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Screening method for phenoxy acid herbicides in ground water by high-performance liquid chromatography of 9-anthryldiazomethane derivatives and fluorescence detection

TOSHINARI SUZUKI* and SATORU WATANABE
Tama Branch Laboratory, Tokyo Metropolitan Research Laboratory of Public Health, 3–16–25, Shibazaki-cho, Tachikawa, Tokyo 190 (Japan)
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ABSTRACT

A high-performance liquid chromatographic (HPLC) method for phenoxy acid herbicides using precolumn derivatization with 9-anthryldiazomethane (ADAM) is presented. The phenoxy acid herbicides investigated were (2,4-dichlorophenoxy)acetic acid, (4-chloro-2-methylphenoxy)acetic acid, 2-(4-chloro-2-methylphenoxy)propionic acid and (4-chloro-2-methylphenoxy)butyric acid. These herbicides reacted with ADAM under mild conditions and were converted into the corresponding fluorescent derivatives. The ADAM derivatives were separated by reversed-phase HPLC and determined using a fluorescence detector. The detection limits were about 500 pg per injection. For the application of ADAM to the determination of these herbicides in ground waters, the recoveries were more than 93% and the average relative standard deviation was 6.0% at 0.5 μg/l. The procedure is useful as a screening method for phenoxy acid herbicides in ground water samples.

INTRODUCTION

Phenoxy acid herbicides are used worldwide in agriculture and forestry for controlling the presence of broad-leaf weeds. In Japan, large amounts of the herbicides, especially (2,4-dichlorophenoxy)acetic acid (2,4-D), (4-chloro-2-methylphenoxy)acetic acid (MCPA), 2-(4-chloro-2-methylphenoxy)propionic acid (MCPP) and (4-chloro-2-methylphenoxy)butyric acid (MCPB), are scattered at golf courses, and it is suspected that these compounds may be leached from the soil into the local ground waters. Therefore, if such ground waters are to be supplied as drinking water, it is necessary to screen them for contamination by these herbicides.

Phenoxy acid herbicides in environmental water samples have been determined by gas chromatography (GC) [1–4] and high-performance liquid chromatography (HPLC) [5–13]. For GC analysis, the compounds must generally be derivatized because of their high polarity and low volatility. Many derivatization procedures, such as esterification [1,2] and silylation [3,4], have been reported.

On the other hand, for the determination of phenoxy acid herbicides by HPLC, no derivatization approach has been reported. Detection has been mainly based on the poor selectivity of UV detection at 230 or 280 nm [5–11], because many of the

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compounds absorb at these wavelengths. Therefore, in order to detect these herbicides in water samples at concentrations lower than 1.0 μg/l, sample enrichment and purification by means of a selective solid-phase extraction cartridge [6] or a complicated HPLC system with on-line column-switching valves [8,9] are needed. As the phenoxy acid herbicides contain carboxyl groups, we considered their precolumn fluorescence labelling for a sensitive and selective HPLC assay.

9-Anthryldiazomethane (ADAM) was developed by Nimura and Kinoshita [14] as a fluorescent reagent for fatty acids. This reagent reacts with carboxyl groups under mild conditions without a catalyst and permits the highly sensitive and selective detection of fatty acids by HPLC. ADAM has been applied to the determination of acidic biological compounds such as prostaglandins [15], carnitine [16] and fatty acids [17].

In this paper, we report the application of ADAM to the determination of 2,4-D, MCPA, MCPP and MCPB by reversed-phase HPLC and the evaluation of a screening method utilizing ADAM for these herbicides in ground water samples.

EXPERIMENTAL

Materials

2,4-D, MCPP were purchased from Gasukuro Kogyo, ADAM from Funakoshi Chemical and 9-hydroxymethylanthracene from Tokyo Kasei Kogyo (all Tokyo, Japan). Oxalyl chloride, silica gel, all organic solvents and other compounds were obtained from Wako (Osaka, Japan). (4-Chloro-2-methylphenoxy)butyric acid (MCPB) was supplied as the herbicide Tropotox (Nissan Kagaku Kogyo, Tokyo, Japan) and extracted and purified in our laboratory.

Instruments

Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained on a Model JNM-FX270 NMR spectrometer (JEOL, Tokyo, Japan) at 270 MHz. Mass spectra were measured with a Model JMS-D300 mass spectrometer (JEOL). The HPLC system consisted of a Model 880-PU pump (Jasco, Tokyo, Japan), a Model 7125 injector with a 20-μl loop (Rheodyne, Cotati, CA, U.S.A.) and a Model 820-FP spectrofluorimeter (Jasco), which was connected to a Model CR-6A chromatographic integrator (Shimadzu, Kyoto, Japan).

Preparation of ADAM derivatives of phenoxy acid herbicides

To 0.25 mmol of phenoxy acid herbicide were added 10 ml of a solution containing 0.25 mmol of ADAM in acetone. The mixture was allowed to stand in the dark for 4 h at 40°C, then evaporated to dryness with a rotary evaporator at 40°C. The residue was purified on a silica gel column with *n*-hexane–ethyl acetate (95:5) as eluent. The main fraction was evaporated to dryness and the residue was purified by recrystallization from *n*-hexane–ethyl acetate. The crystals obtained were used for ^{1}H NMR and mass spectrometric analysis. Chemical shift values (δ) in deuterochloroform were expressed in parts per million downfield from tetramethylsilane as internal standard.

