Journal of Chromatography, 469 (1989) 351–359
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 323

CAPILLARY GAS CHROMATOGRAPHIC–MASS SPECTROMETRIC DETERMINATION OF ACID HERBICIDES IN SOILS AND SEDIMENTS

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(First received January 11th, 1988; revised manuscript received January 11th, 1989)

SUMMARY

A capillary gas chromatographic method with selected-ion monitoring (GC–SIM) was applied to eight common herbicides: 2-(4-chloro-*o*-tolyl-oxy)propionic acid, 3,6-dichloro-2-methoxybenzoic acid , 2-methyl-4-chlorophenoxyacetic acid, 2,3,6-trichlorobenzoic acid, 2,4-dichlorophenoxyacetic acid, 3,5,6-trichloro-2-pyridyloxyacetic acid, 2,4,5-trichlorophenoxyacetic acid and α-(2-methyl-4-chlorophenoxy) butyric acid. The method involves extraction with saturated calcium hydroxide solution, esterification with pentafluorobenzyl bromide, clean-up with a silica gel column and determination by capillary GC–SIM. Recoveries from soil and sediment are over 89% (with the exception of 77% for TBA) with coefficients of variation below 5% ($n = 7$). The method is suitable for the simultaneous determination of the eight herbicides in environmental samples with high sensitivity and accuracy.

INTRODUCTION

Phenoxyalkanoic, benzoic acid-derived and pyridyloxy herbicides have a selective killing effect on broadleaf weeds and are therefore widely used in agriculture and forestry. These herbicides are marketed in the form of esters, salts or acids. After application, they can enter water flow systems, with the possibility of environmental pollution. Hence it is desirable to have a method for the simultaneous determination of multiple components in environmental samples. The purpose of this work was to develop such a method applicable to herbicides in samples at concentrations of the order of micrograms per kilogram.

Many methods[1–15] of analysis have been reported for phenoxyalkanoic and benzoic acid-derived herbicides in soils and sediments. For pyridyloxy herbicides, only one method[16] has been presented for environmental water and none for soil or sediment.

Several methods of extracting herbicides from soils or sediments have been

0021-9673/89/$03.50

proposed. Khan[1] used a mixture of acidified acetone and *n*-hexane, Abbott *et al.*[2] one of dilute sulphuric acid and diethyl ether and Cotterill[3] saturated calcium hydroxide solution.

Herbicides extracted from environmental samples, except esters, are insufficiently volatile or too highly polar for gas chromatographic (GC) analysis. It is therefore necessary to decrease their polarity and to make them more volatile by methylation using diazomethane[1,4,5] boron trichloride–methanol[6], or iodomethane[3,7] Chau and Terry[8] adopted derivatization to halogenated alkyl esters and halogenated aromatic esters such as the pentafluorobenzyl ester to increase the sensitivity of sample herbicides towards electron-capture detection (ECD) in GC. Bertrand *et al.*[15] adopted (cyanoethyl)dimethylsilanization.

In most determinations of extracted herbicides the samples were cleaned up by liquid–liquid partition or column chromatography before being subjected to GC–ECD. Capillary GC–ECD[14] and capillary GC–mass spectrometry (GC–MS) have recently come into use.

This investigation was undertaken in order to establish a suitable method for the simultaneous determination of these compounds in soils and sediments. The examination was focused on the applicability of pentafluorobenzylation as a method for the pre-treatment of environmental samples and the application of high-selectivity GC–selected-ion monitoring (SIM) to the determination of acid herbicides. The method proposed here has proved to have adequate sensitivity, accuracy and selectivity.

