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DETERMINATION OF PHENOXY CARBOXYLIC ACID PESTICIDES BY GAS AND LIQUID CHROMATOGRAPHY

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SUMMARY

Various methods for the chromatographic determination of carboxylic acids are described and compared. These include gas chromatography (GC) after pentafluorobenzylation, high-performance liquid chromatography (HPLC) of the underivatized compounds in the ion-suppression and in the ion-pair mode and HPLC of naphthacyl and methylmethoxycoumarin esters.

The GC method has an excellent sensitivity and all the derivatives can be separated on one of the two columns used. A big disadvantage is the fact that many side-products are formed during the derivatization.

The underivatized acids can all be separated by HPLC and this method is very easy to perform, but the sensitivity is poor and the wavelength of detection is not specific.

With the naphthacyl esters the sensitivity is increased by a factor of 10–20 and with the methylmethoxycoumarin esters the sensitivity is increased by a factor of 5–10. The latter reagent has the advantages that it forms the lowest number of side-products and permits a rather specific detection.

INTRODUCTION

For the determination of phenoxy carboxylic acid pesticides such as 2,4-dichlorophenoxyacetic acid (2,4-D), 2-(2,4-dichlorophenoxy)propionic acid (2,4-DP), 2-methoxy-3,6-dichlorobenzoic acid (dicamba), 2-(2,4,5-trichlorophenoxy)propionic acid (phenoprop), 2-methyl-4-chlorophenoxyacetic acid (MCPA), 2-(2-methyl-4-chlorophenoxy)propionic acid (mecoprop), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2,3,6-trichlorobenzoic acid (TBA), 4-(2-methyl-4-chlorophenoxy)butyric acid (MCPB) and 4-chlorophenoxyacetic acid mainly gas chromatography (GC) with electron capture detection (ECD) is used. However, because of the high polarities of these compounds they first have to be derivatized. A number of derivatization procedures have been described, such as methylation with diazomethane or methanol–boron trifluoride and also chloroethylation, trichloroethylation or pentafluorobenzylation[1]. Methylation has been the method of choice for a number of years because the

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reaction is rather simple with few side-products. Because of the greater sensitivities now needed, reagents are chosen that increase the ECD responses of the compounds to be determined. A number of studies have been carried out to determine which derivative gives the highest increase in sensitivity without the formation of too many side-products[2–4], and in recent years the determination of phenoxycarboxylic acids after pentafluorobenzylation has been reported[5–7].

For the determination of highly polar compounds like these carboxylic acids, high-performance liquid chromatography (HPLC) is much more suitable than GC. The underivatized acids can be very well separated in either the ion-suppression or the ion-pair mode, but the sensitivity can be a problem[8–10]. The sensitivity in HPLC could possibly be improved by labelling the free acids with a suitable chromo- or fluorophore. A number of reagents have been used for this purpose, *e.g.*, 2-naphthacyl bromide (α-bromo-2'-acetonaphthone)[11] and 4-bromomethyl-7-methoxycoumarin[12].

In order to be able to make the best choice for the final step in a procedure for the determination of phenoxy carboxylic acids in plant materials and environmental samples, various methods, *e.g.*, GC after pentafluorobenzylation and HPLC of the free acids or after derivatization, were studied and evaluated with respect to sensitivity, selectivity and ease of operation.

EXPERIMENTAL

Reagents

The carboxylic acids studied were obtained as analytical standards from the manufacturers and were used without further purification. Pentafluorobenzyl bromide (PFBB) was obtained from ICN Pharmaceuticals (Plainview, NY, U.S.A.), 2-naphthacyl bromide (NPB) from Aldrich-Europe (Beerse, Belgium), 4-bromomethyl-7-methoxycoumarin (MMC) from Fluka (Buchs, Switzerland) and caesium carbonate "reinst" from E. Merck (Darmstadt, G.F.R.). Hexadecyltrimethylammonium bromide (cetrimide), K_2HPO_4 and NaH_2PO_4, all reagent grade, were obtained from Baker (Deventer, The Netherlands). All solvents used were of p.a. grade (Merck) and distilled water was used throughout.

