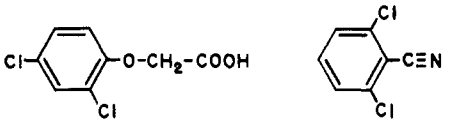
# Determination of (2,4-Dichlorophenoxy)acetic Acid and of 2,6-Dichlorobenzonitrile in Water by High-Performance Liquid Chromatography

William J. Connick, Jr.,* and Jacqueline M. Simoneaux

Direct determinations of the herbicides 2,4-D [(2,4-dichlorophenoxy)acetic acid] and dichlobenil (2,6-dichlorobenzonitrile) in water by reverse-phase high-performance liquid chromatography (HPLC) are described. An ultraviolet absorbance detector at 280 nm gave adequate sensitivity for both compounds, but 236 nm was optimum for 2,4-D as was 215 nm for dichlobenil. The methods are particularly useful for determining release rates of controlled-release formulations into water, where concentrations are low initially but increase appreciably with time. Concentration ranges studied were 0.5–50 ppm for 2,4-D and 0.1–15 ppm for dichlobenil. Significant losses of dichlobenil from dilute aqueous solutions were noted after storage in vials having polyethylene cap liners and after filtering through cellulose acetate–nitrate filters.

The herbicides 2,4-D [(2,4-dichlorophenoxy)acetic acid] (I) and dichlobenil (2,6-dichlorobenzonitrile) (II) are very



I II

effective aquatic weed control chemicals (Cardarelli, 1976). Convenient determinations of each of these herbicides in water were needed to support research on the preparation and evaluation of controlled-release formulations.

Reverse-phase high-performance liquid chromatography (HPLC) was selected because of its inherent simplicity, speed, and sensitivity. HPLC also has the flexibility to ensure that a determination is specific, so that impurities and formulation adjuvants do not interfere.

The method we report here for 2,4-D uses the mobile phase (50% $CH_3CN$–49% $H_2O$–1% HOAc) reported by Edwards et al. (1979) to be satisfactory for detecting 2,4-D as the free acid as well as its dimethylamine salt. Dichlobenil is usually determined by gas–liquid chromatography (Van Rossum et al., 1978), and literature on HPLC methods is scarce (Eichner and Renner, 1980). We report here also a convenient and sensitive determination of dichlobenil by reverse-phase HPLC.

These determinations are particularly useful for obtaining release rates from controlled-release formulations into a fixed volume of water where the concentrations of free herbicide are initially low but increase appreciably with time. The concentration ranges of interest are 0.5–50 ppm for 2,4-D and 0.1–15 ppm for dichlobenil.

## MATERIALS AND METHODS

**Apparatus.** A Waters Associates (Milford, MA) HPLC apparatus was used that consisted of a Model 6000A pump, a U6K injector, and Model 440 fixed-wavelength and Model 450 variable-wavelength ultraviolet (UV) absorbance detectors. The reverse-phase column used for both determinations was a μBondapak $C_{18}$ (Waters Associates), 3.9 mm i.d. × 30 cm, 10 μm, analytical column. Chromatograms were recorded on a Model B5217-1 (10-mV) Omniscribe (Houston Instrument, Austin, TX) strip chart recorder operated at 0.5 cm/min. A Beckman Instruments (Irvine, CA) DB-G grating spectrophotometer and 10-mm quartz cells were used to obtain UV spectra.

**Reagents.** 2,4-D was obtained from Eastman Kodak (Rochester, NY) and recrystallized once from benzene, mp 139–141 °C. Dichlobenil was obtained at 97% purity from Aldrich Chemical Co. (Milwaukee, WI) and recrystallized twice from methanol, mp 144.5–146 °C. Acetonitrile was HPLC grade, and glacial acetic acid (HOAc) was reagent-grade quality. Deionized water was further purified by passage through a series of $C_{18}$ Sep-Pak (Waters Associates) cartridges (one per liter). Prior to use, all solvents were passed through 0.45- or 0.5-μm type HA or FH filters (Millipore Corp., Bedford, MA).

**HPLC Analytical Procedures.** Calibration curves (peak height vs. concentration) that covered the desired range of concentrations were generated for each day's samples by using external standard solutions (usually four) and least-squares regression analysis of the data. Duplicate injections of each standard were made before and after duplicate injections of each sample. Multiple injections ($n$ = 5) of 20 ppm of 2,4-D and 10 ppm of dichlobenil were used to determine standard deviations (Table I). Accepting this measure of precision, and operating in a 95% confidence interval, it was statistically determined that two injections per sample are sufficient.

