CHROM. 9934

GAS–LIQUID CHROMATOGRAPHIC METHOD WITH ELECTRON-CAPTURE DETECTION FOR THE DETERMINATION OF RESIDUES OF SOME PHENOXYACETIC ACID HERBICIDES IN WATER AS THEIR 2,2,2-TRICHLOROETHYL ESTERS

S. MIERZWA and S. WITEK

Institute of Organic and Polymer Technology, Technical University of Wroclaw, 50-370 Wroclaw (Poland)

(Received November 30th, 1976)

SUMMARY

A gas–liquid chromatographic method with electron-capture detection was developed for the determination of residues of 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-methylpenoxyacetic acid (MCPA) in water as their 2,2,2-trichloroethyl esters, and the sensitivity was compared with that for 2-chloro- and 2,2,2-trifluoroethyl esters. The residue is isolated by sorption of the phenoxy acid on Amberlite XAD-4, eluted with benzene and then esterified with 20% (v/v) 2,2,2-trichloroethanol in trifluoroacetic anhydride in the presence of sulphuric acid. The recoveries at the 0.4 ppb* level are 92.1 ± 3.4% (2,4-D) and 87.8 ± 2.1% (MCPA) and the detection limits for a 1-l sample of water are 0.096 ppb (2,4-D) and 0.06 ppb (MCPA).

INTRODUCTION

Herbicide derivatives of phenoxyalkanecarboxylic acids, especially 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-methylphenoxyacetic acid (MCPA), are widely used for weed control in cereals. The problem of the determination of their residues, independently of their formulation (salts, esters), is reduced to the determination of free acids, but these acids are not suitable for direct determination by gas–liquid chromatography (GLC). 2,4-D and MCPA are therefore determined by converting them into chromatographically useful derivatives, mainly esters or phenol derivatives obtained after cleavage of the ether bond of the phenoxy acid. Phenol derivatives can be determined by a variety of methods[1-5], but these methods are of little use in this instance owing to the difficulties that arise from the cleavage of the ether bond of the phenoxy acids.

In the analysis of residues, esters of phenoxyalkanecarboxylic acids are more

___

* Throughout this article, the American billion (10⁹) is meant.

suitable, and determinations of methyl[6–10], chloro and bromomethyl[11], 2-chloro-
ethyl[12,13], n-butyl[14] and trimethylsilyl esters[15] have been reported.

The determination of residues of 2,4-D and MCPA in water as their methyl
esters requires a very large sample in order to attain the appropriate sensitivity[16].
During studies of residues of 2,4-D and MCPA in water from the Oława River[17], we
found that 2-chloroethyl esters are suitable derivatives for their determination, but
the limit of detection for a 1-l sample of water was too high, especially for MCPA
(3 ppb). It was therefore necessary to develop a more sensitive method and to examine
esters that contain a larger number of halogen atoms, which enhances the electron-
capture detector (ECD) signal.

In this paper, we describe work on the use of 2,2,2-trifluoro- and -trichloro-
ethyl esters of 2,4-D and MCPA in the analysis of residues, and a comparison of
their electron-capturing properties with those of 2-chloroethyl esters. Further, we
have developed a method for the esterification, at the residue level, of 2,4-D and
MCPA with 2,2,2-trichloroethanol (TCE) and a method for the isolation of 2,4-D
and MCPA from water at the nanogram level.

EXPERIMENTAL

2,4-D and MCPA were esterified on a preparative scale with 2-chloro- and
2,2,2-trifluoroethanol and with TCE by refluxing 0.05 M phenoxy acid and 0.15 M
alcohol in a solution of benzene (40 ml; the 2,2,2-trifluoroethyl ester was collected
in n-hexane) with the addition of concentrated sulphuric acid and removal of water
by means of a Dean–Stark trap.

After the reaction, the solution was cooled, neutralized with solid sodium
hydrogen carbonate, washed with water and, after drying over anhydrous sodium
sulphate, concentrated in a rotatory evaporator to ca. 10 ml. Chromatographically
pure 2,4-D and MCPA esters were separated by preparative gas chromatography
using a Pye Unicam Model 105 chromatograph, column length 2 m, I.D. 8 mm,
packed with 10% DC-200 + 15% QF-1 (1:1) on 80–100-mesh Gas-Chrom Q at a
column temperature of 210° (180° for 2,2,2-trifluoroethyl esters). The structure of the
esters was confirmed from their nuclear magnetic resonance spectra.