Cetrimide was dissolved in methanol to a concentration of 0.03 *M*, K_2HPO_4 and NaH_2PO_4 were dissolved in water, both to a concentration of 0.25 *M*. Appropriate volumes of these stock solutions of cetrimide and phosphate were mixed with methanol and water to give eluents with the desired concentrations of cetrimide and phosphate. After mixing, the eluents were passed through a 1-μm filter and deaerated ultrasonically.

Derivatization procedure

To 1 ml of a solution of an acid in acetone (100 μg/ml) were added 1 ml of a solution of one of the derivatizing agents in acetone (2–10 mg/ml) and 5–10 mg of caesium carbonate, after which the solution was placed in a water-bath in the dark at 35°C for 45 min. After derivatization this solution was either injected directly onto the gas or liquid chromatograph, or the solvent was evaporated with a stream of nitrogen and the residue redissolved in a suitable solvent. Alternatively the reaction was stopped by adding 100 μl of glacial acetic acid after which the volume was made up to about 5 ml with water and this mixture was extracted twice with 5 ml of light

petroleum (b.p. 40–60°C). The extract was then evaporated to dryness and the residue redissolved in a suitable solvent.

For the preparation of the derivatives on a larger scale, 100 mg of acid and 400 mg of derivatizing agent were dissolved in 100 ml of acetone, caesium carbonate was added to saturation and the mixture was allowed to stand at room temperature for 2 h with occasional shaking.

The pentafluorobenzyl esters were purified by means of partitioning between light petroleum and water. For the purification of the naphthacyl esters, column chromatography on silica gel with toluene as a solvent was used and the methylmethoxycoumarin esters were purified on a column of silica gel, using a mixture of acetone and light petroleum as a solvent.

All compounds yielded a single peak when injected into the gas or liquid chromatograph and their structures were confirmed by IR spectroscopy.

Gas chromatography

A Hewlett-Packard 5880 A gas chromatograph, equipped with a capillary injection system and a ^{63}Ni electron capture detector was used. The glass capillary columns (40 m × 0.3 mm I.D.) were coated dynamically with SE-30 or Superox 04. The injector and detector temperatures were 250°C and after an initial hold of 1 min at 80°C the oven temperature was programmed from 80 to 250°C at a rate of 15°/min with a final hold of 15 min at 250°C. The carrier gas was nitrogen with a column head pressure of 15 p.s.i. The splitless injection technique was used with a purge activation time of 0.4 min and an injection volume of 1 μl.

High-performance liquid chromatography

A component system was used, consisting of a Varian 8500 solvent-delivery system, a Valco loop injector and a Waters 440 UV absorbance detector or a Waters 420 fluorescence detector. The column (15 cm × 4.6 mm I.D.), packed with Hypersil ODS (Shandon Southern, Runcorn, Great Britain), was operated at ambient temperature with a flow-rate of 1 ml/min. The compositions of the solvents used are given in the appropriate tables.

UV spectra

UV spectra were recorded in 65% (v/v) methanol in water with 1% (v/v) acetic acid for the underivatized acids and in 80% (v/v) methanol in water for the derivatives in a 1-cm cell on a Perkin-Elmer 570 spectrophotometer.

RESULTS AND DISCUSSION

Gas chromatography

The pentafluorobenzyl derivatives of the various acids gave symmetrical peaks on the non-polar SE-30 column and on the polar Superox 04 column. Their relative retention times are given in Table I from which it is seen that all the derivatives can be separated on one of the two columns. The sensitivity proved to be excellent; a few pg of each compound could easily be detected. When the reaction mixture of the micro preparation was injected directly into the gas chromatograph many interfering peaks occurred and repeated injections led to severe contamination of the injection port. So a rigorous clean-up is necessary before a quantitation can be carried out.

