

Determination of Pesticides by Derivative Formation

Part IV.* A Sensitive Gas-chromatographic Method for the Determination of MCPA and MCPB Herbicides after Esterification with 1-Bromomethyl-2,3,4,5,6-pentafluorobenzene

Haig Agemian and A. S. Y. Chau

Inland Waters Directorate, Water Quality Branch, Ontario Region, Canada Centre for Inland Waters, P.O. Box 5050, 867 Lakeshore Road, Burlington, Ontario, L7R 4A6, Canada

A sensitive quantitative method for the gas-chromatographic determination of 4-chloro-2-methylphenoxyacetic acid (MCPA) and 4-(4-chloro-2-methylphenoxy)butyric acid (MCPB) in natural water is presented. The 2,3,4,5,6-pentafluorobenzyl (PFB) esters of MCPA and MCPB have longer retention times and provide increased sensitivity over the conventional methyl and 2-chloroethyl esters of these acids. Concentrations as low as $0.1 \mu\text{g l}^{-1}$ of MCPA and $0.2 \mu\text{g l}^{-1}$ of MCPB (for a 1-l water sample) can be determined by this method.

The high polarity or low volatility of the phenoxyalkanoic acid herbicides prevents direct gas-chromatographic determination and necessitates the formation of more volatile derivatives that are suitable for this type of procedure. Alkyl esters, particularly methyl esters, have long been used for this purpose.¹⁻⁷ However, as reported previously,⁸ owing to the similar retention times of several common herbicide esters, the alkyl esters are subject to incorrect identification if several herbicides are present. Also, the sensitivity obtainable by means of electron capture detection of the alkyl esters of some herbicides, such as MCPA and MCPB, is very poor and therefore the method is generally not suitable for the determination of these compounds in waters. In addition, the methyl ester of MCPA has a very short retention time close to the solvent front and is prone to interference from sample co-extractives, which usually appear in this region. In fact, the MCPA methyl ester often cannot be detected even at higher levels because of overlapping with co-extraction peaks when the same gas-chromatographic parameters as for the determination of organochlorine pesticides are used. Further, this type of analysis necessitates the setting aside of a gas chromatograph for the low column oven temperatures required. In order to avoid this inconvenience and to make possible the determination of herbicides and organochlorines using the same gas-chromatographic conditions, and, most important, to lower the detection limits of MCPA and MCPB, other derivatives have been considered.

Among other derivatives, such as those obtained from nitration,^{9,10} bromination coupled with other reactions,^{10,11} silylation¹² and 2-chloroethylation,^{13,14} derivatives obtained from reactions involving 1-bromomethyl-2,3,4,5,6-pentafluorobenzene¹⁵⁻¹⁷ were found to have the most desirable characteristics¹⁸: excellent detector response and longer retention times. 1-Bromomethyl-2,3,4,5,6-pentafluorobenzene has successfully been applied to the determination of carbamates¹⁹ and to the confirmation of organophosphorus pesticides^{20,21} by forming the 2,3,4,5,6-pentafluorobenzyl (PFB) derivatives of the phenolic and thiophenolic hydrolysis products. The conditions for the preparation of PFB derivatives of 2,4-D²² and ten other herbicidal acids have also been studied.¹⁸

This paper reports the application of this reagent in the development of a sensitive method for the determination of 4-chloro-2-methylphenoxyacetic acid (MCPA) and 4-(4-chloro-2-methylphenoxy)butyric acid (MCPB) at concentrations below the nanogram per millilitre level in natural waters.

Experimental

Apparatus

A Hewlett-Packard Model 5713A gas - liquid chromatograph equipped with a nickel-63

* For Part III of this series, see reference list, p. 737.

detector, a 6 ft \times $\frac{1}{4}$ in i.d. coiled glass column and an automatic sampler, Model 7671A, is connected to an Autolab System IV Computing Integrator for data processing. The column used was 3.6% *m/m* OV-101 and 5.5% *m/m* OV-210 on 80–100-mesh Chromosorb W, acid-washed and treated with dimethylchlorosilane and prepared as described by Chau and Wilkinson.²³ The operating conditions were as follows: injector temperature, 220 °C; column temperature, 220 °C, detector temperature, 300 °C; carrier gas, argon - methane (9 + 1) at a flow-rate of 60 ml min⁻¹.

Reagents

The organic solvents used were of pesticide residue grade.