EXPERIMENTAL

Reagents

3,5,6-Trichloro-2-pyridyloxyacetic acid (triclopyr), triethylammonium 3,5,6-trichloro-2-pyridyloxyacetate (triclopyr-TEA) and butoxyethyl 3,5,6-trichloro-2-pyridyloxyacetate (triclopyr-BE) were obtained from Dow Chemical Japan, 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2-methyl-4-chlorophenoxyacetic acid (MCP) from Wako, 2,3,6-trichlorobenzoic acid (TBA) from Takeda Chemical, 3,6-dichloro-2-methoxybenzoic acid (dicamba) from King Chemical, α-(2-methyl-4-chlorophenoxy)butyric acid (MCPB) and 2-(4-chloro-*o*-tolyloxy)propionic acid (MCPP) from Yashima Chemical, 2,3,4,5,6-pentafluorobenzyl bromide (PFB) from Tokyo Kasei and *p*-terphenyl-d_{14} from MSD Isotopes, Canada.

Silica gel (Wako Gel S-1) was activated by heating at 130°C for 12 h before use. Ultrabond 20M, a polyethylene glycol GC packing, was obtained from Ultra Scientific. OV-1 (methylsilicone) and OV-17 (mixed methyl- and phenylsilicone) were obtained in the form of wide-bore capillary columns (15 m × 0.53 mm I.D.), with the silicones chemically bonded, from Gasukuro Kogyo.

n-Hexane, benzene, dichloromethane and acetone were of the grade suitable for the detection of pesticide residues. All the other reagents were of guaranteed grade.

Apparatus

The GC–MS instrument used was a Model JMS-D300 from Japan Electron Optics Laboratory and the ultrasonic cleaner was a Model UT-20 (26 kHz, 300 W) from Kokusai Denki.

Measurement conditions

The conditions for GC were as follows: column temperature, 230°C; injection port temperature, 250°C; and carrier gas (helium) flow-rate, 15 ml/min. Three columns were tested for applicability as described later.

The conditions for SIM-MS were as follows: ion multiplier voltage, 1.8 kV; and ion source and enricher temperature, 250°C. Experiments to select suitable monitor ions and ionization voltages were conducted as described later.

Experiments performed to develop the standard procedure

Extraction conditions were determined as follows: to 20 g of soil sample 5 μg of each acid herbicide were added and after 1 h, 2 g of calcium hydroxide and 100 ml of distilled water were added. The mixture was extracted several times by sonication for various times in the range 10–60 min and the recovery for each herbicide was determined.

To select a suitable esterifying agent, the following three experiments were conducted using 10 μg of each acid herbicide: (1) methylation was conducted using 2 ml of a diethyl ether solution of diazomethane; (2) esterification with trifluoroethanol (TFE) was conducted at 80°C for 1 h using 0.25 ml of boron trifluoride (BF_3)–TFE; (3) esterification with PFB was conducted at 60°C for 3 h and at 25°C for 1–24 h.

To select clean-up conditions, 10 μg of the PFB esterification product of each herbicide was loaded on to a 10 mm I.D. column containing silica gel and eluted with 100 ml of benzene–*n*-hexane (10:90); no ester species were found in the eluate. The esters were completely eluted with 100 ml of benzene–*n*-hexane (55:45).

Interferences from similar substances which might be extracted from the initial soil sample by dichloromethane were examined for the following substances: 2,4-dichlorophenol, 2,4,5-trichlorophenol, pentachlorophenol, salicylic acid, benzoic acid, 2,4-dichlorophenyl 3-methoxy-4-nitrophenyl ether, *p*-nitrophenyl 2,4,6-trichlorophenyl ether and 2,4-dichlorophenyl *p*-nitrophenyl ether. For this examination 100 μg of each substance were added to 100 ml of distilled water prior to extraction with dichloromethane and esterification.

To select the MS conditions, PFB esterification products were prepared, as follows, according to Lee and Chau's method[13] (different from the standard procedure because a larger amount of herbicide was to be handled for preparation purposes). Amounts of 1 mg each of MCPP, dicamba, MCP, TBA, 2,4-D, triclopyr, 2,4,5-T and MCPB were placed in a 5-ml vial, 3 ml of acetone were added and then 30 μl of 30% (w/v) potassium carbonate solution and 200 μl of 5% (v/v) PFB solution in acetone were added. The mixture was allowed to react at 60°C for 3 h and the reaction products were extracted with 20 ml of *n*-hexane. The products were identified by electron impact (EI) MS measurements using the usual technique adopted for the selection of MS conditions.