TABLE I

RETENTION TIMES OF PENTAFLUOROBENZYL ESTERS OF CARBOXYLIC ACIDS, RELATIVE TO THOSE OF THE DICAMBA ESTER ON TWO DIFFERENT CAPILLARY COLUMNS

Acid	*SE-30 column*	*Superox 04 column*
Trichloroacetic acid	0.49	0.39
4-Chlorophenoxyacetic acid	0.96	1.06
Mecoprop	0.97	0.95
Dicamba	1.00	1.00
MCPA	1.00	1.09
TBA	1.02	1.05
2,4-DP	1.02	1.06
2,4-D	1.05	1.22
Phenoprop	1.11	1.15
MCPB	1.16	1.25

High-performance liquid chromatography

In order to optimize the chromatographic conditions for separation of the various underivatized acids, their capacity factors were determined on a C_{18} column in the ion-suppression mode and in the ion-pair mode with various concentrations of pairing ion and counter ion. The conditions and results are given in Table II. The elution order in the ion-suppression mode is different from that in the ion-pair mode. In the ion-pair mode the concentrations of pairing ion and counter ion influence the capacity factors of the various acids in different ways, although these differences are

TABLE II

CAPACITY FACTORS, k', FOR UNDERIVATIZED ACIDS WITH VARIOUS SOLVENTS ON A REVERSED-PHASE (C_{18}) COLUMN

Solvent systems: I, methanol–water (65:35 v/v), 1% (v/v) glacial acetic acid; II, methanol–water (75:25 v/v), 0.001 *M* PO_4^{3-}, 0.005 *M* cetrimide; III, methanol–water (75:25 v/v), 0.01 *M* PO_4^{3-}, 0.005 *M* cetrimide; IV, methanol–water (75:25 v/v), 0.01 *M* PO_4^{3-}, 0.001 *M* cetrimide; V, methanol–water (75:25 v/v), 0.005 *M* PO_4^{3-}, 0.005 *M* cetrimide; VI, methanol–water (75:25 v/v)); 0.005 *M* PO_4^{3-}, 0.001 *M* cetrimide. The PO_4^{3-} concentration is the sum of equal concentrations of KH_2PO_4 and Na_2HPO_4.

Compound	*k'*					
	I	*II*	*III*	*IV*	*V*	*VI*
TBA	0.41	3.31	2.35	1.11	2.77	1.00
Dicamba	0.76	3.38	2.42	1.11	2.77	1.00
4-Chlorophenoxyacetic acid	1.00	3.59	2.60	1.29	2.94	1.19
2,4-D	1.88	5.69	4.52	2.20	5.23	1.91
MCPA	2.12	5.19	4.05	2.09	4.71	1.80
2,4-DP	3.00	7.12	5.64	2.89	6.43	2.44
2,4,5-T	3.24	8.81	7.33	3.63	8.43	3.06
Mecoprop	3.29	6.75	5.22	2.71	6.09	2.28
MCPB	5.00	7.84	6.22	3.40	7.00	2.86
Phenoprop	5.41	11.00	9.00	4.71	10.90	3.89

not as pronounced as they are for nitrophenols[10]. In the ion-suppression mode all acids studied can be separated in a single chromatographic run, except mecoprop and 2,4,5-T, but these two can be separated completely in an ion-pair system. The ion-pair system can also be used for confirmation purposes and it has the advantage that it can be changed rather easily to optimize a particular separation.

A potential disadvantage of pre-column derivatization is that the differences in retention behaviour of various compounds can become smaller upon derivatization. Therefore the capacity factor of the underivatized acids and their naphthacyl (NP) and methylmethoxycoumarin (MMC) derivatives were determined in a reversed-phase system (Table III). The differences in capacity factors have indeed become smaller; the derivatives cannot be separated in a single chromatographic run, but the capacity factors are still different from each other, so that each compound can be identified. No capacity factor for the MMC derivative of 4-chlorophenoxyacetic acid is given, because this ester is formed in only very small amounts.

TABLE III

CAPACITY FACTORS, k', OF UNDERIVATIZED ACIDS AND THEIR NAPHTHACYL (NP) AND METHYLMETHOXY COUMARIN (MMC) ESTERS IN A REVERSED-PHASE SYSTEM

Acid	k'		
	Underivatized 65% (v/v) methanol	*NP derivative 80% (v/v) methanol*	*MMC derivative 80% (v/v) methanol*
TBA	0.41	4.35	2.67
Dicamba	0.76	3.32	2.25
4-Chlorophenoxyacetic acid	1.00	2.16	–
2,4-D	1.88	3.45	1.83
MCPA	2.12	3.45	1.92
2,4-DP	3.00	5.23	2.33
Mecoprop	3.29	5.06	2.83
MCPB	5.00	3.81	3.58
Phenoprop	5.41	8.16	3.42