1-Bromomethyl-2,3,4,5,6-pentafluorobenzene. Obtained from Prince Chemical Company. Prepare a 1% *V/V* solution in acetone in a 100-ml low-actinic calibrated flask.

Caution. This reagent is a strong lachrymator.

Potassium carbonate solution. Prepare a solution of 30 g of anhydrous potassium carbonate, ultrapure grade, in 100 ml of distilled water.

Silica gel. Activate silica gel (Grade 950 for gas chromatography, 60–200-mesh, Fischer Scientific, Canada) by heating at 130 °C overnight (14 h). Then deactivate by adding distilled water, 5% *m/m*.

MCPA and MCPB. Analytical grade. Obtained from May and Baker (Canada) Ltd.

Procedure

Extraction and derivative formation

Stir a 1-l water sample in a 40-oz glass bottle on a magnetic stirrer, using a PTFE stirring bar, so that the vortex formed at the surface almost reaches the bottom of the bottle. Carefully add diluted sulphuric acid (1 + 1) dropwise until the pH is 1 or less (use pH paper).

Add 50 ml of dichloromethane and tightly cover the bottle with the original PTFE- or aluminium-lined cap. After stirring for 45 min transfer the contents of the bottle to a 2-l separating funnel and shake for 1 min. Transfer the organic layer to a clean 500-ml separating funnel. Shake the aqueous layer with two additional 50-ml portions of dichloromethane and transfer the organic layers to the 500-ml separating funnel. Wash the dichloromethane extract by tumbling it a few times with 50 ml of organic-free distilled water (pH 6). Let the layers separate and transfer the lower, organic phase into a 500-ml round-bottomed flask. Wash the aqueous layer in the same manner with two 10-ml portions of dichloromethane and add the organic layers to the rest of the organic extract in the round-bottomed flask.

Evaporate the dichloromethane to 2–3 ml on a rotary evaporator and finish the evaporation on a steam-bath under atmospheric pressure (water-bath temperature kept below 40 °C). Dissolve the residue with several 2–3 ml portions of acetone, each time transferring the acetone quantitatively into a 15-ml graduated centrifuge tube, the total volume of acetone used not to exceed 10 ml. Add 50 μ l of 1-bromomethyl-2,3,4,5,6-pentafluorobenzene solution and 30 μ l of potassium carbonate solution to the centrifuge tube. Stopper the tube and shake it for a few seconds. Let the contents react at room temperature for 5 h. Prolonged reaction should be avoided as extraneous blank peaks would develop. Although heating does increase the rate of the reaction, it was found that this increased the blank owing to the development of additional extraneous blank peaks. In addition, as the reaction takes place in acetone, heating makes it hard to control because of evaporation of the solvent.

After reaction immediately add 2 ml of 2,2,4-trimethylpentane and evaporate the solution to 1 ml with a gentle stream of nitrogen gas. Add another 2 ml of 2,2,4-trimethylpentane and repeat the evaporation to a final volume of about 1 ml. This procedure removes traces of acetone, which can affect the column fractionation.

Column clean-up

Prepare micro-columns by plugging clean disposable pipettes (5 cm \times 5 mm i.d.) with a piece of glass wool that has previously been washed with acetone and dried. Fill the columns with 5 cm of deactivated silica gel and tap them with a pencil or similar device in order to settle the solid.

Pre-wet the columns with 5 ml of hexane and allow the hexane to drain just to the top of the packing material. With a disposable pipette apply the concentrated sample extract, plus the hexane rinsings, to the column. Elute with 15 ml of a 25% *V/V* solution of benzene in hexane in order to remove excess of reagent and contaminants. Elute with 8 ml of a 75% *V/V* solution of benzene in hexane into a clean centrifuge tube. Record the volume collected and analyse this fraction for the PFB esters of MCPA and MCPB by means of gas chromatography.

Results and Discussion

As mentioned earlier in this paper, PFB derivatives of MCPA and MCPB are preferable to the corresponding 2-chloroethyl esters and the commonly used methyl esters. In addition to the longer retention times of the PFB derivatives these esters also show a higher electron-capture response than the same concentration of the corresponding methyl or 2-chloroethyl esters and thus their determination can be carried out under the same gas-chromatographic conditions as for organochlorine pesticides. The detector response obtained for the PFB ester of MCPA is 25 times greater than that obtained for the corresponding 2-chloroethyl ester and 1 000 times greater than that for the methyl ester, whereas the detector response obtained for the PFB ester of MCPB is 500 times greater than that obtained for the corresponding 2-chloroethyl ester and 10 000 times greater than that for the methyl ester. Fig. 1 depicts the relative sensitivity of the method for the three esters of MCPA and MCPB.