Derivatization of phenoxy acid herbicides for HPLC

Phenoxy acid herbicides for working standards were prepared as solutions in acetone. An aliquot (1–500 ng) was dispensed into a 5-ml minivial and evaporated to dryness in a stream of nitrogen. To the residue were added 100 μl of a solution containing 0.025% ADAM in acetone. The reaction mixture was heated in the dark for 60 min at 40°C and then dried in a stream of nitrogen. The residue was dissolved in 75 μl of acetone and 15 μl of the solution were applied to the HPLC system.

Chromatographic conditions

The ADAM derivatives of the acid herbicides were separated by using a TSK-gel ODS120T reversed-phase column (25 cm × 4.6 mm I.D.) (Tosoh, Tokyo, Japan). The eluent was acetonitrile–water (75:25) containing 3% tetrahydrofuran, at a flow-rate of was 1.0 ml/min. The detection wavelengths were adjusted to 365 nm excitation and 412 nm emission.

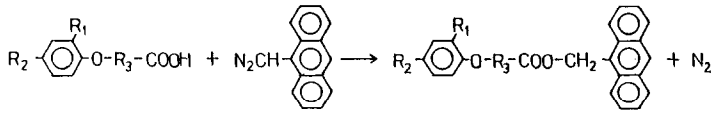
Determination of phenoxy acid herbicides in ground water

A 20-ml volume of ground water was taken in a 25-ml glass-stoppered test-tube, 100 μl of concentrated hydrochloric acid (35%) were added and the phenoxy herbicides were extracted three times by vigorously shaking for 1 min with 2 ml of *n*-hexane–ethyl acetate (20:80). The extracts were collected and concentrated to *ca.* 0.5 ml with a rotary evaporator at 40°C. The resulting solution was transferred to a 5-ml minivial and evaporated to dryness in a stream of nitrogen. The derivatization with ADAM was performed as described above.

RESULTS AND DISCUSSION

Confirmation of ADAM derivatives of phenoxy acid herbicides

The structures of the ADAM derivatives of 2,4-D, MCPA, MCPP and MCPB shown in Fig. 1 were investigated. As an example, The ^{1}H NMR spectral data for the ADAM derivative of MCPP were as follows: δ 1.57 (3H,d,J = 6.60 Hz), 2.06 (3H,s), 4.69 (1H,q,J = 6.60 Hz), 6.18 (2H,s), 6.41 (1H,d,J = 8.57 Hz), 6.74 (1H,dd,J = 2.64, 8.57 Hz), 6.93 (1H,d,J = 2.64 Hz), 7.45–7.55 (4H,m), 8.02 (2H,m), 8.19 (2H,m), 8.50 ppm (1H,s). The methylene protons at the 9-position appeared at 6.18 ppm, which is in good agreement with the value of 6.19 ppm calculated by the method of Shoolery [18].



Phenoxy Acid Herbicides ADAM Esters

	R_1	R_2	R_3
2,4-D	Cl	Cl	CH_2
MCPA	CH_3	Cl	CH_2
MCPP	CH_3	Cl	$CH(CH_3)$
MCPB	CH_3	Cl	CH_2-CH_2-CH_2

Fig. 1. Reaction course of phenoxy acid herbicides with ADAM.

These results indicate a 9-anthrylmethyl ester bond in the derivative. The methylene protons at the 9-position of the ADAM derivatives of 2,4-D, MCPA and MCPB appeared at 6.28 (2H,s), 6.26 (2H,s) and 6.16 ppm (2H,s), respectively. No alteration of the signals other than that of the 9-position in the ^{1}H NMR spectrum was observed after derivatization with ADAM for all the ADAM derivatives of the herbicides.

The mass spectra of the ADAM derivatives of MCPP, 2,4-D, MCPA and MCPB showed the molecular ion (M^+) at *m/z* 404, 410, 390 and 418, respectively. For all ADAM derivatives of the herbicides, the base peak appeared at *m/z* 191; the peak is [9-anthryl–CH_2]$^+$ generated by fragmentation of the 9-anthrylmethyl group.

Further confirmation of the ADAM derivatives of the phenoxy acids was performed. The ^{1}H NMR and mass spectra of each 9-anthrylmethyl ester synthesized with 9-hydroxymethylanthracene and the acid chlorides of 2,4-D, MCPA, MCPP and MCPB, formed by treatment with oxalyl chloride were in good agreement with the spectra of the corresponding derivatives with ADAM (data not shown).

From these results, it is obvious that the phenoxy acid herbicides reacted with ADAM to form the corresponding 9-anthrylmethyl esters as shown in Fig. 1.