The UV spectra of the underivatized acids and of their derivatives were recorded in order to establish the optimum detection wavelength and to determine the increase in sensitivity which could be obtained. The results for 2,4-DP are given in Figs. 1–3. From these spectra the optimum detection wavelengths can be deduced, being 280 nm for the underivatized acids, 250 nm for the NP derivatives and 330 nm for the MMC derivatives. Because a much better signal-to-noise ratio can be obtained with a fixed-wavelength detector than with a variable-wavelength detector, even when the measuring wavelength is not the wavelength of maximum absorption, the minimum detectable quantities of some underivatized acids and their derivatives were determined with a fixed-wavelength detector. The results, given in Table IV, show that with the NP ester the sensitivity is increased by a factor of 10–20 and with the MMC ester by a factor of 5–10.

The MMC esters of various acids have been shown to show a reasonable fluorescence[11] and therefore fluorescence detection of these derivatives was compared to UV detection. Fluorescence detection was found to be a little less sensitive than UV

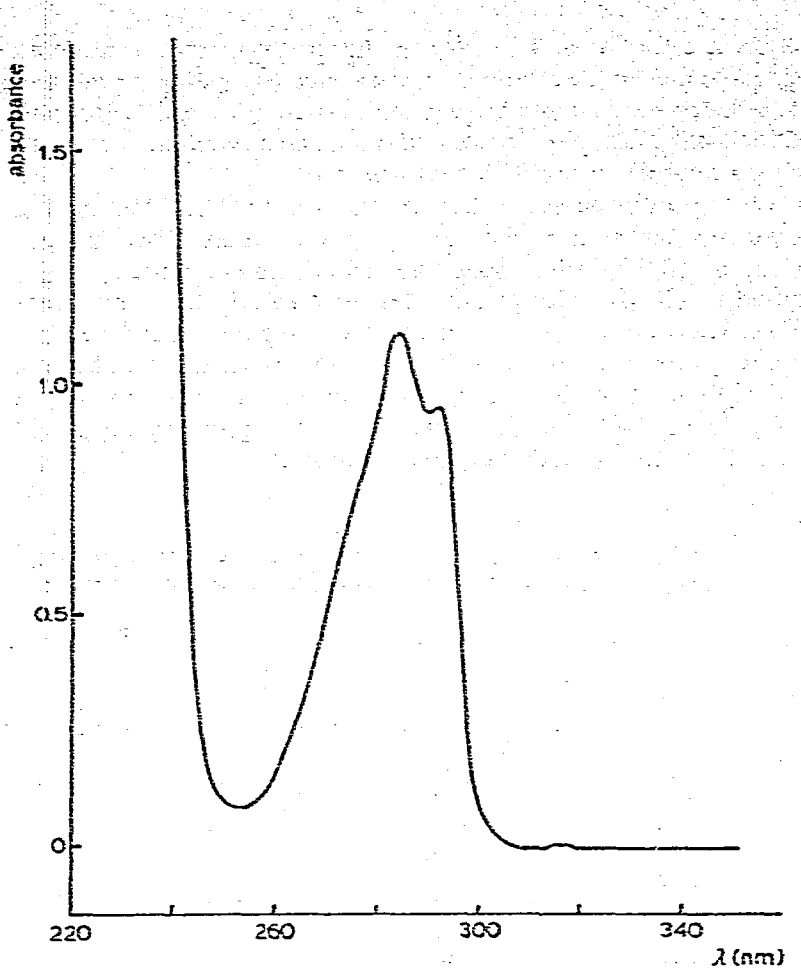

Fig. 1. UV absorption spectrum of underivatized 2,4-DP. Concentration: $60 \cdot 10^{-5}$ *M*.

detection, but it should be kept in mind that the UV detector used is probably the best available absorbance detector, while the fluorescence detector used is a very simple instrument. A more sophisticated fluorescence detector would probably give much better results.

With the aid of the synthesized standards, the yield of the derivatization procedure was determined for a number of acids at a concentration of 10 μg/ml and the results are given in Table V. For most compounds the yield is quite satisfactory, except for 4-chlorophenoxyacetic acid which is obtained in less than 10% yield with pentafluorobenzyl bromide. With naphthacyl bromide the yield seems to be more than 100% for some compounds, and for MCPA the yield could not be determined owing to the presence of a fairly high number of peaks in the chromatogram of the reagent blank.

