Analysis of Dicamba from Water Using Solid-Phase Extraction and Ion-Pair High-Performance Liquid Chromatography

Masood Arjmand, Terry D. Spittler, and Ralph O. Mumma*

Dicamba (3,6-dichloro-2-methoxybenzoic acid) is a postemergence herbicide used for control of broad-leaf weeds and is traditionally analyzed by extraction from acidic aqueous solutions with organic solvents, subsequent methylation, and analysis by gas chromatography. A simple, inexpensive, procedure for analysis of dicamba and its metabolite (3,6-dichlorosalicylic acid) from water has been developed involving solid-phase extraction with amino ion-exchange columns, elution with a salt solution, and subsequent analysis with HPLC utilizing ion-pair elution techniques on reversed-phase columns. The procedure is sensitive (1.6 ppb) and applicable to multiple samples, eliminates the need for costly and environmentally hazardous solvents, and does not require any solvent evaporation steps.

Dicamba (3,6-dichloro-2-methoxybenzoic acid) is a postemergence herbicide used for the control of broad-leaf weeds in cereals and grasslands. Established methods of dicamba analysis involve its extraction from acidic aqueous media with diethyl ether followed by derivatization and gas chromatographic analysis (Devine and Zweig, 1969; Garbrecht, 1970; Chau and Terry, 1976; Agemian and Chau, 1977; Lopez-Avila et al., 1986). However, these procedures are time-consuming and require expensive pesticide-grade solvents. The purpose of this study was to develop a fast, economical method not requiring expensive and environmentally hazardous solvents, suitable for the analysis of a large number of water samples containing dicamba and its 3,6-dichlorosalicyclic acid metabolite (DSA). Solid-phase extraction techniques were evaluated for concentrating the chemicals, and high-performance liquid chromatographic techniques were investigated for quantification.

EXPERIMENTAL SECTION

Chemicals. Dicamba and DSA analytical standards were obtained from Velsicol Chemical Corp. Amino (NH_2) columns (Baker 10 SPE, 3 mL) were purchased from VWR Scientific. All solvents were of analytical grade.

HPLC Conditions. A Waters Associates, Inc., Model ALC/GPC 244 high-pressure liquid chromatograph equipped with Model 6000 A pumps, a WISP 710B automatic injector, a Lambda Max 480 variable-wavelength UV detector, and a Data Module integrator–recorder was used. The metal column (25 cm × 4 mm, i.d.) contained a 5-μm C_{18} bonded phase (Supelco, Inc., Bellefonte, PA) and was preceded with a 2-cm guard column packed with 37–75-μm Porasil B (Waters Associates, Inc.). The mobile phase was methanol/water of varying proportions plus 0.005 M tetrabutylammonium phosphate (low UV PIC reagent A, Waters Associates) at a flow rate of 1 mL/min. The detector was set at 210 nm. Typically a 25-μL aliquot of a 2-mL sample was used for analysis.

Sample Extraction. Amino (NH_2) columns were conditioned and activated by passing 3 mL of 1 N HNO_3 and then 3 mL of deionized water (Milli-Q Water Purification System, Millipore Corp., Bedford, MA) through the column, taking care that the columns never went dry. Water samples (100 mL) were placed in 125-mL Erlenmeyer flasks, and the water was then suction-filtered through the NH_2 columns using a vacuum manifold (Model 5-7030, Supelco, Inc; Bogus et al., 1985). The columns were removed from the extraction apparatus and dried by positioning them vertically with the outlet end down on a vacuum source (20 mmHg) and passing air through them over a 30-min period. NH_2 columns were then removed from the vacuum and rinsed with 2 mL of methanol, dried for 30 min using the vacuum source, and then eluted with 2 mL of either 1 N NaCl or 1M K_2HPO_4 solution. C_{18} SEP-PAK cartridges (Waters Associates, Inc.) were also used and were conditioned by first passing 5 mL of methanol and then 5 mL of water through them. Extraction of the samples were the same as that for NH_2 columns, except in this case the aqueous samples were acidified to pH 1 with HCl and the cartridges were eluted with 2 mL of methanol.

For the absorption and elution studies [^{14}C]dicamba and DSA (sp act. 14.3 mCi/mmol) were used. Samples were prepared by introducing ca. 10^5 dpm ^{14}C-labeled material into 100 mL of water and diluting with the appropriate amount of unlabeled dicamba or DSA. Aliquots of water (1 mL) were taken before and after passing the sample through the solid-phase extraction column, and these aliquots were subsequently counted for ^{14}C label. Columns were then eluted with the corresponding eluant and brought to a final volume of 2 mL from which 20-μL fractions were analyzed for radioactivity. A Beckman Model LS 8000 liquid scintillation counter was utilized to determine the amount of radioactivity in the samples.