For GC using the conditions given above, a column packed with Ultrabond 20M and wide-bore columns with chemically bonded OV-1 or OV-17 were examined. For MS using the predetermined conditions given above, monitor ions and ionization voltages (15–30 and 70 eV) were sought that would be susceptible to as little interference and capable of providing as high sensitivity as possible.

Standard procedure

The standard procedure consists of four steps: extraction, esterification, clean-up and determination.

Extraction. About 20 g of sample are weighed into a 300-ml centrifuge tube and 2 g of calcium hydroxide and 100 ml of distilled water are added. The mixture is stirred with a glass rod and extracted by sonication for 30 min. The sample is centrifuged at 2000 *g* for 10 min and the extract is filtered under suction through a glass-fibre filter paper into a beaker. Extraction is repeated once in the same way with 100 ml of distilled water. The combined extracts are transferred into a separating funnel, acidified to a pH below 1 with 9 *M* sulphuric acid and extracted twice for 3 min with 50 ml of dichloromethane. The dichloromethane extract solution is washed with 50 ml of 5% (w/v) sodium chloride solution, dehydrated with anhydrous sodium sulphate, transferred into a 200-ml oval flask and evaporated to dryness on a rotary evaporator.

Esterification. The residue is dissolved in 4 ml of acetone in the flask, 30 μl of 30% (w/v) potassium carbonate solution and 200 μl of 5% (v/v) PFB solution in acetone are added and the solution is allowed to stand for 5 h at room temperature for esterification to proceed. The solution is evaporated to dryness on a rotary evaporator and the residue is dissolved in about 20 ml of *n*-hexane in a 50-ml separating funnel. The solution is washed twice with 10 ml of 5% (w/v) sodium chloride solution, dehydrated with anhydrous sodium sulphate and concentrated to less than 5 ml in a Kuderna–Danish concentrator.

Clean-up. A silica gel column is prepared by packing a 10 mm I.D. column with a slurry of 3 g of silica gel in *n*-hexane. The concentrate is transferred on to this column, washed with 100 ml of benzene–*n*-hexane (10:90) and eluted with 100 ml of benzene–*n*-hexane (55:45).

Determination. The eluate is concentrated to less than 5 ml in a Kuderna–Danish concentrator and, after adding 0.5 μg of *p*-terphenyl-d_{14} as an internal standard, further concentrated to 1 ml under a gentle stream of nitrogen. The sample is injected into the GC–SIM instrument for determination.

Blank tests are run using the same procedure.

RESULTS AND DISCUSSION

Investigation of extraction conditions for soil samples

The experimental investigation for establishing the extraction condition utilized Cotterill's method[3], which extracts ester-type herbicides under a hydrolysis condition with alkali and may be regarded as efficient with respect to the number of operational steps.

The recovery obtained with extraction times of 30–60 min reached a constant value. One extraction run did not give a sufficient recovery but two successive runs were satisfactory. The extraction conditions adopted were 30-min sonication twice. It was noted that tricopyr-BE is hydrolyzed completely into triclopyr within 5 min.

Investigation of PFB esterification conditions

Diazomethane, which is unstable and explosive, could convert all eight acid herbicides into methyl esters. However, the methyl esters of MCPP, TBA and MCP have such low boiling points that the efficiency of concentration is too low. BF_3–TFE

hardly reacts with MCPP, dicamba, MCP and TBA. PFB was found to effect smooth esterification with the formation of products with relatively high boiling points. It was therefore decided to adopt PFB as the esterifying agent.

