density polyethylene from solutions containing up to 300 ppb of the compounds. Our data also indicate that bromoxynil octanoate is not adsorbed appreciably by glass, although up to 80% of bromoxynil octanoate in aqueous solution can be adsorbed on polyethylene.

Comments. In summary, we have developed an HPLC method for the direct determination of metribuzin and bromoxynil octanoate and their respective metabolites, DADK and bromoxynil, in runoff water from wheat fields when the water contained 5–200 ppb (μg/L) of the compounds. The compounds are partitioned into dichloromethane/acetonitrile after acidifying the water sample with acetic acid. After the extracts are reduced in volume and dried over anhydrous sodium sulfate, the compounds are separated on a reverse-phase octadecyl column by using an acidified water/methanol gradient and determined with a variable-wavelength detector.

The crucial points in utilizing our method are minimization of exposure of samples containing bromoxynil octanoate to room or elevated temperatures, freezing of field samples as soon as possible, and making sure that the pH of the samples is low enough to inhibit ionization of the free phenolic group on bromoxynil during extraction. If samples are stored in polyethylene and probably other plastic containers, a rinsing step with an organic solvent like dichloromethane must be included to recover bromoxynil octanoate adsorbed on the interior surfaces of the plastic containers.

ACKNOWLEDGMENT

We thank L. I. Butler and C. L. Smithhisler of the Yakima Laboratory for helpful discussions and suggestions, T. J. Olson, Mobay Chemical Corp., and R. R. Romaine, Union Carbide Corp., for providing analytical standards, A. W. Taylor, Agricultural Research Service, Beltsville, MD, for technical discussions, and N. J. Milner of the Yakima Laboratory for technical support.

Registry No. Metribuzin, 21087-64-9; bromoxynil octanoate, 1689-99-2; desaminodiketometribuzin, 52236-30-3; bromoxynil, 1689-84-5; water, 7732-18-5.

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Received for review July 15, 1983. Accepted November 14, 1983. Names of companies or commercial products are given solely for the purpose of providing specific information; their mention does not imply recommendation or endorsement by the U.S. Department of Agriculture over others not mentioned.

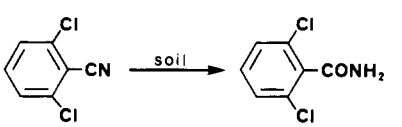
Simultaneous Determination of 2,6-Dichlorobenzonitrile and 2,6-Dichlorobenzamide in Aqueous Samples by a Direct High-Performance Liquid Chromatographic Method

William J. Connick, Jr.,* and Judith M. Bradow

The herbicide dichlobenil (2,6-dichlorobenzonitrile) and its degradation product in soil, 2,6-dichlorobenzamide, have been directly and rapidly determined in aqueous samples by high-performance liquid chromatography (HPLC). Using a radial compression C_{18} column, 50% CH_3CN–50% H_2O mobile phase (2.0 mL/min), 100-μL sample injection, and UV detector at 205 nm, the method described gave a detection limit of 0.01 ppm for each compound without a preliminary extraction or concentration step. This method should be useful in a variety of applications involving dichlobenil formulations and their fate in soil or water over a wide concentration range.

Dichlobenil (2,6-dichlorobenzonitrile) (I) (Scheme I) is a broad-spectrum, somewhat volatile, herbicide that is tolerated well by many established crops. It can be used for total weed control in noncrop situations and is also effective in aquatic weed control (Weed Science Society of America, 1979). Dichlobenil is degraded by soil and hydrosoil microorganisms to 2,6-dichlorobenzamide (II) (Beynon and Wright, 1972; Montgomery et al., 1972; Verloop, 1972). Amide residues in soil are sometimes present in greater amounts than the parent dichlobenil (Beynon and Wright, 1968; Khan and Miller, 1982).

Scheme I



I II

Residue determinations of I and II usually employ an extraction step, column chromatography for extract

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cleanup, and gas chromatography (GC) with electron capture detection (Meulemans and Upton, 1966; van Rossum et al., 1978). The sensitivity limits reported are typically about 0.02–0.05 ppm.

To support our research on controlled-release dichlobenil formulations applied to soil, we wanted to extend our previous high-performance liquid chromatographic (HPLC) method (Connick and Simoneaux, 1982) for determining I so that we could determine I and II simultaneously in aqueous samples over a wide concentration range. We now report such a method that is direct, rapid, and as sensitive as the usual GC methods.

EXPERIMENTAL SECTION

Apparatus. The HPLC equipment was from Waters Associates and consisted of a Model 6000A pump, a Model 450 variable-wavelength ultraviolet (UV) absorbance detector, a WISP Model 710B automatic sample injector, and a radial compression column system composed of a RCM-100 module and a Radial-PAK μBondapak C_{18} cartridge (8 mm i.d. × 10 cm, 10 μm spherical packing) fitted with a Guard-PAK C_{18} precolumn insert preceded by a 2-μm filter unit. Chromatograms were recorded on a 10-mV strip chart recorder operated at 0.5 cm/min.