RESULTS AND DISCUSSION

HPLC offered the potential of direct analysis of dicamba and DSA, without derivatization, and therefore various conditions were evaluated for analysis of these two chemicals. Dicamba and DSA are polar water-soluble molecules that do not adsorb strongly to a reversed-phase support. However, if PIC reagent A (tetrabutylammonium phosphate), an ion-pair reagent, is used along with dicamba and DSA, then good retention and separation of dicamba and DSA can be achieved on a C_{18}-phase HPLC column (Figure 1). DSA formed the double ion-pair in contrast to the single ion-pair formed with dicamba; the two compounds readily separated on HPLC, 16.4 and 7.5 min, respectively. The sensitivity of our system was 2 ng when the ultraviolet detector was operated at 210 nm, which correspond to a lower limit of 1.6 ppb for a normal 25-μL injection from a 2-mL sample derived from 100 mL of water. Figure 2 shows the plot of the detector response versus the amount

The Pesticide Research Laboratory and Graduate Study Center, Department of Entomology, The Pennsylvania State University, University Park, Pennsylvania 16802 (M.A., R.O.M.), and The Analytical Laboratories, Cornell University, Geneva, New York 14456 (T.D.S.).

0021-8561/88/1436-0492$01.50/0

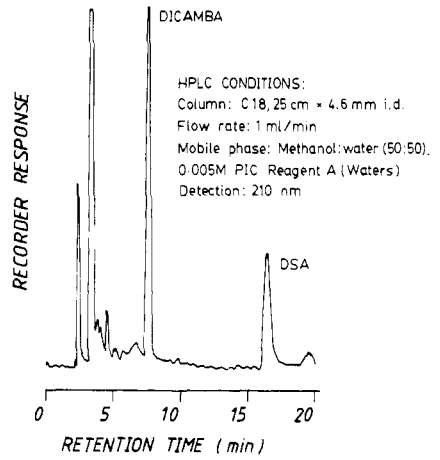

Figure 1. High-performance liquid chromatographic separation of dicamba and DSA utilizing ion-pair techniques.

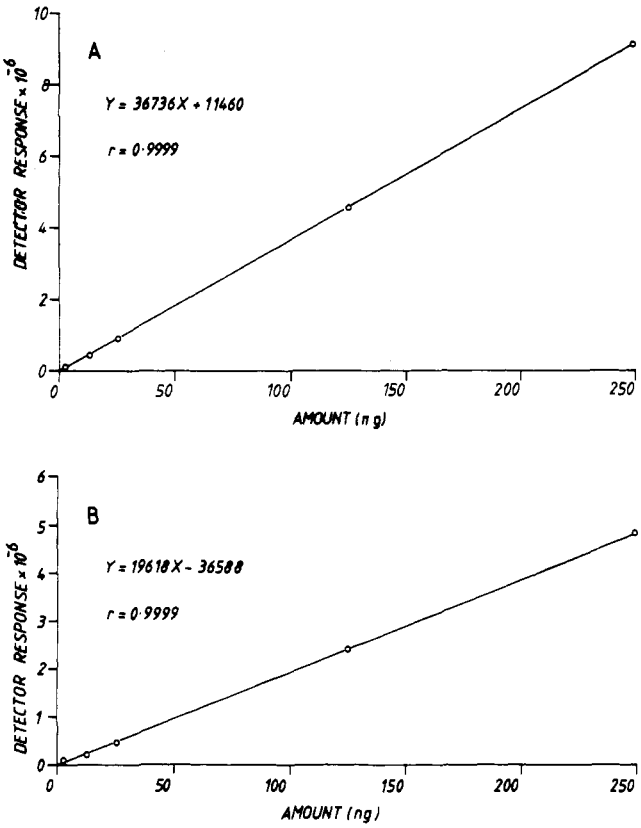

Figure 2. Detector response (210 nm) versus nanograms of dicamba (A) and DSA (B).

of dicamba (A) or DSA (B) over the range of 2.5–250 ng. In each case a linear relationship existed with a correlation coefficient of 0.9999.

The dicamba and DSA eluted from the amino ion-exchange columns with either 2 mL of 1 M NaCl or 1 M K_2HPO_4, and the analysis of the eluant from a dicamba-fortified field water sample is shown in Figure 3. The 1 M K_2HPO_4 buffer caused an increase in background absorbance, so the chromatographic conditions were slightly modified (mobile phase methanol–water (40:60)). This more than doubled the retention time of dicamba, 17.5 min, but this was necessary to reduce the contribution of the phosphate buffer.