Many methods have been reported for the esterification of acid herbicides with PFB. This investigation was conducted according to Lee and Chau's method[13]. However, at 60°C, the heating temperature employed for esterification in their method, additional peaks appeared in the blank chromatogram and the compounds responsible could not be removed from the blank sample by silica gel clean-up. We therefore lowered the heating temperature to 25°C. Heating durations of 4–16 h gave a constant formation ratio whereas heating for 16–24 h caused extraneous peaks at longer retention times. Hence, the optimum conditions were esterification with 5% (v/v) PFB solution in acetone at 25°C for 5 h.

Clean-up of PFB esterification products by silica gel column chromatography

A variety of substances in soils and sediments can be simultaneously extracted and esterified by PFB and cause interference in the determination of acid herbicides. Therefore, the esterification products were purified using a silica gel column. When eluted with 100 ml of benzene–*n*-hexane (10:90) no ester species were found in the eluate. However, the esters were completely eluted with 100 ml of benzene–*n*-hexane (55:45) (Fig. 1).

The clean-up procedure adopted was therefore as follows: the column was washed with 100 ml of benzene–*n*-hexane (10:90) to elute as many of the interfering compounds as possible and the esters were eluted with 100 ml of benzene–*n*-hexane (55:45).

All the phenols, acids and ethers tested with respect to interferences were completely removed by the silica gel clean-up or had sufficiently different GC retention times not to cause interference.

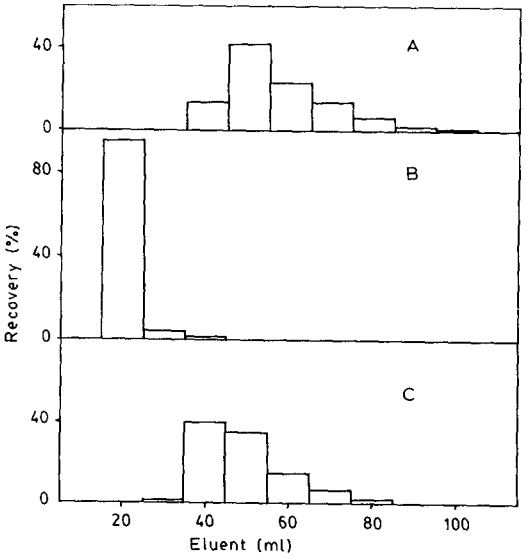

Fig. 1. Elution patterns of (A) MCPB-PFB, (B) TBA-PFB and (C) triclopyr-PFB.

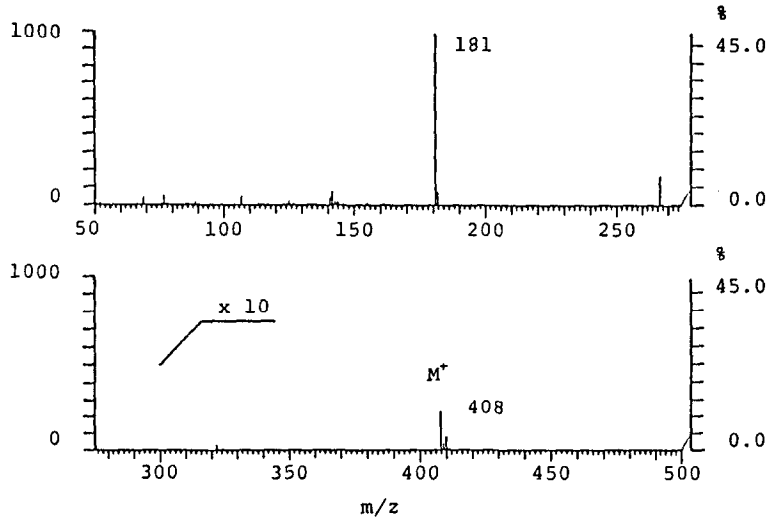

Fig. 2. EI mass spectrum of MCPB-PFB.