Reagents. The dichlobenil was obtained from Aldrich (97%) and recrystallized twice from methanol, mp 144.5–146 °C. The 2,6-dichlorobenzamide was from Aldrich (97%) recrystallized twice from 95% ethanol, mp 200.5–201.5 °C. Solvents were HPLC grade and were passed through a Millipore type FH (0.5-μm) filter before use. Deionized water was further purified by using a Gelman Water-I system.

Soil and Soil Leachate. The soil used in this study was a pine bark mixture (PBM) commonly used in commercial nurseries for raising container-grown ornamental plants. This mixture contained 80% shredded pine bark, 10% sandy loam, and 10% sand. A quantity of aqueous leachate of PBM was obtained by slowly passing 1200 mL of deionized water through 100 g of PBM in a Buchner-type, coarse fritted disk, filter funnel. After the first 200 mL was discarded, the remainder was filtered (slow) through a Millipore type HA filter (47 mm, 0.45 μm) and stored in a freezer. The leachate was thawed and refiltered before use.

HPLC Analytical Procedures. A mobile phase of 50:50 CH_3CN–H_2O was pumped at 2.0 mL/min. Monitored at 205 nm, the amide peak (II) eluted at 2.2 min on the tail of one the peaks due to strongly UV absorbing components of the PBM leachate (Figure 1A). Peak height for II in PBM leachate was measured with a ruler after interpolating the base line just before the peak to the point where it intersected the pen trace after the peak. In water, II eluted just after a negative deflection, but there was enough base-line stabilization for easy quantitation (Figure 1B). Dichlobenil eluted cleanly at 8.2–9.0 min depending on the particular column, temperature, solvent variations, etc. Elution times were highly reproducible for a given column and set of conditions.

The sample size used was 100 μL, but it was determined that no column overload occurred with a 1-ppm solution of I and II up to at least 200 μL. Samples were centrifuged (glass tubes) to remove particulates, if necessary, and not filtered because of the affinity of dichlobenil for some synthetic polymers present in filter materials.

Standard solutions were prepared by diluting a 100 ppm solution of I and II in acetonitrile with water. A calibration curve for each compound (peak height vs. concentration) was generated by calculation of the "line of best fit" using the data obtained from 2-, 1-, 0.1-, 0.05-, and 0.01-ppm

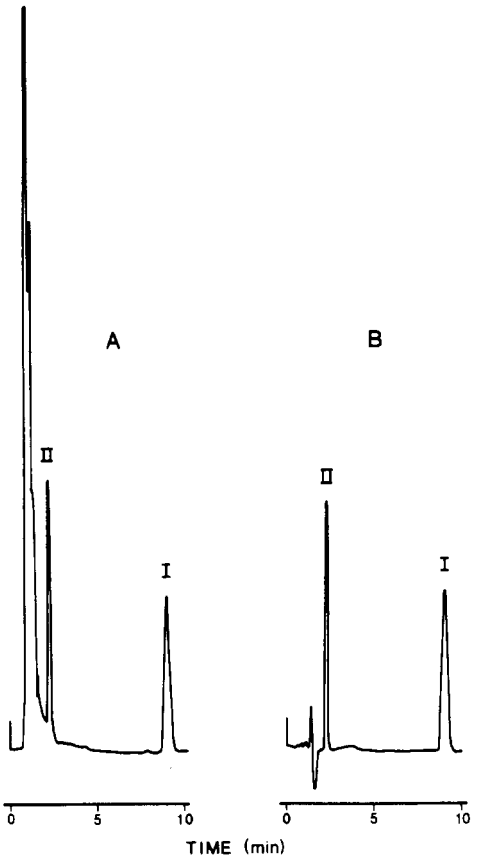

Figure 1. HPLC chromatograms of dichlobenil (I) and 2,6-dichlorobenzamide (II): C_{18} radial compression column; 50% CH_3CN–50% H_2O; 2.0 mL/min; 100-μL injections; UV detection at 205 nm (0.1 AUFS). (A) 0.5 ppm of I and II in PBM leachate. (B) 0.5 ppm of I and II in water.

standards, each injected twice before and after the samples run that day. Correlation coefficients (r) were usually greater than 0.9990. Sample concentration was calculated from the equation of the respective line of best fit. The most sensitive detector setting used was 0.02 AUFS. Calibration curves are linear out to 15 ppm of I and 10 ppm of II, at least. The minimum detectable concentrations of I and II in water using this method are about 0.01 ppm each.

RESULTS AND DISCUSSION

Wavelengths from 196 to 215 nm were investigated to discover the optimum for determining I and II. Figure 2 is a plot of wavelength vs. peak height data obtained from duplicate 70-μL injections of 1 ppm of I and II in PBM leachate. Maximum sensitivity for I occurred at 205–207 nm and at 198 nm for II. We chose 205 nm as a compromise to obtain maximum sensitivity for dichlobenil together with high sensitivity for the amide.