Apparatus
A Perkin-Elmer Model F-11 chromatograph with a borosilicate glass column,
equipped with an ECD and a 10-mCi nickel-63 source with a pulsating voltage
(50 V; 0.5–100 μsec) was used. A Büchi Model SB rotatory evaporator was also
employed.

Reagents and materials
The following reagents and materials were used: pure TCE, re-distilled (Koch-
Light, Colnbrook, Great Britain); trifluoroacetic anhydride (TFA), >99% (Pierce,
Rockford, Ill., U.S.A.) (a 20%, v/v, solution of the alcohol in TFA was prepared
immediately before use); acetone solutions (2 μg/ml) of MCPA and 2,4-D (Poly-
Science, Niles, Ill., U.S.A.; 99% phenoxy acids standards); Kieselgel 60, 70–230 mesh
(Merck, Darmstadt, G.F.R.); and Amberlite XAD-4, 0.3–0.7 mm (Rohm & Haas,
Philadelphia, Pa., U.S.A.). The remaining reagents (p.a. grade) were obtained from

P.O.Ch. (Gliwice, Poland): benzene, acetone, isoctane (re-distilled), concentrated sulphuric acid, 0.5 M potassium hydroxide solution and anhydrous sodium sulphate.

Water

To 1 l of deionised or natural water in a 2-l separating funnel add 0.2-ml portions of 2,4-D and MCPA standard solutions and acidify with sulphuric acid to pH 2. Connect the separating funnel by means of a glass system with a glass column (10 × 1 cm) containing a 6-cm layer of Amberlite XAD-4. Pass the solution through the column at the rate of 15 ml/min, then dry the sorbent in a stream of nitrogen at room temperature and elute the remaining phenoxy acids by passing 40 ml of benzene down the column at the rate of *ca.* 5 ml/min. Collect the benzene eluate in a 100-ml round-bottomed flask and evaporate it to dryness in a stream of air in a rotary evaporator at 40°. Remove the remainder into a test-tube with a ground-glass stopper in three portions (*ca.* 2 ml each) of acetone and evaporate to dryness in a stream of nitrogen at room temperature. Esterify the residue in the test-tube as described below.

Esterification

Esterification is carried out with a 20% (v/v) solution of TCE in TFA, prepared in such volumes as will be used in the course of a few hours after preparation. Concentrated sulphuric acid (0.5%, v/v) is added just prior to esterification, which is carried out as follows.

To a test-tube containing the residues of phenoxy acids, add 1 ml of the esterification solution and 5 μl of concentrated sulphuric acid. Shake the test-tube and place it under a reflux condenser on an oil-bath at 100° for 15 min, or leave it to stand at room temperature for at least 2 h (preferably overnight).

Reduce the volume of the excess of reagents to *ca.* 0.1 ml in a stream of air at room temperature. Add 2 ml of isooctane to the test-tube and, after shaking it, add 6 ml of 0.5 M potassium hydroxide solution, stopper the tube and shake it for 1 min. After the separation of the layers, the content of the phenoxy acid esters is determined by injecting a 2-μl portion of the isoctane layer on to the GLC column.

Clean-up

The direct determination of esters of phenoxy acids in a 2-ml isooctane layer is possible only with pure materials (standards, deionized water). With surface water samples, a clean-up of the isooctane extract is necessary, as follows. A 4-cm layer of silica gel in a column (10 cm × 1 cm I.D.) and a 1-cm layer of anhydrous sodium sulphate above the adsorbent are used. The column is filled with a suspension of the adsorbent in acetone and washed with an additional 10 ml of acetone, followed by 5 ml of isooctane. A 1-ml volume of the isooctane extract is placed on the column and washed with 25 ml of isooctane. Benzene is introduced on to the column and the esters of phenoxy acids are eluted with 15 ml of this solvent.

Depending on the concentration, esters of 2,4-D and MCPA can be determined in this volume or after reducing the volume to 1 ml in a stream of nitrogen.