Sample Extraction. Table I shows the percentage of the adsorption of 10 ppb and 1 ppm solutions of dicamba and DSA and their subsequent elution from amino columns when 1 N NaCl was used as the eluting solvent.

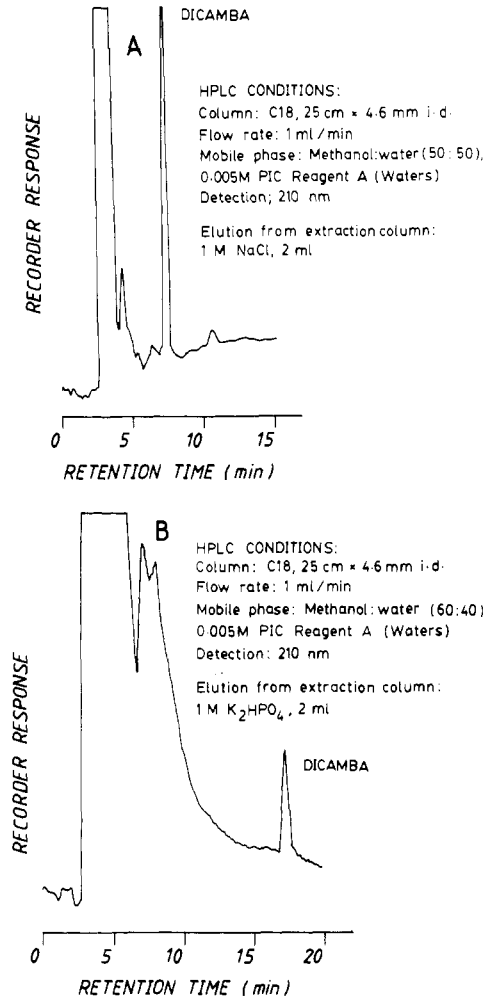

Figure 3. High-performance liquid chromatographic analysis of the 2-mL eluant from the amino ion-exchange columns: A, 1 M NaCl; B, 1 M K_2HPO_4.

Table I. Percentage Adsorption and Elution of Dicamba and DSA from NH_2 Columns When Eluted with 2.0 mL of 1 M NaCl

process	10 ppb dicamba	1 ppm dicamba	10 ppb DSA	1 ppm DSA
adsorption[a]	99.1 ± 0.2[b]	98.6 ± 0.2	98.0 ± 0.8	98.4 ± 0.6
elution	78.2 ± 11.7	82.3 ± 6.2	34.6 ± 7.0	51.1 ± 3.2

[a] Column was conditioned with 1 N HNO_3 prior to adsorption; 100-mL samples of water were used. [b] Data represent the mean of four replications ± SD.

Table II. Percentage Adsorption and Elution of Dicamba and DSA from NH_2 Columns When Eluted with 2 mL of 1 M K_2HPO_4

process	10 ppb dicamba	1 ppm dicamba	10 ppm DSA	1 ppm DSA
adsorption[a]	98.3 ± 0.6[b]	93.3 ± 5.5	97.1 ± 0.9	97.6 ± 0.2
elution	93.9 ± 3.9	92.7 ± 4.3	62.1 ± 2.2	78.6 ± 5.8

[a] Column was conditioned with 1 N HNO_3 prior to adsorption; 100-mL samples of water were used. [b] Data represent the mean of four replications ± SD.

Good adsorption occurred (98.6–99.1%), and dicamba eluted efficiently with 2 mL of 1 M NaCl (78.2–82.3%). DSA also adsorbed well (98.0–98.4%) but did not elute well with the NaCl (34.6–51.1%) These data indicated that the adsorption was complete but the removal of the compounds from the amino column was the limiting factor of recovery. This technique would be very useful if only

Table III. Percentage Adsorption and Elution of Dicamba and DSA from C_{18} Columns When Eluted with 2 mL of Methanol

process	10 ppb dicamba	1 ppm dicamba	10 ppb DSA	1 ppm DSA
adsorption[a]	98.8 ± 0.3[b]	98.3 ± 0.5	95.8 ± 0.2	91.9 ± 0.2
elution	95.5 ± 7.2	98.5 ± 5.2	57.4 ± 18.6	77.3 ± 16.3

[a] Columns were conditioned with methanol prior to adsorption; 100-mL samples of water adjusted to pH 1 were used. [b] Data represent the mean of four replicates ± SD.

dicamba analysis were desired. In order to improve the recovery, different salt solutions were examined. Table II shows the results of the study with the amino columns when 2 mL of 1 M K_2HPO_4 was used as the eluting solvent. Dicamba recovery was 92.7–93.9%. Recovery of DSA ranged from 62.1 to 78.6%, much better than when 1 M NaCl was used.