Standard solutions that were made with water were adequate for our purpose and were used throughout this work. Standard solutions that were made by spiking PBM leachate with I and II to get maximum accuracy at low concentrations, particularly for II, performed well at first but became turbid after a few weeks in spite of refrigeration. Even when sedimentation occurred, these mixtures gave peak heights identical with those of aliquots that had been frozen fresh and thawed as clear solutions.

To determine the precision of the method, solutions containing both I and II were prepared in water and in PBM leachate at concentrations of 1.0 and 0.02 ppm. Each solution was injected 5 times. Correlation coefficients (r) for the resulting calibration curves of I and II were 0.9996

Table I. Statistical Data Obtained Using the HPLC Method

compound	concentration,[a] ppm	mean, $n = 5$	SD	confidence interval, 95%
2,6-dichlorobenzamide (II)				
in water	1.0	0.9923	0.0020	0.9923 ± 0.0025
in water	0.02	0.0246	0.0003	0.0246 ± 0.0002
in PBM leachate	1.0	0.9953	0.0017	0.9953 ± 0.0008
in PBM leachate	0.02	0.0177	0.0012	0.0177 ± 0.0005
dichlobenil (I)				
in water	1.0	0.9816	0.0031	0.9816 ± 0.0039
in water	0.02	0.0267	0.0006	0.0267 ± 0.0003
in PBM leachate	1.0	0.9828	0.0048	0.9828 ± 0.0021
in PBM leachate	0.02	0.0260	0.0008	0.0260 ± 0.0004

[a] Nominal concentration of the standard solutions.

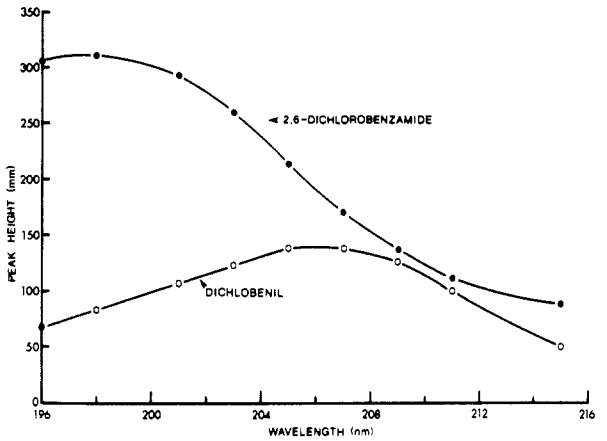

Figure 2. Effect of wavelength on peak height of 2,6-dichlorobenzamide and dichlobenil by HPLC using 70-μL injections of a 1-ppm solution. Peak heights were normalized to 0.04 AUFS.

and 0.9998, respectively. Statistical data are given in Table I. Precision was excellent at each concentration of each compound in water and in PBM leachate. Even without sample concentration, the detection limit could be lowered to about 0.005 ppm (5 ppb) by using a well-tuned instrument and 200-μL sample injections.

Fortunately, extractables from the PBM leachate eluted sufficiently ahead of the amide peak to allow good quantitation even though this involved extrapolation of the base line. The same results were obtained with the leachate of a commercial peat-based growing mix.

The adsorption of I from aqueous solution by several polymers, even from the vapor phase, has been reported (Verloop, 1972; Frank and Comes, 1967; Connick and Simoneaux, 1982) and care should be exercised in all sample handling. To see if II was affected in the same manner, a 1-ppm solution of I and II in water was analyzed before and after storage in a vial with a Poly-Seal polyethylene cap liner and after filtration through a Millex-HA (Millipore) cellulose acetate–nitrate filter unit. Storage of 1 mL for 16 h in a 1-dram vial at 24 ± 2 °C did not affect the peak height of II, but the peak height of volatile I decreased by 25% because it was removed from the vapor phase by the cap liner. Filtration of 1 mL of the solution caused a 6% peak height decrease for II and a 97% decrease for I. The 11th mL of solution passed through the same filter unit lost 0% II and 13% I due to a saturation effect. We used Teflon cap liners without difficulty and preferred to centrifuge in glass tubes where necessary to remove particulates rather than filter.

CONCLUSION

This direct, reverse-phase HPLC method required no extraction and cleanup and greatly facilitated the trace-level determinations of I and II in the horticultural soil leachates we studied. Detection of the compounds by UV was very sensitive (to 0.01 ppm) without sample concentration. To determine only II, detection at 198 nm would give the highest sensitivity. The method may be useful for analyzing technical dichlobenil and its formulations and for monitoring the fate of dichlobenil in other soils, crops, and aquatic environments.

ACKNOWLEDGMENT

We thank M. G. Palazzolo for her excellent technical assistance.

Registry No. I, 1194-65-6; II, 2008-58-4.

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Received for review June 27, 1983. Accepted October 24, 1983. Names of companies or commercial products are given solely for the purpose of providing specific information; their mention does not imply recommendation or endorsement by the U.S. Department of Agriculture over others not mentioned.