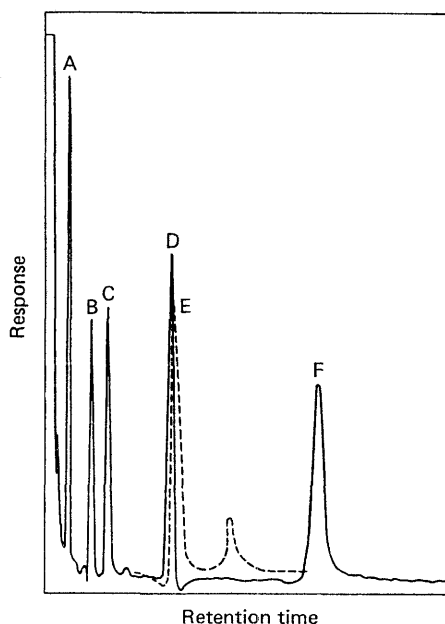


Fig. 1. Chromatograms given by the methyl, 2-chloroethyl and PFB esters of MCPA and MCPB: A, methyl ester of MCPA ($25 \text{ ng } \mu\text{l}^{-1}$); B, methyl ester of MCPB ($500 \text{ ng } \mu\text{l}^{-1}$); C, 2-chloroethyl ester of MCPA ($500 \text{ pg } \mu\text{l}^{-1}$); D, PFB ester of MCPA ($20 \text{ pg } \mu\text{l}^{-1}$); E, 2-chloroethyl ester of MCPB ($25 \text{ ng } \mu\text{l}^{-1}$); F, PFB ester of MCPB ($40 \text{ pg } \mu\text{l}^{-1}$). Sample size, $5 \mu\text{l}$. Attenuation, 16.

Diethyl ether and chloroform used alone^{3,5-7,9,24} or in combination²⁵ are solvents that are commonly used for the extraction of polar herbicidal acids. Owing to the solubility of diethyl ether and ethyl acetate in water, these two solvents were found to be difficult to use. Chloroform and dichloromethane are relatively polar solvents, thus making them desirable solvents for extraction of these polar compounds: their density also facilitates the separation of the

layers. In fact, from a consideration of partition coefficients, chloroform²⁶ is seen to be a good solvent for the extraction of herbicidal acids. However, dichloromethane is preferable. Firstly, it is less toxic and secondly, chloroform should not be used in the proximity of a gas chromatograph with an electron-capture detector because this would result in anomalous responses. (This phenomenon²⁷ was also observed during the development of methods for the determination of organochlorine compounds.) Also, our experiments indicate that dichloromethane has the same extraction efficiency as chloroform.

After extraction with dichloromethane and derivative formation, the extract was cleaned up in order to remove excess of reagent and contaminants introduced by the reagent. The clean-up procedure with silica gel, as reported by Johnson,²² also separates most of the organochlorine pesticides and polychlorobiphenyls (PCBs), if present, from the PFB derivatives (Table I). The few organochlorine pesticides that are eluted in the same fraction (fraction

TABLE I
FRACTIONATION OF PCBs AND ORGANOCHLORINE PESTICIDES
ON A DEACTIVATED SILICA GEL COLUMN

Two micrograms of each PCB and 100 ng of each organochlorine pesticide were added to the column.

Compound	Amount eluted, %	
	Fraction A*	Fraction B†
Aroclor 1254	100	—
Aroclor 1260	100	—
Lindane	98	—
Heptachlor	95	—
Aldrin	95	—
Heptachlor epoxide	102	—
γ-Chlordane	102	—
α-Chlordane	103	2
p,p'-DDE	101	—
α-Endosulfan	101	2
Dieldrin	96	5
o,p'-DDT	100	—
Endrin	95	6
TDE	95	5
β-Endosulfan	—	104
p,p'-DDT	101	—
Methoxychlor	—	105

* Eluted in 25% V/V benzene in hexane.

† Eluted in 75% V/V benzene in hexane.