Chromatography

Quantitative analysis is carried out on a column, 2 m long, 3 mm I.D., packed with 15% QF-1 + 10% DC 200 (1:1) on 80–100-mesh Chromosorb W AW

DMCS, at injector, column and detector temperatures of 220°, 195° and 220°, respectively, with nitrogen as carrier gas at a flow-rate of 80 ml/min. Qualitative confirmation of the results is carried out on a column, 1 m long, 3 mm I.D., packed with 3 % OV-17 on 80–100-mesh Diatomite CQ, at injector, column and detector temperatures of .220°, 195° and 220°, respectively, with nitrogen as carrier gas at a flow-rate of 60 ml/ min.

RESULTS AND DISCUSSION

The 2,4-D and MCPA esters prepared were used for calibration of the ECD and the detection limits for each of these esters were determined as the amount corresponding to 1-cm peaks. The conditions used for the chromatography were as described under Experimental, except that the 2,2,2-trifluoroethyl esters were chromatographed at a column temperature of 170°. The detection limits determined are given in Table I relative to the detection limit of the 2-chloroethyl ester = 100. The sensitivity of the detector was greatest for 2,2,2-trichloroethanol esters, and these esters were chosen for use in the determination of residues.

TABLE I

RELATIVE DETECTION LIMITS OF MCPA AND 2,4-D ESTER DERIVATIVES (2-CHLORO-ETHYL ESTER = 100)

Ester	MCPA	2,4-D
2-Chloroethyl	100	100
2,2,2-Trifluoroethyl	41	66
2,2,2-Trichloroethyl	1.2	12.5

The chromatographic separation of trichloroethyl 2,4-D and MCPA esters does not present difficulties, and they can easily be separated by using various packings, of which two were used here (see Experimental).

While examining the esterification of microgram amounts of 2,4-D and MCPA, the method of esterification of benzoic acids with TCE described by Smith and Tsai[18] was checked, but it proved to be unsuccessful (Table II, No. 4).

Other esterification systems, in which concentrated sulphuric acid is used as catalyst, were studied and some of the results obtained are given in Table II. Esterification with TCE, although concentrated sulphuric acid as catalyst and a high reaction temperature were used, did not give good yields. Esterification with TCE in TFA catalyzed by concentrated sulphuric acid, however, gave good results in various combinations, of which Nos. 5 and 6 in Table II are recommended.

After esterification, the mixture contains trichloroethyl esters and large amounts of TFA and TCE; owing to the strong ECD signal caused by these reagents, direct chromatography cannot be used. Removal of TFA was effected in a stream of nitrogen, the residue was dissolved in a small amount of isooctane and the solution obtained treated with 0.5 M potassium hydroxide solution. Advantage was taken of the high solubility of TCE in potassium hydroxide solutions and, as the results showed, this procedure permits the virtually complete removal of TFA and TCE, giving clean chromatograms (Fig. 1A).

TABLE II

MEAN YIELDS OF 2,2,2-TRICHLOROETHYL ESTERS UNDER VARIOUS ESTERIFICA-
TION CONDITIONS WITH 20 μg OF MCPA

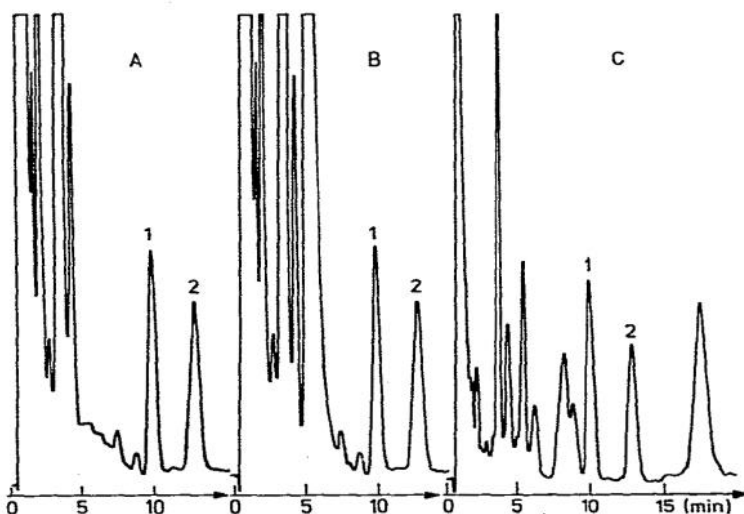| No.	Esterification reagent	Volume (ml)	Reaction time (min)	Amount of H_2SO_4 (%, v/v)	Temperature (°C)	Mean yield (%)
1	Trichloroethanol	1.0	30	50	0–5	30.5
2	Trichloroethanol	0.5	30	0.5	160–165	63.0
3	Trichloroethanol	0.5	30	0.5	180–185	70.5
4	20% trichloroethanol in TFA	1.0	-15	0	100	10
5	20% trichloroethanol in TFA	1.0	15	0.5	100	100
6	20% trichloroethanol in TFA	2.0	120	0.5	20	100