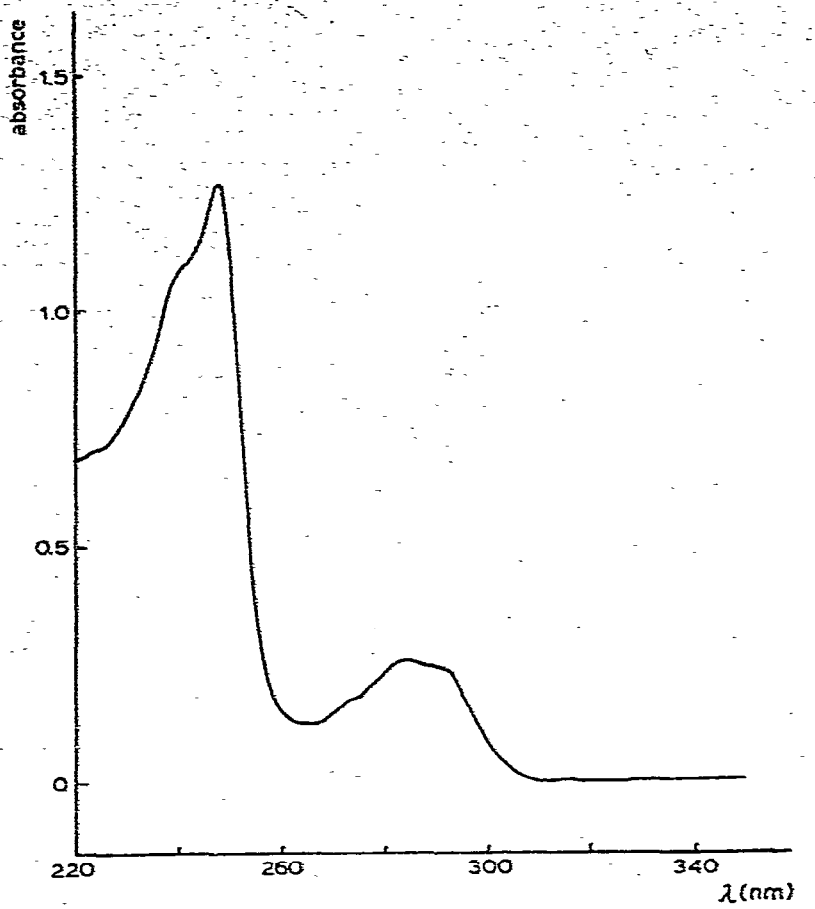

Fig. 2. UV absorption spectrum of the naphthacyl ester of 2,4-DP. Concentration: $2 \cdot 10^{-5}$ *M*.

CONCLUSIONS

When the various methods for the determination of these carboxylic acids are compared it is not obvious which method is the best, but the methylmethoxycoumarin derivative looks the most promising. The reaction can be carried out very easily, the yield is high with little formation of side-products and a sensitive and selective detection is possible. Although the underivatized acids can all be well separated by HPLC, this method has the disadvantage that the sensitivity is poor and that the wavelength of detection is not specific. The pentafluorobenzyl and the naphthacyl esters show a better sensitivity than the methylmethoxycoumarin ester, but with these two reagents so many side-products are formed that a clean-up has to be carried out before the chromatographic determination.