Formation and identification of products from reaction with PFB

Each compound was found to give its corresponding molecular ion as follows: *m/z* 394 (MCPP), 400 (dicamba and 2,4-D), 380 (MCP), 404 (TBA), 406 (MCPB), 434 (2,4,5-T) and 435 (triclopyr). TBA and MCPB had a low tendency to form molecular ions, as shown in Figs. 2 and 3. For this reason ions other than molecular ions, *i.e.* at *m/z* 369 and 267, were adopted as monitoring ions for TBA and MCPB, respectively.

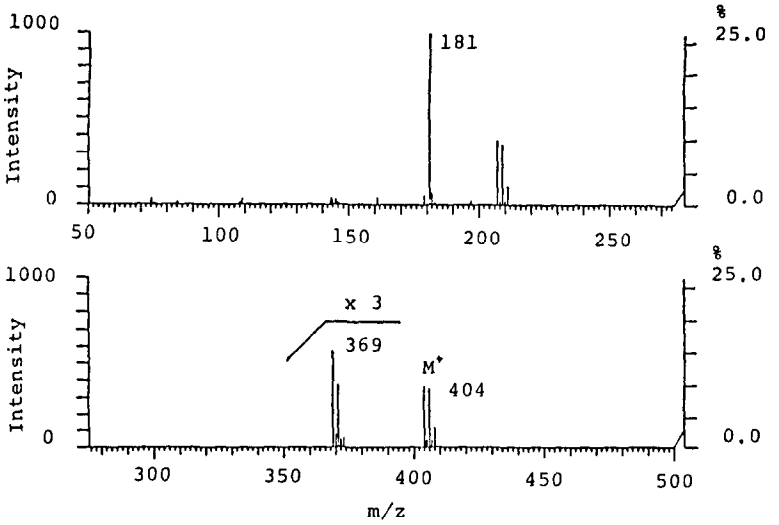

Fig. 3. EI mass spectrum of TBA-PFB.

Chromatography and detection

Lee and Chau[13] used Ultrabond 20M as the GC column packing to separate ten PFB derivatives. We used the same packing for GC–SIM but found that it gave too high a background response to be applicable.

Next, we tried a wide-bore capillary column, which featurs a low background response, high-speed separation and almost the same sample injection volume as a packed column. A wide-bore column (15 m × 0.53 mm I.D.) chemically bonded with OV-17 was capable of almost completely separating all eight acid herbicides in 6 min.

The monitoring ions for determination were selected by measuring the EI mass spectra of each PFB ester and taking into account the fragment intensity and selectivity. The following monitoring ions were selected: *m/z* 267 (MCPB), 369 (TBA), 380 (MCP), 394 (MCPP), 400 (2,4-D and dicamba), 434 (2,4,5-T) and 435 (triclopyr).

The ionization voltage that allows the monitoring ion to give the highest sensitivity was examined in the range 15–30 and 70 eV; 27.5 eV was found to give the highest sensitivity.

Calibration and recovery

The calibration graphs, obtained for 0.05, 0.10, 0.25, 0.50 and 1.0 μg of each acid herbicide, were linear over the concentration range examined. The detection limits were 0.5 μg/kg for MCPP, MCP, TBA and MCPB, 1.0 μg/kg for 2,4-D and triclopyr and 1.5 μg/kg for dicamba and 2,4,5-T.

Recovery experiments were carried out by adding 0.5 μg of each acid herbicide to 20 g of paddy field soil (ignition loss 18.4%) and applying the standard procedure to evaluate the recovery. The recoveries obtained were over 89%, except for TBA (77%), with coefficients of variation being less than 5%.

Table I summarizes the results obtained, indicating that the proposed method is satisfactory.