B in Table I) as the PFB derivatives do not interfere because they have distinct retention times (Table II). Coburn and Chau^{20,21} have shown that organophosphorus pesticides do not interfere and Coburn *et al*¹⁹ have provided data to show that 24 of the most widely used phenols either are eluted in fraction A with the PCBs and organochlorine pesticides or have distinct retention times from those of the PFB esters of MCPA and MCPB.

TABLE II
RETENTION TIMES RELATIVE TO ALDRIN OF COMPOUNDS THAT OCCUR IN FRACTION B

Compound	Relative retention time
Aldrin	1.00*
PFB derivative of MCPA	1.77
Dieldrin	2.02
Endrin	2.30
TDE	2.30
β-Endosulfan	2.58
PFB derivative of MCPB	3.63
Methoxychlor	3.78

* Retention time = 5.2 min.

The whole procedure, *i.e.*, extraction, derivative formation and column clean-up, for 10 determinations of MCPA spiked at a level of $0.5 \mu\text{g l}^{-1}$ in 1 l of distilled water, gave an average recovery of 75% with a coefficient of variation of 15%. Under the same conditions an average recovery of MCPB of 80% was obtained, with a coefficient of variation of 9%. Similar recoveries were obtained at levels of $0.1 \mu\text{g l}^{-1}$ of MCPA and $0.2 \mu\text{g l}^{-1}$ of MCPB.

Analysis at lower levels is limited by the reaction blank although most of the by-products are removed by 15 ml of a 25% *V/V* solution of benzene in hexane. The detection limit of the method is set at $0.1 \mu\text{g l}^{-1}$ of MCPA and $0.2 \mu\text{g l}^{-1}$ of MCPB in water. Fig. 2 shows the chromatographic trace of the reagent blank in relation to the trace of the PFB esters of MCPA and MCPB at 0.1 and $0.2 \mu\text{g l}^{-1}$ levels, respectively. It is obvious from Fig. 2 that the detection limit can be lowered without significant interference from blank peaks and that it can be further reduced if a 2-l instead of a 1-l water sample is taken.

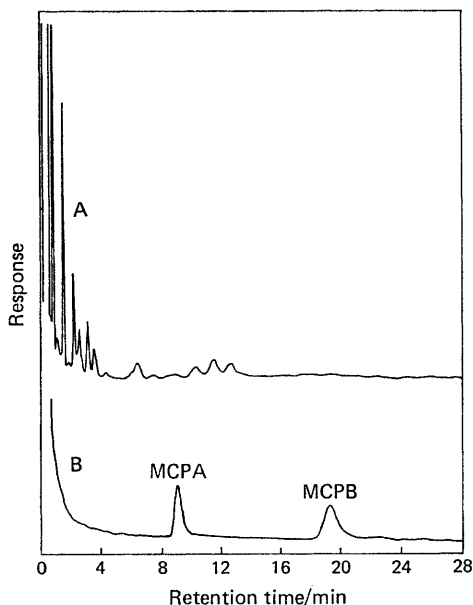


Fig. 2. Chromatograms for: A, reagent blank; and B, PFB esters of MCPA and MCPB at a level of 0.1 and $0.2 \mu\text{g l}^{-1}$, respectively, in water. Attenuation, 32.

In order to test the proposed method on actual samples, Hamilton Harbour waters in 1-l portions were spiked with MCPA and MCPB herbicides at different levels. The recoveries are shown in Table III. In addition, the method was tested on water samples from the Great Lakes, and similar recoveries were obtained. Lower detection limits could be obtained for such cleaner waters, owing to the absence of co-extractives such as those present in Hamilton Harbour waters.

TABLE III

RECOVERIES OF MCPA AND MCPB FROM HAMILTON HARBOUR WATER SAMPLES

Compound				Concentration in 1-l water sample/ $\mu\text{g l}^{-1}$	Recovery, %
MCPA	0.1	80, 82
				0.5	78, 80
MCPB	0.2	80, 82
				1	80, 84

The procedure described in this paper is a much more sensitive method than the procedure based on the formation of methyl esters. The latter method requires extensive concentration

of the sample extract. In view of the volatility, particularly at the microgram per litre level, and the short retention times of methyl esters, the proposed procedure, which involves derivatives that can be determined with greater sensitivity and have lower volatility and longer retention times, is preferable. The longer retention times of PFB esters make it possible to use the same gas-chromatographic conditions as for the determination of organo-chlorine pesticides, thus eliminating the expense of using a gas chromatograph for a specific purpose only.

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NOTE—References 18 and 19 are to Parts III and II of this Series, respectively.

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