Table III shows the percentage adsorption and elution of dicamba and DSA from C_{18} SEP-PAK cartridges. Because the pH had to be adjusted to 1 prior to adsorption, some column packing eluted with the samples. C_{18} SEP-PAK cartridges adsorb many organic compounds and thus are not as selective as the ion-exchange cartridges.

HPLC Analysis. The use of the amino ion-exchange column as a means of solid-phase extraction of dicamba and DSA eliminates the normal organic solvent partitioning of the aqueous phase, and subsequent derivatization, which is used with the gas chromatographic procedures, is time-consuming, and requires expensive environmentally hazardous solvents. The use of solid-phase concentrators was adaptable to multiple samples, and we routinely extracted 12 water samples at the same time. We could detect 2 ng of dicamba or DSA with our detector (25-μL injection), which corresponds to 1.6 ppb when 100 mL of water was used. It is a simple procedure; it does not require expensive chemicals other than PIC reagent A and is easily automated. We have used this method for analysis of over 600 water samples.

ACKNOWLEDGMENT

We appreciate the technical support of Edward Bogus, Mariellen Hardy, and Dave Kurtz. Paper No. 7591 in the Journal Series of the Pennsylvania Agricultural Experiment Station. Support in part by NAPIAP, Northeast Region Research Project NE-115, and Regional Research Funds.

Registry No. DSA, 3401-80-7; H_2O, 7732-18-5; dicamba, 1918-00-9.

LITERATURE CITED

Agemian, H.; Chau, A. S. Y. "Analysis of Pesticide Residues by Chemical Derivatization. V. Multiresidue Analysis of Eight Phenoxyalkanoic Acid Herbicides in Natural Waters". *J. Assoc. Off. Anal. Chem.* **1977**, *60*, 1070–1076.

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.

Chau, A. S. Y.; Terry, K. J. "Analysis of Pesticides by Chemical Derivatization. III. Gas Chromatographic Characteristics and Conditions for the Formation of Pentafluorobenzyl Derivatives of Ten Herbicidal Acids". *J. Assoc. Off. Anal. Chem.* **1976**, *59*, 633–636.

Devine, J. M.; Zweig, G. "Note on the Determination of Some Chlorophenoxy Herbicides and Their Esters in Water". *J. Assoc. Off. Anal. Chem.* **1969**, *52*, 187–189.

Garbrecht, T. P. "Rapid Esterification of Dicamba and Chlorophenoxy Acids with N,O-bis(Trimethylsilyl) Acetamide for Gas Chromatographic Analysis". *J. Assoc. Off. Anal. Chem.* **1970**, *53*, 70–73.

Lopez-Avila, V.; Hirata, P.; Kraska, S.; Taylor, J. H., Jr. "Determination of Dicamba and 2,4-D in Water and Soil by Isotope Dilution GC/MS". *J. Agric. Food Chem.* **1986**, *34*, 530–535.

Received for review February 10, 1987. Accepted November 20, 1987.

Extraction of Soluble Dietary Fiber

Hadden Graham,* May-Britt Grön Rydberg, and Per Åman

The influence of extraction conditions on the solubility of dietary fiber was examined in four cereals (wheat, rye, barley, oats) and four vegetables (potato, carrot, lettuce, pea). The extraction conditions examined were (a) pH 5.0 acetate buffer at 96 °C for 1 h and 60 °C for 4 h during starch degradation, (b) water at 38 °C for 2 h, (c) pH 1.5 HCl/KCl buffer at 38 °C for 2 h, and (d) pretreatment with absolute ethanol at 96 °C for 1 h and extraction with water at 38 °C for 2 h. Although extraction at high temperature gave in general the highest values for soluble fiber, and extraction in acidic buffer the lowest, the yield and composition of soluble fiber varied considerably with extraction conditions and food sample. The use of standardized and physiologically more appropriate extraction conditions is proposed.

Many of the beneficial effects of dietary fiber, such as reduced postprandial glucose response and blood cholesterol, have been attributed mainly to the activity of soluble fibers (Jenkins, 1980; Kay and Truswell, 1980). Thus, many methods for determining fiber allow the extraction and subsequent quantification of a soluble component. Such extraction procedures were often designed to fit conveniently into an analytical procedure rather than to correspond to actual physiological conditions and commonly are preceded by the gelatinization of starch at 96 °C for up to 1 h and further incubation at lower temperatures for up to 16 h (Theander and Åman, 1979; Englyst et al., 1982; Asp et al., 1983; Theander and Westerlund, 1986). This led Åman and Graham (1987) to suggest extraction of mixed-linked β-glucans at body temperature

Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, Box 7024, 750 07 Uppsala, Sweden.

0021-8561/88/1436-0494$01.50/0