Application to actual samples

The proposed method was applied to soils and sediments ($n = 20$). Paddy field soils and sediments were found to contain MCP and 2,4-D at levels of 0.25–3.0 μg/kg,

TABLE I

RECOVERY OF ACID HERBICIDES FROM SOIL

Mean values ($n = 7$)

Herbicide	*Amount of sample (g)*	*Amount of herbicide added (μg)*	*Recovery (%)*	*Coefficient of variation (%)*	*Detection limit (μg/kg)*
MCPP	20.0	0.5	92.5	3.4	0.5
Dicamba	20.0	0.5	92.7	4.3	1.5
MCP	20.0	0.5	94.3	3.5	0.5
TBA	20.0	0.5	77.5	3.2	0.5
2,4-D	20.0	0.5	90.1	4.1	1.0
Triclopyr	20.0	0.5	89.0	3.7	1.0
Triclopyr-TEA	20.0	0.5	90.3	4.3	1.0
Triclpyr-BE	20.0	0.5	89.7	3.5	1.0
2,4,5-T	20.0	0.5	90.4	4.4	1.5
MCPB	20.0	0.5	89.2	4.5	0.5

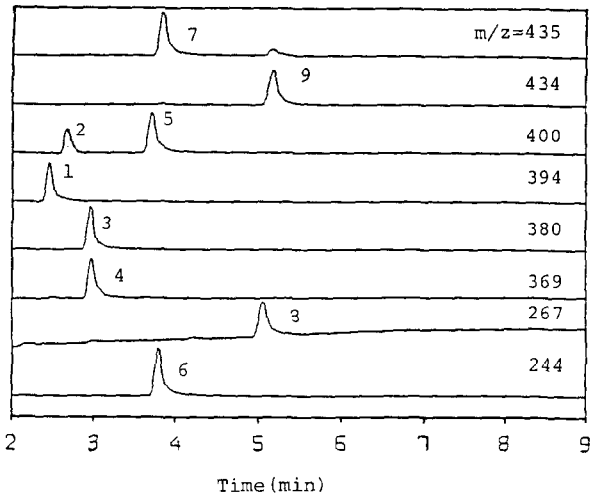

Fig. 4. SIM chromatogram of a standard mixture of acid herbicides. Peaks: (1) MCPP; (2) dicamba; (3) MCP; (4) TBA; (5) 2,4-D; (6) *p*-terphenyl; (7) triclopyr; (8) MCPB; (9) 2,4,5-T.

and turf soils taken from around the laboratory were found to contain TBA, triclopyr, MCP and dicamba at levels of 9–100 μg/kg. Figs. 4 and 5 show examples of SIM chromatograms for acid herbicides and for soil taken from around the laboratory, respectively.

Similar determinations were made using GC–ECD, but the appearance of a large number of interfering peaks required an analysis time for one sample of more than 1 h, with analytical values higher than those given by the proposed method.

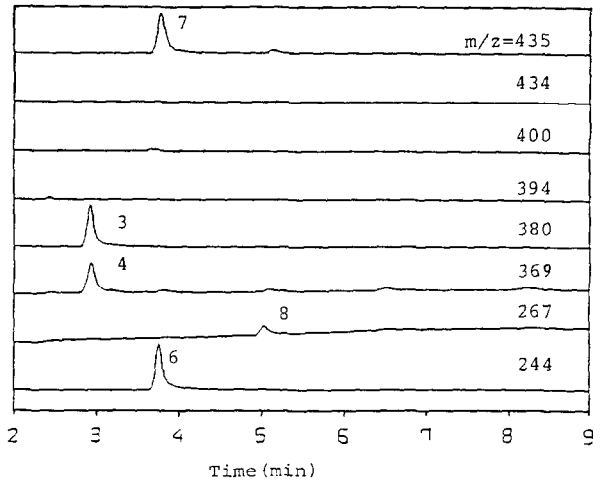

Fig. 5. SIM chromatogram of turf soil from near the laboratory. Peaks as in Fig. 4.

ACKNOWLEDGEMENTS

The authors thank Miss Maria Miyajima and Professor Isao Matuzaki for helpful suggestions.

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