Derivatization of phenoxy acid herbicides for HPLC

The optimum conditions were examined for a reaction volume of 100 μl containing 100 ng of 2,4-D, MCPA, MCPP and MCPB. We first investigated the reaction solvents, using acetone, ethyl acetate and methanol. The fluorescence response of the ADAM ester of each of the four herbicides was the highest with acetone, and the average relative standard deviation (R.S.D.) was 2.4% ($n = 5$). The reaction of the herbicides with 0.025% ADAM at 40°C progressed more than 90% in 15 min and was completed within 60 min. Concentrations of the ADAM–acetone solution of 0.025, 0.05 and 0.1% were examined. The sensitivities of the four herbicides did not change with these concentrations. With 0.1% ADAM solution, the baseline rose owing to the impurities present in the ADAM reagent, hence it was unsuitable when determining a few nanograms of the phenoxy acid herbicides. From the above results, the optimum conditions were fixed as indicated under Experimental.

Chromatographic separation of the ADAM derivatives of phenoxy acid herbicides

A typical chromatogram of the ADAM derivatives of 2,4-D, MCPA, MCPP and MCPB is shown in Fig. 2. The four ADAM derivatives could be separated without clean-up after the derivatization with ADAM. The peaks in front of the peak of 2,4-D were always observed, regardless of the presence of the acid herbicides. The calibration graphs of each of the four herbicides, of amount of herbicide *versus* peak height of the fluorescent response, showed excellent linearity in the range 1.0–100 ng per injection and passed through the origin ($r = 0.998$–0.999). The detection limits of the herbicides under the optimum conditions were about 500 pg per injection (signal-to-noise ratio = 3).

Application to the determination of phenoxy acid herbicides in ground water

Ground water samples spiked with 2,4-D, MCPA, MCPP and MCPB at the 0.5 and 5.0 μg/l levels were extracted with *n*-hexane–ethyl acetate *ca.* pH 1.5. The recoveries of the four herbicides were more than 93% at both levels examined and the average R.S.D. was 6.0% ($n = 5$). These results indicated that the method gave high

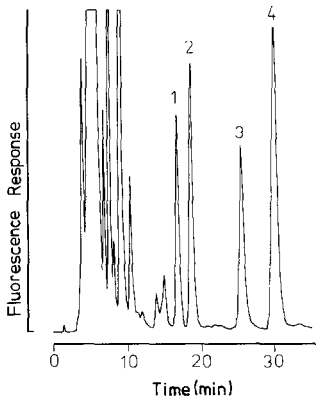


Fig. 2. HPLC separation of the ADAM derivatives of phenoxy acid herbicides. Peaks: 1 = 2,4-D (20 ng); 2 = MCPA (20 ng); 3 = MCPP (20 ng); 4 = MCPB (20 ng per injection). A mixture of the four herbicides (100 ng of each) was esterified with 100 μl of 0.025% ADAM and a 15-μl aliquot from 75 μl was injected.

recoveries and good reproducibility. Under the routine detection conditions used, moreover, the method could determine the phenoxy acid herbicides at concentrations down to 0.2 μg/l (signal-to-noise ratio = 5). These detection limits could be improved by sample pretreatment involving solid-phase extraction with an anion exchanger or a reversed-phase sorbent, and further study of this aspect is required. The chromatogram obtained on sampling 20 ml of a ground water spiked with 0.5 μg/l concentrations of each herbicide is shown in Fig. 3. Clean-up of the extracts from the water sample was unnecessary.

Under the extraction conditions, most acid compounds such as fatty acids and other analogues of phenoxy acid herbicides can be extracted and subjected to reaction with ADAM. For fatty acids, the ADAM derivatives of *n*-butyric, *n*-caproic and

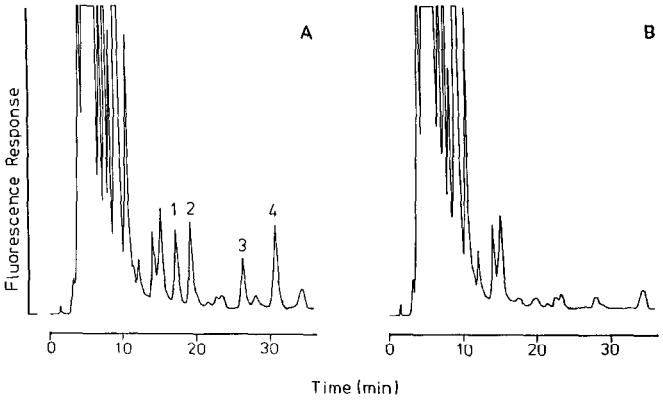


Fig. 3. Chromatograms of ground water extracts treated with ADAM. (A) Ground water sample spiked with 0.5 μg/l concentrations of phenoxy acid herbicides. Numbers on the peaks correspond to those in Fig. 2. (B) Blank ground water sample.

n-caprylic acid were detected at 14.2, 20.4, and 38.0 min, respectively, and under the chromatographic conditions used they were separated from the ADAM derivatives of the four herbicides. The investigation of other phenoxy herbicides is in progress.

In conclusion, the proposed procedure is useful as a rapid screening method for the presence of the herbicides in ground water. By sampling 20 ml of a ground water sample, the method can determine phenoxy acid herbicides at levels lower than 0.5 μg/l.

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