**Determination of 2,4-D.** Standard solutions of 50, 20, 10, 5, 1, and 0.5 ppm in water gave a linear calibration curve over this concentration range with a correlation coefficient ($r$) of 0.9996. The HPLC mobile phase was 50% $CH_3CN$–49% $H_2O$–1% HOAc pumped at a flow rate of 1.5 mL/min which generated a pressure of about 950 psi. The fixed-wavelength UV absorbance detector was set at 280 nm with a sensitivity of 0.02 AUFS for 5–50-ppm standards or 0.005 AUFS for 0.5-1 ppm. The 2,4-D peak eluted at 6.5 min (Figure 1A). A 20-μL sample was selected, which gave a minimum detectable concentration of 0.4 ppm under these conditions or 0.2 ppm by using a variable-wavelength detector set a 236 nm. A larger injection would increase sensitivity, but at 20 μL it was possible to inject each sample and its duplicate about 2 min apart and reduce analysis time for that sample without peak overlap or column overload.

**Determination of Dichlobenil.** Standard solutions of 15, 10, 5, 1, 0.5, and 0.1 ppm prepared in 50% $CH_3CN$–50% $H_2O$ gave a linear calibration curve over this concentration range ($r$ = 0.9997). The HPLC mobile phase was 50% $CH_3CN$–50% $H_2O$ with a flow rate of 1.5 mL/min (1000 psi). The dichlobenil peak eluted at 6.0 min (Figure 1B). The fixed-wavelength detector was operated at 280 nm with a sensitivity of 0.02 AUFS for 1–15 ppm or 0.005 AUFS for 0.1–0.5 ppm. With a 70-μL sample, the minimum detectable concentration was about 0.1 ppm

Southern Regional Research Center, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, New Orleans, Louisiana 70179.

Table I. Accuracy and Precision of HPLC Determinations of 2,4-D and Dichlobenil at Specified Wavelengths

| | 2,4-D, 280 nm | | dichlobenil 280 nm | | dichlobenil 215 nm | |
|---|---|---|---|---|---|---|
| concentration, ppm | 20.00 | 1.00 | 10.00 | 1.00 | 10.00 | 1.00 |
| mean ($n$ = 5) | 20.19 | 0.95 | 10.07 | 1.00 | 10.00 | 0.98 |
| standard deviation | 0.10 | 0.06 | 0.03 | 0.02 | 0.03 | 0.03 |
| 95% confidence interval | 20.19 ± 0.12 | 0.95 ± 0.07 | 10.07 ± 0.04 | 1.00 ± 0.02 | 10.00 ± 0.03 | 0.98 ± 0.03 |
| relative mean error, % | +0.95 | −5.00 | +0.70 | 0.00 | 0.00 | −1.60 |

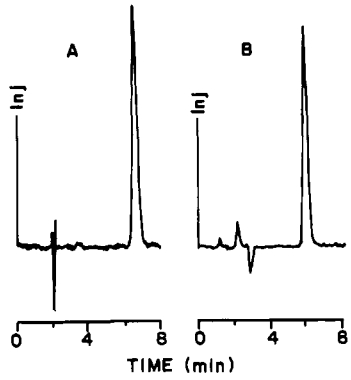



**Figure 1.** HPLC chromatograms using a μBondapak $C_{18}$ column and UV detection at 280 nm (0.02 AUFS). (A) 2,4-D (20 ppm): 50% $CH_3CN$–49% $H_2O$–1% HOAc; 1.5 mL/min; 20 μL injected. (B) Dichlobenil (5 ppm): 50% $CH_3CN$–50% $H_2O$; 1.5 mL/min; 70 μL injected.

under these conditions or 0.02 ppm by using a variable-wavelength detector set at 215 nm.

## RESULTS AND DISCUSSION

Aqueous solutions of 2,4-D with concentrations of 20.00 and 1.00 ppm were prepared by accurate weighing and dilution and analyzed by the method described. Results (Table I) at 280 nm with a fixed-wavelength detector were entirely satisfactory, as the accuracy and precision were adequate for the intended purpose. Determinations made by using a variable-wavelength detector set at the UV absorption maxima of 2,4-D (in $CH_3CN$ vs. $CH_3CN$: 236, 286, and 293 nm) showed higher sensitivity (2×) only at 236 nm.

Besides the free acid form of 2,4-D, we were able to accurately analyze dilute solutions of its water-soluble dimethylamine salt and emulsions of its oil-soluble dodecyl/tetradecylamine salts. The salts were hydrolyzed to the free acid form by the acidic mobile phase, and their chromatograms showed peaks identical with those of 2,4-D acid in appearance and retention time.