Fig. 1. Gas chromatograms of 2,2,2-trichloroethyl esters of phenoxy acids. All peaks correspond to
0.4 μg of phenoxy acids or their content in water at the 0.4 ppb level. Volume injected: 2 μl. 1 =
MCPA; 2 = 2,4-D. A, Standard acids without clean-up; B, fortified deionized water without clean-
up; C, fortified natural water after clean-up.

In the development of a method for isolating 2,4-D and MCPA residues,
extraction with diethyl ether has been employed as the technique used most often[19,20].
During the determination of the recovery of this method (on the basis of the
calibration graph, Fig. 2) using 1 l of water from the Oława River for two phenoxy
acid concentrations (3.0 and 0.6 ppb), a large divergence of the results (especially at
the lower level) and relatively low recoveries of 2,4-D (maximum 65.4%) and MCPA
(maximum 86%) were obtained. Even worse results were obtained when benzene and
chloroform were used for extraction. These poor results may have been caused by
large amounts of impurities extracted from the river water, which was indicated by
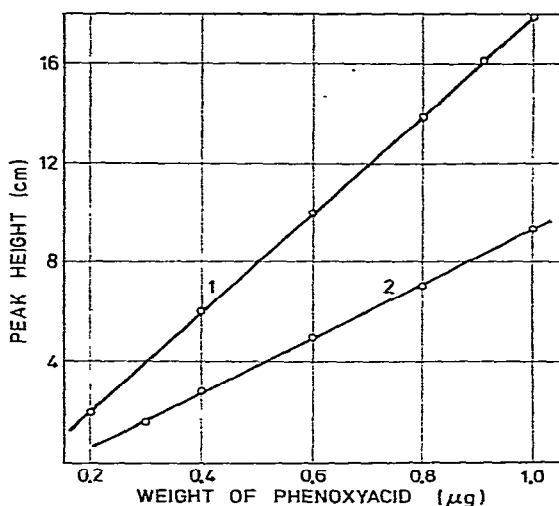darkening of the sample during esterification, an effect not observed when pure

Fig. 2. Calibration graph prepared by esterification of standard amounts of phenoxy acids and injection of 2 μl of isooctane solution. 1 = MCPA; 2 = 2,4-D.

phenoxy acids were used. Therefore, we amended the isolation of phenoxy acid residues by employing sorption on Amberlite XAD-4. A similar method was described by Niederschulte and Ballschmiter[21] for the isolation of 2,4-D and 2,4,5-T (25 μg in deionized water).

The latter method[21] was followed strictly and was checked on 1-1 samples of deionized water containing 0.4 μg each of 2,4-D and MCPA. Recoveries exceeding 92% for each of the herbicides and clean chromatograms (Fig. 1B) were obtained, confirming the suitability of this method of isolation. In order to carry out a statistical analysis, natural water (Oława River) that initially did not contain 2,4-D and MCPA, to which 0.4 μg of each of the phenoxy acids were added, was subjected to the procedure. The residues retained in the column were eluted with acetone as recommended by Niederschulte and Ballschmiter[21]. The level of the simultaneously eluted impurities was so high that the chromatograms obtained were unclear and the recovery was variable. Esterification of residues at room temperature with additional clean-up of the esters on a column containing silica gel did not improve the results.