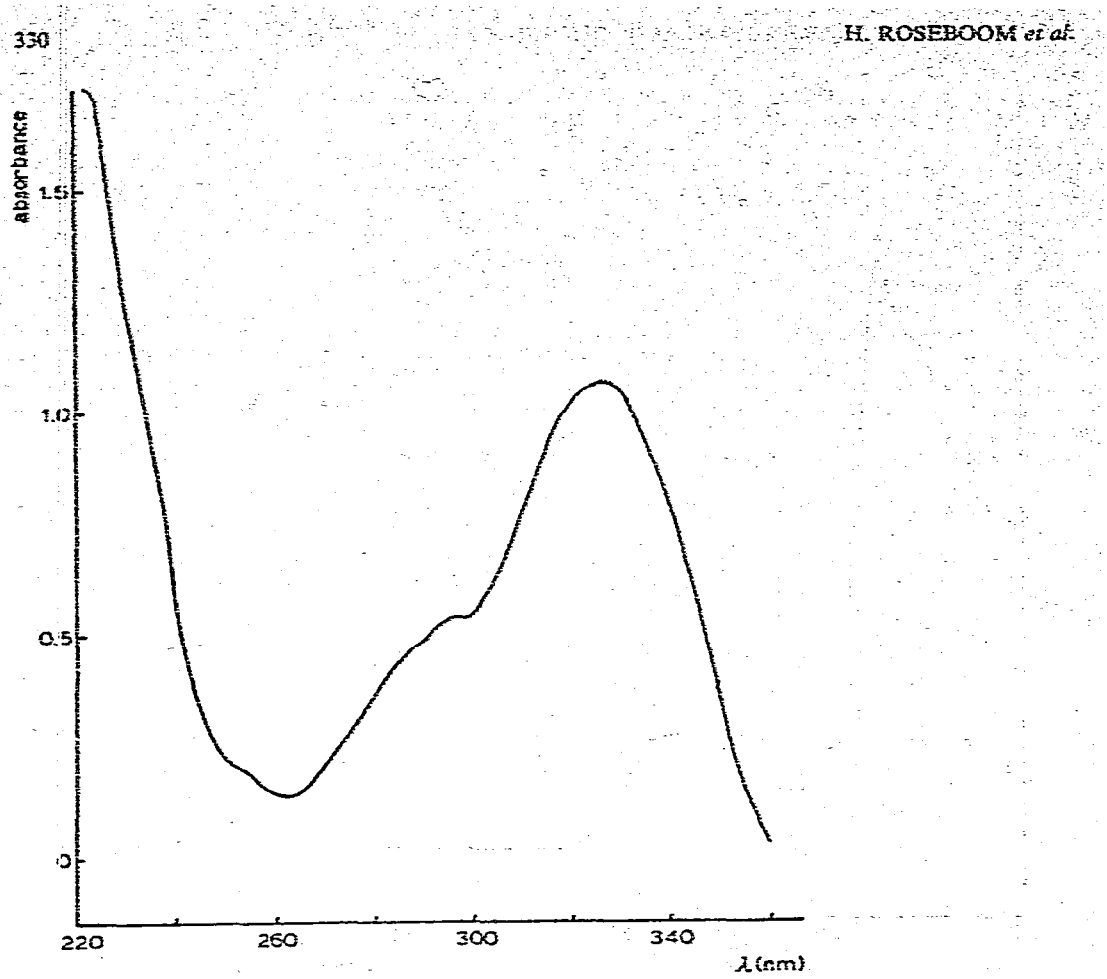

Fig. 3. UV absorption spectrum of the methylmethoxycoumarin ester of 2,4-DP. Concentration: $6 \cdot 10^{-5}$ *M*.

TABLE IV

MINIMUM DETECTABLE QUANTITIES (IN NG) OF VARIOUS ACIDS AND THEIR DERIVATIVES WITH A FIXED-WAVELENGTH DETECTOR

Acid	*Underivatized (280 nm)*	*NP ester (254 nm)*	*MMC ester (340 nm)*
Dicamba	5	0.2	0.5
4-Chlorophenoxyacetic acid	2	0.2	—
MCPA	3	0.2	0.5
2,4-DP	3	0.3	0.5
MCPB	3	0.3	0.7

TABLE V

YIELD OF THE DERIVATIZATION PROCEDURE FOR VARIOUS ACIDS WITH PENTAFLUOROBENZYL BROMIDE (PFBB), NAPHTHACYL BROMIDE (NPB) AND METHYLMETHOXYCOUMARIN (MMC)

Acid	*Yield (%)**		
	PFBB	*NPB*	*MMC*
4-Chlorophenoxyacetic acid	<10	< 10	<10
Dicamba	83	74	92
TBA	88	103	90
MCPB	<10	60	89
2,4-DP	76	103	93
2,4-D	91	99	84
MCPA	87	—**	88

* Each value is the mean from three determinations.
** Not determined, due to interferences.

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