Dichlobenil solutions with concentrations of 10.00 and 1.00 ppm were prepared in 50% $CH_3CN$–50% $H_2O$ and analyzed. The data (Table I) show that good accuracy and precision were obtained at both concentrations. The UV spectrum of dichlobenil in $H_2O$ (vs. $H_2O$) has the following peaks listed in order of decreasing absorbance: 215, 241, 248, 303, and 294 nm. Its spectrum in 50% $CH_3CN$–50% $H_2O$ (vs. air) is the same, except that the 215-nm peak is shifted to 229 nm.

Although the method of determination of dichlobenil was satisfactory by using a fixed-wavelength detector at 280 nm, maximum sensitivity was obtained at 215 nm (Figure 2). Curves for the responses at 248 and 303 nm are not shown but would lie between those of 280 and 241 nm.

Accuracy and precision at 215 nm were determined in the same manner as described above for 280 nm, and the

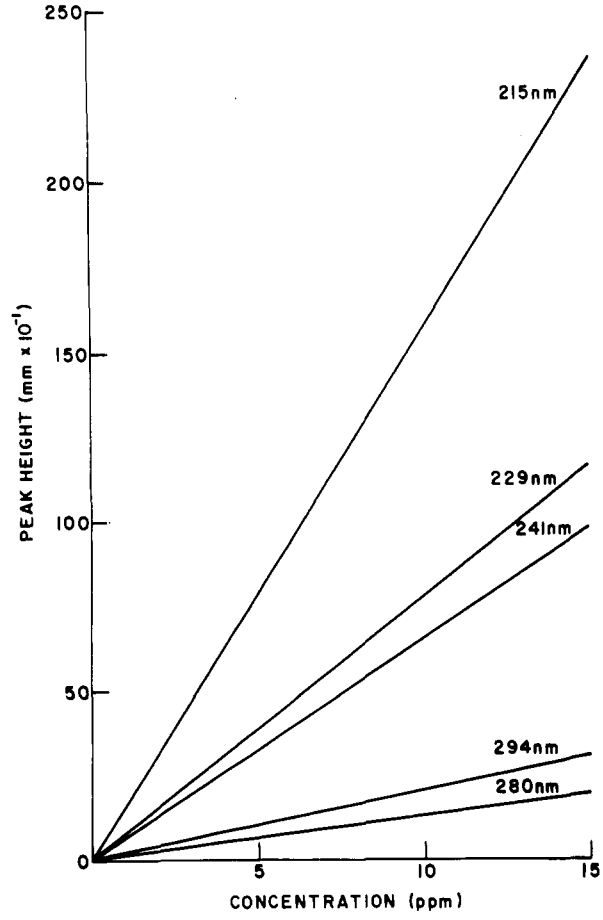



**Figure 2.** Calibration curves ($r$ > 0.9900) of dichlobenil solutions at several wavelengths. Peak heights are normalized to 0.02 AUFS sensitivity.

data (Table I) compare very closely. The minimum detectable concentration was 0.02 ppm at 215 nm by using the variable-wavelength detector (usable routinely at 0.02 AUFS in spite of some base-line irregularity) compared with about 0.1 ppm at 280 nm using the fixed-wavelength detector.

Several observations and caveats regarding dichlobenil were noted. First, its volatility required the storage of solutions in tightly closed containers. Furthermore, dilute solutions of dichlobenil in water that were stored in sample vials containing Poly-Seal polyethylene cap liners lost significant quantities of the herbicide during 24 h. This is probably a result of its adsorption on or solubility in the polyethylene. No losses were encountered when Teflon-lined rubber cap liners were used. Also, in a related problem, it was found that filtering dilute dichlobenil aqueous solutions through type HA cellulose acetate–nitrate filters (0.45 μm; Millipore Corp.) appreciably lowered its concentration. No difficulties of these kinds were encountered with 2,4-D acid solutions.

CONCLUSION

These HPLC determinations are direct, requiring no extraction or derivatization. The microliter-sized samples can be withdrawn with negligible disturbance to the dynamics of a controlled-release experiment, and analysis time for both herbicides is less than 7 min. Good sensitivity is provided by UV detectors, especially the variable-wavelength type. Should other applications require the determination of concentrations of 2,4-D or dichlobenil lower than those studied here, it is suggested that the HPLC methods be optimized by using the guidelines and procedures given by Kirkland (1974) and Macy and Loh (1980).