On the other hand, better results were obtained by elution of the residues from an Amberlite XAD-4 column with benzene, which, being less polar, eluted much smaller amounts of impurities. The solution of isooctane esters obtained nevertheless needed a further clean-up on a silica gel column in order to obtain clean chromatograms (Fig. 1C).

The statistical results are given in Table III. It can be seen that the very low detection limit makes the proposed method superior to other procedures for determining 2,4-D and MCPA. It is characteristic that the detection limit for the 2,2,2-trichloroethyl ester of MCPA (0.15 ng) is lower than that for the 2,4-D ester (0.24 ng), in contrast to other esters (e.g., methyl and 2-chloroethyl) which give detection limits for MCPA many times greater than those for 2,4-D. Hence, the proposed method is especially interesting for the determination of MCPA residues; moreover, it could

TABLE III

CHROMATOGRAPHIC PROPERTIES OF 2,2,2-TRICHLOROETHYL ESTERS AND THE RECOVERY OF THE METHOD

For chromatographic conditions, see Experimental.

Parameter	Acid	
	MCPA	2,4-D
Retention time (min)	10	13
Retention time relative to aldrin = 100	137	178
Detection limit (ng)	0.15	0.24
Detection limit (ppb)	0.060	0.096
Mean recovery (%)	87.8	92.1
Standard deviation for $n = 6$ (%)	2.1	3.4

be suitable for the determination of other phenoxyalkanecarboxylic acid residues (mecoprop, dichlorprop, MCPB, 2,4-DB and others). This method for the determination of phenoxyalkanecarboxylic acid residues might be applied not only to water, but also (after further development) to other biological materials, its advantage being that only small samples are required. This aspect is being studied further.

REFERENCES

1 S. Goren-Strul, H. F. W. Kleijn and A. E. Mostaert, Anal. Chim. Acta, 34 (1966) 322.
2 D. E. Clark, F. L. Wright and L. M. Hunt, J. Agr. Food Chem., 15 (1967) 171.
3 I. C. Cohen, J. Norcup, J. H. A. Ruzicka and B. B. Wheals, J. Chromatogr., 49 (1970) 215.
4 L. L. Sullivan, J. M. Eldridge and J. B. Knak, J. Agr. Food Chem., 15 (1967) 927.
5 J. N. Seiber, D. G. Crosby, H. Fouda and C. J. Soderquist, J. Chromatogr., 73 (1972) 89.
6 M. L. Beall, E. A. Woolson, T. J. Sheets and C. J. Harris, J. Agr. Food Chem., 15 (1967) 208.
7 J. R. Duffy and P. Shelfoon, J. Ass. Offic. Anal. Chem., 50 (1967) 1098.
8 J. M. Devine and G. Zweig, J. Ass. Offic. Anal. Chem., 52 (1969) 187.
9 D. E. Clark and J. S. Palmer, J. Agr. Food Chem., 19 (1971) 761.
10 S. F. Howard and G. Yip, J. Ass. Offic. Anal. Chem., 55 (1972) 1031.
11 C. A. Bachne, L. E. St. John and D. J. Lisk, Anal. Chem., 40 (1968) 1241.
12 B. Kostowska, Ph.D. Thesis, Technical University of Wroclaw, 1971.
13 D. W. Woodham, W. G. Mitchell, C. D. Loftis and C. W. Collier, J. Agr. Food Chem., 19 (1971) 186.
14 C. E. McKone and R. J. Hance, J. Chromatogr., 69 (1972) 204.
15 F. C. Wright, J. C. Riner and B. N. Gilbert, J. Agr. Food Chem., 17 (1969) 1171.
16 D. S. May, J. E. Hindin and G. H. Dunstone, Eng. Bull. Purdue Univ. Eng. Ext. Ser., No. 115 (1963) 321.
17 S. Witek and S. Mierzwa, Pestycydy (Warsaw), No. 3 (1974) 241.
18 R. V. Smith and S. L. Tsai, J. Chromatogr., 61 (1971) 29.
19 D. C. Abbott, H. Egan, E. W. Hammond and J. Thomson, Analyst (London), 89 (1964) 480.
20 T. Bogacka and R. Taylor, Chem. Anal. (Warsaw), 15 (1970) 143.
21 U. Niederschulte and K. Ballschmiter, Z. Anal. Chem., 269 (1974) 360.