on a 5- μ m C_{18} bonded-phase (Supelco) column with a mobile phase of methanol-water (82:18)

(Figure 3). Surprisingly, there was no problem eluting the esters off this C_{18} column. Evidently the introduction of the esters to the HPLC column in the presence of 82% methanol inhibited strong binding as in the case of the C_{18} SPE column. The chlorpyrifos and pendimethalin did not separate on a C_8 HPLC column, but chlorpyrifos eluted approximately 1.0 min ahead of the pendimethalin on the C_{18} Supelco HPLC column (on all lots tried). It was observed that these two chemicals did not always separate with C_{18} columns from other suppliers.

The overall recoveries and lower limits of detection for the seven pesticides spiked in field-collected water are shown in Table II. The recoveries ranged from 82.2% for dicamba to 103% for 2,4-DP. The standard deviation is somewhat high in all cases ranging from ± 10.2 to 17.9%. The lower limit of detection for dicamba is 4 ng, which corresponds to 2.4 ppb, while the lower limit of detection for 2,4-DIOE is 20 ppb. The lower limiting factor of detection is the interfering background absorbance. Table III shows data from analysis of some selected field-collected samples. Chlorpyrifos, pendimethalin, 2,4-DIOE, and 2,4-DPBEE were not detected in the run-off or lysimeter water. Additionally, the acids were not usually detected, except in samples that were obtained when the turfgrass plots were irrigated with 6 in. of water soon after application. These data will be the subject of a future article.

In conclusion, an analytical procedure has been developed involving SPE extraction and reversed-phase and ion-paired HPLC for residue determination of seven pesticides routinely used in commercial turfgrass pest management practices. This procedure will permit a more thorough study of the potential dangers to urban environments from pest management practices on turf.

Registry No. 2,4-D, 94-75-7; 2,4-DP, 120-36-5; 2,4-DIOE, 53404-02-7; 2,4-DPBEE, 53404-31-2; chlorpyrifos, 2921-88-2; dicamba, 1918-00-9; pendimethalin, 40487-42-1; water, 7732-18-5.

LITERATURE CITED

- Anonymous. *SEP-PAK Cartridge: Application Biography*; Waters Associates: 1983.
- Arjmand, M.; Spittler, T. D.; Mumma, R. O. Analysis of Dicamba from Water Using Solid-Phase Extraction and Ion-Pair High Performance Liquid Chromatography. *J. Agric. Food Chem.* **1988**, *36*, 492-494.
- Bogus, E. R.; Gallagher, P. A.; Cameron, E. A.; Mumma, R. O. Analysis of Pesticide Exposure Pads Using Selective Absorption and Elution of Reversed-Phase Solid Support. *J. Agric. Food Chem.* **1985**, *33*, 1018-1021.
- Perry, J. A.; Glunz, L. J.; Szczerba, T.; Hocson, V. S. Reagents for Ion Pair Reversed-Phase HPLC. *Am. Lab.* **1984**, 114-119.

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