LITERATURE CITED

Cardarelli, N. "Controlled Release Pesticides Formulations"; CRC Press: Cleveland, OH, 1976; pp 93–119.
Edwards, R. W.; Nonnemaker, K. A.; Cotter, R. L. *NBS Spec. Publ. (U.S.)* **1979**, *No. 519*, 87–94.
Eichner, M.; Renner, R. *Z. Lebensm.-Unters. -Forsch.* **1980**, *170*, 1–4.
Kirkland, J. J. *Analyst (London)* **1974**, *99*, 859–885.
Macy, T. D.; Loh. A. *Anal. Chem.* **1980**, *52*, 1381–1383.
Van Rossum, A.; de Wilde, P. C.; de Boer, F. G.; Korver, P. K. In "Analytical Methods for Pesticides and Plant Growth Regulators, Vol. X, New and Updated Methods"; Zweig, G.; Sherma, J., Eds.; Academic Press: New York, 1978; pp 311–320.

Received for review July 13, 1981. Revised manuscript received November 5, 1981. Accepted November 5, 1981. 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.

# Isolation and Identification of a Polar Sulfamethazine "Metabolite" from Swine Tissue

Deborah D. Giera,* Riaz F. Abdulla, John L. Occolowitz, Douglas E. Dorman, James L. Mertz, and Robert F. Sieck

The metabolism of orally administered [$^{14}$C]sulfamethazine was studied in swine. A major "metabolite" was isolated from liver and muscle and identified as $N^4$-glucopyranosylsulfamethazine by mass spectral and nuclear magnetic resonance analysis. However, the genesis of the glucose conjugate became suspect when subsequent investigation revealed the drug's spontaneous reaction with endogenous components in control swine tissue extracts in vitro. The glucose adduct and a number of other minor metabolites were formed in vitro by spiking control liver extracts with [$^{14}$C]sulfamethazine.

As sulfonamide animal products come under more intense U.S. Department of Agriculture scrutiny because of the high incidence of violative residues (Trabosh, 1978), additional residue studies can be anticipated. Giera et al. (1982) described the excretion and tissue residue distribution when [$^{14}$C]sulfamethazine was orally administered to swine. Essentially all of the administered drug was excreted 15 days postmedication, depleting total $^{14}$C residues in tissue to levels well below the 100-ppb sulfamethazine tolerance. In this study, the isolation and spectral identification of a major sulfamethazine derivative, $N^4$-glucopyranosylsulfamethazine, in liver and muscle tissue extracts from 1 day postmedicated swine are described.

Further investigation revealed, however, that the analysis of 1 day drug withdrawal tissue samples were complicated by the parent compound's spontaneous derivatization in vitro. The glucose conjugate might have arisen totally or in part from an in vitro reaction between sulfamethazine and an endogenous tissue extract component.

MATERIALS AND METHODS

**Extraction and Purification of Polar Metabolite.** Twenty-five grams of liver tissue from swine dosed orally with [$^{14}$C]sulfamethazine (Giera et al., 1982) was extracted with 80% methanol or acetone (3 × 150 mL) in a blender. Following each extraction, the sample was filtered (Whatman No. 1 paper), and the pooled extracts were evaporated, under vacuum at 40–50 °C, to remove organic solvent. The volume of the aqueous sample was adjusted with water to 100 mL and acidified with 4 mL of 1 N HCl. The aqueous sample was extracted with hexane (3 × 200 mL) which was discarded. The aqueous phase was neutralized with 1 N NaOH and extracted with $CHCl_3$ (3 × 200 mL) and then methyl ethyl ketone (MEK; 2 × 200 mL). The $CHCl_3$, MEK, and spent aqueous extracts were separately taken to dryness under vacuum at 40–50 °C. All extractions were done in a separatory funnel. The MEK and aqueous extracts were separately chromatographed on a Porapak Q (Waters Associates, Inc., Milford, MA), 80–100-mesh, column (2.8 × 20 cm). The column material was soaked overnight in methanol and slurry packed. The column was sequentially prewashed with 250-mL portions of methanol, acetone, methanol, water, and 30% methanol prior to sample loading (30% methanol). The sample was eluted from the column with a methanol gradient under gravity pressure (Figure 1). Twenty-milliliter fractions were collected.

Fractions (12–35), which contained the polar metabolite, were pooled and evaporated under vacuum at 40–50 °C, and the residue was redissolved in methanol and streaked on silica gel 60F-254, 0.25-mm TLC plates (EM Laboratories, Darmstadt, Germany). The plates were developed in benzene–MEK–ethanol–water, 30:30:30:10. The me-

Department of Agricultural Biochemistry, Lilly Research Laboratories, Greenfield, Indiana 46140.

0021-8561/82/1430-0260$01.25/0 